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PREFACE

THE SYMPOSIUM

A total of 208 participants from 78 organizations in 19 countries gathered at the Waikoloa Beach Marriott on the Big Island of Hawaii on September 11-16, 2011 for the XIII International Symposium on Biological Control of Weeds. Following a reception on the first evening, Symposium co-chairs Tracy Johnson and Pat Conant formally welcomed the attendees on the morning of September 12, and introduced Bill Steiner, Dean of the College of Agriculture, Forestry and Natural Resource Management, University of Hawaii at Hilo, who provided opening remarks on future directions of invasive species control in Hawaii and the world. The Symposium keynote address, "Finding the weapons of biomass destruction – identifying potential biocontrol agents by applying principles of chemical coevolution," was delivered by May Berenbaum, Department of Entomology, University of Illinois (Urbana-Champaign).

The Symposium's scientific program included a total of 85 oral and 135 poster presentations organized around nine themes, plus five evening workshops (Table 1). The program was designed to focus on emerging issues affecting invasive plant biocontrol globally and allow colleagues to update one another on specific projects. Our Hawaii venue also provided a unique opportunity to take stock of a century of biocontrol in these islands and begin to build new collaborations to serve the Pacific region. The organizers focused particularly on connecting Hawaii natural resource managers with international biocontrol specialists and raising awareness of Pacific island weeds as potential targets for research.

Table 1. Scientific Program and organizers

Sessions	Organizers
1. Pre-Release Testing of Weed Biological Control Agents	Greg Wheeler
2. Emerging Issues in Regulation of Biological Control	Sharlene Sing
3. Non-Traditional Biological Control Agents	John Goolsby
4. Target and Agent Selection	S. Raghu
5. Prospects for Weed Biological Control in Pacific Islands	Tracy Johnson
6. Integrating Biocontrol and Restoration of Ecosystems	Ted Center
7. Ecological and Evolutionary Processes	Jianqing Ding
8. Social and Economic Assessments of Biological Control	Keith Warner & Martin Hill
9. Post-Release Evaluation and Management	Paul Pratt
Workshops	
1. Is Classical Biocontrol an "Old Science" Paradigm Losing its Way?	Andy Sheppard
2. The International Convention on Protecting Endemic Biodiversity	Dick Shaw & Hugh Gourlay
3. Wild Gingers (<i>Hedychium</i> spp.)	Djami Djeddour
4. Best Management Practices for Communication of Weed Biocontrol	Keith Warner & Darcy Oishi
5. Biological Control of Fireweed: Past, Present and Future Directions	Andy Sheppard & Mohsen Ramadan

Mid-Symposium tours featured natural history and weed biocontrol of the north and south of the Big Island. The North tour ascended the saddle between Mauna Kea and Mauna Loa volcanoes for a hike up the kipuka Pu'u Huluhulu, a biologically diverse, koa-tree forested cinder cone isolated from the surrounding ecosystem by lava flows in 1843 and 1935. Stops were made at two additional kipuka along the Saddle Road to highlight biocontrol efforts against fireweed and gorse and to view native forest plants and birds before descending into Hilo. North tour participants were dropped off at Hawaii Tropical Botanical Garden where they walked to the shoreline for lunch overlooking spectacular Onomea Bay. A hike to view Akaka Falls was the afternoon highlight of the North tour.

The South tour began with a stop at Amy Greenwell Ethnobotanical Garden before proceeding to Punaluu Black Sand Beach and an opportunity to view resident sea turtles. South tour participants then rode to Hawaii Volcanoes National Park where they lunched and hiked along the Kilauea caldera rim to Thurston Lava Tube. South tour buses returned to Waikoloa via Hilo and the Saddle Road, allowing participants to also experience the sights of windward rainforests and Pu'u Huluhulu. Both tour groups met for the last stop of the day on the outskirts of Waikoloa to view an example of successful biological control for species conservation: statewide die-off of the archetypal native Hawaiian dry forest tree wiliwili, *Erythrina sandwicensis*, caused by the 2005 invasion of African eulophid gall wasp *Quadrastichus erythrinae*, has been halted by the introduction in 2008 of the parasitic wasp *Eurytoma erythrinae*.

Symposium presentations ended on the afternoon of September 16, and awards were made for best student talks (winner Ikju Park and runner-up Haley Catton) and posters (winner Wyatt Williams and runner-up Andrea Stephens). The XIII International Symposium on Biological Control of Weeds closed with an evening lu'au featuring traditional and modern island cuisine and Polynesian music and dances performed as the sun set over Anaeho'omalū Bay.

Acknowledgements

The organizers wish to express their sincere gratitude to our Symposium sponsors: USDA Forest Service (including the Pacific Southwest Research Station, Institute of Pacific Islands Forestry; and International Programs); Hawaii Department of Agriculture; Hawaii County Department of Research and Development; Hawaiian Electric Company and Hawaii Electric Light Company; US Fish & Wildlife Service, Pacific Islands Office; USGS Pacific Island Ecosystems Research Center; Landcare Research; Hawaii Forest and Trail; Destination Hilo; Big Island Invasive Species Committee; Maui Invasive Species Committee; and University of Hawaii at Hilo Conference Center. Thanks also to exhibitor CABI.

The organizers are grateful also to the many individuals who contributed to the success of the Symposium.

Program Committee

Tracy Johnson (Chair), Ted Center, Jianqing Ding, John Goolsby, Paul Pratt, S. Raghu, Sharlene Sing, Keith Warner, and Greg Wheeler; and reviewers of abstracts, Pat Conant, Hugh Gourlay, Rich Hansen, Richard Hill, Judy Hough-Goldstein, Ruth Hufbauer, and Link Smith.

Organizing Committee

Tracy Johnson and Pat Conant (Co-Chairs), Franny Kinslow, Hugh Gourlay, George Markin, and the staff of the University of Hawaii at Hilo Conference Center: Judith Fox-Goldstein, Mary Ann Tsuchiyama, Jules Ung, Sharay Uemua, Connie Larsen, Alberta Mehau-Matsu, Robin Black, Kristy Uemura and Kelci Meguro.

Local Arrangements

Renato Bautista, Pat Bily, Adrian Boone, Beverly Brand, Sean Callahan, Vickie Caraway, Stacey Chun, Dave Faucette, Betsy Gagne, Jim Gale, Dean Gallagher, Janis Garcia, Jacqueline de la Garza, Fritz Grevstad, Rob Hauff, Stephen Hight, Richard Hill, Clyde Hirayama, Roger Imoto, Mann Ko, Paul Krushelnycky, Jackie Kozak Thiel, Linda Larish, Christy Martin, Bob Masuda, Shin Matayoshi, Kuponon McDaniel, Walter Nagamine, Darcy Oishi, Jimmy Parker, Bobby Parsons, Lyman Perry, Mohsen Ramadan, Neil Reimer, Brent Sheehan, Mariza Silva and the Hawaii Conservation Alliance, Dean Takabayashi, Ken Teramoto, Marcos Vallejo, Peter Van Dyke, Juliana Yalamar, and Aileen Yeh, as well as Gale Kihoi and all the staff at the Waikoloa Beach Marriott.

Judges for Student Awards

Fritzi Grevstad, Ronny Groenteman, Ruth Hufbauer, John Ireson, Alec McClay and Brian Rector.

Proceedings Committee

Yun Wu, Tracy Johnson, George Markin, Richard Reardon, and Sharlene Sing.

THE NEXT SYMPOSIUM

Delegates voted to return to South Africa for the XIV International Symposium on Biological Control of Weeds. Martin Hill, Fiona Impson and colleagues will convene us next in Kruger National Park in March 2014 to coincide with celebrations in 2013 of the centenary of weed biological control in South Africa.

THE PROCEEDINGS

There are a total of 224 presentations (Table 2) including 36 papers, 183 abstracts, and five workshop summaries in these *Proceedings*, grouped into ten chapters in accordance with the nine sessions and the five workshops at the Symposium (Table 1).

Thanks to Mic Julien, René Sforza and Chuck Benedict for providing advice on submission guidelines; and Tracy Johnson, George Markin, Sharlene Sing, and Richard Reardon for reviewing/revising the guidelines.

Thanks to all the session organizers (Table 1) for their assistance in manuscript collection.

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Yun Wu, Managing Editor
Morgantown, West Virginia, USA
June, 2012

Table 2. Summary of attendance and proceedings of symposia to date

Symposium Details			Attendance numbers by			Number of papers and abstracts in the proceedings
No.	Date	Location	Countries	Participants	Organizations	
I	1969	Delémont, Switzerland	11	22	15	21
II	1971	Rome, Italy	9	37	17	23
III	1973	Montpellier, France	11	25	14	16
IV	1976	Gainesville, FL, USA	11	84	42	45
V	1980	Brisbane, Australia	11	100	52	68
VI	1984	Vancouver, Canada	13	135	59	96
VII	1988	Rome, Italy	20	128	60	96
VIII	1992	Canterbury, New Zealand	18	181	80	139
IX	1996	Stellenbosch, South Africa	25	202	91	165
X	1999	Bozeman, MT, USA	27	308	115	226
XI	2003	Canberra, Australia	20	175	60	177
XII	2007	La Grande Motte, France	32	250	106	226
XIII	2011	Waikoloa, HI, USA	19	208	78	224

Note: Data for I-VII from Proceedings of the VII International Symposium on Biological Control of Weeds, ed. by E. S. Delfosse (1988). Proceedings of Symposia I-XII can be found at: <http://www.invasive.org/proceedings/>; CDs are also available from USDA Forest Service-FHTET (contact Richard Reardon at rreardon@fs.fed.us or Yun Wu at ywu@fs.fed.us).

SYMPOSIUM WELCOME

E komo mai (Welcome) to the XIII International Symposium on Biological Control of Weeds and the beautiful Kohala coast of the Big Island of Hawaii! Extraordinary cultural and natural diversity in Hawaii, and its long history in weed biocontrol, make these islands an ideal site for reflection and discourse on the past, present and future of our field.

Our goal during this symposium has been to help colleagues reconnect, share experiences and plan future collaborations as we examine emerging issues that affect invasive plant management across the globe. This symposium also provided a unique opportunity to take stock of a century of biological control in the Pacific, where our modern history of weed biocontrol began with Albert Koebele and his 1902 introductions for lantana biological control in Hawaii. Looking into the future, the wonderful biodiversity and people of Pacific islands face overwhelming threats, with invasive plants prominent among them. We hope an enduring outcome of this symposium will be new connections between the international community of weed biocontrol specialists and our islands' natural resource managers and scientists, enabling new collaborations that will serve the Pacific region in years to come.

Aloha!

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Opening Address: The Future Challenges of Invasive Species Work

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Introduction

We begin this international gathering of biological control and invasive species experts in high hopes to gain new knowledge, learn about new approaches and technologies, and discover success stories such as described in Asner et al. (2008) that might bolster our own desires to do good. But it would behoove us to also keep a clear eye on the future and to rising conditions that will impact us perhaps in ways unseen. Even though I believe we have enjoyed remarkable success over the past three decades in terms of knowledge learned (if not so much as numbers of species controlled – we are dealing with Nature after all), we still have a long way to go as suggested by the topics addressed at this meeting. So as introduction, let me broach here the topic of special and recently developing challenges we face in an increasingly uncertain future. These may form directions for further research in the future.

Challenge 1. Food and Fuel Insecurity

Since the 1980s, invasive species have been increasingly recognized as a threat to native and indigenous environments, enabling the focus brought by publications like Pimentel et al. (2005). The rapidity at which invasions have been mounted is a direct correlate to the growth of human travel and trade around the globe (Perrault et al., 2003). The last three decades have seen a success in terms of educating and motivating a generation of new scholars and researchers to enter the field in part because the problems brought by invasive species are so interesting, now so widely known, and their impacts so widely touted. But with the increasing challenges brought by these species, comes also changes in society, culture, technology and economics that offer new hope but also new problems. Today, modern societies are themselves challenged by a growing myriad of problems, not the least of which is the continuing spread of alien invasive species.

The growing need for food and fuel security brought on by increasing population, declining petroleum reserves and political unrest serves as major reasons for economic uncertainty. This is summarized in the following statement made by Nobuo Tanaka, CEO of the International Energy Agency (IEA). He was discussing the renewed debate on nuclear energy saying it could have an impact, not only on climate change but also energy security. “The age of cheap energy is over,” Mr. Tanaka said, speaking at the Bridge Forum Dialogue in Luxembourg on 13 April, 2011. “The only question now is, will the extra rent from dearer energy go to an ever smaller circle of producers, or will it be directed back into the domestic economies of the consumers, with the added benefits of increased environmental sustainability?” I would point out, if it is the latter, we stand a chance at success in what we do. The World Energy Outlook, to be published by the IEA in November 2012, will summarize and underscore how serious the situation really is. Achieving projected needs for energy production will place an economic burden on society and direct funding away from invasive species work.

This is important to biological control and invasive species workers because increasing energy costs impact the field in four important ways: (a) by increasing the cost to do business, (b) by destabilizing the economies and countries in which the work has to be done, (c) by increasing reliance on energy crops some of which are invasive in their own right, and (d) by shifting attention away from the problem of invasive species in general. But it has a more insidious effect I discuss below – that of decreasing world trade as

commodity prices become too costly to allow food to be shipped abroad. This might come at a time when invasive species work is most needed but is least affordable.

In Hawaii, biofuel producers are looking at growing guinea grass (*Panicum maximum* Jacq.), elephant grass (*Pennisetum purpureum* Schumach.) and Barbados nut (*Jatropha curcas* L.), biofuel feedstocks that may be invasive in particular environments and especially Hawaii. Already hundreds of acres of Barbados nut have been planted, and guinea grass is a well known invader in Hawaii.

Challenge 2. Loss of Native Species Diminishes Capacity for Cultural Response in Failing Economies

If one were to examine most indigenous cultures prior to their discovery, one would find people who for the most part lived in balance with their environment. Technologies tended to be derived from what the environment had to offer and enable the culture to meet environmental challenges. Food production, if it existed, was highly localized.

This is scarcely the case today where sophisticated technologies drive economies and connect people, and where food may be shipped half way around the world to those who need it. We have moved over the past 10,000 years from cultures which interacted and depended on nearby natural environments to survive, to cultures that interact with each other and depend on their economic means of production in order to trade for what they want/need. This trade off, where culture depended on survival of local fisheries, local prey and locally grown foodstocks to one that depends on intellectual and technological constructs, has within a few generations reduced the value of Nature such that we no longer pay attention to its decline. But Nature does retain inherent value, though shifting. In his seminal paper Vitousek (1990) pointed out that not only were integrated studies of population and ecosystem studies involving alien invasive species called for, but we might expect to find altered properties of ecosystems where aliens had in fact invaded. This latter observation is an extremely important insight.

We have worried in Hawai'i about what to do if "...the ships stop coming." It is true that at one point in Hawaiian history and culture, hundreds of thousands of people were maintained by what was grown in Hawaii. Sophisticated field systems, irrigation systems, fish ponds and technologies were developed to do this. And ocean fisheries were still intact as well.

But since the early 1980s, Hawai'i has increasingly imported its food, until it now imports an estimated 85% (see analysis in Leung and Loke, 2008). The potential for disaster such dependence creates is not limited to the closed environment of these islands. Indeed, scenarios of apocalypse affecting global food production are on the rise, sparked in part by increases in global population, global drought and costs of energy and other inputs, and declines in available fertilizer and water, soil fertility and structure, arable land and more (e.g., see Andreas, 2010; Hogstrandt, 2011).

On top of this, Hawaii is the most energy-dependent state in the union; here 95% of its transportation and electrical energy base is imported (State of Hawaii Energy Resources Coordinator Annual Report, 2006, Department of Business, Economic Development and Tourism, State of Hawaii). Since 1980, the use of electricity on the Big Island of Hawaii alone has increased 2.4 times, with solar, geothermal and wind supplying much of the need in new energy (Davies et al., 2007). Residents of Hawaii pay the highest energy costs in the nation even with a sustainable geothermal source available to us.

The projections I mention have similar impacts on invasive species as Challenge 1 above. But additionally, if in fact costs to ship commodities around the globe become prohibitive, then local cultures may have to again rely on local ecosystems for sustenance and material to support technical innovation. Hawaii is a microcosm of what can happen. Here, the extinction of dozens if not hundreds of species with some 1,120 identified as species of concern, a situation driven by at least 5,138 invading alien species, is enhanced by development in sensitive geographical areas. Can Hawaii, and by comparison other ecosystems, truly return to a level of support of ecosystem services indigenous populations once expected? The answer is

probably no; given the Vitousek effect we can expect to have taken place with introduction of alien invasive species. We can determine the risk of introductions of new species (Daehler et al., 2004), but determining critical alterations in ecosystem function and how this knowledge can be used to offset impacts will also be necessary (Pejchar and Mooney, 2009).

Challenge 3. Motivating Public Opinion to Support Invasive Species Work

Some twelve years ago, Ricciardi et al. (2000) suggested that there existed a strong need to develop global information systems to better understand and share information about alien invasive species. Since then, development of such systems has indeed taken place and continues today. This has happened at a faster rate than was first anticipated. It has been helped by new technologies and by funding from surprisingly sources. Though the recent economic downturn in the US has caused budget cuts to some systems (the U. S. Geological Survey, Biological Resources Division, National Biological Information System is one), most have weathered the crisis fairly well. The need for these information systems is becoming more apparent as invasive species impacts on trade in an era of globalization become more apparent (Meyerson and Mooney, 2007). In fact, this recognition has probably been responsible for much of the global and World Bank funding that has arisen to support its development. It goes without saying that having information available at the fingertips of policy makers is extremely important to winning them over to support alien invasive species research and new approaches to biological control.

But we need to be on the lookout for new approaches as well. The formation of partnerships is one that cannot be mentioned enough. One example is the watershed partnerships formed in Hawaii. We have ten across the islands, composed variously of landowners, state agencies, federal agencies including the Department of Defense, and NGOs. There is room in such partnerships for private businesses and even corporations. Key is having face-to-face meetings where common goals can be recognized, priorities set and workforces mobilized. In these types of meetings, it is paramount to always invite policymakers where they not only can see the partnership at work, but they can be made to feel an important member in helping set priorities.

Broad inclusion of policy makers in science discussions, as advocated by Fleishman et al. (2011), gives a better appreciation of the problems at hand, and helps set clear priorities and responsibilities in the process of implementation, especially when resources are limited. The importance of social context should be emphasized here. This necessarily takes scientist out of the field where s/he is most comfortable, but it is a sacrifice that must be made because it puts a face on any challenge which the policy maker will come to recognize.

Hegamyer et al. (2003) suggest using volunteers to move partnerships in invasive species management forward from discussion to implementation, getting past the problem of paying for labor. Although not all implementation strategies might have room for use of volunteers, many will. The contributions made by these personnel should be tracked in order to demonstrate the importance of citizen inputs. This type of information will also be useful in obtaining matching grants, demonstrating to legislative bodies the interest of the voters and public at large, and attracting the attention of the media.

Challenge 4. Failure to Achieve Adequate and Sustainable Progress Suggests a Need for New Approaches

The problem of funding is one I anticipate will not go away in the future but may worsen instead for obvious reasons, some of which are discussed here. There is simply less money available at a time when society is faced at so many levels by so many different threats. Even in Hawaii, where we can point to a handful of successes on each island, the number of invasive species remaining is daunting, and new ones

arrive at a rate of one per month. Constraints on funding have hurt efforts in Hawaii, and undoubtedly impacted others as well.

This raises the question: can alien invasive species be turned into an economic advantage? For example, in Hawaii I am working on a project to turn invasive tree species into biochar, a soil supplement that could go a long way to helping bring back fertile and healthy soils in underutilized sugar cane fields that are now fallow. Biochar might be used in small, organic farm operations, in government re-seeding operations, and in backyard gardens. As the number of invasive trees is reduced, carbon can go into the soil thus reducing outputs of atmospheric carbon that contribute to global climate warming, and enhancing fertility of the soil to increase food production to benefit local economies. The job creation in this scenario would help stabilize local economies as well. All that is required is to look at each alien invasive species with a more open mind to perhaps come up with ways to reduce their number while helping local society.

Conclusion

So as we begin the work of the XIII International Symposium on Biological Control of Weeds, and as the reader enters these pages for their own edification, Hawaii welcomes your interest as leaders in your field. The contributions you have made and will offer here may serve those of us working in the field of invasive species research and control well; we can only gain from your knowledge. If one examines the list of the world's 100 worst invasive alien species (Lowe et al., 2000), Hawaii has approximately 40% of them, making it an excellent microcosm in which to study impacts, control and eradication procedures and even ecosystem-level approaches. For you, the visitor to our own invaded world, it offers the opportunity to see at close hand not only how we are coping, but what we are doing in the process. We cannot win all the battles, but we know now which are the most important, and we have a better idea of how to move forward.

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SESSION 1: PRE-RELEASE TESTING OF WEED BIOLOGICAL CONTROL AGENTS

Pre-release Studies and Release of the Grasshopper *Cornops aquaticum* in South Africa – a New Biological Control Agent for Water Hyacinth, *Eichhornia crassipes*

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Abstract

The grasshopper, *Cornops aquaticum* Brünner (Orthoptera: Acrididae) has recently been released in South Africa as a biocontrol agent for water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae), the country's worst invasive aquatic weed. The release follows 15 years of pre-release studies to assess *C. aquaticum*'s safety and potential value as a new agent. *Cornops aquaticum* was first introduced into quarantine in South Africa from Manaus, Brazil in 1995. Host specificity testing was completed by 2001 but release of the grasshopper was delayed, initially, due to difficulties in obtaining release permits for weed biocontrol agents. A permit was finally granted in 2007 by which time pre-release efficacy studies had been initiated and new concerns over compatibility of *C. aquaticum* with the *Neochetina* (Coleoptera: Curculionidae) weevils, the most damaging agents in the field in both South Africa and other parts of Africa, had arisen. The efficacy and agent interaction studies were first concluded to guide the decision on whether *C. aquaticum*'s introduction into the country was justifiable. Pre-release impact studies indicated that *C. aquaticum* damage is directly associated with density and that herbivory at relatively low grasshopper densities can disrupt water hyacinth growth and productivity when growing under optimal nutrient conditions. Interaction studies with *C. aquaticum* and *Neochetina eichhorniae* Warner suggested a synergism whereby pairing of these agents, under laboratory conditions, had the greatest negative impact on biomass accumulation compared to the agents alone or other combinations of agents tested. In August 2010, the South African biocontrol community supported a decision to release *C. aquaticum* and field releases began early in 2011. Four initial release sites have been selected to encompass different nutrient and climatic conditions and are being monitored to assess establishment, impact and population dynamics of *C. aquaticum*.

Introduction

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae) is a free-floating perennial herb, native to South America that was introduced into South Africa in the early 1900's via the ornamental plant trade. By the 1970's it had reached pest proportions in many systems around the country and to date remains South Africa's worst invasive aquatic weed (Coetzee et al.,

2011). A biological control programme for water hyacinth was initiated in 1974 with the release of the petiole-mining water hyacinth weevil, *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae). Following this, an additional four arthropod biocontrol agents were released between 1989 and 1996: a leaf-mining mite, *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae) in 1989; another petiole-mining weevil, *Neochetina bruchi* Hustache and, a petiole-mining moth, *Niphograptus albigutallus*

Warren (Lepidoptera: Pyralidae) in 1990; and a sap-sucking mirid, *Eccritotarsus catarinensis* Carvalho (Hemiptera: Miridae) in 1996 (Hill and Cilliers, 1999).

While South Africa's biocontrol programme had a few successes, most water hyacinth sites around the country have been difficult to control biologically. By the end of the 1990's, after almost 30 years of an active biological control programme, it was clear that success was variable and levels of control were deemed unsatisfactory. Hill and Olckers (2001) outlined several factors that were speculated to disrupt or reduce the efficacy of the biocontrol agents already released, the most important of which were eutrophication, where plant growth rates outpace the damage caused by the biocontrol agents, and incompatibility of the agents with the temperate climate of many regions of the country. A potential solution was to consider new agents in the hope that a better agent could be found or that the correct, complimentary suite of agents had not yet been released.

The neotropical grasshopper, *Cornops aquaticum* Br uner (Orthoptera: Acrididae) was considered the most promising candidate based on reports on its damage potential from the native range (Perkins, 1974), and its wide distribution in South America, extending to climatically similar regions to South Africa (Adis et al. 2007). The first collections of the grasshopper took place in Manaus, Brazil in 1995 and subsequent collections were made in Trinidad and Venezuela in 1997 and Mexico in 1997. Oberholzer and Hill (2001) studied the host range of *C. aquaticum* by testing 64 plant species in 32 families and concluded that it is oligophagous within the family Pontederiaceae, with a strong preference for water hyacinth.

Although *C. aquaticum* was considered safe for release in South Africa based on its host specificity, its release was initially delayed due to difficulties in obtaining release permits. Regulatory authorities delayed granting permits as they lacked in-house expertise needed to critically evaluate release applications. A permit was finally granted in 2007, by which time agent efficacy studies had already been initiated. It was decided to complete this research in order to determine whether the grasshopper's introduction into South Africa was justified based on its potential to be an effective biocontrol agent. The efficacy results showed strong

support for the grasshopper's release but new concerns over compatibility of *C. aquaticum* with the *Neochetina* weevils had arisen. The weevils are the most important water hyacinth biocontrol agents in South Africa as well as other parts of Africa so any disruption to their efficacy or populations would have diminished prospects for the grasshopper's release.

This paper presents a subset of results from the efficacy and agent interaction studies that primarily motivated the decision to proceed with the release of *C. aquaticum* into the South African biocontrol programme for water hyacinth. It also summarizes details of the first releases of the grasshopper in South Africa.

Methods and Materials

1. Agent efficacy studies

1.1 Effect of water nutrient levels on the impact of Cornops aquaticum herbivory

Water hyacinth plants were grown in plastic tubs (43 x 31 x 19 cm) in a quarantine glasshouse for a period of four weeks prior to the introduction of the grasshoppers. Each tub contained 15 L of water and two water hyacinth plants and was enclosed with a net canopy. Nutrient levels in the water were manipulated to represent levels of nitrates and phosphates present in South African water bodies. Nitrogen and phosphorus were added as potassium nitrate (KNO₃) and potassium dihydrogen orthophosphate (KH₂PO₄) respectively. Commercial chelated iron was also added at a rate of 1.3g/15L of water. The nutrient levels were classified as eutrophic (high), mesotrophic/eutrophic (medium) and oligotrophic (low) (Table 1) according to the South African Water Quality Guidelines (Holmes, 1996). Water in the tubs was changed once a week to maintain the required nutrient supply to the plants. After the four-week growth period, all daughter plants, dead leaves and stems were removed, and the plants weighed to determine wet weight. Adult *C. aquaticum* grasshoppers were introduced into the experimental tubs at a density of one per plant and one male/female pair per tub. The treatments were replicated six times and the trial was run for a period of ten weeks. Plants were weighed at termination

Table 1. Nutrient concentrations used to represent the range of levels found in South African river systems and impoundments.

	High (eutrophic)	Medium (eutrophic/mesotrophic)	Low (oligotrophic)
Nitrates (mgL ⁻¹)	7.6	2.52	0.034
Phosphates(mgL ⁻¹)	1.37	0.316	0.024

of the trial to determine end wet weight. The effect of nutrient treatment, herbivory by *C. aquaticum* and their combined effect on the difference in wet weight from the start to the end of the trial were analyzed using a two-way ANOVA. Tukey's HSD test was used as a post-hoc comparison of the means.

1.2 Effect of plant nutrient levels on *Cornops aquaticum* (a) survival and (b) fecundity

(a) Twenty-eight newly emerged *C. aquaticum* nymphs were reared on water hyacinth plants grown at the high, medium and low nutrient levels (Table 1) for a period of three months. Water and plant nutrient levels in water hyacinth are highly correlated (Gossett and Norris, 1971) so there was a corresponding increase in plant tissue nutrient levels with an increase in nutrient supply to the plants (Bownes, 2009). The total number of nymphs to survive to adulthood and the proportions of males and females were recorded.

(b) Eight pairs of adult grasshoppers reared in trial (a) were confined in tubs with water hyacinth plants grown at the same nutrient levels on which they were reared. The number of egg packets oviposited by females and the number of nymphs to emerge from each egg packet were recorded. The number of egg packets per female and the number of nymphs per egg packet were compared by one-way ANOVA to test for the effect of nutrient treatment on fecundity of *C. aquaticum*. Tukey's HSD test was used as a post-hoc comparison of the means.

1.3 Density-damage relationships between *Cornops aquaticum* and water hyacinth

The experimental design followed the same protocol as trial 1.1 with the exception that all plants were grown at the high nutrient level, when plant growth and productivity would be optimal. Male and female *C. aquaticum* grasshoppers were introduced into the tubs at a density of 2, 3 and 4 per plant (= four, six and eight grasshoppers per tub). The sexes were separated so that each tub had either only males or only females. Each treatment was replicated six times and two tubs per replicate were used as controls. Water hyacinth plants were weighed at the start and the end of the trial to determine wet weight and the trial was run for a period of eight weeks. The plant biomass data was subjected to a regression analysis to determine the relationship between insect biomass (as the independent variable) and plant biomass (as the dependent variable). Insect biomass was used as a surrogate for insect density since densities of male and female grasshoppers were the same. For this, a random sample of male and female grasshoppers were weighed (males $n = 47$; females $n = 50$) to obtain a mean wet weight (g) for each sex (Bownes et al. 2010a). The biomass and insect data were fitted to a damage curve, similar to that suggested by McClay and Balciunas (2005), and which is used to identify agents that are not sufficiently damaging to their host plant to justify release. The damage curve relates a critical aspect of weed performance such as growth rate or final biomass to increasing densities of the biocontrol agent.

Table 2. Treatments testing interactions between *Cornops aquaticum* and two biocontrol agents already released in south Africa, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*.

Treatment	Species combination (density/plant)
Control	No insects
1	CA (1 female) + NE (2pairs)
2	CA (1 female) + EC (10 adults)
3	NE (2 pairs) + EC (10 adults)
4	NE (2 pairs)
5	EC (10 adults)

Table 3. Effect of nutrient levels on survival of *Cornops aquaticum* from first instar to adult, and sex ratio of survivors.

Nutrient level	Survival to adult	Sex ratio Female:Male
High	82%	65:35
Medium	71%	55:45
Low	64%	39:61

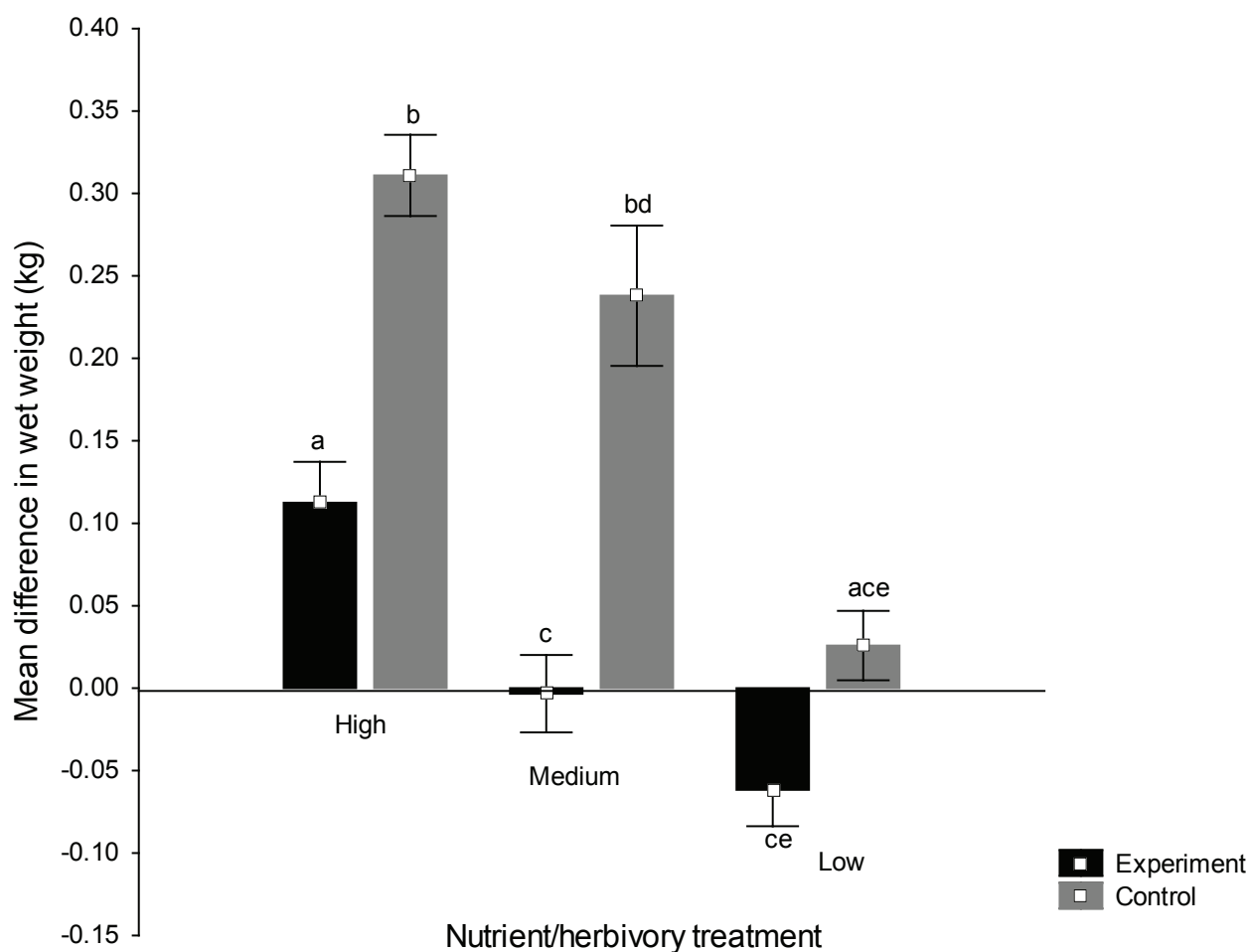


Figure 1. Mean change in wet weight of water hyacinth plants in response to herbivory by *Cornops aquaticum*. Plants grown at high, medium and low nutrient levels for ten weeks. Means with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

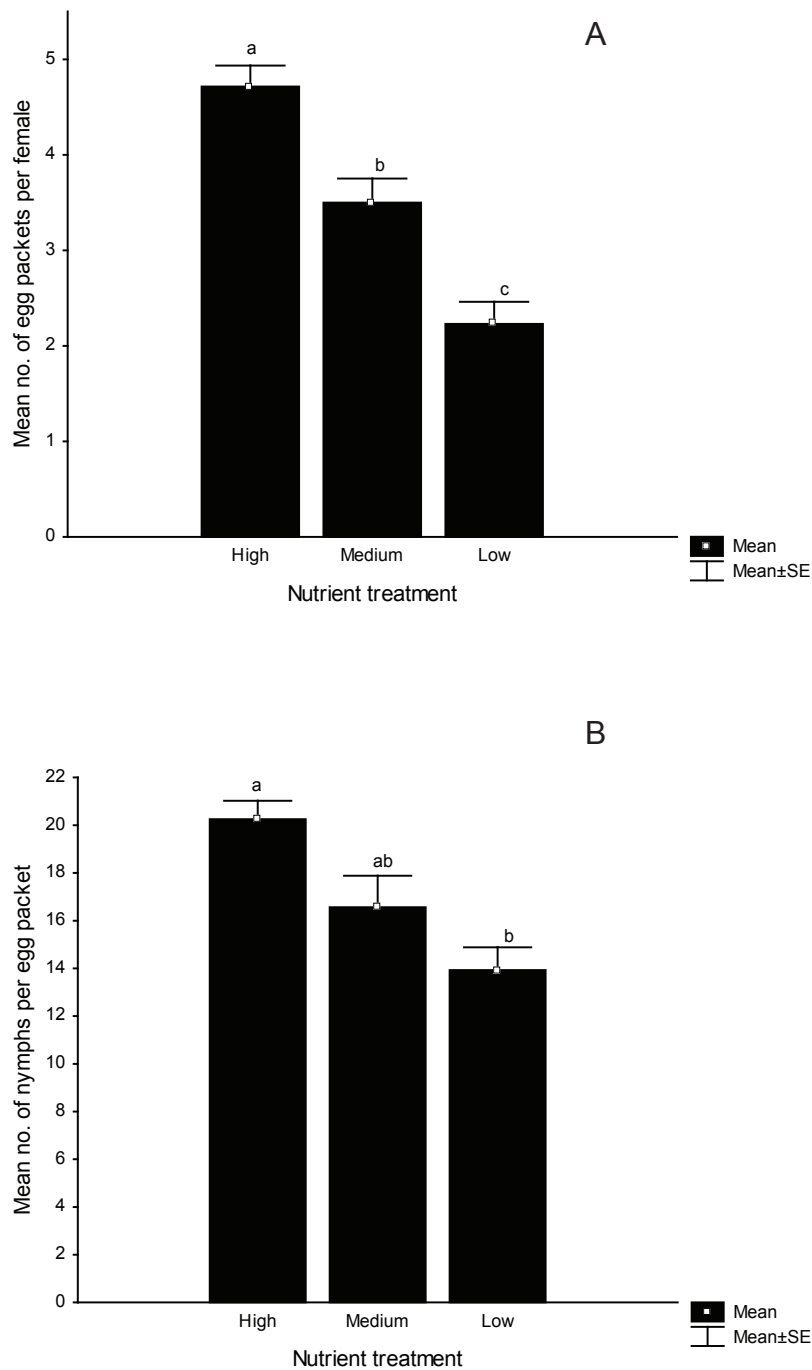


Figure 2. Mean fecundity of *Cornops aquaticum* females measured as (A) the no. of egg packets per female and (B) the number of nymphs to emerge from each egg packet. Means with the same letter are not significantly different (Tukey's HSD test, $P < 0.05$). Error bars represent the standard error of the mean.

2. Agent interaction studies

Effect of *Cornops aquaticum* on populations and feeding damage of the *Neochetina* weevils and the impact of combinations of these agents on water hyacinth growth and productivity

(a) Manipulative, small-scale experiment

One water hyacinth plant per treatment was grown in an 8 L bucket in a temperature controlled quarantine glasshouse. Nitrates and phosphates were added to the water at a rate of 2 mg L⁻¹ and 0.29 mg L⁻¹ respectively. Flowers and ramets were removed from the plants two weeks prior to the start of the trial to allow for the plants to recover and stabilise. Nutrients and water were replaced after the two-week recovery period and 25 days subsequent to this. Table 2 shows the combinations and densities of the insect species that were tested. Plants were weighed at the start and end of the trial to determine wet weight and were sampled weekly to record insect activity such as the number of *N. eichhorniae* feeding scars. Each treatment was replicated 10 times and the trial was run for a period of 50 days. To analyze the effect of the different insect treatments on the change in wet weight of water hyacinth plants, the biomass data were compared by one-way ANOVA. The mean number of weevil feeding scars when in combination with *C. aquaticum* and *E. catarinensis* and alone were also compared by one-way ANOVA. Tukey's HSD test was used as a post hoc comparison of the means.

(b) Pond experiment with an already established weevil population

The trial was conducted in a 1300 L portable pool (215 x 45 cm) housed in a semi-quarantine glasshouse and enclosed with a net canopy to confine the insects to the plants. The pool was 100% covered with water hyacinth and had a combined density of 2.6 ($\pm$ 0.87) adult *N. eichhorniae* and *N. bruchi* per plant that were resident for a period of 3 months prior to the introduction of the grasshoppers. At the start of the trial, 97 adult *C. aquaticum* (46 females: 51 males) were released onto the plants which equated to 0.3 grasshoppers per plant. A random sample of five water hyacinth plants were destructively sampled fortnightly and the following plant and insect parameters were

measured: number of leaves per plant, number of ramets per plant, proportion of petioles mined by *Neochetina* larvae and the total number of larvae recovered. The means of each parameter were compared over time by one-way ANOVA and Tukey's HSD test used as a post-hoc comparison.

Results

1. Agent efficacy studies

1.1 Effect of water nutrient levels on the impact of *Cornops aquaticum* herbivory

Nutrient treatment ($F_{2;29} = 48.53$; $P < 0.0001$) and herbivory ($F_{1;29} = 81.80$; $P < 0.0001$) had a significant effect on the change in wet weight of water hyacinth plants (Fig. 1) from the start to the end of the ten week trial. The interaction between nutrient supply and herbivory was also significant ($F_{2;29} = 5.56$; $P = 0.009$). Plant tolerance to herbivory by *C. aquaticum* increased with an increase in nutrient supply to the plants however feeding by the grasshoppers significantly reduced biomass accumulation at all three nutrient levels.

1.2 Effect of plant nutrient levels on *Cornops aquaticum* (a) survival and (b) fecundity

(a) Survival of *C. aquaticum* nymphs to adulthood was influenced by plant nutrient levels which also had an effect on the proportions of males and females to be reared through to adulthood (Table 3). Higher levels of nitrogen in the plant tissue (Bownes, 2009) elicited higher rates of survival and greater numbers of females survived to adulthood in the high nutrient treatment (Table 3).

(b) Nutrient treatment had a significant effect ($F_{2;20} = 26.06$; $P < 0.0001$) on fecundity of female grasshoppers that were reared and maintained, after pairing at adulthood, on plants grown at the high, medium and low nutrient levels, with fewer egg packets being produced at the low nutrient level. Nutrient treatment had a significant effect ($F_{2;18} = 7.58$; $P = 0.0041$) on the number of nymphs to hatch from egg packets of females (Fig. 2). The mean number of nymphs per egg packet increased with an increase in nutrient supply to the plants although

only the high and low treatments were statistically significantly different from one another.

1.3 Density-damage relationships between *Cornops aquaticum* and water hyacinth

The relationship between plant biomass at the end of the trial as a function of increasing *C. aquaticum* biomass was curvilinear (Fig. 3). Biomass of water hyacinth plants decreased with an increase in feeding intensity by the grasshoppers. Exponential regression best described the relationship between plant yield and insect biomass and was highly significant ($F_{6;43} = 73.20$; $P < 0.0001$) accounting for 75% of the variance (Bownes et al. 2010a).

2. Agent interaction studies

Effect of *Cornops aquaticum* on populations

and feeding damage of the *Neochetina* weevils and the impact of combinations of these agents on water hyacinth growth and productivity

(a) Manipulative, small-scale experiment

A combination of *C. aquaticum* and *N. eichhorniae* was the only treatment to significantly reduce biomass accumulation of water hyacinth plants compared to control plants ($F_{5;54} = 3.62$; $P = 0.0068$). Although the other combinations of insects or treatments with *N. eichhorniae* and *E. catarinensis* alone hampered biomass accumulation relative to insect free plants, none of these differences were statistically significant (Fig. 4). The presence of *C. aquaticum* had no effect on *N. eichhorniae* feeding intensity compared to when the weevils were alone or in combination with the mirid *E. catarinensis*.

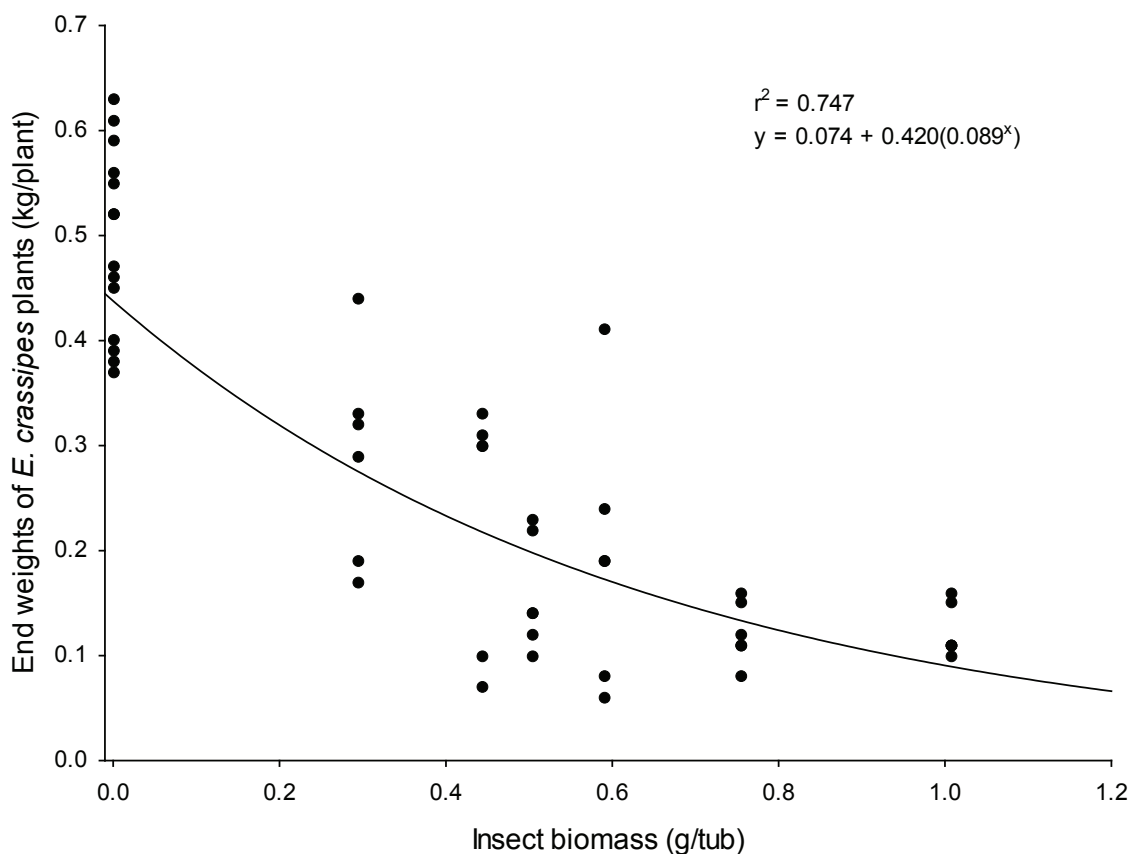


Figure 3. Regression of *Cornops aquaticum* biomass (g) and final weight (kg) of water hyacinth plants after eight weeks. Insect biomass is represented by a mean weight of male or female grasshoppers multiplied by the respective density.

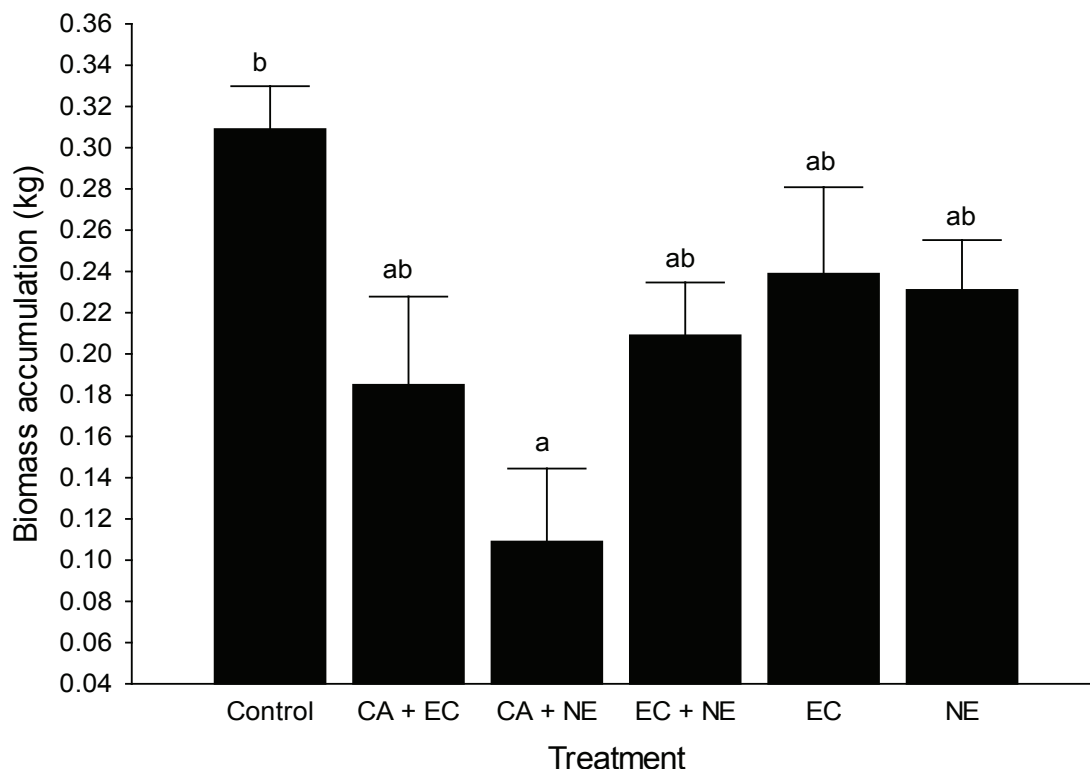


Figure 4. Mean change in wet weight of water hyacinth plants exposed to different combinations of insect biocontrol agents (*Cornops aquaticum*, *Neochetina eichhorniae* and *Eccritotarsus catarinensis*). Means with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

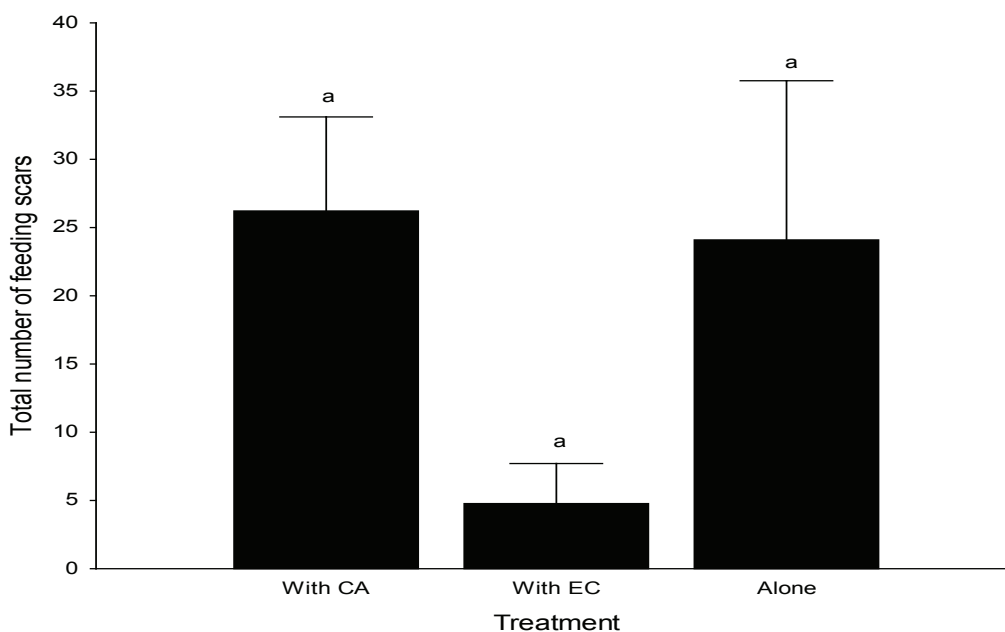


Figure 5. Mean numbers of *Neochetina eichhorniae* feeding scars when tested alone and in combination with *Cornops aquaticum* and *Eccritotarsus catarinensis*. Means with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

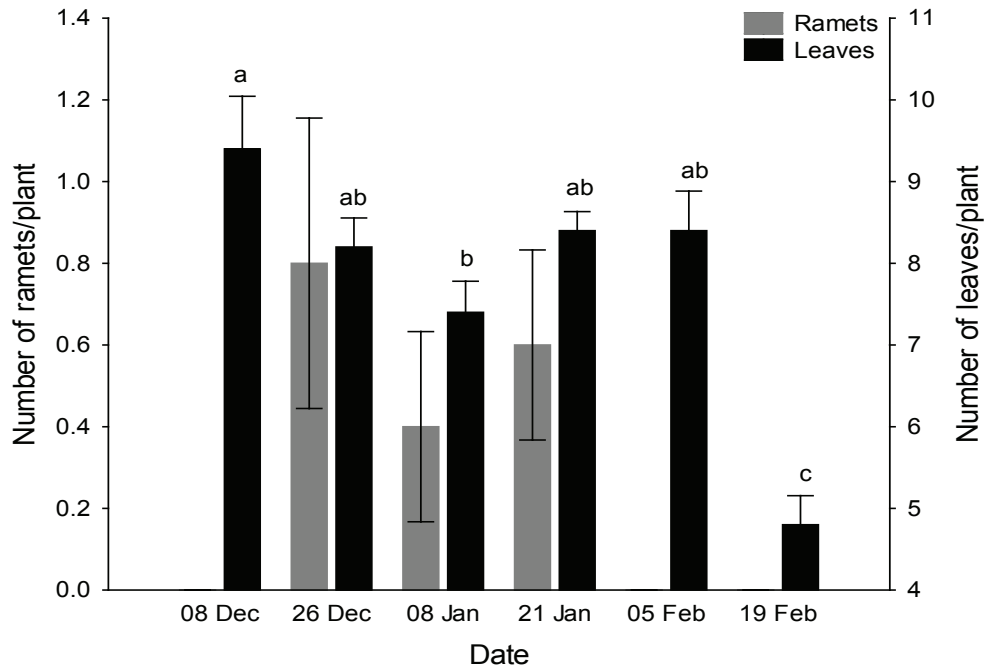


Figure 6. Mean numbers of ramets (daughter plants) and leaves produced by water hyacinth plants over time in response to feeding by the *Neochetina* weevils and *Cornops aquaticum*. Means with the same letter are not significantly different (Tukey's HSD, $P < 0.05$).

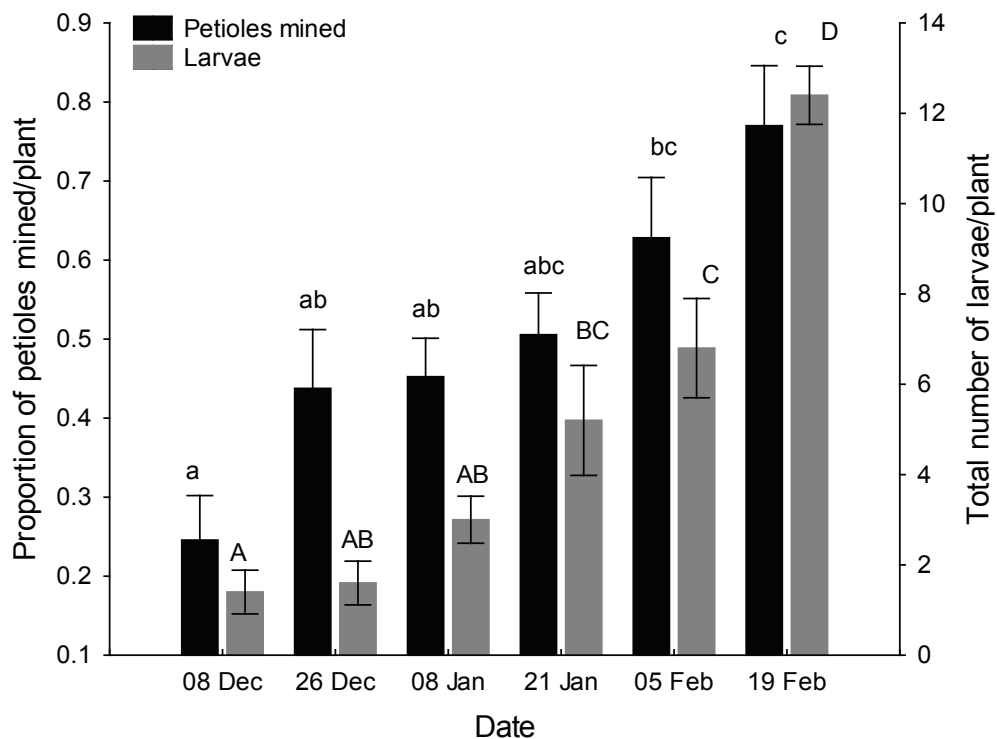


Figure 7. Proportions of petioles mined by *Neochetina* larvae and numbers of *Neochetina* larvae per water hyacinth plant when in combination with *Cornops aquaticum*. Means with the same letter are not significantly different (Tukey's HSD, $P < 0.05$). Error bars represent the standard error of the mean.

Interestingly, the presence of *E. catarinensis* caused a reduction in feeding of *N. eichhorniae*; however the differences in the number feeding scars were not statistically significant (Fig. 5).

(b) Pond experiment with an already established weevil population

Plant productivity, as measured by the number of leaves and ramets per water hyacinth plant decreased over time, although this was only statistically significant for the number of leaves per plant ($F_{5;24} = 12.39$; $P < 0.0001$). Although there were no significant differences, ramet production of the water hyacinth plants ceased after 5-6 weeks (Fig. 6), and by the 10th week, most of the plants in the pool had died back. *Neochetina* larval activity increased over time after the introduction of *C. aquaticum*. There was a significant increase in the proportion of petioles mined ($F_{5;24} = 6.80$; $P = 0.0004$) and in the total number of larvae ($F_{5;24} = 24.38$; $P < 0.0001$) recovered from the plants from the start of the trial to the last sampling event (Fig. 7).

Discussion

The results from these and other studies (Bownes, 2009; Bownes et al. 2010b) strongly suggested that *C. aquaticum* has the potential to be a valuable biocontrol agent for water hyacinth in South Africa and the following conclusions were made: (1) *C. aquaticum* has the potential to reduce populations of water hyacinth under eutrophic nutrient conditions and that these nutrient conditions should have a positive effect on their population dynamics; (2) the damage caused by *C. aquaticum* is density-dependent in that increasing densities will lead to a corresponding reduction in water hyacinth growth and productivity, supporting the conclusion that it would be sufficiently effective to warrant release (McClay and Balciunas, 2005); (3) *C. aquaticum* does not appear to have a negative effect on feeding and populations of the *Neochetina* weevils; and (4) an apparent synergism between *C. aquaticum* and the *Neochetina* weevils could potentially lead to better levels of control of water hyacinth in South Africa. On the basis of these conclusions and on the fact that a substantial amount of time and resources had been

invested in developing this agent, a decision was made in August 2010 to proceed with the release of the grasshopper.

For the initial releases and monitoring of *C. aquaticum*, four sites were selected to encompass a range of both nutrient and climatic conditions such as high or low water nutrient conditions and temperate to sub-tropical climates. All four sites were monitored for at least nine months prior to release in order to evaluate site-specific conditions such as microclimate, water nutrient conditions and the status of the plants and insect biocontrol agents already present. The first release took place in January 2011 followed by a second release in March 2011. Three hundred adult and late instar nymphs were released at each site. Sites were monitored three months post-release, but to date evidence of establishment of the grasshoppers has not been found.

With assistance with mass rearing from the South African Sugar Research Institute (SASRI) which has a specialized rearing facility for insect biocontrol agents, repeated releases will take place during the summer of 2011/2012. All sites will be monitored on a quarterly basis to determine establishment and efficacy of *C. aquaticum* as well population dynamics of the insect biocontrol agents already present on water hyacinth in South Africa.

Acknowledgements

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Australia's Newest Quarantine for Weed Biological Control

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Abstract

Two of Australia's leading weed biological control groups, from the Queensland Government's Alan Fletcher Research Station and CSIRO's Long Pocket Laboratories, recently relocated to the new world-class Ecosciences Precinct at Dutton Park, Brisbane. There they share a purpose built quarantine facility of QC3 standard. Built on the level 5 rooftop of the Precinct, the 400 m² quarantine facility has six research suites, each consisting of an 11 m² laboratory and a 30 m² air-conditioned glasshouse. These research areas are supported by controlled environment rooms, storage areas, a room for controlled environment cabinets and an unpacking room. One research area is isolated for working with plant pathogens or extremely small arthropods requiring "shower out" procedures. Special features of this state-of-the-art facility include double glazing of laminated glass of the glasshouses, HEPA filtration, "pass through" autoclaves and fumigation chamber, and a heat transfer, continuous treatment system for liquid waste. The QC3 facility includes dedicated mechanical services rooms on the loft above and the floor below for easy access for maintenance. Approximately 3000 m² of non-quarantine on-site plant growth facilities support the QC3. The facility was approved as a QC3 facility at its first inspection. The immediate approval of the facility was attributed to several factors, including benchmarking existing quarantine facilities world-wide, high standards of materials and building expertise, and detailed communication between project staff, certifying agencies, construction and specialist consultants and scientists throughout the whole process. Within the next few years, this facility will become one of only two quarantines in Australia designed for weed biological control research.

Introduction

Secure quarantine facilities are a cornerstone requirement of any classical biological control program, allowing researchers to undertake host testing of foreign organisms and ensuring security control of any unintended organisms in imported packages. Quarantine requirements have changed

over time and will continue to change into the future, probably with tighter controls. When Koebele sent his first insects (without any host testing) to Hawaii in 1902 (Perkins and Swezey, 1924) the packages were first opened in the corridor before someone suggested that it might be better to open them in a room with the door closed! By the time insects for prickly pear were brought to Australia in the 1920s,

a special quarantine area (though crude by today's standards) had been constructed at what became the Alan Fletcher Research Station in Queensland (Dodd, 1940). Today a very high standard of quarantine containment is required in all countries with significant biological control programs (Radcliffe et al., 2003; Agostino et al., 2004; Ferrar et al., 2004; Anon, 2005; Adair and Irwin, 2008).

Quarantine facilities have also assumed greater importance with time because budgetary constraints have resulted in a greater proportion of total research being undertaken in the home country and because there is a much higher requirement to test against native plant species (Fisher and Andres, 1999).

Strong investment in new quarantine facilities over the last decade or so has resulted in new facilities for biological control being constructed in Canada (De Clerck-Floate et al., 2000), England, South Africa, New Zealand, Brazil and the United States.

This paper describes a new quarantine facility constructed within the \$270 million Ecosciences Precinct, which replaced quarantine facilities at the Queensland Government's Alan Fletcher Research Station and CSIRO Ecosystem Science's Long Pocket Laboratory. The Ecosciences Precinct itself is a newly constructed, state-of-the art science facility housing approximately 1000 scientists and staff from CSIRO, two Queensland Government agencies, and the University of Queensland. It was developed to replace several aging research facilities in the Brisbane area and forms part of a science corridor within South East Queensland.

Quarantine Philosophy

Quarantine Level

Very early in the planning and after advice from the regulating agencies it was decided to build the entire quarantine at the Australian Quarantine and Inspection Service (AQIS) defined Quarantine Containment level 3 (QC3). A major consideration was that future quarantine requirements may be more stringent and retrofitting an existing facility to a higher standard is very undesirable. Essential features of QC3 are that there is an interlock space at the entrance, exhaust air is HEPA-filtered (meaning that glasshouses can't use evaporative air conditioning systems), liquid waste must be

sterilized by heat and areas within the quarantine envelope are built to air-tight standards and are held at negative air pressure. While QC3 gives a high level of certainty of containment, it has the disadvantages that it is sophisticated, expensive-to-build and incurs high running and maintenance costs.

Pathogen (Micro) area

Regulatory authorities advised that QC4 level was necessary for certainty of approval to import exotic pathogens but that pathogens might be approved on a case by case basis for a QC3 quarantine, providing certain extra features were present. Those features included a shower-out for exiting staff and heat treatment of liquid waste. It was therefore decided to separate one suite of the facility from the others and to provide this suite with its own entrance (Figure 1). This suite was to be reserved firstly for pathogens, tiny arthropoda such as mites or thrips, or any other agent requiring extra caution or separation from the other projects.

Insect (Macro) area

This area, with one common entrance and five independent research suites, is designated for standard insect work (Figure 1). Each suite includes a small laboratory attached to a 30 m² glasshouse with refrigerated air conditioning. In addition to these suites, the Macro area has shared spaces including three controlled environment rooms (CERs), a room housing four controlled temperature cabinets, an unpacking room, and a storage room.

Waste Disposal

We were advised by the regulators that heat sterilization was the preferred option for treatment of liquid waste. This was problematic because of energy costs and particularly because a facility with several glass houses could generate considerable quantities of liquid waste. There were then issues such as continuous versus batch systems to consider. The best option appeared to be the selection of an Actini® with its continuous flow, heat transfer system.

Most solid waste would be treated by sterilization in pass-through autoclaves. Each area has such an autoclave.

Some solid wastes, laboratory equipment such as insect cages, books or other paper products can be removed from the quarantine through a 3-door,

pass through fumigation chamber. This chamber is of generic design and is capable of using any one of several fumigants. A hazard and operability study (HAZOP) is presently being undertaken before the fumigant of choice, methyl bromide, is used.

Management

The Ecosciences Precinct quarantine facility replaces two facilities; the Queensland Government's Alan Fletcher Research Station and the CSIRO's Long Pocket Laboratory and now houses teams from both agencies. The new facility is managed as an integrated shared facility to maximize resources and functionality of the facility.

The Quarantine Manager, jointly funded by CSIRO and the Queensland Government, is responsible for the functioning of the infrastructure and adherence to protocols of all personnel using the facility, regardless of their affiliation.

Standard Operating Procedures (a 100 page document) detail quarantine protocols and procedures for quarantine users and maintenance staff.

Collaboration

This QC3 facility is one of the most innovative, leading-edge structures built within the Ecosciences Precinct. Successful commissioning of the facility means CSIRO and Queensland Government scientists now work together to develop safe and sustainable methods to manage the spread and impact of the worst weeds and insect pests which threaten the environment and Australia's rural industries.

Because of the structural complexity in QC3 laboratories and glasshouses, the need to meet very high containment standards and requirement for certification through the AQIS, these types of facilities have a high risk of building failure.

Collaboration and cohesive communication were critical success factors for the design, construction, certification and commissioning processes for the QC3 facility at the Ecoscience Precinct. This was achieved by continual engagement and regular feedback loops, scheduled workshops and programmed visual inspections by CSIRO and Queensland Government scientists (users), consultants (architects, mechanical, electrical, hydraulics engineers and other specialists) and

construction teams throughout the six years from project brief to construction completion. In addition, scientists were actively engaged in benchmarking world's best practices in QC3 construction and working with consultants and the construction team to test and source suitable materials and construction methodologies, including problem solving with regard to infrastructure technologies such as heat treatment and material containment. A List-server, which is still operating, was set up to facilitate email discussion about quarantine problems with quarantine managers around the world.

Maintenance

Discussions with other quarantine facility managers taught us the importance of building a structure with easy access to all associated mechanical, electrical and hydraulic equipment. We built a floor above the facility in which some of this equipment is housed. A floor below the facility houses much of the remaining equipment.

Supporting infrastructure

The quarantine is supported on the rooftop by both airconditioned and evaporatively cooled non-quarantine glasshouses and shadehouses. These are used to grow test plants and also to mass rear insects approved for release. All of the rooftop infrastructure is supported by potting areas in the basement.

Some Design Details

The Actini® liquid waste system

Liquid waste from the quarantine facility is decontaminated by passing through an Actini® system that treats the liquid by holding it at 145°C for 260 seconds. Energy consumption is minimized by a heat transfer system. The system is capable of treating 4000 L per week, which generously allows for contingencies. In addition a storage tank can hold 4000 L of untreated waste.

Autoclaving

Pass-through Getinge® autoclaves with 415 L sterilizing chambers service both Micro and Macro areas. They are programmed so that the outer door can only be opened after a sterilizing event has taken place. The requirement for sterilization is 121°C for

15 minutes if the core temperature is measured and 121°C for 30 minutes where the core temperature is not measured.

Fumigation

The fumigation chamber has been built to use a number of approved fumigants. It is a custom built, pass-through chamber that services both Macro and Micro areas. This is achieved by the three door design. These doors are sequenced electronically to prevent cross contamination between Macro and Micro areas and to ensure that contaminated material is fumigated before the outer door is opened. In essence, once either of the inner doors is opened a fumigation event must occur before any of the other doors can be opened. Once a fumigation event has occurred and the chamber has been unpacked from the outside, it can be used as a pass back facility to take materials and equipment back into the quarantine areas.

Air handling system

There are 18 air handler units in the facility, with all glasshouses and all CERs having independent units. This design, and also the manipulation of supply and exhaust dampers, allow each suite to be fumigated separately by either injecting gas (through dedicated fumigation ports) or heating liquid formaldehyde (using dedicated power points) while allowing the remainder of the facility to operate normally.

Negative pressures are achieved with a Variable Speed Device system, maintained at -15 Pa in the external corridor, -25 Pa in the airlocks, -50 Pa in the internal corridor, -65 Pa in laboratories and Macro CERs and -75 Pa in the glasshouses and Micro area CER.

All exhaust air is passed through HEPA filters situated in plant rooms on Level 4, Level 5 and the loft above and outside the quarantine envelope. There are 39 HEPA filters mounted in 21 stainless steel HEPA boxes that can be opened on the clean side for annual integrity testing.

Glasshouses

The glasshouses are each 30 m² in size and are provided with compressed air, carbon dioxide and reverse osmosis water. Liquid waste drains from the

centre of each room to the Actini® treatment system. The roof of the glasshouse has a 27° slope from 4.5 m high on the south side to 2.7 m high at the north side.

The roof is shaded by a retractable internal blind controlled by a pneumatic device. Its operation is controlled from the Building Management System (BMS) using an algorithm based on ambient temperature, humidity and solar radiation measured by an independent weather station in the building. The BMS can be over-ridden by the user. External roll up blinds have been retrospectively fitted on the vertical glass walls to intercept radiant heat, which was a problem particularly in the winter months.

The glasshouses are fully enclosed in a double glazing system. This allows a panel of the inner or outer glass to be replaced, in the event of damage, without disruption to the building function. An entire prototype glasshouse was built to test the system before construction. The internal laminated pane of glass consists of one 4 mm thick layer of glass on each side of a 1mm PVB layer. The external laminated pane of glass consists of one 5 mm thick layer of glass on each side of a 4mm PVB layer. A replaceable desiccant prevents condensation in the 300 mm space between the glass panes.

Laboratories

Each laboratory is fitted with a sink, benches, under bench cabinets, above bench shelves, and a space for an item of equipment such as a refrigerator. Each laboratory is 11 m² except the Micro laboratory, which is 18 m² to fit the various additional pieces of equipment needed for working with plant pathogens. Each laboratory is provided with compressed air and carbon dioxide.

Controlled Environment Rooms

There are four CERs, varying in size from 9 to 12 m². Each has its own air handling and dehumidifying unit and is illuminated by 24 metal halide lamps housed in a space above the room, separated by a glass barrier ceiling which allows the light to penetrate but isolates the heat generated by the lamps. Maintenance of the lamps is from outside the quarantine envelope. We have experienced excellent growth of tropical plants in the rooms. Each CER is provided with compressed air and reverse osmosis water.

Entry and Exit

Each quarantine area is accessed by passing through the airlock and change room, which are accessed from a foyer outside the quarantine envelope. On entry, the first room (airlock) in each quarantine area is blackened and is kept dark except when in use. This airlock is fitted with a black light insect trap. The outer door of this airlock is considered the boundary of the quarantine envelope. The next room is a changing room. Electronic locks ensure that only one door in a set of airlocks can be opened at any one time. An air curtain operates above the door from each change room to the corridor within their respective quarantine areas.

Toilets

A toilet is provided only in the Micro area because staffs are more likely to remain in quarantine for long periods when shower out protocols apply. The toilet is for urination only because the Actini® system cannot handle paper.

Safety

Emergency door release buttons for all interlocking doors, push buttons to isolate reticulated services such as gas, water and electricity, automatic exhaust of the fumigation if a leak is detected in the fumigation chamber, and visible and audible alarms for pressure deviations have been installed to enhance personal safety. All these devices and other sensors throughout the areas of the quarantine are monitored through the BMS. High priority BMS alarms are sent to the SMS alarm messaging service, resulting in callouts any time during the day or night. In addition, an intercom system provides immediate communication to the security desk.

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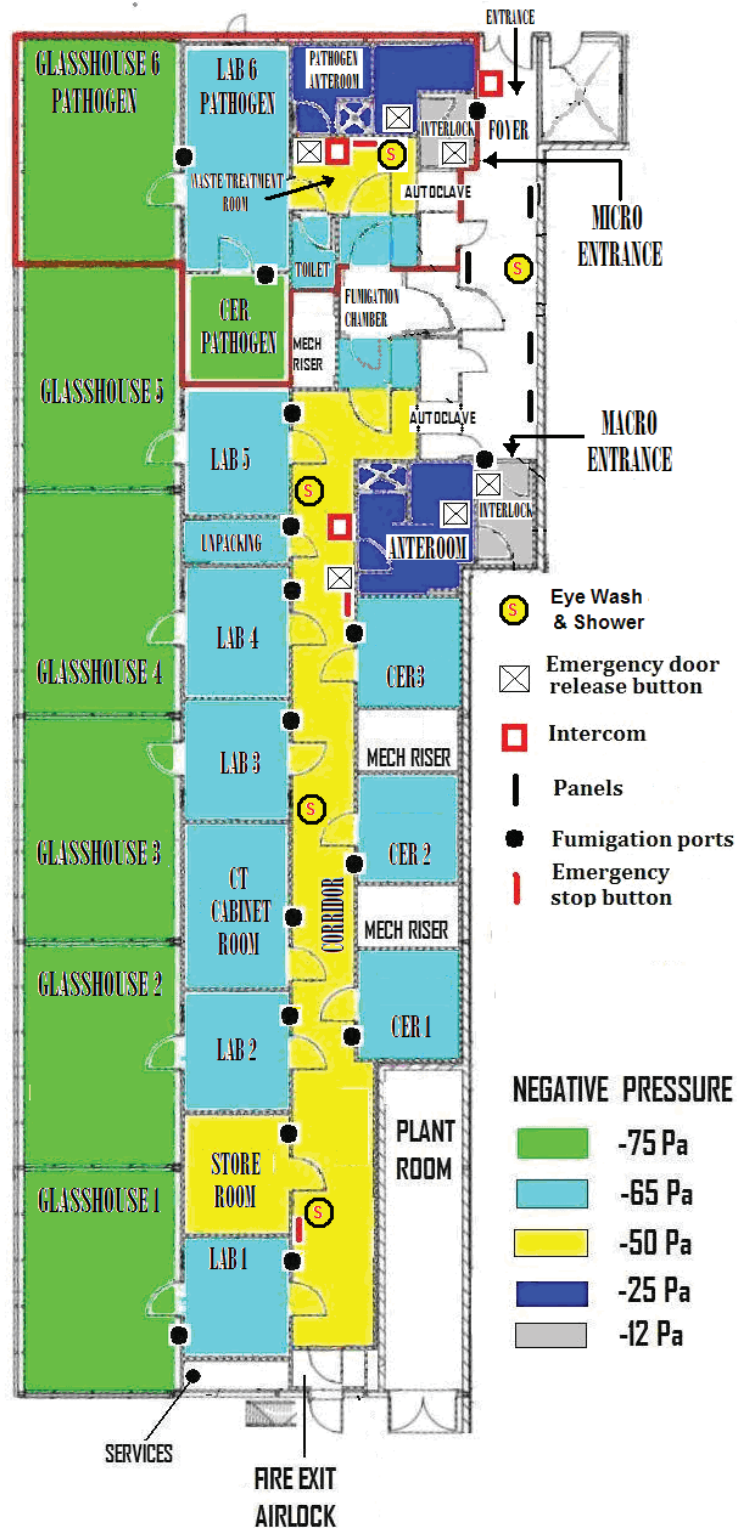


Figure 1. Schematic diagram of the quarantine facility

Host Specificity of an Italian Population of *Cosmobaris scolopacea* (Coleoptera: Curculionidae), Candidate for the Biological Control of *Salsola tragus* (Chenopodiaceae)

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Summary

Russian thistle, *Salsola tragus* L. (Chenopodiaceae) is a troublesome weed infesting the drier regions of western North America. It is native to Central Asia and infests rangelands and semi-arid pasture lands, croplands, residential, disturbed and industrial areas. *Cosmobaris scolopacea* (Germar) is a weevil distributed in Eurasia and North America, and generally associated with plant species of the family Chenopodiaceae. The larvae feed and pupate within the stems of the host plant, and the adults emerge in the following late spring. From preliminary host range testing carried out at the ENEA-BBCA facilities in Rome, Italy, it appeared that *C. scolopacea* might harbour different host races, one being potentially more specific to the target and only present in Sicily, Italy. To determine species boundaries and reveal population structure at the intraspecific level, a phylogeographic study using the mitochondrial COI gene was conducted on specimens collected in the native range (Italy, Spain, Iran, Bulgaria, Turkey) and the U.S.A. The study confirmed the presence within the species of a highly divergent Sicilian lineage that has only been reared from *Salsola kali* L. The degree of specificity of this particular lineage and hence host race status is being tested through host specificity testing. Preliminary results seem to indicate that this Sicilian lineage can be at least a true *Salsola* host race, opening doors for further testing as a biological control agent for Russian thistle.

Introduction

Salsola tragus L. (*sensu lato*), together with other closely related Russian thistle species, is a troublesome weed in the drier regions of western North America (Young, 1991). It infests range and semi-arid pasture lands as well as cropland, agricultural, residential and industrial areas. As a crop weed it can cause

yield losses of greater than 50% in spring wheat (Young, 1988). It is also a host for several crop pests, and the tumbling plant skeletons can fill irrigation canals and pile against fences (Goeden and Ricker, 1968). A biological control program started during 1970s and there is still a need for effective agents (Goeden and Pemberton, 1995; Smith et al., 2006).

Larvae of *Cosmobaris scolopacea* (Germar,

1819) (Coleoptera: Curculionidae) were recorded and reared from plants of *Salsola kali* L. (Chenopodiaceae) near Catania (Sicily, Southern Italy) by Gaetano Campobasso (pers. comm., 2000). Despite the fact that the stem boring weevil is known as a cosmopolitan pest species of several Chenopodiaceae crops, a screening for the evaluation of the host range of this population was started in the early 2000s by G. Campobasso, performing mainly open field host range observations by dissecting native and crop Chenopodiaceae occurring in sympatric conditions with the target weed.

Starting in 2008, we decided to continue the screening of the weevil, by the combination of three different approaches: morphological taxonomy, genetic analysis and ecological bioassays. The purpose of the present study was to compare for these two last aspects different populations of *C. scolopacea* to reveal the existence of cryptic species and /or host races within the species, and to determine if any are potentially suitable as a biological control agent for the target weed.

Material and methods

Field sampling

Weevil larvae were collected from *Salsola* spp. in Eurasia, i.e. Sicily, Central Italy, Central Spain, Central Turkey, Bulgaria, Iran, and in the US (California) from 2008 to 2010. In most of the sites, larvae were collected also in the stems of other Chenopodiaceae (often *Chenopodium album* L. and rarely on *Halimione* spp.). Stem dissection was conducted *in situ*, with some of the larvae preserved in absolute ethanol for genetic studies and others transferred to artificial diet (Tomic-Carruthers, 2009) for adult emergence to use for morphological study. Adults have been collected in two locations in Sicily (Simeto and Eraclea Minoa, respectively) to carry out host range tests in laboratory conditions at the BBKA facilities.

Molecular and phylogenetic analysis

Weevils were collected as adults and larvae from a total of i) 29 populations throughout the Eurasian native distribution range from Italy to Northern

Iran and ii) 2 populations in North America, and iii) across three major host plants in the Chenopodiaceae family, i.e. the Russian thistle (*Salsola* spp.), *C. album* and *Halimione* spp. Also included in this study as an outgroup was a dried specimen of *Cosmobaris discolor* (Boheman, 1836) collected by E. Colonnelli in South Africa in 2007 on *Chenopodium* sp. Weevils were preserved in absolute ethanol and stored at -20°C before DNA extraction. Genomic DNA was extracted from single specimens using either the CTAB protocol (Doyle and Doyle, 1987) or the DNeasy Blood and Tissue DNA extraction kit (Qiagen S.A, Courtaboeuf, France) following the manufacturer's protocol. A ~830 bp section of the mitochondrial cytochrome oxidase c subunit I (COI) gene was amplified through Polymerase Chain Reaction (PCR) in a 9700 Perkin Elmer thermal cycler (Applied Biosystems) using primers C1-J-2183 and TL2-N-3014 (Simon et al., 1994) and PCR profile: 5 min at 94°C, 5 cycles of 30s at 92°C, 30s at 48°C, 1 min at 72°C, followed by 25 cycles of 30s at 92°C, 30s at 52°C, 1 min at 72°C, and 7 min at 72°C. PCR products were sequenced on both strands at Genoscreen (Lille, France) on ABI 3130XL automatic sequencers (Applied Biosystems, Foster City, CA, USA). Alignments of consensus sequences were manually edited with Bioedit 7.09 (Hall, 1999). A dataset of 79 sequences of 638bp of length was obtained for *C. scolopacea* sensu lato. To determine species boundaries and reveal discontinuities among lineages at the intraspecies level, an analysis method based on haplotype relationships (i.e. statistical parsimony) was chosen (TCS; Clement et al., 2000). Under the 95% parsimony criterion haplotype network resulted in three unconnected networks (data not shown). To provide a framework for understanding the evolutionary relationships between all populations and between these haplotype networks, a phylogenetic analysis was performed on the same dataset. Modeltest version 3.7 (Posada and Crandall, 1998) was used to determine the model of nucleotide substitution that fitted the data best. The hierarchical likelihood ratio (hLRT) test as implemented in Modeltest selected the HKY+G model as the best fit for our dataset. The Maximum Likelihood analysis was conducted under PhyML 3.0 (Guindon et al., 2010), and bootstrapping was calculated from 100 replicates. Genetic divergence levels within and between networks and species were

determined by calculating un-corrected p distances in PAUP*4.0 (Swofford, 2002).

Host range experiments

Laboratory host range choice-tests were carried out during 2010, testing one population from *Eraclea Minoa*, Western Sicily. Bioassays were carried out in Petri dishes in a climatic cabinet at 21–26°C and with a 14:10 h L/D cycle, confining one female with one stem of *S. kali* (SAKA) plus one stem of one of the following plant species: *Kochia scoparia* (L.) Schrader (KOSC), *Chenopodium album* (CHAL), *Suaeda taxifolia* (P. C. Standley) P. A. Munz (SUTA) and *Bassia hyssopifolia* (Pallas) Volk (BAHY).

Results and Discussion

The ML phylogenetic tree obtained on the basis on the 79 COI sequences of *C. scolopacea* sensu lato that was rooted with *C. discolor* as an outgroup is presented in Figure 1. Three major highly diverged mitochondrial clades supported with high bootstrap values above 93% can be observed, and are equivalent to the three unconnected haplotype networks obtained under TCS. One clade (A) contained the nine North American specimens from one site in California and one site in Prince George's County, Beltsville, Maryland, corresponding to two haplotypes. Specimens from *S. tragus* and *C. album* at the Brentwood, CA site shared the same haplotype. Genetic divergence within this clade was very low (0.06%). A second clade (B), also named the Sicilian clade, contained the 18 samples collected in Sicily and that were associated with *S. kali* and *Halimione* sp. hosts. It contained three haplotypes, and its

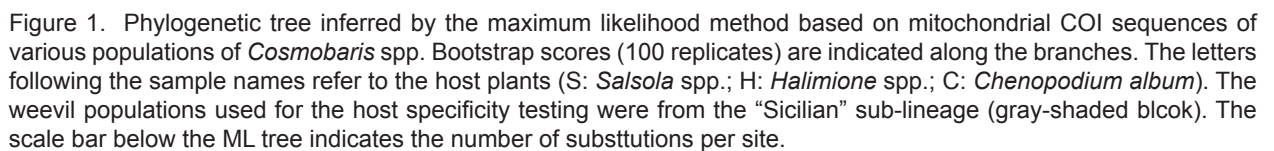
intraclade genetic divergence averaged 0.25%. The third clade (C) contained the remaining 52 samples distributed across Eurasia and that were associated with *Salsola* and *Chenopodium* host plants. A total of 24 haplotypes belong to this clade which has the highest intraclade genetic divergence among the three, averaging 0.56%. The genetic divergence between the three clades ranged from 8.5% between the "American" clade (A) and the "C" clade to 9.2% between the Sicilian clade (B) and the "C" one. These values are nearly of the same order as those observed between the outgroup species *C. discolor* and any of the three clades. First described by Casey in 1920, the American lineage has been assumed to be *C. americana* Casey (Casey, 1920; O'Brien and Wibmer, 1982). In the present day, from gathered morphological data, it is admitted that *C. americana* Casey is a synonym of *C. scolopacea* and should not retain its separate name (Colonelli, pers com.). However, to determine whether the extent of the divergence is sufficient for the three clades to be considered cryptic species, sub-species, host races or biotypes, further research is likely required in the future.

The presence of a highly divergent lineage of *C. scolopacea* in Sicily that was collected mainly on *S. kali* supported Campobasso's hypothesis that Sicilian populations may be more host specific than other populations of *C. scolopacea* in Eurasia. We therefore conducted host range testing of specimens from the Sicilian clade, collecting individuals as adults on *S. kali* (the Sicilian subclade highlighted in grey in the ML tree). Two-way choice oviposition experiments carried out during 2010 in Petri dishes showed a clear preference of the weevil for *S. kali*, with occasional oviposition on *B. hyssopifolia*, *C. album* and *S. taxifolia* (Table 1).

Table 1. Oviposition preference by *Cosmobaris scolopacea* in two-choice tests.

Plant spp. ¹ A+B	No. of reps	eggs on A (mean)	Std. Error	eggs on B (mean)	Std. Error	Wilcoxon Signed Ranks Test (Z)	Asymp. Sig. (2 tailed)
SAKA+BAHY	7	2.00	0.76	0.57	0.43	-1.279	0.201
SAKA+KOSC	6	3.00	0.82	0.00	0.00	-2.226	0.026
SAKA+CHAL	6	2.33	0.61	0.17	0.17	-2.214	0.027
SAKA+SUTA	9	1.78	0.43	0.11	0.11	-2.354	0.019

¹BAHY = *Bassia hyssopifolia*, CHAL = *Chenopodium album*, KOSC = *Kochia scoparia*, SAKA = *Salsola kali*, SUTA = *Suaeda taxifolia*.



As recently and extensively reviewed by Gaskin et al. (2011), for weed biological control practitioners, there is a reasonable consensus that specialized lineages, including morphocryptic ones, whatever the stage of speciation they represent, are now considered one of the best routes for efficacy as regards their host specificity and perhaps safety. Hence, depending upon future results, the *Cosmobaris* weevil could be yet another example of phytophagous weevils that have several host races or cryptic species, some being strictly specific to a targeted weed, and hence opening the door for potential use in biological control (Fumanal et al., 2004; Antonini et al., 2008; Gaskin et al., 2011).

Acknowledgements

We want to remember and thank Gaetano Campobasso, USDA ARS Research Entomologist, passed away 3 years ago, who recorded for the first time the weevil damage on Russian thistle in Sicily. We gratefully thank Alessio De Biase (University of Rome "La Sapienza") and René Sforza (USDA-ARS EBCL) for reviewing the manuscript; and Fatiha Guermache (USDA-ARS EBCL) for her assistance with the molecular analysis.

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Biological Control of Chilean Needle Grass (*Nassella neesiana*, Poaceae) in Australasia: Completion of Host Range Testing

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Abstract

Nassella neesiana (Trin. and Rupr.) Barkworth (Chilean needle grass, CNG, Poaceae) is a Weed of National Significance in Australia and a declared pest plant in parts of New Zealand. Studies have been conducted in Argentina to identify potential biological control agents (pathogens) for this species. The rust *Uromyces pencanus* Arthur & Holw has been selected for having the greatest potential: it is highly host specific and can cause significant damage to the target weed. Most of the host range testing for the selected isolate (UP27) is now complete. No pustules have developed on any of the test species other than the target. However, there has been some development of the rust, with formation of haustoria, in *Piptatherum miliaceum* (L.) Cosson and several *Austrostipa* spp. In addition, some of the inoculated plants of *P. miliaceum* showed peculiar macrosymptoms ("blisters") somewhat resembling pustules. Resistance mechanisms were observed to occur in all these species, probably explaining why the rust could not develop further to produce spores. As *Austrostipa* and *Piptatherum* are very closely related to *Nassella* (all in the tribe Stipeae) these results are not unexpected. It is surprising though that penetration, with little further development, was observed in other congeneric *Nassella* spp. tested. Testing of various isolates of *U. pencanus* revealed they varied greatly in their ability to attack different populations of CNG. *U. pencanus* isolate UP27 is able to infect eight out of the nine tested Australian populations and one out of three from New Zealand. An isolate has yet to be found that is able to infect those CNG populations resistant to UP27: those tested so far have failed. Authorities in New Zealand have recently approved the importation of *U. pencanus* for release in that country. An application to release the rust in Australia will be prepared soon.

Introduction

Nassella neesiana (Trin. and Rupr.) Barkworth (Chilean needle grass, Poaceae) is a perennial tussock-forming grass that is indigenous to Argentina, Bolivia, Chile, Ecuador, southern Brazil

and Uruguay (Rosengurt et al. 1970). In Australia, it is both a serious environmental weed (Carr et al. 1992; McLaren et al. 1998) and a problem weed of agriculture (Grech 2007) and is a Weed of National Significance (WONS) (Thorp and Lynch 2000). It is widespread in Victoria, NSW and the ACT with

recent outbreaks occurring in Queensland, SA and Tasmania (Snell et al. 2007). *N. neesiana* is also a serious weed in New Zealand (Bourdôt and Hurrell 1992). Small populations occur in the North Island but the worst infestations occur in the Marlborough region, near the top of the south island.

Difficulties in controlling Chilean needle grass by chemical and cultural methods have led to investigations into the possible use of pathogens for biological control of this species in Australia and New Zealand. The rust *U. pencanus* has the greatest potential as: it is relatively easy to manipulate; it persists as urediniospores; it is highly host specific; and, can cause significant damage to the target weed (Giordano et al. 2009, Anderson et al. 2010a). We discuss the results of the host range testing carried out to study the specificity of the rust. We also report the outcome of the application to introduce the rust to New Zealand.

Materials and Methods

A host specificity test list of 58 grass species was developed that included significant Australian and New Zealand native and commercially important grass species selected according to their taxonomic relatedness to *N. neesiana*. Testing was conducted in part at CERZOS in Bahía Blanca, Argentina, where the project is based, and part in a quarantine facility located at IMYZA-INTA, Castelar, Buenos Aires, Argentina, where all species exotic to Argentina had to be tested (Anderson et al., 2010b).

An isolate (UP27) of the rust fungus that originated from a field site in Bahía Blanca was selected on the basis of its virulence against Australian accessions of *N. neesiana* (Anderson et al. 2006). Batches of 4-5 test plant species were screened at one time, with a total of 8 plants being tested for each test species whenever availability permitted. Dry urediniospores mixed in talcum powder (ratio 1:30) were brushed onto the adaxial side of two leaves per plant, which were later sprayed with water. Accessions of *N. neesiana* from the Australian Capital Territory (ACT) were included in each test as positive controls. Inoculated plants were maintained at 18-20°C under a 12h (D:L) photoperiod and 100% relative humidity (RH) for 48h, after which they were kept under the same conditions, but at 70% RH for four weeks. Four

weeks was selected because there is generally a two-week gap between inoculation and spore production on positive control plants. All inoculated plants were then inspected for external symptoms of infection. Also, samples were taken for internal microscopic examination at one week after inoculation and at the end of each experiment (four weeks). These samples were cleared and stained so as to make it easier to distinguish between fungal and plant tissues under the microscope using a modification of the Bruzzese and Hasan (1983) method (Flemmer et al., 2010). Each species was screened at least twice.

Results

Most Australian accessions of *N. neesiana* proved to be susceptible to isolate UP27, with development of normal uredinia on infected leaves. However, a collection of *N. neesiana* from Ballarat (Victoria) did not become infected. Plants from only one out of the three tested accessions from New Zealand (Marlborough) proved to be susceptible, while those from the other two (Hawke's Bay and Auckland) did not become infected. There were no pustules formed on any of the other species tested. Peculiar symptoms that looked like blisters, and somewhat resembled pustules, were formed on some of the inoculated leaves of *Piptatherum miliaceum*. Microscopic examination revealed these "blisters" were composed of plant rather than fungal tissues and were formed in response to rust penetration at some infection sites. Here both hyperplasia and hypertrophy seem to occur. On a few other species leaf spots were formed on inoculated leaves i.e., small yellow specks on congeneric *Nassella* species and small black spots on several *Austrostipa* species. Table 1 shows a summary of the results obtained on 50 tested species. These are listed in decreasing order of taxonomic relatedness with *N. neesiana*, the target weed. Details of the interactions of the rust and host cells at a microscopic level and the resistance mechanisms recorded will be presented elsewhere.

Discussion

As a result of the inoculation experiments using *U. pencanus* isolate UP27, no pustules developed on any test species other than the target *N. neesiana*.

Table 1. Host specificity *Uromyces pencanus* on Poaceae species.

Species	Macroscopic Symptoms	Microscopic Symptoms*									
		1	2	3	4	5	6	7	8	9	10
<i>Nassella neesiana</i> (Trin. & Rupr.) Barkworth [ACT]	Pustules	X		X						X	X
<i>N. neesiana</i> [Bacchus Marsh, Vic]	Pustules	NE									
<i>N. neesiana</i> [Ballarat, Vic]	None	NE									
<i>N. neesiana</i> [Clifton Springs, Qld]	Pustules	X		X						X	X
<i>N. neesiana</i> [Fitzroy flats, NSW]	Pustules	X		X						X	X
<i>N. neesiana</i> [Goulburn, NSW]	Pustules	NE									
<i>N. neesiana</i> [Laverton, Vic]	Pustules	NE									
<i>N. neesiana</i> [Thomastown, Vic]	Pustules	NE									
<i>N. neesiana</i> [Truganina, Vic]	Pustules	NE									
<i>N. neesiana</i> [Auckland, NZ]	None	X		X	(X)		(X)	X	X		
<i>N. neesiana</i> [Hawke's Bay, NZ]	None	X		X			X				
<i>N. neesiana</i> [Marlborough, NZ]	Pustules	X		X						X	X
<i>N. charruana</i> (Arechav.) Barkworth	None	X	(X)	X	(X)				X		
<i>N. hyalina</i> (Nees) Barkworth	Yellow leaf spots	X		X	(X)	X					
<i>N. leucotricha</i> (Trin. & Rupr.) R.W. Pohl in Barkworth	Yellow leaf spots	X	(X)	X	(X)			(X)	X		
<i>N. tenuissima</i> (Trin.) Barkworth	None	X		X	(X)	X					
<i>N. trichotoma</i> (Nees) Hack. ex Arechav. [Dalgety, NSW]	Yellow leaf spots	X		X		X					
<i>N. trichotoma</i> [N. Canterbury, NSW]	None	X				X					
<i>Achnatherum caudatum</i> (Trin.) S.W.L. Jacobs & J. Everett	None	X		X	(X)		X	(X)			
<i>Austrostipa aristiglumis</i> (F. Muell.) S.W.L. Jacobs & J. Everett	None	X		X	X		X				
<i>A. bigeniculata</i> (Hughes) S.W.L. Jacobs & J. Everett	None	X		X	X		(X)		X		
<i>A. breviglumis</i> (Hughes) S.W.L. Jacobs & J. Everett	Black leaf spots	X		X	X		X		X	(X)	
<i>A. elegantissima</i> (Labill.) S.W.L. Jacobs & J. Everett	Black leaf spots	X		X	(X)			X	X		
<i>A. eremophila</i> (Reader) S.W.L. Jacobs & J. Everett	Black leaf spots	X		X	X		X		X	(X)	
<i>A. flavescens</i> (Labill.) S.W.L. Jacobs & J. Everett	Black leaf spots	(X)	(X)	(X)	(X)			(X)	X		
<i>A. mollis</i> (R.Br.) S.W.L. Jacobs & J. Everett	None	X	(X)	X	(X)			X	X		

Species	Macroscopic Symptoms	Microscopic Symptoms*									
		1	2	3	4	5	6	7	8	9	10
<i>A. nitida</i> (Summerh. & C.E. Hubb.) S.W.L. Jacobs & J. Everett	Brown leaf spots	X		X	(X)			(X)	(X)	(X)	
<i>A. nullanulla</i> (J. Everett & S.W.L. Jacobs) S.W.L. Jacobs & J. Everett	Black leaf spots	X	(X)	X	(X)			X	X	(X)	
<i>A. rudis</i> (Spreng.) S.W.L. Jacobs & J. Everett	None	X		X	(X)			X	(X)		
<i>A. setacea</i> (R.Br.) S.W.L. Jacobs & J. Everett	None	X	(X)	X	(X)			X	(X)		
<i>A. scabra</i> (Lindl.) S.W.L. Jacobs & J. Everett	None	X		X	(X)		X	(X)	(X?)		
<i>A. verticillata</i> (Nees ex Spreng.) S.W.L. Jacobs & J. Everett	None	(X)		(X)					(X)		
<i>Piptochaetium napostaense</i> (Speg.) Hack.	Yellow leaf spots	X		X	X			(X)			
<i>Piptatherum miliaceum</i> (L.) Coss	Yellow leaf spots, "blisters"	(X)		X					X	(X)	(X)
<i>Avena sativa</i> L.	None	(X)		(X)		X					
<i>Brachypodium distachyon</i> (L.) P. Beauv.	None	X	X	(X)	(X)				(X)		
<i>Bromus catharticus</i> Vahl.	Yellow leaf spots	X		X	X		X				
<i>Dichanthium aristatum</i> (Poir.) C.E. Hubbard	None	X		(X)	X		X	X	X		
<i>Elymus scabrifolius</i> (Döll) J.H. Hunz.	Yellow leaf spots	X		(X)	X			(X)	(X)		
<i>Eragrostis curvula</i> (Schrud.) Nees	None	X		(X)	(X)	X					
<i>Festuca arundinacea</i> Schreb.	None	X		X	X		X		(X)		
<i>Hordeum vulgare</i> L.	Yellow leaf spots	X		X			X				
<i>Lolium perenne</i> L.	None	X		X	X		X				
<i>Phalaris aquatica</i> L.	Yellow leaf spots	X		X			X	(X)	(X)		
<i>Poa ligularis</i> Nees ex Steud.	None	X		X	X		(X)				
<i>Secale cereale</i> L.	None	X		X	X		X	X			
<i>Triticum aestivum</i> L. cv. ACA 303	None	X		X	X		X	X	X		
<i>T. aestivum</i> cv. Arriero	None	X		X	X		X	X	X		
<i>T. aestivum</i> cv. Guapo	Yellow leaf spots	X		X	X		X	X	X		
<i>T. aestivum</i> cv. Liquén	Yellow leaf spots	X		X	X		X	X	X		
<i>T. aestivum</i> cv. Malevo	Yellow leaf spots	X		X	(X)		X	X	X		
<i>T. aestivum</i> cv. Sureño	None	X		X	X		X	X	X		

Species	Macroscopic Symptoms	Microscopic Symptoms*									
		1	2	3	4	5	6	7	8	9	10
<i>T. aestivum</i> unknown cv.	Yellow leaf spots	X		X	X	X					
<i>Microlaena stipoides</i> (Labill.) R.Br.	None	(X)		(X)	(X)				(X?)		
<i>Oryza sativa</i> L.	None	X		X	X		X	(X)			
<i>Phyllostachys aurea</i> Riviere & C. Riviere	None	X		(X)	(X)	X					
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	None	X		X	X	X					
<i>Austrodanthonia geniculata</i> (J.M. Black) H.P. Linder	None	(X)	(X)	(X)	X				(X)		
<i>Chloris gayana</i> Kunth.	None	X		(X)	X	X					
<i>Cynodon dactylon</i> (L.) Pers.	None	X		X		X					
<i>Sporobolus rigens</i> (Tr.) Desv.	None	X		(X)		X					
<i>Aristida pallens</i> Cav.	Yellow leaf spots	X		(X)		X					
<i>Bothriochloa springfieldii</i> (Gould) Parodi	None	(X)		(X)			(X)	(X)			
<i>Cymbopogon citratus</i> (DC.) Stapf.	None	(X)					X				
<i>Paspalum dilatatum</i> Poir.	Yellow leaf spots	X		(X)			X	X	(X)		
<i>Pennisetum clandestinum</i> Hochst. ex Chiov.	None	X		X			X	X	X		
<i>Sorghum halepense</i> (L.) Pers.	None	X		X			X				
<i>Zea mays</i> L.	None	X		X	X		X	X	X		

* 1= normal spore germination; 2= abnormal spore germination; 3= normal appresoria; 4= abnormal appresoria or non-stomatic appresoria; 5= penetration not observed; 6= penetration, two to four infection hyphae formed from substomatal vesicle, growth cessation; 7= penetration + contact with plant cells, growth cessation; 8= penetration + contact with plant cells + thickening of cell walls, growth cessation; 9= haustoria; 10= abundant intercellular mycelia. ()= observation was infrequent; ?= observation was doubtful; NE= not examined.

Moreover, one of the tested Australian accessions of *N. neesiana* and two from New Zealand (Auckland and Hawke's Bay) did not become infected either. It should be noted that results regarding these two accessions from New Zealand are not conclusive as only four plants from Auckland were available for testing and those from Hawke's Bay belonged to only one site. Fortunately, the Marlborough population of *N. neesiana*, which is susceptible to UP27, is the infestation in New Zealand that most requires a biological control agent. An isolate has yet to be found that is able to infect plants from populations not susceptible to UP27.

Different types of leaf spots were formed on inoculated leaves of several test species but there appears to be no direct relation between the presence of these symptoms and the level of development reached by the rust within inoculated leaves. Leaf spots were formed on leaves where no penetration was recorded, and conversely, there were instances of no apparent symptoms on leaves where penetration by the rust was later confirmed under the microscope (Anderson, unpublished). There was some development of the rust within the leaves of *P. miliaceum* and several *Austrostipa* spp, in which a few haustoria and some development of intercellular mycelium was observed, but no such development was recorded in congeneric *Nassella* species. This was unexpected, as typically, the taxa most susceptible to a pathogen are the plants most closely related to its preferred host (Wapshere 1974). Still, development of the rust within leaf tissues only occurred in grasses belonging to the same tribe as the target weed and the pathogen could only complete development on some populations of *N. neesiana*. Conducting host range studies in artificial conditions can predispose plants to infection (Parker et al., 1994), and lead to an over estimation of the field host range. Overall, our results suggest that the rust is very unlikely to cause any damage to non-target plants in the field. Several different resistance mechanisms were observed during these studies which will be discussed in detail elsewhere. Such mechanisms include: abnormal germination; incorrect appressorium positioning; inhibition of growth shortly after penetration; thickening of host cell wall in response to the presence of, or contact with, fungal hyphae; necrosis of cells in the proximity of the penetration area; and, encasement of haustoria

by deposition of cell material (Heath, 1981, Heath 1982 & Heath, 1997). In most cases, more than one mechanism was recorded on samples of a single inoculated leaf. These plant defenses probably account for the failure of the rust to produce pustules on all of the tested species. On the basis of these findings, authorities in New Zealand have recently approved the importation of *U. pencanus* isolate 27 for the control of *N. neesiana* in that country. This is a historic achievement as this is the first time a pathogen has been approved for release on a grass.

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Finding the Weapons of Biomass Destruction—Identifying Potential Biological Control Agents by Applying Principles of Chemical Co-Evolution

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Abstract

The history of the biocontrol of weeds has many parallels with that of biocontrol of insects in that the practice began without an extensive conceptual or theoretical framework. Among the first scientific attempt at using natural enemies to control weeds was undertaken in Hawaii. Soon after *Lantana camara* L. was introduced into Hawaii in 1858 for ornamental purposes, it became a noxious weed. Shortly thereafter, lantana was suffering from the effects of infestation by the exotic scale *Orthezia insignis* Browne and by the turn of the century Hawaiian Sugar Planters' Association members were transporting the scale all over the islands. Albert Koebele was sent by the Association in 1902 to Mexico, from which he forwarded 23 insect species to Honolulu, 8 of which, representing six families in three orders, became established. For decades thereafter, suites of prospective agents were imported to areas of non-indigeneity in the hope that, either individually or collectively, they would have the desired effect of restricting weed growth and expansion. In the intervening century, a deeper understanding of phytochemical constraints on hostplant utilization has developed and principles derived from studies of plant-herbivore chemical coevolution have considerable potential for informing the design and implementation of weed biocontrol programs, in particular in anticipating nontarget risks. Among the predictive indicators, reflective of coevolutionary adaptations, are: 1. phylogenetic patterns of host usage, as evidenced by literature records; 2. behavioral adaptations that express dependence (e.g., taxonomic restriction of kairomones); 3. physiological limitations of plant response (e.g., galls); 4. ecological dependence on unique phytochemistry for defense against predators (e.g., sequestration); and 5. dependence on abiotic activators of plant defenses. Studies of coevolutionary interactions between herbivorous insects and their hostplants, independent of the economic status of the plants, thus can contribute meaningfully to the construction of a theoretical framework to aid the weed biocontrol community.

Molecular Analysis of Host-Specificity in Plant-Feeding Insects: Phylogenetics and Phylogeography of *Fergusonina* Flies on Australian Paperbarks

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Abstract

Molecular phylogenetics has been widely used by evolutionary biologists to explore patterns of host-plant specificity in phytophagous insects. This approach has also been used in biological control research where it can provide critical information during pre-release exploration of the potential utility of an insect against a weed target. Most importantly, molecular phylogenetics can resolve species limits, reveal cryptic species, and assess host-specificity within and among closely related species. Intra-specific phylogeographic analysis can provide additional information on the suitability or lack thereof of particular species for use in biological control. *Melaleuca quinquenervia* (Cav.) S.T.Blake., the “broad-leaved paperbark,” is an Australian wetland tree that has become an important invasive weed within Florida, including the Everglades. The search for potential biological control agents within Australia, discovered undescribed *Fergusonina* gall flies (Diptera: Fergusoninidae) feeding on *M. quinquenervia* and its relatives. Using molecular phylogenetics, we explored species limits and host-specificity in this group of flies from nine species of Australian paperbarks with the aim of assessing host specificity. In most cases, species delimited by molecular data were monophagous, feeding on a single host species. Further analysis of Australian populations of *Fergusonina turneri* Taylor the species on the invasive paperbark, suggest that pre-release agent selection may also need to consider phylogeographic structure of natural populations of potential agents.

Selection of Test Plant Lists for Weed Biological Control with Molecular and Biochemical Data

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Abstract

The initial steps of weed biological control programs involve the determination of the host range of a prospective agent prior to consideration for release. Accurately predicting the host range of a potential agent is fundamental to this process. This may be conducted first in the country of origin in open field testing (Briese et al., 2002) and later under controlled environmental conditions in quarantine (Zwölfer and Harris, 1971; McFadyen, 1998). Initially a plant test list is established composed of species that are taxonomically related to the weed and species of economic and ecologic importance from the area where the weed is a problem (Wapshere, 1974). This centrifugal / phylogenetic testing procedure involves “testing plants of increasingly distant relationship to the host until the host is circumscribed” (Wapshere, 1974) and is based upon the assumption that host shifts occur to plants of similar taxa (Ehrlich and Raven, 1964; Mitter and Farrell, 1991). Typically rare species are also included in the plants tested. As useful as this process is it potentially overlooks unrelated plant taxa that share similar secondary plant metabolites. Recent evidence indicates that chemical similarity may be a better predictor of host use than are phylogenetic relationships (Becerra, 1997; Wahlberg, 2001). Although little evidence may exist from weed biological control projects (Schaffner, 2001), species with secondary metabolites similar to the target weed should be included in the test list as they may contain the behavioral cues used by these specialized herbivore species to locate hosts and initiate feeding (Wheeler, 2005). As useful as the centrifugal / phylogenetic testing procedure may be, it potentially overlooks distantly unrelated plant taxa that share similar secondary plant metabolites.

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Successfully Eliminating Parasitic Gregarines from *Neolema ogloblini* (Coleoptera: Chrysomelidae) - a Biological Control Agent for *Tradescantia fluminensis* (Commelinaceae)

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Abstract

Tradescantia fluminensis Vell. (Comelinaceae) was introduced into New Zealand (NZ) as a house plant but is now a serious under storey weed of indigenous forest. Surveys for potential biological control agents in SE Brazil, starting in 2005, identified a rich natural enemy biota including herbivorous insects and plant pathogens. Routine screening of the first insect agent to be host range tested, the leaf beetle *Neolema ogloblini* (Monros) (Chrysomelidae), revealed high levels of a gregarine (sporozoan protozoan) gut parasite. This appeared to reduce beetle fecundity, longevity and general vigor, potentially compromising its biological control efficacy. Depending on the host specificity of the gregarine, it could also threaten NZ fauna. In NZ biological control agents can only be released from containment if they are shown to be free from unwanted associated organisms. We report on two years of increasingly intensive attempts to obtain a gregarine-free population of *N. ogloblini* including use of highly hygienic field collection methods in Brazil to get clean material at source, surface sterilization of eggs, use of cages with HEPA-filtered air in containment, and attempts to improve our gregarine detection methods by gut dissection and DNA probes (both of which proved less easy, and more expensive than anticipated). In December 2010 we finally released *N. ogloblini* from containment after showing we had three consecutive generations of beetles tested negative for gregarines. Success was achieved by repeated sub-culturing. Firstly, eggs were collected as hygienically as possible from single female beetles (each having been paired with a single male). Then each larva was reared in solitary containment but with poor hygiene to ensure that any low level of gregarine infection would be expressed sufficient to minimize the risk of getting false negatives in subsequent testing. All lines testing positive were eliminated. Final crossing of lines before release from containment was carried out in an attempt to restore lost heterozygosity and overcome any inbreeding depression or adaptation to laboratory conditions.

Metabolic Profiling: A New Tool in the Prediction of Host-Specificity in Classical Biological Control of Weeds?

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Abstract

Current host-specificity testing for the selection of environmentally safe weed biological control agents is based on the molecular phylogeny of the weed. According to the centrifugal phylogenetic theory, non-target species closely related to a target weed should be at greatest risk of attack by a biological control agent, as they are biochemically and morphologically more similar to the target, and therefore more likely to share the cues used by specialists to select their host. However, a molecular phylogeny is not always a suitable surrogate for phenotypic traits at the species level. For example, the potential weed biological control agent *Ceutorhynchus cardariae* Korotyaev (Coleoptera: Curculionidae) investigated for the invasive Brassicaceae plant *Lepidium draba* L., attacks plant species distantly related to *L. draba* under no-choice conditions, revealing a disjunct fundamental host range. The aim of this study is to compare the reliability of a phenotypic phylogram with a genetically based one for predicting host use by *C. cardariae*. We used data of feeding and oviposition trials for 23 test plant species/populations, differing in susceptibility to *C. cardariae* attack. Host preference of *C. cardariae* was assessed using different phylograms based either on genetic distance between test plant species or various combinations of phenotypic traits, such as chemical profile and physical attributes. Patterns of susceptibility to *C. cardariae* among the different trees were compared using different measures of phylogenetic correlation. Principles discovered could be used to explain and potentially predict the host range of other biological control agents.

Individual Variation in Insect Response Causes Misleading Interpretation of Host Specificity Tests

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Abstract

Host specificity tests, conducted prior to the introduction of broom seed beetles (*Bruchidius villosus* (F.)) to New Zealand for biocontrol of *Cytisus scoparius* (L.) Link, failed to detect their ability to oviposit and develop into adults on the closely related non-target host plant *C. proliferus* L.f. Tests conducted with individual beetles indicated that the failure of the original host specificity tests resulted from high levels of variation in beetle oviposition preference and relatively low levels of replication. The occurrence of a host range expansion was discounted, but New Zealand beetles showed a higher preference for *C. proliferus* than newly imported UK beetles, indicating some adaptation to the novel host. Although beetles still strongly preferred the target host *C. scoparius*, beetles reared from *C. proliferus* scored more highly on several key performance criteria than beetles reared from *C. scoparius*, indicating the potential for increasing use of the non-target host. Implications for host-testing species with high levels of individual variation are that individuals, rather than groups, should be tested and that higher levels of replication are needed to ensure detection of low-level effects. Also, a strong preference for the normal host shown in laboratory testing may not be a sufficient indication of host specificity in the field. It is suggested that changes to host specificity testing protocols are needed, including a more conservative approach to interpreting low levels of non-target use.

Simulated Herbivory May Underestimate the Effects of Natural Herbivory: A Case Study with Dyer's Woad

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Abstract

Weed biological control critics and advocates alike have expressed a strong desire for improved predictive ability in the selection of effective agents. Consequently, studies on plant response to herbivory have become increasingly important in risk assessments. The first objective of our study was to assess the response of Dyer's woad (*Isatis tinctoria* L.) to damage by a root-crown mining weevil (*Ceutorhynchus rusticus* Gyllenhal.) currently investigated as a biological control agent for North America. Manipulating phytophagous insects can be logistically challenging and simulated herbivory is frequently advocated as a technique for replacing natural herbivory. However, mechanical damage does not always produce the same response as herbivore feeding, in particular when trying to mimic internal feeding organisms. A second objective was therefore to evaluate whether artificial herbivory can reproduce *C. rusticus* attack on dyer's woad. In addition, both natural and simulated herbivory were combined with two levels of plant competition from the North American grass *Festuca idahoensis* Elmer. Dyer's woad reacted to both types of herbivory by an increased production of secondary shoots. These shoots were however thinner and shorter and both biomass and seed production were reduced compared to control plants. Simulated herbivory caused similar effects as natural herbivory, but the magnitude of impact was lower compared to natural herbivory. *F. idahoensis* only had a weak effect on Dyer's woad, while Dyer's woad reduced biomass of *F. idahoensis*. Weevil attack on Dyer's woad increased the biomass of *F. idahoensis*, while simulated herbivory had no effect on grass biomass. In conclusion, the results of our study confirm 1) the potential of *C. rusticus* as an effective biological control agent for Dyer's woad and 2) revealed that simulated herbivory was able to mimic effects of natural herbivory, but that it underestimated the magnitude of effect in our study system.

Does Nitrogen Influence Host Choice by a Biological Control Insect?

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Abstract

Previous studies have demonstrated the importance of nitrogen as a component of host plant quality for phytophagous insects, with some insects preferring and performing better on hosts of higher nitrogen content. Although some researchers have investigated the role of nitrogen in host species choice by insect pests, there has been little exploration of how nitrogen may influence host choice patterns, and thus risk assessment, for insects used in weed biological control. The root weevil, *Mogulones crucifer* Pallas (Coleoptera: Curculionidae), was first released in Canada in 1997 against the rangeland weed, houndstongue (*Cynoglossum officinale* L.) (Boraginaceae). Both pre and post-release investigations have documented non-target attack on closely-related Boraginaceae species, albeit at a lower level than on houndstongue. Field and laboratory studies also have shown how fertilization of houndstongue with nitrogen can increase *M. crucifer* population size and weevil feeding and oviposition. As a next investigative step, laboratory studies were conducted using houndstongue and the native North American borage species, *Hackelia floribunda* (Lehm.) I.M.Johnst., to determine how the addition of nitrogen may alter non-target choice by *M. crucifer*. Two single-choice experiments (adult feeding and oviposition) were conducted using greenhouse grown houndstongue and non-target plants of either low or high nitrogen content. Leaves from individual plants were paired in small containers so that all possible combinations of plant species and nitrogen level were replicated for each experiment. Laboratory-reared female weevils at their ovipositional peak were added to each container and left for 24 hours to feed (1 female) or 48 hours to oviposit (2 females), before data collection. The results showed no effects of either species or nitrogen level on the amount of feeding by weevils. Although there was some oviposition preference shown for high nitrogen houndstongue, overall, the preference for houndstongue was greater than for the non-target species regardless of nitrogen level.

Neoclassical Biological Control: Will the Introduction of a New Association Contribute to the Control of *Myriophyllum spicatum* in South Africa?

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Abstract

While South Africa has a long and successful history of classical biological control of floating aquatic weeds, investigations into biological control against submerged plant invasions have only recently been initiated. *Myriophyllum spicatum* L. was first recorded in South Africa in the 1880s, yet the need to control it only became obvious in the 2000s, following its explosive growth on the Vaal River, one of South Africa's largest and most important rivers. *M. spicatum*, which is indigenous to Europe, Asia and North Africa, is one of the most important waterweeds in continental USA, causing millions of dollars to be spent on its control. Despite surveys for natural enemies in its regions of origin, no suitable agents have been found. However, a successful biological control program based on a new association has been implemented in the USA with a native North American weevil, *Euhrychiopsis lecontei* Dietz. which prefers *M. spicatum* over its natural host plant. Based on the experience in the USA, *E. lecontei* has been imported to South Africa as a candidate agent for *M. spicatum*. The use of new associations is inherently risky and is appropriate only where the target weed has few or no native relatives in the area of introduction. There is only one indigenous plant in the milfoil family in South Africa, *Laurembergia repens* P.J. Bergius (Haloragaceae), and host specificity tests show that *E. lecontei* cannot complete development on it. Further, this is a unique program for weed biological control, because preliminary amplified fragment length polymorphism (AFLP) analysis of the ITS region showed the South African *M. spicatum* populations to be genetically distinct from US samples, which is mirrored by differences in performance of *E. lecontei* on these populations. Implications for neoclassical biological control are discussed.

A Review of Interactions between Insect and Fungal Biological Control Agents of Water Hyacinth and Our Recent Studies

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Abstract

A large number of biological control agents have been released for the management of water hyacinth, *Eichhornia crassipes* (Mart.) (Solms-Laubach) (Pontederiaceae), an aquatic weed of socio-economic importance. Integrated biological control is a holistic approach aimed at minimizing weed population while simultaneously maintaining the integrity of the ecosystem and reducing reliance on any single agent. In South Africa six arthropod and one fungal biological control agents have been released and several indigenous fungal agents have been reported. With the presence of these biological control agents interaction between host plants, their herbivores and pathogens could play an important role in control of the weed. Such studies have been largely ignored and impacts of biological control have been mostly analyzed separately, thereby neglecting mutualistic or antagonistic interactions between these bioagents and possible joint effects on the host. We studied the possible mutualistic or antagonistic effect between mirid, *Eccritotarsus catarinensis* (Carvalho) and phytopathogen, *A. zonatum* (Sawada) W. Gams. at different insect inoculation load and culture age of pathogen respectively. Our 21 day old culture of *A. zonatum* was found to be the most virulent for all inoculation loads of *E. catarinensis*. At lower inoculation loads of 5 and 10 *E. catarinensis* per plant, the disease initiation and disease index was significantly higher than that at higher insect inoculation loads of 15 and 20 mirids per plant at same culture age of the fungi. The study brings to light the fact that the co-existence of the arthropod and fungal biological control agents can be both beneficial and detrimental to each other depending on their interactions with the host plant. Successful combination of the biological control agents can be applied under field conditions with higher chances of success.

Host-Specificity Testing of *Liothrips tractabilis* (Thysanoptera: Thripidae), a Candidate Biological Control Agent for *Campuloclinium macrocephalum* (Asteraceae) in South Africa

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Abstract

Pompom weed, *Campuloclinium macrocephalum* (Less.) DC. (Asteraceae), originates from Central and South America and was first detected in South Africa in 1962. In the 1980s *C. macrocephalum* started slowly extending its range and in the 1990s and 2000s it entered a dramatic expansion phase. An invasive of grasslands, savannas and wetlands, *C. macrocephalum* reproduces and spreads via numerous wind-dispersed seeds. Studies have highlighted the significant negative impact the weed has on biodiversity. A biological control program was initiated against the weed in 2003. Two rust fungi and nine insect species have been found to be associated with the plant in its native range. Of these, two insect species, *Liothrips tractabilis* Mound & Pereyra (Thysanoptera: Thripidae) and *Cochylis campuloclinium* Brown (Lepidoptera: Tortricidae), and one pathogen, *Puccinia eupatorii* Dietel (Uredinales: Pucciniaceae) were rated (based on damage, range and abundance) as having the most potential. The stem-deforming thrips, *L. tractabilis*, was selected as the first agent to be tested. Field host range surveys (15 species - one Lamiaceae and 14 Asteraceae) and laboratory host-specificity testing (43 species in 11 tribes in the Asteraceae) were conducted in Argentina and quarantine in South Africa, respectively. In the native range, no signs of thrips activity were recorded on any of the species surveyed. In laboratory no-choice trials, feeding damage and/or oviposition was recorded, albeit at lower levels than on the *C. macrocephalum* controls, on 14 test species in four tribes. Paired-choice trials were conducted on the 14 species that were positive in the no-choice trials. No feeding or oviposition was recorded on any of the test species, whereas the control plants were heavily attacked. *Liothrips tractabilis* is therefore considered to be suitably host-specific to *C. macrocephalum* and permission for its release is currently being sought.

Developing Biological Control for Common and Glossy Buckthorn

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Abstract

Rhamnus cathartica L.(common buckthorn) and *Frangula alnus* L.(glossy buckthorn) (Rhamnaceae) are both shrubs and small trees of Eurasian origin which have become invasive in North America. Over the past nine years, eight potential common buckthorn biocontrol agents have been studied and discarded due to lack of host-specificity, including five leaf-feeding moths. Among the other 15 leaf-feeding moths known from buckthorn in Europe, only one or two species might be specific enough to deserve further attention. There will be no further research on glossy buckthorn biocontrol agents as initial research found no promising agents. The most specific species studied so far is the leaf-margin curl galler psyllid *Trichochermes walkeri* Foerster which is known only from *R. cathartica* in Europe. Other potentially specific candidate species are the psyllids *Cacopsylla thamnocola* Foerster. and *Trioza rhamni* Schrank. and the seed-feeding cecidomyiid fly *Wachtiella krumbholzi* Stelter. Thus there are only a few candidate agents left. In addition, the detection of ‘*Candidatus Phytoplasma rhamni*’ Marcone et al. in *T. walkeri* adults raises several questions that will need to be addressed before further considering sap-suckers for biological control of *R. cathartica*. Populations of the psyllid species as well as *Rhamnus* spp. in Europe and North America have been collected to detect the presence of phytoplasma. First results indicate the presence of the phytoplasma in most psyllid populations and its host plant in Europe. We are planning in a second step to determine whether *T. walkeri* transmits ‘*Candidatus Phytoplasma rhamni*’. Studies of buckthorn seedling mortality in Europe may identify additional potential biocontrol agents, such as pathogens.

Evaluating the Potential for Biological Control of Swallow-Worts (*Vincetoxicum nigrum* and *V. rossicum*) in Eastern North America

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Abstract

Two European species of swallow-worts, *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopov) Barbarich are now naturalized in eastern North America, and considered invaders of natural areas and abandoned pastures. Herbivore surveys conducted since 2006 in Switzerland, France, Germany and Ukraine located four potential biological control agents on *V. hirundinaria* (L.) Pers.: the leaf-feeding noctuid *Abrostola asclepiadis* Denis & Schiffermüller, the leaf-feeding chrysomelid *Chrysolina aurichalcea asclepiadis* (Villa), the root-feeding chrysomelid *Eumolpus asclepiadeus* Pall., and the seed-feeding tephritid *Euphranta connexa* Fabricius. Surveys also documented the first occurrence of the leaf feeding moth noctuid *Hypena opulenta* Christoph. on *V. rossicum* in Ukraine. No herbivores have been found in Europe on *V. nigrum*. A petition is currently being prepared for release of *H. opulenta* against *Vincetoxicum* in North America (Weed et al., in these proceedings) while *C. a. asclepiadis* has been found to be too polyphagous to be considered as a potential agent. *E. asclepiadeus* overwinters as larvae in the soil; generation time ranged from one to three years in common garden experiments in Switzerland. A few native North American non-target species in the genus *Asclepias* support complete larval development of this species. In no-choice adult feeding and reproduction tests with potted plants, naïve *E. asclepiadeus* females were able to produce fertile eggs on two *Asclepias* species. *E. asclepiadeus* will occasionally oviposit in the vicinity of non target plants in the presence of *Vincetoxicum* although very little or no adult feeding was recorded from potted non-target plants under choice conditions. In an impact study with *V. rossicum* and *V. nigrum*, root herbivory at larval densities of 20 and 60 larvae/plant stunted shoot height by 4 and 6 cm and reduced plant biomass by 30% and 70% respectively. We are planning to screen additional populations of *E. asclepiadeus* and to continue working with *E. connexa* and *A. asclepiadis*. Additional surveys on *V. rossicum* and *V. nigrum* are also being considered.

Laboratory and Open-Field Tests on *Abia sericea* (Hymenoptera: Cimbicidae) – a Candidate for Biological Control of Teasels (*Dipsacus* spp.)

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Abstract

Invasive teasels (*Dipsacus* spp.) are widespread in the USA (43 states) and listed as noxious in five states. The cimbicid sawfly *Abia sericea* L. (Linné, 1758) is under evaluation as a potential agent for biological control of teasels. *A. sericea* lays its eggs under the epidermis of the leaves of *Dipsacus* plants and the larvae feed on the leaves. Laboratory and open-field experiments to evaluate the host specificity of the sawfly were conducted from 2007-2010 at Agricultural University of Plovdiv, Bulgaria. In the laboratory, potted plants from twelve plant species belonging to the families Dipsacaceae, Caprifoliaceae, Valerianaceae, Apiaceae, Asteraceae, and Brassicaceae were tested in multi-choice oviposition and feeding tests. They were arranged in plastic cages measuring 40x40x20 cm, with each cage containing one *Dipsacus laciniatus* L. plant and seven plants of different species. Individual females were released in each cage to oviposit. Number of eggs laid, number of larvae hatching and larval feeding were recorded. Eggs were laid only on *D. laciniatus* plants with one exception – on *Valeriana officinalis* L., although no larvae hatched from the latter. Larval feeding was observed only on *D. laciniatus*, *Knautia arvensis* (L.) Coult. and *Scabiosa* sp. (all Dipsacaceae). An open field test was conducted in 2010 with seven plant species from the families Dipsacaceae, Caprifoliaceae, Valerianaceae, Apiaceae, and Brassicaceae arranged in a pseudo Latin square design with a distance of 70 cm between the plants within rows. Third- and fourth-instar larvae were released in June at a rate of 1 or 2 per test plant. Adults were released on the plants several times in June-July to lay eggs. In the open-field test eggs were laid and larvae fed only on *D. laciniatus*.

Biology and Fundamental Host Range of the Stem Boring Weevil *Apocnemidophorus pipitzi* (Coleoptera: Curculionidae), a Candidate Biological Control Agent for Brazilian Peppertree

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Abstract

Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae), was introduced into Florida, USA, from South America as an ornamental in the 1840s. It eventually escaped cultivation and has become a serious threat to the state's biodiversity, especially over large areas of the Everglades. In the 1980s, this invasive weed was targeted for classical biological control because of the extent of the infestation and the absence of native congeners in the continental USA. In March 2006, a survey for new natural enemies of Brazilian peppertree was conducted in southeastern Paraguay. A stem boring weevil identified as *Apocnemidophorus pipitzi* (Faust) was collected from the plant at several locations. The insect also has been reported from Argentina, Brazil and Uruguay. Adults are defoliators and feed mainly on the upper surface of subterminal leaflets, where they produce a characteristic notching pattern. Weevils were transported under permit to the Florida Biological Control Laboratory in Gainesville, FL. A laboratory colony of *A. pipitzi* was established in April 2007 by caging the adults on cut branches of Brazilian peppertree supplemented with leaf bouquets. This insect is the first stem borer of Brazilian peppertree successfully reared under laboratory conditions. To date, over nine generations of the weevil have been produced in the laboratory, with over 10,000 adults emerging in the fifth generation. Females deposit eggs singly inside the stems and larvae feed under the bark where they damage the vascular cambium. There are five instars, pupation also occurs inside the stem, and a new generation of adults emerges in 3-4 months. Host specificity tests were conducted with 77 plant species in 39 families and 7 orders. The results showed that *A. pipitzi* can reproduce only on Brazilian peppertree and the congeneric Hardee peppertree, *Schinus polygamus* (Cav.) Cabrera, which is invasive in California. The results of laboratory host range tests indicate that *A. pipitzi* is a *Schinus* specialist. A petition to release this insect in Florida for classical biological control of Brazilian peppertree was submitted to the federal interagency Technical Advisory Group for Biological Control Agents of Weeds.

Biology, Host Specificity, and Larval Impact of *Hypena opulenta* (Lepidoptera: Noctuidae): A Promising Biological Control Agent of Swallow-Worts (*Vincetoxicum*) in North America

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Abstract

A classical biological control program has been initiated against the invasive European swallow-worts *Vincetoxicum nigrum* (L.) Moench and *V. rossicum* (Kleopov) Barbarich in North America. After its discovery in southeastern Ukraine attacking leaves of *V. rossicum*, the noctuid moth *Hypena opulenta* (Christoph) was transported to quarantine to initiate studies on its life history, host specificity, and larval impact. In the laboratory, adults of *H. opulenta* begin oviposition two days after emergence and produce approximately 600 eggs. Larvae develop through five instars and overwinter as pupae. Pupal diapause is facultative, resulting in at least two generations per year. Longevity and fecundity of females raised on *V. nigrum* and *V. rossicum* were similar and they showed no oviposition preference among *Vincetoxicum* species. Of the 74 plant species tested (distributed among 43 genera within 9 families), *H. opulenta* larvae completed development only on *Vincetoxicum*. *H. opulenta* averaged over 75% survival on all *Vincetoxicum* species, indicating that both target weeds are suitable hosts. Partial development occurred on two plants in the Urticaceae. In the impact study, feeding by two larvae per plant caused reductions in aboveground biomass to *V. rossicum* resulting in decreased reproductive output (flower, seedpod, and seed production). Only flower production of *V. nigrum* was negatively affected by larval feeding. The results of this study indicate that *H. opulenta* apparently poses little risk to native North American plants and is a promising agent against forested populations of *V. rossicum*. A petition is currently being prepared for release against *Vincetoxicum* in North America.

Phenotypes of Common Crupina (*Crupina vulgaris*), Synchronization of Bolting, and Yield Effects of Leaf Removal and Inoculation by *Ramularia crupinae*

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Abstract

Common crupina (*Crupina vulgaris* Cass.) is an annual plant of major importance in the Western United States. There are two varieties of crupina, *i.e.*, var. *vulgaris* and var. *brachypappa*, that occur in North America. Only by artificial plant vernalization, is it possible to synchronize bolting between varieties for comparative studies. Successful vernalization was achieved in this study by germinating seeds and growing transplants at 10°C with an 8 hr photoperiod for a minimum of one month. Typical plant phenological development, *i.e.*, seedling, rosette, bolt, bud, flowering, and seed stages, results for both varieties. Use of this protocol has enabled comparative studies on susceptibility of both varieties at the same time. Because crupina reproduces only by seed, an attempt was made to determine which plant part (or parts) provides photosynthate for seed fill. If such can be identified, then climatic conditions that occur at that stage of growth can be estimated and used to determine if conditions would be favorable for disease when the plant is most vulnerable. Either selected leaf removal or inoculation of various plant parts (or growth stages) by *Ramularia crupinae* Dianese, Hasan & Sobhian was used in these tests. Clear evidence of the importance of cauline leaves was found in two leaf removal experiments. Although reductions in seed yield and other parameters resulted from inoculations with *R. crupinae*, the importance of plant part was less clear than in the detached-leaf experiments. One reason for this difference is that symptom development under greenhouse conditions requires from 10 days to 2 weeks, so effects from infection of crupina on yield parameters may be manifested at a slower rate than when leaves are detached. Although *R. crupinae* was damaging and caused seed yield loss in these studies, more profound effects may result from inoculations, either at earlier stages of plant development or after multiple inoculations.

An Update on Biological Control of Invasive Hawkweeds in North America

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Abstract

European hawkweeds (*Pilosella* spp.) have been introduced into New Zealand and North America where several species have become problematic. In the early 1990s, CABI Europe-Switzerland initiated a biological control project on behalf of the *Hieracium* Control Trust in New Zealand for mouse-ear hawkweed, *Pilosella officinarum* (L.) F.W.Schultz & Sch. Bip. (= *Hieracium pilosella*). Five insect species were eventually released in New Zealand. Since 2000, CABI has also been investigating natural enemies for use against invasive alien hawkweeds in North America, namely meadow hawkweed, *P. caespitosa* Chiov. (= *Hieracium caespitosum*) and orange hawkweed, *P. aurantiaca* (L.) F.W.Schultz & Schultz-Bip. (= *Hieracium aurantiacum*). In contrast to New Zealand, where all existing hawkweeds are naturalized, native *Hieracium* spp. occur in North America, thus limiting the number of species that can be considered for introduction. A gall wasp, *Aulacidea subterminalis* Niblett, which attacks the stolon tips of several *Pilosella* species has proven to be very specific. Regulatory authorities in the USA and Canada have recently approved release of the agent in time for releases in spring 2011. A TAG petition for a root-feeding hoverfly, *Cheilosia urbana* Meigen. is currently being drafted. One additional candidate agent investigated is *Aulacidea pilosellae* Kieffer. which galls the midrib of leaves, stolons and flower stalks of several *Pilosella* spp. Two forms of the wasp that differ in life history are known. Wasps located in the northern distribution range are univoltine, whereas wasps in the southern range are bivoltine. The two forms also appear to differ in their host range. Molecular analyses are currently underway to determine the level of genetic differentiation between the two forms. Apart from the candidate agents listed above, we believe that the prospects for finding further effective and safe European insects for hawkweed biological control in North America are unlikely. Should further agents be required, we suggest that pathogens, especially the rust *Puccinia hieracii* (Röhl.) H. Mart. should be re-evaluated.

Searching for New Potential Agents for an Old Problem: Field Bindweed (*Convolvulus arvensis*)

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Abstract

In the 1980s, two biological control agents were released for field bindweed (*Convolvulus arvensis* L.) management in North America: the bindweed moth *Tyta luctuosa* Denis & Schiffermüller (Lepidoptera: Noctuidae), and the gall mite *Aceria malherbae* Nuzzaci. (Acari: Eriophyidae). While establishment for the moth has not been confirmed, the mite is established in several U.S. states and in Canada, but impact is variable. In 2009, the search for additional potential agents for the US was revived. We currently focus on the stem-boring fly *Melanagromyza albocilia* Hendel. (Diptera: Agromyzidae) and the root-mining flea beetle *Longitarsus pellucidus* Foudras. (Coleoptera: Chrysomelidae). The agromyzid has two generations per year and field observations revealed that attacked shoots often dry up and die. Unfortunately, we were not able to obtain oviposition of the fly under lab conditions in 2010, and so no host-specificity tests could be conducted. Adults of the flea beetle readily laid eggs, and we started to conduct no-choice larval transfer tests with ten test plant species, eight native to North America. Adults emerged from at least three test species; these will be exposed under multiple-choice conditions in 2011. Apart from the agromyzid and the flea beetle, there are at least five additional insects with biocontrol potential, i.e. a defoliating leaf beetle, two leaf and flower feeding moths, and two root-mining sesiid moths. We are also revising the original test plant list. The mite had been tested on 48 plants species in a wide range of families, including many economically important, but unrelated, crop species. Taking into account changes in the emphasis of host range testing since the 1980s and new information on the phylogeny of the family Convolvulaceae, the revised list will mainly contain native North American species and ornamentals and crop plants in the family Convolvulaceae.

Field Garden Experiments to Assess the Host Specificity of *Aceria solstitialis* (Acari: Eriophyoidea), Potential Biological Control Agent for *Centaurea solstitialis* (Asteraceae)

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Abstract

Centaurea solstitialis L. (yellow starthistle) is an annual noxious weed that currently infests millions of acres of rangelands, non-cultivated and natural areas in the Western USA. It displaces native plant communities reducing plant diversity and forage production for livestock and wildlife. *Aceria solstitialis* L. is an eriophyoid mite found exclusively in association with *C. solstitialis* in Turkey and Bulgaria. This mite damages bolting plants causing stunting, leaf curling and incomplete flower development. During 2008 and 2009, two open field tests were conducted in Bulgaria, to study the mite's dispersal behavior and host range. The experiments were conducted on plots of 100 m² at the experimental field of Agricultural University of Plovdiv. Five plant species were included in the experiment: *C. solstitialis* (infested and not-infested), *C. diffusa* Burm.f., *C. cyanus* L., *Carthamus tinctorius* Mohler, Roth, Schmidt & Boudreaux, and *Cynara scolymus* L. The plants were infested with mites before transplanting them in the field. An infested leaf cutting, with at least 30 mites, was placed on each test plant except on the negative control (*C. solstitialis* not-infested). Results of these field experiments showed that *A. solstitialis* mites were present in high population densities only on intentionally infested *C. solstitialis* and *C. cyanus*.

Open Field Experiment to Assess the Host Specificity of *Lixus cardui* (Coleoptera: Curculionidae), a Potential Candidate for Biological Control of *Onopordum acanthium* (Asteraceae)

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Abstract

Scotch thistle *Onopordum acanthium* L. (Asteraceae) is native to Europe and Asia and has been introduced to temperate climates elsewhere, including North America and Australia. In the US the weed is most problematic in the semi-arid parts of the Northwest, California and Nevada. *Lixus cardui* Olivier is a weevil that lays its eggs in the flowering stem of Scotch thistle in cavities chewed by ovipositing females. The larvae burrow, feed and pupate within the stem. An open field experiment, to evaluate the host specificity of the weevil, was conducted on a small experimental plot at the Agricultural university of Plovdiv, Bulgaria, in 2010. Nine plant species, belonging to the family Asteraceae, were arranged in a pseudo Latin square design with a distance of 1 m among the plants in the rows. Most plants were provided as rosettes, which were transplanted from the field in Southern Bulgaria during April and early May (*O. acanthium*, *Cirsium arvense* (L.) Scop., *Arctium lappa* L., *Carduus acanthoides* L., *Carthamus tinctorius* L. and *Centaurea cyanus* L.). Some were sown in the lab and then transplanted to the experimental plot (*Cynara scolymus* L., *Silybum marianum* (L.) Gaertn. and *Helianthus annuus* L.). Adult *L. cardui* were collected in May and June in the area around Plovdiv and were released in the experimental field, one or two on each plant. At the end of August the plants were dug out, except those which did not bolt (*C. scolymus* and *A. lappa*). The stems were dissected and examined for larvae, pupae or adults of *L. cardui*. The results of dissections showed that all the Scotch thistle plants were damaged by the weevil, while its presence was never registered in any other test species. Specimens from these experiments are currently undergoing genetic and morphological studies to understand if we are in the presence of different genetic entities not distinguishable by morphological traits.

Targeting Ecotypes of *Hydrellia lagarosiphon* in Pre-Release Studies Using Adult Longevity, Reproductive Performance and Temperature Tolerance

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Abstract

A leaf-mining fly, *Hydrellia lagarosiphon* Deeming (Ephydriidae) was identified as a potential biological control candidate for the submerged aquatic weed *Lagarosiphon major* Ridl. Moss ex Wager (Hydrocharitaceae). Larvae feed on the leaves and cause significant damage to shoot tips reducing the photosynthetic potential of the plant. Three populations of *H. lagarosiphon* were collected from different sites across the native geographic range in South Africa varying in altitude. As part of the pre-release testing the variation in adult longevity, reproductive performance and extreme temperature tolerance of the three fly populations were assessed under laboratory conditions maintained at 22:16° C in a day:night photoperiod of 15:9h. The mismatch between performance in native and introduced ranges in classical biological control is considered an important factor reducing the efficacy of biocontrol agents. To assess temperature tolerance for different fly populations critical minima trials were run using a range of pre-treatments and plunge protocol into discriminating temperatures before scoring survival. The implications for targeting specific populations in the native range as a better ecological match to conditions in Ireland and parts of Europe are discussed.

Developing Biological Control for Perennial Pepperweed in the U.S.: Progress So Far

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Abstract

Perennial pepperweed, *Lepidium latifolium* L. (PPW), is a mustard of Eurasian and Central Asian origin that is invading natural and cultivated habitats in North America and is difficult to control with conventional means. A project investigating the potential for biological control of PPW was started in 2004. Based on field collections in various countries within the native range of PPW, 113 phytophagous organisms were sampled or reared, five of which were prioritized as potential biological control agents: the root-mining weevil *Melanobaris* sp. n. pr. *semistriata* Boheman (Coleoptera, Curculionidae), the gall-forming weevil *Ceutorhynchus marginellus* Schultze (Coleoptera, Curculionidae), the stem-mining flea beetle *Phyllotreta reitteri* Heikertinger (Coleoptera, Chrysomelidae), the gall-forming eriophyid mite *Metaculus lepidifolii* Monfredo & de Lillo (Acari, Eriophyidae) and the stem-mining fly *Lasiosina deviata* Nartshuk (Diptera, Chloropidae). Host-specificity testing in quarantine at CABI Europe-Switzerland started in 2006 with the first three of the potential agents. In addition, several field tests were conducted in the native range of the organisms in Russia and Turkey. A summary of results so far will be presented and the potential of the organisms as biological control agents discussed.

What's Been Happening in Our Containment Facility? The Old and the New

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Abstract

Landcare Research, formerly Department of Scientific and Industrial Research (DSIR), has worked on the biological control of weeds for the past 90 years. During this time 63 species of insects have been imported into our containment facility for re-phasing, host testing, pathogen screening and rearing before being released into the New Zealand environment. Some species were imported and not released for various reasons. These included, inherent diseases, population collapse in containment, used for experiments only, regulations allowing host testing only. Table 1 (See the URL below) shows which species have been assessed for establishment, which species were tested for diseases and those which tested positive. Additionally, the table shows which importations were approved for release, when and by which authority, and which importations were never released. Families of insects introduced into our quarantine facility:

Acari=2, Chrysomelidae=16, Curculionidae=9, Tephritidae=5, Tenthredinidae=2, Pyralidae=3, Oecophoridae=3, Tortricidae=3, Nymphalidae=2, Scythrididae=2, Tingidae=2, Cerambycidae=1, Psyllidae=1, Agromyzidae=1, Syrphidae=2, Cecidomyiidae=2, Bruchidae=1, Eupterotidae=1, Crambidae=1, Cynipidae=1, Pterophoridae=2, Arctiidae=1, Cosmopterigidae=1. Parts of the plant targeted for attack by these potential biological control agents were: stems, 15; leaves, 30; roots, eight; and flowers, one. Nine insect species were released to attack seeds. Six seed-feeding agents, three root feeders, one stem feeder and four foliage feeders, were shown to have an impact on the target plant either in the laboratory or in the field. During 1920 to 2011 most introductions have occurred during the 1980s (16), 1990s (24), and 2000s (23).

There have been 18 weed species (listed below) that these insects have been imported to attack as biological control agents. Of the weed species selected as targets for biological control 11 were environmental (i.e. not productive sector weeds), three solely pastoral while four were both pastoral and environmental weeds. The 18 weed species include: *Cytisus scoparius* (L.) Link, *Jacobaea vulgaris* Gaertn., *Ulex europaeus* L., *Cirsium arvense* (L.) Scop., *Carduus nutans* L., *Cirsium vulgare* (Savi) Ten., *Passiflora tripartite* (Juss.) *mollissima* (Kunth) Holm-Niesen & P.M. Jørg., *Hieracium pillosellae* (L.) F.W.Schultz & Sch.Bip., *Chrysanthemoides monilifera* (L.) Norlindh, *Lonicera japonica* Thunb., *Tradescantia fluminensis* Vell., *Araujia hortorum* E. Fourn, *Calluna vulgaris* (L.) Hull, *Clematis vitalba* L., *Alternanthera philoxeroides* Griseb, *Solanum mauritianum* Scop., *Ageratina riparia* (Regel) R. M. King & H. Rob., and *Hypericum perforatum* L. Twenty-one potential agents were imported but not released and 10 of these were not released because they failed host range testing experiments. Only three species were not released because of disease risk. Up until 1991, 21 species were internally approved for release from quarantine by the director of DSIR. Only one species was refused approval for release in

1991 by an external agency, the Ministry of Agriculture and Fisheries (MAF). From 1991 to 1998 MAF approved the release of 8 species from quarantine before the existence of the Environmental Risk Management Authority New Zealand (ERMA NZ). ERMA NZ (now the Environmental Protection Authority, EPA) was formed and began presiding over releases of exotic organisms in 1998 and has since approved the release of 16 species of biological control agent for seven weed species.

Table 1 can be located and viewed at the following URL:

http://www.landcareresearch.co.nz/publications/researchpubs/biocontrol_spp_list_hugh_gourlay.pdf

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Biological Control of Garlic Mustard, *Alliaria petiolata*, with the Root and Crown-Boring Weevil *Ceutorhynchus scrobicollis*

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Abstract

Biological control of garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande, testing indicates potential for long-term management of this invasive biennial weed. Extensive host specificity trials with the potential biological control agent *Ceutorhynchus scrobicollis* Nerensheimer and Wagner, a root and crown-boring weevil, have been conducted at CABI Europe-Switzerland and at the University of Minnesota. To date, we have tested native Brassicaceae species representing the majority of Brassicaceae tribes present in North America, as well as tribes containing cultivated or ornamental species, a total of 72 species in all. Representative species from twenty-two additional plant families have been tested, primarily plants growing in the same habitat as garlic mustard. Results of these tests indicate that *C. scrobicollis* is a highly specific herbivore. Female *C. scrobicollis* do not exhibit normal oviposition behavior on plant species outside of the Brassicaceae. Within the Brassicaceae, *C. scrobicollis* has been found to have a narrow host range. After applying to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) for approval to field release *C. scrobicollis*, we are currently testing additional native mustards at the reviewers' request. We hope to complete additional tests by May 2011 and submit supplemental data to TAG this summer.

Pre-release Efficacy Assessments of the Leaf-Mining Fly *Hydrellia lagarosiphon*, a Candidate Biological Control Agent of the Submerged Weed *Lagarosiphon major*

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Abstract

Releasing biological control agents that suppress plant populations is essential in achieving effective control. Pre-release efficacy assessments were conducted on the leaf-mining candidate agent *Hydrellia lagarosiphon* Deeming (Diptera: Ephydriidae) to ensure that the agent will incur sufficient damage to control *Lagarosiphon major* Ridl. Moss ex Wager (Hydrocharitaceae) in Ireland. Larval densities were manipulated with limited resources available to determine the carrying capacity of shoot-tip fragments and the resultant impact on the growth and viability of *L. major*. Both potted plants and shoot-tip fragments of varying lengths were used representing rooted field infestations and fragments that are essential for short and long-distance dispersal. The results indicate that leaf damage increased with larval densities, but that competition for limited resources resulted in a maximum of between 3-4 larvae being supported by each shoot tip. Of the parameters measured for the effects on subsequent growth of *L. major*, shoot tip length and shoot biomass were negatively affected by larval damage at all densities and at higher damage levels in particular, failed to recover over time. Plants compensated for larval damage through the initial production of side-shoots and roots. However over time, at larval medium (3) and high (5) densities, these parameters were negatively affected and failed to recover. Establishment of *L. major* was compromised at medium (3) and high densities (5) of larval damage and of those that did establishment at these densities were significantly impacted in terms of root and shoot biomass. Sustained damage induced by consecutive generations of *Hydrellia* showed increasing negative effects on rooted plants at both low and high larval densities in terms of root and shoot biomass. The pre-release efficacy studies suggest that *H. lagarosiphon* will contribute to the suppression of plant growth at relatively low larval densities per shoot-tip.

Biology and Preliminary Host Range of *Hydrellia lagarosiphon*, a Potential Biological Control Agent against *Lagarosiphon major*

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Abstract

A recently discovered fly, *Hydrellia lagarosiphon* Deeming (Diptera: Ephydriidae) was investigated in South Africa as a possible biological control agent for *Lagarosiphon major* (Ridley) Moss (Hydrocharitaceae) in Ireland and other parts of the world where it is invasive. Impact studies under laboratory conditions show that *H. lagarosiphon* larvae destroy approximately 20 leaves of *L. major* before pupation, and restrict the formation of side branches, an important aspect of vegetative spread of the weed. Investigations in the field showed that *H. lagarosiphon* was the ubiquitous and most abundant species associated with *L. major* in South Africa as it was found at every field site (n= 29) where *L. major* was recorded. High larval abundance was also recorded, with a majority of shoots investigated containing larvae (58%) at densities of up to ten larvae per shoot. Furthermore, examination of its field host specificity showed *H. lagarosiphon* to be specific to *L. major* as it was not recorded from any other co-occurring aquatic or semi-aquatic species. Due to the difficulty in managing *L. major* in its invaded range, *H. lagarosiphon* could be considered a valuable candidate biological control agent.

Host Range of Two Chrysomelid Beetles, *Zygogramma signatipennis* and *Z. piceicollis*, Biological Control Candidates for *Tithonia rotundifolia*

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Abstract

The weedy red sunflower *Tithonia rotundifolia* (Mills) S.F. Blake (Asteraceae: Heliantheae), originally from Mexico, has become invasive throughout the humid and sub-humid tropics of South America, South East Asia and tropical and subtropical Africa, including South Africa. In South Africa, *T. rotundifolia* is declared a category 1 weed, and was targeted for biological control in 2007. Host-specificity tests showed that the two leaf-feeding beetles, *Zygogramma signatipennis* Stål and *Z. piceicollis* Stål (Chrysomelidae: Chrysomelinae), were the most damaging and promising biological control agents for *T. rotundifolia*. During no-choice tests on 29 plant species in eight plant families, *Z. signatipennis* laid overwhelmingly on *T. rotundifolia*, with 79.67 eggs deposited on the target weed versus 33.5 and 2.5 deposited on its congener *Tithonia diversifolia* (Hemsl.) A.Gray and *Helianthus annuus* L. (Asteraceae), respectively. *Tithonia rotundifolia* was also found to be the most suitable host for the other chrysomelid, *Z. piceicollis*, during no-choice tests, depositing 56 eggs on this plant versus 29 and 7.5 eggs on *T. diversifolia* and *H. annuus*, respectively. Further larval survival tests showed that both *Zygogramma* species were able to complete development only on *T. rotundifolia* but not on *T. diversifolia* and *H. annuus*. During multi-choice tests including nine plants species in three plant families, both *Zygogramma* spp. strongly preferred *T. rotundifolia* to other plant species. Based on host-specificity tests and surveys conducted in the native range, the two *Zygogramma* spp. appear to be sufficiently host-specific to be released against *T. rotundifolia* in South Africa.

Biological Control of Silvery Threadmoss (*Bryum argenteum*) in Turfgrass, Nursery Crops, and Hardscapes

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Abstract

Silvery threadmoss (*Bryum argenteum* Hedw.) has become an increasingly problematic weed of golf courses, particularly since the loss of mercury and other heavy metal based pesticides. Though not labeled for moss control, they were used extensively on golf course putting greens as fungicides and at the same time controlled moss. To meet golfer demand for firmer, faster playing surfaces superintendents have decreased mowing heights, requiring increased passes of equipment over the green. This, along with decreased nutrient inputs and an open turf canopy contributes to moss encroachment on putting greens. Currently, few labeled products exist for moss control driving turf managers to use off-label substances including peroxides, baking soda, and detergents. These dessicate moss and may severely injure turfgrass even with careful applications. Hand removal of moss is also a common practice. The only commercial herbicide labeled for control is carfentrazone applied at 6.7fl oz/A, which does not completely eradicate moss, so sequential applications are required once moss recovers. Aside from turf, silvery threadmoss can also be a weed problem of containerized nursery crops as well as nursery growth pads and stone hardscapes. With no professional products labeled for moss control in these systems there are several potential niche markets for an effective biological control of silvery threadmoss. A naturally occurring microorganism has been discovered that effectively controls moss on putting greens without causing injury to the most commonly managed turf species, creeping bentgrass and annual bluegrass. We are evaluating this organism for all three niche markets. Testing includes fulfillment of Koch's postulates, pathogen characterization to determine the site of action on silvery threadmoss and evaluation of host specificity in *Bryum* and related genera. Studies conducted this season will evaluate non-target effects on desirable plant species in the turfgrass and nursery industries and naturally occurring mosses in the landscape.

Estimating Density Dependent Impacts of the Arundo Scale, Biological Control Agent for the Invasive Giant Reed

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Abstract

The sap-feeding armored arundo scale (*Rhizaspidiotus donacis* (Leonardi)) has been permitted for use as a biological control agent for giant reed (*Arundo donax* L.), a non-native, highly invasive woody grass that infests waterways and riparian areas of the southwestern US and Mexico. We used a nested factorial design within a controlled greenhouse setting to (1) test the hypothesis that pressure from natural enemies can interrupt the net primary production of giant reed by disrupting water and nutrient transport and detrimentally affecting the photosynthetic ability of the plant and (2) build a predictive model of the density dependant impacts of the arundo scale on plant growth to inform a biological control program. Different densities of the immature stages of two distinct genotypes of the arundo scale were administered to individually-potted ramets of the same genotype of the target weed. Growth parameters of plant such as shoot height and number of nodes, number of shoots, number and length of side shoots were measured monthly for six months, or after one generation of the scale. Insect-induced plant physiological stress was estimated with monthly measurements of light reflectance using a spectroradiometer, and by analyzing differences in leaf gas exchange among the different treatments at the end of the experiment. At six months, all plants were destructively sampled to count the density of mature scale adults and to extrapolate biomass accumulation and allocation of the test plants. Initial results suggest a scale density-dependent effect on both total plant biomass and water use efficiency. *Arundo* plants with severe infestations of scale insect exhibit reduced photosynthesis and tend to have a slower rate of growth than control plants with no insects or plants with low levels of scale density. If this trend continues, this biological control agent may prove to be an effective tool to curb the negative ecological and social impacts of this weed, especially if present in high densities. These results may help inform an inundative approach to weed biological control.

Morphological and Molecular Identification of White Blister Rust Collected from Perennial Pepperweed in Nevada and California

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Abstract

Perennial pepperweed (PPW, *Lepidium latifolium* L.) is a cruciferous plant native to Eurasia that is a noxious weed in the western USA. In northern Nevada, PPW plants in the field are commonly infected with white rust fungus (*Albugo* sp.), exhibiting white pustules on the leaves and stems of mature plants in summer. Molecular taxonomic identification of the *Albugo* species encountered on PPW in Nevada and preliminary host-specificity tests were performed to assess the potential of this fungus as a bioherbicide for control of PPW. Using genus-specific PCR primers (DC6 and LR-0), a region of rDNA including sections of ITS1, 5.8S ribosomal RNA gene, and ITS2 were amplified, subcloned into the pGEM[®]-T vector, and sequenced using the T-7 promoter and SP6 upstream primers. A BLAST search matched DNA sequences of the Nevada isolate of *Albugo* sp. with five voucher isolates of *Albugo candida* (Pers.) Kuntze (98% identity, E value=0.0) as well as three isolates of *Albugo lepidii* A.N.S. Rao (99% identify, E value=0.0). Thus, this Nevada isolate has significant variations within its rDNA sequence from that of both *A. candida* and *A. lepidii* and its identity remains somewhat ambiguous. Preliminary host-range tests under both greenhouse and growth chamber conditions have shown that the Nevada isolate infected PPW but there were no symptoms on any of 12 varieties of cruciferous vegetables during tests of up to one month. These preliminary results suggest that the Nevada *Albugo* isolate may represent a previously unknown pathotype with high host-specificity on PPW. Further studies on the host-specificity and pathogenicity of Nevada white rust isolates, particularly for early-season application to new PPW growth, will be necessary to better understand the potential benefits and risks of using this fungus as a bioherbicide against PPW in Nevada.

**Preference and Damage by the Stem-Boring Moth,
Digitivalva delaireae – a Potential Biological Control Agent
of Cape-Ivy, *Delairea odorata*, on its Two Varieties in California, USA**

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Abstract

Cape-ivy, *Delairea odorata* Lem. (Asterales: Asteraceae), is a perennial vine native to South Africa, and was introduced in the eastern United States (U.S.) in the 1850s as an ornamental. The plant is now well established in natural areas and has become a serious pest in coastal regions of California and upland Hawaii, as it is an aggressive climbing vine that can form solid covers which can block light and smother native vegetation. The U.S. Dept. of Agriculture, Area Research Service (USDA-ARS) has initiated a biological control project targeting Cape-ivy, and host-range testing of a potential agent - the stem-boring moth *Digitivalva delaireae* Gaedike & Krüger (Lepidoptera: Acrolepiidae), is nearly complete. In both South Africa and California, two morphological varieties (stipulate and astipulate) of Cape-ivy exist. The stipulate variety is most common in both South Africa and California, therefore host-range tests of Cape-ivy were conducted on plants of this variety. We are currently studying preference, as well as the effect of infestation by *D. delaireae* on the development of both Cape-ivy varieties found in California, and whether preference and damage inflicted on Cape-ivy by *D. delaireae* differs between varieties. Results of choice preference tests showed that *D. delaireae* infested 4% more leaves on the astipulate variety, though this difference is minimal, it is significant ($P = 0.01$). Tests assessing the effect of damage by *D. delaireae* on Cape-ivy development, on both varieties are on-going.

Potential of the Seed-Feeding Weevil *Cissoanthonomus tuberculipennis* for Biological Control of Balloon Vine *Cardiospermum grandiflorum* in South Africa

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Abstract

Balloon vine *Cardiospermum grandiflorum* Swartz (Sapindaceae), originally from South and Central America and now invasive in South Africa, was one of the five emerging weeds targeted for biological control in 2003. In search of potential biocontrol agents, exploratory surveys were conducted in northern Argentina from 2005 to 2009. These surveys, which included other plant species in the genus *Cardiospermum* and other native Sapindaceae, were aimed at determining the distribution and ecological host ranges of the natural enemies associated with balloon vine. The seed-feeding weevil *Cissoanthonomus tuberculipennis* Hustache (Coleoptera: Curculionidae) was one of the two insect species found to be restricted to balloon vine, and was widespread throughout the north eastern part of Argentina, particularly in Misiones province. Open-field tests, conducted under natural conditions in the native range, also showed that *C. tuberculipennis* was restricted and highly damaging to its natural host *C. grandiflorum*, with up to 54% of balloon vine fruits damaged by the beetle between September 2008 and April 2010. Host-specificity tests, including no-choice, paired-choice and multi-choice tests, showed that *C. tuberculipennis* fed and reproduced only on *C. grandiflorum*. Failure of *C. tuberculipennis* to feed and reproduce even on the three congeners of *C. grandiflorum* during host range studies proves beyond reasonable doubt that the weevil is highly host-specific to the target weed, and therefore poses no threat to non-target plant species. As *C. tuberculipennis* is monophagous and has a very short life cycle with a highly damaging larval stage, it is the best candidate for biological control of this weedy creeper in South Africa and elsewhere. It is strongly recommended that permission be granted for the release of *C. tuberculipennis* from quarantine for the biological control of *C. grandiflorum* in South Africa.

Artificial Diet for Completing Development of Internal Feeding Insects of Plant Stems and Roots as an Aid for Foreign Exploration

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Abstract

Internal-feeding insects can be effective biological control agents of invasive alien weeds, but it is usually difficult to rear field-collected immature stages to the adult stage to facilitate identification and establishment of laboratory colonies. The development of effective diets and rearing systems could greatly aid the discovery and evaluation of root- and stem-feeding insects for biological control. We developed and tested a system for rearing adult insects from field-collected larvae that is useful for foreign exploration. We adapted a previously developed artificial diet for *Hylobius transversovittatus* Goeze, the purple loosestrife root weevil, and tested the system on a root-feeding weevil, *Ceratapion basicorne* (Illiger), dissected from *Centaurea solstitialis* L. (yellow starthistle) plants in Turkey. The diet ingredients were modified to reduce microbial contamination, and the container size and style of top were chosen for ease of use and to reduce diet desiccation. Gouging the diet at the container sides facilitated insect survival and permitted easier monitoring of developmental progress. The method also worked with varying success for a variety of other beetles (Buprestidae, Cerambycidae, Chrysomelidae, Curculionidae), moths (Noctuidae, Pyralidae) and flies (Chloropidae) dissected from a variety of plant species (Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Elaeagnaceae). However, the diet was not successful for rearing adults of the yellow starthistle stem-boring flea beetle, *chalconera* Illiger, which normally pupates in soil. The diet probably can be further modified to better suit insects associated with a particular plant species and/or plant parts that differ in critical physical and/or chemical properties.

First Insect Agents Evaluated for the Biological Control of *Parthenium hysterophorus* (Asteraceae) in South Africa

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Abstract

The annual herbaceous plant, *Parthenium hysterophorus* L. (Asteraceae: Heliantheae) (parthenium), has wide-ranging negative impacts on crop and animal production, biodiversity conservation, and human and animal health in Africa, Asia and Australia. Parthenium is an increasing problem in southern and eastern Africa, causing severe economic losses in some countries. In 2003, South Africa became only the third country globally, after Australia and India, to implement a biological control program against this weed. Relying on the experience of the Australian program, three agents were selected and imported into quarantine in South Africa, for evaluation of their suitability for release. They included the leaf rust fungus *Puccinia xanthii* Schwein. var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero (Pucciniaceae), that, after evaluation, was released in South Africa in 2010, the leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) and the stem-boring weevil *Listronotus setosipennis* Hustache (Coleoptera: Curculionidae). No-choice tests were conducted and, later, multiple choice tests resolved results of non-target feeding and/or oviposition under no-choice conditions for several plant species, for both *Z. bicolorata* and *L. setosipennis*. However, up to eight *Helianthus annuus* L. (Asteraceae: Heliantheae) (sunflower) cultivars were accepted by *Z. bicolorata* and *L. setosipennis* for feeding and/or oviposition under choice conditions, necessitating the investigation of larval development. Finally, further analyses were used to quantify the risk, which was demonstrated to be very low, to non-target plant species that were suitable for feeding and/or oviposition under laboratory conditions. Both agents have since been provided to Ethiopia for a biological control program there. The stem-galling moth *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) and the seed-feeding weevil *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae) have recently been imported for evaluation in South Africa, as experience elsewhere has demonstrated that a suite of agents is required to achieve effective biological control of parthenium under different environmental conditions and in different regions.

Host Specificity Testing of *Archanara geminipuncta* and *A. neurica* (Lepidoptera: Noctuidae), Candidates for Biological Control of *Phragmites australis* (Poaceae)

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Abstract

Two European stem-boring moth species (*Archanara geminipuncta* Haworth and *A. neurica* Hübner.) are evaluated as possible biological control agents for introduced *Phragmites australis* (Cav.) Trin.ex Steud. in North America. A particular challenge in the *Phragmites* biocontrol program is the existence of a native subspecies *P. australis* (Cav.) Trin.ex Steud. subsp. *americanus*. We have developed a sequence of quarantine-testing procedures, first transferring neonate larvae onto young shoots for five days. If plants are successfully attacked, larvae are given a range of stem sizes and tested for ability to complete larval development. This approach quickly eliminates most plant species as unsuitable hosts with the exception of several native haplotypes and a few test plant species which are exposed for larval development. In addition, open-field tests are underway at CABI in Switzerland concentrating on potential oviposition preference of the moths between introduced *P. australis* and the native subspecies.

Foreign Exploration and Host Testing of Brazilian Pepper (*Schinus terebinthifolius*) Biological Control Agents

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Abstract

Brazilian pepper is among the worst environmental weeds in Florida and other areas of the US. This species occupies diverse habitats causing many environmental problems including decreased biodiversity of the infested areas. Although chemical controls are known and used to control this invasive species, biological control presents an attractive alternative when practiced safely. The native range of this species includes eastern Brazil, northeastern Argentina, and eastern Paraguay. The USDA/ARS Invasive Plant Research lab with colleagues at the Universidade Regional de Blumenau in Brazil, and the South American Biological Control Lab in Argentina have been searching for and testing insects that will be safe and effective at controlling this weed in the US. Surveys in South America have discovered many new insects including new moth, wasp, and caterpillar species. Several of these species are undergoing testing to determine suitability and safety for release in the US. These include the Phlaeothripidae thrips, *Pseudophilothrips ichini* Hood, the Attelabidae beetle *Omolabus piceus* (Germar), two Gracillariidae moths *Eucosmophora schinusivora* Davis and Wheeler and *Leurocephala schinusae* Davis and Mc Kay, the Notodontidae moth *Tecmessa elegans* Schaus, the Geometridae moth *Oospila pallidaria* Schaus, and the Gelechiidae moth *Crasimorpha infuscat* Hodges. Additionally several new species have yet to be described including the Braconidae wasp *Allorhogas* n. sp. and an unknown Gelechiidae stem galling moth.

Foreign Exploration and Host Testing of Chinese Tallow Biological Control Agents

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Abstract

Chinese tallow, *Triadica* (=Sapium) *sebifera* (L.) Small (Euphorbiaceae) or popcorn tree is among the worst environmental weeds in the southeastern US. This species occupies diverse habitats causing many environmental problems including decreased biodiversity of the infested areas. Although chemical controls are known and used to control this invasive species, biological control presents an attractive alternative when practiced safely. The native range of this species primarily includes central and southern China. The USDA/ARS Invasive Plant lab, colleagues at the Australian biological control lab, and the Chinese Academy of Science have been conducting foreign surveys searching for insects that will be safe and effective at controlling Chinese tallow in the US. Surveys have revealed many new herbivores throughout the native range of these species. These include many new weevil, thrips, psyllid, eriophyid mites and lepidopteran species. Several of these species are, or have undergone preliminary testing to determine suitability for release. These include the Attelabidae beetle *Heterapoderopsis bicallosicollis* (Voss), the Chrysomelidae beetle *Bikasha collaris* (Baly), and the Noctuidae moth *Gadirtha inexacta* Walker. Host testing conducted in China indicates all three species have narrow host ranges. Quarantine testing is underway in the US and indicates the defoliator/root feeding *B. collaris* herbivore is the most suitable biological control agent. Additionally, a new stem gall wasp (Eulophidae; Tetrastichinae) has been colonized in China and will undergo testing as available.

Performance of *Hydrellia pakistanae* (Diptera: Ephydriidae) and *Hydrellia* sp. on the South African Biotype of *Hydrilla verticillata* (Hydrocharitaceae)

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Abstract

Hydrilla, *Hydrilla verticillata* L.f. is a submerged aquatic plant of Asian and Australian origin that is invasive in the U.S.A. and South Africa. Two leaf-mining flies, *Hydrellia pakistanae* Deonier. and *Hydrellia* sp., are currently under evaluation as candidate biological control agents for *H. verticillata* in S.A. Because of high genetic diversity within the species, biotype matching for the biological control program was an important consideration. *H. pakistanae* originates on a dioecious biotype of hydrilla from India and *Hydrellia* sp. originates on monoecious hydrilla from Singapore, close to the region of origin of S.A. *H. verticillata*. Based on this, it was assumed that *Hydrellia* sp. is the more suitable candidate however any differences in performance between the two cultures needed quantification before rejecting *H. pakistanae*. Various fitness parameters were measured in a quarantine glasshouse with a min/max of 22/28 °C and a growth chamber with a constant temperature of 27°C and a 16:8 day: night period. *Hydrellia* sp. had a shorter development time and significantly higher egg to adult survival, fecundity and adult longevity compared to *H. pakistanae* under both experimental conditions. *H. pakistanae* fitness parameters were also compared to those measured for this species in the U.S.A. By this comparison, *H. pakistanae* doesn't perform as well on S.A. monoecious *H. verticillata* as it does on the U.S. dioecious form. The results from this study in conjunction with results from host specificity testing suggest that *Hydrellia* sp. is the better candidate for release against *H. verticillata* in S.A. However, the damage potential of each candidate agent will be measured through impact trials before making a final decision on whether *H. pakistanae* should be rejected as a biological control agent for *H. verticillata* in S.A.

SESSION 2: EMERGING ISSUES IN REGULATION OF BIOLOGICAL CONTROL

Why the New Zealand Regulatory System for Introducing New Biological Control Agents Works

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Abstract

For the past 12 years, New Zealand has operated the most comprehensive and internationally admired regime for regulating the introduction of new organisms. Using this system, which is based on the premise that the benefit of introduction must outweigh any risks, 21 species of biocontrol agent have been approved, and no applications have been declined. Over the same period the rate of introduction of biocontrol agents into other jurisdictions worldwide appears to have declined. This paper discusses why the Hazardous Substances and New Organisms Act has been an effective and efficient statute for managing the introduction of new organisms, including: founding documents that defined the initial standards for decision-making, the use of formal analysis to weigh the risks and costs of a proposed introduction against the benefits, legal deadlines that bind the regulator, the independence of the regulator, commitment to genuine public participation in decision-making, and the cooperative culture of the regulatory agency. Despite these successes, some practitioners claim that it may encourage a conservative approach, limiting investigation of agents to those that are sure to be approved. The regulations may also be limiting the types and number of biocontrol agents being introduced into New Zealand. This paper therefore discusses new developments that will facilitate the introduction of agents while still satisfying the regulatory requirements.

Introduction

New regulations for the introduction of new biocontrol agents into New Zealand came into effect in 1998. It was initially thought that the new process would be onerous and expensive for applicants, resulting in a decline in biological control activity (Fowler et al., 2000). In fact, for applicants seeking to introduce biocontrol agents for weeds, the process has evolved into a stable, effective and manageable (although still costly) regulatory regime. This paper identifies and discusses the features that have been largely responsible for the success of this regime,

namely the nature of the legislation and the decision-making framework, the use of formal risk assessment and cost-benefit analysis, statutory time frames for decision-making, independence of the regulator, and public participation in decision-making.

While the regulatory process has been particularly helpful for the biological control of weeds, others have found the process less accessible, and recent changes to accommodate their concerns are discussed. The work of Environmental Risk Management Authority (ERMA) has recently been subsumed into a new, wider-reaching Environmental Protection Authority (EPA). The powers and operations of ERMA are expected to transfer

seamlessly, but how the rise of the EPA might affect the current regulatory environment is also discussed.

The regulatory framework

The Hazardous Substances and New Organisms Act (HSNO Act) became law in 1996 (Goldson et al., 2010; Pottinger and Morgan, 2008). The central tenet of the Act is that the same risk assessment criteria can be applied to a wide range of human activities that impact on the New Zealand environment and society. As a result, the Act regulates not only the introduction of all exotic organisms (for whatever purpose) and all genetic modification, but also activities as diverse as the licensing of pesticides, and the storage of explosives and fireworks. The 'new organisms' provisions of the HSNO Act that regulate biocontrol agents came into force in 1998.

The Act required the establishment of:

1. A six to eight member authority (the Environmental Risk Management Authority, or ERMA) appointed by the government. The Authority had the considerable powers of a commission of inquiry, and members had the same immunities and privileges as those of a lower court judge when making decisions. These powers and responsibilities have now passed to the EPA. Its decisions can be challenged judicially, but only on points of law. In other words, a court cannot overturn a decision, only refer it back for reconsideration if it deems that correct processes have not been followed.
2. A Māori advisory board (Ngā Kaihautū Tikanga Taiao), tasked with advising the Authority and ensuring that the regulatory process reflected the concept of partnership between the Crown (the government) and the indigenous people as implied in the Treaty of Waitangi of 1840.
3. An agency of the public service (called ERMA New Zealand, but not to be confused with the Authority itself) to facilitate and evaluate applications, manage the regulatory process, and make recommendations to the Authority.
4. A methodology interpreting the requirements of the HSNO Act for applicants and for the Authority. The key features of the methodology are listed in Table 1.

Table 1. Features of the Hazardous Substances and New Organisms (Methodology) Order 1998 that must be applied to decisions to introduce a new organism. The costs and benefits are those that relate to New Zealand and would arise as a consequence of approving the application.

When considering an application the decision-maker must recognise and take into account a range of risks, costs, benefits and other impacts including:
• The safeguarding of the life-supporting capacity of air, water, soil and ecosystems • The maintenance and enhancement of the capacity of people and communities to provide for: ○ Their own economic, social and cultural wellbeing ○ Reasonably foreseeable needs of future generations • The sustainability of all native and valued introduced flora and fauna • The intrinsic value of ecosystems • Public health • The relationship of Māori and their culture and traditions with their ancestral lands, water, sites, wahi tapu (special places), valued flora and fauna, and other taonga (important spiritual and material values) • The economic and related benefits • New Zealand's international obligations • The ability of the organism to establish an undesirable self-sustaining population anywhere in New Zealand, and the ease with which it could be eradicated

When evaluating the risks, costs and benefits of the organism, the decision-maker must:

- Use recognized risk identification, assessment, evaluation, and management techniques, and must take into account.
 - ☐ The nature of the effect, including monetary and non-monetary costs and benefits
 - ☐ The probability of occurrence and the magnitude of each risk, cost and benefit
 - ☐ The distributional effects of the costs and benefits over time, space and groups in the community
 - ☐ The uncertainty bounds on the information
 - ☐ Any submissions received, and the applicant's assessment and proposals for managing the risks
 - ☐ Consider the scientific evidence, and the degree of uncertainty associated with the evidence

The decision-maker must be cautious if there is:

- Unresolved scientific or technical uncertainty relating to potential adverse effects
- Disputed scientific or technical information

The decision-maker must have regard to the following risk characteristics:

- Exposure to the risk is involuntary
- The risk will persist over time
- The risk is subject to uncontrollable spread
- The potential adverse effects are irreversible
- The risk is not known or understood by the general public

The decision-maker may approve an application where:

- The organism poses negligible risks to the environment and human health and safety
- OR The risk and costs of the organism are outweighed by the benefits

The decision-maker must decline an application where the organism is likely to cause:

- Significant displacement of any native species within its natural habitat
- Significant deterioration of natural habitats
- Significant adverse effects on human health
- Significant adverse effects on New Zealand's inherent genetic diversity
- Disease, be parasitic, or become a vector for human, animal, or plant disease (unless this is the aim).

Attributes contributing to the success of the regulatory process

At first, the new regime proved difficult for both applicants and agency staff as they came to grips with the requirements of the legislation. This appears to remain a deterrent for those seeking to establish invertebrates as control agents of insect pests as only four such applications have been submitted since 1999. However, applicants seeking to introduce agents for weed control persevered through this

period, working collaboratively with the agency in the evolution of what has become a highly effective regulatory process. Since 1999 application has been made to introduce 21 agents to attack ten weeds and four pest insects (Table 2). Five of those targets have been novel (not previously targeted elsewhere in the world) and one of the agents was a pathogen. Weed biocontrol researchers were also involved in the recent application to introduce 11 species of scarabaeid beetles to improve the efficiency of dung removal from New Zealand's pastoral landscape. No applications have been declined (Table 2).

Table 2. Applications approved under the HSNO Act 1996 for the release into New Zealand of biocontrol agents for weeds, insect pests and ruminant dung, and time to decision.

Target Category/Species	Agents	Year of approval	Working days to approval
Biocontrol of weeds:			
<i>Ageratina riparia</i>	<i>Procecidochares alani</i>	2000	109
<i>Pilosella</i> spp.	<i>Macrolabis pilosellae</i>	2001	135
	<i>Cheilosia urbana</i>		
	<i>Cheilosia psilophthalma</i>		
<i>Buddleja davidii</i>	<i>Cleopus japonicus</i>	2005	473
<i>Chrysanthemoides monilifera</i>	<i>Tortrix s.l.sp.</i>	2005	111
<i>Jacobaea vulgaris</i>	<i>Cochylis atricapitana</i>	2005	119
	<i>Platyptilia isodactylis</i>		
<i>Cytisus scoparius</i>	<i>Agonopterix assimilella</i>	2006	93
	<i>Gonioctena olivacea</i>		
<i>Cirsium arvense</i>	<i>Cassida rubiginosa</i>	2007	71
	<i>Ceratapion onopordi</i>		
<i>Tradescantia fluminensis</i>	<i>Neolema ogloblini</i>	2008	210
<i>Solanum mauritianum</i>	<i>Gargaphia decoris</i>	2009	109
<i>Nassella neesiana</i>	<i>Uromyces pencanus</i>	2011	68
<i>Tradescantia fluminensis</i>	<i>Lema basicostata</i>	2011	77
	<i>Neolema abbreviata</i>		
Biocontrol of insect pests:			
Mealy bug	<i>Pseudaphycus maculipennis</i>	2000	83
<i>Heliothrips haemorrhoidalis</i>	<i>Thripobius semiluteus</i>	2000	45
<i>Sitona lepidus</i>	<i>Microctonus aethiopoides</i>	2005	121
<i>Uraba lugens</i>	<i>Cotesia urabae</i>	2010	55
Removal of ruminant dung:			
Ruminant dung	11 scarabaeid species	2011	77

Documents defined the initial standards for making decisions

The methodology order (ERMA, 1998) set out the standards by which the regulatory framework was to operate. It laid out guiding principles and protocols implicit in the Act for environmental risk assessment, and a set of minimum standards with which applications must comply. In particular, it defined what issues applicants should address to produce a credible application (Table 1). It also let

the agency assist applicants in the preparation of applications. A range of detailed technical guides were prepared on a wide range of topics, such as how to assess qualitative risks and benefits, or how to measure potential benefits. The methodology and other publicly available policy documents provided a stable and coherent initial reference point. This documentation prevented the early development of arbitrary interpretations of the Act by decision-makers, and fixed standards through time. This was vital to the development of the process initially.

However, the culture of application and evaluation has evolved, and case history has accumulated. EPA staff now consider some of these documents to be too prescriptive to govern modern decision-making, and these have been withdrawn.

Decisions must be based on recognized techniques for risk–cost–benefit (RCB) analysis

At the core of the New Zealand system is the principle that if the benefit of an introduction sufficiently outweighs the adverse effects, then that action should be allowed. This differs from many other jurisdictions worldwide where the quantum of risk is the primary factor considered, with little or no regard for potential benefit. The HSNO Act requires the Authority and the agency to use recognized risk identification, assessment, evaluation, and management techniques in their consideration of applications.

The regulator must meet deadlines set by legislation

Statutory time frames remove delay as a means of avoiding decisions. Once an application has been lodged, the Act compels the regulator to meet a set of five deadlines in the evaluation process. The regulator does not have the option of not making a decision. A decision on an application must be made within 100 working days of submission (Table 3). If the agency finds that it is likely to breach the deadlines for any of the steps in the evaluation process, it must seek the permission of the applicant to extend the period. In the only exception to this, the Authority may decide that additional information is required to make a rational decision, and may ‘stop the clock’ while this information is obtained. This provision has been used only rarely (Kay et al., 2008).

Independence of the decision-making authority

Although government appointed, the Authority acts autonomously, advised (but not instructed) by the agency, with input from experts if required. Decisions made by the Authority cannot be overturned by the courts. This independence gives the Authority

strength to resist rogue political, economic, conservationist or other activist influences that might affect the decision-making process. This is reinforced by the instructions of the Act about how the Authority should behave.

The regulator promotes genuine public participation in decision-making

From the outset, the HSNO Act has required that the application and evaluation process should be open, transparent, and public (ERMA, 1998). The agency has encouraged applicants to engage with affected parties long before the application is written to ensure that all issues are identified and addressed in the application process. In particular, applicants are required to consult with the indigenous people (Māori) to ensure that their specific concerns are addressed, and that the application process conforms to the principles of the Treaty of Waitangi. In addition, all applications to release biocontrol agents are then open for public submission for 30 working days (Table 3). This allows those consulted to assess whether their concerns have been adequately presented or not, and gives any other organizations or members of the public the opportunity to make a submission in favor or in opposition to the application. If any submitter asks for it, a public hearing before the Authority must be convened.

This high degree of public involvement, coupled with the professionalism of the agency and the acknowledged independence of the decision-making body, has established a high degree of trust between applicants, the public and the decision-makers. However, the finality of the Authority’s decision then provides an absolute endpoint to public involvement. The process leaves no stone unturned, but in the end, the regulator’s decision is final.

The culture of the agency has facilitated applications

The Authority has a responsibility to do what is best for New Zealand. In accordance with the Act, the agency actively assists applicants in the development of applications, but critically, this assistance focuses on helping an application to succeed, as long as conditions are met. The risk profiles of applications to introduce control agents for weeds are very

Table 3. Maximum time available for steps in the regulatory process to introduce a new organism under the Hazardous Substances and New Organisms Act 1996.

Regulatory step	Working days per step	Working days following submission
Application officially received, checked and accepted. Application widely advertised to seek submissions from the public, and made available on the Web (epa.govt.nz)	10	10
Public submissions closed	30	40
Evaluation and review by EPA staff, advice provided to the decision-maker, public hearing held if required	30	70
Consideration of the application by the decision-maker, and public notification of written decision	30	100

similar. As case history has grown, the agency and the Authority have become increasingly confident in their ability to assess the risk of these applications, without the support of detailed information. A constant in its recent evolution has been the commitment of the agency to making the application process easier and less expensive for all.

Has the HSNO Act been universally positive for applicants?

The culture of the agency has been to remove barriers to applications, and a range of potential applicants were recently interviewed to identify factors that limit their willingness to make applications. The key issues identified were the cost and the bureaucratic nature of the regulatory process, the level of evidence required to complete an application, attitudes to uncertainty, and the consequences of a submitted application failing (Campbell, 2010).

The agency currently covers approximately 80% of the cost of a proposal, and the applicant the remaining 20%. The application fee was recently halved, and the evolution towards a simpler system has continued the downwards trend in both application cost and processing time. The fees for submitting proposals to decision-makers are still considerable (typically NZ \$18,000) and smaller entities regard cost as a significant barrier to application.

The current regime was instituted during a time when the regulatory atmosphere was conservative. This led to a very technical and bureaucratic process.

An example of such is the technical guides and methodology mentioned earlier. At the time these helped define expectations of applicants and the process that would be followed. The documents were complex, dense and voluminous but at the same time they were stable and were used for almost a decade without significant change. It now appears what once was useful is now holding back the further evolution of the legislation in practice. On the other hand, the system has provided certainty and assurance. An applicant knows that if historic methods are used, the approval of a proposal is highly likely.

Costs of application arise not only from the regulatory process, but also from preparation and technical input. The most prohibitive of these has been the use of complex safety-testing experiments. Though not necessarily a requirement, a culture has developed among researchers and decision-makers that ensures scientifically validated host-range testing is now an expectation of all proposals. The Act allows for the possibility of making an application without such information, but this is an option that has not yet been explored by applicants.

However, the way the legislation, including the methodology, is drafted also creates problems for applicants. The legislation takes a strong position around uncertainty. It asks that a precautionary approach is taken which many have taken to mean that an absence of evidence is evidence of risk.

Finally, among researchers there is a large belief that having a proposal declined would inflict reputational damage, and many do not want to take the risk.

If you combine prohibitive cost, a requirement for specialist and technical knowledge, conservative decision-makers, conservative legislation and conservative researchers you undoubtedly end up with a very conservative system. The jury is still out, but there is the possibility that this has led to New Zealand missing out on useful biocontrol agents. At a time when biodiversity loss is increasing, the cost of not releasing biocontrol agents to control pests and weeds is certainly not something that should be ignored.

Future of the regulatory process

The EPA wants to make the innovation chain, from picking a potential agent through to its release into the environment, more efficient by reducing barriers to successful application. For example, ERMA/EPA recently agreed to a broad approval for importing into containment any invertebrate that is a prospective weed biocontrol agent. Species-by-species approvals are no longer required. Work is underway to extend this to biocontrol agents for all pests and their hosts. This will reduce waiting time and the burden of fees.

Work is continuing to reduce the costs associated with a release application. This focus has recently been to move away from much of the past practice. This practice has been technical in nature and attempted to take a one size fits all approach. The EPA sees reliance on host range testing as a case in point. While necessary for many cases, the regulators are questioning the perception that host range testing is a prerequisite for an application. For example, it has been used in instances to test the host range of generalist predators to little if any benefit. Allowing the application process to become more case specific and less of a box-ticking exercise is aimed at reducing the burden of time and cost.

However, perhaps of more importance to the EPA is building a stronger applicant community. Bringing practitioners together gives their voice much more power than being separated as individuals. It also identifies common and recurring issues that can be solved. In future this group may also become the applicant, rather than using individual organizations. This allows the reputational risk to be shared among the group.

Finally, for this good work to continue, both the EPA and applicants consider it vital to continue

building trust in the decisions made. This is a very public process in a very democratic society. People need to feel comfortable with the decisions made. In order to make this a reality the EPA strives to make all documentation simple, readable and plain. Everyone needs to be able to understand the issues before confidence can be built. In future, greater emphasis will also be placed on working with stakeholders outside the formal process. Interacting with government agencies can be bureaucratic, intimidating and a very slow process. Working with people outside these formal channels allows more freedom for discussion and the ability to generate a genuine dialogue.

Discussion

Globally, despite well-developed regulatory processes (Sheppard et al., 2003; De Clerck-Floate et al., 2006) and active revision of those processes (e.g., Hunt et al., 2008; Palmer, this volume), gaining approval for the introduction of a biocontrol agent into at least some jurisdictions appears to be subject to delays, or is becoming increasingly difficult (Sheppard et al., 2003; Klein et al., 2011). The key impediments vary from country to country, but include: preoccupation with the harm that biological control can do rather than the benefits it can provide; increasing demands for evidence of safety; lack of capacity or appropriate focus in key decision-making positions; the involvement of a multitude of organizations that must consider applications sequentially (or at least separately) and the need for dual approvals; reliance on the goodwill and/or knowledge of individuals within the regulatory process rather than a firm framework; and the influence of federalism and the need to take account of continental neighbors.

By contrast, the HSNO Act and ERMA/EPA have provided 13 years of stable, rational and open decision-making about biocontrol agents. In recent cases involving control agents for weeds there has been sufficient trust by the public in both the applicants and the regulatory process that the level of public participation has actually declined, and there has only been one public hearing required for the five applications lodged since 2007 whereas hearings were the norm before this. Apart from the

key characteristics of the process discussed in this paper, it is likely that other factors contribute to the efficiency of the regulatory process in New Zealand, including small population size and efficient networking, a pragmatic conservation lobby, the simplicity of unicameral government, and isolation from neighboring countries.

The demise of ERMA and the assumption of its roles by the EPA pose threats to the integrity of this approval process for biocontrol agents. However, the HSNO Act remains, and so key tenets of the Act such as the information requirements, statutory time frames, and the use of RCB analysis also remain. The revision of the process and its translation to a new authority must protect the features of the process that have made it so successful. 'New organisms' applications will become a smaller part of the business of environmental risk management under the EPA, and may be susceptible to changes in culture. Similarly, the regulatory process is being simplified to remove barriers to applicants and to reduce costs. This has seen the withdrawal of the written guidance, the simplification of applications, and increased reliance of applicants on agency staff for advice. This risks inconsistency in approaches with changes in agency personnel, and reduced public trust if the amount of information immediately available to the public reduces.

As New Zealand's regulatory process for new biocontrol agents evolves, the challenge for applicants and EPA staff will be to guard against variation over time in standards for judging applications, loss of institutional memory and corporate culture as processes migrate to the new organization, loss of a public framework to guide applicants and regulators, and loss of public trust in the process. EPA staff and applicants are working together to ensure this transition occurs safely.

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Australia's Current Approval Procedures for Biological Control with Particular Reference to its *Biological Control Act*

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Abstract

In 2010 Biosecurity Australia introduced new procedures for evaluating applications to approve release of biocontrol agents in Australia under the *Quarantine Act 1908*. The first organism treated under the new system was the leaf beetle, *Plectonycha correntina* Lacordaire, for biocontrol of Madeira vine, *Anredera cordifolia* (Tenore) Steenis. This case is used to describe Australia's current protocols for release. Conflicts of interest can still be resolved by the *Biological Control Act 1984* which was introduced to allow continuation of the project for Paterson's curse (*Echium plantagineum* L.). After Paterson's curse was declared a target organism and eight insect species declared agent organisms in 1987, blackberry (*Rubus fruticosus* L.) and rabbits (*Oryctolagus cuniculus* L.) were later declared as target organisms in 1987 and 1995 respectively. No other cases have been considered under the Act. A case is presently being prepared by Queensland to declare mother-of-millions, *Bryophyllum delagoense* (Eckl. and Zeyh.) Schinz, as a target and the weevil *Osphilia tenuipes* (Fairmaire) as an agent under the Act because the weevil may attack closely related exotic ornamentals. This test case will provide perspective about the usefulness of the Act for weed biocontrol in Australia and internationally.

Introduction

As in other countries, Australia's protocols for approving the release of biological control agents are ever evolving. Early biological control projects were lantana (Day et al., 2003) and prickly pear (Dodd, 1940), both of which began before the First World War. The first four lantana insects were introduced and released in Australia on the basis that they appeared host specific and had not attacked non-targets in Hawaii after their release there a decade earlier. Host testing was undertaken for the prickly pear insects in the 1920s but the Prickly Pear Board decided that it would give the approvals to release, rather than the officer-in-charge of the scientific

investigation (Anon., 1923). No regulatory agency was involved at that stage and the Board also requested legislation to prevent individuals making their own importations and releases (Anon, 1923). As time progressed the approval process became more rigorous (McFadyen, 1997; McLaren et al., 2006).

This paper describes Australia's current arrangements using the leaf beetle *Plectonychya correntina* Lacordaire for illustration. The paper also provides an update on the use of the *Biological Control Act 1984*, which provides a mechanism for resolving conflicts of interest, whether regarding the target weed or the candidate biological control agent.

Standard procedures leading to release

Approval of a weed as a target for biological control

Regulatory agencies (and often funding bodies for the research) require that a weed be approved as a target for biological control to ensure that any beneficial uses of the plant are noted. Generally a weed would not be approved as a target if it had significant beneficial uses or if it were a native plant. The process has been that an agency makes written application to the Australian Weeds Committee, which considers the application and also notifies all of the Australian jurisdictions. It then makes a recommendation to the Standing Committee (a committee of departmental heads of all relevant federal and state jurisdictions) for approval by Standing Committee. In 2011 Standing Committee delegated responsibility to approve targets to the Australian Weeds Committee.

Madeira vine, *Anredera cordifolia* (Tenore) Steenis, is a serious weed along the east coast of Australia and elsewhere in the world (Vivian-Smith et al., 2007). It was approved as a target in May 2007 by the Natural Resource Management Standing Committee following an application submitted in April 2005 and subsequent recommendation from Australian Weed Committee.

Approval to import agents into Australian quarantine facilities

Two federal agencies, the Australian Quarantine and Inspection Service (AQIS), acting on recommendation by Biosecurity Australia (BA) (both part of the Biosecurity Services Group, Department of Agriculture, Fisheries and Forestry) and the Department of Sustainability, Environment, Water, Population and Communities (SEWPC) issue permits for biological control agents to be imported into approved quarantine premises. These actions are made under the authority of the *Quarantine Act 1908* and the *Environment Protection and Biodiversity Conservation Act 1999* respectively. Obtaining these permits is a relatively straightforward procedure, with the major consideration being the security of the quarantine facilities and associated arrangements.

Following approval of Madeira vine as a target, the leaf feeding beetle, *Plectonycha correntina* Lacordaire, was selected as the first candidate for testing. This chrysomelid was known to have a limited host range, to be multivoltine, and to attack the plant in both larval and adult stages (Cagnotti et al., 2007). Permits from both agencies were obtained within 2 months of application.

Approval to release agents from quarantine facilities

AQIS approves releases of biological control agents under the *Quarantine Act* but does so following a recommendation from BA. Until 2009, applications to release agents were reviewed for BA by 21 cooperators, drawn from all the jurisdictions and including representatives of all the weed biocontrol groups. Essentially the release application needed the support of all the cooperators before BA would recommend release.

In 2009, BA adopted new procedures. All applications are now reviewed within a formal risk assessment framework for imported goods (Australian Government Department of Agriculture, Fisheries and Forestry, 2011). Within that framework they have so far been treated as non-regulated cases of existing policy rather than as standard Import Risk Analyses (IRA). The essential steps of the non-regulated process are similar to an IRA and they are: (1) provisional assessment and recommendation by BA, (2) posting of the application and provisional assessment on the web for 50 working days, (3) consideration by BA of any objections to the release and (4) recommendation to AQIS to approve (or deny) the release.

SEWPC independently ascertains whether the proposed agent will harm Australia's flora or fauna. The SEWPC process is to post the application on their web site for stakeholder comment, to write to all appropriate jurisdictions, and to consider the recommendation of BA before deciding upon further import and release of the agent. Under the terms of the *Environment Protection and Biodiversity Conservation Act 1999* the Minister must sign the approval within 30 business days and there is provision for the decision to be tabled as a disallowable instrument in both Houses of Parliament for 15 sitting days. This tabling has been

a concern for biocontrol practitioners as it can result in an additional delay of 6-9 months (depending on when Parliament sits) in the release of the agent.

It should be emphasized that both BA and SEWPC conduct risk analyses and not benefit/cost analysis. They do not take into account the potential benefits of introducing the biocontrol agent and reject agents if there is significant risk to Australia's economy or Australia's flora or fauna.

Host testing of *P. correntina*, using 37 plant species, showed that the insect was specific to Madeira vine (Palmer, 2009). Some adult feeding and very occasional larval development occurred on the closely related Ceylon spinach, *Basella alba* L. This was interesting because Ceylon spinach is commonly grown as a vegetable by our Asian communities though there is little commercial trade.

Application to release *P. correntina* was made to both agencies in December 2009, and this agent was the first to be considered under BA's new procedures. In November 2010, AQIS advised that the insect could be released. The 11 months taken to process the application was longer than might be expected in the future. The approval from SEWPC was received in February 2011. A very important change made was that SEWPC did not require the decision to sit in the Parliament before the approval took effect and that this requirement may no longer be necessary in non-controversial cases. A final departmental approval was then obtained and the insect was released from quarantine in early April 2011, some 16 months after application.

The Biological Control Acts

Madeira vine is an uncontroversial weed and *P. correntina* is a host specific agent. Together they provide a clear cut example with which to demonstrate Australia's usual protocols for biocontrol agents. In some instances there may be conflicts of interest associated with either the target weed or the specificity of the agent, such as the examples provided by Palmer (2003a; -2003b). Australia can resolve these issues through the *Biological Control Act 1984 (Cwlth)* and the mirror legislation of each of the state jurisdictions.

The *Biological Control Act 1984* was enacted as a response to the conflict that developed between most

graziers, who regarded Patterson's curse (*Echium plantagineum* L.) as a serious weed and apiarists who valued the plant for its honey. Use of the Act allowed resolution of the *Echium* conflict (Anon., 1985) in 1987, the blackberry issue in 1992 and the rabbit calici virus issue in 1995 (Anon., 1996). These have been the only times the Act was applied over 16 years ago.

The mother-of-millions case

Mother-of-millions, *Bryophyllum delagoense* (Eckl. & Zeyh.) Schinz, and its hybrid *B. houghtonii* (D. B. Ward) P. I. Forst. (Crassulaceae), became approved targets for biological control in 2001. Exploration for agents in Madagascar and southern Africa resulted in consideration of four agents as potential biological control agents (Witt et al., 2004; Witt and Rajaonarison, 2004). Detailed host specificity testing of two agents and preliminary testing of the other two indicated that none was completely specific and all had narrow host ranges. The host range of the weevil *Osphilia tenuipes* (Fairmaire) provides a typical profile. Host testing indicated that *O. tenuipes* could utilize all *Bryophyllum* spp. found in Australia (all exotic), several of the closely related exotic *Kalanchoe* spp. and possibly a few other exotic Crassulaceae (Palmer, 2003a; -2003b). It does not attack any native Australian plant.

Queensland's actions

Because Queensland believes that the benefits of controlling mother-of-millions far outweigh possible costs to the nursery industry and private gardeners, it has recently proposed to the Primary Industries Ministerial Council that all *Bryophyllum* spp. be declared target organisms and that the four insect species be declared agent organisms under the *Queensland Biological Control Act 1987*. This proposal was unanimously supported by the Ministerial Council in April 2011.

Future course

The Queensland Minister for Agriculture, Food and Regional Economies who is the 'Biological Control Authority' for Queensland under the Act

will now ascertain whether there is significant community dissent about proceeding with biological control by releasing any of the insect species. Should objections be significant, the Biological Control Authority would appoint a commission of enquiry to ascertain the relationship between the potential benefits of the biological control of the weed and the potential costs to the nursery industry should the insect become a pest.

Should there either be no significant objections to the releases or the commission of enquiry finds that the benefits outweigh the costs, the Biological Control Authority would recommend that the declarations be approved by unanimous agreement of Ministerial Council.

After the weeds and the insects are declared target organisms and agent organisms, respectively, under the *Biological Control Act 1987*, application would then be made to AQIS and SEWPC to approve the releases under the *Quarantine Act 1908* and the *Environment Protection and Biodiversity Conservation Act 1999* respectively.

New ground

This application through a Biological Control Act breaks new ground in three aspects. All previous applications under the Act have been taken retroactively after agent organisms had been released or had escaped. This is the first application to be made completely *a priori*.

Previous applications have involved conflicts of interest with the target organisms, essentially when some stakeholders regarded the targeted species as beneficial. This is the first application where the potential conflict about the lack of specificity of the candidate agents.

Australia's first legislation providing resolution of these conflicts of interest was the Commonwealth's *Biological Control Act 1984* which was used for all three previous cases. All of the state jurisdictions enacted mirror legislation (e.g. Queensland's *Biological Control Act 1987*) but this will be the first use of state legislation and a state minister acting as the Biological Control Authority.

Discussion

Australia remains well placed to practise classical biological control, having appropriate legislation and protocols (summarized in Table 1) to assess applications in a reasonably rigorous and transparent fashion. Over the decades the process has become more rigorous (and onerous). In the early 1980s it took 2-3 months to receive permission to release and that permission was given by a single entity. Today we hope for permission in 9 to 12 months but quite often it takes longer.

It is of course highly desirable for approvals to be obtained as quickly as good evaluation will allow. There are significant costs and risks associated with the long term culturing of agents in quarantine, including the occupation of very valuable quarantine space, the labor costs associated the continued culture, the cost of continued control by stakeholders in the absence of the biocontrol, the risk that the culture could be lost or genetically modified, and even the risk that it might escape. It is therefore hoped that further modifications can be made to Australia's procedures to reduce the time taken by the decision making processes.

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How Specific is Specific Enough? - Case Studies of Three Rust Species under Evaluation for Weed Biological Control in Australia

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Abstract

Host specificity is one, if not the most critical attribute for any biological control agent to be considered for introduction into a new environment. Nevertheless, specificity is not always an absolute measure. The acceptability of any potential non-target effects will depend on a number of factors such as the extent of the non-target damage, the status of the affected species and the inherent characteristics of the receiving ecosystem; and, any decision for introduction will have to be based upon an encompassing risk – benefit analysis. The concept of “acceptable levels of host specificity” is illustrated using the case studies of three rust species which are currently under evaluation as potential classical biological control (CBC) agents for Australia: *Puccinia lantanae* Farlow from Peru for lantana, *Lantana camara* Linnaeus; *Phakopsora jatrophiicola* Cummins from Mexico for bellyache bush, *Jatropha gossypifolia* Linnaeus; and *Ravenelia acaciae-arabicae* Mundkur & Thirumalachar from India for prickly acacia, *Acacia nilotica* ssp. *indica* (Linnaeus) Wildenow ex Delile. Based on the research conducted to date, the risks associated with the potential release of each individual pathogen as a CBC agent for Australia and, in the case of *P. lantanae* also for New Zealand and South Africa, are assessed. Finally, comparisons are drawn with the risk evaluation undertaken for two other rust pathogens, *Maravalia cryptostegiae* (Cummins) Y. Ono and *Puccinia xanthii* Schweinitz var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero, previously introduced for weed control into Australia.

Introduction

For a natural enemy to be employed as a classical biological control (CBC) agent against an invasive alien weed, it is essential to elucidate its potential host-range prior to consideration for release. Specificity testing of both arthropods and pathogens has routinely been undertaken according to the centrifugal phylogenetic procedure (Wapshere, 1974), and constitutes a major component in a CBC

program. More recently, Briese (2003) suggested that the degree of phylogenetic separation between test species and the target weed should also be taken into account when compiling test lists. Standard host-range testing of fungal pathogens is conducted under optimum ambient conditions for host infection and disease development with respect to temperature and the length of the initial dew period, and by applying high inoculum loads of the infective spore stage. The aim of such an experimental set-up is to

establish the fundamental host range of a candidate agent as the widest possible range of hosts that a pathogen could infect and utilize to complete its life cycle. Based on results of this experimental host-range screening, predictions can be made about the anticipated field host-specificity or ecological host range of a pathogen. Frequently, these have been shown to be narrower than fundamental host ranges of CBC agents, as optimum experimental conditions can create so-called “false positives” (Bruckart et al., 1985; Evans et al., 2001).

Risk analyses which are undertaken before the introduction of an agent into a new environment are based largely, but not exclusively, on the data generated during host-range testing. These analyses aim to weigh the perceived risks associated with such introductions, i.e. to the environment, the economy and/or the cultural background of the geographic region, against the anticipated benefits or positive impacts the agent will exert i.e. by controlling the target weed. Equally, the risks posed through the introduction of a CBC agent will need to be balanced against the current and future environmental and/or economic consequences due to the invasive weed, or the cost and impact of management with conventional control methods, such as herbicide applications (Harris, 1990; McFadyen, 1998). A risk assessment of a fungal pathogen needs to be based on its predicted ecological host range rather than its fundamental host range, by asking: “How likely is any non-target to come under attack in a field situation?”. Fungal pathogens which exhibit a fundamental host range limited exclusively to the target weed can easily be evaluated. More commonly encountered, however, are cases where pathogens have the potential to damage a limited number of non-target species under optimum conditions for infection and disease expression. In those cases, the “status” of a potentially affected non-target species becomes relevant i.e. whether the attacked plant species is native, naturalized or introduced; whether it is of ecological, economic and/or cultural importance; whether it occurs geographically separated from the target weed or grows sympatrically. Furthermore, the extent of the anticipated impact on a non-target species will be of importance; the likelihood that exposure to infective propagules (e.g. airborne spores) will occur in the field and that conditions for infection and disease development will be met given

prevailing geographic and climatic conditions, also needs to be considered. For example, an endemic plant species, of high biodiversity importance, growing in close proximity to stands of the targeted invasive weeds, will raise more concern if damaged by a newly introduced CBC agent, than a non-native species of minor economic value, cultivated in a separated geographic region with different climatic conditions.

Thus, infection of a non-target species under controlled or artificial conditions does not necessarily preclude the introduction of the agent. The following case studies of rust species undergoing, or having undergone evaluation as CBC agents for Australia - and for one case also for New Zealand and South Africa - illustrate the concept of “acceptable level of host specificity” and highlight how this depends on specific circumstances.

Case studies

Rust pathogens currently undergoing evaluation as potential biocontrol agents

Phakopsora jatrophiicola for control of bellyache bush, *Jatropha gossypifolia*

Jatropha gossypifolia L. (Euphorbiaceae), native to the Caribbean rim and its islands, is an invasive weed in Queensland (QLD), as well as in Western Australia (WA) and the Northern Territory (NT) in Australia (Bebawi et al., 2007). In the native range, the macrocyclic rust *Phakopsora jatrophiicola* Cummins is a widespread and damaging pathogen of bellyache bush, as well as of other selected *Jatropha* species, i.e. *Jatropha curcas* Linnaeus and *J. integerrima* Jacquin (Leahy, 2004; Farr and Rossman, 2011). Since 2008, the rust has been evaluated as a potential biocontrol agent for Australia under quarantine conditions at CABI UK (CABI Europe - UK), using a urediniospore accession ex *J. gossypifolia* collected in the Mexican State of Veracruz (IMI 397220). Following confirmation that all major Australian biotypes of bellyache bush tested were susceptible to this rust accession, preliminary host range testing commenced and included the biofuel species *J. curcas*, the ornamental species *J. multifida* Linnaeus, *J. integerrima* and *J. podagrica* Hooker,

as well as rubber (*Hevea brasiliensis* (Willdenow ex A. Jussieu) Müller Argoviensis and cassava (*Manihot esculentum* Crantz) as economically important members of the *Euphorbiaceae*. Results showed *J. multifida* to be 'fully susceptible' (consistent sporulation) and *J. curcas* to be 'partially susceptible' (restricted sporulation) to the rust accession. *Jatropha integerrima* was classed as 'resistant' (macroscopic necrotic symptoms, but no sporulation) or 'partially susceptible', depending on biotype, while *J. podagrica* was rated as 'resistant'. Both rubber and cassava were immune (no symptoms).

Despite *P. jatrophiicola* being able to attack three non-target *Jatropha* species, full host-range testing of the rust was proposed, endorsed by the Queensland government, based on the following considerations. The genus *Jatropha* is introduced to Australia and *J. integerrima*, *J. multifida* and *J. podagrica* are ornamentals of minor importance. The important crop plants rubber and cassava are not susceptible. Infection of *J. curcas*, as having potential economic value as a biofuel crop, could cause concern; however, this species is a declared weed in QLD, NT and WA as well as an approved target for biocontrol nominated by the NT government. Importation of *J. curcas* into Australia is now prohibited by the Australian Quarantine and Inspection Service (AQIS). The species is currently not cultivated in Australia and, in view of its weed status, it is unlikely that *J. curcas* would become a major biofuel crop in Australia in the future.

While *P. jatrophiicola* is associated with different *Jatropha* species in the centre of origin, host-specificity studies indicated the existence of host-specialized accessions within this rust species. *Jatropha curcas*, a reported field host of the rust, was only partially susceptible towards the accession IMI 397220 ex *J. gossypiiifolia*. Similarly, cross-infectivity studies using a Mexican rust accession of *P. jatrophiicola* ex *J. curcas* (IMI 397097) against *J. gossypiiifolia* caused limited sporulation on the latter host. Based on these observations, it was considered prudent to survey a range of different geographic locations for accessions of *P. jatrophiicola* ex *J. gossypiiifolia* potentially less virulent to *J. curcas*. A rust accession ex *J. gossypiiifolia* from Trinidad (IMI 397 973), selected

due to comparatively low virulence to *J. curcas*, is currently undergoing full host-range testing.

Ravenelia acaciae-arabicae* for control of prickly acacia, *Acacia nilotica* ssp. *indica

Prickly acacia, a member of the Leguminosae - Mimosoideae, is a major invader of arid and semi-arid land in QLD. Survey work conducted in the Indian native range of this weed species found the macrocyclic galling rust species *Ravenelia acaciae-arabicae* Mundkur & Thirumalacha to be virulent to *A. nilotica* ssp. *indica* (Linnaeus) Delile, while not attacking any other subspecies or other *Acacia* species (Dhileepan et al., 2010). Based on the damage inflicted on its host and its apparent field host-range, the rust was selected for further evaluation as a potential biocontrol agent. Preliminary host-range testing using a urediniospore accession of *R. acaciae-arabicae* from Tamil Nadu, India (IMI 398973) against 17 selected *Acacia* species commenced under quarantine conditions at CABI UK in June 2010. Susceptibility of the assessed *Acacia* species towards the rust was variable. Of those tested, 16 species showed macroscopic symptoms ranging from mild to severe leaf chlorosis and/or necrosis, sometimes accompanied by strong polyphenolic plant reactions. Critically, the rust was able to sporulate with viable, infective urediniospores on the Queensland native species, *Acacia sutherlandii* (F. Mueller) F. Mueller. Although sporulation on *A. sutherlandii* was always accompanied by dark necrotic lesions, indicating that this non-target species is not a natural host, its susceptibility is, nevertheless, cause for concern.

The risks posed by *R. acaciae-arabicae* to the important Australian native genus *Acacia*, particularly *A. sutherlandii* that grows sympatrically with the target weed prickly acacia in the field, was considered unacceptably high, and further assessments were put on hold. Instead, the focus has shifted towards a second rust species, *Ravenelia evansii* Sydow & P. Sydow, associated with prickly acacia in its native range, but predominantly present in north-western parts of India (Dhileepan et al., 2010; Shivas et al., 2011). The susceptibility of *A. sutherlandii* to this rust species is currently being evaluated.

Puccinia lantanae* for control of *lantana*, *Lantana camara

Lantana (Verbenaceae), native to Central and South America, is one of the most widespread invasive plant species and has been a target for CBC for over a century (Day et al. 2003). In Australia, 31 biological control agents have been introduced, including the highly host specific rust *Prospodium tuberculatum* (Spegazzini) Arthur in 2001 (Ellison et al., 2006; Thomas et al., 2006). A second rust species, the microcyclic rust *Puccinia lantanae* Farlow, is currently being screened in quarantine at CABI UK for potential introduction into Australia, New Zealand and South Africa. A damaging rust accession from the upper Amazon in Peru (IMI 398849), causing not only the typical leaf infection but also infection of the petioles, stems, and systemic infection of meristems, was assessed for its infectivity and virulence towards 30 weedy lantana forms from Australia, five from New Zealand and six from South Africa. A qualitative scoring system was used, based on the number and size of telial pustules formed after inoculation with a standardized spore dose. Of those screened, 18 forms were found to be fully susceptible (with examples from all three target countries); three as moderately susceptible and six as weakly susceptible (supporting limited telia formation); and 14 were rated as immune or resistant (unable to complete life cycle).

Host-range testing of the rust for Australia focused on plant species in the Verbenaceae. None of the non-target species were found to be fully susceptible under optimum experimental conditions. However, one species (*Lippia alba* [P. Miller] N.E. Brown) was weakly susceptible and two (*Phyla canescens* [Kunth] Moldenke and *Verbena officinalis* Linnaeus [var. *africana* and var. *gaudichaudii*]) were weakly to moderately susceptible. Only the infection of the two varieties of *V. officinalis* is considered relevant, since these two varieties are regarded as native to Australia and occur sympatrically with lantana; the other two susceptible species are introduced weeds. It was not possible to maintain a culture of the rust on these non-target species and no infection was achieved when a low concentration of spores was applied. In addition, there was variation in the susceptibility of individual *V. officinalis* test plants, suggesting that in the field, natural resistance within wild populations is likely to

mitigate any potential impact of the rust. For New Zealand, where there are no native species within the plant family Verbenaceae, host-specificity testing has focused on native non-target species belonging to the closely related families Lamiaceae and Bignoniaceae, as well as other families in the Lamiales. All of the test species were classed as immune or resistant. Host-range testing undertaken for South Africa is still on-going. Encouragingly, the native species *Lantana rugosa* Thunberg and three *Lippia* species potentially the most at risk, are rated as resistant to the rust.

Given the variation in susceptibility of weedy lantana forms and the results of the host-range testing, the position on risks associated with a potential introduction of *P. lantanae* versus the anticipated benefits is likely to differ between the three countries concerned. For New Zealand, no apparent hurdles concerning introduction of the rust are anticipated and an application to release this rust is under preparation by Landcare Research (L. Hayes, pers. comm.). The same may apply to South Africa, providing none of the non-target species still to be assessed are attacked by the rust. Australia, however, has to deal with a more complex risk analysis, given the infection of the two native varieties of *V. officinalis*. The risk, albeit low, posed to this non-target species needs to be weighed against the likely impact the rust would have on lantana, given the resistance expressed by some of the forms. Further work under quarantine is planned, particularly to assess the impact of the rust on the growth of *V. officinalis*, prior to the preparation of an application to AQIS for its introduction.

Rust pathogens previously evaluated and introduced into Australia

A number of fungal pathogens considered as safe, based on a risk analysis have previously been introduced as CBC agents for weed biological control into Australia (Julien and Griffiths, 1998). Despite known non-target effects, the rubber-vine rust, *Maravalia cryptostegiae* (Cummins) Y. Ono, and the parthenium summer rust, *Puccinia xanthii* Schweinitz var. *parthenii-hysterophorae* Seier, H.C. Evans & Á. Romero, were regarded as having an "acceptable level of host specificity" to be utilized as biocontrol agents in Australia.

***Maravalia cryptostegiae* for control of rubber-vine**

Rubber-vine *Cryptostegia grandiflora* (Roxburgh ex. R. Brown) R. Brown, a Madagascan endemic belonging to the Apocynaceae - Asclepiadoideae, has been described as “the biggest single threat to natural ecosystems in tropical Australia” (McFadyen and Harvey, 1990). As part of a CBC program, the rust species *M. cryptostegiae* (Roxburgh ex R. Brown) R. Brown), infecting the weed in its native range, was evaluated as a promising biocontrol agent during a five-year study. Under optimum ambient conditions for infection and disease development, and by applying high urediniospore inoculum loads, the rust was able to infect and produce limited sporulation on the endangered Australian-native asclepiad species, *Cryptolepis grayi* P.I. Forster (Evans and Tomley, 1994). However, when simulating more realistic field conditions for infection, by using a wind tunnel for urediniospore dispersal, *C. grayi* showed no rust sporulation (Evans and Tomley, 1996). Based on these results, it was concluded that the fundamental host range of *M. cryptostegiae* is wider than the anticipated ecological host range and, thus, that *C. grayi* is unlikely to come under attack in the field. Furthermore, the habitats of this Australian endemic and of the target weed rubber-vine have no geographic overlap, while the ecosystem which harbors *C. grayi* is itself under threat and likely to disappear in the foreseeable future (Evans, 2000). Weighing these considerations against the threat posed by the invasive rubber-vine to entire tropical ecosystems, the risk presented by the rust to *C. grayi* was considered acceptable and importation of the pathogen was approved by AQIS in 1994. Since its release, *M. cryptostegiae* (IMI 331455) has not been reported to attack any non-target species (Barton, 2004) and the cost-benefit ratio for agriculture in QLD has been calculated as 108:1, with an accrued benefit of AUS\$ 232.5 million up to 2004 (Page and Lacey, 2006). Saving Australian ecosystems from rubber-vine will be priceless.

***Puccinia xanthii* var. *parthenii-hysterophorae* for control of parthenium weed**

Following the introduction of the parthenium winter rust, *Puccinia abrupta* var. *partheniicola*

(H.S. Jackson) Parmelee into Australia (Dhileepan and McFadyen 1997), a second microcyclic rust species, *Puccinia xanthii* var. *parthenii-hysterophorae* (Roxburgh ex R. Brown) R. Brown) (formerly *P. melampodii* Dietel & Holway) or parthenium summer rust, was evaluated as a complement for control of the highly invasive and allergenic weed parthenium, *Parthenium hysterophorus* Linnaeus (Asteraceae), in the more tropical regions of QLD. Comprehensive host-range testing of 80 non-target species under optimum conditions, showed that the asteraceous Australian-native species *Flaveria australasica* Hooker, as well as one commercial variety of *Zinnia elegans* Jacquin, were highly susceptible, supporting abundant sporulation of the summer rust. *Helianthus argophyllus* (D.C. Eaton) Torrey & A. Gray, *Parthenium confertum* A. Gray and two commercial varieties of *Calendula officinalis* Linnaeus proved to be moderately susceptible showing restricted sporulation (Seier et al., 1997). Subsequent wind-tunnel experiments conducted with *H. argophyllus*, and one susceptible variety of both *Z. elegans* and *C. officinalis*, resulted in restricted infection on *C. officinalis* only (Seier et al., 1997). As previously done for the rubber-vine rust, the risk analysis for *P. xanthii* var. *parthenii-hysterophorae* was based on its apparent ecological host range as established in the wind-tunnel rather than its fundamental host range. Of further consideration was the fact that the susceptibility of crop and ornamental species to the rust was highly dependent on the variety, an observation which had already been documented by Morin et al. (1993) for an accession of *Puccinia xanthii* Schweinitz attacking the invasive weed *Xanthium strumarium* Linnaeus (Bathurst burr). This latter accession had previously been introduced into Australia by accident and had subsequently been noted to attack some cultivars of sunflower as well as *F. australasica* in the field (Alcorn and Kochman, 1976). Commercial damage to Australian sunflower crops, however, has not been recorded (J.K. Kochman, pers. comm.) and infection rates on *F. australasica* are generally low without any apparent impact on this species (K. Dhileepan, unpublished data). Based on the results of the host-range testing for the parthenium summer rust, and the “unintentional” field experience with the accidentally introduced *P. xanthii* accession attacking Bathurst burr, *P. xanthii* var. *parthenii-hysterophorae*

(IMI 379934), then named *P. melampodii*, was approved for introduction into Australia in 1999. To date, no non-target effects have been reported (K. Dhileepan, unpublished data).

Conclusions

The case studies presented here highlight the lack of an absolute measure for the required host specificity of a CBC agent, and raise the concept of an “acceptable level of host specificity”, which is very much dependent on a variety of factors unique to each case. The status of, or the value put upon, a non-target species potentially under attack by a biocontrol agent, is of high relevance as illustrated in the case of *Phakopsora jatrophiicola*. Australian authorities regard the risk posed to *Jatropha curcas* - a declared weed species in the country - and two ornamental *Jatropha* species of minor economic importance, as less critical when compared to the potential positive impact the rust may have on the invasive bellyache bush populations. Conversely, the anticipated damage to *Acacia sutherlandii* - a native species growing sympatrically with the target weed - by the rust *Ravenelia acaciae-arabicae*, is unlikely to be acceptable regardless of any potential benefit of the CBC agent. A risk analysis may also conclude that the level of damage to a desirable non-target is acceptable. For example, AQIS approved the introduction of the parthenium summer rust, despite the attack of one variety of *Calendula officinalis*. However, India, where *C. officinalis* is a highly valued species due to its cultural importance, is likely to view this differently. The importation of *Puccinia xanthii* var. *parthenii-hysterophorae* is unlikely to be endorsed, therefore, although *Parthenium hysterophorus* is an equally problematic weed as in Australia (Evans, 2000). The importance of geographic separation is demonstrated for the case of the rubber-vine rust. The introduction of the pathogen was approved despite potential damage to the endemic *Cryptolepis grayi*, partly because the target weed rubber-vine and the non-target have no overlap in their geographic ranges. The benefit to Australian ecosystems affected by invasive rubber-vine was thus considered to outweigh the risk to an Australian endemic species.

It can be critical for a risk analysis to predict the

ecological host range of a CBC agent as accurately as possible. This was demonstrated with rubber-vine rust and the parthenium summer rust, where wind-tunnel experiments were conducted to provide more realistic field conditions for spore dispersal and infection. Inherent characteristics of the receiving environment, such as climatic conditions, can further narrow the ecological host range of an agent and need to be considered.

Overall, the benefits of the introduction of a CBC agent have to significantly outweigh the associated risk to justify its introduction into a new environment. It is the aim of the risk analysis to establish this, and the decision is case specific. In the example of the lantana rust, from an Australian perspective, the fact that *Puccinia lantanae* only infects some of the important weedy lantana varieties, may influence the decision to introduce the rust, given its ability to cause limited sporulation on the two native varieties of *Verbena officinalis*. Conversely, for New Zealand and South Africa, where there is no apparent risk to non-target species, it is likely that the rust will be released despite it only attacking some of the weedy forms in these countries.

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Weed Biological Control in Europe: A Reality

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Abstract

2010 saw the release of the psyllid *Aphalara itadori* (Shinji, 1938) against Japanese knotweed, *Polygonum cuspidatum* Sieb. & Zucc. (= *Fallopia japonica* (Houtt. Dcne.), in England, arguably the first foray into weed biological control by any EU country. This project took 21 years from concept to license and has cut a path through the regulations for future bio-controllers to follow. This paper summarizes the lessons learned during the research and licensing phase of the project before providing the latest data from the on-going post-release monitoring plan. Progress with the research on other European weed targets is presented and the reasons for their selection are discussed in an attempt to determine what the real drivers are in this new area of operation.

Successes We Might Never Have Had: A Retrospective Comparison of Predicted Versus Realized Host Range of Established Weed Biological Control Agents in North America

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Abstract

The aim of host range testing for biological control agents prior to their release is to assess the risk of non-target effects. The most robust tests are conducted under no-choice conditions and describe the fundamental host range of a species, which is generally broader than the realized host range assessed following the release of the agent. Therefore, it may overestimate the risk of non-target attack. Retrospective analyses of predicted versus realized host range of successfully established agents can provide insights into 1) the accuracy of pre-release predictions and 2) which test designs best predict an agent's realized host range. This in turn may not only improve pre-release testing procedures and further increase safety but should also be acknowledged in current regulatory policies for the evaluation of classical biological control agent releases. We review examples of several successful biological control agents in North America and compare results of pre-release studies (predicted host range) with post-release results (realized host range) as far as data exists. We conclude that predictions on potential non-target effects were generally accurate to conservative. In most instances non-target attack was transitory, and either ceased with distance from mass outbreak areas of the agent or after successful control of the target. Under the current more stringent regulations with regard to weed biological control, many of the successful agents we reviewed would most likely not be released in the USA. Often, systematic post-release monitoring on potential non-target impacts or even target impacts has not been conducted. These data are limited because their collection is not necessarily mandated in regulatory policies or it may be mandated but without resource allocation. Systematic post-release monitoring of target and potential non-target impacts is, however, critical for balanced data-driven benefit-risk decisions regarding biological control implementation.

Recent Issues and New Challenges Regarding the Permitting of New Weed Biological Control Agents

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Abstract

In order to be approved by governmental regulatory agencies in USA, prospective biological control agents must be found to have no significant impact on non-target organisms or the environment. Host specificity experiments are used to assess the risk that an agent poses to non-target plants. However, the results of such experiments can vary widely depending on the experimental conditions, which range from highly artificial no-choice laboratory oviposition or development trials to natural field experiments. The USDA-APHIS Technical Advisory Group (TAG) Reviewer's Manual provides some guidance on how to interpret such data. A recent example of a petition for *Ceratopion basicorne* (Illiger), a weevil that develops inside the root crown of yellow starthistle (*Centaurea solstitialis* L.), shows how such data can vary. TAG interpreted the results to indicate that the insect would be safe to release. However, the observation that some individuals can complete development under no-choice conditions on a crop plant, safflower, caused APHIS to deny a permit despite absence of attack during field experiments. This raises the question of how reliable are the results of field experiments, and what data are necessary to show "no significant impact".

SESSION 3: NON-TRADITIONAL BIOLOGICAL CONTROL AGENTS

The Case for Biological Control of Exotic African Grasses in Australia and USA Using Introduced Detritivores

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Abstract

Many species of African grasses were introduced into Australia and the USA to improve the quality and biomass of green pastures for domestic livestock. However, a range of non-target environmental impacts eventuated from these introductions, including spatial displacement of indigenous ecosystems, and induced changes to soils and nutrient re-cycling. The accumulation in biomass of flammable and senescing grasses predisposes grassland and woodland ecosystems to increasing impacts from wildfires, threatening indigenous plants and animals, and the recovery after fires of natural ecosystems. Very few detritivores have adapted to feed on and decompose detritus from African grasses in Australia and the USA, resulting in accumulation of dead leaves and a build-up of fuel increasing the risks from wildfires. In grasslands and woodlands of the Northern Hemisphere, epigeic detritivores and leaf-shredders include groups of invertebrates such as earthworms and isopods. In Australia, larvae of leaf-shredding moths, beetles and several other insects are important detritivores including oecophorid moths, cryptocephaline beetles, termites and cockroaches; some specifically adapted to breakdown of sub-surface plant materials in dry and moist ecosystems. In grasslands and woodlands the range of epigeic insects are likely to reduce accumulating dead biomass and fuel loads that contribute to flame height and intensity of fires. We propose that detritivores of African grasses may be potential biological control agents for senescing and dead biomass and meet the specificity requirements as agents, to control invasive African grasses in the USA and Australia.

Introduction

African grasses were introduced into Australia (Tothill and Hacker, 1983), the USA and other countries in the mid to late 1900s, to improve the quality of pastures and forage for grazing by livestock. Introductions aimed to increase green biomasses to enable high stocking rates, or maintain seasonal growth of pastures with drought tolerant species, to suppress weeds and produce protective covers to reduce soil erosion and nutrient losses. In Africa,

indigenous grasses have co-existed with grazing by abundant large herbivores and respond positively to regular schedules of burning. After fires and rain copious re-growth referred to as “green pick” and favoured by livestock, is more palatable and contains higher concentrations of nutrients when compared with unburned, unpalatable and senescing grass or its detritus.

African grasses spread into natural ecosystems in Australia and USA where layers of senescing leaves and detritus accumulate, accompanied by little or no decomposition attributable to indigenous

herbivores. Grass seeds or vegetative fragments are often carried into natural areas by livestock, on clothing or on vehicle tires. They spread from rangelands into native grasslands, woodlands, and rainforests (Table 1), where they displace non-flammable or weakly flammable plant communities with understories of highly flammable grass monostands. Invasive grasses (especially signal grass, *Brachiaria decumbens* Stapf.) advance growth from the margins of paths and roads, into undisturbed natural vegetation.

Introductions of African species have been mostly beneficial as fodder for livestock but several have detrimental impacts or have become serious weeds, e.g., molassesgrass (*Melinis minutiflora* Beauv.) is unattractive to stock (Elliott, 2008) and guineagrass, *Megathyrsus maximus* (Jacq.) R. Webster (= *Panicum maximum*, *Urochloa maxima*) (Henty, 1969) can be poisonous (cyanogenic). buffelgrass (*Pennisetum ciliare* (L.) Link) is a host and potential transmitter of sugarcane whitefly (*Neomaskellia bergii* Signoret) in Australia (Palmer, 2009) while gamba grass (*Andropogon gayanus* Kunth) produces dense, tall and flammable stands (Table 1) that limits mustering or location of stock in northern Australia. Several grasses (e.g. *M. maximus*, *B. decumbens*) invade forestry plantations in Australia and New Guinea (Henty, 1969) and are difficult to manage. In eastern Australia, African grasses increase the access to hosts by the paralysis tick (*Ixodes holocyclus* Neumann), facilitating the higher transfer of nymphs and adults from tall grass species to humans and livestock.

African grasses displace plant communities

In Australia and USA in the absence of natural enemies, African grasses spread and will out-compete most indigenous grasses, shrubs and low plant communities. African grasses inhibit vegetative growth and reduce or prevent seedling recruitment, by shielding them against entry of light. Those grasses with rhizomes compete for root space and moisture, replacing indigenous grasses and sedges with surface roots. For example, African lovegrass (*Eragrostis curvula* (Schrud.) Nees) in southern Australia, and Buffelgrass (*P. ciliare*) in Australia and southern Texas, USA, displace beneficial and indigenous plants and often destroy the integrity of

whole natural ecosystems (Everitt et al., 2011). In the USA, east African guineagrass, *Megathyrsus infestus* (Andersson) B. K. Simon & S. W. L. Jacobs, and *P. ciliare* are similarly invasive, as are other invasive species in many parts of Australia (Table 1).

Impacts of African grasses on invertebrate biodiversity

The high flammability of some African grasses (e.g., molassesgrass, *M. minutiflora*) (Tothill and Hacker, 1983) adds to detrimental impacts on ecosystems, but the combination of competition and flammability (e.g., of guineagrass, *M. maximus*; buffelgrass *P. ciliare*) is not well documented. Root balls and rhizomes of African grasses modify the soil texture, chemistry and inhibit activity by sub-surface invertebrates.

Some indigenous animals use African grasses, particularly in the absence of indigenous species, for example in Australia, wallabies and kangaroos will feed on and use dense stands for alluding predators. Small vertebrate animals and birds will use the grasses for food or shelter, for example, some finches will feed on seeds of *M. maximus* and wrens will construct nests in the dense thickets. A few polyphagous invertebrates feed on African grasses and maintain their abundance in the absence of indigenous plant hosts, for example, grass-feeders including the larvae of moths and butterflies (Braby, 2004; Zborowski and Edwards, 2007) will feed on African grasses; especially when the densities of indigenous grass food plants cannot alone sustain breeding.

While having serious impacts on many plants, invasive grasses reduce or prevent invertebrates occupying the shrub and ground cover plants, using their fallen limbs and rock shelters, or the decomposing organic materials. Most insects, for example, occupy and breed in the understory on shrubs, "sub-surface" plants, under bark or logs and in fallen leaves. Representing the majority of invertebrate species in temperate and tropical ecosystems, they regulate the architecture of plants, break down decomposing vegetation and re-cycle nutrients, and form part of the food webs and food chains for small vertebrates. Some grasses (e.g., *B. decumbens*) appear to be repellent to indigenous

invertebrates that normally breed on, visit or shelter in grasslands.

Most indigenous insects require spatial access to their understory food plants for oviposition and particular phenotypic expressions of their food plants. In Australia, phytophagous Lepidoptera of grasslands are mostly monophagous or oligophagous and require particular densities of their food plants. They are often very 'local' and restricted in their breeding sites and patrolling sites, such as hilltops, to undisturbed small areas containing their food plants or supporting specific vegetation types. For example, some Hesperidae always congregate on isolated hilltops with particular rock formations or plant communities. Hilltops and slope ecosystems are particularly vulnerable to displacement from signalgrass (*B. decumbens*), buffelgrass (*P. ciliaris*) and guineagrass (*M. maximus*) and there is strong evidence of losses of rare species from protected areas in south-eastern Queensland (unpublished). Buffelgrass is a particularly serious threat to many insect species in the inland areas of Central Queensland, and across Central Australia to northern Western Australia.

African grass and fire interactions

Frequent-lit fires in indigenous ecosystems are known to have significant impacts on some species of invertebrates in Australia (Greenslade and Smith, 2010), but the exacerbating effects from invasive flammable grasses have until recently (Sands and Hosking, 2005) not been well documented. African grasses are either much more flammable than indigenous species (Table 1) or the densities and uniformity of their communities enhances fuel loads. The heat generated and flame height mostly exceeds those from flammable indigenous species, for example, the flames from gamba grass often exceed 100 m, sufficient to kill trees, prevent their re-sprouting and change the structures of all woodland into grassland species. When accompanied by sufficient soil moisture, re-growth of African grasses after fires is more rapid and vigorous than indigenous species, with mono-stands out-competing indigenous species for light, root space and by changing soil composition and texture. The high flammability of some African grasses (e.g., molasses grass, *Melinis minutiflora*) (Tothill

and Hacker, 1983) adds to detrimental impacts on ecosystems, but the combination of competition and flammability (e.g., of guineagrass, *M. maximus*; buffelgrass *P. ciliaris*) is not well documented. Root balls and rhizomes of African grasses modify the soil texture, chemistry and inhibit activity by sub-surface invertebrates.

After each fire event, re-growth of exotic grasses is invariably more aggressive than prior to being burned; African grasses out-compete the slower re-growth and recruitment by the indigenous plants. After being burned and re-sprouting from root stocks, African grasses grow more rapidly than indigenous grasses and can prevent re-sprouting of indigenous plants or germinating seedlings. The most serious impacts on ecosystems occur when African grasses are burned frequently and the indigenous plants have no time to recover. After each fire, invasive grasses advance progressively into intact areas taking advantage of entry of light to stimulate rapid seed germination, rhizome advancement and to shade out of indigenous plants.

Plant species and plant communities vary in their responses to fire ranging from highly flammable to weakly flammable, and fire adapted to fire sensitive. Several grasses (e.g. buffelgrass) will invade the understory of dry rainforests and other weakly and non-flammable plant communities (e.g., brigalow, *Acacia harpophylla* F. Muell.), replacing indigenous flora with mono-stands of highly flammable grass and its senescing products. The biomass becomes more uniform in flammability in any given area without the variability in height and architecture of most indigenous ecosystems. Most Australian grassland species are adapted to being burned where the recovery of species and rate of recovery varies with the frequency, season and the scale of each fire event. The intensity of each fire also determines biodiversity recovery and may limit the densities of plants and animals able to re-colonize.

When burned, the vegetative re-growth of the exotic grasses replaces indigenous re-growth, or is followed by stagnation, senescence and damping off. By displacing whole plant communities, the exotic grasses can extirpate or reduce the distribution and densities of indigenous arthropod food plants, and eliminate the hosts, prey, and shelters used as habitats. For example, repeated burning of gamba grass has had widespread impacts on biodiversity

including losses of threatened species including the iconic Leichardt's Grasshopper, now nearly extinct in Northern Territory (D. Rentz, pers. comm.). Absence of unburned refuges enabling escape by mobile stages makes most surface-dwelling invertebrates even more vulnerable (Lindenmayer et al., 2011), especially if they are not sufficiently mobile to escape from the radiant heat by sheltering underground. The intensity, scale, frequency and seasons of fires directly affects invertebrate survival if they have no fire-adapted strategy, unburnt refuges, or are not sufficiently mobile to escape from being burnt. African grasses when frequently burned can lead to extirpations and have possibly caused extinctions of terrestrial invertebrates including the invertebrates that depend on shrubs, sedges and grasses for food and shelter.

Frequent burning may promote seed germination but it rapidly exhausts the seed bank when recruiting plants have no time to mature between fire events. Frequent fires in plant communities occupied by African grasses may rapidly deplete seed banks and lead to the development of "landscape traps" (as defined by Lindenmayer et al., 2011) and are likely to cause in-breeding depression in some fauna and flora. The flammability of some African grasses has been recognized (Tothill and Hacker, 1983) but the effects on ecosystems, especially small animals (e.g., by molasses grass, *Melinis minutiflora*, guineagrass, *M. maximus*; buffel grass *P. ciliare*) have not been well documented. Within 12 months of being burned, regrowth of several grasses (e.g., *B. decumbens*) will exceed the pre-burning biomass. Burning for "fuel reduction" or "hazard reduction" becomes impractical when burning schedules favour re-growth of the African grasses and thus, frequent burning schedules can *increase* the fuel loads in short time periods following burns.

The case for biological control of African grasses using detritivores

Targeting African grasses by biological control is not without difficulties when considering the importance of green forage to farmers and equestrian groups. Classical biological control for some grass targets using insects (Moran and Goolsby, 2009) or fungi (Anderson et al., 2010) is showing promise comparable with biological

control of dicotyledonous plants. Several insects are known to be grass specialists, for example the Elachistidae (Lepidoptera) with larvae that mine leaves and stems of grasses, may contain candidate agents for the vegetative parts of grasses (Mercadier et al., 2010). Documented biological control projects targeting exotic grasses are few (Julien and Griffiths, 1998) but there are notable recent targets, including Mediterranean giant reed, *Arundo donax* L., currently invading riparian parts of the Rio Grande Basin in the southwestern USA (Moran and Goolsby, 2009) and Chilean needlegrass, *Nassella neesiana* (Trin. & Rupr.) Barkworth, in southern Australia (Anderson et al., 2010; Faithful et al., 2010). A conventional approach to biological control has been used against the vegetative parts of these grasses using insects or fungi as control agents.

Very few detritivores have adapted to feed on and decompose detritus from African grasses in Australia and the USA, resulting in accumulation of dead leaves and a build-up of fuel that increases the risks of wildfires. In grasslands and woodlands of the Northern Hemisphere, epigeic detritivores and leaf-shredders include groups of invertebrates, such as earthworms. In Australia, predominant groups include oecophorid, tortricid and hepialid moths and cryptocephaline beetles (Clytrinae), termites and cockroaches, springtails (Collembola), Protura, and Diplura. Epigeic earthworms are not significant detritivores in Australian ecosystems (Baker, 1996) but many insect groups adapted to breakdown of sub-surface plant materials are well adapted in indigenous Australian ecosystems (Table 2). Other potential detritivore agents would appear to include isopods, earthworms and fungi. While the roles of Australian detritivores are not well understood, these observations are useful for revealing the hosts and habitats of indigenous species, and might aid the prospects for locating suitable grass-adapted agents in Africa (Table 2).

There are more than 5,000 species of oecophorid moths, comprising about ¼ of the known moth fauna, in Australia. The larvae break down fallen leaves in all Australian eucalypt ecosystems and in many types of grasslands (Common, 1996; Zborowski and Edwards, 2007). The larvae of many species decompose leaves and reduce accumulation and thus the flammability of dead leaves. Their activity binds sub-surface organics with soils and

prevents erosion; they re-cycle nutrients (first stage breakdown), some may reduce the surface tension of soil surface layers of decomposing leaves and help retain moisture (unpublished). Leaf litter oecophorid moths provide an essential source of ground-level prey for insectivorous animals and birds throughout Australia. They are winter breeders, the adult moths are poorly mobile and susceptible to extirpations from too-frequent burning of their habitats. The larvae of leaf eating beetles, *Cryptocephalini* (> 500 species) (Coleoptera: Chrysomelidae) are also an important group of detritivores in Australian ecosystems.

Epigeic agents would appear to be desirable candidates for biological control of African grasses but assessment for non-target impacts such as competition with native species is as important as for any conventional biological control agents. For example, in Tennessee an epigeic Asian earthworm, *Amyntas agrestis* Goto and Hatai, competes with indigenous detritivores for forest floor organic litter and reduces millipede biodiversity, both species and their abundance (Snyder et al., 2011). Some isopods are known to reduce the leaf litter of indigenous grasses and show degrees of host preferences, for example in California, the isopod, *Porcellio scaber* Latreille, had a substantial effect on litter loss of annual grasses but it had no effect on an exotic species, common velvetgrass, *Holcus lanatus* L. (Bastow et al., 2008).

Discussion

Increased frequency of fire is believed to have had a major impact on small animals in northern Australia (Woinarski et al., 2010), a trend probably Australia-wide that has led to extirpations and sometimes extinctions of fire sensitive species (unpublished). Increasing impacts by fire can be attributed to African grasses wherever the scale is sufficient to displace and increase the flammability of natural ecosystems. In all natural areas throughout Australia, threats to small animal biodiversity are exacerbated by increases in the scale, frequency and inappropriate seasons (e.g., cool seasons when insect stages are immobile) of fires, wherever African grasses are invasive.

Without natural re-growth following each burn,

prolific growth and seasonal senescence of exotic grasses, followed by the accumulation of copious dry leaves and detritus, is providing a major threat to biodiversity, particularly of invertebrates, in Australia and the USA. For more than 10 years extreme weather events in eastern Australia have influenced the growth of African grasses and build up in fuel loads in dry periods, exacerbated by increases in periods of prolonged drought and heavy rainfall events. Some grass species have become more invasive in natural ecosystems, for example *Megathyrsus maximus* is becoming more competitive and *Melinis minutiflora* appears to be increasing in distribution in northern New South Wales (unpubl.). As a result of climate changes, these two species as well as *B. decumbens*, are becoming the most serious invasive threats to understory-breeding invertebrates in the coastal, subtropical parts of Australia.

Targeting for biological control the decomposing leaves (sometimes reaching 2 m in depth in *M. minutiflora*) and detritus from African grasses, is least likely to be of concern when the livestock industries promote grazing of green pastures. Prospects for finding leaf shredders or detritivores to control dead and flammable African grasses is comparable to the challenge of finding African dung beetles to control dung breeding flies using dung from introduced large animals in Australia (Waterhouse, 1974; Waterhouse and Sands, 2001).

Control methods for African grasses are often difficult, costly and polluting. Moreover, fuel reduction practices, including micro-mosaic methods recommended for short-term fire management to protect invertebrate biodiversity (New et al., 2010; Greenslade and Smith, 2010), are becoming more difficult in the presence of rapidly accumulating detritus from African grasses. In the longer term, biological control of the grasses is the desired and only option for controlling invasive grasses. In the case of detritivores to be tested as agents, before introductions they must first be shown to have no detrimental impacts on indigenous detritivores, or interfere with other ecological processes

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Table 1. Highly flammable African grasses affecting biodiversity in the tropics and subtropics of Australia and USA

GRASS	REGIONS AFFECTED	ESTIMATED DISPLACEMENT + FUEL VALUES *
gamba grass (<i>Andropogon gayanus</i>)	Tropical northern & inland; moist & dry tropical woodlands & grasslands	+++
buffelgrass (<i>Pennisetum ciliare</i>)	Tropical & subtropical northern & inland: central & western; woodlands & grasslands	+++
lovegrass (<i>Eragrostis curvula</i>)	Subtropical & temperate sub- coastal & inland; woodlands & grasslands	++
guineagrass (<i>Megathyrsus maximus</i>) and east African guineagrass (<i>Megathry- sus infestus</i>)	Tropical & subtropical coastal: eastern, wet woodlands & forests	++
signalgrass (<i>Brachiaria decumbens</i>)	Subtropical coastal: moist & wet woodlands	++
molassesgrass (<i>Melinis minutiflora</i>)	Tropical & subtropical; eastern & coastal wet woodlands & forests	+++
South African pigeongrass (<i>Setaria sphacelata</i>) (Schumach.) Stapf & C.E. Hubb.	Tropical & subtropical eastern, dry & coastal woodlands & grasslands	+++
giant Parramatta grass (<i>Sporobolus fertilis</i>) (Steudel) Clayton	Tropical & subtropical inland; woodlands & grasslands	+++

* estimated values contrasted with the widespread Australian kangaroo grass, *Themeda triandra* Forssk. (rated +)

Table 2. Insect detritivores and leaf shredders commonly associated with Australian ecosystems

ECOSYSTEM	ASSOCIATED DETRITIVORES/LEAF SHREDDERS
Dry eucalypts & woodlands	leaf litter moths (Oecophoridae, Tortricidae: Epitymbiini) leaf beetles (Cryptocephalini), Isoptera (<i>Microcerotermes</i> , <i>Ephelotermes</i> , <i>Hesperotermes</i> , <i>Nasutitermes</i>)
Rainforests & moist forests	cockroaches (Blattoidea; <i>Geoscapheus</i> , <i>Cryptocercus</i>), moths (Oecophoridae: <i>Barea</i>)
Grasslands	leaf litter moths & “mallee” moths (Oecophoridae), termites (Isoptera: <i>Drepanotermes</i> , <i>Lophotermes</i> , <i>Nasutitermes</i> , <i>Tumulitermes</i>)
Heathlands	leaf litter moths (Oecophoridae & Tortricidae); Isoptera & others but not well documented

Rhizaspidiotus donacis* (Hemiptera: Diaspididae), an Armored Scale Released for Biological Control of Giant Reed, *Arundo donax

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Abstract

Non-native, invasive perennial grasses have not been widely targeted for classical biological control with insects, despite their global prevalence and damaging effects, in part because of a perceived paucity of host-specific insect herbivores. Armored scales (Hemiptera: Diaspididae) have not been used for biological weed control. However, over 250 armored scale species occur on grasses, of which 87% feed only on this family and 58% feed on only one grass genus, suggesting that armored scales may have unrealized biological control potential. We selected the armored scale *Rhizaspidiotus donacis* Leonardi as a candidate agent against the exotic, invasive, water-consuming grass known as giant reed (*Arundo donax* L.), based on literature records and our collections indicating host-specificity to the genus *Arundo* and its broad geographic range in the Mediterranean basin. Observations of reduced giant reed vigor at scale-infested sites in Spain and France were confirmed in native range field studies showing a 50% reduction in lateral shoot growth rate and rhizome weight on scale-infested versus non-infested *A. donax*, as well as significant reductions in photosynthesis rates in quarantine laboratory studies. Specialized procedures were developed using gelatin capsules to isolate females from host tissues and neonate crawlers from females. Based on laboratory and native range field studies, the host range of *R. donacis* is limited to *Arundo* spp., and the life cycle requires 5–6 months. A scale accession from eastern coastal Spain established larger populations on rhizomes of two invasive *A. donax* accessions from this same region than on rhizomes representing a separate, geographically isolated introduction. In 2011, *R. donacis* became the first armored scale released for biological weed control, establishing robust rearing colonies and reproductive field populations with evidence of lateral shoot deformities at the first release site along the Rio Grande in Texas, USA.

Introduction

Arundo donax L, known as arundo, giant reed, or carrizo, is native from the Mediterranean Basin to India, and invasive in North and South America, South Africa, and Australia. *Arundo* has colonized at least 30,000 ha along rivers, reservoirs and irrigation canals in the Lower Rio Grande Basin of south Texas and northern Mexico (Yang et al., 2009, 2011), removing economically-significant amounts of water, displacing native plants, altering flood and fire regimes, harboring cattle fever ticks, and hindering border security activities (Moran and Goolsby 2009; Racelis et al., 2011), with similar ecological impacts in California (Coffman et al., 2010; Lambert et al., 2010) and in other arid riparian ecosystems. Non-native, invasive perennial grasses (Poaceae) and sedges (Cyperaceae) are among the world's most widespread weeds (Witt and McConnachie, 2003), attaining densities in riparian and rangeland habitats that exceed the capacity of chemical and mechanical control. However, few biological control projects have targeted perennial grasses, due to a perceived paucity of genus- or species-specific insect herbivores (Evans, 1991). Pathogenic fungi have been considered for inoculative (Yobo et al., 2009; Anderson et al., 2011) or inundative releases (Yandoc et al., 2004). Surveys of herbivorous insect fauna on common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) (Tewksbury et al., 2002), giant reed (*Arundo donax*) (Tracy and DeLoach, 1998; Kirk et al., 2003), *Sporobolus* spp. (Witt and McConnachie, 2003), *Calamagrostis* spp (Dubbart et al., 1998) and multiple grass genera (Tschardt and Greiler, 1995) have shown that oligo- and monophagous herbivores exist on grasses, but exhibit feeding patterns and taxonomic affiliations that are non-traditional in weed biological control. Examples include shoot-galling wasps (Hymenoptera: Eurytomidae), stem-boring flies (Diptera: Chloropidae), galling and non-galling leafminers (Diptera: Cecidomyiidae) and, in the case of giant reed, an armored scale (Hemiptera: Diaspididae).

Armored scales are immobile besides the short-lived neonate crawler (first instar) and adult male stages. These small, sometimes cryptic insects generate cumulative damage to perennial plants over several generations of an often prolonged life cycle (McClure, 1990). Over 200 pest species are

known, most with broad host ranges (Miller and Davidson, 1990). No armored scales have been released for weed control, although two adventive *Chionaspis* species are widespread and damaging on saltcedars (*Tamarix* spp.) in North America (Wiesenborn, 2005). Armored scales are commonly found on grasses (Evans and Hodges, 2007), with over 250 species known (<http://www.sel.barc.usda.gov/scalenet.html>), and 222 of these (87.5%) occur only on hosts in the Poaceae; 58% (128) are found on only one grass genus. Armored scales may thus have unrealized potential for biological control of perennial grasses.

A biological control program targeting *A. donax*, led by the USDA-Agricultural Research Service, is the first to release multiple non-native insect agents to control a grass weed. We selected *Rhizaspidiotus donacis* Leonardi as a candidate agent on the basis of literature records (reviewed in Goolsby et al., 2009a) and our collections indicating specificity to the grass genus *Arundo*, which has no native members in North or South America. The geographically-broad native range of this scale, as well as the thin, brittle arundo shoots observed at sites with dense scale populations, also favored its selection for further testing.

Methods and Materials

Determination of native distribution

Collections to survey insects on *A. donax* were made at over 330 sites in 19 countries between 2000 and 2007 during the spring, summer and fall. Rhizome samples were taken to the USDA-ARS European Biological Control Laboratory to detect the presence of *R. donacis* and natural enemies (Kirk et al., 2003).

Evaluations of impact

In the Province of Alicante, in southeastern coastal Spain, 15 shoots at each of five sites were treated with foliar and root drench insecticide monthly for 12 months and 15 shoots were left untreated. Growth rates were compared across the two treatments and between monthly measurements (Cortés et al., 2011a). To examine arundo scale

effects on rhizome weight, nine sites with and nine without *R. donacis* were selected in the Languedoc region of southwestern France and the Province of Alicante in Spain (Cortés et al., 2011b). In a three-month quarantine laboratory study, potted, fertilized arundo stems were infested with *R. donacis* at a rate of 158 crawlers per week, and also received arundo wasps (*Tetramesa romana* Wallker) at a rate of 18 per week. Shoot and selected leaf lengths were compared to wasp-only and control treatments (Goolsby et al., 2009b). In a six-month lab study, 500 to 700 *R. donacis* crawlers were released per stem and leaf gas exchange measured 24 weeks later to infer effects on photosynthetic processes (Moore et al., 2010). In a six-month greenhouse study, rhizomes and crowns of young arundo shoots were infested with 1,000–5,000 crawlers and shoot elongation and biomass examined (Racelis et al., this volume). In greenhouse studies, crawlers were released onto rhizomes either fertilized with urea plus slow-release nitrogen-phosphorous-potassium pellets, or given only pellets (Moran and Goolsby, this volume).

Determination of biology and host range

Adult females were shipped on arundo rhizomes from 15 sites in southwestern France and Mediterranean Spain. Females were removed from rhizomes and stored in 1.5-cm gelatin capsules at 27 °C, 60% RH, 14:10 L:D. Crawlers were collected in gelcaps, which were screened microscopically for *Aphytis acreulatus* Rosen and DeBach ectoparasites and other contaminants. Capsules with crawlers were pinned to shoots of 2-month old arundo shoots or non-target plants. Destructive dissections at varying time points and isolation of crawlers, adult males and females were used to determine duration and survival of life stages (Moran and Goolsby 2009). For host range studies, shoots of *A. donax*, 40 other grass species, and 5 non-grasses received 200 crawlers each and were dissected three months after infestation (Goolsby et al., 2009a). Field studies were conducted in Spain as a follow up to examine laboratory non-target development on *Leptochloa* spp. and *Spartina alternifolia* Loisel grasses. Sub-specific host range was examined by infesting arundo rhizomes from two Texas sites (Austin and Laredo) that genetically match several populations in eastern and southern coastal Spain (D. Tarin, A.

Pepper, J. Goolsby, P. Moran, A. Contreras Arqueta, A. Kirk, and J. Manhart, unpublished), and from one site (Balmorhea) with a distant point of origin.

Mass-rearing and field release

A permit from the USDA-APHIS-PPQ to release the arundo armored scale into the field was received on 16 December 2010. Mass-rearing was conducted in 700-L plastic inner tubs filled to one-half depth with pea gravel and elevated 10 cm above an outer tub modified with drain holes to maintain the water level below the rooted arundo rhizomes, which were kept dry and partially exposed on the gravel surface. A similar subsurface watering system was used for rhizomes planted in outdoor and greenhouse trenches. Releases at a field site in Del Rio, Texas on the floodplain of the Rio Grande began in January 2011 (Goolsby et al., 2011).

Results

Native distribution

Our collections and literature records indicate that the geographic range of *R. donacis* includes eastern and southern Spain, extreme southern France, Italy, Crete, southern and western coastal Turkey, and coastal Algeria, with no collections in the Balearic Islands, Corsica, Sicily, Croatia, Bulgaria, Israel, Egypt, Morocco, the Canary Islands, China, Nepal, or India. Some of the most robust *R. donacis* populations, in eastern and southern Spain, were found on arundo accessions matched using microsatellites to the invasive arundo genotypes in the Lower Rio Grande Basin.

Impact on target weed

Infestation by *R. donacis* reduced both lateral shoot growth and rhizome weight of *A. donax* by 50% in the native range (Table 1). In quarantine, the arundo armored scale slightly enhanced the negative effect of the arundo wasp on main shoot growth, and independently reduced the ability of the plant to absorb light energy to convert nitrogen into protein by over 60% (Table 1). In a 6-month greenhouse study, high levels of arundo scale infestation (5,000 crawlers)

reduced main shoot growth by 50% or more (Table 1). Urea fertilization increased female reproduction and settling of second-generation crawlers, but results were inconsistent across two rearing environments (Moran and Goolsby, this volume).

Biology

At 26 °C, arundo scale crawlers lived less than two days without food, or, on arundo shoots, settled to an immobile 'whitecap' phase to complete the first instar within 10–13 days of emergence. Males completed second-instar development within an additional 20 days and emerged as adults by the 40th day after emergence. Non-feeding winged males lived less than two days. Females completed the second instar

by the 50th day post-emergence, with a brief period beforehand during which adult females had eclosed but were still inside the second instar nymphal cuticle. Adult females fertilized by males required a total of 170 total days from emergence as crawlers to reproductive maturity. Survival to adulthood (both sexes combined) was 20-25%. Each reproductive female produced an average of 85 crawlers. More details may be found in Moran and Goolsby (2010).

Host range

The arundo scale readily completed development on *A. donax*, with significantly less development on *A. formosana*, and none on closely related common reed, *Phragmites australis* or 37 other

Table 1. Evaluations of impact of the arundo armored scale *Rhizaspidiotus donacis* on giant reed (*Arundo donax*), based on pre-release observational and manipulative field studies in Mediterranean Europe, quarantine laboratory studies, and a post-release greenhouse study in Texas, USA.

Study Environment	Length of Time of Scale Infestation	Variable	Reduction Associated with Scale Infestation	Reference
Field-Spain	12 months	Daily rate of shoot growth (cm day ⁻¹)	61%	Cortés et al., 2011a
Field-France and Spain	Unknown	Rhizome weight (g)	46%	Cortés et al., 2011b
Laboratory	3 months	Stem and leaf length ¹	5–10%	Goolsby et al., 2009b
Laboratory	6 months	Maximum rate of electron transport ²	61%	Moore et al., 2010
Non-quarantine greenhouse	6 months	Shoot elongation rate and above-ground biomass	50%–60%	Racelis et al., this volume

¹Effect of scale over and beyond that of the arundo wasp *Tetramesa romana*. Differences between arundo wasp infestation alone and wasp+scale were not significant.

²Measure of efficiency of conversion of light energy in photosynthesis.

native grasses. Very limited development to adult occurred on three species in the genus *Leptochloa* and on *Spartina alterniflora* in quarantine, but no settling by *R. donacis* was observed in dissections of these grasses when found in populations sympatric with *A. donax* in Spain and France, even when potted plants were maintained in stands of heavily scale-infested arundo for 6 months. More details may be found in Goolsby et al. (2009a). Sub-specific host specificity tests in quarantine indicated development of significantly larger second-generation populations on invasive Texas rhizomes matched to Spanish locations from which crawlers were obtained than on Texas rhizomes representing a geographically distinct source of introduction (J. Goolsby, E. Cortés, P. Moran, J. Adamczyk, M. Marcos García and A. Kirk unpublished).

Mass-rearing and field release

Mass-rearing of *R. donacis* began in December 2010 and open field releases in January 2011. On 20 July 2011, whitecaps indicative of reproduction in the field were observed at the Del Rio, Texas site. Reproductive females and a new generation of immatures were found on rhizomes at this site in August 2011 (Goolsby et al., 2011). Colony-reared females produced similar numbers of crawlers as did females collected in Europe (P. Moran, unpublished).

Discussion

The arundo armored scale *R. donacis* exhibits a broad native geographic range with substantial, measurable impacts on the growth of giant reed, but narrow fidelity to the target genus *Arundo*, with full population growth potential only on *A. donax*. The biological attributes of this scale are suitable and adaptable for mass rearing, given sufficient attention to geographic matching of scale accessions from the native range to the known geographic sources of the giant reed populations that are invasive. After only one generation in greenhouse studies, the arundo scale reduced lateral and main shoot growth and alters photosynthetic processes. Feeding by multiple generations is likely necessary to reduce rhizome weight and shoot recruitment in the field. Improvements in visibility through dense giant reed

stands along the Rio Grande and decreases in water consumption by giant reed are key benchmarks in this biological control program, and are being examined as arundo armored scale populations develop.

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***Fergusonina turneri*/*Fergusobia quinquenerviae* (Diptera: Fergusoninidae/Nematoda: Tylenchida: Sphaerulariidae),
a Bud-Gall Fly and its Obligate Nematode Released for the
Australian Paperbark Tree, *Melaleuca quinquenervia***

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Abstract

The gall fly *Fergusonina turneri* Taylor and the nematode *Fergusobia quinquenerviae* Davies & Giblin-Davis form a mutualist association on *Melaleuca quinquenervia* (Cav.) S.T. Blake, an Australian tree that has invaded south Florida. Together they form multi-locular galls that compromise meristems thereby curtailing growth and reproduction of the targeted plant. Flies oviposit and nematodes oviposit into vegetative and reproductive *M. quinquenervia* buds. Nematodes initiate cecidogenesis producing hypertrophied tissue prior to fly egg hatch. The maggots then feed on the primed nutrient-rich tissue while presumably inducing further enlargement of the galls. Meanwhile, the parthenogenetic nematodes produce a second generation of amphimictic individuals. The mated female nematodes invade the hemocoel of fully grown female (3rd instar) fly larvae. The fly pupates and the female nematodes produce juveniles that invade the rudimentary ovaries of the developing female flies. The adult fly then emerges from the gall carrying juvenile nematodes in their ovaries. All female flies contain nematodes, which are deposited in buds during oviposition allowing the cycle to begin anew. Molecular analyses of related *Melaleuca* species and host range studies proved this fly-nematode combination to be specific to *M. quinquenervia*. A permit for their release as biological control agents was subsequently granted. They were released in south Florida beginning in 2005 and temporarily established, but disappeared completely after about three generations. To date, these two agents have not established self-sustaining field populations in Florida. The more recent release and establishment of *Lophydiplosis trifida* Gagné, a gall-forming midge, produces similar effect thus precluding the need for the fly/nematode combination. Nonetheless, this is the first time that a mutualistic combination of two agents has been approved and attempted for use in a biological control program.

Tetramesa romana* (Hymenoptera: Eurytomidae), a Parthenogenic Stem-Galling Wasp Released for Giant Reed, *Arundo donax

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Abstract

Plant development and growth are controlled by a series of independent processes that are determined by physiological and genetic mechanisms. Gall-inducing organisms can interrupt these processes and modify normal plant growth, often via plant species-specific interactions, and thus should be considered seriously for biological control. In the case of the arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), larval development on giant reed (*Arundo donax* L.) induces gall formation, interrupting meristematic activity and stimulating lateral budding. Since egg laying females prefer to oviposit on phenotypically labile tissues, gall formation has a negative influence on stem elongation, which in turn can limit the competitive ability of aggressive giant reed. Apparent host range of the arundo wasp was tested in quarantine by recording the occurrence of ovipositor probing events of the female, which occurred on 15 of 35 different test plants. However, its actual host range, indicated by successful larval development, was restricted to two species in the *Arundo* genus. With its host specificity, relatively the short generation time (an average of 33 days) and prolific parthenogenic reproductive output (an average 21 offspring per female), *T. romana* has promise as a control agent for giant reed and was released in the spring of 2009 in the Lower Rio Grande Basin of Texas and Mexico. The USDA-ARS has developed an adaptive, mass-rearing protocol for multiple arundo wasp genotypes based on responses to ambient light, temperature, and host-plant conditions. A series of sequential greenhouse studies reveal superior performance by Spanish genotypes of the wasp over French genotypes, in terms of greater reproductive output and compatibility with abiotic conditions. A mass release program for *T. romana* has been initiated using fixed-wing aircraft, and post-release field evaluations, including field studies of population establishment based on genotype and climate matching, are ongoing.

SESSION 4: TARGET AND AGENT SELECTION

Biological Control of *Senecio madagascariensis* (fireweed) in Australia – a Long-Shot Target Driven by Community Support and Political Will

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Abstract

Fireweed (*Senecio madagascariensis* Poir.) biological control has a chequered history in Australia with little to show after 20 plus years. Plagued by local impacts, sporadic funding, a poor understanding of its genetics and its origins, and several almost genetically compatible native species, the fireweed biological control program has been faced with numerous hurdles. Hope has risen again, however, in recent years through the staunch support of a very proactive team of local stakeholders and their good fortune of finding themselves in a key electorate. The Australian Department of Agriculture, Fisheries and Forestry has recently funded an extendable two year project for exploration in the undisputed native range of fireweed in South Africa and a detailed search for agents that are deemed to be both effective and unable to attack closely related Australian *Senecio* species. This will be a tall order, but nevertheless is essential to conclude once and for all whether biological control has the potential to reduce the negative effects of the plant in south eastern Australian grazing and dairy country.

Introduction

Fireweed (*Senecio madagascariensis* Poir.) is a toxic, short-lived, perennial, temperate to subtropical climate pasture weed of South African origin that has established and spread in Australia, the USA (Hawaii), Japan, Brazil, Argentina, Venezuela, Columbia and Uruguay (Sindel et al., 1998). It is the focus of a biological control program in Australia (Julien et al., 2012; Radford, 1997) and

Hawaii (Ramadan et al., 2010). The plant contains genotoxic carcinogens in the form of pyrrolizidine alkaloids which cause cumulative chronic liver damage and fatality, especially in monogastric livestock like horses (Sindel et al., 1998; Bega Valley Fireweed Association, 2008). Such toxins can enter the human food chain through nectar incorporated into honey or via dairy products from animals grazing on infested land. The estimated control costs averaged around \$9000 per farm per annum

in Australia, and pasture productivity and profits on infested land were reduced by between 15-50% (Bega Valley Fireweed Association, 2008). Owners of infested land also are at risk of suffering from reduced morale and stress-associated social costs via; a) perception of declining property values, b) increased potential for neighbourhood conflicts, c) regulatory obligations around weed control, d) animal health issues and costs, and e) longer-term business viability and succession issues. There also remains significant debate about whether and when the weed can be controlled using traditional pasture management approaches, such as herbicides and controlled grazing. A high number of land owners, including commercial grazing businesses, have found such interventions to be impractical and the price of chemicals too costly.

In Australia, fireweed is spreading from a core coastal strip in the south-east to the north and to more upland and inland areas, and has certainly not yet spread to all suitable regions of Australia, being still largely absent from the states of Victoria, Tasmania, South Australia and Western Australia (Sindel et al., 2008). As a target for weed biological control, fireweed has many factors in its favour. Its taxonomy and native range are now well understood, being part of a complex of *Senecio* species in the KwaZulu-Natal region of South Africa (Radford et al., 2000; Lafuma et al., 2003). It exhibits much lower abundance in its native range, where competition from native perennial grasses in summer, largely confining the plant to isolated pockets in disturbed fields, dunes and along roadsides (A. Sheppard, pers. obs.). While a community of more than 30 natural enemies can be found on fireweed in Australia (Holtkamp and Hosking, 1993), none of these are very damaging and none of the natural enemies from the native range are already present in Australia, except for a rust fungus that appears to be of Australian origin anyway (Morin et al., 2009). Various other weeds of similar biology and impacts to fireweed have been successfully controlled in Australia using classical biological control, particularly Paterson's curse (*Echium plantagineum* L.) and ragwort (*Jacobaea vulgaris* Gaertn.) (Julien et al., 2012). Finally fireweed, as a target for biological control, has very strong community support in both Australia (Sindel et al., 2011) and Hawaii (M. Ramadan, pers. obs.) which has opened up new opportunities for

international collaboration on this target weed.

Community support in Australia comes from an active landholder group in Bega, in south-east New South Wales (the Bega Valley Fireweed Association; www.fireweed.org.au), which is fortunate to have found itself in a key electorate at both the state and federal level. The federal Member of Parliament for this electorate has since become the Parliamentary Secretary for Agriculture, and has been highly supportive of the fireweed issue, which has led to fireweed biological control gaining funding in the last few years and to the recent successful nomination of fireweed as a Weed of National Significance in Australia.

What makes this plant a difficult target for weed biological control in Australia is the taxonomic close proximity of fireweed to a group of Australian native species in the *Senecio pinnatifolius* (= *S. lautus*) group (Scott et al., 1998; Pelser et al., 2007). Hybridization can occur between these native species and fireweed, even if the progeny are sterile (Prentis et al., 2007). Any natural enemies selected as potential biological control agents for Australia therefore need to be monospecific to *S. madagascariensis*.

Biological control history

Fireweed was declared a target for weed biological control by the Australian Weeds Committee in 1991. At the time, the taxonomy of the target was poorly understood and all the earlier surveys for natural enemies were undertaken in south and eastern Madagascar (Marohasy 1989). These early surveys recorded two moths; a flower-feeding pyralid (*Phycitodes* sp.) and a stem-boring tortricid (*Lobesia* sp.), which were brought into quarantine in Australia. Both, however, failed to be specific enough for release in Australia (McFadyen and Sparks, 1996). One 18-day visit was also made to KwaZulu-Natal province in South Africa as part of these activities in 1991 and identified 11 species of insects feeding on the plant (Marohasy unpublished report - see Table 1 below).

In 2002, Meat and Livestock Australia funded a short project to look at the potential of strains of rust on fireweed in South Africa as potential biological control agents. One such rust, *Puccinia lagenophorae* Cooke, is considered to be native to Australia, but is

Table 1. Natural enemies found on *S. madagascariensis* in South Africa (KwaZulu-Natal) and Swaziland, South Africa.

Nature of attack	Natural enemy type (family)	Scientific name (current knowledge)
Stem-borers	Weevil (Curculionidae)	<i>Gasteroclisus tricostalis</i> (Thunberg)
	Moth (Tortricidae)	<i>Lobesia</i> sp.
	Fly (Tephritidae)	<i>Coelopacidia strigata</i> Bezzi
	Fly (Agromyzidae)	<i>Melanagromyza</i> spp.
Flower feeder	Moth (Pyrilidae)	<i>Homeosoma stenotea</i> Hampson
	Moth (Pyrilidae)	<i>Phycitodes</i> sp.
	Moth (Pterophoridae)	undetermined
	Fly (Tephritidae)	<i>Cryptophorellia peringueyi</i> (Bezzi)
	Fly (Tephritidae)	<i>Trupanea inscia</i> Munro
	Fly (Tephritidae)	<i>Sphenella austrina</i> Munro
	Fly (Tephritidae)	<i>Telaletes ochraceus</i> (Loew)
	Fly (Tephritidae) small	undetermined
	Fly (Cecidomyiidae)	undetermined
Root feeders	Weevil (Curculionidae)	<i>Proictes longehirtus</i> Fairemaire
	Leaf beetle (Chrysomelidae)	undetermined
Sap suckers	Leaf hopper (Tettigometridae)	<i>Hilda elegantula</i> Gerstaecker
	Plant bug (Miridae)	<i>Ellenia</i> sp.?
	Lace bug (Tingidae)	undetermined
Plant pathogens	Yellow rust (Pucciniaceae)	<i>Puccinia lagenophorae</i> Cooke & hybrids
	White rust (Albuginaceae)	<i>Albugo</i> nr <i>tragopogonis</i>
	Flower smut (Ustilaginaceae)	<i>Ustilago</i> sp.
	Stem blotch fungus	undetermined

cosmopolitan in distribution on a range of *Senecio* species. It is also found in both Australia and South Africa on *S. madagascariensis*. Surveys in KwaZulu-Natal, South Africa were used to collected rust samples at 14 of 25 sites and accessions were tested from samples from eight of these sites (Morin et al., 2009). Taxonomic and genetic sequencing evidence confirmed that these accessions were a mixture of both *P. lagenophorae* and interspecific hybrids with *P. lagenophorae* as one of the parents. These rust accessions were found to attack Australian fireweed plants and some failed to develop on the native species tested, *S. lautus* subsp. *lanceolatus*, in laboratory trials. However, all the South African accessions were either comparable to Australian *P.*

lagenophorae rust isolates, or less virulent, in their response to Australian fireweed plants (Morin et al., 2009).

Biological control – future activities

The scientific case for a continuing biological control program against fireweed in Australia has been quite challenging, driven by the need for both monospecific and effective agents. Although more than 50–85% of national weed biological control programs have led to significant or permanent weed control (Myers and Bazely, 2003), these constraints and the high associated chance of non-target impacts,

suggests a very much lower chance of success. To be conservative, we have presented to the stakeholders that the chances of success might be in the order of 20%. The arguments in favour are as follows:

- a. There have been at least six weed biological control programs in Australia where there are native species in the same genus as the target weed. At least two of these have been successful to some degree; i.e. the programs against *Rumex pulcher* L. and *Jacobaea vulgaris* Gaertn..
- b. Monospecific agents are not hard to find in weed biological control programs. Most targets that are widespread in their native range have at least some natural enemies that are monospecific. Also most plant pathogens used as biological control agents are monospecific and indeed many are weed-genotype or biotype-specific pathotypes. Researchers have also found biotype-specific arthropod agents (e.g. two strains of *Dactylopius opuntiae* (Cockerell) on *Opuntia ficus-indica* (L.) Mill. or *O. stricta* (Haw.) Haw. in South Africa).
- c. No in-depth studies have been completed in the native range of fireweed in South Africa.

Based on these arguments and on a workshop at the first National Fireweed Conference in Bega in 2008, the continuation of the fireweed biological control program was supported by the stakeholders and the federal Department of Agriculture, Fisheries and Forestry. Funding was made available for this in 2009.

The re-started biological control program is now based in South Africa at the University of KwaZulu-Natal and will focus on studies of the following issues:

- a. The community and population ecology of fireweed in its native range pastures, particularly the roles of inter- and intra-specific

plant competition, climate and soil type.

- b. Taxonomic and genetic variation in the target, through surveying the morphological variation of fireweed in KwaZulu-Natal and measuring the associated local genetic variation.
- c. The natural enemy community across sympatric *Senecio* species in South Africa, looking specifically at host use across the different *Senecio* species and natural enemy distribution and abundance.
- d. Experimental manipulations of both fireweed and the natural enemy densities to identify agents that are resource limited and capable of causing high levels of impact. Through this, fireweed growth and reproductive effort, with and without natural enemies, will be measured.

Based on surveys carried out to date and historical surveys made by Marohasy in 1991 and by Mohsen Ramadan on more recent trips to South Africa for Hawaii, Table 1 shows the current list of known natural enemies.

Conclusion

Fireweed biological control in Australia was started nearly 20 years ago, but the amount of investment and effort has been quite limited to date. Work has consisted of a few surveys to a poorly defined native range and the importation and basic testing of two moths and one pathogen, of which only the latter was from the true native range. Two years ago, a strong local support group – the Bega Valley Fireweed Association – organised a National Conference on fireweed and were lucky enough to be heard and find themselves with some political advantage at the 2007 federal election. This has led to some renewed federal funding for fireweed biological control, despite the fact that the prospects for success are seriously constrained by the very close taxonomic affinity of fireweed to a group of native

Senecio species. Scientists argued that the chances of success may be as little as 20%, but a lack of any in-depth studies in the native range of the weed suggested that continuation of the program may still be worthwhile. The program has, therefore, recently moved to the plant's native range in South Africa to seek effective monospecific biological control agents for fireweed.

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Prospects for the Biological Control of Tutsan (*Hypericum androsaemum* L.) in New Zealand

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Abstract

The feasibility for biological control of tutsan, *Hypericum androsaemum* L., in New Zealand (NZ) was assessed. Conventional control methods are impractical and tutsan is not valued by any groups of society. It therefore makes a potentially good candidate for biological control. However, the lack of information about potential agents and the existence of four indigenous *Hypericum* spp. in NZ, including two endemics, are likely to prove challenging.

Introduction

Tutsan, *Hypericum androsaemum* L., is an evergreen or semi-evergreen shrub (up to 1.5 m) of the family Clusiaceae (alternatively Guttiferae). In New Zealand (NZ) tutsan has become a common weed in higher rainfall areas, growing in open forest, forest margins, scrub, waste places and garden surroundings. Tutsan is shade tolerant, unpalatable to stock, and tends to infest areas in which mechanical and/or chemical control options are impractical.

Tutsan's extensive native range includes Europe, Caucasus, Turkmenistan, Iran, Syria, Turkey, north-west Africa and temperate Asia (Davis, 1967; USDA ARS, 2009). The naturalised range includes Australia, NZ, Southern Africa, continental Chile and possibly part of the US (Thomas, 2007).

A climate similar to that of southern France, with average annual temperature of 13°C and annual rainfall of 910 mm, appears optimal for tutsan; however, tutsan can tolerate a wide temperature range (Van Der Veken et al., 2004). It is also tolerant of various soil types and acidity levels (e.g., Hutchinson, 1967). Tutsan is a shade-tolerant species and, in its native range is a component of mature forests (Olano et al., 2002). These findings suggest that large parts of NZ could prove to be

suitable habitat for this species.

Tutsan is a garden escapee in NZ (Healy, 1972) and was first recorded as naturalised here in 1870 (Owen, 1997). The plant is well established throughout NZ (North and South Islands, Stewart Is, Chatham Islands, and Campbell Islands) (Sykes, 1982). It is currently of greatest concern in the Taumarunui District in the North Island of NZ.

In NZ tutsan is considered a major pest in a range of bioclimatic zones from warm- to cool-temperate (ranging from latitude 31° to 50° S, maritime climate, below 600 m with average annual temperatures ranging between 12.5 and 22.5°C). Plant community types identified as prone to invasion by tutsan include shrublands, tussock grasslands and bare land. Tutsan can impact on the structure (i.e., on the dominant growth form of forest, shrubland etc.), or have a "major effect on many native species or on the composition or density of dominant species" (Owen, 1997).

A 1995 survey of weeds of conservation land determined its national distribution status as: "established, widely distributed throughout NZ and extending its range into new habitats and areas". Tutsan is a problem in regenerating forest (Sullivan et al., 2007). Its biological success is mainly attributed to the high seeding ability per plant, seedbank persistence of >5 years, and its tolerance of semi-

shade conditions, hot or cold temperatures, high to moderate rainfall, damage and grazing. In addition, its fleshy fruits are effectively dispersed by birds, and possibly also by goats, possums, and soil and water movement (Whatman, 1967; Owen, 1997). Classical biological control is therefore a desirable option.

In NZ there are four indigenous *Hypericum* spp. (Webb et al., 1988; Heenan, 2008, 2011):

- *Hypericum involutum* (Labill.) Choisy, native to NZ, Australia, Tasmania and New Caledonia
- *Hypericum pusillum* Choisy, native to NZ, Australia and Tasmania.
- *Hypericum rubicundulum* Heenan, endemic to the South Island of NZ (and known from one locality in the North Island) and considered naturally uncommon
- *Hypericum minutiflorum* Heenan, endemic to NZ, restricted to the central North Island Volcanic Plateau and considered nationally critically endangered

A high degree of host specificity would be required of any agent introduced against tutsan, if we were to avoid significant non-target risks to the indigenous *Hypericum* species. There are no other indigenous representatives in the Clusiaceae family in NZ.

History of biological control of tutsan in NZ

Biological control of tutsan in NZ was attempted opportunistically in the late 1940s, using a St John's wort biological control agent *Chrysolina hyperici* (Forst.) (Julien and Griffiths, 1998). Adult beetles were observed feeding on tutsan, and subsequently, an attempt was made to release *C. hyperici* in areas where tutsan was considered a problem. Beetles released on tutsan between 1947 and 1950 all failed to establish on the weed (Miller, 1970).

Early instar larvae of both the lesser and greater St. John's wort beetles, *C. hyperici* and *Chrysolina quadrigemina* (Suffrian) suffered high mortality when offered tutsan in recent no-choice laboratory feeding experiments, and the survivors' development was severely impeded (Groenteman et al., 2011),

confirming that tutsan is a sub-optimal host for the beetles, and explaining why beetles released on tutsan in the late 1940s quickly died out.

History of biological control of tutsan world-wide

The state of Victoria, Australia, initiated a biological control programme against tutsan in the early 1990s. This programme was discontinued at an early stage, prior to any surveys in the native range of the weed being carried out, after the rust fungus *Melampsora hypericorum* (De Candolle) Winter was discovered to have self-introduced there. While the use of *M. hypericorum* as a biological control agent has generated mixed results, the fungus has largely successfully controlled tutsan in Victoria (Bruzzeze and Pascoe, 1992; McLaren et al., 1997; Casonato et al., 1999; David McLaren pers. comm.).

Objectives

Given the difficulties to control tutsan using conventional methods, and given it is rapidly expanding its range, classical biological control emerges as an attractive option. The objectives of the current study were, therefore, a) to review the literature to identify potential biocontrol agents for tutsan and assess the feasibility of their release in NZ and, b) to assess the prospects of achieving successful biological control of tutsan in NZ.

Methods

Identifying fungal pathogens of tutsan

The information was obtained by searching online databases and Internet sites. Online databases searched were:

USDA Fungus-host database or FDSM (which includes most NZ plant disease records): http://nt.ars-grin.gov/fungal_databases/fungushost/FungusHost.cfm

Fungal Records Database of Britain and Ireland or FRDBI (Cooper, 2006): <http://www.feldmycology.net/FRDBI/assoc.asp>

IMI fungal herbarium (CABI Bioscience, 2006) <http://194.203.77.76/herbIMI/index.htm>

NZ fungi and bacteria database or NZFUNGI

(Landcare Research, 2009): <http://nzfungi.landcareresearch.co.nz/html/mycology.asp>; this database was also used to determine which species were already present in NZ

In addition, CAB abstracts, Current Contents, ISI Proceedings, Web of Science, Agricola, Science Direct, Google and Google Scholar were searched, using the terms “*Hypericum androsaemum* or tutsan” and sub-searched using the terms “pathogen* or fung*”. Once a list had been created, further information about each fungus was sought in the published literature as well as in the following online databases:

Index Fungorum database (Index Fungorum, 2004): <http://www.indexfungorum.org/Names/Names.asp>

Global Biodiversity Information Facility or GBIF (Global Biodiversity Information Facility, 2009): <http://data.gbif.org/species/>

Identifying arthropod biological control agents for tutsan

Unlike for fungal pathogens, comprehensive online databases for all arthropod herbivores do not exist. However, the following databases were searched:

For Lepidoptera, the Natural History Museum's world listing (Natural History Museum London, 2007): <http://www.nhm.ac.uk/jdsml/research-curation/research/projects/hostplants/>

Database of Insects and Their Food Plants Biological Records Centre (UK) (Biological Records Centre (BRC), 2009) http://www.brc.ac.uk/dbif/Interpreting_foodplant_records.aspx

Plant-SyNZ™ <http://www.crop.cri.nz/home/plant-synz/database/hostplant.php>

In addition, CAB abstracts, Current Contents, ISI Proceedings, Web of Science, Agricola, Science Direct, Google and Google Scholar were searched using the terms “*Hypericum androsaemum* or tutsan” and sub-searched using the terms “invertebrate* or herbivor*”. Checklists of NZ fauna were referred to, to determine whether any of the species recorded feeding on/infecting tutsan already occurs in NZ.

Results

Extensive searches of the literature and online databases yielded very few records of organisms attacking tutsan. This could reflect scarcity of herbivores and pathogens attacking tutsan; but it could also reflect lack of interest in tutsan on behalf of entomologists and plant pathologists, and consequently a potential array of agents to discover. All but one of the organisms recorded from tutsan were not specific to this species (see also Groenteman, 2009).

Fungi

Only 10 species of fungi have been reported in association with tutsan (Table 1). One was an endophyte, which does not cause disease symptoms. Five others could not be considered either because their host range is too broad or they are unlikely to be sufficiently damaging.

Four other pathogens may hold some potential as biological control agents. The powdery mildew *Erysiphe hyperici* (Waller.) Fr. attacks various *Hypericum* species, and is troublesome for *H. perforatum* L. where the latter is cultivated for its medicinal values (e.g., Radaitienė et al., 2002). It may be worthwhile investigating whether a virulent tutsan-specific strain exists.

Another powdery mildew, *Leveillula guttiferarum* Golovin, has only been recorded from three *Hypericum* spp. That it has not been recorded from the highly studied *H. perforatum* suggests, perhaps, a relatively narrow host range. There is no information regarding the virulence of this pathogen and, its native range is not well matched to NZ climate.

The brown leaf spot *Diploceras hypericinum* (Ces.) Died. was recorded from tutsan in NZ and Japan, and in the Netherlands in the form of *Pestalotia hypericina* Ccs. It attacks other *Hypericum* species and can cause severe dieback in *H. perforatum*. The virulence of this pathogen to tutsan in NZ is not known, but could relatively easily be tested. In the Netherlands, conditions of nearly 100% relative humidity were necessary to create

infection on tutsan in the laboratory (Van Kesteren, 1963) so conditions for natural infection in the field might rarely be met. Developing this pathogen into a bioherbicide is an avenue that could potentially be explored to overcome this limitation; however, this is an expensive pathway, unlikely to be economically viable for tutsan.

Finally, the rust *M. hypericorum* was the most common species recorded from tutsan, including in NZ. *M. hypericorum* was first recorded in NZ in 1952 (Baker, 1955). It is unclear how the fungus has arrived here, and its effectiveness in controlling tutsan is variable (Baker, 1955; Whatman, 1967).

M. hypericorum is also found in Australia, first recorded in Victoria in 1991. By 1992 it had already shown phenomenal potential as a biocontrol agent of tutsan (Bruzzese and Pascoe, 1992). Once a very common and invasive weed in south-western Victoria, by 1997 tutsan was difficult to find in that region, resulting in “possibly the most spectacularly successful example of weed biocontrol ever witnessed in Victoria” (McLaren et al., 1997).

Further attempts to use the rust as a biocontrol agent had mixed results: genetic variation between tutsan populations suggested intrinsic resistance, and various rust isolates varied in virulence (Casonato et al., 1999).

The findings from Australia highlight the importance of compatibility between genotypes and strains of fungal pathogens and their weedy hosts, and suggest that as part of a biological control programme against tutsan in NZ it should be determined what strains of tutsan and *M. hypericorum* are present here and how they compare to those known from Australia. The hypothesis that observed variation in the impact of the rust against tutsan is attributed to genetic variability of the weed, the rust, or both should be examined. In addition, if rust strains from Australia are absent from NZ, their virulence against NZ tutsan should be tested.

Arthropods

Only nine species of insects have been recorded from tutsan, four of which can be immediately precluded as potential agents due the breadth of their host range (Table 2).

The remaining five insect species are oligophagous, but restricted to the genus *Hypericum*.

Four of these species are chrysomelid beetles, two of which, *Chrysolina quadrigemina* Suffrian and *Chrysolina hyperici* Forster, are well established in NZ and their performance on tutsan is poor. *Chrysolina varians* Schaller failed to establish in Australia and North America as a biological control agent against *H. perforatum* (Currie and Garthside, 1932; Currie and Fyfe, 1938; Coombs et al., 2004). San Vicente (2005) mentions tutsan and as host of *C. varians* in Spain, yet does not explicitly treat *H. perforatum* as a host. Whether the Spanish *C. varians* is a biotype adapted to tutsan is perhaps an avenue to pursue. Lastly, *Cryptocephalus moraei* L. thrives on *H. perforatum* but not on tutsan (Tillyard, 1927).

Concluding remarks

Available information about prospective biological control agents for tutsan is slim, and makes it difficult to assess the prospects of successful biological control at this time. However, it is clear that tutsan has never been the target of any extensive surveys, and it is possible that a suite of potentially useful agents would be discovered should such a survey take place.

The genus *Hypericum* has four indigenous representatives in NZ, therefore highly specific agents are likely to be required.

Opposition to biological control of tutsan is unlikely. It is not grown here for medicinal purposes, nor is it highly valued as a garden plant. It is highly unpalatable to stock and therefore not valued for fodder, nor is it valued for beekeeping.

In a significant part of its range in NZ, tutsan is a problem on terrain where mechanical and chemical control methods are impractical. Therefore, bioherbicides are not likely to be a practical (or economic) solution and are not recommended as an avenue of future research for this weed.

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Table 1. Fungi recorded on tutsan, *Hypericum androsaemum*, and their potential usefulness for biological control.

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsaemum</i> ²	Likely to be damag- ing?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Botryosphaerales Botryosphaeriaceae	<i>Guignardia endophyl- licola</i> Okane, Nakagiri & Tad. Ito	Japan	Endophytic. Does not cause disease symp- toms in <i>H. androsae- mum</i>	No. Recorded from a wide range of hosts from various plant families	Not yet re- corded here / No
Ascomycota Erysiphales Erysiphaceae	<i>Leveillula taurica</i> (Lév.) G. Arnaud (= <i>Erysiphe taurica</i>)	Iran	Insufficient informa- tion	No. Attacks multiple genera in multiple fami- lies	Yes, exotic / No
Ascomycota Erysiphales Erysiphaceae	<i>Leveillula guttiferarum</i> Golovin	Iran	Insufficient informa- tion	Possibly genus specific. Two records in FDSM ³ : one from <i>H. androsaemum</i> , one from <i>H. helian- themoides</i> ; one record in IF ⁴ from <i>H. scabrum</i>	No / ?
Basidiomycota Pucciniales Melampsoraceae	<i>Melampsora hypericorum</i> (DC.) J. Schröt.	UK, Ireland, Scotland, Canada (BC), Australia, Bulgaria, Japan, New Zealand, USSR	Yes. Has been highly successful in control- ling <i>H. androsaemum</i> in Victoria, Australia. Highly damaging in parts of New Zealand.	Yes, highly specific (to <i>H. androsaemum</i> strains). Note that the species had been recorded from various other <i>Hypericum</i> spp., including <i>H. perforatum</i> ; however, the <i>H. an- drosaemum</i> strain failed to infect <i>H. perforatum</i> in Australia	Yes, since early 1950s / Offers partial con- trol in some areas
Chromista Oomycota Pythiales Pythiaceae	<i>Phytophthora cinnamomi</i> Rands	Japan	Yes, a highly aggres- sive species	No. Attacks many unrelated woody plant spe- cies. Not classified strictly as a fungus anymore, due to a mobile life stage (akin to brown algae). Highly invasive (classified as a 'Key process threatening biodiversity in Australia')	Yes / No!

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsaemum</i> ²	Likely to be damaging?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Erysiphales Erysiphaceae	<i>Erysiphe hyperici</i> (Wallr.) S. Blumer	England, Scotland	Yes, considered a troublesome disease of <i>H. perforatum</i> and gets sprayed with fungicides where the latter is cultivated for its medicinal values. A powdery mildew	Yes, at the genus level. FRDBI ⁵ has 8 records from <i>H. androsaemum</i> but 39 from <i>H. perforatum</i> and additional 91 from various other <i>Hypericum</i> spp. FDSM has 149 records from various <i>Hypericum</i> spp., none from <i>H. androsaemum</i>	No / Possibly, if a specific strain is found
Ascomycota Xylariales ⁶	<i>Melomastix mastoidea</i> (Fr.) J. Schröt.	Ireland	No, saprobe ⁷	No, associated with plants from various families	No / No
Ascomycota Xylariales Amphisphaeriaceae	<i>Diploceras hypericinum</i> (Ces.) Died. <i>Pestalotia hypericina</i> Ces. <i>Hyaloceras hypericinum</i> (Ces.) Sacc. <i>Seimatosporium hypericinum</i> (Ces.) B. Sutton	Netherlands (as <i>P. hypericina</i>), New Zealand, Japan	Causes leaf blight and severe stem dieback in <i>H. perforatum</i> . Not virulent to <i>H. androsaemum</i> - requires 100% RH post-inoculation to produce symptoms (in the form of <i>Pestalotia hypericina</i>). Brown leaf spot	Attacks other <i>Hypericum</i> spp. Had been collected from <i>Fragaria</i> (strawberry) plants in Canada (as <i>P. hypericina</i>)	Found in New Zealand as <i>D. hypericinum</i>
Basidiomycota Hymenochaetales Hymenochaetaeaceae	<i>Hymenochaete cinnamomea</i> (Pers.) Bres.	New Zealand	Probably not. Wood rot (attacks dead and decaying wood, but also live wood). Not likely to be very damaging.	No. Attacks hosts from multiple families.	Yes/No

Phylum Order Family	Species (and other names) ¹	Range on <i>H. androsae- mum</i> ²	Likely to be damaging?	Likely to be host specific? (and comments)	Found in New Zealand/ biocontrol potential?
Ascomycota Hypocreales Monolinaceae	<i>Verticillium</i> sp. Needs [stat. anam.]	New Zealand	Insufficient information. Plant-pathogenic <i>Verticillium</i> spp. exists in various strains with variation in virulence and host range. They are known to cause severe wilting in susceptible hosts, but no symptoms in tolerant hosts.	Possibly not. <i>Verticillium</i> spp. attack woody hosts of various plant families. A number of <i>Verticillium</i> spp. are listed on the Unwanted Organism register. http://www1.maf.govt.nz/uor/searchframe.htm	Yes/No

¹Many fungi have more than one Latin name because they can produce more than one type of spore. The name given when they are producing 'sexual' spores is called the teleomorph, whereas the stage producing 'asexual' spores is called the anamorph. The two stages often look completely different. Fungi are classified according to their 'teleomorph' name, unless the 'anamorph' is the only form known. So, Table 1 gives the taxonomy of the teleomorph, but column 2 uses whichever name/names were recorded when the fungus was found on *H. androsaeumum*. If a fungus was listed under an out-of-date name (synonym) this is also stated in column 2.

²Only the places where the organism was found associated with *H. androsaeumum* are listed here. It may also be found elsewhere on other hosts.

³FDSD = USDA Fungus-host database at <http://nt.ars-grin.gov/fungal/databases/fungushost/FungusHost.cfm>

⁴IF = Index Fungorum, World database of fungal names at <http://www.indexfungorum.org/Names/Names.asp>

⁵FRDBI = the Fungal Records Database of Britain and Ireland (FRDBI) at <http://194.203.77.76/fieldmycology/FRDBI/FRDBI.asp>

⁶Insertae sedis = of uncertain taxonomic position within a higher taxonomic order (e.g. Phylum known, but order within that phylum uncertain).

⁷Saprobe: An organism using dead organic material as food and commonly causing its decay (Kirk et al. 2001). Unlikely to cause disease and therefore probably insufficiently damaging to be useful for biocontrol.

Table 2. Records of invertebrates feeding on tutsan *Hypericum androsaemum*.

Order and Family	Species	Type of organism	Range	Likely to be sufficiently host specific?
HEMIPTERA				
Aleyrodidae	<i>Aleyrodites fragariae</i> Walker (=loniceriae)	Whitefly	Europe, Northern Asia, Mediterranean Basin	No. Polyphagous. A pest of strawberry.
HETEROPTERA				
Lygaeidae	<i>Kleidocerys truncatulus</i> ericae (Walker)	Ground bug	Isle of Wight (UK), Dutch West Frisian Isles (as <i>K. ericae</i>)	No. Feeds on Erica spp. and Calluna spp.
LEPIDOPTERA				
Nepticulidae	<i>Ectoedemia</i> (=Fomoria) <i>septembrella</i> (Stainton)	Leaf mining moth	Palearctic	Possibly not. Feeds on various <i>Hypericum</i> spp. Possibly more common on <i>H. perforatum</i> .
Tortricidae	<i>Ctenopseustis herana</i> Felder & Rogenhofer and <i>C. obliquana</i> Walker	Leafrollers	New Zealand (endemic), Australia (introduced)	No. Highly polyphagous. Pests of many crops
	<i>Planotortrix excessana</i> Walker and P. Octo Dugdale			
COLEOPTERA				
Chrysomelidae	<i>Chrysolina varians</i> (Schaller)	Leaf beetle	Europe (from Spain to West Siberia)	Possibly not. Feeds on various <i>Hypericum</i> spp., and more common on <i>H. perforatum</i> . Possibility of an <i>H. androsaemum</i> biotype in Spain?
	<i>Cryptoccephalus moraei</i> (L.)	Leaf beetle	Europe	Possibly not. Feeds on various <i>Hypericum</i> spp. Does not thrive on <i>H. androsaemum</i>
	<i>Chrysolina quadrigemina</i> (Suffrian)	Leaf beetle	From North Africa to Denmark. Introduced to Australia, New Zealand and North America	No. Prefers <i>H. perforatum</i> . Performs poorly on <i>H. androsaemum</i> . Reported feeding on <i>H. involutum</i> (indigenous to Australia and New Zealand) in Australia
	<i>Chrysolina hyperici</i> (Forster)	Leaf beetle	Native to northern and central Europe and western Asia. Introduced to Australia, New Zealand and North America.	No. Prefers <i>H. perforatum</i> . Performs poorly on <i>H. androsaemum</i>

The Use of *Ascochyta caulina* Phytotoxins for the Control of Common Ragweed

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Abstract

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage. Previous research had lead to the identification of a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., having high toxicity against both host and non-host plant leaves, with lack of antibiotic and zootoxic activities. Recently, a two-year research project named ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop the technologies to obtain a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the possibility of its practical use. Among the selected target weeds, *A. artemisiifolia* showed an evident response to the toxin application, probably due to the presence of trichomes on the leaves that allow a quick absorption of the metabolites, with fast appearance of leaf necrosis and plant desiccation. Greenhouse bioassays as well as open field experiments clearly indicate the lethal effects on the young ragweed plants.

Introduction

Common ragweed, *Ambrosia artemisiifolia* Bess. is an alien weed of North American origin that became invasive in Europe. Although the main concern regarding this plant is its impact on human health, due to abundant production and easy spread of its highly allergenic pollen, *A. artemisiifolia* is also increasingly becoming a major weed in agriculture, being very competitive with crops and difficult to manage.

Previous research had lead to identify a mixture of three toxins from the culture filtrates of *Ascochyta caulina* (P. Karst.) Aa & Kestern, a fungal pathogen of *Chenopodium album* L., showing a wide range of phytotoxicity against different weed species.

Recently, a two-year research project named the ECOVIA has been approved and financially supported by the Regional Governorate of Lombardy (Italy). The main aim of the project is to develop technologies for obtaining a natural herbicide based on the bioactive toxins produced by *A. caulina*, and to study the potential of its practical use.

During 2010 and 2011, in the framework of the ECOVIA research project, among the 16 weed species we tested, *A. artemisiifolia* was evaluated both in laboratory and semi-field conditions.

Materials and Methods

Toxin production

The fungus, *A. caulina*, a biological control agent of the weed *C. album*, was grown on a liquid defined mineral medium (named M1-D) for 4 weeks in static conditions as previously described (Evidente et al., 1998). Toxins were purified as described (Evidente et al., 1998; 2000) and obtained as a pure mixture containing three main phytotoxic compounds (i.e., ascaulitoxin, its aglycone, and trans-4-aminoproline; Fig. 1).

Laboratory and screen house bioassays

Young potted ragweed plantlets were sprayed uniformly with an 8 mg/ml water solution of the purified fungal toxins by uniform nebulisation of leaf surface. A relative humidity of 100% was maintained for 24 hours after each application. The experiment was successively repeated with a lower concentration of toxins solution (2 mg/ml) on cut leaves in Petri dishes and on potted plantlets.

In order to enhance the efficacy of the toxins, further bioassays were performed by adding a wetting agent to the toxin solution. The following four commercial products, which appear to best fit our needs, were used at the labelled concentration: Biopower®, Adigor®, Etravon® and Codacide®.

Results

A. artemisiifolia showed an evident response to the toxin application. In particular, the 8 mg/ml solution showed a high herbicide effect on young plantlets with 100% leaf damage within 3-5 days after the application (Fig. 2). The sensitivity of the target

weed is likely due to the presence of high numbers of trichomes on the leaves (Grangeot et al. 2006) that allow a quick absorption of the metabolites, with the fast appearance of leaf necrosis and plant desiccation. We obtained a significant damage with cut leaves treated with the 2 mg/ml solution in Petri dishes (Fig. 3). The same solution (2 mg/ml) caused a leaf damage of less than 50% when sprayed on the plantlets, but its phytotoxicity has been significantly improved using the toxin in combination with the Codacide®.

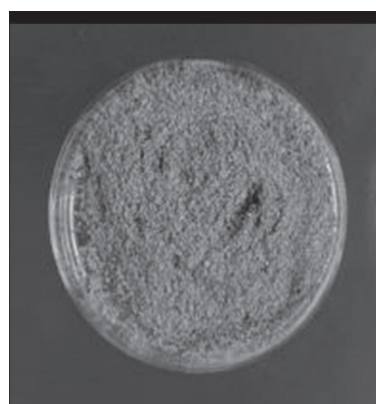
Results presented here show promise that these toxins can be effective in controlling common ragweed. Bioassays are in progress to further develop methods of application of the toxin mixture in order to achieve higher efficacy as a natural herbicide for the control of common ragweed.

Acknowledgements

We thank A. Evidente (University of Naples, Italy) for chemical extraction of fungal toxins, F. Vidotto (University of Turin, Italy) for providing us ragweed seeds, L. Smith and T. Widmer (USDA-ARS) for reviewing the paper draft, and L.M. Padovani, P. Carrabba and C. Tronci (ENEA C.R. Casaccia, Rome, Italy) for their support in ECOVIA project.

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The culture filtrate can be purified by using a simple and cheap method, obtaining a toxic hydrophilic and almost pure mixture of the three metabolites as a yellowish powder.

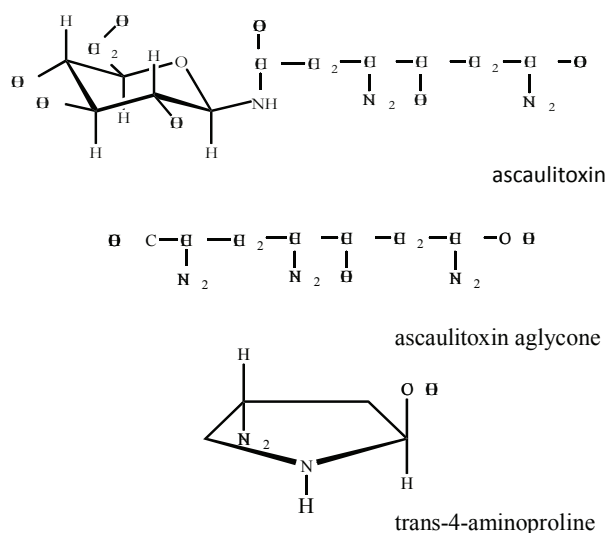


Figure 1. The toxin mixture is a yellowish powder containing three compounds: a caulitoxin, ascaulitoxin aglycone, and trans-4-aminoproline.

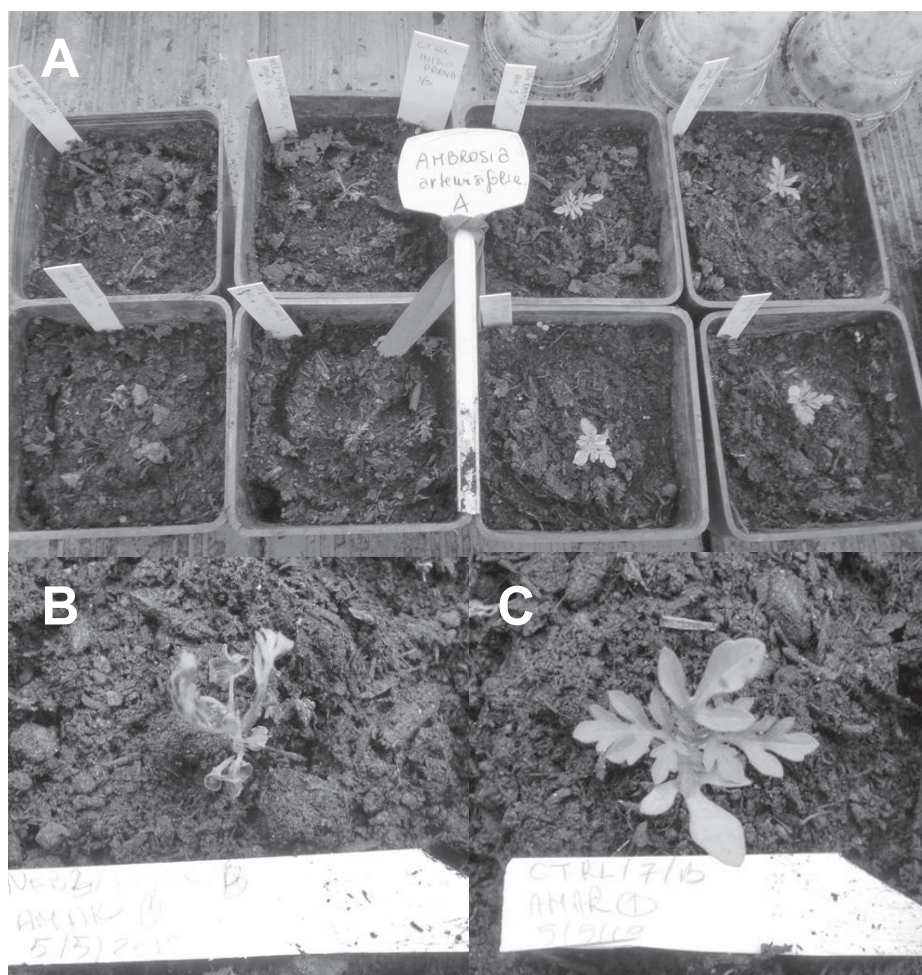


Figure 2. Phytotoxicity of an 8 mg/ml toxin solution on ragweed plantlets: A) treated plants (left) and control plants (right); B) treated plant, C) control plant.



Figure 3. Effects of a 2 mg/ml toxin solution on ragweed cut leaves (control - left, treated - right).

Biological Control of Hygrophila: Foreign Exploration for Candidate Natural Enemies

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Abstract

Hygrophila, *Hygrophila polysperma* (Roxb.) T. Anders (Acanthaceae) is an invasive aquatic weed of lotic habitats in Florida, USA. This rooted submerged or emergent plant is typically found in flowing fresh water channels and structured shorelines. Hygrophila forms dense vegetative stands that occupy the entire water column, interfering with navigation, irrigation and flood control activities. It is listed as a Federal Noxious Weed and a Florida Exotic Pest Plant Council Category I invasive species. A visible increase in the number of water bodies invaded by hygrophila since 1990 suggested that current methods employed to control this weed are inadequate. A previous study confirmed that hygrophila is a good candidate for classical biological control. However, little information was available on natural enemies affecting hygrophila in its native range. Exploratory field surveys were conducted in a range of habitats in India and Bangladesh during 2008 and 2009. In total, 41 sites were surveyed, including 28 sites in the states of West Bengal and Assam, India and 13 sites in Mymensingh, Bangladesh. The geolocation and altitude of each survey site were recorded. Several collection techniques, e.g. hand picking, Berlese funnel extraction, as well as sweep and clip vegetation sampling, were used to collect natural enemies. A number of insects, including two caterpillars (*Precis alamana* L., Nymphalidae and an unidentified noctuid moth, Lepidoptera) that defoliate emerged plants, an aquatic caterpillar (*Parapoynx bilinealis* Snellen, Crambidae, Lepidoptera) feeding on submerged hygrophila, and a leaf mining beetle (*Trachys* sp., Buprestidae, Coleoptera) were collected during these surveys. In addition, a very damaging aecial rust fungus was collected.

Introduction

Florida historically has been vulnerable to invasion by exotic animals and plants. The aquatic weed hygrophila, *Hygrophila polysperma* (Roxb.) T. Anderson (Acanthaceae), is one such plant. It is listed as a federal noxious weed (USDA, 2006),

and a Florida Exotic Pest Plant Council Category I invasive species (FLEPPC, 2009). This aquatic plant escaped cultivation by the aquarium trade and is now causing serious problems by displacing native aquatic vegetation (Spencer and Bowes, 1985), primarily in lotic habitats (Spencer and Bowes, 1985; Sutton, 1995). Hygrophila is believed

to be native broadly to the southeastern Asiatic mainland (Les and Wunderlin, 1981; Spencer and Bowes, 1985; Schmitz, 1990; Angerstein and Lemke, 1994). This herbaceous perennial weed is capable of forming dense stands and can occupy the entire water column, causing disruption in irrigation and flood control systems (Schmitz and Nall, 1984; Sutton, 1995). Conventional control techniques do not provide effective control of this invasive weed. Mechanical methods may be useful for removing the floating mats, but harvesting increases the number of viable stem fragments that can be transported to other areas where they can infest new water bodies (Sutton, 1995). Established hygrophila infestations are also difficult to control with registered aquatic herbicides (Sutton et al., 1994a; Sutton et al., 1994b). Due to presence of cystoliths (calcium carbonate pustules) in its leaves and stems, hygrophila is not a preferred food plant for triploid grass carp *Ctenopharyngodon idella* Val (Cuvier & Valenciennes) (Pisces: Cyprinidae) (Sutton and Vandiver, 1986). Considering several biological and economic attributes of this weed, biological control may be a viable option for managing hygrophila (Pemberton, 1996, Cuda and Sutton, 2000).

A noticeable increase in the number of public lakes and rivers with hygrophila has occurred in Florida since 1990 (Langeland and Burks, 1999). One of explanations for the recent hygrophila problem is simply a lack of host specific natural enemies attacking the plant, which would give it a competitive advantage over native flora. Surveys of the natural enemies of hygrophila are needed because there is no information available on potential biological control agents of this aquatic plant (Cuda and Sutton, 2000). The objectives of this study were to (i) catalog and georeference historical populations of hygrophila from herbaria records in India; and (ii) identify candidate natural enemies associated with hygrophila in its native range.

Materials and Methods

Cataloging and Geopositioning Herbaria Records

In order to identify areas for field surveys, locality information of hygrophila specimens was collected

from herbaria in the plant's native range. In India, herbaria label information was obtained from The Central National Herbarium, Howrah, India under the Botanical Survey of India. In addition, label information was collected from the Royal Botanic Garden Herbarium, Kew, United Kingdom.

Exploratory Field Surveys in Hygrophila's Native Range

Exploratory field surveys were conducted in September 2008 and September 2010 in a range of habitats in India and Bangladesh to collect natural enemies of hygrophila. In total, 41 sites were surveyed, including 28 sites in the states of West Bengal and Assam, India and 13 sites in Mymensingh, Bangladesh (Fig. 1). The geoposition and altitude of each survey site were recorded. Several collection techniques, e.g., hand-picking, Berlese funnel extraction, sweep and clip vegetation sampling, as well as dissection of plant parts, were used to collect natural enemies. Arthropods collected during surveys were preserved according to standard methods. Specimens were submitted for identification to cooperating systematists with expertise on specific taxa.

Results and Discussion

Cataloging and Geopositioning Indian Herbaria Records

In total, 64 herbaria records of hygrophila were examined and the locality information/ ecological notes recorded from the Central National Herbarium at Kolkata, India. The herbarium's records indicated that hygrophila was collected from 12 Indian states and the majority of samples (26 of 64, or 41%) were from the state of West Bengal in the eastern part of India. The earliest record was dated 1910 and at least one sample was collected at an altitude of 1200 m.

Label information from 41 specimens was collected from the Kew Herbarium, Richmond, Surrey, UK. Records were collected from specimens dating back to the 1800s and early 1900s from Pakistan, Burma, Vietnam, Taiwan, Sri Lanka and Malaysia. This information was helpful in delimiting the native range of hygrophila.

Exploratory Field Surveys in the Native Range

Insects collected from *hygrophila*'s native range belonged to the Orders: Coleoptera (Anthicidae, Buprestidae, Carabidae, Chrysomelidae, Coccinellidae, Curculionidae, Dytiscidae, Hydrophilidae, Noteridae, Scarabaeidae, and Staphylinidae), Lepidoptera (Crambidae, Noctuidae and Nymphalidae) and Hemiptera (Cicadellidae, Delphacidae, Meenoplidae and Tingidae). In comparison to its invasive range, substantially higher numbers of herbivores were associated with *hygrophila* in its native range (Mukherjee, 2011). Table 1 lists the insects collected along with their approximate abundances, trophic status or feeding guild and methods of collection.

Coleoptera

In total, 19 genera of beetles in 11 families were collected during surveys in India and Bangladesh (Table 1). The most important species observed to cause direct damage to *hygrophila* was a leaf mining buprestid beetle *Trachys* sp. (Family: Buprestidae, Fig. 2). This insect was collected in both Assam and West Bengal in India, as well as the Mymensingh area of Bangladesh from terrestrial population of *hygrophila*. Based on field observations, the buprestid completes its life cycle within a single leaf of *hygrophila*. The dorsoventrally flattened, wedge-shaped larva has a characteristic buprestid form with large flattened head and thoracic regions. The larva mines entirely inside *hygrophila* leaves, feeding on leaf tissue from side to side without damaging the upper and lower leaf epidermises, forming a transparent leaf cavity. Larval duration is ~3 weeks. Pupation occurs within the leaf pocket and the pupal duration is ~7 days. The pupa is brownish in color and ~6-7 mm long and 2-3 mm broad. The adult beetle is metallic black in color, 3-4 mm in length and 1-1.5 mm in width. It is important to note that the occurrence of this insect was rare. In total, only 19 larval specimens were collected during the all the surveys. As this insect completes its life cycle within a single leaf, the size of the *hygrophila* leaf may be an important factor limiting its abundance, and damage was usually observed in broader and longer lower leaves of the plant. The average length and width of the leaves attacked by this insect ranged between 3.0 ± 0.5 cm and 1.2 ± 0.2 cm (mean $\pm$ SD)

, respectively. Further studies on *Trachys* sp. will be required in order to understand its life cycle and host range. This information will be essential for determining its usefulness as a potential biological control agent of *hygrophila*.

A weevil, *Bagous luteitarsis* Hustache (Curculionidae) also was collected during these surveys (Table 1) (O'Brien and Askevold, 1995). This is a semi-terrestrial species in the usually aquatic genus and to date there is no available host plant information (Charles O'Brien, personal communication). The genus *Bagous* has been important for classical biological control of aquatic weeds (O'Brien and Askevold, 1995). *B. luteitarsis* only was collected once during the surveys, suggesting that this is not an abundant species. However, considering that this is a semi-terrestrial species, enabling it to attack both terrestrial and submerged forms of *hygrophila*, further studies are needed to evaluate its biological control potential.

Several other phytophagous leaf beetles (family: Chrysomelidae) were collected during this surveys (Table 1). Congeners of some of these insects have been considered as potential biological control agents for other weeds (for example, Kok, 2001). However, as indicated in Table 1, these beetles were collected during sweep sampling or Berlese funnel extraction. Therefore, there is no direct evidence that these insects feed directly and/or exclusively on *hygrophila*.

Hemiptera

In total, ten species in the order Hemiptera, including suborders, Auchenorrhyncha (families Cicadellidae, Delphacidae and Meenoplidae) and Heteroptera (family Tingidae) were collected during these surveys (Table 1). All the Auchenorrhyncha genera are known to be phloem feeders and are major pests of agricultural crops including rice (*Oryza sativa* L). These insects were collected during sweep sampling and probably used *hygrophila* as an alternate host. No information on host range is available on the two tingid species, *Belenus bengalensis* Distant and *Cantacader quinquecostatus* Fieber, collected during this survey (Distant, 1902;1909). It is noteworthy that several tingids have been used as biocontrol agents or reported as feeding on invasive weeds in several classical

weed biological control programs (summarized in Julien and Griffiths, 1998). The above information suggests that tingids could be successful biological control agents. Further studies will be necessary to confirm the feeding damage of *B. bengalensis* and *C. quinquecostatus* on *hygrophila*.

Lepidoptera

Three lepidopteran species, an aquatic leaf cutter *Parapoinx bilinealis* Snellen (Crambidae: Acentropinae) (Fig. 3), and two foliage feeders, *Nodaria* sp. (Noctuidae: Herminiinae) (Fig. 4) and *Precis almana* L. (Nymphalidae) were collected during the surveys. All three species were observed to cause direct feeding damage to *hygrophila*.

Larvae of *P. bilinealis* make cases with *hygrophila* leaves and feed internally. The mature larva is cream colored, ~15 mm in length and with conspicuous branched tracheal gills on all body segments except the prothoracic region (Fig. 3). Presence of branched gills along the body segments is the diagnostic character for this genus (Habeck, 1974). Duration of the larval stage was ~3 weeks. The larva feeds by scraping leaf tissue from the inner surface of the case, rendering it transparent. In some cases, they were observed to extend outward from the larval case to feed on nearby leaves. However, no feeding damage to the stem was observed. Pupation occurs within the leaf case, and the leaf case containing the pupa floats on the water surface. The duration of the pupal stage was ~7 days. The adult moth (wing span ~10 mm, Fig. 3B) is yellowish brown in color with conspicuous white wavy strips on the wings. The insect caused substantial damage to the submerged *hygrophila* plants. Available host records suggest that insects of this genus tend not to be host specific (for example, see Habeck, 1974). Based on available information, it seems unlikely that *P. bilinealis* is specific to *hygrophila*, although our suspicions should be confirmed through host range tests.

A noctuid moth, *Nodaria* sp., which attacks both emergent and terrestrial populations of *hygrophila*, was also recorded during these surveys (Fig. 4). This moth was found at all locations surveyed and was observed to cause complete defoliation of *hygrophila* plants (Fig. 4C). The fully grown semilooper larva is ~35-40 mm long and ~2-3 mm wide with a reddish-brown dorsal surface and green ventral surface (Fig.

4B). The dorsal surface of the larva changes color from green to reddish-brown with successive molts. Based on field and laboratory observations, this insect has five instars and total duration of the larval stadium is ~14 days. The larva consumes the entire leaf, leaving only the midrib intact (Fig. 4C). Multiple larvae have been observed feeding on the same plant. Pupation occurs on the plant within a pupal case constructed of 3-4 *hygrophila* leaves tied together by silk. The pupa is brownish-black in color, ~10 mm in length and 5 mm in width. Duration of the pupal stage is ~7 days. The adult moth is grayish-brown with indistinct blackish antemedial, postmedial and subterminal wavy lines on the wings; the wingspan ~25 mm. Field observations confirmed that this insect can be very damaging, and is probably specific to *hygrophila* as no feeding was observed on other species of nearby plants. Further studies are needed to confirm its host range and determine its biological control potential.

In addition, a nymphalid butterfly, *Precis almana* L., commonly known as the Peacock Pansy butterfly, was found feeding on *hygrophila*. This insect, which was collected from both India and Bangladesh, also caused complete defoliation. However, because it is a polyphagous species known to feed on wide range of plants (Kehimkar, 2008), it has no value for importation biological control of *hygrophila*.

Pathogens

In addition to the aforementioned insects, a very damaging aecial rust fungus (Pucciniales: Pucciniaceae, hereafter *hygrophila* rust) that completely killed *hygrophila* plants was found during surveys in India and Bangladesh (Fig. 5). Rust infections were observed at all locations surveyed, suggesting that it could be an important natural enemy of *hygrophila*.

Historically, rust fungi have been used effectively in classical weed biological control programs (summarized in Charudattan, 2001). Most rust fungi have a complex life cycle, involving both sexual (aecial and pycnial spores) and asexual (uredenia, telial and basidial spores) stages (see Kolmer et al., 2009 for detailed description of a rust life cycle). A prerequisite for a rust fungus to be a potential classical biological control agent is that it must be autoecious, whereby the rust completes its life cycle on a single host species. In case of the

hygrophila rust, only the aecial (Fig 5B) and pycnial (Fig. 5C) stages were observed in the field. Repeated attempts to inoculate healthy hygrophila plants with aeciospores from infected plants failed to initiate infection. However, laboratory observations confirmed that aeciospores germinated on tap-water agar medium, producing a normal germ tube (CAE, personal observation). If this rust were cycling through aecia alone (microcyclic), then it would be expected that the aecia would be behaving as aeciod-telia, germinating to produce basidiospores. However, more work is required to elucidate the germination process since this can be influenced by temperature. If this rust is a full-cycled autoecious rust, then it would be expected that the aeciospores would infect the hygrophila, and produce uredinia. Thus, either the conditions following inoculation were not conducive to infection, or the rust is indeed heteroecious, requiring a primary host to complete its life cycle.

In an earlier study, Thirumalachar and Narasimhan (1954) reported that the aecial stage of the heteroecious leaf rust, *Puccinia cacao* McAlp. occurs on *Hygrophila spinosa* (Acanthaceae), a congener of *H. polysperma*. The common grass *Hemarthria compressa* (L.f.) R.Br. (Poaceae) is the primary host of *P. cacao*. They reported that on *H. spinosa*, the rust develops a systemic infection and the infected shoots are paler in color. Interestingly, similar observations were made on hygrophila. Laundon (1963) provided descriptions of all rust fungi infecting *Hygrophila* spp., at that time. When compared, the measurements of aecial and pycnial spores, collected from *H. phlomoides* Nees, *H. salicifolia* (Vahl) Nees and *H. spinosa* T. Anders as reported in Laundon (1963), match closely with those of the hygrophila rust that we collected (Table 2). These results also suggest the rust from *H. polysperma* could be the sexual stage of a different pathotype of *P. cacao*. Cross-inoculation studies to test the ability of aeciospores from *H. polysperma* to initiate infection (uredinia) in a currently unknown primary host (possibly a grass such as *Hemarthria compressa*) is necessary to confirm the identification of this rust fungus. However, field observations in Assam, over two growing seasons and at multiple natural sites, have not resulted in the discovery of a grass infected with uredinia, growing in the vicinity of aecia-infected *H. polysperma* (pers.

comm., K.C. Puzari, 2011). Considering the high level of aeciospore inoculum on *H. polysperma*, and assuming that the rust is the full cycled *P. cacao*, then it would be expected that uredinia would have been found on a native grass, after such long term and intensive observations. Clearly, a more in depth study of the life cycle is required before this potential biological control agent is rejected.

Overall, a number of natural enemies, including two caterpillars (*P. almana* and *Nodaria* sp.) that defoliate emerged plants, an aquatic caterpillar (*P. bilinealis*) feeding on submerged hygrophila, and a leaf mining beetle (*Trachys* sp.) were collected during surveys in India and Bangladesh, part of the native range of hygrophila. Some of these insects, in particular, *P. bilinealis*, *Nodaria* sp. and *Trachys* sp. hold promise as potential biological control agents of hygrophila. Further studies are necessary to determine their host ranges and specificity to hygrophila. In addition, a very damaging aecial rust fungus (*Puccinia* sp.) was collected. Although initial studies suggested that this rust could be the aecial stage of the heteroecious *P. cacao*, detailed cross-inoculation studies, involving its primary host *H. compressa*, are necessary to confirm its identity.

Acknowledgements

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Table 1. Insects collected from *hygrophila* during surveys in India and Bangladesh, native habitats of *Hygrophila polysperma*.

Taxonomy	Approx. abundance ¹	Trophic level or feeding guild ²	Methods of collection ³
Anthicidae			
<i>Anthelephila mutillaria</i> Saunders	R	Scavenger?	Sweep
Buprestidae			
<i>Trachys</i> sp.	U	Leaf miner	Hand coll.
Carabidae			
<i>Bembidion</i> sp.	R	Predator	Sweep
<i>Clivina</i> sp. Latreille	U	Predator	Sweep
<i>Tachys</i> sp.	R	Predator	Sweep
Chrysomelidae			
<i>Altica</i> sp.	U	Leaf feeder	Sweep
<i>Aspidomorpha</i> sp.	R	Leaf feeder	Sweep
<i>Cassida</i> sp. A	U	Leaf feeder	Sweep
<i>Cassida</i> sp. B	U	Leaf / root feeder	Sweep
<i>Chaetocnema</i> sp.	R	Leaf feeder	Sweep
<i>Lema</i> sp. A	R	Leaf feeder	Sweep
<i>Lema</i> sp. B	R	Leaf feeder	Berlese
<i>Pachnephorus</i> sp.	U	Leaf feeder	Sweep
<i>Philipona</i> sp.	U	Leaf feeder	Sweep
Coccinellidae			
<i>Harmonia</i> sp.	C	Predator	Sweep
Curculionidae			
<i>Bagous luteitarsis</i> Hustache	R	?	Berlese
Hydrophilidae			
<i>Helochaers</i> sp.	U	Predator	Berlese
<i>Paracymus</i> sp.	U	Predator	Berlese
<i>Regimbartia</i> sp.	U	Predator	Berlese
Scarabaeidae			
<i>Rhyssomus</i> sp. Mulsant	R	Scavenger?	Sweep
Staphylinidae			
<i>Philonthus</i> sp. Stephens	R	Predator	Sweep
Hemiptera			
Cicadellidae			
<i>Nephotettix</i> sp.	C	Sap feeder	Sweep
<i>Cofana spectra</i> Distant	C	Sap feeder	Sweep
<i>Cofana unimaculata</i> Signoret	C	Sap feeder	Sweep

Table 1. Insects collected from hygrophila during surveys in India and Bangladesh, native habitats of *Hygrophila polysperma*.

Taxonomy	Approx. abundance ¹	Trophic level or feeding guild ²	Methods of collection ³
<i>Hecalus</i> sp.	U	Sap feeder	Sweep
<i>Scaphomonus indicus</i> Distant	U	Sap feeder	Sweep
Delphacidae			
<i>Nilaparvata</i> sp.	C	Sap feeder	Sweep
<i>Perkinsiella</i> sp. Kirkaldy	C	Sap feeder	Sweep
Tingidae			
<i>Belenus bengalensis</i> Distant	R	Sap feeder	Sweep
<i>Cantacader quinquecostatus</i> Fieber	R	Sap feeder	Sweep
Lepidoptera			
Crambidae			
<i>Parapoynx bilinealis</i> Snellen	R	Leaf cutter	Hand coll.
Noctuidae			
<i>Nodaria</i> sp.	C	Leaf feeder	Hand coll.
Nymphalidae			
<i>Precis almana</i> L.	C	Leaf feeder	Hand coll.

¹Approximate abundance: R = Rarely collected, 3 times or less; U = Uncommonly collected, 4-10 times; and C = Commonly collected, >10 times

²Trophic level and feeding guild information for herbivorous species does not imply that species included on this list were actually observed using hygrophila as a food source

³Methods of collection: Sweep = Sweep net sampling; Berlese = Berlese funnel extraction and Hand coll. = larva collected from field and reared to adult

Table 2. Comparison of aecial rust collected from *Hygrophila polysperma* with that collected from *H. phlomoides*, *H. salicifolia* and *H. spinosa* by Laundon, (1963)

	Aecia (diam.)	Aeciospores (diam.)	Pycnia (diam)	Pycniospores
<i>Puccinia</i> sp. collected from <i>hygrophila</i>	228.1 - 398.1 μm	18.43 - 28.4 μm	103.4 - 120.1 μm	Paraphysate
Laundon (1963)	200 - 400 μm	15 - 30 μm	80 - 100 μm	Paraphysate

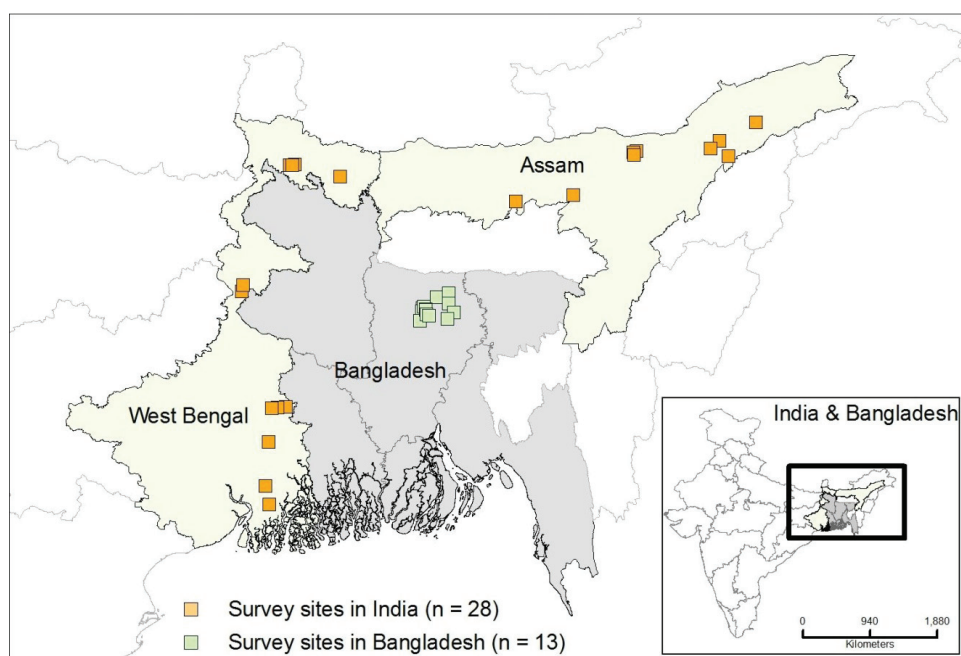


Figure 1. Survey sites in India (n = 28) and Bangladesh (n = 13). In India, surveys were conducted in the states of West Bengal (n = 15) and Assam (n = 13).

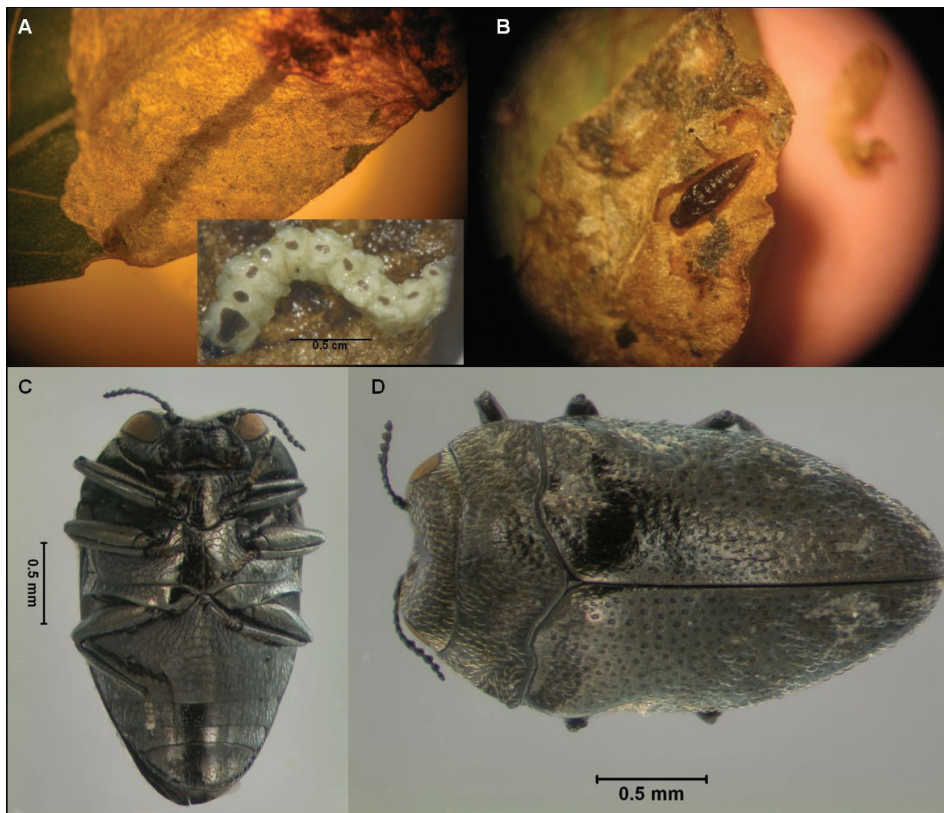


Figure 2. *Trachys* sp. (Coleoptera: Buprestidae) collected from *H. polysperma*; Larva mining inside the leaf cavity, inset: close-up of larva (A); Pupa inside the leaf cavity (B); Ventral and dorsal views of the adult beetle (C-D). Photo credit A. Mukherjee

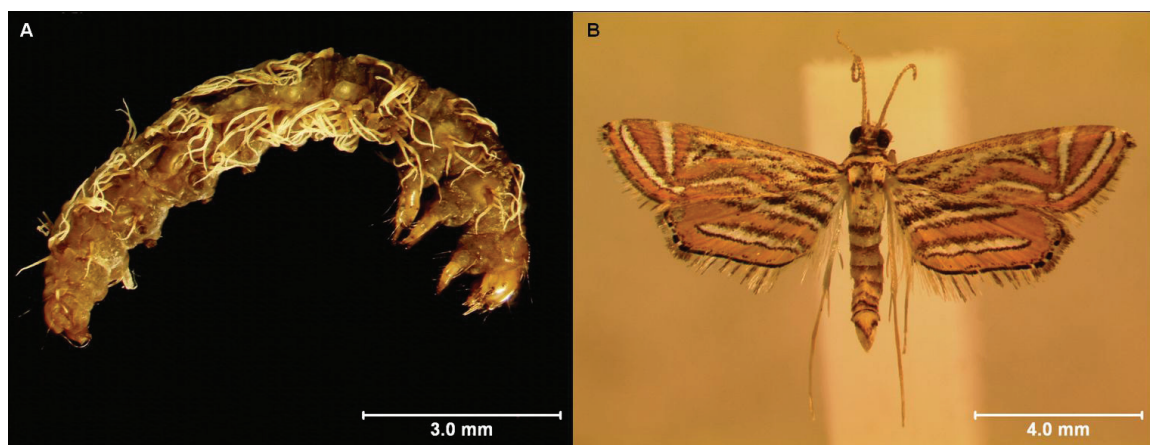


Figure 3. Larva (A) and adult (B) of *Parapoynx bilinealis* (Lepidoptera: Crambidae) collected from *H. polysperma*. Note the presence of branched tracheal gill on larval body – a characteristic of the genus. Photo credit A. Mukherjee

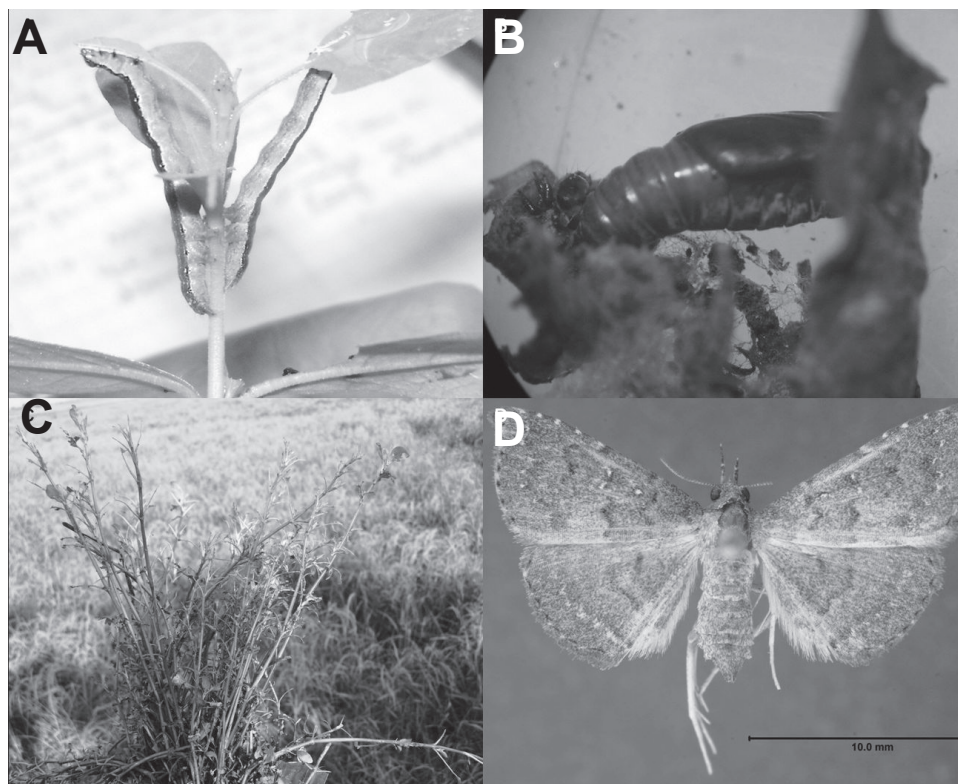


Figure 4. *Nodaria* sp. (Lepidoptera: Noctuidae) collected from *H. polysperma*; Larva feeding on hygrophylla leaves (A); Pupa (B); Feeding damage (C); Adult moth (D). Photo credit A. Mukherjee

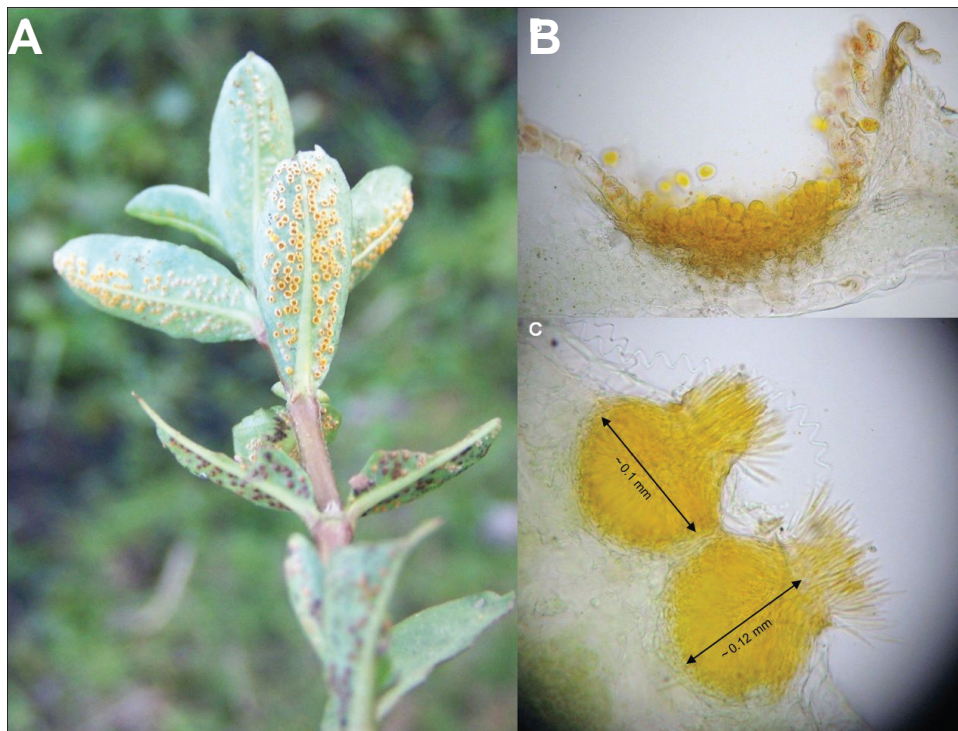


Figure 5. Rust fungus (*Puccinia* sp.) collected from *H. polysperma*. Rust infected hygrophylla plant (A); Cross section of aecia (B); Cross section of pycnia (C). Photo credit A. Mukherjee

Biological Control of *Rubus alceifolius* (Rosaceae) in La Réunion Island (Indian Ocean): From Investigations on the Plant to the Release of the Biological Control Agent *Cibdela janthina* (Argidae)

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Abstract

The giant bramble (*Rubus alceifolius* Poir.: Rosaceae), native to Southeast Asia, is one of the most invasive plants in La Réunion. A ten year research program was launched in 1997 with three components: i) genetic diversity, ii) development strategy, and iii) selection of biological control agents. Introduced populations in La Réunion, Mauritius, Mayotte and Australia were clonal and far from the highly variable native populations in Asia, while Madagascar populations appeared intermediate. Seed production is by apomixis in La Réunion Island and by allogamy in the native habitat. Fruit production occurs up to 1,100 m elevation while vegetative multiplication is possible up to 1,700 m. The plant grows in well lighted places, invading forest edges, and all open areas. From surveys of *Rubus* natural enemies in its native range, the sawfly *Cibdela janthina* (Klug) (Argidae) was selected as the most promising biological control agent and studied. The first population was thus released in La Réunion in early 2008 with the agreement of the local authorities for the biological control of *R. alceifolius*. It is now naturalized, spreading and under evaluation.

Introduction

Giant bramble (*Rubus alceifolius* Poir.: Rosaceae) is an invasive Southeast Asian bramble introduced to La Réunion Island (Indian Ocean) in the mid 19th century. In 1892, it was already cited as fatal for the island (Lavergne, 1978), and Rivals (1960) said “it was a real pain for natural environment. Density was so high that regeneration of indigenous forest plants was impossible under *Rubus* thickets.” At that time, he also mentioned that “only a biological solution could destroy this plant and solve the problem”.

Since the 16th century, the native ecosystems of La Réunion Island have undergone rapid

transformation with the introduction of more than 2000 plant species, of which 628 have become naturalised (Lavergne et al., 1999). During their evaluation of the threat posed by invasive plants to the island, Macdonald et al. (1991) underlined that 62 exotic species were major threats, among them was *R. alceifolius*, which topped the list.

For many years, the *Office National des Forêts* attempted to bring the weed under control. But neither mechanical weeding nor chemical control was successful. Control was possible only on small surface areas, and needed to be repeated regularly. Also, the cost was extremely high (at present, €2 million is spent annually on the control of invasive plants) and the use of herbicides in native forests

has raised ecotoxicological concerns. In 1992, the *Conseil Régional* of La Réunion made *R. alceifolius* a priority and decided to fund a research program to develop integrated control methods, with the emphasis placed on biological control. This program started in 1997 and ended in 2006 with the selection of a biological control agent. It was then followed by complementary action funded by the DIREN (Direction Régionale de l'Environnement) of La Réunion until 2009 for the introduction, acclimation and release of the selected biological control agent *Cibdela janthina* (Klug) (Hymenoptera: Argidae).

While much research has been done on the biological control of other species of *Rubus* (Bruzze and Lane, 1996; Evans et al., 1999; Gardner et al., 1997; Groves et al., 1997; Julien and Griffiths, 1998; Nagata and Markin, 1986), little work has been done on tropical *Rubus* species or *R. alceifolius* itself. It concerned its distribution, seed production and spread in La Réunion (Sigala and Lavergne, 1996; Strasberg, 1995; Thebaud, 1989). The chrysomelid *Phaedon fulvescens* Weise, originating in Northern Vietnam, was mentioned as a potential candidate for biological control (Jolivet, 1984). Other natural enemies were an unidentified stick insect and two rust fungi, *Hamasporea acutissima* P.Syd. & Syd. and *Gerwasia rubi* Racib. recorded in Thailand by Taysum et al. in 1991 (unpublished document). All previous experiments carried out worldwide in the biological control of *Rubus* spp., used pathogens.

The project was built around three components. The first concerned the plant's genetic diversity: i) to compare the diversity between the area of introduction and the native range in order to match genetic and geographic origins of the invader, and ii) to determine the degree of specificity needed by biological control candidates and the opportunity for their use throughout the area of introduction. The second component was the biological study of the weed under La Réunion environmental conditions in order to highlight factors for growth, multiplication and spread. The third component aimed to select and study the biological control candidates suitable for introduction and release in La Réunion.

This paper presents a compilation of the project's main findings leading to the proposal for a biological control of the giant bramble in La Réunion using the sawfly *Cibdela janthina*.

Materials and Methods

La Réunion Island (2,512 km² in area) is a volcanic island in the Indian Ocean rising to 3,061 m a.s.l., with considerable climate variations. Rainfall ranges from 8,000 mm per year on the east coast to 500 mm per year on the lowland west coast (Robert, 1986). The island's vegetation comprises four main types that are determined by a combination of rainfall and temperature: semi xerophytic tropical forest, lowland humid tropical forest, mountain humid tropical forest and ericoid vegetation at high elevations (Cadet, 1977).

The giant bramble, *Rubus alceifolius* Poir. (Rosaceae, subgenus *Malachobatus*) was first described as a subspecies of *R. moluccanus* L., but is now considered a separate species (Kalkmann, 1993). It is a shrub with arching or climbing branches up to 5 m long in native areas or up to 15 m in length in areas of introduction. Branches and leaves bear curved prickles and yellow hairs. Stipules are large, orbicular, and deeply digitately divided. Leaves are simple, orbicular to broadly ovate, 10-30 cm in diameter, with 5-7 lobes, and cordate at the base. Inflorescence is terminal, consisting of up to 4 racemes with up to 8 flowers. Flower bud is globular, petals are orbicular, white. Collective fruit is globular, succulent, 1 cm in diameter and containing many red drupelets (Kalkmann, 1993). The giant bramble is exotic in Australia (Queensland), Mauritius, Mayotte, Madagascar and La Réunion Island but is native to Southeast Asia (southeast China, Hainan, Taiwan, Myanmar, Thailand, Laos, Cambodia, Vietnam, Indonesia/Sumatra, Java, Malaysia, Lesser Sunda Islands, Borneo and Sulawesi) (Friedmann, 1997; Kalkmann, 1993; Parsons and Cuthbertson, 1992). At La Réunion it frequently occurs as large stands along forest edges, road and river sides from sea level up to 1,700 m of elevation (Baret et al., 2004).

Genetic diversity studies of *R. alceifolius* were based on 224 specimens: 116 from introduced populations (La Réunion (75), Mayotte (8), Mauritius (7), Madagascar (19) and Australia/Queensland (7), and 108 from native populations (Thailand (59), Vietnam (30), Laos (1), Java (4), and Sumatra (14). Thirty specimens of other *Rubus* species were also evaluated (La Réunion (3), Thailand (12), Vietnam (10), Laos (2), and Sumatra (3). Plant DNA was

extracted and processed following the protocol of Bousquet et al. (1990). Genetic differentiation was assessed by amplified fragment length polymorphism (AFLP), using 4 primer pairs and the restriction enzymes *EcoRI* and *MseI*, as detailed by Amsellem et al. (2000). The genetic distance between individuals was calculated and expressed as the Simple Matching distance (Rohlf, 1993). A tree was constructed according to the Neighbour-Joining Method (Felsenstein, 1993). The study focused on two levels of comparison: individuals and areas (Amsellem et al., 2000).

The reproductive biology of *R. alceifolius* in the native and introduced habitats was assessed and compared using microsatellite markers specific for *Rubus* species (Amsellem et al., 2001a). We compared the reproductive system (fruit set) of *R. alceifolius* in its native range (nine half-sib seedlings derived from different fruits on a single plant sourced from Vietnam), in its area of introduction in Madagascar (three individuals and their half-sib progeny from two localities), and in La Réunion where 44 flowers from several parents were manually fertilized with pollen from other plants (Amsellem et al., 2001b).

Developmental patterns were then studied at La Réunion by conducting architectural and morphometric analyses that described individuals at the five specific growth stages from seedlings to mature plants (Baret et al., 2003a; Baret et al., 2003b). Altitudinal variations in fertility and vegetative growth were assessed by counting flowers, fruits, seeds and leaves in eight randomly located quadrats at six sites from 50 m to 1,500 m a.s.l., and by estimating the soil seed bank (Baret et al., 2004).

Biological control agents were selected from surveys carried out in the native range (Vietnam, Laos, Thailand, China, Indonesia (Sumatra) and Singapore) from 1997 to 2004, and in the area of introduction at La Réunion Island (1998). Altogether, stands of *Rubus* were examined at 309 different locations subject to different climatic conditions, ranging from the lowlands to the highest elevations (2,500 m a.s.l. in Doi Inthanon Mountains, Northern Thailand) and from equatorial (Toba Lake, Northern Sumatra) to tropical climates with fairly cold winters (Guangdong and Hainan mountains in China). *Rubus* natural enemies (arthropods or pathogens) were collected and identified. Symptoms and environmental conditions were recorded. The

most promising ones were collected alive and reared in laboratories in Sumatra, Montpellier and La Réunion for further biological and specificity studies in order to select those most suitable for biological control of *R. alceifolius* at La Réunion.

Results

Rubus genetic diversity and putative origins of *R. alceifolius*

The genetic study of *R. alceifolius* revealed two well-separated groups, both distinct from the out-group corresponding to other *Rubus* species. The first group consisted of all the samples taken within the native range and the second group, all the samples from the area of introduction. A study of the within-area diversity showed relatively marked genetic diversity between individuals from countries of the native range. On contrary, each population sampled in the Indian Ocean islands (La Réunion, Mayotte, Mauritius, and Australia), with the exception of Madagascar, was characterized by a single different *R. alceifolius* genotype. All these genotypes were closely related to individuals from Madagascar where polymorphism was intermediate between the situation in areas of introduction and the native range.

Many authors have discussed the different hypothetical origins of this exotic plant. E. Jacob de Cordemoy (1895) mentioned that it may have been introduced at La Réunion in 1846. Nevertheless a previous text “*Ralliement du 17 août 1892*” cited by Lavergne (1978) specified that this plant came from the Botanical Garden in Calcutta. Other authors thought it was sent from Vietnam (Jolivet, 1984), or that it may either have been introduced first in Mauritius by Commerson on his return from Java in 1768 (Rivals, 1960), or introduced into Mauritius from Madagascar (Vaughan, 1937) and then spread to La Réunion in the 1850s (Rivals, 1960). Our results suggest that *R. alceifolius* was first introduced into Madagascar, perhaps on multiple occasions. The Madagascan individuals were thus the immediate source of the plants that colonized other areas of introduction. Successive nested founder events appear to have resulted in the populations in the area of introduction showing a cumulative reduction

in genetic diversity. The marked genetic differences between the populations in the native range and in the area of introduction prevented us from determining the geographical origin of the alien plant (Amsellem et al., 2000).

Genetic diversity and the weed's biological strategy as pointers for control

The low diversity of the populations found in most areas of introduction suggested that a biological control agent efficient against one individual should be able to attack the entire population on the island.

Both the genetic diversity patterns and differences between half-sib progeny and their maternal parents (revealed by microsatellite markers) showed that in the native range, seeds are produced sexually (Amsellem et al., 2001a; Amsellem et al., 2001b). By contrast, in Madagascar, over 85% of the half-sib progeny resulting from open pollination gave multilocus genotypes identical to those of their respective maternal parents. Seeds thus appear to be produced mostly or exclusively by apomixis in Madagascar. We therefore suggest that Madagascan populations resulted from the hybridization of an introduced *R. alceifolius* and native populations of presumably *R. roridus* Lindley that Kalkman (1993) considered similar and synonymous to *R. alceifolius*. Apomixis was therefore a consequence of this hybridization. In Reunionese populations of *R. alceifolius*, seeds obtained in controlled pollination experiments were all genetically identical to the maternal parents. While genetic variation (microsatellite markers) in Reunionese populations was low, it was sufficient to demonstrate that seeds could not have resulted from fertilization by the pollen donors chosen for controlled pollinations, or from autogamy, but were produced exclusively by apomixis (Amsellem et al., 2001b). This phenomenon was responsible for the clonal population in the area of introduction.

The architectural and morphological studies showed five developmental stages for *R. alceifolius*, differing by several markers such as internode length and diameter, pith diameter, and plant shape. A heteroblastic developmental pattern was thus revealed for the plant, midway between that of a bush and a vine. The results also showed that this species taps environmental resources early in its

development, whereas it “explores” the environment during the adult stage (Baret et al., 2003b).

To determine the invasive capacity of *R. alceifolius*, fertility and vegetative growth were studied at different altitudes on La Réunion Island (Baret et al., 2005). Flowering period duration, seed production, and the seed bank were found to be negatively correlated with elevation (50 – 1,500 m a.s.l.). At a lowland site, fruit production averaged between 30 and 80 fruits m⁻², while no fruits were observed above 1,100 m. The seed bank was greater under *R. alceifolius* patches (>10,000 seeds.m⁻²) than in understories not colonized by the bramble (approximately 3,000 seeds.m⁻²). Seed dispersion in forest was mainly by running water. Although the number of leaves per unit area was similar along the entire gradient studied, the reduced fruiting in upland areas might be offset by an increase in vegetative growth. Monospecific bramble patches in lowland areas may serve as the sources of seed for the colonization of new areas by bird dissemination. Once established at high elevations, the weed grows vegetatively without flowering and multiply by layering, cutting or sucker (Baret et al., 2004).

Selection of potential biological control agents

Fifty one arthropods and four pathogens were recorded and collected during the surveys conducted in the native range and in La Réunion Island. Particular care was taken to select agents on the basis of a combination of criteria (type of damage to and impact on *R. alceifolius*, host specificity, life traits etc.) for further biological and specificity studies. Of the leaf feeders, sawflies from Sumatra and China (*Cibdela janthina*, *C. chinensis* Rohwer, and *Arge siluncula* Konow) appeared to be the most promising. They caused complete defoliation of the weed and seemed to be highly specific. The beetles *Phaedon fulvescens* and *Cleorina modiglianii* Jacoby, found respectively in Vietnam and Sumatra, were also promising. The rust fungus *Hamaspora acutissima* was observed and collected in many places throughout Asia. Only a few common insects were reported in La Réunion Island, but never damaging *R. alceifolius*.

Phaedon fulvescens Weise (Coleoptera: Chrysomelidae) was collected in Northern Vietnam

while feeding on *Rubus* spp. leaves on the edge of mountain forests at 900 m a.s.l. Field observations coupled with biological and host specificity studies in Montpellier generated a wealth of new information about this species. Although not encountered frequently, individuals were very numerous in the populations observed and both adults and larvae damaged the plant by leaf skeletonizing. Eggs were laid separately on the underside of leaves and coated with feces. We also noted that the insect undergoes a two-month summer diapause. Therefore, this insect may only complete a single generation in a year, not three or four as initially thought. Field observations indicated that the beetle was highly specific to *Rubus* species of the *Malachobatus* subgenus. But host specificity tests carried out on *R. apetalus* (indigenous of La Réunion) of the *Ideobatus* subgenus showed that the insect can also feed on this plant and survive throughout its lifecycle. We therefore rejected *P. fulvescens* for the biological control of *R. alceifolius* in La Réunion (Le Bourgeois et al., 2004).

Cleorina modiglianii Jacoby (Coleoptera: Chrysomelidae) was found in Sumatra on several *Rubus* spp. in the shade provided by *Pinus merkusii* Jungh. & de Vriese forests from 700 to 1,200 m. This beetle caused leaf skeletonizing damage to *R. alceifolius* and *R. moluccanus* L. We were unable to find eggs or larvae despite numerous field surveys. Host specificity tests were carried on adults in the laboratory, comparing *R. alceifolius* (Réunion), *R. alceifolius* (Sumatra), *R. apetalus* (L Réunion) and *R. fraxinifolius* (La Réunion). The adults only fed and survived on *R. alceifolius* (Sumatra) and *R. apetalus* (La Réunion). This insect was therefore rejected as a potential candidate for biological control.

Cibdela janthina (Klug) (Hymenoptera: Argidae) was recorded in Sumatra. The insect's behavior was observed in the field while its biological traits were assessed in the laboratory in Sumatra. Mating happened in full light at 30°C and 80% humidity two days after female emergence. Then eggs were inserted into the main nerves of the plant's upper young leaves not yet fully opened. Average fertility was 58 eggs per female, with 84% viability. The eggs hatched after 10 days of incubation. The larvae completed seven instars within 25 to 30 days, and then pupated in a silk cocoon under the leaf litter. Larvae were gregarious during the major part of their development and presented a typical S-shape.

The full life cycle ranged from 48 to 62 days. The insect may complete six generations per year without any diapause. Adult life span was only 7-14 days and they were found not to be feeding under Sumatran environmental conditions; only drinking dewdrops on leaves, while the larvae were feeding on *R. alceifolius* leaves consumed systematically along the branches in a top-down process. Host range tests were conducted on 41 plant species from 13 botanical families chosen on the basis of phylogeny and economic or conservation issues for La Réunion. *Cibdela janthina* appeared to have a very narrow host range, feeding only on *Rubus* species. Starvation tests showed some feeding on certain subspecies of *R. moluccanus* not present in La Réunion, on *R. fraxinifolius* (exotic to La Réunion) and on *R. apetalus* (indigenous in La Réunion). Choice tests showed that the insect mainly prefer to feed on *R. alceifolius*. Temperature conditions that impact on the insect's development should keep *C. janthina* under 1,000 m of elevation while *R. apetalus* is present from 700 m to 1,700 m. Considering these results and the insect's biological features, *C. janthina* was considered as a good potential biological control agent against *R. alceifolius* in La Réunion. Accordingly, a petition was made for permission to introduce and release it.

Hamaspora acutissima P.Syd. & Syd. (Uredinales: Phragmidiaceae) was observed at many locations in Vietnam, Thailand and Indonesia (Sumatra). It was visible on the upper face of leaves as small brown to yellowish spots and on the lower face as bunches of orange paraphyses containing teliospores. Spots were found on isolated leaves and plants, or as intense infestations covering all parts of *Rubus* plants. In cases of marked contamination, leaves were drying and withering. All the *Rubus* mentioned as infected by this fungus belonged to the *Malachobatus* subgenus indicating the narrow host range of the rust. Biological and host specificity studies showed that Asian *Rubus* species belonging to the *Malachobatus* subgenus could sometimes be inoculated. *R. alceifolius* from La Réunion was inoculated but the pathogen stopped growing at the mycelium stage without producing new teliospores. Rust fungi are known to be highly specific (Evans and Gomez, 2004). Our results confirmed that the Reunionese *R. alceifolius* is genetically too different from those in the native range to allow *H. acutissima* attack.

Conclusion

The alien invasive giant bramble found in La Réunion and other areas of introduction is genetically different from those in the native range and probably resulted from hybridization in Madagascar. It has a high growth potential and produces apomictic fruits in the area of introduction. With its marked phenotypic plasticity, the plant is able to fruit below 1,100 m a.s.l. and can grow and multiply vegetatively up to 1,700 m.

Of the biological agents collected in the bramble's native range, the sawfly *C. janthina* showed the best biological and ecological traits and host specificity that could justify its introduction into La Réunion to regulate *R. alceifolius* populations under 1000 m a.s.l. It also appeared to have the most severe impact on weed growth.

Therefore, a petition form to introduction and release of *C. janthina* was submitted in 2006 to the *ad hoc* scientific committee and was accepted by local authorities. Cocoons from Indonesia/Sumatra were introduced in mid 2007 for the rearing of the sawfly and acclimatization at La Réunion. The first population was released on the east coast of La Réunion in early 2008. It is now spreading well, and controlling populations of the giant bramble. Studies of the spread dynamics and impact of *C. janthina* on *R. alceifolius* thickets at the island level are ongoing, as is a study of the impact of the decline of populations of the giant bramble on natural vegetation dynamics.

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Beyond the Lottery Model: Challenges in the Selection of Target and Control Organisms for Biological Weed Control

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Abstract

Biological control scientists have long tried to pick winning combinations of target and control organisms, while minimizing adverse effects on nontarget organisms. What have we learned from recent work and what should be focus on moving forward? Establishment rates for new control organisms are nearing 100% with little room for improvement; however, improving rates of control given establishment and minimizing off-target effects are two areas for improvement. Two ways to keep control organisms effectively on target are to (1) diagnose and exploit weed vulnerabilities using targeted life-cycle disruption (thereby achieving effective biological using fewer control organism individuals and species), and (2) investigate the mix of evolutionary and ecological forces enabling control organisms to exploit new habitats and hosts (thereby better forecasting outcomes of biological control). Continuity of program support, learning from experience, rational regulations and policies, plus developing and exchanging new technology will help achieve these goals.

Bottom-Up Effects on Top-Down Regulation of a Floating Aquatic Plant by Two Weevil Species: The Context-Specific Nature of Biological Control

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Abstract

Predicting the efficacy of prospective biological control agents, the holy grail of weed biological control, while often advocated, is rarely implemented. We examined, *a posteriori*, whether it would have been possible to predict which of two introduced weevil species, *Neochetina eichhorniae* Warner or *N. bruchi* Hustciche, would have been the superior choice for controlling *Eichhornia crassipes* (Mart.) Solms-Laubach. Plant nutrition and competition can alter a plant's ability to sustain or compensate for herbivory and affect a phytophagous insect's ability to reproduce. These factors could also influence efficacy predictions. We therefore conducted three outdoor mesocosm experiments to compare the performance of these two weevils, independently and together, among five fertilizer treatments. A low initial plant density experiment examined their ability to reduce growth and flowering but allowed for density to increase. A high plant density experiment evaluated their ability to lessen biomass and reduce surface coverage. The third experiment began with low plant density but plants were maintained at low density by harvesting a portion whenever coverage exceeded 50% of the water surface. This was intended to minimize intraspecific competition. The effects varied between weevil species, among fertilizer treatments, and among experiments. Interactions between herbivory and fertilizer treatments were apparent and the nature of these interactions varied among experiments. Efficacy therefore seemed nuanced and context specific, requiring extensive assessments of multiple evaluation criteria across a wide range of environmental and ecological conditions. Overly simplistic evaluations risk rejection of effective agents capable of mediating adverse impacts from invasive plant populations. These results also argue against the concept that a single best agent can be identified to control a weed that inhabits a broad range of habitats and conditions.

Predicting Parasitism of Weed Biological Control Agents

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Abstract

We conducted a nationwide survey of parasitism of weed biological control agents in New Zealand (NZ) and found that 19, mostly native, parasitoid species attack 10 weed biological control agent species. Fifteen of these parasitoid species were confined to five agents that possessed “ecological analogues”, defined as a native NZ insect that belongs to the same superfamily as the agent and occupies a similar niche on the target weed. Parasitoid species richness in NZ was positively correlated to richness in the area of origin. However, only agents with ecological analogues contributed significantly to this pattern. Our results support Lawton’s (1985) hypothesis that, to find enemy-free space, selected agents should “feed in a way that is different” and “be taxonomically distinct” from native herbivores in the introduced range.

Learning from Experience: Two Weed Biological Control Programs with Rust Fungi Compared

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Abstract

Rust fungi are the type of pathogens most widely used in classical biological control of weeds. This is primarily because of their typically high level of specificity, the severe damage they can inflict on plants, and efficient wind dispersal capability. They are not, however necessarily the easiest and most effective solutions to pursue for all weed problems. To illustrate the potential pitfalls that can be encountered with rust fungi as biological control agents, I will compare various aspects of the recent programs against bridal creeper (*Asparagus asparagoides* (L.) Druce) (Kleinjan et al., 2004; Morin and Edwards, 2006; Morin et al. 2002, 2006; Turner et al., 2010) and European blackberry (*Rubus fruticosus* L. aggregate) (Evans and Bruzzese, 2003; Evans et al., 2011; Gomez et al., 2008; Morin et al., 2011) in Australia. For example, specificity in rust fungi can be too high from a biological control point of view, necessitating the release of a range of rust pathotypes to affect the different genotypes of the weed that exist in the introduced range. Leaf-age resistance of the weed to the rust fungus can drastically limit its impact on individual plants, leading to only minor changes in the weed population dynamics. The growing season and preferred habitat of the target weed, as well as prevailing climatic conditions, can also influence the development of severe epidemics of the rust fungus and consequently its efficiency as a biological control agent.

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Potential Benefits of Sourcing Biological Control Agents from a Weed's Exotic Range

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Abstract

Specialist herbivores that establish in the exotic range of a weed provide a potential source of biocontrol agents. Such agents are more likely to adapt successfully to another novel environment and to be devoid of parasitoids and disease. In addition, in a simplified system lacking many key phytophages, assessment of the impact of potential agents may be easier than in the native range. In a South African program for control of weedy *Acacia* species *Bruchophagus acaciae* (Cameron) has been collected from New Zealand where, having established without parasitoids, it is much more abundant than in its native Australia. New Zealand has accumulated many specialist herbivores of *Acacia* from Australia and one of these, the weevil, *Storeus albosignatus* Blackburn, might have provided a useful biocontrol agent for South Africa's weedy *Acacia* species. This weevil was first recorded in New Zealand in the 1930s where it is now widespread and relatively common, feeding on seeds of several *Acacia* species. However, it was not associated with *Acacia* in Australia until 2009. Although seed-feeding agents of target *Acacia* species were sought in Australia intermittently over a period of more than 30 years and five species of *Melanterius* weevils were introduced to South Africa for control of different weedy *Acacia* species, the program did not collect *S. albosignatus* from *Acacia* species. *Storeus albosignatus* could possibly have provided a more parsimonious solution by limiting seed production of several of the target *Acacia* species. Searching for biocontrol agents in the exotic range will be most useful in areas where potential agents naturally accumulate in the exotic range through relative proximity to the native range, but it is also applicable in areas where establishment occurs through accidental transfer by human agency.

Plant-Mediated Interactions among Herbivores: Considerations for Implementing Weed Biological Control Programs

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Abstract

Complex trophic interactions are common, both in natural and managed ecosystems. One such interaction that has important implications for biological control of weeds involves plant responses to feeding by an herbivore which then impacts one or more other herbivores. Effects may be positive or negative, and mechanisms can be chemical or structural. Knowing if, and to what extent, these indirect plant-mediated interactions occur prior to importing new biological agents can assist with decisions about candidate selection, thus reducing economic and environmental costs, and increasing the overall success rate of weed biological control programs. We examined whether feeding by the musk thistle weevil *Trichosiromus horridus* (Panzer), which attacks the vegetative crown early in the plant's development, alters musk thistle as a resource for the later-arriving weevil *Rhinocyllus conicus* Frölich, which infests flower heads. Minor infestations of musk thistle by *T. horridus* had no effect on *R. conicus* oviposition and subsequent production of new adults. In contrast, heavy infestations of *T. horridus* reduced 1) *R. conicus*-musk thistle synchrony, 2) acceptability of musk thistle to ovipositing *R. conicus*, 3) the quantity and 4) the quality of resource available to *R. conicus* larvae. As a result, the production of new *R. conicus* adults was reduced 63%. Thus, even spatially- and temporally-isolated herbivores can affect one another negatively and in multiple ways. Nevertheless, musk thistle seed reduction was still greater when both weevils were present. Hence, the outcome for biological control programs may not necessarily be adverse because of compensatory trade-offs concerning the relative impacts of the two herbivores on the weed. Recommendations for incorporating protocols to assess potential indirect plant-mediated impacts on weed biological control programs will be given.

The Use of Chemical Ecology to Improve Pre-Release and Post-Release Host Range Assessments for Potential and Released Biological Control Agents of *Cynoglossum officinale*

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Abstract

Unlike their extensive use in pest insect management systems, chemical ecological techniques are not commonly used in biological weed control programs, despite their potential benefits to improve host range predictions. Semiochemicals are the principal communication in insect-plant interactions and are especially important mediators of host selection behavior in response to different chemical cues. While much of pre-release host range testing focuses on differing test conditions to either assess the fundamental host range (no-choice) or the realized host range (choice and field tests), little research is directed at underlying host plant cues triggering or preventing a potential agent female's host choice. This is true for two insects currently used or proposed as biological control agents for *Cynoglossum officinale* L., the root-mining weevil *Mogulones cruciger* Hbst. and the seed-feeding weevil, *Mogulones borraginis* (Fabricius). The former has been released for the control of *C. officinale* in Canada in 1997 but a pest alert has been issued for the insect by regulatory authorities in the USA in 2010 because of risks of non target plant feeding. *M. borraginis*, has a narrower host range and is being proposed for introduction into the USA. Pre-release host range evaluations for *C. officinale* agents are complicated by the fact that several native confamilials are rare and endangered or cannot be grown under greenhouse conditions. To assess the risk of non-target feeding by *M. cruciger* in the USA and predict the environmental safety of *M. borraginis*, we developed a portable system to collect headspace volatiles of native confamilials of *C. officinale*. We use combined gas chromatography and electroantennogram detection (GC-EAD) to test whether the volatile headspace or particular components of it, identified using gas chromatography with mass spectrometry (GC-MS), triggers responses of the antennas of either weevil species. We then verify preliminary GC-EAD results in behavioral trials using a four-area olfactometer. We assert that a major advantage of this approach is the non-destructive assessment of the attractiveness of rare non-target plant species to either weevil species.

Shooting Straight: What Weeds Should We Target Next?

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Summary

Biocontrol is the only realistic option for managing some of our most serious weeds, but projects are typically long-term and costly to develop. Therefore it is critical that targets are objectively selected by assessing the likely return on investment. This can be difficult given that both stakeholders and biocontrol practitioners can have very different views on which weeds should be targeted. Further, the relative importance of a particular weed problem, and how feasible and realistic biocontrol is for the particular species, is frequently not considered. Despite these challenges, little attention has been given to the process of target selection within the biocontrol community.

Target prioritisation can be considered within a matrix that includes weed impact, the feasibility of biocontrol (the process constraints for the establishment and execution of a biocontrol program) and the likelihood of successful outcomes (for example the chance that biological control can mitigate the identified impact). Weed impact can be extremely difficult to evaluate and predict, and any assessment needs to consider the context in which the weed occurs, whether the weed is a passenger or driver of environmental change, what it is impacting (in terms of production, biodiversity and ecosystem services), and the temporal and spatial dimension of invasions. Assessment of feasibility includes an in-depth analysis of constraints and opportunities, ranging from the practical (e.g. access to the native-range), social or political (e.g. acceptance of biocontrol as a management solution), and ecological (e.g. the genetic integrity of the target within its native range and the availability of host-specific natural enemies). Likelihood of success includes determining whether biocontrol can address clearly defined criteria for the successful management of the weed, and whether weed impacts are likely to be mitigated by the available natural enemies.

Australian examples were used to illustrate how such a prioritisation matrix can help identify the next generation of biocontrol targets by (a) helping achieve the right balance between targeting weeds of moderate to high impact where potential agents are readily available and high impact weeds where they may not be, and (b) focusing research around key knowledge gaps preventing the adequate prioritisation of new and existing targets. The biocontrol “industry” needs to demonstrate continued success against important targets if it is to survive, and that this will require science-driven target selection, a balanced portfolio of targets, quantification of potential weed impact, and continued improvement in science and technology.

Does Rise and Fall of Garlic Mustard Eliminate the Need for Biological Control?

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Abstract

Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara and Grande), a European biennial herb introduced to North America 150 years ago is now widespread in temperate forests from East to West and as far north as Alaska and south to Georgia. Garlic mustard's rapid spread, high local abundances, and lack of any beneficial effects spurred development of a biological control program in the late 1990's, which included a long-term monitoring program to develop baseline data on life history and population dynamics of *A. petiolata*. We anticipated that before and after comparisons would allow us to assess changes in *A. petiolata* performance associated with biological control agent releases. After a decade of studies in the East and Midwest, we have seen declines of *A. petiolata* to very low abundance at our permanent monitoring locations (but only in the absence of management efforts). Detailed evaluations of potential mechanisms point to negative soil feedback, i.e. the build-up of soil pathogens that appear to suppress *A. petiolata* while having no apparent negative effect on associated forest understory vegetation. While these effects appear widespread in the oldest invaded areas, similar population declines are not as widespread or apparent in the more recently invaded areas further west. We are now ready to petition the introduction of the first host specific control agent – but is this the right choice of action? At the present time the lack of clear and extensive negative ecosystem impacts of *A. petiolata* and the apparent decline without management intervention suggests that adding biological control agents to the North American fauna will not aid in restoration of herbaceous forest communities.

Unravelling the Identity of *Tamarix* in South Africa and its Potential as a Target for Biological Control

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Abstract

The Old World genus *Tamarix* has become naturalized and invasive in much of the rest of the world. *Tamarix usneoides* E. Mey. ex Bunge is native to south western Africa and indigenous to South Africa, where it is being used for phytoremediation of acid mine drainage from tailings storage facilities on gold mines. However, *T. chinensis* Lour., *T. parviflora* DC. and *T. ramosissima* Ledeb. are all exotic to South Africa, and are hypothesized to be hybridizing among themselves and with *T. usneoides*. Correct specific identification is therefore essential; to clone the indigenous species for phytoremediation use, and to investigate the possibility of biological control of the alien species. *Tamarix* remains one of the more taxonomically difficult genera to identify and when in the vegetative state many taxa are almost indistinguishable. The high incidence of hybridization in *Tamarix* also plays a role in the taxonomic confusion. The Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA) were successfully used to identify the local *Tamarix* species and their hybrids. Insect abundance and diversity were found to be higher on the indigenous *T. usneoides* than on the exotic *T. ramosissima* and its hybrids, suggesting that a potential biological control agent might distinguish between the alien and indigenous species. The potential for successful biological control of *T. ramosissima* in South Africa is discussed.

Origins and Diversity of Rush Skeletonweed (*Chondrilla juncea*) from Three Continents

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Abstract

Rush skeletonweed (*Chondrilla juncea* L.) is an invasive apomictic perennial plant in Australia, South- and North America, accidentally introduced from Eurasia, which shows differential resistance/tolerance to some herbicides and classical biological control agents. Rush skeletonweed biotypes have been locally described using morphology, phenology, isozyme patterns, and resistance to control agents, but studies comparing invasions on different continents and determining exact origins of invasive genotypes do not exist or are lacking in detail. Commonly available molecular tools and bulk analysis capacity now make it possible to determine genetic diversity within invasions and their origins. We investigated over 1000 plants from three invaded continents using highly variable AFLP (Amplified Fragment Length Polymorphism) markers, and found 13 distinct genotypes (three from Australia, three from Argentina, and seven from North America). No genotypes were shared between continents. Certain regions in North America, such as California, contain only one genotype of the weed. We then investigated over 1000 plants from the native Eurasian range to determine, as accurately as possible, origins of the invasive genotypes, including those that are currently resistant to strains of rust (*Puccinia chondrillina* Bubak & Syd.) used in biological control programs. This information can be used to screen for pathogens and other agents that will not be resisted or tolerated by certain rush skeletonweed genotypes. Understanding global intraspecific diversity and exact origins can improve management of differentially-resistant/tolerant weed biotypes, enhance efficacy of future agent selection, and increase cooperation between invaded regions.

Comparing the Population Biology of *Isatis tinctoria* in its Native Eurasian and Introduced North American Range under Different Experimental Treatments

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Abstract

Isatis tinctoria L. is an annual, biennial, or short-lived perennial mustard native to Eurasia. The weed was culturally introduced to North America where it has naturalized and become a noxious weed in several western US states. The goals of this study are to identify the sensitive phenostages of *I. tinctoria* to improve management strategies for the invasive plant and to identify most effective biological control agents. Permanent study plots were established in 2010 at an experimental and a close by natural infestation of *I. tinctoria* in southeastern Idaho. In Europe, the experimental site was established in 2008 in southern Germany. The study site in Germany includes the following treatments: Presence and absence of 1) interspecific competition and 2) biological control insect herbivory. The experimental site in Idaho includes presence and absence of 1) interspecific competition and 2) the native rust pathogen (*Puccinia thlaspeos* Schub.), as treatments. Established with each experimental site were seed bank plots with presence and absence of interspecific competition and seed longevity experiments. In both ranges, interspecific competition had so far the strongest and most consistent effect on plant numbers. In Europe, specific insect herbivores negatively impacted individual plant parameters. We hope that the continuation of the study will allow us to demonstrate effects of treatments on life stages of *I. tinctoria*. Results will ideally help prioritizing candidate insect biological control agents currently studied in the native range and assessing the net effect of the native rust present in the introduced range.

Invasive Exotic Plant Species in Tennessee, USA: Potential Targets for Biological Control

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Abstract

Numerous invasive exotic plant species are well established in Tennessee, located in the southeastern United States, where many of these plant species pose serious economical and environmental threats to agriculture, forestry, natural areas, and urban areas. The threat of some invasive plant species, such as musk thistle (*Carduus nutans* L.), multiflora rose (*Rosa multiflora* L.), and kudzu (*Pueraria montana* var. *lobata* (Willd.) Maesen and S. Almeida), is well documented, and these species are well recognized by growers and landowners. Management programs, primarily focused on chemical, mechanical and cultural controls, have been developed to limit the spread of these exotic plant species, and biological control is a major component of integrated pest management of musk thistle. However, the threat of other species, such as purple loosestrife (*Lythrum salicaria* L.), spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos*), and Canada thistle (*Cirsium arvense* (L.) Scop.), in Tennessee is not as well known, and growers and landowners are not as familiar with these exotic species. In most cases, the state-wide distribution of many of these invasive plant species has not been clearly defined. In addition, the diversity of native and exotic insect herbivores that utilize many of these introduced plant species has not been investigated. Biological control will be explored as a management tactic against selected exotic weeds based upon the 'best fit' for the climate and geography of Tennessee. This poster details invasive exotic plant species established in Tennessee and examines the potential for the integration of biological control into pest management programs directed against selected weed species.

Genetic Variation in a Biological Control Target Weed: The Strawberry Guava Species Complex

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Abstract

Our objective is to characterize the genetic variation in strawberry guava populations in Hawaii, with the goal to inform biological control efforts currently being developed to counter this invasive species complex in native forests. Specimens collected on the islands of Hawaii, Maui, and Oahu were evaluated for fruit and vegetative morphology, ploidy as determined by flow cytometry, and microsatellite variation at three chloroplast SSR loci and three nuclear SSR loci. Results supported three previously recognized taxa and one new category. *Psidium littorale* Raddi was uniform with regard to fruit morphology (yellow, spindle-shaped), ploidy (8x), and SSR polymorphisms, suggesting that it may be a fertile allo-octoploid or a sterile apomict. Similarly, *P. lucidum* Hort. was uniform in fruit morphology (yellow, spherical fruits) and SSR genotype, but showed minor ploidy variation about 6x, suggesting a mostly fertile allo-hexaploid or an apomict with some residual sexual function. *P. cattleianum* Afzel. ex Sabine displayed a single uniform chloroplast and nuclear SSR genotype, but ploidy variation between 6.5x and 7.1x, and red fruit color of variable hue and intensity, suggesting that sexual reproduction is operative in this nominally heptaploid form and that it produces mainly aneuploid progeny. A fourth form (*Psidium* "X") with fruit color (orange) and ploidy range (6.4x to 6.8x) intermediate between those of *P. lucidum* and *P. cattleianum* originally suggested derivation through interspecific sexual crossing or possibly elimination of genetic material in the aneuploid sexual progeny of hybrids or of self- or sib-mated *P. cattleianum*. However, the presence of a unique chloroplast SSR allele found in the orange-fruited forms and not in either of the putative parent species indicates that it is not recently of hybrid origin or directly derived from *P. cattleianum*. The orange-fruited form represents a new taxon not previously described in Hawaii. The SSR uniformity within the four strawberry guava taxa may reflect predominantly apomictic seed production, or simply that our survey employed an inadequate number of marker loci to detect polymorphisms. This apparently modest level of genetic variation may suit the strawberry guava complex in Hawaii to target status for a host-specific biological control agent, such as *Tectococcus ovatus* Hempel.

Demographic Matrix Model for Swallow-Wort (*Vincetoxicum* spp.)

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Abstract

Demographic matrix modeling of plant populations can be a powerful tool to identify key life stage transitions that contribute the most to population growth of an invasive plant and hence should be targeted for disruption (weak links) by biological control and/or other control tactics. Therefore, this approach has the potential to guide the selection of effective biological control agents. We are in the process of parameterizing a five life-stage matrix model in order to generate pre-release agent recommendations for the swallow-wort biological control program. Pale swallow-wort (*Vincetoxicum rossicum* (Kleo.) Barb.) and black swallow-wort (*V. nigrum* L.) are herbaceous, perennial, viny milkweeds introduced from Europe (Apocynaceae-subfamily Asclepiadoideae). Both species are becoming increasingly invasive in a variety of natural and managed habitats in the northeastern United States and southeastern Canada. Black swallow-wort appears restricted to higher light environments, whereas pale swallow-wort infestations occur from the high light environments of open fields to low light forest understories. We are quantifying demographic transitions over 3-4 years of both swallow-wort species in field and, for pale swallow-wort, forest habitats in New York State (N = six populations). Vital rates estimated include seed survival, germination, plant survival to reproductive maturity, and fecundity (viable seeds produced per plant). Data will be presented on model parameters derived to date.

How Many Species of *Salsola* tumbleweeds (Russian Thistle) Occur in the Western USA?

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Abstract

Russian thistle or common tumbleweed, *Salsola tragus* L. (sensu lato), is an alien weedy annual plant that infests over 41 million hectares in the western United States. The taxonomy of this plant has had a long confusing history, with frequent misapplication of the species names *kali* and *australis*. Recent studies based on morphology, allozymes and molecular genetics indicate that “Russian thistle” comprises seven distinct species in North America. *Salsola tragus* is probably the most widespread species. *Salsola collina* Pall. occurs primarily east of the Rocky Mountains, *S. paulsenii* Litv. primarily in deserts, and *S. kali* is restricted to ocean shores and is not a rangeland weed. *Salsola australis* R., sometimes reported as “type B”, occurs primarily in California, South Africa and Australia, but has never been documented to occur in Eurasia. Almost all uses of this name before 2008 are probably misapplications. Polyploid hybrids include *S. x gobicola* (includes *S. tragus* and *S. paulsenii* ancestry), which is known from western USA and central Asia, and *S. x ryanii* (includes *S. tragus* and *S. australis* ancestry), which is known only from California. A gall forming midge, *Desertovelum stackelbergi* Mamaev, from Uzbekistan (Sobhian *et al.* 2003. Biol. Control 28: 222-228) and a fungal pathogen, *Colletotrichum gloeosporoides* (Penz), from Hungary (Bruckart *et al.* 2004. Biol. Control 30: 306-311) had much higher rates of attack and damage to *S. tragus* than *S. australis*. Although it was previously believed that all species in the *kali* section of *Salsola* originated in Eurasia, the presence of 4 indigenous species in Australia suggests a separate clade (Borger *et al.* 2008. Aust. J. Bot. 56: 600–608). It is likely that *S. australis* is native to Australia.

An Initial Focus on Biological Control Agents for the Forest Invasive Species *Prosopis juliflora* in the Dry Zone of Myanmar

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Abstract

Prosopis juliflora (Sw.) DC (*Prosopis* hereafter) was introduced into Myanmar for dry zone greening about 1950 from Israel by Agriculture Research and Development Cooperation (ARDC). *Prosopis* is an aggressive weed of forests in the dry zone of Myanmar. Though regarded useful for fuel wood for rural people, this deep rooted species forms thickets, outcompetes native vegetation, has hard and sharp thorns. It is also capable of vegetative reproduction and produces many coppices when cut, and is hence difficult to eradicate. Its distribution is expanding and control is needed in some areas. Investigation for biological control agents were conducted in Pyawbwe in January, 2010. *Prosopis* appeared to struggle to compete with the climber *Combretum roxburghii* D. Don, and the shrubs *Azima sarmentosa* Benth. and *Lantana camara* L. The use of these plants as competitors to suppress *Prosopis* growth is not desirable as *Prosopis* has benefits for rural people, while these competitors do not. Damage to *Prosopis* was detected in the form of yellowing foliage and damage from pathogens around the cuts made to the woody tissue during harvest of fuel wood. *Fusarium* sp., *Tubercularia* sp. and *Nectria* sp. were identified from these damaged trees. Small-scale trials have been initiated to examine the potential for these fungal pathogens to aid in the biological control of *Prosopis*.

Potential for the Biological Control of *Crassula helmsii* in the U.K.

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Abstract

Crassula helmsii (Kirk) Cockayne, also known as Australian swamp stonecrop or New Zealand pigmyweed, is native to Australia and New Zealand as the common names suggest. Since being introduced to the UK in 1911 as an 'oxygenating' plant for garden ponds, it has been gradually increasing its range both in the U.K. and parts of Europe by escaping gardens and through incorrect disposal by aquarium and pond owners. It has now spread to at least 2000 sites in the UK, particularly threatening conservation sites that are home to rare and endangered organisms. With no dormant period and a high tolerance to a range of temperatures, it can dominate static and slow moving water bodies, as well as bank sides, growing in dense mats as an emergent, submerged or terrestrial form. Once established its impacts can be serious; affecting native biodiversity and impeding water flow. With limited possibilities for chemical control in the EU and the plant's ability to re-grow from fragments as small as 1cm, this weed is particularly difficult to manage. An estimation of the cost of treating 2000 infested sites was estimated to be between €5.8 - 12 million. Little is known of the natural enemy complex on *C. helmsii* populations in the native range, so CABI and collaborators initiated scoping surveys in New Zealand and Australia in 2009. Despite the limited nature of the initial surveys, considerable pathogen and herbivore damage were observed, revealing an assortment of natural enemies associated with this weed. The discovery of two highly damaging stem-mining weevils that were previously unrecorded, suggests more species may be identified in the native range in following future surveys. The discovery of additional natural enemies, to those specified in the literature, bodes well for the future biological control of this species.

The Road Less Taken: A Classical Biological Control Project Operated Through an NGO

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Abstract

Historically, most classical weed biological control projects have been initiated and managed by government departments or agencies. We have successfully developed a project along an alternative route, where the main sponsor is a non-governmental organization. The Alberta Invasive Plants Council (AIPC) was established in 2006 to raise the awareness of invasive plants as a problem in Alberta and to promote cooperative efforts to manage these plants in the province. Later that year, AIPC obtained funding to start a biological control project against common tansy (*Tanacetum vulgare* L.), a toxic, perennial weed native to Europe and Asia which is spreading rapidly in pastures and riparian areas in western Canada and the northern USA. This is a joint US-Canadian project, with funding from a number of state, provincial, federal, and industry sponsors in both countries. The US funding is coordinated by the Minnesota Department of Agriculture, and most Canadian funding is handled through AIPC. The CABI laboratory in Delémont, Switzerland, conducts exploration and agent testing under contract with AIPC, and a private consultant (the first author) coordinates the project, also under contract with AIPC. The project coordinator prepares proposals for ongoing funding, reports on progress to project sponsors, promotes public awareness of the project, and works with CABI to select agents for study and oversee the project. AIPC provides administrative services to the project including contract management, billing, and accounting. An annual consortium meeting is held to review progress and plan future work. This approach has allowed us to enhance the existing Canadian capacity for managing overseas biological control research.

A Reassessment of the Use of Plant Pathogens for Classical Biological Control of *Tradescantia fluminensis* in New Zealand

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Abstract

Tradescantia fluminensis Vell. is an herbaceous plant of the family Commelinaceae, native to South America, and commonly known as small-leaf spiderwort. In 1910, it was introduced into New Zealand and became an aggressive invasive weed in natural ecosystems, causing serious environmental imbalances: in particular, preventing the regeneration of native species, and thus undermining the local biodiversity. The problem caused by *T. fluminensis* quickly led to the use of conventional control measures, but without success. As a result, biological control is considered to be the most promising alternative to mitigate the problems generated by *T. fluminensis*. Since 2003, surveys have been conducted in search of plant pathogens in south and southeastern Brazil. In the first phase of surveys, five new fungal species were added to the known pathogenic mycobiota of *T. fluminensis*: *Ceratobasidium* sp., *Cercospora apii* Fresen., *Kordyana* sp., *Uredo* sp. and *Mycosphaerella* sp. In 2009, a second phase of surveys was performed, and resulted in the addition of four more species of pathogenic fungi to the list: *Colletotrichum falcatum* Went, *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc. and *Septoria* sp. During this period, *Kordyana* (Brachybasidiaceae = Exobasidiales) - commonly associated with species of Commelinaceae, causing symptoms equivalent to those of white smuts such as *Entyloma ageratinae* Barreto and eVans, the successful biological control agent of *Ageratina riparia* in Hawaii and New Zealand, was assessed to have the most potential, based on field data. Subsequently, pathogenicity tests were conducted to evaluate its potential use as a biological control agent. Besides confirmation of its infectivity to *T. fluminensis*, its specificity to this target species was also demonstrated, among the 70 plant species that were tested. Based on these results, further studies are in progress to clarify the identity of *Kordyana* sp. and to develop a protocol for handling the introduction of the pathogen into New Zealand.

European Insects as Potential Biological Control Agents for Common Tansy (*Tanacetum vulgare*) in Canada and the United States

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Abstract

Common tansy (*Tanacetum vulgare* L., Asteraceae), an herbaceous perennial native to Europe, was introduced into North America as a culinary and medicinal herb. Now widely naturalized in pastures, roadsides, waste places, and riparian areas across Canada and the northern USA, tansy is also spreading in forested areas. It contains several compounds toxic to humans and livestock if consumed, particularly α -thujone, and is listed as a noxious weed in several states and provinces. A biological control program for common tansy is being coordinated by a Canadian-US consortium led by the Alberta Invasive Plant Council and the Minnesota Department of Agriculture, with CABI Switzerland Centre identifying and testing potential agents for efficacy and host specificity. Collection efforts are focused on Eastern Europe (Russia and Ukraine) to maximize the climatic match with the infested areas in North America. Several potential agents are under study, the most promising agent at present being a stem-mining weevil, *Microplontus millefolii* (Schltz.). A root-feeding flea beetle, *Longitarsus noricus* Leonardi, also shows promise, and DNA barcoding is being used to separate this species from morphologically similar species that may emerge as contaminants in host-specificity tests. The leaf-feeding tortoise beetle *Cassida stigmatica* Suffr. is specific to *Tanacetum* but is able to complete development on the North American native *T. bipinnatum* ssp. *huronense* (Nutt.) Breitung; further evaluation of the risk to this species is needed. Life history studies on a stem-mining moth, *Isophrictis striatella* (Denis & Schiffermüller), suggest that it develops mainly in the previous year's dead stems. This may reduce its potential impact as a biological control agent. The effects of chemical and genetic variation in tansy on the feeding and oviposition responses of insects are being studied, and molecular methods are also being used to evaluate the relationships between *T. vulgare* and other species.

The Potential for the Biological Control of Himalayan Balsam Using the Rust Pathogen *Puccinia* cf. *komarovii*: Opportunities for Europe and North America

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Abstract

Himalayan balsam (*Impatiens glandulifera* Royle) is a highly invasive annual herb, native to the western Himalayas, which has spread rapidly throughout Europe, Canada and the United States since its introduction as a garden ornamental. The plant can rapidly colonize riparian systems, damp woodlands and waste ground where it reduces native plant diversity, retards woodland regeneration, outcompetes native plants for space, light and pollinators and increase the risk of flooding. Current control methods are fraught with problems and often unsuccessful due to the need to control the plant on a catchment scale. Since 2006, CABI and our collaborators have surveyed populations of Himalayan balsam throughout the plants native range (the foothills of the Himalayas, Pakistan and India) where numerous natural enemies have been collected and identified. Agent prioritization, through field observations and host range testing has narrowed the potential candidates down to the rust pathogen, *Puccinia* cf. *komarovii* Tranzschel. This autoecious, monocyclic pathogen shows great promise, not only due to its impact on the host but also due to its high specificity as observed in the field and preliminary host range testing. The aecial stage infects the hypocotyl of young seedlings as they germinate through leaf litter containing teliospores. This initial infection severely warps the structure of the developing plant. The aeciospores then infect developing leaves to produce the cycling phase (uredia). This severely affects the photosynthetic capacity of the maturing plant, with the potential of reducing seed-set. Late in the season, teliospores are produced which overwinter in the leaf litter. This paper will review the research conducted to-date, including a molecular comparison of *P. cf. komarovii* with other closely related species, the life cycle and infection parameters of the rust and an up-date on the current host specificity testing under quarantine conditions in the UK.

The Scotch Broom Gall Mite: Accidental Introduction to Classical Biological Control Agent?

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Abstract

The gall mite, *Aceria genistae* (Nal.) Castagnoli s.l., an accidentally introduced natural enemy of Scotch broom (*Cytisus scoparius* (L.) Link), was first discovered in the Portland OR and Tacoma WA region in 2005. It has since been reported from southern British Columbia to southern Oregon. Observationally, the mite appears to reduce Scotch broom seed production and at high densities can cause extensive stem die-back and plant mortality. In order to utilize the mite as a classical biological control agent, a study of its host range and potential nontarget attack was started in 2006 and continued in 2008-2010. Over 20 ecologically- and economically-valued species were tested in greenhouse and open-field studies. Surveys of confamilial nontarget plant species naturally co-occurring with mite-infested Scotch broom were also assessed. Mites and gall formations were noted on hybrids and ornamental species of Scotch broom. Under greenhouse tests, gall-like growth and eriophyid mites were found on *Lupinus densiflorus* Benth. (*L. microcarpus*), a species listed as endangered in Canada. One unidentified eriophyid mite and no deformed growth was detected on *L. densiflorus* at naturally occurring populations growing sympatrically with mite-infested Scotch broom on Vancouver Island. The ambiguous taxonomy of the mites found on Scotch broom, gorse (*Ulex europaeus* L.) and *L. densiflorus* has added further complications to the study. The overall project and plans for developing a petition for its approval as a biological control agent will be discussed.

The Impact of the Milfoil Weevil *Eubrychius velutus* on the Growth of *Myriophyllum spicatum* and Other Watermilfoils Native to Europe

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Abstract

Eurasian milfoil *Myriophyllum spicatum* L. (Haloragaceae) is an aquatic submerged macrophyte that has become invasive in several countries outside its native range. In the United States a native weevil *Euhrychiopsis lecontei* (Dietz) (Coleoptera: Curculionidae) is being used as a natural enemy of *M. spicatum*. In Europe, a similar aquatic weevil *Eubrychius velutus* Beck develops on several native milfoil species. Impact studies were conducted on three milfoil species including *M. spicatum* to assess the growth impact of the largely leaf-feeding habit of *E. velutus*. The results indicate that the adult and larval damage significantly reduces the growth rate of plants, even at low beetle densities. Although it may not be specific enough to release in regions that have closely related native milfoils, *E. velutus* may be utilized as a candidate agent elsewhere in the world. The implications of the levels of damage are discussed and compared with the damage characteristics of *E. lecontei*, informing the potential use of the weevil as a classical biological agent.

Field Explorations in Anatolia for the Selection of Specific Biological Control Agents for *Onopordum acanthium* (Asteraceae)

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Abstract

Scotch thistle, *Onopordum acanthium* L. (Asteraceae), is a biannual thistle of Eurasian origin, accidentally introduced in North America in the late 1800s. It occurs in most of the western states especially in rangelands, pastures and disturbed soils. When abundant, it reduces forage availability for livestock and wildlife. Western Europe has previously been explored for prospective biological control agents, and seven species of insects have been released in Australia. However, host specificity requirements in N. America are more stringent for these agents because of the presence of many native *Cirsium* species. Some of the agents released in Australia do not appear to be sufficiently specific to use in N. America. Therefore we decided to conduct foreign exploration further east in areas not previously explored. Since 2007 we conducted several explorations and survey trips to discover new potential biological control agents in Turkey. Among the most promising candidates are three weevils *Larinus latus* (Herbst.), *L. gigas* Petri and *L. grisescens* Gyllenhal that were collected in Central Anatolia. The larvae of these species develop in the flowerheads and destroy most seeds. Other candidates are *Psylliodes* cf. *chalconera* Illiger and *Lixus cardui* Olivier, whose larvae develop inside stems and leaves. Preliminary laboratory host specificity tests show a narrow host range of some biotypes of both potential biological control agents. Specimens from these experiments are currently undergoing genetic and morphological study to understand if there are distinct genetic entities not distinguishable by morphological traits. A new species of eriophyoid mite, *Aceria* sp., was recorded for the first time in the vicinity of Isparta, Western Turkey associated with the target weed. It causes stem atrophy and flower bud abortion with a consequent decrease of seed production. An unidentified nematode species causing visible blisters on Scotch thistle leaves was found in Central Turkey.

Potential Biological Control of Invasive Tree-of-Heaven (*Ailanthus altissima*)

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Abstract

The highly invasive tree-of-heaven, *Ailanthus altissima* (Mill.) Swingle, was introduced into Pennsylvania (PA), USA, in 1784 and has since spread across PA and most of the USA. Wherever it is found, *Ailanthus* often dominates a site at the expense of native plant species. However, in 2002-2003, we discovered several stands of dead and dying *Ailanthus* trees within oak-dominated, mixed-hardwood forests in south-central PA. Isolations from symptomatic *Ailanthus* seedlings, root sprouts, saplings, and canopy trees in the field consistently yielded the naturally occurring, soil-borne, wilt fungus *Verticillium albo-atrum* Reinke & Berthold. Identification was based on morphological characteristics, and confirmed using molecular techniques. Potted *Ailanthus* seedlings in the greenhouse and canopy *Ailanthus* trees in the field were inoculated with a randomly selected isolate of *V. albo-atrum*. Classic wilt symptoms quickly developed on inoculated plants, from which *V. albo-atrum* was recovered, fulfilling Koch's Postulates and illustrating that *V. albo-atrum* was highly virulent on *Ailanthus*. As of 2011, *V. albo-atrum* has killed thousands of canopy *Ailanthus* trees in south-central PA. Intermingled non-*Ailanthus* tree species and understory shrubs have been generally unharmed. We hypothesize that a naturally occurring strain of *V. albo-atrum* has become host-adapted to *Ailanthus*. As part of risk assessment, we inoculated more than 80 non-*Ailanthus* species (potted seedlings in the greenhouse, as well as trees in the field) with a randomly selected strain of *V. albo-atrum*. Inoculated non-*Ailanthus* species were generally unharmed, again indicating that a pathogenic strain of *V. albo-atrum* may have become host-adapted to *Ailanthus*. In addition, past forest management practices in the area (e.g., clear-cutting large blocks of oaks killed by insect infestations) favored development of dense, nearly monoculture stands of clonal *Ailanthus*, which in turn. Short-range dissemination of *V. albo-atrum* within infected stands of *Ailanthus* likely occurs *via* intraspecific root grafts between diseased and healthy trees within these dense stands. Long-range dissemination of *V. albo-atrum* may be facilitated by *Euwallacea validus* (Eichhoff), an introduced ambrosia beetle that is epidemic on *Ailanthus* trees under stress from *V. albo-atrum* infections.

***Abrostola clarissa* (Lepidoptera: Noctuidae), a New Potential
Biological Control Agent for Invasive Swallow-Worts,
Vincetoxicum rossicum and *V. nigrum***

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Abstract

Pale and black swallow-worts (*Vincetoxicum rossicum* (Kleopow) Barbar. and *V. nigrum* (L.) Moench; Apocynaceae, subfamily Asclepiadoideae), perennial vines native to Eurasia, are now invading natural and anthropogenic habitats in the northeastern U.S.A. and southeastern Canada, threatening natural biodiversity and increasing control costs for land managers. Chemical and mechanical methods have not been adequate to control swallow-worts. In addition, no local American herbivores or pathogens cause significant damage to these weeds. Several potential biological control agents associated with *Vincetoxicum* spp. in Europe have been found and investigated, but none of them have yet been introduced. During explorations for herbivorous insects feeding on *Vincetoxicum* species in the Russian North Caucasus, we discovered a new potential biological control agent, *Abrostola clarissa* Staudinger (Lepidoptera, Noctuidae). *A. clarissa* inhabits low mountains and dry hills, having 1 – 2 generations per season. The biology of this species is similar to that of the closely related *A. asclepiadis*: eggs are laid on the undersurface of the host plant leaves, and larvae feed on the foliage and pupate in the soil. In natural conditions, larvae of this noctuid moth were collected only on *Vincetoxicum* spp. No-choice tests conducted under laboratory conditions showed that larvae of *A. clarissa* voluntarily fed and successfully pupated on *Vincetoxicum nigrum*, *V. rossicum*, *V. hirundinaria* (L.) Pers., and *V. laxum* (Bartl.) C.Koch. Neither feeding nor survival was recorded on other Apocynaceae (11 species of *Amsonia*, *Apocynum*, *Asclepias*, and *Cynanchum*) or on plants from other, more distantly related, families (Rubiaceae, Scrophulariaceae, and Convolvulaceae). We conclude that *A. clarissa* can be considered a highly specific potential biological control agent that undoubtedly deserves further study.

Suitability of Using Introduced *Hydrellia* spp. for Management of Monoecious *Hydrilla verticillata*

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Abstract

Two species of introduced leaf-mining flies, *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock, suppress dioecious hydrilla by reducing photosynthesis, thereby impacting biomass production, tuber production, and fragment viability. To determine the flies' suitability for monoecious hydrilla management several studies were conducted. When reared on monoecious hydrilla, *H. pakistanae* survival was reduced by 40 and 26 percent during bioassays and greenhouse rearing, respectively. *Hydrellia* spp. also exhibited a 9 day increase in developmental time when reared on monoecious hydrilla. *Hydrellia* spp. colonization and percent leaf damage between biotypes also differed significantly using small-scale tank experimentation. Initial fly stocking rates were equal, but four weeks after release fly levels and percent leaf damage in dioecious tanks were 5.3 and 2.4 fold higher than on monoecious. Small pond experimentation revealed similar results with fly levels 50-fold higher on dioecious in comparison to monoecious. Most importantly, release of close to 2,000,000 *Hydrellia* spp. at sites on Lake Gaston, NC since 2004 have failed to provide convincing evidence of establishment let alone population increase and impact. Recent anecdotal information from Guntersville Reservoir, AL suggests that shifts from a dioecious hydrilla dominated system to one composed mainly of the monoecious biotype may have been, in part, caused by differential feeding by *Hydrellia* spp. Experiments and field studies conducted since 2004 indicate that the monoecious biotype is not as suitable a host for introduced *Hydrellia* spp. as is dioecious hydrilla. This conclusion is based in part on reduced survival and longer developmental time in bioassay experiments and greenhouse rearing, lower colonization success and population growth in larger outdoor systems, and lack of establishment on Lake Gaston. Underlying mechanisms for such differences are unknown but may be due to overwintering strategies, density of the canopy at the water surface, and lowered nutritional value between the biotypes.

Natural Enemies of Floating Marshpennywort (*Hydrocotyle ranunculoides*) in the Southern USA

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Abstract

The aquatic plant, floating marshpennywort, is invasive to many areas where it has been introduced, including Australia and Europe. Because traditional methods of control are often costly, ineffective, or unsustainable, biological control is being considered as a viable alternative. The first step in initiating a successful biological control program is a survey of natural enemies of the target plant in its home range. Since it is considered native to the United States, floating marshpennywort was surveyed in the Gulf Coast States for insect herbivores and pathogens from 2007-2011. A total of ten insect and five potential pathogenic species were recovered during the surveys. Insects recovered included three weevil (Coleoptera: Curculionidae), two caterpillar (Lepidoptera), and at least one grass fly (Diptera: Chloropidae). Although many of the insect species collected are known to be generalist herbivores, several had unknown diets. The chloropid, *Eugaurax floridensis* Malloch, and nymphalid, *Enigmogramma basigera* (Walker) show the greatest promise as biological control agents because both species are able to cause damage and complete their life cycles on floating marshpennywort. Host specificity testing is needed to determine diet for both species. Potential pathogen genera collected included *Alternaria*, *Pestalotiopsis*, *Colletotrichum*, and *Phoma*. None of the pathogen species collected are likely to be host-specific, but several appear to cause considerable leaf damage to floating marshpennywort and may limit its growth and reproduction, at least to some degree. The potential for these species to be used as mycoherbicides will require further research.

Can We Optimize Native-Range Survey Effort through Space and Time?

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Abstract

Native range surveys are an expensive and time consuming step in biological control projects but are crucial for determination of the arthropod and fungal fauna which provides the basis upon which agent selection can take place. A key challenge when designing and conducting native-range surveys is to decide how best to allocate survey effort through space (the species-native range distribution) and through time (at any one particular site or region). For example, should more effort be spent searching the same sites more extensively or on searching elsewhere, and when should surveying come to an end? *Parkinsonia aculeata* L. is a weed of Australia's rangelands that occurs naturally over a large part of the American tropics and subtropics (USA to Argentina). Native range surveys have been conducted over many years in many parts of this range but in an uneven way with some parts intensively surveyed, some parts minimally surveyed and other parts not surveyed at all. A study commenced in 2008 which used the existing results of surveys to predict where further surveys would be most fruitful. Information used in this study included 1) biogeographic zoning of the Americas according to the insect fauna, 2) a *P. aculeata* climate model, 3) *P. aculeata* species distributions, and 4) the insect fauna collected. We used generalized dissimilarity modelling of *P. aculeata* fauna to provide the data for a technique called survey gap analysis. The survey gap analysis predicted areas which should yield the greatest number of new species. In addition, we used species accumulation curves to predict which existing regions further surveying would yield most new species. We discuss the potential for these and other approaches to improve the allocation of native-range survey effort.

Potential Agent *Psectrosema noxium* (Diptera: Cecidomyiidae) from Kazakhstan for Saltcedar Biological Control in USA

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Abstract

Research was conducted on eight native populations in southeastern Kazakhstan during 1995-2009. This narrow-oligophagous, univoltine, stem-galling, midge fly attacks saltcedar (*Tamarix* spp.) where both are native in Kazakhstan, Turkmenistan, Uzbekistan and western China. It is a candidate for biological control of saltcedars in the western United States and northern Mexico. In nature, the adults of *Psectrosema noxium* Marikovskij emerged from overwintered galls in late April, mated, and laid 1-30 eggs on each of several new foliage buds. The larvae emerged in 3-4 days and immediately entered the same buds. They developed slowly as the buds developed into stems, and formed colonial galls of 1-30 cells visible in early summer that became ligneous by autumn. The larvae pupated in spring in synchrony with *Tamarix* bud-break. Where numerous, they killed branches or even the entire *Tamarix* plant. Outbreak populations were seen only each 5-7 years, reduced between peaks by severe attack by four species of egg and larval parasitoids. In Kazakhstan, we observed galls of *P. noxium* mostly on *T. ramosissima* Ledeb. and sometimes on other saltcedars but never on other genera of Tamaricaceae. In outdoor, uncaged tests at Almaty, females readily accepted accessions of *T. ramosissima* from five U.S. states, for oviposition and the larvae completed their development on it. However, close synchronization between *Tamarix* bud-break and adult midge emergence is critical so that oviposition can take place on just erupted buds. Eggs laid on young shoots produce only undersized galls that fall from the plant by autumn and the larvae die. Females did not oviposit in on athel, *T. aphylla* (L.) H.Karst., a beneficial exotic shade tree in northern Mexico and the U.S., that is not a target for biological control. Methods of culturing *P. noxium* in the laboratory and in field cages were developed if it is introduced into the USA.

Fungi Pathogenic on *Paederia* spp. from Northern Thailand as Potential Biological Control Agents for Skunkvine *Paederia foetida* (Rubiaceae)

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Abstract

The skunkvine *Paederia foetida* L. (known locally as Maile Pilau) is one of the major invasive environmental weeds on the Hawaiian Islands. Due to its rapid spread to remote and inaccessible areas, its ability to cause substantial damage to natural ecosystems and the increasing cost of conventional control methods, its management by biological control seems to be the most appropriate means with high success potential. A survey was conducted in its native range in northern Thailand in the fall of 2010, with the aim of locating and identifying potential agents for classical biological control. Diseased tissues of *Paederia* species exhibiting symptoms of necrotic spots/lesions, galls and rusts were imported into the Hawaii Department of Agriculture's Plant Pathogen Containment Facility (HDOA-PPCF) for evaluation. Most of the infected tissues were derived from *Paederia pilifera* Hook.f., from which several fungi were subsequently isolated. Among these fungi were the gall rust (Basidiomycota) *Endophyllum paederiae* (Dietel) F. Stevens & Mendiola, the Hyphomycetes *Pseudocercospora paederiae* Sawada ex Goh & W.H. Hsieh, and two isolates of the Coelomycetes *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Repeated attempts to establish *E. paederiae* on the local skunkvine *P. foetida* failed, indicating that this rust fungus may be too host specific to infect the local species. However, the remaining fungi *P. paederiae* and isolates of *C. gloeosporioides* were amenable to laboratory culture on standard potato dextrose agar. Separate inoculation tests on the local *P. foetida* plants with conidia from each fungal culture showed leaf lesions or necrotic spots, where the respective fungus could subsequently be reisolated. One of the isolates of *C. gloeosporioides* was relatively aggressive, causing leaf chlorosis, defoliation and even shoot tip dieback on the infected *P. foetida* plants. Further studies on the potentials of these fungi as biological control agents on *P. foetida*, such as their effects on other economic plants (host range), culture and pathogenic enhancements by environmental factors etc., are underway inside the HDOA-PPCF.

Preliminary Surveys for Natural Enemies of the North American Native Delta Arrowhead (*Sagittaria platyphylla*, Alismataceae), an Invasive Species in Australia

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Abstract

The perennial, North American native delta arrowhead (*Sagittaria platyphylla* (Engelm.) J.G.Sm., Alismataceae) was introduced to Australia as an ornamental pond plant in the 1950s. Today, it is a serious invader of irrigation channels and natural waterways in south-eastern Australia with up to \$2 million being spent annually on control in the worst affected regions. Delta arrowhead is free from attack by herbivores and pathogens in Australia, where it seeds prolifically for nearly seven months of the year. Recent preliminary surveys in the southern USA identified a number of curculionids in the genus *Listronotus* associated with delta arrowhead, none previously reported on this host-plant. These weevils and a chloropid fly, *Eugaurax* sp. were all found to attack flowers and fruits. Further surveys are planned to complete the catalogue of natural enemy flora and fauna across the native range. Upcoming genetic studies to identify the genetic origin(s) of the Australian populations will underpin future research on the selection of biological control agents for delta arrowhead.

Prospects for Biological Control of *Berberis darwinii* (Berberidaceae) in New Zealand: What are its Seed Predators in its Native Chilean Range?

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Abstract

A native of South America, Darwin's barberry, *Berberis darwinii* Hook, (Berberidaceae) has become an invasive species in New Zealand. It has invaded many habitat types from grazed pastures to intact forests, due in part to its large reproductive capacity. Consequently, as an early step in a biocontrol solution surveys were conducted in its native range for damaging invertebrates utilizing flower buds and seeds. Sampling for potential agents was conducted at 35 sites in southern Chile between Concepción (36°57' S.) and Chiloé (42° 52' S.). At suitable sites flowering or fruiting plants of *B. darwinii* were beaten 5 times onto a beating tray and all weevil species observed were collected. The insect surveys yielded four weevil species on Darwin's barberry. *Berberidicola exaratus* (Blanchard) was the most common and widely distributed seed predator. It was detected at 29 of the 35 sites. *Anthonomus kuscheli* Clark was the most common flower bud feeder and was detected at 13 of the 35 sites. Damage to the seeds and flower buds by these weevils is obvious. Host-testing studies of these two weevil species is continuing in Chile.

Surveys for Potential Biological Control Agents for *Pereskia aculeata*: Selection of the Most Promising Potential Agents

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Abstract

New biological control agents are required for the control of *Pereskia aculeata* Mill. in order to reduce the weed's density to acceptable levels. Data from eight surveys for natural enemies of *P. aculeata* in the native distribution were used to compile a list of insect species associated with the plant. Sixty-two sites were surveyed resulting in a list of 40 insect species associated with the plant. Six prioritization categories were used to identify the most promising potential biological control agents from the suite of insects associated with the plant. Prioritization categories were i) the presence of feeding damage, ii) insect incidence measured as the number of sites at which the insect species was present divided by the total number of sites, iii) the host range of the insect observed in the field, iv) the similarity of the climate where the insect species was found to the climate at the weed's introduced distribution, v) the similarity of the weed genotype to the genotype on which the insect species developed in the native distribution and vi) the mode and levels of damage in the native distribution. The most promising potential biological control agents for *P. aculeata* identified using the various criteria of the prioritization categories are the released biological control agent, *Phenrica guérini* Bechyné (Chrysomelidae), two species of Curculionidae and *Maracayia chlorisalis* Walker (Crambidae). The method used to prioritize the most promising potential biological control agents for future research may be useful when surveying for natural enemies for use as biological control agents for other weed species.

Predicting the Feasibility and Cost of Weed Biological Control

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Abstract

We reviewed previous work related to selection and prioritisation of weed biological control targets and developed and tested hypotheses regarding which attributes make weeds more or less amenable to biological control. Our analyses revealed that biocontrol impacts have, on average, been greater against, biennial and perennial versus annual weeds, plants capable of vegetative reproduction versus plant that reproduce solely by seed or spores, aquatic and wetland weeds versus terrestrial weeds, and plants that are not reported to be weedy in the native range versus those which are known to be weedy in the native range. We incorporated these criteria affecting biological control success into a feasibility scoring framework that should enable practitioners to better prioritise targets for biological control in the future.

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Abstract

The staff of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Australian Biological Control Laboratory (ABCL) conduct exploration in the natural areas of Australia and Asia for insects and other organisms that feed on pest insects and plant species that are invasive in the USA. Based at the Ecosciences Precinct in Brisbane, Queensland, Australia, the ABCL is operated by the USDA-ARS Office of International Research Programs (OIRP) while personnel and facilities in Australia are provided through a co-operative agreement with the Commonwealth Scientific and Industrial Research Organization (CSIRO). Many invasive weeds in the USA such as the broad-leaved paperbark tree, *Melaleuca quinquenervia* (Cav.) S.T. Blake; Old World climbing fern, *Lygodium microphyllum* (Cav.) R.Br.; hydrilla, *Hydrilla verticillata* (L.f.) Royle; and Australian pine, *Casuarina* spp. are native to Australia. However, the native distribution of many of these weed species extends into tropical and subtropical Southeast Asia, including Indonesia, Malaysia, Thailand, Vietnam, Papua New Guinea, India and southern China, as well into the Pacific Islands. With excellent collaborators in these regions, ABCL has the capability to explore these countries to find the most promising biological control agents for these and other targets, and evaluate them under quarantine conditions in Brisbane. Research conducted at ABCL includes determination of the native range of a target, exploration for natural enemies, molecular typing of herbivores, ecology of the agents and their hosts, field host-range surveys and ultimately preliminary host-range screening and impact assessments of candidate agents. The data we gather on potential agents is combined with information about the ecology of the target where it is invasive. Our research seeks to determine what regulates the target in its native environment and evaluates all potential biological control agents particularly those that can mitigate the weed's impact. Organisms with a narrow host range and good regulatory potential are prioritized and intensively investigated. In collaboration with US-based ARS scientists, agents are selected and shipped to the United States for further quarantine studies and possible release.

Potential Biological Control Agents of Skunkvine, *Paederia foetida* (Rubiaceae), Recently Discovered in Thailand and Laos

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Abstract

The skunkvine, *Paederia foetida* L., also known as Maile pilau in Hawaii, is an invasive weed that smothers shrubs, trees, and native flora in dry to wet forests. It disrupts perennial crops and takes over landscaping in moist to wet areas on four Hawaiian Islands. Skunk-vine is considered a noxious weed in southern United States (Alabama and Florida) and also an aggressive weed in Brazil, New Guinea, Christmas and Mauritius Islands. Chemical control is difficult without non-target damage as the vine mixes up with desirable plants. Biological control is thought to be the most suitable option for long term management of the weed in Hawaii and Florida. Skunk-vine and most species of genus *Paederia* are native to tropical and subtropical Asia, from as far as India to Japan and Southeast Asia. There are no native plants in the tribe Paederieae in Hawaii and Florida and the potential for biological control looks promising. A recent survey in October-December 2010, after the rainy season in Thailand and Laos, confirmed the presence of several insect herbivores associated with *P. foetida* and three other *Paederia* species. A leaf-tying moth (Lepidoptera: Crambidae), two hawk moths (Lepidoptera: Sphingidae), a herbivorous rove beetle (Coleoptera: Staphylinidae), a chrysomelid leaf beetle (Coleoptera: Chrysomelidae), a sharpshooter leafhopper (Hemiptera: Cicadellidae), and a leaf-sucking lace bug (Hemiptera: Tingidae) were the most damaging to the vine during the survey period. The beetles are being investigated at the HDOA Insect Containment Facility as potential candidates for biological control of Maile pilau in Hawaii. Initial findings on host specificity, biology, and their potential for suppressing this weed are discussed.

Towards Biological Control of Swallow-Worts: The Ugly, the Bad and the Good

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Abstract

Native to Eurasia, swallow-worts (“the ugly:” *Vincetoxicum rossicum* (Kleopov) Barbarich and *V. nigrum* L. - Apocynaceae) have invaded forested landscapes and prevented native plant regeneration in eastern North America. We first aimed to understand where the invasive populations of both species come from, and then evaluated the impact of potential biological control agents (BCAs). The following phytophagous BCAs have been studied since 2009: *Chrysochus asclepiadeus* (Pallas) (Col., Chrysomelidae), *Abrostola asclepiadis* (Denis & Schiffermuller) and *Abrostola clarissa* (Staudinger) (Lep., Noctuidae). Adults of the beetle feed on leaves while larvae are root feeders, and *Abrostola* spp. larvae are foliage feeders. Genetically, none of the native *V. nigrum* populations analyzed to date possesses exactly the major multilocus genotype detected in the invasive North American populations, in contrast to *V. rossicum*, for which source populations of the invasion are found to be in Ukraine. We performed choice and no-choice specificity tests with French and Russian populations of *C. asclepiadeus*. We evaluated adult herbivory in no-choice tests on three *Vincetoxicum* spp., as controls, and seven *Asclepias* spp.: *C. asclepiadeus* fed on controls but also on *Asclepias tuberosa* L., a monarch butterfly host plant. Choice tests revealed no herbivory outside the genus *Vincetoxicum*. Larval herbivory in choice tests was noticed on all controls, plus *A. tuberosa* and *Asclepias syriaca* L. Similar results were obtained for both populations of *C. asclepiadeus*. Although *C. asclepiadeus* has a severe impact on swallow-worts, herbivory on several *Asclepias* spp. lead us to consider it a “bad” BCA. No-choice tests with larvae of *Abrostola asclepiadis* from France revealed that they died in 5d on all the *Asclepias* spp., but developed to pupa in 23d on *Vincetoxicum hirundinaria* Medik., in 20.6d on *V. rossicum*, and only reached the 3rd instar in 17.8d on *V. nigrum*. Similar results were obtained with *Abrostola clarissa* of Russian origin. Thus, data with *Abrostola* spp. appear promising, and we consider the two *Abrostola* species to have good potential as BCAs against all genotypes of swallow-worts.

Genetic and Behavioral Differences among Purported Species of *Trichosirocalus* (Coleoptera: Curculionidae) for Biological Control of Thistles (Asteraceae: Cardueae)

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Abstract

Trichosirocalus horridus (Panzer) was introduced to North America, New Zealand and Australia for biological control of *Carduus nutans* L. Since then two more species of *Trichosirocalus* have been described (Alonso-Zarazaga and Sánchez-Ruiz. 2002. Aust. J. Entomol. 41: 199-208), and the three species are thought to have different host plant associations: *T. horridus* on *Cirsium vulgare* (Savi) Ten. and possibly on other Cardueinae, *T. mortadelo* Alonso-Zarazaga & Sanchez-Ruiz on *C. nutans*, and *T. briesei* Alonso-Zarazaga & Sanchez-Ruiz on *Onopordum* spp. This raises the question of which species were previously released for biological control of *C. nutans*. Subsequent studies by Groenteman et al. (2009, XII International Symposium on Biological Control of Weeds, pp. 145-149.) raises uncertainty about which species are in New Zealand and whether *T. mortadelo* was a valid species. *Trichosirocalus briesei* was introduced to Australia to control *Onopordum* spp. and is being evaluated for introduction to North America. We analyzed part of the mtDNA cytochrome oxidase I (COI) gene sequence of adult specimens representing the three species collected in Spain, Italy, USA, New Zealand and Australia. All specimens morphologically identified as *T. briesei* formed one clade that was clearly distinct from all the other specimens. The COI sequences for specimens of *T. horridus* and *T. mortadelo* were intermixed within the same clade, suggesting that they represent one heterogeneous species. Furthermore, the morphological characters attributed to *T. mortadelo* are of little significance to really isolate two different species, so that we combine them under the name of *T. horridus*. In laboratory choice experiments, specimens from Spain identified as *T. briesei* preferred *Onopordum acanthium* L. to *Carduus* or *Cirsium* spp., whereas those from North America, identified as *T. horridus* preferred *Carduus* spp. but also attacked *Cirsium* spp.

Survey of Dispersal and Genetic Variability of *Tectococcus ovatus* (Heteroptera: Eriococcidae) in the Regions of Natural Occurrence of *Psidium cattleianum* (Myrtaceae)

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Abstract

The species *Psidium cattleianum* L. is considered one of the greatest threats to the ecosystem and biodiversity of the islands of Hawaii. Seeking to control its dissemination, techniques of biological control were used. Among the various species studied, as a biological agent control, *Tectococcus ovatus* Hempel showed a higher level of specificity. This work had as aim to verify the existence of genetic variability among and inside the different populations of *T. ovatus*, using the technique of PCR-RAPD. The analyses were made from females collected in the states of Rio de Janeiro, Paraná, Santa Catarina and Rio Grande do Sul in Brazil. From the eight initiators of PCR-RAPD tested, four were used in the analyses, revealing monomorphic and polymorphic markers with a variable frequency, to the individuals of one place as well as to the individuals of different places. Through the analysis of the grouping of molecular characterization it was possible to verify a formation of two distinctive groups A and B presenting a genetic variability/variable of 44%. The results obtained through the analysis of markers RAPD were useful in the verifying of variation and provided safe information about the levels of variability and similarity amongst and inside the different populations of *T. ovatus*.

***Arundo donax* – Giant Reed**

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Abstract

Arundo donax L., giant reed, carrizo cane, is an exotic and invasive weed of riparian habitats in the southwestern U.S. and northern Mexico. Giant reed dominates these habitats, which leads to: loss of biodiversity; stream bank erosion; damage to bridges; increased costs for chemical or mechanical control along irrigation canals and transportation corridors; and impedes access for law enforcement personnel. Most importantly this invasive weed competes for water resources in an arid region where these resources are critical to the environment, agriculture and urban users. Biological control using insects from the native range of giant reed may be the best option for long-term management. *A. donax* is a good target for biological control because it has no close relatives in North or South America, and several of the plant feeding insects from Mediterranean Europe and known to only feed on *A. donax*.

Foreign Exploration for Biological Control Agents of Giant Reed, *Arundo donax*

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Abstract

Collections of insect-infested *Arundo donax* L. have been made 2000 - 2011 by scientists at the USDA/ARS EBCL facility in Montpellier, France. Pieces of *A. donax* stem, leaves and rhizomes were placed in double wrapped paper bags and kept in tight mesh covered boxes in the EBCL insect quarantine. Emergence of insects was noted and specimens sent to relevant authorities for identification. Samples were taken at the same time for genetic characterization of *A. donax*. Surveys have been made in appropriate areas of Croatia, Bulgaria, Slovenia, France, Spain, Portugal, Canary Isles, Italy, Greece, Crete, Turkey, Morocco, Tunisia, Egypt, South Africa, Namibia, Kenya and Australia. Surveys were in fall and spring to cover the most important growth periods. Site details, locality, altitude, GPS position were recorded. Giant reed rhizomes were unearthed and dissected at some sites for natural enemies. Lengths of rhizome and cut stems and leaves were placed in moisture absorbent bags, cooled, and returned to the EBCL quarantine for emergence. Quadrats (50x50cm) of *A. donax* have been sampled from 6 stands each week for 15 weeks (starting May 5 2003 in 2004 and 2005) in the Montpellier and Perpignan areas of southern France. All *A. donax* within the quadrats was cut, taken back to the laboratory, examined, dissected and documented. Organisms found were where possible reared and adults passed on to appropriate taxonomists. The arthropod herbivores collected from *A. donax* were (in order of most to least common) *Tetramesa romana* Walker (Hymenoptera: Eurytomidae); *Rhizaspidotus donacis* (Leonardi) (Hemiptera: Diaspididae); *Cryptonevra* spp. (Diptera: Chloropidae); *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae); *Cerodontha phragmitidis* Nowakowski (Diptera: Agromyzidae); *Melanaphis donacis* (Passerini) (Hemiptera: Aphidae); *Aclerda berlesii* Buffa (Hemiptera: Aclerdidae); *Siteroptes* sp. (Acarina: Pyemotidae); and *Hypogaea* sp. (Hemiptera: Aphididae). Only the first four species were found to be sufficiently host specific to warrant further host range testing.

SESSION 5: PROSPECTS FOR WEED BIOLOGICAL CONTROL IN PACIFIC ISLANDS

Weeds of Hawaii's Lands Devoted to Watershed Protection and Biodiversity Conservation: Role of Biological Control as the Missing Piece in an Integrated Pest Management Strategy

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Summary

Despite Hawaii's reputation as an extinction icon, significant biological resources remain, especially in watersheds, natural areas, and specialized edaphic sites (e.g., lava dry forest, coastal). While direct habitat destruction by humans continues, human-facilitated biological invaders are currently the primary agents of continuing degradation. The ability of invasive plants to have prolific seed production, efficient dispersal systems, and to become established in dense vegetation, complicated by Hawaii's rugged topography, appears to render mechanical and chemical control as mere holding actions. Costly, 'environmentally unfriendly', and often ineffective, strategies using chemical and mechanical control on a large scale, despite the most valiant of efforts, can be viewed simply as attempts to buy time. Without increased levels of safely tested biological control, the seemingly inevitable result is the landscape level transformation of native forests, with potentially catastrophic consequences to cultural, biological, water, and economic resources. Increased levels of effective biological control for certain intractable invasive species appear to comprise a conspicuous 'missing piece' in our efforts to protect Hawaiian watersheds and other conservation lands.

Evolution of Hawaiian Biota

The Hawaiian Islands are comprised of eight major high islands (19-22°N) and scattered small islands stretching northwest to 28°N. Hawaii is a world biodiversity hotspot and much like the Galapagos archipelago provides abundant textbook examples of evolutionary adaptive radiation in isolation, for decades having been a premier study site for evolutionary processes (Givnish et al., 2009; Baldwin and Wagner, 2010; Lerner et al., 2011). Substantial archipelago age, a highly isolated geographical position (ca. 4,000 km) from the nearest continent, and a remarkably wide range of microclimates from dry to very wet (with 200-10,000 mm mean annual precipitation)

are all apparent drivers of the development of Hawaii's renowned biota and perhaps its relative vulnerability to invasions (Loope, 2011). Among animals, honeycreeper birds, drosophilid flies, long-horned and proterhinid beetles, crickets, microlepidoptera, and several groups of land snails are notable radiations, while among plants, the silversword alliance, lobelias, mints and gesneriads are conspicuous radiations. Among the most notable groups are the over 50 species of the extremely diverse Hawaiian honeycreepers evolved from a common cardueline finch ancestor, believed to have arrived from Asia just over 5 million years ago (Lerner et al., 2011). Biodiversity conservation challenges of the islands are perhaps best epitomized by these birds (Pratt et al., 2009), though similar challenges exist for many other endemic plants and animals.

Value of Remaining Natural Areas and Watersheds

Though Hawaii is world renowned for its tremendous losses to biodiversity and natural communities, montane watersheds, natural areas, and specialized edaphic sites such as dry forest on lava and coastal vegetation on saline sands still continue to support communities of native species.

Losses of Hawaiian biota are significant not only biologically but also culturally. In Hawaiian culture, native biota and forests are critically important in both utilitarian and spiritual regards. They serve as indispensable sources of indigenous material culture, providing feathers, medicines, wood, fibers, and flowers and ferns for garlands (*lei*). The original native Hawaiian belief system recognized multiple gods, some dwelling in forested areas and taking earthly manifestations (*kinolau*) as particular plants within the forest. To Hawaiians, dense mountain forests were termed '*wao 'akua*', literally 'place (of) god'.

In terms of human economics, the density of human populations living at first world standards on a limited land base in a highly isolated location has made Hawaii's watersheds, which are the sole sources of its potable and agricultural water, especially critical resources. With populations growing rapidly (predicted to double on Maui in the next 15-20 years), an increased and consistent water supply, especially in times of coming climate change, appears to be one of the islands' most important limiting factors. Not surprisingly, watershed conservation has traditionally received much attention in Hawaii, and the issue is now widely recognized as crucial. One recent economic study focused on aquifer recharge in Oahu's Koolau Mts. as the most direct benefit from tropical watershed conservation. The study calculated that if recharge to the aquifer from the Koolau Mts. ceased altogether, the reduction of inflow to the aquifer would be approximately 133 MGD/day, with a lost net present value of \$4.6 to \$8.6 billion (Kaiser et al., n.d.). In most Hawaiian watersheds, annual precipitation averages 4,000-6,000mm/yr. Watershed forest cover and composition is thought to largely determine how much of the water will run off, how much sediment it will carry, and how much it will recharge regional

aquifers. Public agency awareness of the importance of watersheds was crucial to the establishment of Hawaii's Watershed Partnerships beginning some 20 years ago. The intent of these regional coalitions is to protect upland watersheds in order to facilitate water collection, promote water recharge and conservation, and prevent watershed degradation through erosion and siltation (<http://hawp.org>). An important concurrent goal is to protect native biological diversity to the extent practical.

Threats to Remaining Natural Areas and Watersheds

Arriving about a millennium ago, Hawaiians deliberately introduced about 32 plant species for utilitarian purposes (Nagata, 1985) and, presumably inadvertently, about eight species of minor weeds (Wester, 1992). Since arrival of Europeans in 1778, approximately 13,000-15,000 species of non-native plants have been introduced; 1,200 of these are now naturalized, compared to a total native flora of approx. 1,200 species. Of introduced non-native species, about 100 non-native plant species in Hawaii are causing significant damage in natural areas (Smith, 1985; Stone et al., 1992). Invasive alien species may reach such high densities that they become community dominants, threaten entire native ecosystems and their component biodiversity, and threaten ecosystem services (Meyer and Florence, 1996; Denslow and Hughes, 2004). The International Union for the Conservation of Nature (IUCN) Invasive Species Specialist Group (ISSG) published a list of "100 of the World's Worst Invasive Alien Species" (Lowe et al., 2000). Of the 32 species among the hundred classified as 'land plants', Hawaii has 22.

While direct habitat destruction, first by Polynesians and now by modern humans, continues (especially of lowland and leeward ecosystems), human-facilitated biological invaders (weeds, non-native ungulates, rodents, and predators) are the primary agents of continuing degradation of biodiversity in Hawaii. A limited number of invasive plant species constitute the most important threat to extensive remaining native '*ohi'a lehua* (*Metrosideros polymorpha* Gaudich.) forests in Hawaii, which dominate moist, higher elevation areas (>ca. 900

m elevation). As a consequence, in many areas, this community is being progressively displaced by non-native plant species (Denslow, 2003; Medeiros 2004). Besides serving as refugia for native biota, 'ohi'a lehua forests are also critically important economically as their distribution largely coincides with source areas of potable water. Research is only beginning to document the impacts of individual species (Asner and Vitousek, 2005; Kagawa et al. 2009) but three important vignettes are presented here to illustrate the magnitude of the problem.

Rapid invasion of Hawaiian watersheds by the quick growing Neotropical tree miconia (*Miconia calvescens* DC.) in the 1990s raised concerns about potential adverse hydrological impacts, especially on the steep slopes characteristic of Hawaii's watersheds (Conant et al., 1997). In part, these concerns were based on the behavior of the species in Tahiti – notably the formation of dense monotypic stands with little or no ground-covering vegetation (Meyer and Florence, 1996). Miconia's dense, large foliage dramatically reduces sub-canopy light levels and strongly inhibits survival and establishment of other plant species – often leaving near-barren soil surfaces. Giambelluca et al. (2009) demonstrated that the large leaves produce large through-fall drops during and after rain storms that reach high levels of kinetic energy and can result in substantial impacts to the soil, likely creating high rates of soil detachment, erosion, and reduced rates of infiltration.

The Brazilian tree strawberry guava (*Psidium cattleianum* Sabine) was introduced nearly two centuries ago and since has arguably achieved greater local dominance in Hawaiian watersheds and more negative effects on endemic species than any other invasive plant species (Medeiros, 2004; Asner et al., 2009). Invasion of *P. cattleianum* is currently being locally facilitated by the N-fixing invasive tree, *Falcataia moluccana* (Miq.) Barneby & J.W.Grimes (Hughes and Denslow, 2005). Takahashi et al. (2011), in a pioneering though preliminary study in Hawaii Volcanoes National Park, compared hydrologic properties of a *P. cattleianum* dominated stand to a comparable stand nearby comprised of native *M. polymorpha*. Cloud water interception at the native site was higher than in the invaded stand, likely because the characteristics of the native *M. polymorpha* tree facilitate more effective harvesting of cloud water droplets. Species invasion results in

a lower proportion of precipitation reaching the forest floor and becoming available for groundwater recharge, suggesting that invasion by *P. cattleianum* may have significant negative effects on Hawaii's aquatic ecosystems and water resources.

Species brought from continental areas, having been separated from predators or pathogens of their native habitat (DeWalt et al., 2004), are often able to substantially outgrow Hawaii's endemic species. One dramatic well-documented example is the invasive Australian tree fern, *Cyathea (Sphaeropteris) cooperi* (Hook. Ex F. Muell.) Domin., that has been shown to be extremely efficient at utilizing soil nitrogen and is enabled to grow 6x as rapidly in height (15cm annually vs. 2-3cm), maintain 4x more fronds, and produce significantly more fertile fronds per month than the native Hawaiian endemic tree ferns, *Cibotium* spp. (Durand and Goldstein, 2001a, 2001b). Additionally, whereas *Cibotium* spp. provide an ideal substrate for epiphytic growth of many understory ferns and flowering plants, *C. cooperi* has the effect of impoverishing the understory and fails to support an abundance of native epiphytes with consequent reduction of local biological diversity (Medeiros and Loope, 1993).

Conclusions

Current strategies of management agencies in Hawaii tasked with control of invasive plant species have focused on chemical and manual control methodologies, sometimes in conjunction with technical advancements such as directed herbicide application by helicopter. However, the ability of invasive plants to have prolific seed production, efficient dispersal systems, and to become established in dense vegetation, complicated by Hawaii's rugged topography, appears to render these primarily mechanical and chemical controls as mere holding actions for established species. An integrated pest management approach towards Hawaii's invasive species problems would include as its components: exclusion of new weed species and genotypes via quarantine and inspections, traditional management (fences and ungulate removal), expedient eradication/control of incipient invaders, research, public education, and a biological control program for the most problematic species.

In the Hawaiian Islands, invasive plant species which are beyond chemical and mechanical control yet are amongst the most serious habitat modifying species include the trees *Miconia calvenscens*, *Morella* (*Myrica*) *faya* (Aiton) Wilbur (fayatree), *Psidium cattleianum*, *Falcataria moluccana* (albizia), and *Schinus terebinthifolius* Raddi (Christmas berry); tree fern *Cyathea cooperi*; shrubs *Clidemia hirta* (L.) D. Don (Koster's curse), *Ulex europaeus* L. (gorse), and *Rubus ellipticus* Focke (yellow Himalayan raspberry); forbs *Cortaderia jubata* (Lemoine ex Carrière) Stapf (pampas grass), *Hedychium gardnerianum* Ker-Gawl. (kahili ginger), *Rubus argutus* Link (Florida blackberry), *Tibouchina herbacea* (DC.) Cogn (cane tibouchina), and *Verbascum thapsus* L. (mullein); and the vine *Passiflora tarminiana* Coppens & Barney (banana poka).

Without increased levels of safely tested biological control, the seemingly inevitable result is landscape level transformation of native forests with potentially catastrophic consequences to cultural, biological, water, and economic resources. Increased levels of effective biological control for certain intractable invasive species appear to be the most conspicuous 'missing piece' in efforts to protect Hawaiian watersheds.

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**Biology, Field Release and Monitoring of the Rust Fungus
Puccinia spegazzinii (Pucciniales: Pucciniaceae),
a Biological Control Agent of *Mikania micrantha* (Asteraceae) in
Papua New Guinea and Fiji**

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Abstract

Mikania micrantha Kunth (Asteraceae), mikania or mile-a-minute, is a neotropical plant species now found in all lowland provinces of Papua New Guinea (PNG) and all major islands of Fiji. The weed invades plantations and cropping areas, thereby reducing productivity and threatening food security of rural communities. As part of an Australian Government-funded biological control program, the rust fungus *Puccinia spegazzinii* de Toni was imported into PNG and Fiji in 2008 and released. Life cycle studies were conducted in PNG and inoculation techniques were evaluated. Field releases were made in areas where *M. micrantha* was abundant and monthly sampling at three sites determined the impact of the rust on *M. micrantha* in the field. *P. spegazzinii* is a microcyclic, autoecious rust, with a life cycle of 15-21 days. The most efficient inoculation method was to place 3-4 week old plants under infected plants in a perspex inoculation chamber for 48 hours at 26±1°C and 98% relative humidity. The most efficient field release method was to transplant about five infected 3-4 week old plants in amongst *M. micrantha* growing densely under canopy or in gullies where there is adequate water and humidity. The rust fungus has now been released at nearly 560 sites in 15 provinces in PNG and over 80 sites on four islands in Fiji. In PNG, the rust has established at over 180 sites in 11 provinces, spreading up to 40 km from some sites; while in Fiji, it has established at 25 sites on two islands. Detailed field monitoring has shown that *P. spegazzinii* reduces mikania density and therefore has the potential to control this weed in many parts of both countries.

Introduction

Mikania micrantha Kunth (Asteraceae), mikania or mile-a-minute, is an aggressive neotropical vine. It has become a major weed throughout Asia and the South Pacific (Waterhouse and Norris, 1987; Waterhouse, 1994). It grows about 1 m/month and flowers profusely, producing thousands of light-weight barbed seeds. The seeds are spread by wind, by people on clothing and possessions, and by animals on their fur. *M. micrantha* is a particular problem on subsistence farms, where slash and burn agriculture is practiced. The plant can quickly invade cleared lands and smother crops. The main control method on subsistence farms is hand-pulling or slashing, as the use of herbicides is not economically feasible for resource-poor farmers (Day et al. 2011).

Plants can reshoot from broken stems and the vine's rapid growth rate means that land holders have to constantly clear land of the weed (Waterhouse and Norris, 1987; Holm et al., 1991). In subsistence farms, *M. micrantha* can grow over and kill crops such as taro, banana and papaw, while in plantations, it can smother cocoa, coconut seedlings or young oil palm trees, reducing flowering and productivity (Day et al., in press).

Biological control of *M. micrantha* was first attempted in the 1980s, with the introduction of the thrips *Liothrips mikaniae* (Priesner) (Thysanoptera: Phlaeothripidae) into the Solomon Islands in 1988 and Malaysia in 1990 (Cock et al., 2000). However, it failed to establish in either country (Julien and Griffiths, 1998; Evans and Ellison, 2005). The thrips was also sent to Papua New Guinea (PNG) in 1988 but the colony died out in quarantine before field releases could be conducted (Cock et al., 2000).

Renewed efforts into the biological control of *M. micrantha* commenced in 2005 with the release of the rust *Puccinia spegazzinii* de Toni (Pucciniales: Pucciniaceae) into India, mainland China and Taiwan, following exploration and host specificity testing by CABI Europe-UK (Evans and Ellison, 2005; Ellison et al., 2008; Ellison and Day, 2011). However, the rust appears to have established only in Taiwan (Ellison and Day, 2011).

Biological control of *M. micrantha* in the Pacific recommenced in 2006 following meetings of the Pacific Plant Protection Organization held in 2002 and 2004, where *M. micrantha* was rated as the

second most important weed in the region behind the vine *Merremia peltata* L. (Convolvulaceae) (Dovey et al., 2004). A project, funded by the Australian Government and managed by the Queensland Government, aimed to introduce into PNG and Fiji safe biological control agents that were effective elsewhere (Pene et al., 2007).

Two butterfly species, *Actinote antea*s (Doubleday and Hewitson) (Lepidoptera: Arctiidae) and *A. thalia pyrrha* Fabr., were imported into Fiji in 2006 from Indonesia, where they were reported to aid the control of chromolaena, *Chromolaena odorata* (L.) King and Robinson (Asteraceae), and also damage *M. micrantha* (R. Desmier de Chenon pers. comm. 2006). However, colonies of both species died out before additional host-specificity testing could be completed. The rust *P. spegazzinii* was introduced into both PNG and Fiji in November 2008, following testing by CABI of additional plant species of concern to both countries. This paper reports on the biology, field release and monitoring of *P. spegazzinii* in PNG and Fiji.

Materials and Methods

Biology and culturing

Four small bare-rooted *M. micrantha* plants, each infected seven days prior with *P. spegazzinii* collected from Ecuador, were imported from CABI Europe-UK into quarantine facilities at the National Agriculture Research Institute, Island Regional Center, at Kerevat, East New Britain, PNG and the Secretariat of the Pacific Community, Fiji in November 2008. Plants were potted into clean pots with sterilized soil and held in a laboratory to aid recovery.

When the pustules on the imported plants reached maturity (about 15 days after inoculation), the infected plants were placed on a stand in a perspex inoculation chamber (60cm x 60cm x 45cm) within a quarantine laboratory (26±1°C, natural lighting). Healthy 3-4 week old *M. micrantha* plants, grown from 3cm long cuttings and containing 2-4 pairs of leaves, were placed under the infected plants. The chamber was sealed and the plants left for 48 hours for sporulation to occur and the fresh plants to be inoculated. After two days, the newly inoculated

plants were removed and placed under a light bank in the laboratory for 48 hours prior to being placed in a shade house for pustules to develop. Plants were watered as necessary.

A culture of *P. spegazzinii* was maintained by repeating the above steps, using plants with mature pustules to inoculate young healthy plants. The life cycle of the rust was determined through daily observations of developing pustules.

To facilitate an increased number of field releases, small 3-4 week old plants were placed for 4-5 days in a field site where the rust had been established previously. The plants were then returned to the shade house for the pustules to develop. Using this field inoculation technique, in contrast with the laboratory procedure, many more plants can be inoculated.

Field release and monitoring

Field releases of *P. spegazzinii* were conducted by transplanting infected potted plants amongst patches of actively growing *M. micrantha* such that the infected plants trailed over the field plants to encourage the inoculation process. Releases were generally conducted in areas where there was greater shade and damp soil to help keep the potted plants alive until infection of the field plants occurred. Release sites were inspected about three months after the rust was released, by which time the pustules had a chance to develop on the field plants and be more easily seen.

Two release sites near the research station at Kerevat were monitored in detail every month. At each site, ten 1m² quadrats were placed randomly and the percent plant cover by *M. micrantha* was recorded in each. The number of infected leaves, petioles and stems in each quadrat was recorded.

Results

Biology

P. spegazzinii has a life cycle of 15-21 days. Tiny white spots appear on the upper leaf surface about six days after inoculation and the pustules continue to develop and grow, turning yellow by 11 days. Mature pustules become brown by day

15-17 when they are ready to infect other plants.

Field release and monitoring

In PNG, the rust was released at over 560 sites in all 15 provinces infested with *M. micrantha*. Of the sites which were re-checked, the establishment rate was about 50%, with pustules being found at over 180 sites in 11 provinces. The rust established better in the wetter provinces of Oro (100% of release sites), Western (82%) and East New Britain (78%). Although the rust established at only three release sites out of seven checked in East Sepik Province, it spread up to 40 km in 16 months. The rust also spread widely in Oro and West New Britain provinces (Fig. 1).

Establishment was poor in Northern Solomons (4% of release sites), Sandaun (11%), Gulf (13%) and Madang (22%) provinces. To date, the rust has not established in Milne Bay, Morobe and New Ireland provinces, as well as around Port Moresby but recent release sites still need to be revisited (Fig. 1).

In Fiji, *M. micrantha* was found on all major islands. The rust was released at over 80 sites on the islands of Viti Levu, Vanua Levu, Taveuni and Ovalau. Pustules were found at 39% of sites checked on Viti Levu and 38% of sites on Vanua Levu (Fig. 2). Establishment was better on the wetter eastern side of Viti Levu than the drier western side of the island. On the eastern side of Viti Levu, the rust has begun to spread to other sites. No spread has been reported on Vanua Levu to date. Release sites on Taveuni and Ovalau are yet to be checked (Fig. 2).

In the field at one site near Kerevat, PNG, *P. spegazzinii* suppressed the growth of *M. micrantha*, allowing the growth of other plants (such as clycine *Glycine wightii*) over the *M. micrantha*, further reducing its cover. During a subsequent long dry season in 2010, the rust was not detected and *M. micrantha* began to increase again. However, the rust re-appeared after rains in early 2011 and the growth of *M. micrantha* is again beginning to be checked (Fig. 3).

At Tavilo, East New Britain (Fig. 4), the rust is suppressing *M. micrantha*, with cover decreasing from 100% to 40% following the release of the rust. Monitoring at both sites in PNG is continuing.

Discussion

M. micrantha is considered a major weed in most wet lowland areas of both PNG and Fiji (Day et al., in press). The rapid growth rate of *M. micrantha* and its ability to smother crops and reduce productivity is a concern for land holders. Conventional control methods such as hand-pulling and slashing is time-consuming and the plant can re-shoot from the broken fragments left behind (Waterhouse and Norris, 1987; Holm et al., 1991). Biological control therefore is seen as a viable strategy.

Following additional host specificity testing by CABI, *P. spegazzinii* was approved for release in both countries. Laboratory trials suggested that the rust has the ability to reduce the growth rate of *M. micrantha*, which should reduce its competitiveness and limit its ability to smother crops (Day et al., 2011). Field monitoring at two sites confirmed that plant density has decreased since the release of the rust.

The rust has been widely released in PNG and has established in most provinces, where it is beginning to disperse from the release sites. At sites where it is currently in low abundance due to being

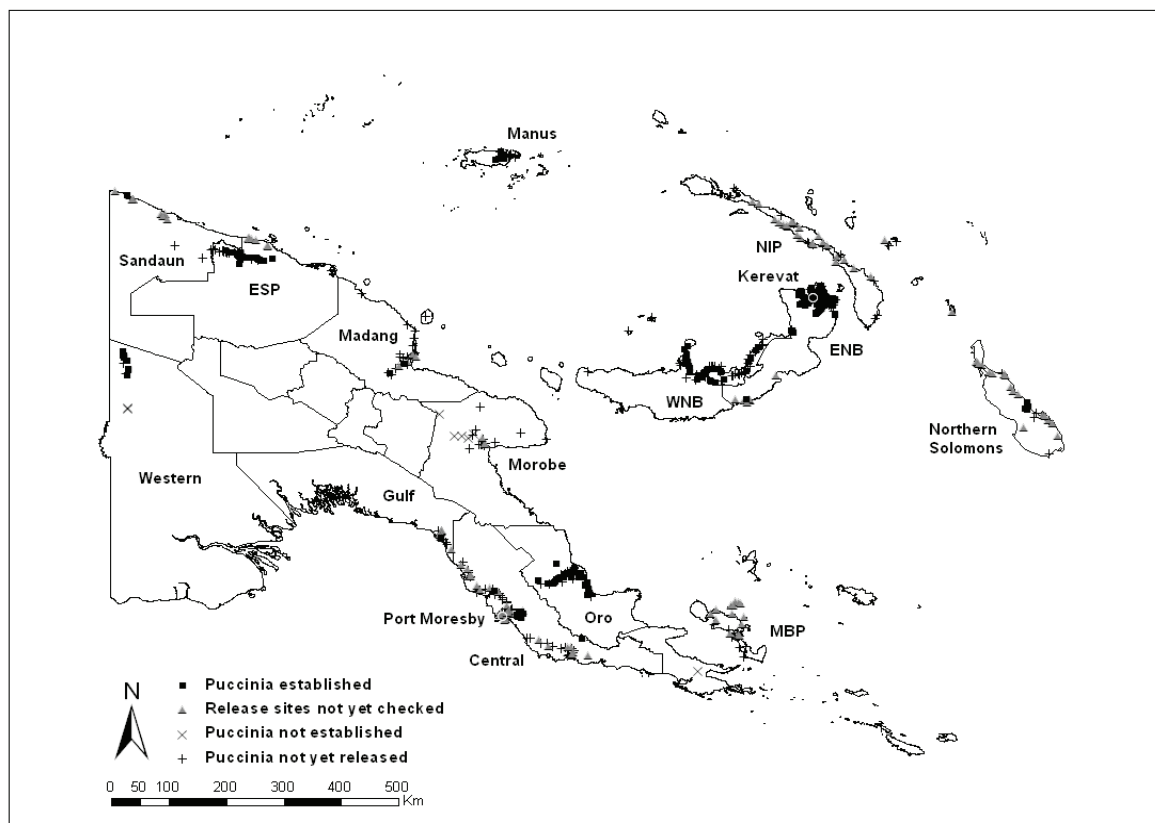


Figure 1. The distribution of *M. micrantha* in Papua New Guinea (all symbols); and the sites where *P. spegazzinii* is established, released but establishment unconfirmed, not established, and not yet released. ESP = East Sepik Province, ENB = East New Britain Province, MBP = Milne Bay Province, NIP = New Ireland Province and WNB = West New Britain.

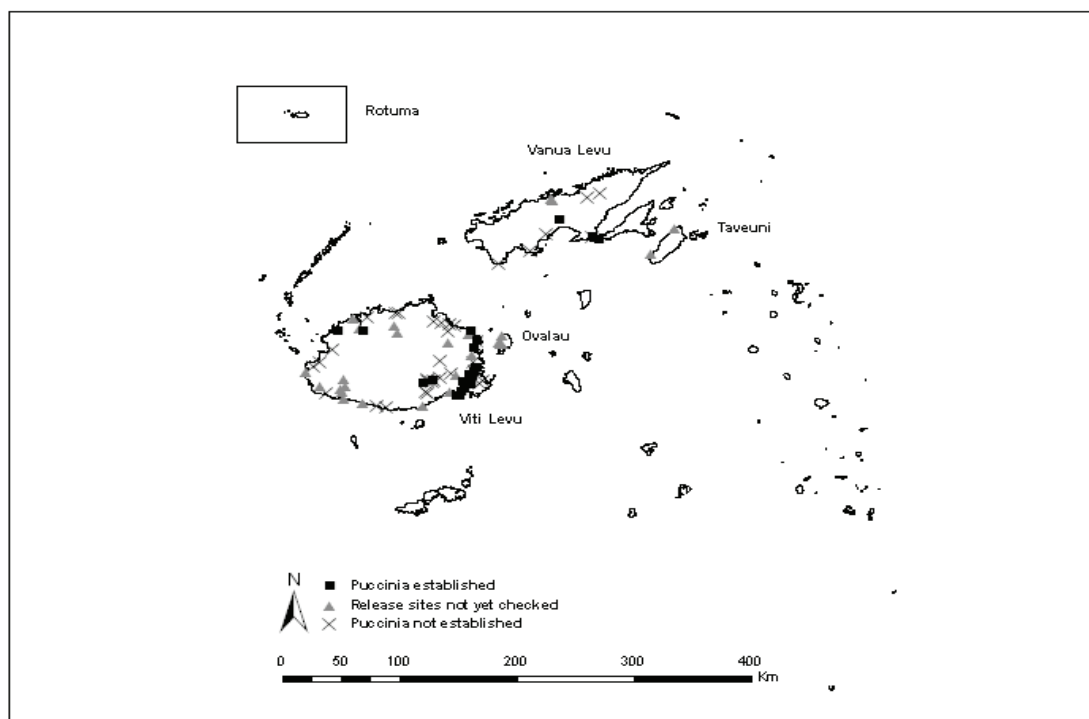


Figure 2. Sites in Fiji where *P. spegazzinii* is established, released but establishment unconfirmed, and not established.

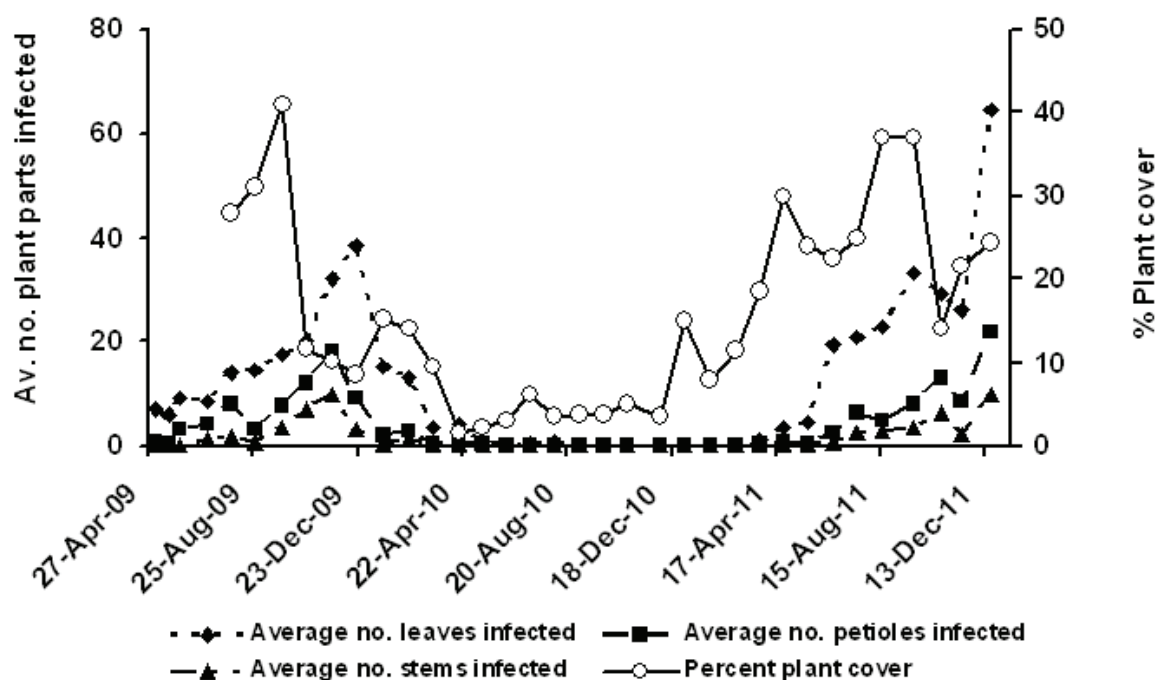


Figure 3. The mean number of leaves, petioles and stems of *M. micrantha* infected by *P. spegazzinii* per 1 m² and the percent plant cover of *M. micrantha* at site 1, Kerevat, East New Britain, PNG (release date: 10 January 2009).

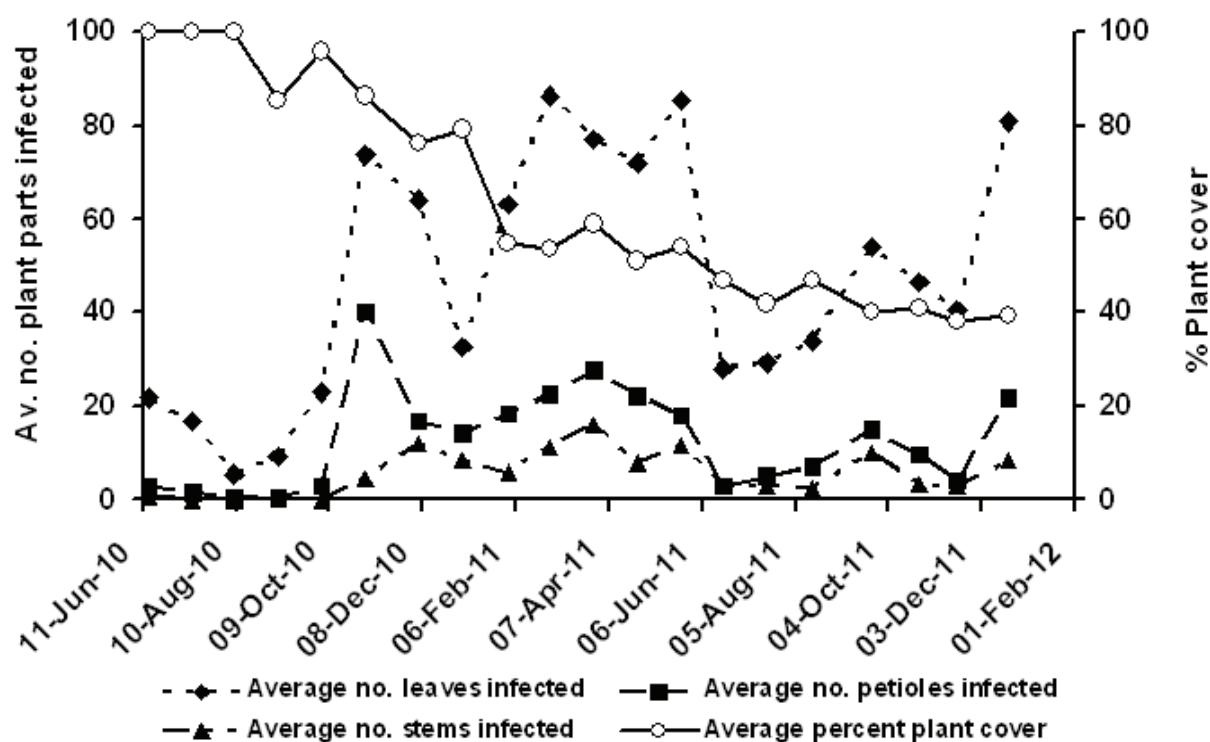


Figure 4. The mean number of leaves, petioles and stems of *M. micrantha* infected by *P. spegazzinii* per 1 m² and the percent plant cover of *M. micrantha* at site 2, near Kerevat, East New Britain, PNG (release date: 31 March 2010).

recently released, it is anticipated that populations will increase with time and it is expected that *P. spegazzinii* will have similar impacts on *M. micrantha* at these sites as at the monitoring sites. However, at some sites which are drier, such as in parts of Gulf and Morobe provinces and around Port Moresby, the impact of the rust may be less.

There are still many release sites that need to be checked and still a few sites where the rust has not yet been released, especially in more remote regions. The release and monitoring program will therefore continue in an effort to get the rust established in all parts of PNG where *M. micrantha* occurs.

In Fiji, *P. spegazzinii* has not been as widely released. There were lengthy delays in obtaining approval to release the rust; and many sites, particularly those away from the main islands of Viti Levu and Vanua Levu, are difficult and costly to reach. The rust has established well in the eastern areas of Viti Levu, which are much wetter than the western part of the island. It has also established

at several sites on Vanua Levu. Field monitoring continues, with a slight decrease in plant density observed following the release of the rust.

In other countries where *P. spegazzinii* has been released, establishment has been patchy. The rust has appeared to have established in Taiwan but does not appear to have established in mainland China or India (Ellison and Day, 2011). Following the promising results seen in PNG, plans are underway to introduce *P. spegazzinii* into Vanuatu and Guam and re-introduce it into mainland China.

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The Invasive Alien Tree *Falcataria moluccana*: Its Impacts and Management

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Abstract

Falcataria moluccana (Miq.) Barneby and Grimes is a large tree that has become invasive in forests and developed landscapes across many Pacific islands. A fast-growing nitrogen-fixing species, it transforms invaded ecosystems by dramatically increasing nutrient inputs, suppressing native species and facilitating invasion by other weeds. Individuals rapidly reach heights of 35 m, and their massive limbs break easily in storms or with age, creating significant hazards in residential areas and across infrastructure corridors such as roads and power lines. Their management is extremely costly for landowners, utilities, and local governments, since removal of hazardous trees can cost several thousand dollars apiece. Although efficient mechanical and chemical controls are being used with some success against incipient invasions of *F. moluccana*, biological control is needed to manage spread of populations and the massive seedling recruitment that occurs once mature individuals have been killed. The benefits of a biological control program for *F. moluccana* would likely extend to tropical islands throughout the Pacific, helping prevent further loss of native forest biodiversity and saving many millions of dollars in damage and maintenance associated with these trees growing near utilities, roads, homes and workplaces.

Introduction

Alien species have caused untold damage to the ecology and economies of areas they have invaded (Elton, 1958). Where such species introduce new biological processes or disturbance regimes into ecosystems, they have the potential to profoundly alter both community characteristics and ecosystem functions, often to the extreme detriment of the native flora and fauna being invaded (Vitousek et al., 1987; D'Antonio and Vitousek, 1992). The invasive alien *Falcataria moluccana* (Miq.) Barneby and Grimes (synonyms: *Paraserianthes falcataria* (L.) I.C. Nielsen, *Albizia falcataria* (L.) Fosberg, *Albizia falcata* auct) is a very large, fast-growing, nitrogen-fixing tree in the legume family (Fabaceae) (Wagner et al., 1999). Recognized as the world's fastest growing tree species, it is capable of averaging 2.5 cm gain

in height per day (Walters, 1971; Footman, 2001). Individuals reach reproductive maturity within four years and subsequently produce copious amounts of viable seed (Parrotta, 1990) contained within seed pods that can be wind-dispersed over substantial distances (i.e., > 200 m up- and down-slope during windy conditions). Mature trees can reach heights over 35 m, with the canopy of a single tree extending over a one-half hectare area. The broad umbrella-shaped canopies of multiple trees commonly coalesce to cover multiple hectares and even up to square kilometers (Hughes and Denslow, 2005). An important constraint to *F. moluccana* seedling recruitment is light availability; seedlings are very sensitive to shade and germinate in abundance only where the overstory canopy is open enough to allow sufficient light penetration (Soerianegara et al., 1994).

Although valued by some, *F. moluccana* has

become invasive in forests and developed landscapes across many Pacific islands. Native to the Moluccas, New Guinea, New Britain, and Solomon Islands (Wagner et al., 1999), *F. moluccana* was exported widely across the Pacific, typically for the purpose of providing shade and nutrients (via litterfall) to crop species. It is currently considered invasive in the Republic of Palau, Pohnpei, Yap, New Caledonia, Fiji, Independent Samoa, American Samoa, the Cook Islands, the Society Islands, and the Hawaiian Islands, and it is present though not yet considered invasive on Guam, Wallis and Futuna, and Tonga (USDA Forest Service, 2012). Given the widespread presence of *F. moluccana* across the Pacific Islands, it poses a serious threat to the highly diverse biological hotspot that these islands collectively constitute (Myers et al., 2000). An archetypical early successional (i.e., pioneer) species, *F. moluccana* is generally found in mesic to wet forest and favors open, high-light environments such as disturbed areas; its capacity to readily acquire nitrogen via its symbiotic association with *Rhizobium* bacteria allows it to colonize even very young, nutrient-limited lava flows such as those found on Hawaii Island (Hughes and Denslow, 2005).

Falcataria moluccana was first introduced to the Hawaiian Islands from Borneo and Java in 1917 by the explorer botanist – and champion of native Hawaiian species – Joseph F. Rock (Rock, 1920). He noted the rapid growth rate of *F. moluccana*, stating that it is capable of reaching a height of over 35 m in 25 years time and that, “trees nine years old had reached a height of over a hundred feet, a rapidity of growth almost unbelievable.” Ironically, Rock also commented on the life cycle of *F. moluccana* that “the only objection to the tree is its short-lived period, but as it is an abundant seeder, there should always be a good stand of this tree present” (Rock, 1920), yet individuals planted by Rock in 1917 remain living today, nearly 100 years later, on the grounds of the Lyon Arboretum on Oahu, Hawaii. Following its introduction, *F. moluccana* was one of the most commonly planted tree species in concerted, long-term, and wide-ranging non-native tree establishment efforts conducted by Hawaii Territorial and State foresters during the early to mid-1900s; approximately 140,000 individuals were planted throughout the Forest Reserve systems across the Hawaiian Islands, and populations have

spread extensively from those intentional plantations (Skolmen and Nelson, 1980; Woodcock, 2003).

Ecological Impacts

Previous research on the impacts of *F. moluccana* on native forests in Hawaii has demonstrated that this species profoundly transforms invaded forests by dramatically increasing inputs of nitrogen, facilitating invasion by other weeds while simultaneously suppressing native species. Hughes and Denslow (2005) described the impacts of *F. moluccana* invasion on some of the last intact remnants of native wet lowland forest ecosystems in Hawaii. They found that primary productivity in the form of litterfall was more than eight times greater in *F. moluccana*-dominated forest stands compared to stands dominated by native tree species. More importantly, nitrogen (N) and phosphorus (P) inputs via litterfall were up to 55 and 28 times greater in *F. moluccana* stands compared to native-dominated forests (Hughes and Denslow, 2005), and rates of litter decomposition – as well as rates of N and P release during decomposition – were substantially greater in *F. moluccana* invaded forests relative to native-dominated forests (Hughes and Uowolo, 2006). These inputs of up to 240 kg N ha⁻¹ y⁻¹ in *F. moluccana* stands exceed typical application rates of N fertilizer documented for industrial, high output corn cropping systems of the US Midwest (Jaynes et al., 2001). As a consequence, soil N availability was 120 times greater in *F. moluccana* forests relative to native-dominated forests on comparably-aged lava flow substrates. Simultaneously, *F. moluccana* invasion increased soil enzyme – particularly acid phosphatase – activities and converted the fungal-dominated soil communities of native stands to bacteria-dominated soil communities (Allison et al., 2006). These profound functional changes coincided with dramatic compositional and structural changes; *F. moluccana* facilitated an explosive increase in densities of understory alien plant species – particularly strawberry guava (*Psidium cattleianum* L.). Native species – particularly the overstory tree, ‘ohi’a lehua (*Metrosideros polymorpha* Gaud.) – suffered widespread mortality to the point of effective elimination from areas that they had formerly dominated. Based on these findings,

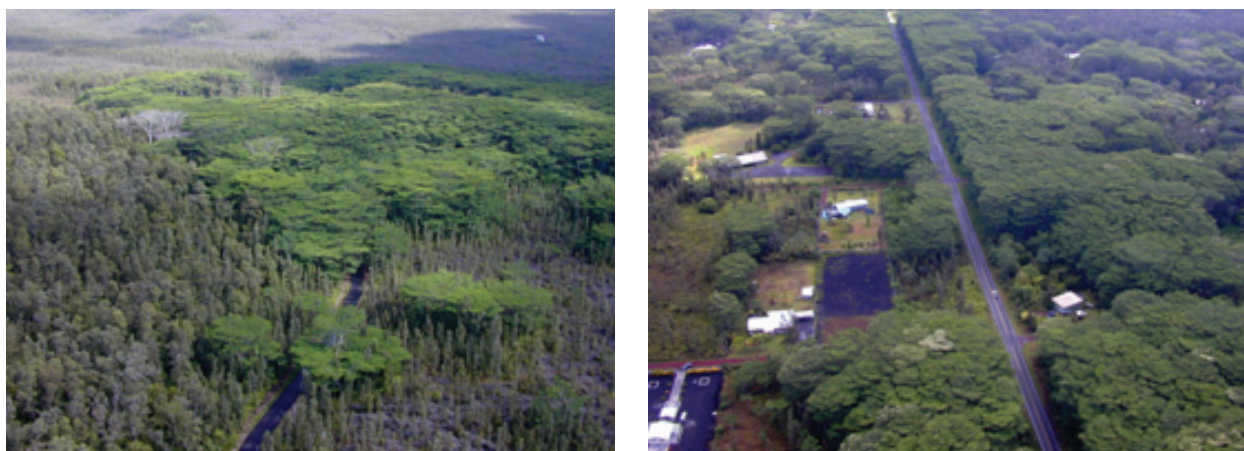


Figure 1. Invasion by *Falcattaria moluccana* on Hawaii Island within Keauohana Forest Reserve (left) and in a residential area (right).

Hughes and Denslow (2005) concluded that the continued existence of native-dominated lowland wet forests in Hawaii largely will be determined by the future distribution of *F. moluccana* (Figure 1).

In American Samoa, where *F. moluccana* (locally known as tamaligi) was introduced in the very early 1900s and was present across 35% of the main island of Tutuila by 2000, an aggressive campaign has been undertaken by federal, state, and local groups to control and ideally eradicate this invasive species from the island (Hughes et al., 2012). Research addressing the role of *F. moluccana* in Samoan native forest communities supports the need for control as well the feasibility of eradication in these ecosystems. Results indicate that *F. moluccana* displaces native trees: although aboveground biomass of intact native forests did not differ from those invaded by *F. moluccana*, greater than 60% of the biomass of invaded forest plots was accounted for by *F. moluccana*, and biomass of native species was significantly greater in intact native forests. Following removal of *F. moluccana* (i.e., killing of mature individuals), the native Samoan tree species grew rapidly, particularly those which exhibit early successional, or pioneer species traits. The presence of such pioneer-type tree species appeared to be most important reason why *F. moluccana* removal is likely a successful management strategy; once *F. moluccana* is removed, native tree species grow rapidly, exploiting available sunlight and the legacy of increased available soil N from *F. moluccana* litter. Recruitment by shade intolerant *F. moluccana* seedlings was severely constrained to the point of being non-existent, likely a result of the shade cast

by reestablishing native trees in management areas (Hughes et al., 2012). Thus, although *F. moluccana* is a daunting invasive species, its ecological characteristics and those of many of Samoa's native trees actually create conditions and opportunities for successful, long-term control of *F. moluccana* in lowland forests of American Samoa.

Socio-Economic Impacts

Falcattaria moluccana is also a roadside, urban forest and residential pest of major significance. Because individuals rapidly and routinely reach heights near 35 m and their weak wood breaks easily in storms or with age, catastrophic failure of massive limbs creates major hazards in residential areas and across infrastructure corridors such as roads and power lines. For example, on April 16, 2010, a 25-30 m tall *F. moluccana* tree fell across a residential street in the Puna District of Hawaii Island, destroying power lines and fences and landing in a backyard area where children often play (Hawaii Tribune Herald, May 6, 2010). Management of these large hazardous trees is extremely challenging for landowners, utilities, and local governments (Figure 1).

The potential economic burden posed by *F. moluccana* is staggering. In 2009 on the island of Kauai, the Hawaii Department of Transportation (HDOT) was compelled to act on two unconfirmed near fatalities involving large branches of *F. moluccana* dropping onto cars and a house located close to the road right-of-way. In response, the HDOT spent

one million dollars to remove approximately 1,500 *F. moluccana* individuals growing along a single mile of roadway. Because *F. moluccana* has such soft wood and unstable branches, arborists were forced in this case to employ expensive cranes and lifts to remove these trees. As a consequence the larger trees cost in excess of \$10,000 per individual to remove safely. Across the state of Hawaii, it has been estimated that over 40% of HDOT damage claims involving falling trees and branches are due to *F. moluccana* and between 50 and 100 miles of state roads have maturing *F. moluccana* populations (personal communication, Christopher A. Dacus, Landscape Architect and Certified Arborist, Hawaii State Department of Transportation). Even where *F. moluccana* grow at some distance from roads, they are considered problematic and hazardous because limbs can fall into waterways and accumulate against bridges, potentially causing flooding and physical damage to critical infrastructure. In addition, natural events such hurricanes or storms often cause extreme damage to *F. moluccana* stands which in turn contribute to road closures, electrical outages, and property damage, thus exacerbating post-storm and cyclone cleanup and repair work. With no natural predators to constrain them, *F. moluccana* populations are increasing in both stature and area, with concomitant maintenance costs increasing annually.

Control Measures

Successful efforts to control *F. moluccana* populations within the National Park of American Samoa (NPAS) and adjacent lands employed a girdling method. Field crews of 2–6 people incised the bark of each mature individual at its base using bark spuds and manually peeled up the bark in large strips around the entirety of the trunk, resulting in a 1–3 m wide girdled section. Individual trees died gradually but inevitably, six months to a year following treatment. NPAS field crews have killed over 6,000 mature trees, thus restoring approximately 1,500 ha of native Samoan forest. This approach has been successful for three main reasons. First, significant funding was available to implement *F. moluccana* control across the targeted areas. Second, overwhelming public support for

the *F. moluccana* control effort has been cultivated through outreach and informational meetings with local village leadership, employment of villagers from areas adjacent to infestations, and use of media outlets on a consistent basis. Third, *F. moluccana* exhibits characteristics that make it vulnerable to successful control: it is easily killed by girdling or herbicides, and its seeds and seedlings are exceedingly shade intolerant, while many of the common native Samoan tree species recover quickly from disturbance, and the shade they cast preempts subsequent *F. moluccana* seedling recruitment (Hughes et al., 2012).

Herbicides also have proven to be effective in controlling saplings and larger, mature *F. moluccana*. On the Hawaiian Island of Molokai, the Molokai-Maui Invasive Species Committee spearheaded a multi-agency effort in 2008 to eliminate a large stand of *F. moluccana* with extensive root systems threatening sensitive cultural sites (Wianecki, 2011). Field crews girdled the trees with chainsaws and applied Garlon 3A mixed with crop oil. Significant canopy defoliation was noted within weeks of treatment. Mortality of treated trees was 98% one year following application, and 100% with no subsequent seedling recruitment in the 3 years since treatment. As of this writing, all known populations of *F. moluccana* on Molokai have been killed, providing a compelling example of island-wide eradication of this highly invasive tree. As in American Samoa, the *F. moluccana* control project was successful in bringing together a diversity of community members, agency staff, and cultural practitioners. Participants are determined to use this project as a model for community involvement and creating a proper emphasis on Hawaiian cultural practices.

Encouraging recent advances in the development and use of another herbicide, Milestone® (EPA reg. no. 62719-519; active ingredient aminopyralid), have provided a highly effective means to quickly and efficiently kill mature *F. moluccana*. Milestone is administered by injection of very low volume, metered doses of the undiluted formulation. Trials indicate that very low dosage treatments resulted in near 100% mortality in less than one month. This new method – demonstrated to be safer and more effective than current conventional methods – appears to be a “game changer,” allowing efficient

control of *F. moluccana* populations across broad landscapes of Hawaii (personal communication, James Leary, Invasive Weed Management Specialist, University of Hawaii; <http://www.ctahr.hawaii.edu/LearyJ/videos/albizia.html>).

Biological Control

While girdling and herbicide can provide effective means to kill *F. moluccana* saplings and mature trees, more challenging is control of the massive seedling recruitment that occurs once mature individuals have been killed. This is particularly true in Hawaii, where fast-growing pioneer-type tree species are not common in the native flora (Wagner et al., 1999). Identifying appropriate biological control agents is a logical and compelling solution to this challenge. Already at least one natural enemy of *F. moluccana* appears worth investigation: the gall rust *Uromycladium tepperianum* (Sacc.) McAlpine has been identified as a damaging pest of *F. moluccana* grown in plantations of Southeast Asia (Rahayu et al., 2010).

Recent biological control programs targeting alien *Acacia* species in South Africa have met with considerable success by focusing on agents that attack reproduction and reduce spread of trees from existing stands (Hoffmann et al., 2002; Post et al., 2010). In another successful effort in South Africa, seed feeders have been employed to control a close relative of *F. moluccana*, the Australian tree *Paraserianthes lophantha* (Willd.) I.C. Nielsen (Donnelly, 1992; Dennill et al., 1999). Seed predators make sense as a potential biological control agent for *F. moluccana* given that ongoing herbicide trials demonstrate the ease of killing mature trees: if post-control seedling recruitment could be minimized through seed predation, effective control of *F. moluccana* populations in Hawaii might be feasible. The benefits of a combined chemical and biological control program for *F. moluccana* would likely extend to tropical islands throughout the Pacific. Further loss of native forests and biodiversity, as well as extremely high costs in damage to private property and public infrastructure, can be expected from *F. moluccana* invasion if chemical and biological control work is not initiated.

Conclusions

Previous research and recent experience demonstrate that unchecked invasion by *F. moluccana* poses significant threats to native ecosystems and human health and welfare across the Pacific Islands. Successful containment of *F. moluccana* by self-perpetuating biological control agents, along with improved chemical control measures, are needed to sustainably manage native ecosystems and to save many millions of dollars in damage and maintenance costs associated with these trees growing near utilities, roads, homes and workplaces.

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Effective Biological Control Programs for Invasive Plants on Guam

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Abstract

Several biological control agents were imported and released in Guam for the control of siamweed (*Chromolaena odorata* (L.) King & H.E. Robins), ivy gourd (*Coccinia grandis* (L.) Voigt), and the giant sensitive weed (*Mimosa diplotricha* C. Wright ex Sauvalle). Substantial control of *C. odorata* was achieved using the moth (*Pareuchaetes pseudoinculata* Rego Barros) (Lepidoptera: Arctiidae) and the gall fly (*Cecidochara connexa* Macquart) (Diptera: Tephritidae). There was a remarkable reduction of plant height caused by *C. connexa*. A biological control program on *C. grandis* has been effective, following the success achieved in Hawaii, through the introduction of the natural enemies *Melittia oedipus* Oberthür (Lepidoptera: Sesiidae) and *Acythopeus cocciniae* O'Brien and Pakaluk (Coleoptera: Curculionidae). At this moment, there are no infestations of *C. grandis* seen in Guam. Although *Heteropsylla spinulosa* Muddiman, Hodkinson & Hollis (Homoptera: Psyllidae) established successfully at release sites on Guam, it has yet to provide significant control of *M. diplotricha*. Presently, a biological control program has been initiated against *Mikania micrantha* (L.) Kunth. (Asterales: Asteraceae), using the rust fungus *Puccinia spegazzinii* De Toni (Basidiomycotina: Uredinales). Additionally, the biological control programs will be extended to neighboring Micronesian islands.

Introduction

Invasive and exotic pest plant species have become an escalating problem in Guam and other Micronesian islands. Non-native plant invasions can be seen in agricultural and residential areas, roadsides, rangelands, pastures, forests, wetlands and parks (Reddy, 2011). Control of invasive, non-native plant species involves difficult and complex procedures. Reddy (2011) listed the top 20 invasive plant species which have impacted Guam greatly and a strategic plan with several possible control measures was suggested.

Siam weed, *Chromolaena odorata* (L.) King &

H.E. Robins (Asterales: Asteraceae), is one of the most severe invasive weeds in Guam and other Micronesian Islands. It is a problem mostly apparent in plantations, pastures, vacant lots and disturbed forests (Cruz et al., 2006). It grows extremely rapidly, invading a wide range of vegetation types, forming dense monospecific stands and smothering other vegetation (Zachariades et al., 2009). Biological control using insects is considered an effective component of an Integrated Pest Management (IPM) program for this weed and has been incorporated into the IPM strategy in several countries where this weed is a problem. The moth *Pareuchaetes pseudoinculata* Rego Barros (Lepidoptera: Arctiidae) was introduced from India and Trinidad and became established on

Guam in the 1980s (Zachariades et al., 2009). The gall fly *Cecidochara connexa* Macquart (Diptera: Tephritidae) was introduced from Indonesia in 1998 and established on Guam in 2002 and other Micronesian islands in succeeding years (Cruz et al., 2006; Zachariades et al., 2009).

Ivy gourd or scarlet gourd, *Coccinia grandis* (L.) Voigt (Violales: Cucurbitaceae), is an invasive and perennial vine which grows best under conditions of adequate rainfall and high humidity (Muniappan et al., 2009). Introduction of this vine in the 1980s resulted in invasion of over 100 hectares in different parts of Guam and almost one-third of Saipan (Bamba et al., 2009). It is also invading neighboring islands, Rota and Tinian (Muniappan et al., 2009). Based on the success of biological control in Hawaii, a program was initiated in the Mariana Islands using the natural enemies *Acythopeus cocciniae* O'Brien and Pakaluk (Coleoptera: Curculionidae), *Acythopeus burkhartorum* O'Brien and Pakaluk (Coleoptera: Curculionidae) and *Melittia oedipus* Oberthür (Lepidoptera: Sesiidae) (Reddy et al., 2009a, b). The two weevil species, *A. cocciniae* and *A. burkhartorum*, were field released in various locations on Guam in 2003 and 2004, respectively (Bamba et al., 2009 and Raman et al., 2007), and *M. oedipus* was released in 2007 (Reddy et al., 2009b).

The giant sensitive weed, *Mimosa diplotricha* C. Wright ex Sauvalle (Fabales: Fabaceae), also referred to in the literature as *M. invisa*, is a serious weed occurring mainly in vacant lots, roadsides, and crop lands (Kuniata, 2009). It has invaded most of the islands in Micronesia and the South Pacific (Esguerra et al., 1997). Recently, it has become established and spread to approximately two hectares in Guam, 120 hectares in Rota, 150 hectares in Tinian and 140 hectares in Saipan. After host specificity tests were conducted in Australia, *Heteropsylla spinulosa* Muddiman, Hodkinson & Hollis (Homoptera: Psyllidae) was released in Australia, Papua New Guinea, Samoa, Fiji, Cook Islands, Pohnpei, Yap and Palau (Esguerra et al., 1997; Wilson and Garcia, 1992), and it has effectively suppressed the weed in all the introduced countries. Because herbicidal control of *M. diplotricha* is expensive, labor intensive and requires frequent application, it is not a viable technique in the Marianas, and it was decided in 2005 to initiate biological control. Nymphs and adults of *H. spinulosa* were collected from Pohnpei and Palau and

were field released on Guam in 2008.

The objective of this project is to assess the impact and interaction of the established natural enemies of *Chromolaena odorata*, *Coccinia grandis* and *Mimosa diplotricha* at various locations on Guam.

Methods and Materials

Several sites with well-established stands of the three invasive plant species were selected for this study from villages in northern, central and southern Guam to represent the entire area of the island (Table 1). The sites were selected to include forested areas, suburban areas, waysides, and agricultural areas. A 12-channel global positioning system (GPS) (Garmin Corp., Taiwan) device was used to record longitude and latitude coordinates of each study site. Vegetation of each target weed was examined in randomly placed quadrats (1m²) (Reddy, 2011), with 2-4 replicates per site (Brower et al., 1998). The number of stems, leaves, and plant height of *Chromolaena odorata* was measured, *C. connexa* galls in each quadrat were counted, and number of *P. pseudoinculata* larvae and adults and their feeding damage in terms of larval holes and yellow leaves were counted. Similarly, holes in leaves and stems of *Coccinia grandis* caused by *A. cocciniae* and *M. oedipus*, respectively, and number of larvae or adults of *A. cocciniae*, *A. burkhartorum* and *M. oedipus* were counted. Damaged leaves and dead branches of *M. diplotricha* caused by *H. spinulosa* feeding were counted, and number of nymphs and adults of *H. spinulosa* were counted. Sites were visited monthly.

All data were analyzed using the GLIMMIX procedure in SAS v.9.2. For yield data (by site), a one-way ANOVA was performed, and if treatment effects were significant ($P < 0.05$), mean pairwise comparisons were performed by the least-squares difference method. If the treatment and/or month effects were significant, pairwise mean comparisons were performed with log-transformed LSMEANS.

Results

Effect of natural enemies on *C. odorata*

There has been a significant increase in mean number of *C. connexa* gall formations on *C. odorata*

over the years and a corresponding significant decrease in growth of *C. odorata* (Figure 1). The average height of *C. odorata* was 65.2 cm in 2006 and decreased to 18.3 cm in 2010. Although larvae of *P. pseudoinsulata* were present in all five years we monitored, there was no significance in population build up (Table 2), except in the case of small larva holes, which were significantly higher in 2010 than in the previous years ($P < 0.05$). However, the leaves of *C. odorata* turned yellow due to larval feeding by *P. pseudoinsulata* in all the years.

Effect of natural enemies on *C. grandis*

The weevil *A. burkhartorum* has not become established, but population levels of *A. cocciniae* have increased significantly over the years (Table 3), and damage in terms of feeding holes on the leaves was observed to be significant in each year ($P < 0.05$). Similarly, the damage to the stems caused by larvae of *M. oedipus* increased significantly over the years ($P < 0.05$; Table 3), even though *M. oedipus* adults were not consistently observed.

Effect of natural enemies on *M. diplotricha*

Populations of nymphs and adults of *H. spinulosa* and incidence of damaged branches of *M. diplotricha* increased consistently between years, significantly from 2009-2010 (Table 4). However, incidence of dead branches remained low.

Discussion

Worldwide, biological weed control programs have had an overall success rate of 33 percent, with success rates considerably higher for programs in individual countries (Culliney, 2005). According to Reddy (2011), *C. odorata* has been rated the eighth most invasive weed on Guam. The two introduced natural enemies, *C. connexa* and *P. pseudoinsulata*, were well established on all parts of Guam. First and foremost, *C. connexa* has significantly affected

the height of *C. odorata*. Our results agree with Zachariades et al. (2009), who suggested that *C. connexa* may provide good control of *C. odorata*. The impact of *C. connexa* on growth and reproduction, in addition to the defoliation by *P. pseudoinsulata*, should provide successful control of *C. odorata* in other parts of the world. Although *P. pseudoinsulata* was established on Guam in 1985 followed by *C. connexa* in 2005, *C. odorata* is still among the top invasive weeds in Guam. Therefore, it is advisable to mass rear *P. pseudoinsulata* and release them at various locations in Guam to suppress *C. odorata*.

Coccinia grandis agents *A. cocciniae* and *M. oedipus* established well and provided effective control. Currently, there are no infestations of *C. grandis* on Guam. Although *H. spinulosa* was established at release sites, it has not yet provided a significant control of *M. diplotricha*. Esguerra et al. (1997) reported that a few months after release, *H. spinulosa* became well established and assisted in the control of this weed in both Pohnpei and Yap. Similarly, it is expected that *H. spinulosa* will reduce *M. diplotricha* populations by affecting all aerial parts, causing damage that can lead to the death of the entire plant. A biological control program is underway for mile-a-minute weed, *Mikania micrantha* (L.) Kunth. (Asterales: Asteraceae), the ninth most invasive weed in Guam, using a rust fungus *Puccinia spegazzinii* De Toni (Basidiomycotina: Uredinales) (Reddy, 2011).

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Table 1. Monitoring sites on Guam

Site	Invasive plant	Coordinates	No. of samples per site (n)
Yigo	<i>Chromolaena odorata</i>	13°31.869' N, 144°52.291' E	4
Agat		13°21.789' N, 144°39.001' E	4
Dededo		13°30.700' N, 144°51.173' E	4
Hagatña		13°28.161' N, 144°44.817' E	4
Mangilao		13°26.978' N, 144°48.737' E	4
Talofofo		13°23.025' N, 144°46.342' E	4
Inarajan		13°15.259' N, 144°43.300' E	4
Merizo		13°15.058' N, 144°43.071' E	4
Marbo Cave	<i>Coccinia grandis</i>	13°49.789' N, 144°87.001' E	3
Barrigada		13°28.385' N, 144°48.132' E	3
Inarajan Bay		13°28.395' N, 144°75.885' E	3
Yaña		13°24.359' N, 144°46.352' E	2
Merizo		13°15.063' N, 144°43.074' E	3
Mangilao		13°26.978' N, 144°48.737' E	3
Hagatña		13°28.598' N, 144°44.313' E	3
Tarja Falls	<i>Mimosa diplotricha</i>	13°24.348' N, 144°46.363' E	4
AES, Yigo		13°31.872' N, 144°52.297' E	2

Table 2. Effect of *Pareuchaetes pseudoinsulata* on *Chromolaena odorata*

Year	Mean number $\pm$ SE (n=8) / one m ² quadrat				
	Larvae	Adults	Small larval holes	Large larval holes	Yellow leaves
2006	2.4 $\pm$ 0.8a	0.0 $\pm$ 0.0a	8.0 $\pm$ 0.4a	3.2 $\pm$ 0.8a	3.0 $\pm$ 0.1a
2007	3.3 $\pm$ 1.2a	0.0 $\pm$ 0.0a	8.5 $\pm$ 2.8a	4.0 $\pm$ 0.3a	2.0 $\pm$ 0.6a
2008	1.6 $\pm$ 1.4a	0.0 $\pm$ 0.0a	7.0 $\pm$ 0.9a	3.0 $\pm$ 0.5a	1.4 $\pm$ 0.3a
2009	1.8 $\pm$ 0.5a	0.0 $\pm$ 0.0a	4.5 $\pm$ 1.2a	0.0 $\pm$ 0.0a	2.5 $\pm$ 0.4a
2010	4.5 $\pm$ 0.6a	3.5 $\pm$ 0.7a	12.4 $\pm$ 0.3b	8.5 $\pm$ 0.6a	4.5 $\pm$ 0.8a

Means within each column followed by different letters are significantly different at the P<0.05 level.

Table 3. Effect of *Acythopeus coccinae*, *Acythopeus burkhartorum* and *Melittia oedipus* on *Coccinia grandis*

Date	Mean number $\pm$ SE (n=6) / one m ² quadrat					
	<i>Acythopeus coccinae</i>		<i>Acythopeus burkhartorum</i>		<i>Melittia oedipus</i>	
	Feeding holes on leaves	Adults	No. of galls formed	Adults	Larval damage on stems	Adults
2006	43.2 $\pm$ 2.2a	22.5 $\pm$ 3.4a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	not released	not released
2007	120.9 $\pm$ 4.1b	34.2 $\pm$ 1.8b	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	2.4 $\pm$ 2.7a	0.0 $\pm$ 0.0a
2008	223.6 $\pm$ 3.4c	46.4 $\pm$ 0.6c	2.4 $\pm$ 1.3a	2.0 $\pm$ 0.4a	12.5 $\pm$ 1.2b	2.0 $\pm$ 0.2a
2009	436.2 $\pm$ 1.8d	48.6 $\pm$ 4.2c	4.0 $\pm$ 0.6a	0.0 $\pm$ 0.0a	22.6 $\pm$ 3.1c	0.0 $\pm$ 0.0a
2010	all plants died					

Means within each column followed by different letters are significantly different at the P<0.05 level.

Table 4. Effect of *Heteropsylla spinulosa* on *Mimosa diplotricha*

Date	Mean number $\pm$ SE (n=4) / one m ² quadrat		
	Nymphs/Adults	Damaged branches	Dead branches
2008	8.4 $\pm$ 1.8a	1.5 $\pm$ 0.2a	0.0 $\pm$ 0.0a
2009	11.6 $\pm$ 2.4a	6.4 $\pm$ 0.1a	0.5 $\pm$ 0.2a
2010	28.0 $\pm$ 0.3b	13.0 $\pm$ 2.8b	4.2 $\pm$ 1.2a

Means within each column followed by different letters are significantly different at the P<0.05 level.

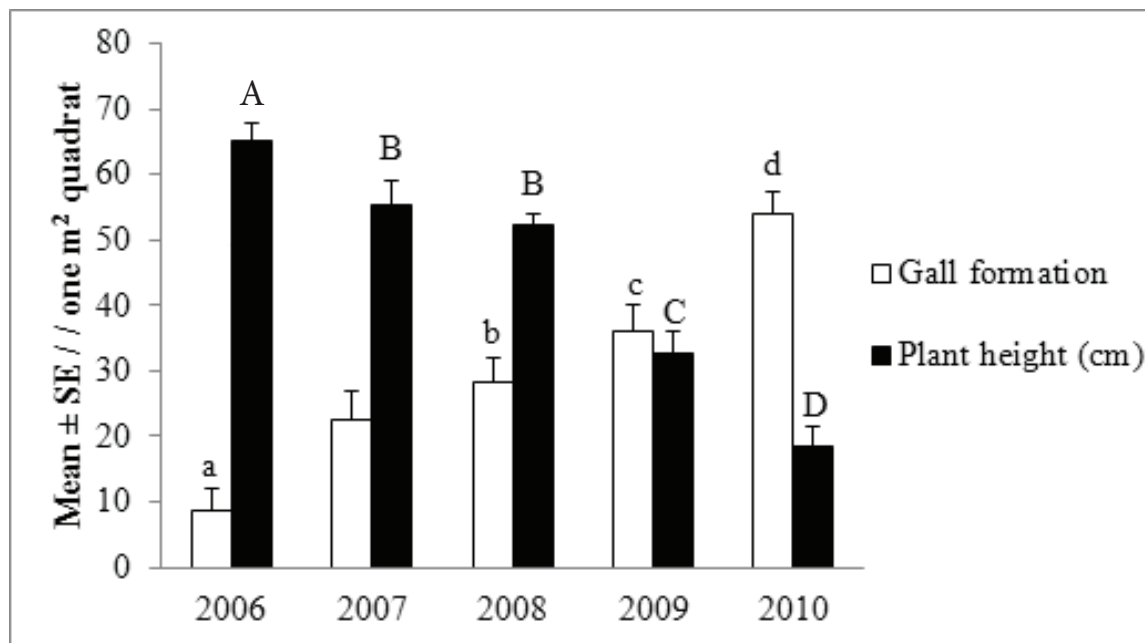


Figure 1. Effect of *Cecidochares connexa* on gall formation and plant height (n=8) of *Chromolaena odorata*. Means marked by different letters are significantly different at the P<0.05 level.

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Releases of Natural Enemies in Hawaii since 1980 for Classical Biological Control of Weeds

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Abstract

A comprehensive review of biological control of weeds in Hawaii was last published in 1992, covering 74 natural enemy species released from 1902 through 1980. The present review summarizes releases of 21 natural enemies targeting seven invasive weeds from 1981 to 2010. These projects were carried out by Hawaii Department of Agriculture (HDOA), USDA Forest Service (USFS), University of Hawaii (UH), and US Geological Survey Biological Resources Discipline. An appendix summarizing the chronology and outcomes of releases is included (Appendix 1).

Introduction

The practice of classical biological control of weeds began in Hawaii in 1902, with the release by the Territory of Hawaii of a tinging lacebug (*Teleonemia scrupulosa* Stål) for control of lantana (*Lantana camara* L.) (Swezey, 1924). Since that time, Hawaii has witnessed several spectacular successes in weed biological control, and today continues the science of managing weeds at the landscape level using natural enemies introduced from the target's native range after thorough testing and evaluation. The most recent comprehensive review of weed biological control in Hawaii (Markin et al., 1992) summarized 74 introductions of natural enemies between 1902 and 1980. Additional information for introductions through the mid-1990s was included in the worldwide compilation by Julien and Griffiths

(1998). Hawaii's weed biological control projects from 1902 to 1980 resulted in establishment of 42 insects and one fungal agent on 19 target weeds.

Our objective here is to provide an updated report of agents released for weed biological control in Hawaii. We briefly summarize results for each natural enemy released since 1981, and we include an appendix listing all weed biological control agents released in Hawaii from 1902 to 2010 (Appendix 1).

Methods

Data on weed targets and weed biological control agents released since 1981 were compiled from published and unpublished sources, including records of the Hawaii Department of Agriculture and personal observations of biological control specialists and weed management partners.

Results

***Passiflora tarminiana* Coppens & V. E. Barney (Passifloraceae), banana poka**

Passiflora tarminiana (formerly *P. mollissima*) is a vine native to the South American Andes, apparently introduced into Hawaii in the early 1900s (Wagner et al., 1990). It is invasive in native forests on the islands of Hawaii and Kauai, and is found in alien forest habitat in the Kula area of Maui (Hauff, 2006). Funding for the biological control program in Hawaii came primarily from the USFS and National Park Service. *P. tarminiana* also is invasive in New Zealand, where a biological control program is being implemented (Williams and Hayes, 2007).

***Scea* (= *Cyanotricha*) *necyria* (Felder & Rogenhof) (Notodontidae)**

Scea necyria was imported from Colombia and subsequently released on the islands of Hawaii, Kauai and Maui, but failed to establish. Despite repeated releases between 1988 and 1992 totaling over 15,000 individuals (including adult moths, defoliating larvae, and eggs), no evidence of establishment was ever observed. Larvae and pupae collected from the field exhibited 17% and 9% parasitism, respectively, while no parasites were recovered from field collected eggs (Campbell et al., 1995). It was suspected that *S. necyria* pupae may have been significantly preyed upon by birds. Research in Colombia has suggested that adult moths may be missing an obligate nectar source in the Hawaii environments where this species was released (Campbell et al., 1995).

***Pyrausta perelegans* Hampson (Crambidae)**

Pyrausta perelegans imported from Venezuela was released on Hawaii, Maui, and Kauai islands in 1991-1992, but established only on Hawaii and Maui. Larvae feed inside flower buds, consuming the ovary, anther, gynophore, inner flower tube, and petals (Ramadan et al., 2008). *P. perelegans* populations have remained at low levels, with vine infestation rates averaging 2-11% in post-release monitoring in 1992-1993 by Ramadan et al. (2008), and around 2% or less of flower buds infested a decade later (M.T. Johnson, unpublished data). While generalist lepidopteran parasitoids were

known to be active in the study area and could utilize *P. perelegans* in lab tests, no field-collected larvae or pupae were found to be parasitized (Ramadan et al., 2008). *Trichogramma chilonis* (Trichogrammatidae) parasitism affected from 0 to 26% of field collected eggs (Ramadan et al., 2008), and may be a significant factor suppressing *P. perelegans* (Campbell et al., 1995). Several common predators of lepidopteran larvae were noted also, but definitive evidence of their impacts on *P. perelegans* was lacking (Campbell et al., 1995; Ramadan et al., 2008).

***Septoria passiflorae* Syd. (Mycosphaerellaceae)**

Septoria passiflorae, a fungus originally from Colombia, attacks *P. tarminiana* leaves, first forming distinct yellow spots which eventually spread to cover much of the leaf and cause premature abscission. It was released on Hawaii Island in 1996 (Trujillo et al., 2001), and quickly controlled a large infestation of banana poka, which had smothered native forest canopy at approximately 2,000 m elevation. *S. passiflorae* was much less effective in the drier habitat of Kula, Maui (G. Shishido, pers. comm.). In Kokee, Kauai, defoliation of vines was observed at inoculation sites, but the clumped distribution of the weed in the forest may have inhibited dispersal of the pathogen (G. Kawakami, pers. comm.). Furthermore, the climate in Kokee is somewhat drier than the windward inoculation sites on the island of Hawaii, where infection and control results were substantial (Trujillo, 2005).

***Coccinia grandis* (L.) Voigt (Cucurbitaceae), ivy gourd**

Ivy gourd is probably native to central East Africa, and was most likely moved to the Indo-Malayan region in centuries past. It may have come to Hawaii as a food or medicinal plant, via immigrants from that region. Ivy gourd was first reported on Oahu in 1968, and has since spread to Hawaii, Maui, Kauai and Lanai. This climbing vine blanketed large areas of alien wayside trees and shrubs on the island of Oahu, and less so in drier Kona (Hawaii). Three insects were released for biological control between 1996 and 1999 (Chun, 2002).

***Melittia oedipus* Oberthür (Sesiidae)**

Melittia oedipus, a clear-winged moth from

Kenya, was released from 1996 through 2002. Larvae feed in both roots and stems of the vine, forming galls. Galls can grow larger than 1.5 cm and cause breakage of the vine, so that the foliage above in the tree canopy dies. *M. oedipus* appears to be responsible for a widespread reduction in ivy gourd foliage density in tree and shrub canopies on Oahu. Development of land for housing also contributed to the decline of ivy gourd in the Kona area on Hawaii Island. Rat predation on *M. oedipus* larvae and pupae appeared to be significant, but the potential for biotic interference has not been studied further (Chun, 2002).

***Acythopeus coccineae* O'Brien and Pakaluk, *Acythopeus burkhartorum* O'Brien and Pakaluk (Curculionidae)**

Two weevil species of the genus *Acythopeus* were first released in 1999. *A. burkhartorum*, a stem gall former, failed to establish, despite many releases in different habitats. *A. coccineae*, whose larvae are leaf miners, established on Oahu and in Kona, and appears capable of some impact. Ivy gourd has been a target of physical containment on Kauai, Lanai and Maui, and these efforts, on Maui in particular, likely contributed to lack of establishment of *A. coccineae* there. The parasitoid *Eupelmus* prob. *cushmani* (Eupelmidae) has been reared from *A. coccineae* larvae and pupae, and may contribute to the low numbers of *A. coccineae* in field populations (M. Ramadan, pers. comm.). Both *Acythopeus* species were also released for the biological control of ivy gourd in Guam, however, biotic interference by an unidentified hymenopteran parasitoid emerging from *A. coccineae* pupae was noted (Muniappan et al., 2009).

Miconia calvenscens* DC. (Melastomataceae), *miconia

Miconia was introduced to Hawaii as an ornamental tree around 1961 and planted on four of the major Hawaiian Islands before it was recognized as a serious invasive threat to native forests and watersheds (Conant et al., 1997). *Miconia* containment programs have been in effect since 1991 on Hawaii and Maui, and eradication programs continue on Oahu and Kauai. The containment effort on Maui has been very expensive, entailing aerial application of herbicide and chemical/

mechanical control on the ground. On Hawaii, the task is even more daunting, given the large area invaded, and resources are presently not available to maintain extensive control. A single fungal pathogen has been released for *miconia* biological control, in both Hawaii and Tahiti. Additional agents are under development (Johnson, 2010).

***Colletotrichum gloeosporioides* (Penz.) f. sp. *miconiae* Killgore et al. (Glomerellaceae)**

This anthracnose fungus, released in 1997, is currently the only natural enemy approved for *miconia* control in Hawaii (Killgore, 2002). Although laboratory tests by Meyer et al. (2008) found significant mortality of *miconia* seedlings infected with *C. g. f. sp. miconiae* in Tahiti, the effects of the fungus on flowering and fruiting remain unknown. Post-release evaluation studies found this agent causing premature leaf drop in wild sapling plants in Hawaii (Brenner, 2000). At higher elevations in Tahiti, partial defoliation of large monospecific stands have increased light levels penetrating the canopy so that some limited regeneration of native flora is occurring (Meyer et al., 2011).

Lantana camara* L. (Verbenaceae), *lantana

Lantana has been a target of weed biological control work worldwide since 1902, when the first ever natural enemy was released here in Hawaii (Swezey, 1924). A total of 25 agents have been released to control this weed in Hawaii, with only one release made after 1980.

***Septoria* sp. (Mycosphaerellaceae)**

Septoria sp. collected from *lantana* in Ecuador was first released in 1997 by Dr. Eduardo E. Trujillo (UH) on the islands of Hawaii and Kauai (Trujillo, 2005). He reported excellent control of *lantana* at inoculated sites at Kokee on Kauai. There has been no further update published on the impact of the fungal pathogen on its host.

Clidemia hirta* (L.) D. Don (Melastomataceae), *Koster's curse

Clidemia hirta was first collected in Hawaii in 1949, but was known to occur on Oahu since 1941 (Wagner et al., 1990). It is now highly invasive on all

the islands except Niihau and Kahoolawe, which are probably too dry. It is a large shrub that dominates the subcanopy of both native and alien wet forests, and also can be a serious pest of windward pastures. Effectiveness of biological control agents released before 1981 is thought to be limited to open, sunny habitats (Reimer and Beardsley, 1986; Reimer and Beardsley, 1988). As a result of HDOA exploration and testing in the Republic of Trinidad and Tobago, four more insect agents were released between 1988 and 1995. Additional natural enemies are still needed for sufficient control.

***Lius poseidon* Napp (Buprestidae)**

Larvae of *Lius poseidon*, released in 1988, are leaf miners of *C. hirta*, while adult beetles are leaf feeders (Conant, 2002). Larvae are parasitized by at least one natural enemy, intentionally released for the control of agromyzid leaf miners (Conant, 2002). Combined foliar damage by adults and larvae to wild plants has been minimal.

***Antiblemma acclinalis* Hübner (Noctuidae)**

Antiblemma acclinalis was first released in 1995 (Conant, 2009). This leaf feeder is recorded as established on Oahu and Kauai, but appears to never have become common, perhaps due to parasitism.

***Carposina bullata* Meyrick (Carposinidae)**

Carposina bullata, first liberated in 1995 failed to establish, possibly due to the low number of pupae that survived shipment from Tobago (Conant, 2009). Poor survival resulted in only about 140 adults being released, primarily on Hawaii and very few on Oahu.

***Mompha trithalama* Meyrick (Coleophoridae)**

Mompha trithalama survived shipment from Tobago much better than *C. bullata*, resulting in a higher number of individuals released on Hawaii and Oahu in 1995 (Conant, 2009). *M. trithalama* was subsequently moved to Kauai and Maui. Larvae can now be found statewide, commonly feeding on the seeds of green fruit. It is possible that *M. trithalama* damage can result in premature fruit drop, but the overall impact of this agent on seed production has not yet been quantified. A

pteromalid wasp parasitoid has been reared from the young fruit of Koster's curse, suggesting possible biotic interference (T. Johnson, unpublished data).

***Colletotrichum gloeosporioides* f. sp. *clidemiae* Trujillo (Glomerellaceae)**

This pathogen was imported by Trujillo (2005) from Panama and released in 1986. Repeated annual inoculations were observed to reduce *C. hirta* cover in one area, and similar mycoherbicidal application has been pursued elsewhere on a limited basis. More recently, natural defoliation events affecting as much as 90% of *C. hirta* cover were observed on the wet windward side of Kauai, but plants appeared to recover with time (N. Barca, pers. comm.). This phenomenon has been commonly observed on several islands at unpredictable intervals once the fungus became well established in the 1990s. Cool, rainy, windy weather conditions appear conducive to outbreaks.

***Morella* (= *Myrica*) *faya* (Aiton) Wilbur (Myricaceae), firetree**

Morella faya is thought to have been introduced by Portuguese plantation workers who emigrated from Atlantic islands in the 1800s. Firetree was used for reforestation in Hawaii in the 1920s, and now occurs on the islands of Hawaii, Maui, Lanai, Oahu and Kauai (Whiteaker and Gardner, 1992). *M. faya* is considered one of the worst invasive species in Hawaii Volcanoes National Park, since its ability to fix nitrogen allows it to thrive in low-nutrient lava soils (Whiteaker and Gardner, 1985). A tortricid moth released for biological control of *M. faya* by HDOA in 1956 is considered ineffective, because it only affected a minor population of *Morella cerifera* (L.) Small (Markin, 2001). The National Park Service provided most of the funding for *M. faya* biological control efforts in the 1980s and early 1990s. Two natural enemies were released since 1980, but neither appears to have any noticeable impact (G. Markin, pers. observation).

***Caloptilia coruscans* (Walsingham) [= *C. sp. nr. schinella* (Walsingham)] (Gracillariidae)**

Caloptilia coruscans, whose larvae are blotch miners and leaf edge rollers, was released in 1991 from

collections made in the Azores and Madeira (Markin, 2001). Populations have remained low, and foliar damage has been minimal. Field collected larvae were infected by a fungal pathogen, hymenopteran parasitoids emerged from pupae, and lab tests found that local parasitoids attack its egg stage (Markin, 2001). Evidence of biotic interference also includes high levels of predation of *C. coruscans* larvae within leaf rolls (P. Yang and D. Foote, unpublished data).

***Septoria hodgei* Gardner
(Mycosphaerellaceae)**

Septoria hodgei, a fungus collected from wax myrtle, *Morella cerifera*, in North Carolina, was tested for host specificity at the HDOA Plant Pathogen Containment Facility in Honolulu and was subsequently released and briefly monitored on Hawaii Island in 1997 (Culliney et al., 2003). *S. hodgei* was found established, but was never abundant in the experimental area, and no follow up work has been done. Smith (2002) mentions a personal communication from Trujillo, which suggested that acid rain near the volcano could be inhibiting spore germination, and that the fungus should be released elsewhere.

***Ulex europaeus* L. (Fabaceae), gorse**

Ulex europaeus was introduced to Hawaii in the 1800s possibly as a cultivated hedge row plant from Europe, and may also have been used as a grazing plant for sheep. It now occupies over 2,000 ha on Mauna Kea (Hawaii) and over 2,000 ha in the Olinda area on Maui. The first biological control effort against gorse was launched by HDOA in 1927 and more recently has been a cooperative effort among HDOA, USFS and Landcare Research of New Zealand. In total, eight agents have been released, but only four became established (Markin and Conant, in these proceedings).

***Agonopterix ulicetella* (Stainton) (Oecophoridae)**

Agonopterix ulicetella was first released in 1988 and became established on both Hawaii and Maui (Markin et al., 1995). This species is univoltine and overwinters as adults. In the spring larvae feed on terminal shoots, sometimes causing considerable damage, which can reduce

flowering (Markin, unpublished data). Three species of parasitoids attack *A. ulicetella* pupae, but no larval parasitoids had ever been found, even with intensive searching, and egg parasitism by *Trichogramma* sp. appeared low (Markin et al., 1996).

***Stenopterapion scutellare* (Kirby) (Brentidae)**

Stenopterapion scutellare was first liberated in 1961 (Markin and Yoshioka, 1989) on Maui (186 released), but failed to establish and so was re-released in 1989 on the island of Hawaii (788 released). None of the characteristic galls caused by the weevil were ever seen outside the field release cages in the 1990s, nor have they been seen since then.

***Sericothrips staphylinus* Haliday (Thripidae),
gorse thrips**

Two biotypes of this thrips, both a winged and brachypterous form, were released in Hawaii between 1991 and 1994 (Markin et al., 1996). The thrips feed on the mesophyll of gorse foliage, producing pale stippled areas on leaves, spines, and stems (Hill et al., 2001). Both biotypes became established and widespread in the gorse infestation on Hawaii Island, but finding visible feeding damage in the field on mature plants is difficult. Seedlings may be more affected, given that *S. staphylinus* at high densities in lab experiments can damage and kill gorse seedlings (Fowler and Griffin, 1995). Populations of this agent may be limited by predators (Markin and Conant, in these proceedings).

***Tetranychus lintearius* Dufour
(Tetranychidae), gorse spider mite**

First released in 1995 (Culliney and Nagamine, 2000), *Tetranychus lintearius* dispersed rapidly throughout the gorse infestation on Hawaii Island, and was subsequently released on Maui. *T. lintearius* populations increased tremendously during summer seasons, and resulted in considerable damage to gorse foliage in disjunct patches. *T. lintearius* feeding induces mottled chlorosis with stunting, and flowering may be aborted on damaged shoots in spring. Some shoots may even die from heavy feeding (Hill et al., 1991). However, after the year 2000, annual *T. lintearius* population explosions

ceased, presumably caused by an accumulation of predacious mite populations.

***Pempelia genistella* (Duponchel)**

(Pyralidae), gorse hard shoot moth

Releases of *Pempelia genistella* on Hawaii were conducted by USFS 1996-1997, but after initial establishment all populations apparently were eradicated as a result of a 2001-2002 control effort using herbicide and burning (Markin et al., 2002; Markin and Conant, in these proceedings). In its native habitat in Europe, larvae feed during fall and winter months, followed by pupation and adult emergence in early summer. Larvae often feed gregariously within webbing on older spines and may girdle the terminal shoots (Markin et al., 1996).

***Uromyces pisi* (DC.) Otth f. sp. *europaei* Wilson and Henderson (Pucciniaceae)**

This rust fungus was released on Hawaii Island in 2000, and was seen once soon after, but never recorded again during occasional searches over several years (Markin et al., 2002; Markin and Conant, in these proceedings).

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Appendix 1. Agents introduced to Hawaii for biological control of weeds 1902-2010, compiled from Julien and Griffiths (1998), recent publications cited in this review, and unpublished records of the Hawaii Department of Agriculture.

Target Weed	Agent Origin	First Release	Agent Family	Agent Species	Impact
Lantana	Mexico	1902	Agromyzidae	<i>Ophiomyia lantanæ</i> (Froggatt)	Ineffective
<i>Lantana camara</i> L. (Verbenaceae)	Mexico	1902	Brentidae	<i>Apion</i> , 2 spp.	Not established
	Mexico	1902	Cerambycidae	<i>Parevander xanthomelas</i> (Guérin-Méneville)	Not established
	Mexico	1902; 1955	Cerambycidae	<i>Aerenicopsis championi</i> Bates	Not established
	Mexico	1902 ¹ ; 1953	Chrysomelidae	<i>Ocotoma scabripennis</i> Guérin-Méneville	Partial
	Mexico	1902	Gracillariidae	<i>Cremastobombycia lantanella</i> Busck	Partial
	Mexico	1902	Hepialidae	<i>Hepialus</i> sp.	Not established
	Mexico	1902	Lycaenidae	<i>Strymon bazochii gundlachianus</i> (Bates)	Ineffective
	Mexico	1902	Lycaenidae	<i>Tmolus echion</i> (L.)	Ineffective
	Mexico	1902	Pterophoridae	<i>Lantanophagapusillidactyla</i> (Walker)	Ineffective
	Mexico	1902	Tephritidae	<i>Eutreta xanthochaeta</i> Aldrich	Ineffective
	Mexico; Cuba; Belize; Florida, USA; Trinidad; Brazil	1902; 1952; 1953; 1954; 1954; 1954	Tingidae	<i>Teleonemia scrupulosa</i> Stål	Partial
	Mexico	1902	Tortricidae	<i>Crociosema lantana</i> Busck	Partial
	Cuba	1953	Chrysomelidae	<i>Ocotoma gundlachi</i> Suffrain	Not established
	Mexico	1953; 1965	Crambidae	<i>Pseudopyrausta santatalis</i> (Barnes & McDunnough)	Not established
	Honduras	1954	Chrysomelidae	<i>Ocotoma championi</i> Baly	Not established
	Panama; Mexico	1954; 1962	Noctuidae	<i>Diastema tigris</i> Guenée	Not established
	California	1955	Noctuidae	<i>Neogalia sunia</i> (Guenée)	Partial
	Florida, USA; Cuba	1956	Crambidae	<i>Salbia haemorrhoidalis</i> Guenée	Substantial
	Kenya/Zimbabwe; Philippines	1957; 1965	Noctuidae	<i>Hypena laceratalis</i> Walker	Substantial
	Mexico	1960	Cerambycidae	<i>Plagiohammus spinipennis</i> (Thomson)	Partial
	Brazil	1961	Chrysomelidae	<i>Uroplata girardi</i> Pic	Partial
	Colombia/Peru via Australia	1970	Tingidae	<i>Leptobyrssa decora</i> Drake	Ineffective
	Ecuador	1997	Mycosphaerellaceae	<i>Septoria</i> sp.	Uncertain ²

Target Weed	Agent Origin	First Release	Agent Family	Agent Species	Impact
Purple nutsedge <i>Cyperus rotundus</i> L. (Cyperaceae)	Philippines	1925	Curculionidae	<i>Athesapeuta cyperi</i> Marshall	Ineffective
	Philippines	1925	Tortricidae	<i>Bactra venosana</i> (Zeller)	Ineffective
Gorse <i>Ulex europaeus</i> L. (Fabaceae)	England; England via New Zealand; France	1927 ¹ ; 1949 ¹ ; 1955	Brentidae	<i>Exapion ulicis</i> (Forster)	Partial
	Portugal	1958	Brentidae	<i>Apion</i> sp.	Not established
	Portugal; Spain; France	1961; 1989; 1990; 1991	Brentidae	<i>Stenopterapion scutellare</i> (Kirby)	Not established
	England; Portugal	1988; 1991	Oecophoridae	<i>Agonopterix ulicetella</i> (Stainton)	Partial
	England; Portugal; France	1991; 1992; 1992	Thripidae	<i>Sericothrips staphylinus</i> Haliday	Uncertain ²
	England, Portugal, Spain via New Zealand via Oregon, USA	1995	Tetranychidae	<i>Tetranychus lintearius</i> Dufour	Partial
	Portugal	1996	Pyralidae	<i>Pempelia genistella</i> (Duponchel)	Not established
	England	2000	Pucciniaceae	<i>Uromyces pisi</i> (DC.) Oth f. <i>europaei</i> Wilson and Henderson	Not established
Maui pamakani <i>Ageratina adenophora</i> (Spreng.) King & H. Rob. (Asteraceae)	Mexico	1945	Tephritidae	<i>Procecidochares utilis</i> Stone	Substantial
	Mexico	1955	Tephritidae	<i>Xanthaciura connexionis</i> Benjamin	Not established ³
Prickly pear <i>Opuntia</i> spp. (Cactaceae)	Mexico via Australia	1949	Dactylopiidae	<i>Dactylopius opuntiae</i> (Cockerell)	Substantial
	Texas, USA	1949	Pyralidae	<i>Melitara dentata</i> (Grote)	Not established
	Texas, USA	1949	Pyralidae	<i>Melitara prodentialis</i> Walker	Not established
	Texas, USA	1950	Cerambycidae	<i>Monilema armatum</i> LeConte	Not established
	Argentina via Australia	1950	Pyralidae	<i>Cactoblastis cactorum</i> (Berg)	Substantial
	Mexico via Australia	1951	Cerambycidae	<i>Lagocheirus funestus</i> Thomson	Partial

Target Weed	Agent Origin	First Release	Agent Family	Agent Species	Impact
Koster's curse <i>Clidemia hirta</i> (L.) D. Don (Melastomataceae)	Trinidad via Fiji	1953	Phlaeothripidae	<i>Liothrips urichi</i> Karny	Partial
	Trinidad; Puerto Rico	1970	Crambidae	<i>Ategumia matutinalis</i> Guenée	Ineffective
	Panama	1986	Glomerellaceae	<i>Colletotrichum gloeosporioides</i> f. sp. <i>clidemiae</i> Trujillo	Partial
	Trinidad	1988	Buprestidae	<i>Liua poseidon</i> Napp	Ineffective
	Tobago	1995	Carposinidae	<i>Carposina bullata</i> Meyrick	Not established
	Tobago	1995	Coleophoridae	<i>Mompha trithalama</i> Meyrick	Partial
Christmas berry <i>Schinus terebinthifolius</i> Raddi (Anacardiaceae)	Tobago	1995	Noctuidae	<i>Antiblemma acclinalis</i> Hübner	Ineffective
	Brazil	1954	Tortricidae	<i>Episimus unguiculus</i> Clarke	Ineffective
	Brazil	1960	Bruchidae	<i>Lithraeus atronotatus</i> (Pic)	Ineffective
	Brazil	1961	Gelechiidae	<i>Grasimorpha infusata</i> Hodges	Not established
	Florida/Georgia, USA	1956	Tortricidae	<i>Strepsicrates smithiana</i> Walsingham	Ineffective
	Azores/Madeira, Portugal	1991	Gracillariidae	<i>Caloptilia coruscans</i> (= <i>C. sp. nr. schindella</i>) (Walsingham)	Ineffective
Sourbush <i>Pluchea carolinensis</i> (Jacq.) G. Don (Asteraceae)	North Carolina, USA	1997	Mycosphaerellaceae	<i>Septoria hodgesii</i> D.E. Gardner	Uncertain ²
	Mexico	1957	Gelechiidae	<i>Dichomeris aenigmatica</i> (Clarke)	Ineffective
	Guatemala	1959	Tephritidae	<i>Acinia picturata</i> (Snow)	Ineffective
Emex <i>Emex australis</i> Steinheil <i>Emex spinosa</i> (L.) Campd. (Polygonaceae)	South Africa	1957	Brentidae	<i>Perapion antiquum</i> (Gyllenhal)	Substantial
	Morocco	1962	Brentidae	<i>Perapion neofallax</i> (Warner)	Not established
	Portugal	1962	Brentidae	<i>Perapion violaceum</i> (Kirby)	Not established

Target Weed	Agent Origin	First Release	Agent Family	Agent Species	Impact
Asian melastome <i>Melastoma septemnerium</i> Lour. (Melastomataceae)	Philippines	1958	Crambidae	<i>Ategumia fatualis</i> (Lederer)	Ineffective
	Malaysia	1965	Crambidae	<i>Ategumia adipalis</i> (Lederer)	Ineffective
	Malaysia	1965	Noctuidae	<i>Rhynchopalpus brunellus</i> (Hampson)	Partial
Elephant's foot <i>Elephantopus mollis</i> Kunth (Asteraceae)	Trinidad via Fiji	1961	Tephritidae	<i>Tetraeuaresta obscuriventris</i> Loew	Ineffective
Puncture vine <i>Tribulus cistoides</i> L. <i>Tribulus terrestris</i> L. (Zygophyllaceae)	Italy via Calif./Arizona, USA	1962	Curculionidae	<i>Microlarinus lareynii</i> (Jacquelin du Val)	Substantial
	Italy via Calif./Arizona, USA	1963	Curculionidae	<i>Microlarinus lypriformis</i> (Wollaston)	Substantial
Florida blackberry <i>Rubus argutus</i> Link (Rosaceae)	California, USA	1963	Schreckensteiniidae	<i>Schreckensteiniella festaliella</i> Hübner	Partial
	Oregon, USA	1963	Sesiidae	<i>Pemisetia marginata</i> (Harris)	Not established
	Mexico	1964	Tortricidae	<i>Croesia zimmermani</i> Clarke	Partial
	Oregon/California, USA	1966	Tenthredinidae	<i>Priophorus morio</i> (Lepeletier)	Ineffective
Klamath weed <i>Hypericum perforatum</i> (Hypericaceae)	Missouri, USA	1969	Chrysomelidae	<i>Chlamisus gibbosa</i> (Fabricius)	Not established
	France via California, USA via Australia/New Zealand	1965	Cecidomyiidae	<i>Zeuxidiplosis giardi</i> (Keiffer)	Substantial
	France via Australia via California, USA	1965	Chrysomelidae	<i>Chrysolina quadrigemina</i> (Suffrain)	Substantial
Hamakua pamakani <i>Ageratina riparia</i> (Regel) King & H. Rob. (Asteraceae)	Mexico	1973	Pterophoridae	<i>Oidaematophorus beneficus</i> Yano & Heppner	Partial
	Mexico	1974	Tephritidae	<i>Procecidochares alani</i> Steyskal	Partial
	Jamaica	1975	Entylomataceae	<i>Entyloma ageratinae</i> Barreto & Evans	Substantial
Russian thistle <i>Salsola tragus</i> L. (Chenopodiaceae)	Pakistan via California, USA	1980	Coleophoridae	<i>Coleophora klimeschiella</i> Toll	Not established
	Egypt/Pakistan via Califor- nia, USA	1980	Coleophoridae	<i>Coleophora parthenica</i> Meyrick	Not established

Target Weed	Agent Origin	First Release	Agent Family	Agent Species	Impact
Banana poka <i>Passiflora tarminiana</i> Coppens & V.E. Barney (Passifloraceae)	Colombia/ Ecuador	1988	Notodontidae	<i>Scea</i> (= <i>Cyanotricha</i>) <i>necyria</i> (Felder & Roggenhofer)	Not established
	Venezuela	1991	Crambidae	<i>Pyrausta peregans</i> Hampson	Ineffective
	Colombia	1996	Mycosphaerellaceae	<i>Septoria passiflorae</i> Syd.	Partial
Ivy gourd <i>Coccinia grandis</i> (L.) Voigt (Cucurbitaceae)	Kenya	1996	Sesiidae	<i>Melittia oedipus</i> Oberthür	Substantial
	Kenya	1999	Curculionidae	<i>Acythopeus burkhardtorum</i> O'Brien and Pakaluk	Not established
	Kenya	1999	Curculionidae	<i>Acythopeus coccinae</i> O'Brien and Pakaluk	Partial
Miconia <i>Miconia calvescens</i> DC. (Melastomataceae)	Brazil	1997	Glomerellaceae	<i>Colletotrichum gloeosporioides</i> (Penz.) f. sp. <i>miconiae</i> Killgore et al.	Partial

¹ These early releases failed to establish.

² Established, but population and/or distribution limited, and evidence on impact lacking.

³ Agent apparently was released on both *Ageratina* spp.

Gall Nematode of *Miconia*: A Potential Classical Biological Control Agent for Weedy Melastomataceae

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Abstract

Miconia (*Miconia calvenscens* DC.) is a small tree or shrub from the neotropics that was introduced into Pacific Islands as an ornamental plant and, released from its natural enemies, became a devastating invader of native forests. Exploratory assessments of plant pathogens to be used for biological control have yielded a variety of possible agents, including two nematode species belonging to the genus *Ditylenchus*, namely: *D. drepanocercus* Goodey and a new species *Ditylenchus* sp. nov. The latter forms abundant galls and deformations on aerial tissues of several Melastomataceae, including *M. calvenscens* and Koster's Curse (*Clidemia hirta* (L.) D. Don), which also is a noxious invader in Pacific islands. The disease caused by this nematode can be very severe and therefore it was intensively investigated to elucidate: aspects of its biology and ecology; gall ontogeny; host range; and impact on *M. calvenscens*. Its host range is restricted to plants belonging to the Melastomataceae, with members of the genus *Miconia* appearing to be the most susceptible. *Ditylenchus* sp. nov. was pathogenic both to the Brazilian and the Hawaiian biotypes of *M. calvenscens*. Population dynamics of this nematode species were studied in Viçosa, and it was found capable of surviving in a state of anhydrobiosis, in dehydrated plant materials, for up to six months. There was a decrease in the development of plants of *M. calvenscens* with increased concentration of the inoculum, indicating the potential for significant impact by the nematode. Considering that there are no native members of the Melastomataceae in the Hawaiian flora and that other members of this family introduced into Hawaii are either weedy or economically insignificant, we conclude that *Ditylenchus* sp. nov. has clear potential for use as a classical biological control agent against *M. calvenscens* and possibly also *C. hirta*.

See our recent publication: Oliveira, R. D. L., Santin, A. M., Seni, D. J. Dietrich, A., Salazar, L.A., Subbotin, S. A., Mundo-Ocampo, M., Goldenberg, R., Barreto, R. W. (2012) *Ditylenchus gallaeformans* sp. n. (Tylenchida: Anguinidae) - a neotropical nematode with biocontrol potential against weedy Melastomataceae. Nematology, DOI: 10.1163/15685411-00002670

Lepidopterans as Potential Agents for the Biological Control of *Miconia calvenscens*

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Abstract

Miconia (*Miconia calvenscens* DC.) (Melastomataceae) is a severe weed found in rainforest ecosystems on oceanic islands, including French Polynesia, Hawaii and New Caledonia, and Australia, where it was introduced as ornamental plant. This plant is native to Central and South America and is classified among the 100 worst invasive species around the world. To select agents for biological control of *M. calvenscens*, surveys of the arthropods that attack this weed in Brazil have been carried out since 2001. Eight species of Lepidoptera were found attacking *M. calvenscens*, including six defoliators: *Salbia lotanalis* Druce (Pyralidae), *Druentia inscita* Schaus (Mimallonidae), *Antiblemma leucocyma* Hampson (Noctuidae) and three unidentified Limacodidae species; a fruit borer: *Carposina cardinata* Meyrick (Carposinidae); and a flower feeder: *Pleuroprucha rudimentaria* Guenée (Geometridae). We evaluated the damage, host specificity and population dynamics of these Lepidoptera species and the field occurrences of their natural enemies. Based on host specificity and damage caused to plants, *S. lotanalis* and *D. inscita* are the most promising species for biological control of *M. calvenscens*. If *C. cardinata* and *P. rudimentaria* prove host-specific in future tests, they may also be appropriate as biological control agents.

Can Wild Gingers Ever be Tamed? The Search for Natural Enemies Hots up

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Abstract

Kahili ginger, *Hedychium gardnerianum* Sheppard ex J. B. Ker Gawler, and yellow ginger, *H. flavescens* W. Carey ex W. Roscoe (Zingiberaceae), stunning and fragrant ornamental herbs native to the Eastern Himalayan foothills, have escaped cultivation to become aggressive colonizers of indigenous and intact forest habitats, smothering unique and delicate ecosystems and threatening specialized communities. In the worst affected countries such as the US (Hawaii) and New Zealand, Kahili ginger continues its range expansion through seed spread to new pristine sites, while large monotypic infestations are deemed lost causes, with management efforts largely restricted to outlier populations. In 2008, consortium funding allowed an exploratory survey to the states of Assam, Meghalaya and Sikkim in India, with reviews of the scientific and botanical literature, as well as historical herbarium records providing the geographical focus. Since then, repeated field trips across the season have been conducted in Sikkim, where the most natural populations of Kahili ginger were identified. Whilst literature studies highlighted a dearth of damaging species associated with wild gingers in the introduced range, the plant was always subject to significant natural enemy pressure in India, from a diverse entomofauna occupying a range of niches/guilds as well as from pathogenic fungi. Here we report the results of surveys with emphasis on those agents which have shown the most promise as biological control agents based on identifications, field observations and preliminary specificity studies. Future prospects and opportunities are discussed in the light of the access and benefit sharing challenges faced thus far.

Determining the Origin of African Tulip Tree, *Spathodea campanulata* (Bignoniaceae), Populations in the Pacific Region Using Genetic Techniques

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Abstract

African tulip tree, *Spathodea campanulata* Beauv. (Bignoniaceae), is a problematic invasive weed in the Pacific region for which a biological control program has been initiated. The species is native in western and central Africa where three distinct subspecies are recognized. The polymorphic nature of the species increases the likelihood that natural enemies collected for biological control will have local adaptation to different variants of the plant. One of the potential biological control agents for *S. campanulata* is a gall forming eriophyid mite that is likely to complete multiple generations on a single host plant individual, and is therefore likely to develop local adaptations to certain plant variants. The African region where the Pacific *S. campanulata* population originated is expected to be the most appropriate region in which to collect biological control agents because natural enemies will be adapted to the same variant of *S. campanulata* that is present in the Pacific region. Morphological characteristics are unreliable for identification to the subspecies level, making it difficult to determine the origin of the Pacific population. DNA sequencing of five non-coding regions and Inter-Simple Sequence Repeats were used to determine the origin of the introduced *S. campanulata* population in Fiji. The closest relatives to the Fijian *S. campanulata* were plants from Ghana indicating that the *S. campanulata* population in Fiji originated from the West African subspecies, *S. campanulata* subsp. *campanulata* Beauv. West Africa is therefore the most appropriate region to survey for potential biological control agents for the management of *S. campanulata* in the Pacific.

Managing *Miconia calvenscens* in Hawaii: Biology and Host Specificity of *Cryptorhynchus melastomae*, a Potential Biological Control Agent

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Abstract

Cryptorhynchus melastomae Champion (Coleoptera: Curculionidae) is a stem boring weevil from Costa Rica under evaluation as a potential biological control agent for the invasive tree miconia, *Miconia calvenscens* DC. (Melastomataceae). Adult *C. melastomae* feed externally on miconia foliage and stems, and larvae bore stems. Under lab rearing the life cycle of *C. melastomae* averages 218 days from egg deposition until death. Eggs hatch within two weeks; larvae undergo rapid growth for the first 70 days and pupate around day 111. Adult eclosion occurs at day 140, and the mean adult lifespan is 75 days, although it is not unusual for adults to survive 4-6 months. Adults reach sexual maturity at one month, and females lay large eggs at a rate of 3-6 per week up until death. Larval feeding can result in death of the distal portion of stem, and adult feeding can severely impact growing tips and leaf veins. Thirty two plant species, including a variety of natives and non-natives within the order Myrtales, were tested to assess potential non-target impacts of this weevil. No-choice and multi-choice tests with adult *C. melastomae* revealed a host range restricted to melastomes (family Melastomataceae), all of which are invasive weeds in Hawaii. In addition to miconia, adults fed mainly on arthrostemma (*Arthrostemma ciliatum* Pav. ex D. Don), Koster's curse (*Clidemia hirta* (L.) D. Don), false meadowbeauty (*Pterolepis glomerata* (Rottb.) Miq.), princess flower (*Tibouchina urvilleana* (DC.) Cogn.), Asian melastome (*Melastoma septemnerivium* Lour.), pearlflower (*Heterocentron subtriplinervium* (Link & Otto) A. Braun & Bouché), and cane tibouchina (*Tibouchina herbacea* (DC.) Cogn.). Egg laying was largely restricted to a subset of these species. Ideally, *C. melastomae* might contribute to management of several species of weedy melastomes, but the actual consequences of such interactions with multiple hosts are difficult to predict.

Biological Control for Management of Cane Tibouchina and Other Weedy Melastome Species in Hawaii

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Abstract

Syphraea uberabensis Bechyné (Coleoptera: Chrysomelidae) is a South American flea beetle whose adults and larvae feed externally on foliage and soft stems of *Tibouchina* spp., causing enough damage to kill small plants. Under quarantine evaluation as a potential biological control agent for cane tibouchina, *Tibouchina herbacea* (DC.) Cogn. (Melastomataceae), *S. uberabensis* has been tested on a variety of native and non-native species within the order Myrtales to identify its expected host range in Hawaii. Multi-choice behavioral tests with adult beetles and no-choice tests with adults and larvae indicated a host range restricted to several species within the tribe Melastomeae, all of which are invasive weeds in Hawaii. Preferences were found for feeding and egg laying on cane tibouchina, longleaf glorytree (*Tibouchina longifolia* (Vahl) Baill. ex Cogn.), false meadowbeauty (*Pterolepis glomerata* (Rottb.) Miq.) and Asian melastome (*Melastoma septemnerium* Lour.), and all four of these species were suitable hosts for the complete life cycle of *S. uberabensis*. Beetles appeared unlikely to impact other seriously invasive melastomes including princess flower (*Tibouchina urvilleana* (DC.) Cogn.), miconia (*Miconia calvenscens* DC.) and Koster's curse (*Clidemia hirta* (L.) D. Don). We consider the potential for using this biological control agent in management of multiple weedy melastomes.

Biological Control of *Solanum mauritianum*: South African Experiences and Prospects for the Pacific Islands

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Abstract

Solanum mauritianum Scop. (Solanaceae), a fast-growing tree with a high seed output, threatens commercial activities and natural habitats in several tropical, subtropical and warm temperate regions worldwide. The plant's high reproductive output, long-distance seed dispersal by birds and short-lived seed banks, suggest that it is an appropriate target for biological control using agents that can reduce its rapid growth rates and high levels of fruiting. South Africa was the first country to explore this possibility and has pursued it for 26 years. The program was recently extended to New Zealand, and several other countries (e.g., Pacific Islands) also may be able to benefit from South African experiences. Although surveys in South America have revealed several promising candidate agents, there have been major difficulties in securing their release because of the conservative nature of host-specificity testing and several cultivated and native plant species in the genus *Solanum*. Despite these hurdles, two agents have been released and established in South Africa and are now pending release in New Zealand. Guidelines for countries wishing to target *S. mauritianum* for biological control include: (i) conducting pre-introduction surveys of the plant and any native or cultivated *Solanum* species to record any host-range extensions of native insect herbivores and natural enemies that could affect introduced agents; (ii) selecting host-range testing methodologies that are less likely to yield ambiguous results; (iii) determining the economic or conservation status of all cultivated and native congeneric plants; (iv) quantifying the economic and environmental damage already caused by the weed in relation to any potential risks; and (v) conducting quantitative prerelease studies on the impact of promising agents. Although *S. mauritianum* is a difficult target for biological control, there is potential for success.

Future Prospects for Biological Control of Weeds in Fiji Islands

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Abstract

Fiji has been a strong supporter of biological control of weeds and insect pests. Biological control has been found to be a sustainable and environmentally friendly method, and a viable alternative to the steadily growing use of pesticides. In the past, the use of biological control has achieved great success in control of many weeds in Fiji. Taking this into account, biological control will be needed in the future for many introduced weeds, including African tuliptree (*Spathodea campanulata* P. Beauv.), fire plant (*Clerodendrum quadriloculare* (Blanc.) Merr.), noogora burr (*Xanthium pungens* Wallr.), merremia (*Merremia peltata* (L.) Merr.) , wedelia (*Sphagneticola trilobata* (L.) Pruski) and water lettuce (*Pistia stratiotes* L.). These weeds are major problems for crop cultivation, and are difficult to control using herbicides in some cases. Water lettuce, recently introduced through floriculture, is becoming a problem for waterways.

Defoliation and Leaf-Rolling by *Salbia lotanalís* (Lepidoptera: Pyralidae) Attacking *Miconia calvenscens* (Melastomataceae)

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Abstract

Salbia lotanalís Druce (Lepidoptera: Pyralidae) is a leaf-roller that attacks miconia (*Miconia calvenscens* DC., Melastomataceae) in Costa Rica and Brazil (Morais et al., 2010). We monitored population dynamics of *S. lotanalís* and quantified defoliation and leaf-rolling in *M. calvenscens* leaves at a field site in central Costa Rica. Larval populations peaked during the transition from rainy season to dry season and were lowest at the end of the dry season. Leaf rolling and defoliation both greatly reduced leaf area exposed to sunlight, likely reducing photosynthetic capacity. Damage by *S. lotanalís* was very high compared to other leaf feeders attacking *M. calvenscens* elsewhere in the native range, including larvae of the sawfly *Atomacera petroa* Smith (Hymenoptera: Argidae) and the moth *Antiblemma leucocyma* Hampson (Lepidoptera: Noctuidae) (Badenes-Pérez and Johnson, 2007; Badenes-Pérez and Johnson, 2008).

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Survey for Natural Enemies of *Bocconia frutescens* in Costa Rica

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Abstract

Bocconia (*Bocconia frutescens* L.) (Papaveraceae) are small trees up to 6 m tall native to the Neotropics. *Bocconia* has established populations on the islands of Maui and Hawaii, spreading from dense groves in disturbed sites to invade native dryland and mesic forests, where it is considered a threat to rare endemic organisms. *Bocconia* is recognized as a noxious weed by the State of Hawaii, making it a target for eradication on islands where it is not already established. Mechanical and chemical controls have been pursued in some areas, but are prohibitively expensive over its present wide distribution. *Bocconia* is found in a variety of habitats in biodiversity-protected land of Costa Rica, typically colonizing disturbed areas such as tree falls and landslides but without displaying invasiveness. Plants in this native range were explored for insects and other natural enemies between December 2008 and September 2009. On monthly survey trips habitat and plant conditions were noted, and its natural enemies were photographed and collected for rearing and identification. A total of 38 species of natural enemies have been documented (35 insects, one mite, one phytoplasma and one fungus). Thus far only three potential control agents are recognized: a gregarious leafminer (*Liriomyza* sp.) (Agromyzidae), a treehopper (*Ennya pacifica* Fairmaire) (Membracidae) which deposits egg masses in leaf veins and inflorescence stems, and an unidentified leaf tier moth (Tortricidae) whose larvae damage young leaves. These insects appear to merit further study, but there may be serious constraints (for example, low impact and possible biotic interference) on their efficacy as biological control agents. Additional exploratory field work, perhaps in other areas of *Bocconia*'s native range, might discover more promising biological agents.

SESSION 6: INTEGRATING BIOLOGICAL CONTROL AND RESTORATION OF ECOSYSTEMS

Integrating Biological Control and Native Plantings to Restore Sites Invaded by Mile-A-Minute Weed, *Persicaria perfoliata*, in the Mid-Atlantic USA

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Abstract

Successful biological control can significantly reduce the competitive ability and population density and/or distribution of an invasive weed. However, in some cases the target weed is replaced by other nonnative weeds. If this “invasive treadmill effect” occurs, the biodiversity of the site will not improve. Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross, is an annual vine from Asia that has invaded natural areas in the eastern U.S. The host-specific weevil *Rhinoncomimus latipes* Korotyaev was approved for release in 2004, and is showing considerable success. However, in some sites the suppressed mile-a-minute weed has been replaced by other invasive species, such as Japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus. Two integrated weed management experiments were conducted to determine best practices for breaking the invasive treadmill cycle and restoring native plant communities at sites invaded by mile-a-minute weed. In one experiment, sites received a combination of the biological control weevil, plantings of competitive native vegetation, and a pre-emergent herbicide application. Native plantings consisted of plugs of flat-top goldentop, *Euthamia graminifolia* (L.) Nutt., and seedlings of Dutch elm disease tolerant American elm, *Ulmus americana* L. Integrating these treatments decreased mile-a-minute seedling numbers and prevented Japanese stiltgrass from becoming the dominant vegetation at sites where this weed was abundant. The sites with the greatest pressure from invasive species had a higher percentage of native cover when herbicide and planting treatments were combined compared to the control. In the second experiment, sites were seeded with a mix of native warm and cool season grasses and forbs, with and without weevils, which were excluded using a systemic insecticide. This fully factorial experiment showed reduced mile-a-minute weed cover and greater native plant richness in the treatment with both restoration seeding and biocontrol than for either treatment alone. The results of these experiments suggest that integration of control methods can suppress mile-a-minute weed and help restore a diverse native plant community.

Introduction

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross, is a temperate annual vine of Asian origin that has invaded natural areas in the eastern U.S. Seeds of *P. perfoliata* were accidentally imported into a nursery in York County, PA, in the 1930s (Moul, 1948) and the plant has subsequently spread to sites throughout much of the eastern U.S. from Massachusetts to North Carolina and west to Ohio (EDDMapS, 2011). A biological control program was initiated by the U.S. Forest Service in 1996 (Wu et al., 2002). The host-specific weevil *Rhinoncomimus latipes* Korotyaev, originally collected in China, was approved for release in North America in 2004 (Hough-Goldstein et al., 2008). This weevil develops at least three to four overlapping generations during the growing season in the mid-Atlantic region of the U.S., increasing in population size and dispersing to new patches (Lake et al., 2011). It can reduce populations of mile-a-minute weed substantially within one to three years (Hough-Goldstein et al., 2009).

The primary mechanism for mile-a-minute weed suppression is through release of apical dominance and production of “stacked” nodes in response to adult and larval (internal) feeding, resulting in shorter vines, which are less competitive with surrounding vegetation (Hough-Goldstein et al., 2008). In some areas, the resident plant community is diverse and consists of mostly native species, and as mile-a-minute is suppressed, these desirable plants become dominant. At other sites, however, additional non-native invasive plant species are replacing mile-a-minute, a phenomenon that has been termed the “invasive species treadmill” (Thomas and Reid, 2007).

One possible way to enhance plant competition, increase the effectiveness of the biocontrol agent, and at the same time avoid the invasive species treadmill effect is through restoration planting using native species. Two different experiments were conducted to test the effects of restoration planting on mile-a-minute weed populations. The first studied the impact of planting a vigorous native perennial, flat-top goldentop (*Euthamia graminifolia* (L.) Nutt.), with and without use of a pre-emergent herbicide, along with release of the biocontrol agent. In the second experiment, a seed mix of native grasses and forbs was tested with and without the biocontrol weevil.

Methods and Materials

For the first experiment, 6.1 x 6.1 m plots of *P. perfoliata* were treated with post-emergent herbicide, cleared of woody and herbaceous debris, and plantings were established in October 2008 (details in Lake, 2011). Treatments included a low-density planting of 100 *E. graminifolia* plugs per plot; a high-density planting of 400 *E. graminifolia* plugs per plot; a low-density planting of *E. graminifolia* plus 25 Dutch elm disease-tolerant elm tree seedlings in each plot; and a control treatment with no planting (Fig. 1). These treatments were replicated at three different sites in southeastern PA (the Laurels, Waterloo Mills, and Crosslands). In April, 2009, a pre-emergent herbicide was applied to half of each treatment plot, randomly assigned. Although the biocontrol weevil was already present at all sites in low numbers, 500 additional weevils obtained from the rearing facility at the NJ Department of Agriculture Phillip Alampi Beneficial Insect Laboratory in Trenton, NJ were added to each plot in June, 2009. Plots were monitored in five 1 m² quadrats randomly and permanently established within each half of each plot (Fig. 1). The plant community in these plots was assessed in the fall of 2010.

The second experiment was conducted at a single site on the grounds of Longwood Gardens in Kennett Square, PA (details in Cutting, 2011). At this site, four treatments consisting of combinations of weevils and no weevils, with and without a native seed mix, were applied to 2 x 2 m plots of *P. perfoliata* with five replicates, beginning in April, 2009. The seed mix consisted of a combination of three native perennial grasses, big bluestem (*Andropogon gerardii* Vitman), Canada wildrye (*Elymus canadensis* L.), and switchgrass (*Panicum virgatum* L.); and two native perennial forbs (both Asteraceae), blackeyed Susan (*Rudbeckia hirta* L.) and oxeye sunflower (*Heliopsis helianthoides* (L.)). Weevils were present in low numbers at this site, but were supplemented by releasing 100 weevils per plot in weevil treatments in May 2009. Additional weevils were allocated to plots, with insect numbers standardized by percent mile-a-minute cover in each plot in August 2009, May 2010, and June 2010 (Cutting, 2011). Weevils were excluded from plots to produce a no-weevil treatment using a soil drench with the systemic insecticide, dinotefuron (Safari®

20 SG, Valent U.S.A. Corp., Walnut Creek, CA). The percent cover of mile-a-minute, number of weevils, and seed production were monitored weekly within the entire plots. Plant species richness was evaluated twice each season. A destructive harvest to obtain *P. perfoliata* biomass occurred at the end of year two.

Results and Discussion

For the *E. graminifolia* planting experiment, mile-a-minute seedling counts for spring, 2009, were significantly lower on the side of the plots where the pre-emergent herbicide had been used (Fig. 2A). This was expected, because the pre-emergent herbicide kills seeds that are in the process of germinating, while having no effect on live vegetation. However, this difference persisted in 2010 (Fig. 2B) and 2011 (Fig. 2C) even though no additional herbicide was used and the original herbicide would no longer have been active. Mile-a-minute seedling numbers were much lower in all of the plots in both the herbicide

and no-herbicide treatments in 2010 compared to 2009, and this was also true of mile-a-minute cover during the growing season (data in Lake, 2011). Although a no-weevil control was not included in this experiment, it is likely that the mile-a-minute weed in these plots was suppressed by the weevils, as has occurred in other studies (Hough-Goldstein et al., 2009). Mile-a-minute was most effectively suppressed in this experiment where it was initially reduced by the pre-emergent herbicide (Fig. 2).

A total of 127 plant species from 48 families were identified in these plots in the fall of 2010, and more than 60% of plant species at each site were native. At one of the sites (the Laurels) most of the vegetation cover consisted of native plants. At this site, weevils plus pre-emergent herbicide use effectively restored native vegetation, and the planting treatments had no additional effect. At the other two sites, however, native plant cover was higher in the treatments with restoration plantings compared to the control plots, both where herbicide had been applied and where no herbicide had been applied (Fig. 3A). Native

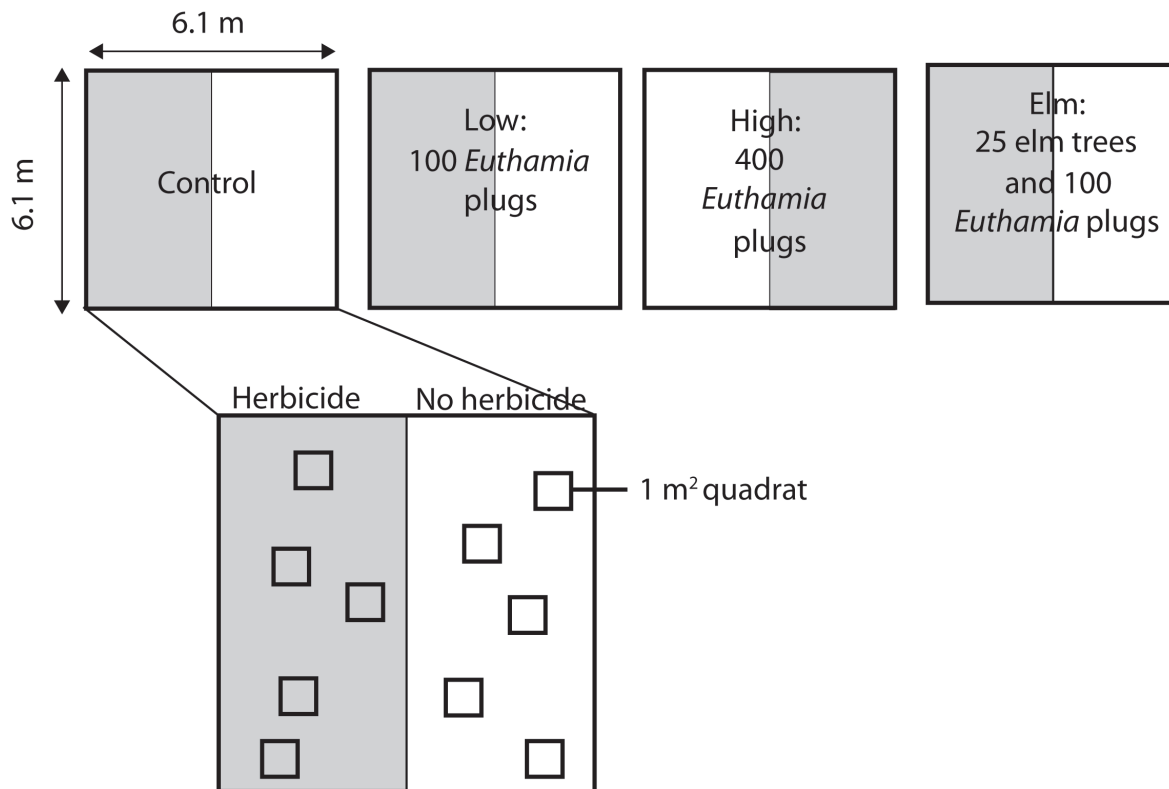


Figure 1. *Euthamia graminifolia* restoration experiment site design. Each of three sites had four planting treatment plots as shown. The inset illustrates the herbicide and no herbicide treatment areas and the five monitoring quadrats that were established randomly and permanently within each herbicide and planting treatment combination.

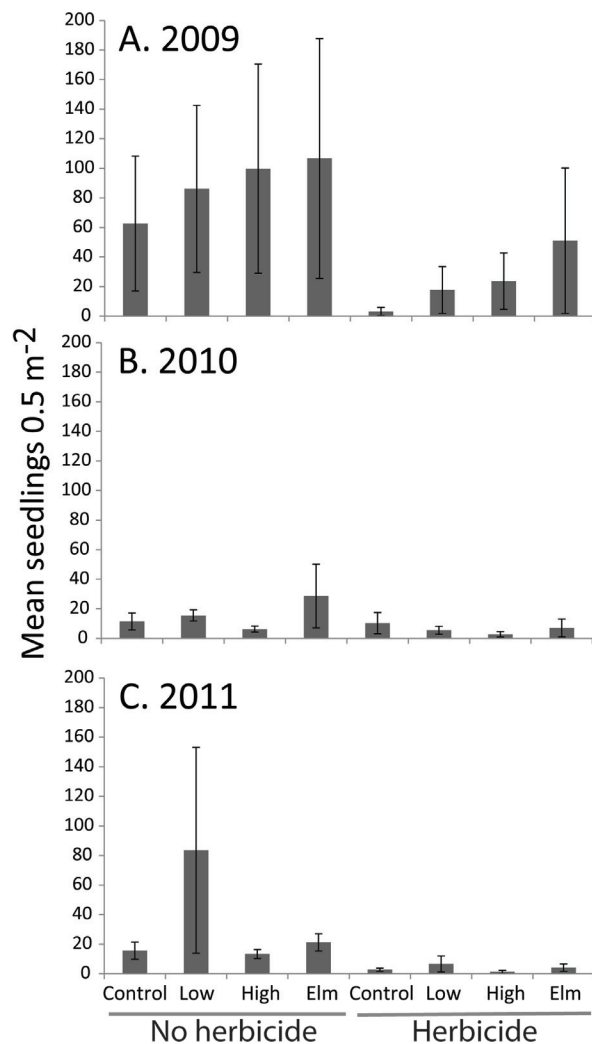


Figure 2. *Persicaria perfoliata* spring seedling counts (mean $\pm$ SEM) in (A) 2009, (B) 2010 and (C) 2011. Counts were conducted in 0.5 m² of the 5 monitoring quadrats in each herbicide and planting treatment combination at three sites. There were significantly more seedlings in the no herbicide than herbicide plots in 2009 ($P = 0.0001$), 2010 ($P = 0.0056$), and 2011 ($P = 0.0040$), but no significant differences by planting treatment.

cover at these sites was much higher in the herbicide than in the no-herbicide plots (Fig. 3A), indicating that the restoration plantings established poorly and resident native plants were not released where no herbicide was used, despite reductions in mile-a-minute weed cover. At these sites, Japanese stiltgrass largely replaced mile-a-minute weed on the no-herbicide side (Fig. 3B). The one-time application of

a pre-emergent herbicide (which killed germinating Japanese stiltgrass seeds as well as mile-a-minute seeds) along with restoration planting prevented the reinvasion of stiltgrass, resulting in 80-90% native plant cover with virtually no Japanese stiltgrass (Fig. 3).

In the native seed mix experiment, integrating the biocontrol weevils with restoration seeding significantly reduced mile-a-minute weed cover compared with the control, to about 20% by the second year (2010). In plots where weevils were excluded using dinotefuron, mile-a-minute cover remained at about 80% (Fig. 4). The treatments with weevils but no seed mix produced intermediate mile-a-minute cover in 2010. Two additional measures of plant productivity, mile-a-minute seed cluster production and dry biomass showed similar results (Fig. 5) to mile-a-minute cover. Native plant species richness in 2010 was highest in the weevil plus seed mix treatment (Cutting, 2011).

Conclusions

For both experiments, an integrated weed management approach was more effective than the biological control weevil alone for suppressing mile-a-minute weed and restoring the native plant community. The use of a pre-emergent herbicide in the *E. graminifolia* planting experiment improved the establishment of planted perennial vegetation and helped to prevent Japanese stiltgrass from taking over where mile-a-minute weed had been suppressed. In both experiments, enhanced competition via native restoration plantings improved the effectiveness of the biocontrol agent and increased native plant biodiversity following suppression of the target weed. Where practical and necessary, depending on the resident plant community, both selective herbicides and restoration planting should be considered for use in conjunction with biological control of invasive weeds.

Acknowledgements

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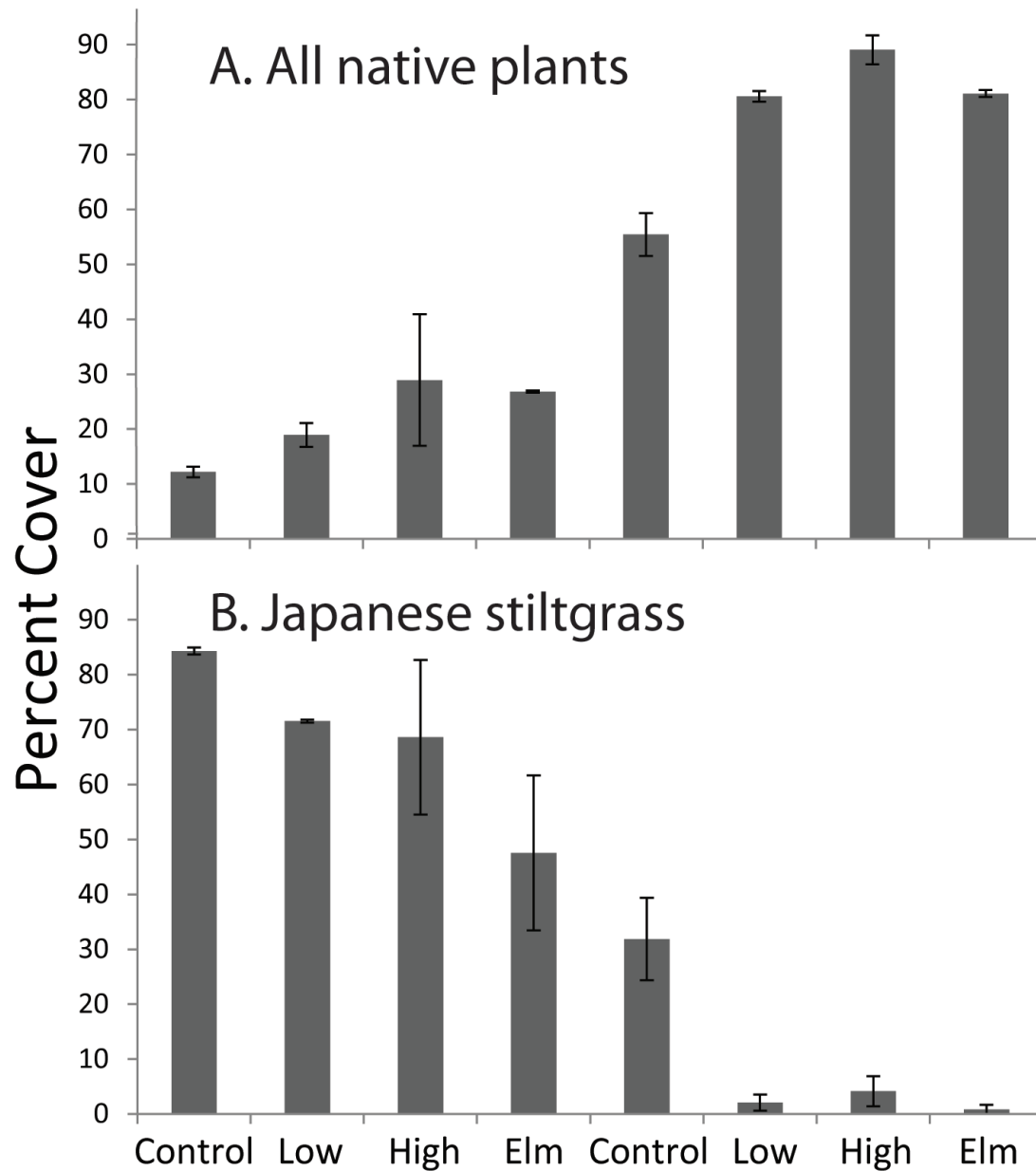


Figure 3. Cover (mean $\pm$ SEM) of (A) native plants, including *Euthamia graminifolia* (significantly higher in the herbicide than no herbicide plots, $P < 0.0001$) and (B) Japanese stiltgrass (significantly lower in the herbicide than no herbicide plots, $P < 0.0001$), at Crosslands and Waterloo Mills, Fall of 2010.

Persicaria perfoliata percent cover

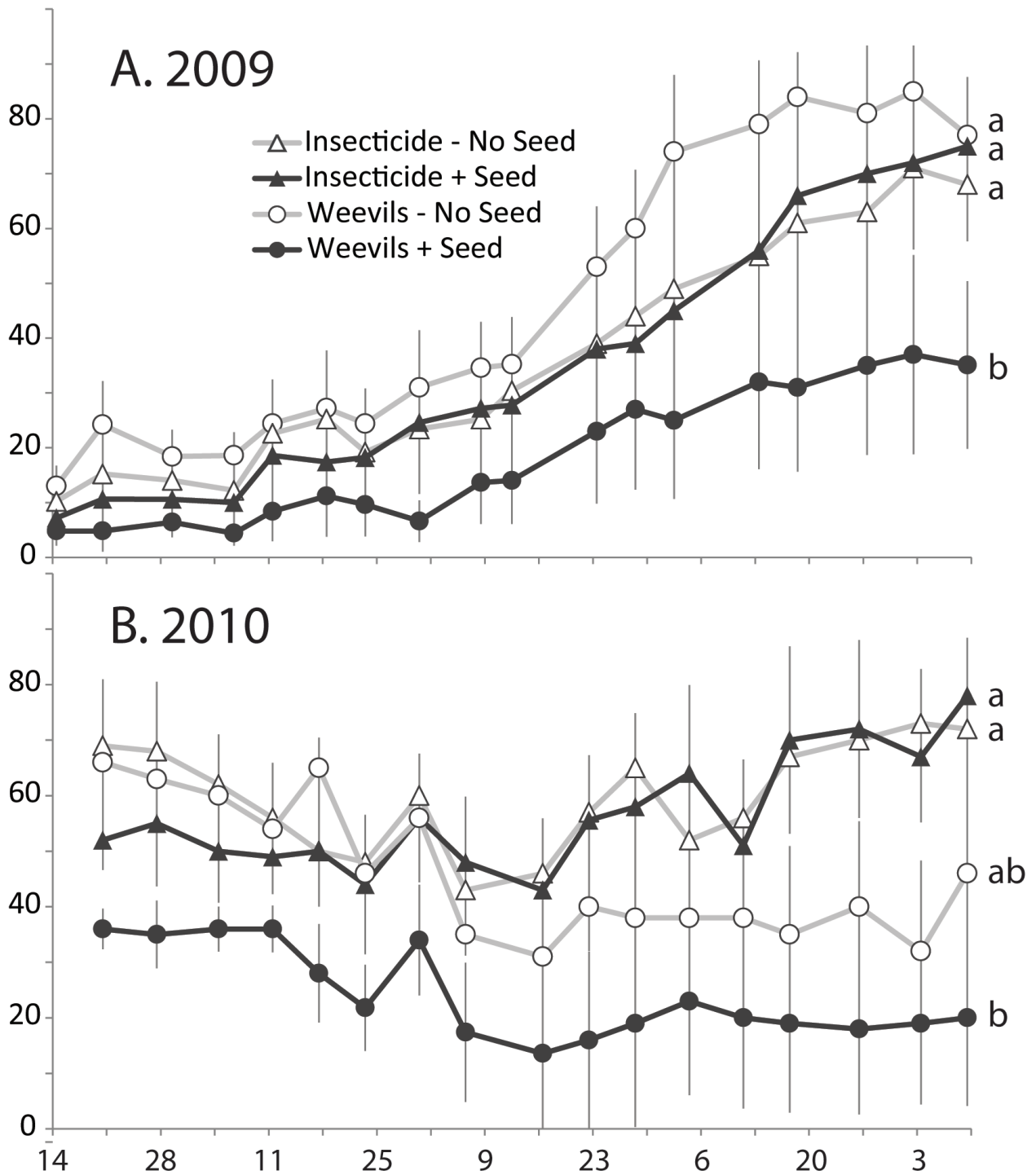


Figure 4. Cover (mean $\pm$ SEM) of *Persicaria perfoliata* in (A) 2009 and (B) 2010, native seed mix experiment. Means for the last date within each year followed by the same letter are not significantly different (two-way ANOVA, Tukey's test).

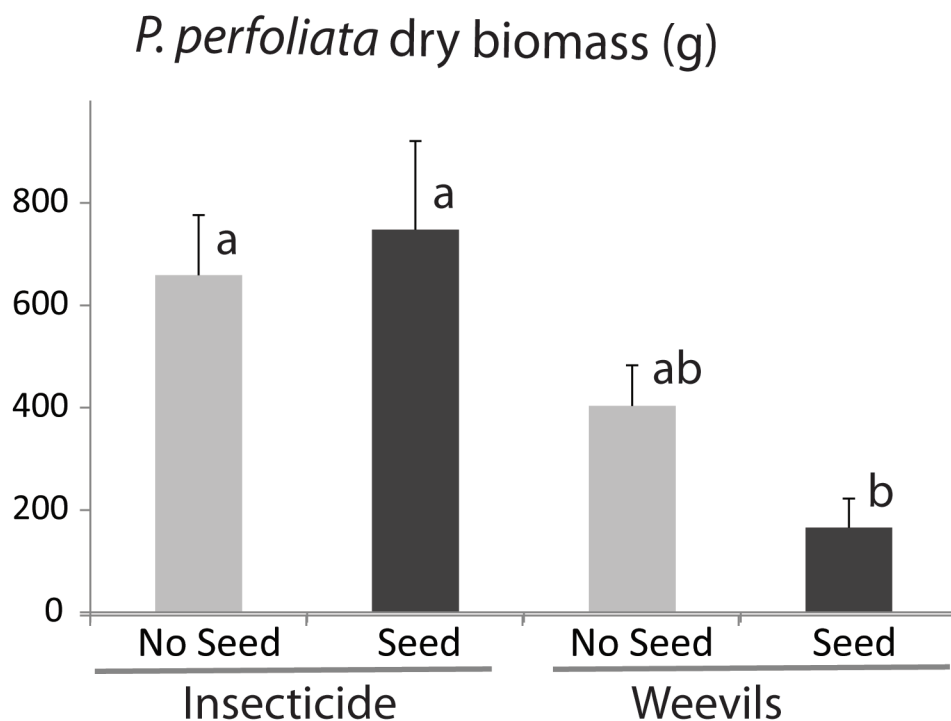


Figure 5. Dry biomass (mean $\pm$ SEM) of *Persicaria perfoliata* harvested from the native seed experiment in 2010 ($P = 0.0039$). Means with the same letter are not significantly different (Tukey's test).

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Rehabilitation of Melaleuca-Invaded Natural Areas through Biological Control: A Slow but Steady Process

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Abstract

Natural areas invaded by invasive exotic plants can develop dense populations that displace native plants. The Australian tree *Melaleuca quinquenervia* (Cav.) Blake (melaleuca) disrupts natural areas in southern Florida by developing into near monocultures typified by low species diversity. Environmental plasticity coupled with fire resistance, high reproductive potential, and deposition of large quantities of slowly degrading litter on forest floors affects species diversity in melaleuca dominated habitats. Intentionally released biological control agents *Oxyops vitiosa* Pascoe (weevil), *Boreioglycaspis melaleucae* (Moore) (psyllid) and adventive natural enemies (rust-fungus *Puccinia psidii* G. Wint, and a transitory lobate-lac scale *Paratachardina pseudolobata* Kondo & Gullan) accelerated defoliation, crown thinning and mortality of melaleuca trees over a 14-yr (1997-2011) study period. Following the long-term effects of two biological control insects and the rust fungus, melaleuca stem density decreased significantly and reversed some of the effects of its invasion. The decreased melaleuca densities correlate with the increased diversity and abundance of mostly native plants; this trend observed during the first 7 yrs continued during the second 7-yr period. Species diversity remained similar during the second 7-yr period, but sub-canopy coverage by sawgrass and native woody plants increased. Melaleuca continued to occupy the top canopy albeit with reduced density and lessened ability to recruit seedlings. These melaleuca-degraded lands are not fully restored to their pre-invasion status but rehabilitation is progressing as indicated by the return of many native plant species.

Introduction

Invasive plants, by definition, overrun native plant communities (OTA, 1993; Wilcove et al., 1998; Myers and Bazely, 2003) and reduce the biodiversity of natural systems (D'Antonio and Vitousek 1992). For instance, a majority (ca 77%) of invasive plants in Florida reportedly alter biotic communities (Gordon, 1998). The Australian tree *Melaleuca quinquenervia* (Cav.) Blake (Family: Myrtaceae, hereafter referred as "melaleuca") is among the most problematic weeds in Florida (Austin, 1978; Center et al., 2011). The mitigation and ultimate restoration of invaded sites typically involves the suppression of

the targeted weed. Classical weed biological control offers a supplemental method of control that can be strategically integrated with conventional mechanical and herbicidal control tactics to more fully suppress densities of invasive plants and thereby rehabilitate degraded natural communities. The enemy release hypothesis (Keane and Crawley, 2002) suggests that the reunification in the adventive range of an exotic plant with specialized, host-specific natural enemies from its native range may suppress the pest plant population and ultimately allow desirable vegetation to return (McEvoy and Rudd, 1993).

A biological control program targeting melaleuca was initiated in 1986 (Balciunas et al., 1994), with

the expectation that introduced herbivores would weaken trees through defoliation, and thereby limit melaleuca's invasive potential. With this objective in mind, surveys were conducted to enumerate the natural enemies of melaleuca in Australia. Over 450 species of arthropods were recorded (Balciunas et al., 1994). To date, 5 species have been released after rigorous host range testing (Center et al., 2011). Three of these established. The weevil *Oxyops vitiosa* Pascoe was released during 1997. Its larvae feed on leaves of newly developed shoots (Center et al., 2000). The injury resulting from the feeding of this insect did not become widespread until 2001. A second biological control agent, the psyllid *Boreioglycaspis melaleucae* (Moore), was released during spring 2002 (Center et al., 2006). The psyllid prefers young shoots but also attacks older foliage. Localized impacts had become apparent by 2003. The third agent, the gall fly *Fergusonina turneri* Taylor, in conjunction with a mutualistic nematode, induces galling of vigorously growing vegetative and reproductive tissues. It was released in 2005 but never established despite series of releases (Center et al., 2011). More recently, a stem-gall midge *Lophodiplosis trifida* Gagné was released in 2008 that has already widely established (Center et al., 2011). In addition, an adventive scale-insect *Paratachardina pseudolobata* Kondo & Gullan infested numerous phylogenetically unrelated plant species including melaleuca (Pemberton, 2003). An unidentified sooty mold (indiscriminately covering foliage and green stems) also became abundant usually in association with heavy infestations of this scale (Rayamajhi et al., 2007). In addition, an adventive rust fungus *Puccinia psidii* G. Wint, which infects young foliage of plant species in the family Myrtaceae (Laundon and Waterston, 1965; Marlatt and Kimbrough, 1979), became prevalent on melaleuca during this time (Rayachhetry et al. 1997). All these released biological agents and adventives pest species caused substantial damage to melaleuca trees (Fig. 1).

Various studies have reported the impacts of the biological control agents on melaleuca trees (Pratt et al., 2003, 2005; Franks et al., 2006; Rayamajhi et al., 2007, 2008, 2009; Center et al., 2011). Herein, we provide an overview of long-term studies that demonstrate concomitant increases in plant species diversity and abundance associated with the decreased density of melaleuca.

Materials and Method

During 1996, we established permanent plots in melaleuca stands representing three hydrologically differentiated habitat types: permanently flooded (year-around wet), seasonally flooded (seasonally wet) and non-flooded (or flooded only for a few days after severe rain-storms) in southern Florida, USA. We gathered data on stand density, standing biomass, litterfall dynamics, seed quality and reproductive potential using these permanent plots as well as sites outside the plots. Detailed materials and methods have been presented in Rayachhetry et al. (1998 and 2001), Rayamajhi et al. (2002, 2006, 2007 2008 and 2009) and Van et al. (2000, 2002, and 2005). The general discussions presented herein are based on the data published in the aforementioned articles as well as observations made in April 2011.

Results and Discussion

Among natural enemies, the weevils and psyllids caused premature abscission of variously aged leaves (Pratt et al., 2005; Morath et al., 2006); while rust-fungus pustules induced abscission of immature leaves (Rayachhetry et al., 2001). The combination resulted in trees that appeared progressively more denuded (Fig. 1) as natural enemy attacks continued unabated. Following field release, the impacts of introduced natural enemies on target plants are rarely quantified by biological control practitioners (Denslow and D'Antonio, 2005). These post-release evaluations are often difficult because the impacts can take decades to be realized and replicated treatments are difficult to maintain considering the dispersive nature of natural enemies. Our pre- and post- biological control release data showed that stem density of melaleuca trees across all diameter classes decreased, the highest (16.3%/yr between 2002-2005) being among trees of smaller diameter at the periphery of the stands (Rayamajhi et al., 2007). A biomass allocation study conducted in 1996 and 2003 showed a significant reduction in the melaleuca foliage, fruit and seed biomass by 55%, 85% and 74%, respectively between 1996-2003 albeit the total biomass (348 Mg/ha in 1996 to 331 Mg/ha in 2003) remained virtually unchanged during the same period (Rayamajhi et al., 2008). This apparent

anomaly resulted because the largest trees, although defoliated and severely stressed, remained alive and made up the bulk of the standing biomass. However, most of these stressed trees were dead by the 2011 evaluation (data not presented). Overall, the natural enemy impacts have caused substantial reductions in melaleuca tree density and seed production. This has drastically reduced seedling recruitment in southern Florida (Rayamajhi et al., 2007; Tipping et al., 2012).

Canopy gaps created by uprooted, dead or dying trees provide space on the forest-floors for seedling recruitment (Kneeshaw and Bergeron, 1998). Rayamajhi et al. (2009) have linked corresponding increases in the diversity and abundance of native plant species in mature melaleuca stands to the crown thinning and reduced density of the dominant melaleuca trees that have created canopy openings. A total of 54 plant species of plants were recorded representing 38 families from within 14 plots located in two non-flooded study sites containing mature melaleuca stands (Table 1).

Our observations suggest that sites dominated by melaleuca in the past will be rehabilitated, resulting in more stable and diversified plant communities dominated mostly by natives; but the process may take several years as melaleuca is not declining at the same pace throughout its range owing to site-specific differences in the impact of biological control agents. For example, melaleuca in the sandy soils of the west coast of Florida appeared to decline at a faster rate than those in the organic soil of the east coast of Florida (personal observation). Overall, melaleuca tree defoliation, crown thinning, and mortality has continued, and its seedling recruitment and survival ability has substantially diminished (Fig. 1). Long-term field-research data and experience on various aspects of melaleuca biocontrol suggest that biodiversity in sites degraded by melaleuca will steadily improve with biological control as a long-term management tool.

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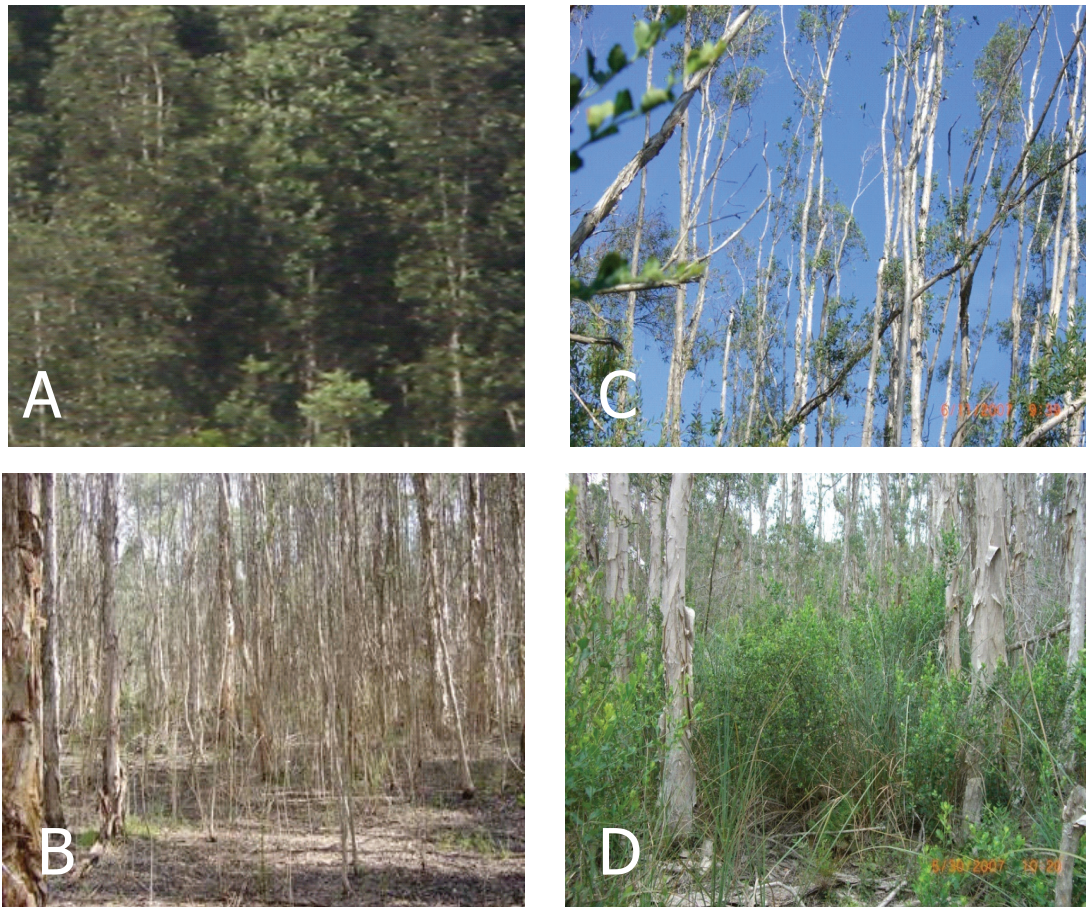


Figure 1. Transition of natural-enemy impacted *Melaleuca quinquenervia* stands in non-flooded sites from near monocultures to diversified plant communities comprised of mostly native plant species. (A) a typical melaleuca stand with full green foliage prior to *Oxyops vitiosa* release in 1996; (B) thinned melaleuca stand after severe defoliation by the weevil, psyllid and rust fungus as observed in 2004, note plants emerging from the forest floor; (C) thinned melaleuca crowns by 2007; (D) forest floors covered by various non-melaleuca plants such as *Cladium jamaicense*, *Baccharis*, *Myrsine* and *Illex* species by 2007.

Table 1. Mean density of the major perennial plant species in mature *M. quinquenervia* forest stands in southern Florida.

Plant Species	Density (plants ha ⁻¹)		
	1997	2005	2011
<i>Baccharis glomeruliflora</i> Pers.	1597	5375	5811
<i>Casuarina</i> sp.	100	100	100
<i>Cephalanthus occidentalis</i> L.	200	300	200
<i>Cladium jamaicense</i> Crantz	6913	3238	38263
<i>Ficus aurea</i> Nutt.	100	225	150
<i>Ilex cassine</i> L.	0	500	13087
<i>Melaleuca quinquenervia</i> Cav. S.T. Blake	28513	18900	5125
<i>Myrica cerifera</i> L.	2638	650	2112
<i>Myrsine floridana</i> A. D.C.	525	650	2412
<i>Persea palustris</i> (Raf.) Sarg.	200	300	960
<i>Schinus terebinthifolius</i> Raddi	300	875	3425
<i>Thelypteris</i> sp.	1240	3624	2950
<i>Trema micrantha</i> (L.) Blume.	0	300	6100

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Twenty-five Years of Biological Control of Saltcedar (*Tamarix*: Tamaricaceae) in the Western USA: Emphasis Texas – 1986-2011^a

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Abstract

Four species of leaf beetles, *Diorhabda carinulata* (Desbrochers) from Kazakhstan and China (released in the USA in 2001), *D. elongata* (Brullé) from Greece and *D. carinata* (Faldermann) from Uzbekistan (released in 2004), and *D. sublineata* (Lucas) from Tunisia (released in 2009), have been utilized for control of highly invasive saltcedars (*Tamarix* spp., SC), small trees from Asia and the Mediterranean area that are destructive to native riparian vegetation in the western USA and northern Mexico. The beetles' field ecology, biology and host ranges were determined overseas and in quarantine in Texas, California (CA) and New Mexico. These beetles are restricted to species of *Tamarix*, none of which are native in the western hemisphere. By 2010, the China/Kazak beetles had defoliated about 2,400 stream km of SC in Utah, and NW New Mexico and NW Arizona and 550 km in Nevada, the Crete beetles defoliated about 125 km in central-west Texas and 80 km in CA, and the Tunisian beetles defoliated about 130 km along the Rio Grande of western Texas. "Spillover" populations of *D. sublineata* defoliated athel shade trees (*Tamarix aphylla* (L.) Karsten in 2010, but these plants revegetated within 2 months. Record cold weather severely depressed beetle populations throughout north and central Texas during February 2011, killing most beetles and damaging SC top growth; the cold caused little mortality to the beetles along the Rio Grande but caused 95 to 100% dieback of the athel.

Introduction

Exotic saltcedars (SC), highly invasive small trees introduced from Asia and the Mediterranean area beginning in 1823, (mainly *Tamarix ramosissima* Ledebour, *T. chinensis* Loureiro, and in California, also *T. parviflora* de Condolle) are severely damaging native ecosystems along streams and lakeshores throughout the western United States and northern Mexico. No *Tamarix* species are native in the Western Hemisphere. Athel, *T. aphylla* (L.) Karsten), another exotic species, (a large 20 m-tall, evergreen, cold-intolerant tree), is used as a shade tree and windbreak in Mexico and the southwestern USA and is not a target for control (DeLoach, et al., 1997, 2000). However, since recent floods, it is becoming more invasive and damaging to native ecosystems along the Rio Grande of Texas and is hybridizing with other *Tamarix* spp. in the western USA.

The impetus for biological control (BC) was by Lloyd Andres (ARS, Albany, CA), who initiated overseas explorations for natural enemies in the 1960's-1970's, that produced reports of ca. 350 herbivorous insect species, with information on biology and host range, in Israel and the Middle East (Gerling and Kugler, 1973), Turkey (Pemberton and Hoover, 1980), Pakistan (Habib and Hassan, 1982), and Kazakhstan (Mityaev and Jashenko, 2007), summarized by Kovalev (1995).

Biological control experimentation began in 1986 by one of us (DeLoach) with literature review and risk assessment, and from 1991 to 1998 with overseas exploration for natural enemies with collaborators within the native range of SC in France (A. Kirk, R. Sobhian, L. Fornasari), Israel (D. Gerling), China (B.P. Li, R. Wang, Q. G. Lu, and H. Chen), Kazakhstan (I.D. Mityaev and R.V. Jashenko) and Turkmenistan (S. Myartseva) (DeLoach, et al., 2003; Carruthers, et al., 2008) and supplementary funding from USDI-Bureau of Reclamation. A petition for introduction and testing in quarantine at Temple, TX of a leaf beetle from China and Kazakhstan, then identified as *Diorhabda elongata* (Brullé) ssp. *deserticola* Chen, was submitted to the Technical Advisory Group for Biological Control of Weeds (TAG) of the US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA-APHIS-

PPQ) and state departments of agriculture in May 1994. This petition initiated the requirement for a Biological Assessment (BA) to the US Department of Interior, Fish and Wildlife Service (USDI-FWS) for consultation under Section 7 of the Endangered Species Act, (DeLoach et al., October 1997). Release into field cages for 1 year, then into the open environment, was approved by FWS via Letter of Concurrence, 3 June 1999, and APHIS-PPQ via a "Finding of No Significant Impact" (FONSI), 7 July 1999 for 10 specified sites in Texas (TX), Colorado (CO), Wyoming (WY), Utah (UT), Nevada (NV) (3) and California (CA) (3). The beetles were placed in large cages during the summer of 1999 and released beginning in May 2001. The USDA-ARS Temple project was joined by one of us (Carruthers) in early 1998, who organized the ARS Exotic and Invasive Weeds Research Unit (EIWRU), Albany, CA with several additional scientists and obtained a USDA-CSREES-NRI-IFAFS grant for area-wide biologically based control of SC that provided supplemental support for all projects for 4 years. Carruthers coordinated the projects in CA and NV and DeLoach in the other states.

These *Diorhabda* spp. beetles were identified by chrysomelid taxonomic authorities as *D. elongata* (Brullé). However, morphological studies by Tracy and Robbins (2009); cross-mating experiments by David Thompson; DNA analyses by David Kazmer; and pheromone comparisons (Cossé, et al., 2005) demonstrated that these beetles were actually five valid, separate species – *D. carinulata* (Desbrochers) from Fukang (44.8° N. Lat.), China and Chilik (43.3° N.), Kazakhstan; *D. elongata* from Crete (35° N) and Posidi Beach near Thessaloniki (40.6° N), Greece; *D. sublineata* (Lucas) from Mareth (33.4° N), Tunisia; *D. carinata* (Faldermann) from Karshi (38.5° N), Uzbekistan, and *D. meridionalis* Berti and Rapiilly from coastal Iran (not introduced).

Results and Discussion

Field Ecology, Biology and Behavior

Diorhabda spp. adults and larvae feed on the foliage and flowers of SC. The larvae pupate under litter on the ground and the adults overwinter there. The northern Fukang/Chilik beetles have

two generations north of the 38th parallel, the Crete beetles three to four generations in central TX and along Cache Creek, CA, and the Tunisian beetles five to six generations along the w TX Rio Grande and Pecos River. Diapause is regulated by daylength after June (Lewis, *et al.*, 2003b; Milbrath *et al.*, 2007; Bean *et al.*, 2007). Since 2009, the Chilik beetles appear to be adapting to areas farther south and by September 2011 were reported at Algodones, NM (35.4° N Lat.) These beetles have a high reproductive rate, especially in more southern areas with several generations. Females lay an average 280 eggs (net reproductive rate 80.9), generation time is about 38.3 days, and the population can double in 6.1 days (Milbrath *et al.*, 2007). Major predators on the ground are ants (and possibly small mammals) that feed on the pupae and adults; and in the trees are assassin bugs and spiders on adults and larvae, and ladybird beetles on eggs. No parasitoids have been observed in the USA, but a tachinid fly (*Erynniopsis antennata* (Rondani) was reared from larvae and adults, and a eulophid wasp parasitoid of larvae and a *Nosema* pathogen were often reported by our overseas collaborators. Adult beetles congregate in mating swarms when food is scarce, regulated by a male aggregation pheromone (Cossé, *et al.*, 2005) and can fly 50 km or more to find fresh food and to avoid predators.

Host Specificity

Intensive testing at Temple and Albany demonstrated that these four *Diorhabda* species are restricted in host range to the genus *Tamarix*. The *D. carinulata* beetles from Fukang and those from Chilik were tested in quarantine at Temple, TX from 1992 and at Albany, CA from 1998 (DeLoach *et al.*, 2003; Lewis *et al.*, 2003a; Milbrath and DeLoach, 2006 a, b; Herr *et al.*, 2009). At Temple, reproductive index (% larval survival in no-choice tests in sleeve bags on plants growing outdoors multiplied by % eggs laid in multiple-choice tests on potted plants in large outdoor cages) for Fukang, Crete, and Tunisian beetles was 12.4, 20.5, and 12.6, on *T. ramosissima*/*T. chinensis*, 8.4, 8.2, and 3.0 on athel, and 0.103, 0.70, and 0.00 on *Frankenia*, respectively. The most highly selective life stage was the female searching for a plant on which to oviposit. Athel and *Frankenia* spp. were included in nearly all tests. In outdoor, uncaged tests in 2005 at Big Spring by DeLoach, 65.14% of

the adults were found on SC, 34.63% on athel, and 0.23% on *Frankenia*; for eggs, 82.2% were on SC, 12.8% on athel and 0.0% on *Frankenia* (Moran *et al.*, 2009). At Cache Creek (near Rumsey), CA, *D. elongata* beetles laid 3.7% of their eggs on *Frankenia salina* (Molina) I.M. Johnson in a paired choice field test with *T. parviflora*, 4.3% in a multiple-choice cage, and 1.2% in a multiple-choice open field test (Herr, *et al.*, in revision). Thus, we expected heavy damage to and control of SC, light to moderate damage to athel, and no or only minor damage to *Frankenia*.

Releases in Northern States

After regulatory approvals, the Fukang beetles were released into the field beginning in May 2001-2002 in Texas, Colorado, Wyoming, Utah, Nevada and California and the Chilik near Delta, UT. For those in desert areas of NV, UT and western CO, control has been spectacularly successful (Carruthers *et al.*, 2008). By fall 2009, populations released near Lovelock and Schurz, NV had coalesced and defoliated the SC along 550 stream km in NV, which is nearly all the SC in that state (Jeff Knight, NV Dept. Agric., personal comm.). Also, the Chilik beetles released in 2001 near Delta, UT, had defoliated about 80 km of SC along the Sevier River and were redistributed to several other sites in Utah in August 2005. Those released along the Colorado River near Moab, UT had defoliated a total of about 2,400 stream km by fall 2010: to Lake Powell, and into western CO along the Delores River and along the Green River to Dinosaur National Monument (Jamison, 2010); along the San Juan River, UT into NW NM and to Navajo Lake by Autumn 2011 (Thompson, personal comm.), then probably along the Jemez River 200 km to Algodones (35.4° N) on the Rio Grande 37 km N of Albuquerque (DeLoach, personal obs.). In many areas of CO, native willows have increased and the beetles defoliate the SC annually, keeping it suppressed. A different redistribution near Saint George, UT (probably in 2006 but under unknown circumstances), has defoliated SC along the Virgin River for about 150 km in SW Utah and NW Arizona (Bean, personal comm.).

Releases of the Fukang beetles near Pueblo, CO and Lovell, WY in 2001 have been less successful but are established, spreading, and still may have the potential for rapid increase and control (D.

Eberts, Bureau Reclamation, Denver, personal comm.). Those released near Pueblo, by 2011 had dispersed upstream along the Arkansas River to Cañon (Bean, personal comm.). Those along the Bighorn River near Lovell by 2007 had defoliated 95% of the SC along 50 river km; in Montana, repeated releases of several thousand beetles from Lovell have failed to establish at Lake Fort Peck, probably because of flooding around the lake (D. Kazmer, J. Gaskin, K. Delaney, personal comm.).

Releases in North and Central Texas

The *D. carinulata* (Fukang) beetles (adapted to long northern daylength were released at Seymour, TX (33.7° N) but were not adapted to the short and decreasing summer daylength after June (a sign of approaching winter), which caused premature entry into diapause in July, and failed to overwinter or establish (Lewis *et al.*, 2003b, Milbrath *et al.*, 2007, Bean *et al.*, 2007). Beginning in September 2001, more southern *D. elongata* beetles (adapted to shorter summer daylength) were collected in Greece by Carruthers and Kashefi (the ARS cooperator there). Sobhian and Kirk (ARS European Biological Control Laboratory, Montpellier, France) collected *D. carinata* from Uzbekistan and *D. sublineata* from Tunisia. In 2003, FWS allowed unrestricted releases of *Diorhabda* in Texas but only at the original release sites in other states; however, state or private workers could make intrastate releases within their own state except for restrictions by FWS near critical habitat for endangered species.

All four *Diorhabda* species had been released in Texas by 2009, at a total of about 64 sites. On 22 April 2004, one of us (DeLoach) released 28 Crete adults, after they overwintered in on-site nursery cages, along Beals Creek near Big Spring, that defoliated two SC bushes by mid-June, and another 2,200 adults were released by September. ARS (DeLoach with technicians Tracy, Robbins and summer students) conducted detailed weekly or biweekly monitoring of the beetles and SC damage along transects of 200 m in 2005 to 9.5 km in 2009. These Crete beetles defoliated 70-98% of the SC stand two or three times annually – 1 ha by October 2005, 10 ha in 2006, 20 ha plus 3 km along Beals Creek in 2007, and 60 ha along 4 km of the creek plus 10 satellite colonies over a 10×23 km area by October 2008. During 2009, the

Crete beetles produced large populations from late May that began rapid, large scale dispersal that by August extended for 56 km and defoliated nearly all the SC along Beals Creek plus numerous outlying satellite colonies. By 2010, defoliation extended for 125 km along Beals Creek west to Mustang, Buzzard, and Silver Spring draws, to Stanton town, and along the Colorado River from Lake Thomas (with gaps) east to Colorado City. After 3 years of twice annual defoliation (2005-2007), canopy cover and green biomass of SC had been reduced by 85-95%, and about 20 to 25% of the trees had died. The local grasses and forbs revegetated naturally and abundantly, usually within 1 year after canopy defoliation.

Starting in 2006, two of us (Knutson and Muegge) began an implementation program to redistribute the Crete beetles in western Texas watersheds to more rapidly expand the area of control and to develop improved methods of release. Only 10% established when 300 beetles were released per site, but ant control plus releasing large numbers increased success to 80%. During 2008, they developed an insecticide baiting material that was very effective in controlling predaceous ants in the field and allowed a 10-fold increase in *Diorhabda* populations that could be released from the field cages. The large beetle populations then overwhelmed the ants (and other predators), spread rapidly and defoliated the SC. This method then was used routinely at new release sites. Several redistributions where 10,000 to 20,000 beetles were released rapidly produced large populations that defoliated several trees the first year, then several ha afterward. In 2009, about 340,000 adults were collected from the Big Spring area and released mostly in the Colorado River watershed. Releases along the Pecos River in 2006 had defoliated 29 stream km by 2010. Tunisian beetles also were released at a different location along the Pecos in 2010, and only those could be found after the February 2011 freeze.

In north Texas, *D. elongata* from Posidi, Greece were released along the Canadian River in 2003 and the Uzbek beetles in 2005 by one of us (Michels), and along the Wichita and Pease Rivers (Knutson) in 2008. They increased rapidly and defoliated 0.5 to 1.0 ha on these rivers, then declined, and did not survive the 2011 freeze. However, releases of Crete beetles at White River Lake (70 km E of Lubbock) by Knutson

in 2009 had established by 2010 and survived the 2011 cold. The Fukang beetles (that had failed to establish at Seymour, TX in 2001) were obtained from Pueblo, CO and released in June 2009 near Lake Meredith north of Amarillo, TX but did not establish. The Kazak beetles from northern NM have adapted to shorter day lengths and now may be the most promising for north Texas, if or when the ongoing APHIS moratorium is lifted on new releases, related to transient impacts of SC defoliation on the federally endangered bird, the southwestern willow flycatcher (*Empidonax trailii extimus*) near St. George, UT.

Releases along the Rio Grande, West Texas

The Rio Grande valley from old Ft. Quitman (115 km downstream from El Paso) through Big Bend National Park (BBNP), including the "Forgotten River" area from Ft. Quitman to Presidio, contains the largest SC stands in Texas and probably the second largest in the USA. After several meetings with Mexican scientists, caged overwintering tests were made of the Crete, Uzbek and Tunisian beetles species along the Rio Grande from October 2006 to May 2007. Only the Crete beetles were released from field cages by three of us (Fain, Donet, DeLoach) on five private ranches from 21-76 km upstream from Presidio to Candelaria on 29 June 2007. At first, they defoliated 10-100 trees per site but survived only 1-2 years at only three of the sites. In September 2009, 11,000 Crete adults from Big Spring were released at each of two of these sites. These defoliated several small to medium-sized trees but since have maintained only a weak population.

The *D. sublineata* beetles from Tunisia were shipped to quarantine at Temple, TX, and then to two of us (Thompson and Bean) for heat treatment to destroy a *Nosema* pathogen in the beetles. They were released in three nursery cages at Ruidosa (58 km upstream from Presidio) by one of us (Ritzi) and Andrew Berezin (also SRSU) from October 2008. They increased greatly by spring and were released from the cages on 19 May 2009, and rapidly defoliated about 1 ha of SC. Adults were placed in cages on a private ranch at Alamito Creek (10 km below Presidio), and in Big Bend Ranch State Park (BBRSP) at Madera Canyon (59 km) and Lajitas (78

km) downstream from Presidio in June 2009 by park personnel with ARS cooperation. They defoliated 2 ha at Alamito Creek and a few trees at the other sites by October 2009.

The Ruidosa site burned in a wildfire in March 2010, destroying all the beetles, but populations at Alamito Creek, Madera Canyon and Lajitas continued increasing rapidly. Redistributions of Tunisian beetles from Alamito Creek were made on 1-4 June 2010 at three additional sites downstream to Lajitas. Upstream, beetles were released at four sites from 21 km to 78 km NW of Presidio to Candelaria by Ritzi, DeLoach and Donet on 16 June 2010 before the moratorium on further releases was imposed by APHIS and FWS on 10 July 2010. SC trees from Alamito Creek to Lajitas near the river were very large (to 10-12 m tall, up to 0.5 m trunk diameter). During 2010, the Tunisian beetles at nearly all the sites increased rapidly and the defoliation coalesced among several sites during the summer.

In BBNP in 2010, two of us (Knutson and Muegge) together with Joe Sirotnak (Botanist, BBNP) established the Crete beetles at Santa Elena Canyon, which by 2011 have dispersed ca. 8 km downstream along the Rio Grande. The Tunisian beetles were established at the Gravel Pit site (164 stream km) and at the private Adams Ranch (203 km) downstream from Presidio and just beyond BBNP. By late September 2011, they had defoliated most of the SC for 27 km along the Rio Grande from Boquillas to Mariscal Canyons and at the Adams Ranch.

During the population pulse of 2010, the beetles moved rapidly out from the release sites, and by late August had defoliated most of the SC from Alamito Creek upstream to Ruidosa and downstream to Lajitas, a total of about 130 km with only a few gaps left untouched. Beetle defoliation was seen on the Mexican side of the river near Alamito Creek in March 2010, and mirrored the movement and spread of beetles on the US side of the river.

Large athel trees have been used for many years as shade around houses and as windbreaks in the warm southwestern US and northern Mexico. Clumps of large athels also grew along TX Highway 170 and were common (but less than 10% of the shade trees) in Presidio. Until mid-August, no damage had been seen on athel. However, by 24 August, with nearly 100% of the SC defoliated for a long distance, the

hungry adults left the SC, flew to nearby green athel trees only a few meters or a few blocks away, and defoliated almost all athel trees in Presidio, Ojinaga, and downstream along Hwy. 170. This caused great concern among residents in Presidio and especially in Mexico. An intensive sampling program was begun by Anne Marie Hilscher (SRSU) and two of us (Ritzi and Moran). Also, Don Grossman (Texas Forest Service, College Station, TX) initiated experimental chemical treatments of athel trees that protect them from the beetles. By mid-October 2010 most of the athel trees had refoliated to 90-95% of the original green canopy. This transient defoliation of athels was a “spillover” event (sometimes seen in other biological control of weeds projects, but usually not seen again after the first year).

In mid-February 2011, after an extremely dry year, the Big Spring area, the Pecos River, and the western Rio Grande, TX experienced 3 days of very warm weather (29° to 32°C), followed by record cold temperatures for 4 days (lows of -7° to -16°C). This killed 95-100% of the canopies of athel trees. The beetles in 2010 had not yet dispersed upstream beyond Ruidosa, but the athels there were killed back the same as athels with beetles plus freeze damage near Presidio, demonstrating that the athel dieback during 2011 was caused by the freeze, regardless of presence of the beetles. During 2011, the beetle populations were large along the river, again causing heavy defoliation of SC. The recovery of athel was demonstrated by shoots emerging from the base on the trunks or from a few large branches. Beetle populations on athel were tolerable and not causing notable defoliation or damage through September 2011.

The above information indicates that the introduced *Diorhabda* beetles can provide spectacular control of SC in large areas, but not yet in all areas, of the western USA. Also, we have learned much about their behavior and field ecology. No non-target feeding has been seen on any other plant species, although minor spillover damage from adult feeding may occur on *Frankenia salina* in California if nearby saltcedar is damaged. Increasing growth and abundance of willows and other native plants have been seen at several locations after saltcedar defoliation.

Additional Information Needed

1. How far and how rapidly will the beetles disperse and defoliate SC across the southwestern USA? From 2005 to September 2011, the Chilik beetles have moved about 240 km south of their presumed 38° lat. limit to Algodones on the Rio Grande, NM and the Tunisians have moved upstream from Alamito Creek 85 km to Candelaria toward El Paso. Will they meet along the Rio Grande, NM or will they be restricted by day length/climate?
2. Are additional natural enemies from Asia (such as stem or foliage-galling insects under study in Kazakhstan) needed to control SC in the northwestern tier of US states?
3. Will the Tunisian beetles establish on the *Tamarix* hybrids along the Texas coast?
4. Why do the Tunisian and Chilik beetles appear to provide more rapid control than the Crete beetles?
5. What will happen genetically when the Chilik, Crete, and/or Tunisian beetles meet?
6. What will be the effect of SC/BC on native plant and animal communities in relation to environmental and economic interests?
7. What will be the effect of SC/BC on survival and reproductive rate of the endangered southwestern willow flycatcher and the 40 other T & E plant and animal species harmed by SC?
8. Will feeding on athel decrease in the future (as in other BC examples of “spillover”) and cause little damage?
9. What will be the effect of SC/BC in Mexico?

^aThe section on saltcedar biological control vs southwestern willow flycatcher habitat in the oral presentation at this symposium will be published elsewhere.

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Tamarix Biological Control and the Restoration of Riparian Ecosystems

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Abstract

The introduction of the tamarisk leaf beetle, *Diorhabda carinulata* Desbrochers (Chrysomelidae), into the Virgin River watershed encompassing portions of Utah, Arizona and Nevada brings this controversial biological control agent for the first time into the critical habitat range for the southwestern willow flycatcher. This federally listed sub-species nests to some extent in tamarisk (*Tamarix* spp.), risking exposure if defoliation occurs during breeding. A collaborative monitoring program by university and federal researchers is documenting the process of tamarisk biological control and the ecosystem and biodiversity responses to the anticipated suppression of this dominant invasive plant. Recent data indicate that willow flycatchers were able to switch reproductive behavior by using restored native habitat patches in the upper watershed. We have initiated a series of restoration trials to develop the methods for facilitating riparian recovery, which were initially successful but were destroyed by a flood event in December 2010. We thus have proposed a restoration design that incorporates site suitability for re-vegetation with hydrologic modeling to predict locations with low probability of flood disturbance, along with a spatial evaluation to determine where 'propagule islands' of native vegetation should be created to provide sufficient seed dispersal to facilitate natural recruitment of native plant. Once conflicts related to the flycatcher are resolved, we anticipate that legal and regulatory restrictions on Tamarisk biological control can be lifted, and effective management of this weed can be resumed.

Searching for Microbial Biological Control Candidates for Invasive Grasses: Coupling Expanded Field Research with Strides in Biotechnology and Grassland Restoration

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Abstract

Highly invasive grasses (*e.g.*, *Bromus* spp., *Pennisetum ciliare* (L.) Link., *Taeniatherum caput-medusae* (L.) Nevski) are largely unabated in much of the arid Western U.S., despite more than 70 years of control attempts with a wide array of tools and management practices. The development and sustained integration of new approaches and potentially new tools is warranted in order to combat these destructive species. An expanded program of field exploration for microbial biological control agents is needed. However, any biological control agent must display a high level of efficacy, specificity and genetic stability to preclude any host range extension to native grasses or valued introduced species, especially cereals. A principle limitation to this research in the past – the seemingly insurmountable hurdle of characterizing the full genetic variation among target invasive species and their potential biological control agents – has been much reduced by impressive and continuing strides in sequencing technology (*e.g.*, 454 sequencing, RAD tag sequencing). These rapidly developing tools can enormously speed the screening of effective pairings between host and pathogen. Any biological control program would be only one part of a holistic integrated management against these invaders. Effective restoration of these vast grasslands also includes massive and sustained re-introduction of native grasses and the cryptobiotic crust – essential aspects of effective control that should proceed even now.

The Southwestern Willow Flycatcher – Saltcedar/Willow – Saltcedar Biological Control Debate: Popular Concepts – How Realistic?

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Abstract

The southwestern Willow Flycatcher, *Empidonax trailii* (Audubon) subspecies *extimus* Phillips (“flycatcher” hereafter) is a small, insectivorous, obligate riparian bird that breeds in Arizona (AZ) and parts of adjoining states and winters in Central America. It breeds in broad floodplains, in dense thickets of willows (occasionally in other native small trees), near or over water. Since saltcedars (SC) (*Tamarix* spp.) have displaced the willow, it now nests in SC, preferentially so in AZ but not in other areas, even though willows may be present or abundant. Its populations have decreased since 1948 as SC has increased. It was federally listed as endangered in 1995; total population in 1997 was about 540 adults at 62 sites, mostly with five or fewer adult pairs per site, and more than 20 at only a few sites. Major populations are at the San Pedro/Gila River confluence and Roosevelt Lake, AZ and at Topock Marsh, CA/AZ (all in SC); and on the Gila River at Cliff, and Rio Grande at San Marcial/Elephant Butte Reservoir (SM/EB), NM (both in willow dominated habitat). Former large breeding areas have been lost since SC came to dominate (the lower Colorado and Gila rivers, CA/AZ), but it never bred in large pure stands of SC (the Pecos River, NM/TX). The largest population now is at a site that began in 1996 at SM/EB and in 2009 with 356 fledglings in 294 nests by 224 adult pairs. Of the 1355 nests censused over 11 years (1999-2009), 79% were in willow, 17% in mixed, and 4% in SC dominated territories; cowbird parasitism was 11.7% in willow, 22.0% in exotic and 18.1% in mixed. Populations at some sites have partially shifted from SC to cottonwood/willow (C/W) or vice versa or moved along the river as the habitat changed. A substantial breeding population also is present in the Virgin/Muddy river system, including Pahrnagat NWR; recent restoration of native willows on the Virgin adjacent to St. George resulted in increased nest productivity as birds previously nesting in SC made a major behavioral switch to nesting in willows. Saltcedar biological control (SC/BC) was begun by Lloyd Andres (USDA-ARS, Albany, CA) in the 1960s, and Bob Pemberton (ARS, Ft. Lauderdale, FL) in the 1980s. Research began by ARS Temple, TX in 1986 (see DeLoach *et al.*, this Symposium). It met strong opposition from the US Fish and Wildlife Service (FWS), Region 2, Albuquerque, NM, when the *Diorhabda* leaf beetles from Asia were proposed for release in 1994. After 7 years of discussions, the beetles were released in May 2001, but not within 322 km of known flycatcher nesting in SC. By 2011, these beetles had defoliated 3,100 river km of SC in NV, UT, and CO and 100 to 200 km in WY, AZ, NM, by others from Crete and Tunisia in TX (along each of 3 river valleys). The local grasses, forbs and willows had recovered at many locations. The debate between the SC/BC workers and the flycatcher biologists and associated

ecologists has centered on evaluating the impact of SC. According to a 2008 paper by two of the leading flycatcher biologists, "Recent research on southwestern Willow Flycatchers has found no negative effects from breeding in *Tamarix* habitats." However, factors degrading the C/W habitat are many: 1) the shift along controlled rivers from the natural spring snow-melt floods on which the early spring reproduction of C/W is synchronized, to the more constant flows below dams that favor establishment of SC that reproduces from spring through fall, 2) shallow-rooted C/W grows only near streams but deep-rooted SC grows across the entire valley, using much more water than C/W; 3) SC lowers water tables, dries up springs and small streams, and increases soil-surface salinity and wildfires that kill or stunt C/W but to which SC is tolerant; and 4) C/W has many natural enemies in the US but SC has no major enemies, allowing its unrestrained growth and spread. The direct impact of SC on flycatcher mortality includes cowbird nest parasitism (twice as high in SC as in willow) and nest site temperatures above the lethal 43° C that kill bird eggs. Less well documented factors are 1) diversity of food insects high in C/W but low in SC, with no immatures for example but with many pollen/nectar-feeding adults in SC all produced on nearby native plants; 2) possible stress on females caused by producing multiple broods after nesting failures and possibly death during the next migration, accounting for the ca. 25% shortage of females on the breeding territories, and 3) in some areas (but not in other areas) higher predation in SC than in C/W, related to canopy density and/or surface flooding. A misinterpretation of the saltcedar impact on C/W and the flycatcher has implied a high value of the many male territories along the Rio Grande near Yuma, AZ, where no females, no nests and no reproduction occurs, thus of no value. Most reports make no mention of other bird surveys with different conclusions; or of the large, new and thriving flycatcher population at SM/EB, NM; or of the effects of high temperatures on the loss of previous breeding areas on the lower Colorado, Gila, AZ or Rio Grande of western Texas; or if SC is good habitat, why the flycatcher does not breed on the Pecos River. The information presented provides answers to these questions and refutes the concept that no negative effects occur when the flycatcher breeds in SC.

Biological Control as a Tool in Restoration and Conservation Programs and for Reducing Wildfire Risk

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Abstract

Non-native plant invasion in western North America riparian systems has caused wide-ranging impacts to ecosystems including reduced wildlife habitat for native species, increased wildfire frequency and intensity, and increased evapotranspiration rates in this arid region. The spatial extent of these invasions makes traditional control and management techniques economically and logistically unfeasible. Programs targeting invasive plant reduction as a key element to restoring critical habitat and reducing impacts to ecosystems should consider biological control as an essential tool in the management of invasive plant populations. We provide examples of how biological control is being implemented in large-scale riparian restoration, conservation, and wildfire management programs in western North America.

Benign Effects of a Retardant Dose of Glyphosate on the Biological Control Agents of Water Hyacinth and Amphibians

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Abstract

Water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach has a major impact on aquatic ecosystems in South Africa despite biological control, which remains hampered by high nutrient levels and low temperatures. Often, the biological control agents are unable to overcome rapid weed growth, necessitating intervention by herbicidal control. However, lethal doses of herbicides have harmful environmental consequences and kill the biological control agents by removing their habitat. A dose of glyphosate 0.8% retards the growth and vegetative reproduction of the weed without detrimental effects on the biological control agents. However, glyphosate is known to interfere with nitrogen (N) metabolism in plants. Depleted nitrogen resources after herbicide application have consequences for insect survival and capacity to reproduce. Moreover, glyphosate has garnered bad press for its non-target ecological impacts on amphibians. In this study, the application of 0.8% of glyphosate did not affect the nitrogen and phosphorous levels in herbicide treated water hyacinth leaves and crown samples. Moreover, water hyacinth, alone or coupled with an application of glyphosate herbicide, is potentially lethal to aquatic amphibians. All *Xenopus* larvae died in the treatments containing water hyacinth, regardless of whether they were unsprayed, or sprayed with a retardant dose or a lethal (to the plant) dose of glyphosate, while glyphosate alone was not as harmful. This study, under laboratory conditions, has shown for the first time that an invasive aquatic weed was more lethal to an aquatic vertebrate than the herbicide advocated for its control.

Hydrilla Integrated Pest Management Risk Avoidance and Mitigation Project (Hydrilla IPM RAMP)

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Abstract

The University of Florida / IFAS Entomology and Nematology Department is conducting the Hydrilla Integrated Pest Management Risk Avoidance and Mitigation Project (Hydrilla IPM RAMP). This project is designed to tackle one of the United State's most troublesome invasive plants: *Hydrilla verticillata* (L. f.) Royle. Hydrilla is an invasive freshwater plant common in Florida. It was probably brought to the Tampa and Miami areas as an aquarium plant in the late 1950s. By the 1970s, it was established throughout Florida and much of the southern United States. If left unmanaged, hydrilla is capable of creating damaging infestations which can impede waterway navigation and increase flooding. In addition, hydrilla is showing resistance to fluridone, a systemic herbicide used to manage it for the past 20 years. According to the University of Florida / IFAS Center for Aquatic and Invasive Plants, millions of dollars are spent each year on herbicides and mechanical harvesters in Florida in an effort to place hydrilla under "maintenance control." Thanks to a new 4-year grant from the USDA National Institute of Food and Agriculture, University of Florida/IFAS research and extension faculty, FAMU Faculty and an Army Corps Engineer are tackling the hydrilla problem head-on. This team is studying new chemical and biological control methods as part of an overall hydrilla integrated pest management (IPM) plan. The central hypothesis of this project involves integrating herbivory by a naturalized meristem mining midge, *Cricotopus lebetis* Sublette (Diptera: Chironomidae), with the native fungal pathogen *Mycoleptodiscus terrestris* (Gerd.) Ostaz., (1968) and low doses of a new acetolactate synthase (ALS) inhibiting herbicide (imazamox) as a viable strategy for long-term sustainable management of hydrilla. Researchers expect this IPM strategy will safely control fluridone resistant hydrilla biotypes in Florida watersheds and in other locations in the US where the resistant biotypes are expected to become established.

Biological Control of Old World Climbing Fern by *Neomusotima conspurcatalis* in Florida: Post-Release Impact Assessment and Agent Monitoring

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Abstract

Old World climbing fern, *Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae), is one of the most problematic invasive weeds impacting natural areas in southern Florida. The brown lygodium moth, *Neomusotima conspurcatalis* Warren (Lepidoptera: Crambidae), was introduced in early 2008 and rapidly developed large populations. Large larval populations caused substantial defoliation of lygodium that reduced ground cover by about 50%. As populations of the moth have fluctuated over recent years, some re-growth of lygodium has occurred, although recent data indicate that ground cover of lygodium is still lower than before the agent was released. *N. conspurcatalis* is a tropical insect and populations decline substantially during Florida's cool winter season. This affords a period in spring and early summer when lygodium can grow in the absence of larval feeding pressure. Populations of the moth increase during late spring. By late summer, larval densities on lygodium foliage in areas experiencing moth population outbreaks may reach 2,000 larvae per square meter of ground area and may sometimes exceed 16,000 larvae per square meter. At these densities larvae cause complete defoliation and significant suppression of lygodium. Parasitic wasps were first recovered from field-collected *N. conspurcatalis* larvae in autumn 2008. Six species of parasitoids have been reared from *N. conspurcatalis*, although the majority of individuals belong to a single native braconid species, *Rhygoplitis choreuti* (Viereck). Across 22 collections of *N. conspurcatalis* larvae over a 27-month period, overall emergence of adult parasitoids was 6.8% while emergence of *N. conspurcatalis* adults was 73.6%. Despite parasitism, densities of *N. conspurcatalis* larvae observed on foliage during autumn 2010 were comparable to densities recorded at the same sites during autumn 2008. Results suggest that under favorable environmental conditions, *N. conspurcatalis* is capable of contributing to suppression of lygodium in south Florida, although long-term impacts on the population dynamics of the weed are not yet known.

SESSION 7: ECOLOGICAL AND EVOLUTIONARY PROCESSES

Ecological Data Key to Building Successful Biocontrol Programs: A Case Study Using *Chrysochus asclepiadeus* (Coleoptera: Chrysomelidae) Against *Vincetoxicum* spp. (Apocynaceae)

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Summary

Native from Eurasia, *Vincetoxicum rossicum* (Kleopow) Barbar Moench and *V. nigrum* (L.) Moench (Apocynaceae) invade forested landscapes of eastern North America where they prevent regeneration of native species. A third species, *Vincetoxicum hirundinaria* Medik., was also introduced but never reached invasive status. The Eurasian phytophagous beetle, *Chrysochus asclepiadeus* (Pallus) (Chrysomelidae), was studied as a potential biocontrol agent because both adults (leaves) and larvae (roots) feed preferentially on *Vincetoxicum*. In the framework of developing a biocontrol programme in North America, we asked three key questions: 1) How does the beetle disperse in its environment?; 2) How does the beetle impact plants under different natural light conditions?; and 3) How do plants react to natural or simulated herbivory? Using mark-release-recapture, we determined that most beetles were flightless and dispersed <15 m after 25 d. Under differential light conditions, beetles defoliated all three *Vincetoxicum* spp. significantly less in full sun than in partial or full shade, suggesting that herbivory was influenced by either temperature on beetle behavior, light on leaf quality, or both. *Vincetoxicum hirundinaria* was clearly able to compensate for loss of leaf tissue by increasing its dry root biomass relative to its shoot biomass reflecting a long relationship with *C. asclepiadeus*. Both *V. nigrum* and *V. rossicum* were unable to compensate for leaf tissue lost to herbivore feeding in partial and no sun and in all light conditions, respectively. Simulated herbivory reduced *V. rossicum* growth by 30% and root herbivory and plant resistance by 60%, and the combined effect of leaf and root herbivory reduced sexual reproduction of *V. rossicum*. Data on natural herbivory showed that while *V. hirundinaria* increased allocation of resources to roots, *V. nigrum* did not. If *C. asclepiadeus* was introduced for controlling *V. rossicum* in North America, reductions in plant biomass and spread would be greatest if beetles were released on forest edges at low plant densities. In the case of *V. nigrum*, beetles could be released irrespective of plant density, but populations in forest understories would be more impacted than those in full sun. For both species, multiple beetle releases would facilitate dispersion and improve biocontrol.

Introduction

Over the past three decades two Eurasian species of swallow-wort, *Vincetoxicum rossicum* (Kleopow) Barbar Moench and *Vincetoxicum nigrum* (L.) Moench. (Apocynaceae), have become invasive in northeastern North America (NA) and now are a significant threat to native biodiversity. Both species are herbaceous, perennial vines related to native North American milkweeds. Swallow-worts have been present in NA for over a century, but have started to spread only recently. *Vincetoxicum rossicum* tends to be shade-tolerant and is found further into forest understories than *V. nigrum*, which tends to be limited to habitat edge and open areas (Milbrath 2008). Invasive *Vincetoxicum* spp. impact native faunal and plant species by out-competing endangered herbaceous species and preventing natural tree species regeneration (Tewksbury et al., 2002). There is currently no effective method to regulate populations, and biological control has been suggested as one of the few options available to provide long-term control (Miller et al., 2008). From a list of potential natural enemies in Eurasia (Weed and Casagrande, 2010; Sforza, 2009), the chrysomelid beetle *Chrysochus asclepiadeus* Pallas (Col.: Chrysomelidae) was selected for study because all developmental stages feed on *Vincetoxicum* spp.: as larvae on the roots and as adults on the foliage.

In order to evaluate the potential to use a biocontrol candidate such as *C. asclepiadeus*, we initiated ecological studies to examine the impact of beetle density on herbivory (Maguire et al., 2011) in conjunction with host specificity testing (see Poster - Sforza et al., this volume). Here, we report results from three experiments that asked key questions addressing beetle dispersal and herbivory: 1) How does *Chrysochus* disperse in its environment? (Experiment 1); 2) How does it impact plants under different natural light conditions? (Experiment 2); and 3) How do plants react to natural or simulated herbivory? (Experiment 3). Specifically, the third experiment evaluated the impact of leaf and/or root herbivory on *V. rossicum* under controlled environmental conditions.

Material and Methods

Plant material

Three species of *Vincetoxicum* were tested at the USDA's European Biocontrol Laboratory in Montpellier, France (EBCL). To address the first two experimental questions (Experiments 1 and 2), *V. hirundinaria* (widespread in Eurasia but not invasive in NA) was collected from native and non-invasive field populations in Mont Ventoux, France in November 2009. For Experiment 2, *V. rossicum* was collected from invasive field populations in Wehle State Park, New York, USA in July 2009, and *V. nigrum* was collected from invasive field populations in Bear Mountain State Park, New York, USA in September 2009. *Vincetoxicum rossicum* and *V. nigrum* rootstocks were shipped to EBCL and arrived on 17 March 2010. To address the third question (Experiment 3), all *V. rossicum* were field collected in April 2010 from High Park, Toronto, Ontario, Canada.

Insect source

For Experiments 1 and 2, *Chrysochus asclepiadeus* beetles (sex ratio 1:1) were collected in June 2010 on their host plant *V. hirundinaria* from Jura, France. The beetles were stored in cardboard cylinders for transportation to EBCL and then maintained under laboratory conditions on plants of *V. hirundinaria* until use.

Experimental design, data collection and analysis

Experiment 1: Forty groups of five *V. hirundinaria* plants were out-planted in a 60-m diameter circle at EBCL every 5 m from a center point. Additional groups of plants were similarly distributed along a diagonal axis, every 5, 10, and 25 m in the same field. Three hundred beetles were marked with colored and numbered stickers (bee marking kit, Thorne ®) to identify them individually and released in the field at the centre of the circular experimental site on 12 July

2010. Beetles were counted twice daily at 7 and 9am during the 4-week field experiment.

Experiment 2: A 3x3x2 factorial experiment in an outdoor randomized complete block design was used at EBCL using light intensity, plant species, and herbivore density as the factors. The three light level treatments were: full sun, partial sun, and no sun. Full sun conditions were achieved by placing plants directly in the field while partial sun conditions were achieved by placing them under trees. No sun conditions were achieved by placing a tarp over the plants in the field 1.5m off the ground either under fully blocked sun (full) or under trees (partial). On 21 June 2010, beetles (sex ratio 1:1) were randomly placed on potted plants (three pots per block) in the field where they remained for three weeks. They were monitored every second day throughout the experiment and total dry biomass was compared between treatments with and without (controls) beetles under the three different light conditions. The Scheirer-Ray-Hare test was used for all data that did not meet assumptions of parametric statistics.

Experiment 3: In Toronto, Canada, 200 plants were divided in April 2010 into four treatments corresponding to simulated herbivory on leaves (L): 90% of each leaf cut, on roots (R): 90% of roots removed, and on both leaves and roots (RL). Fifty plants were randomly assigned to each treatment and divided into five plots of ten plants each; another 50 plants were left untreated as controls. After one month of growth, plants were measured in terms of the number of stems per pot, the number of nodes, and flowering stage. Foliage and roots were cut 21 days later, immediately following measurements on the roots. After 30 days, the plants were harvested and the number of folicles noted. Non-parametric Wilcoxon test and Tukey's HSD were used for statistical analysis.

Results

Results from each experiment illustrated the effective use of ecological approaches in developing a successful biological control program. Working indoor and outdoor under summer climatic conditions, we were able to compare different biotic and abiotic factors that are important for optimizing the release of a biocontrol agent against the target weed.

Experiment 1: Beetle dispersion

Beetle dispersal from the release point was observed on four different dates (Fig. 1). At $t+1d$, 84% of the 179 beetles were found 5 m away from the release point, with four beetles (3%) already at 15 m from the release. For the duration of the experiment, most insects dispersed no further than 15 m, and 67% (4/6) were found at that distance at $t+25d$, while 33% remained at 5 m near the circle center (initial release point). Only two beetles were ever found at over 15 m from release, including one at $t+14d$; both of these were recovered at 25 m.

Experiment 2: Impact of light on herbivory

Scheirer-Ray-Hare tests indicated that dry total mass was significantly different among species ($p<0.001$), light levels ($p<0.001$), and herbivory levels ($p<0.001$) (Fig. 2). Addition of beetles did not significantly affect dry total biomass of either *V. hirundinaria* under any light conditions, *V. nigrum* under full sun conditions, or *V. rossicum* under full or no sun conditions. Herbivory by beetles significantly lowered dry mass of *V. nigrum* under partial and no sun conditions ($p=0.002$ and $p<0.038$) (Fig. 2) and significantly lowered dry mass of *V. rossicum* under partial sun conditions ($p=0.001$) (Fig. 2), according to Wilcoxon rank sum tests. Total dry mass of *V. hirundinaria* control (untreated) plants was significantly higher in full sun than in no sun ($p=0.040$), and *V. nigrum* control plants had significantly higher total dry mass in partial than in no sun ($p=0.038$).

Experiment 3: Simulated herbivory

The number of folicles harvested was compared according to the treatment applied (Fig. 3). Control plants had the highest number of folicles (24.5 ± 1.81 folicles/plant) with RL plants having the lowest number of folicles (5.7 ± 0.70 folicles/plant). L and R plants had a similar number of folicles, with 17.3 ± 1.40 and 16.0 ± 1.52 folicles per plant, respectively. Follicle number recorded for control and RL plants was also significantly different from the other two treatment groups. Follicle density for L and R plants was not significantly different.

Discussion

The main purpose of our study was to understand the capacity for *C. asclepiadeus* to disperse in a natural environment, and its responses to climatic factors in the context of developing a biological program for its control in North America. Our results show that adult *C. asclepiadeus* had a low dispersion rate with limited flight capability. Dispersal, primarily through walking, has been observed in several Eumolpinae family members and may be due either to atrophy of wing muscles (Tourniaire et al., 2000) as in this case, or due to winglessness, as seen in some other genera of the family (Flowers, 2004). Only two adult insects were found at 25 m from their release point and most of the others were found no further than 15 m by the end of the experiment. When an insect was found on a plant, it was generally observed to stay there until the plant was completely defoliated, often with up to 15-20 beetles congregating on the same plant. Insects could walk 5-15 m in 24 h, but only around 50% of 103 insects after 48 h had found a host plant. This suggests that if *C. asclepiadeus* were to be released in NA, several release points would be necessary to effectively cover the target area.

In addition to beetle dispersal, our study shows that *C. asclepiadeus* will defoliate more leaf tissue area under partial and no sun than under full sun conditions. There are two main reasons why beetles might be consuming more plant material under shady conditions, namely, the effect of temperature and/or the effect of light. Temperature can have a serious effect on small poikilothermic animals like beetles. Some beetle species bury themselves in soil to maintain a lower body temperature, thereby conserving water and avoiding desiccation (Cloudsley-Thompson, 2001; Gunn, 1942). As *C. asclepiadeus* appeared to bury themselves under full sun conditions, it would seem they were spending fewer hours aboveground than beetles in shady conditions. Fewer hours aboveground would mean that *C. asclepiadeus* in full sun had fewer hours to feed, resulting in lower defoliation of leaf tissue area. Cooler and moister temperatures in shady conditions would allow *C. asclepiadeus* to remain aboveground for longer, continuing to eat leaf tissue. Sunny conditions decrease the number of hours available to feed, but not the rate at which *C. asclepiadeus* feeds. Beetle consumption of leaf tissue area was

also dependent on species: *V. nigrum* experienced less damage than *V. rossicum* or *V. hirundinaria*. It is likely that these differences are based on differences in size rather than *C. asclepiadeus* herbivory preference. The large size of *V. nigrum* compared to *V. rossicum* and *V. hirundinaria* suggests that the *V. nigrum* plants used in the experiment were older. As *Vincetoxicum* spp. are perennial, it is difficult to ensure that all plants collected are of the same age. If *C. asclepiadeus* were eating the same amount of leaf per capita on all three plants, but *V. nigrum* was larger, damage levels on *V. nigrum* would be expected to be lower. *Chrysomus asclepiadeus* were not eating less total mass of *V. nigrum*, but rather inflicted lower damage levels because defoliation was measured as a percentage of aboveground tissue lost.

In the third experiment, herbivore pressure on the plants also had an effect on the production of fruits and seeds, which can be described as an indirect effect because herbivory in this case was not directed against these specific organs. The treatments had an effect on fruit size of *V. rossicum* and on seed development (data not shown). Given the number of fruits produced per plant, the separate treatments on leaves and roots (L and R) had a negative impact, which is similar, and this impact was even greater when both treatments were combined. The effect of the two treatments appears to be synergistic. Treatments can then be arranged according to the importance of their impact on follicle development with herbivory on roots and leaves having the greatest effect, followed by treatment on roots only, and finally by leaves only. Herbivory had the same effect on seed production and development (data not shown).

Our results show an indirect effect of herbivory on the target plants, an effect not always seen in ecological studies of biocontrol agents and this has implications for successful implementation of biological control strategies. For example, if a seed feeder such as *Euphranta connexa* (Fab.) (Dipt.: Tephritidae) was to be used for control of swallow-worts, it should not be released into shady areas where the number of follicles is small or where the root and leaf herbivory is already sufficient to impact plant sexual reproduction. In shady areas it would seem more important to focus on phytophagous insects affecting leaves and roots than on those that will impact reproductive structures.

Classical biological control with herbivorous insects to control the expansion of an invasive plant requires many years of study, but once set up, can be more effective than repeated short-term measures (McFadyen, 1998). For targets such as swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*), high plant densities and large areas covered in regions of introduction present a major obstacle to success; however, such challenges could be met by releasing herbivorous insects in small numbers strategically at targeted locations. Thus, it is important to couple comparative studies on the ecology of the system and potential for biological control that include simulated herbivory in the invaded area (Milbrath, 2008; Doubleday and Cappuccino, 2011) as well as natural herbivory in the native area (Gassman et al., 2010; Sforza, 2009; Weed and Casagrande, 2010; Maguire et al., 2011) in order to make a final decision as to whether a biocontrol agent can be developed for use. Such studies should include measuring the potential for an insect to control the target plant, but also to limit its unintentional risks to non-target species. The stakes for success in systems such as swallow-worts are very high because these plants, currently a major problem in forested ecosystems, particularly in Canada and the northeastern USA, continue to invade new habitats outside of forests throughout North America, including grasslands, farmlands and urban areas.

Acknowledgements

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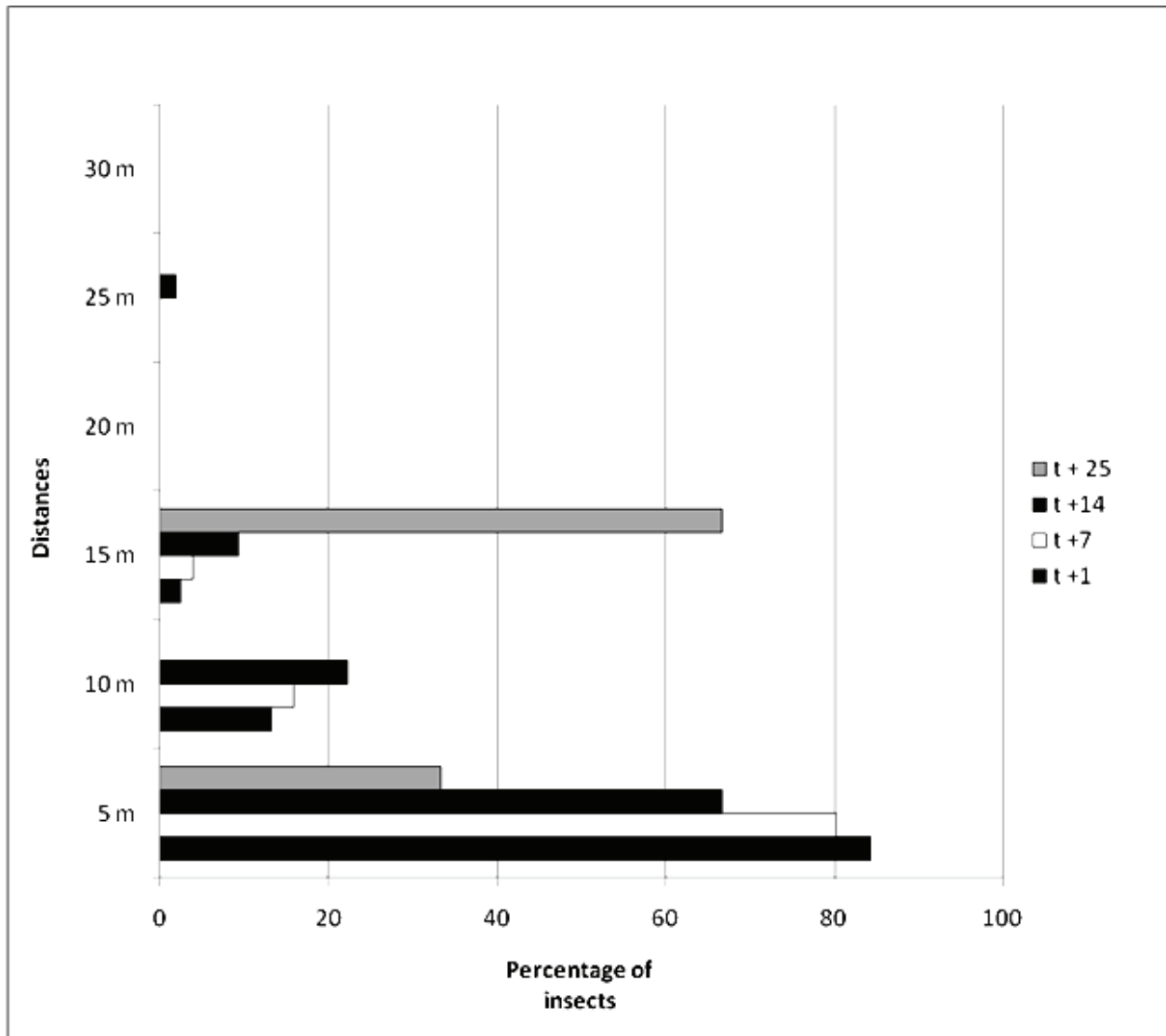


Figure 1. Dispersal activity of adults of *Chrysochus asclepiadeus* (Col.: Chrysomelidae) observed from 14 July to 9 August 2010, in percentage of alive insects observed for both sexes at 4 selected observations (t+1d, t+7d, t+14d and t+25d).

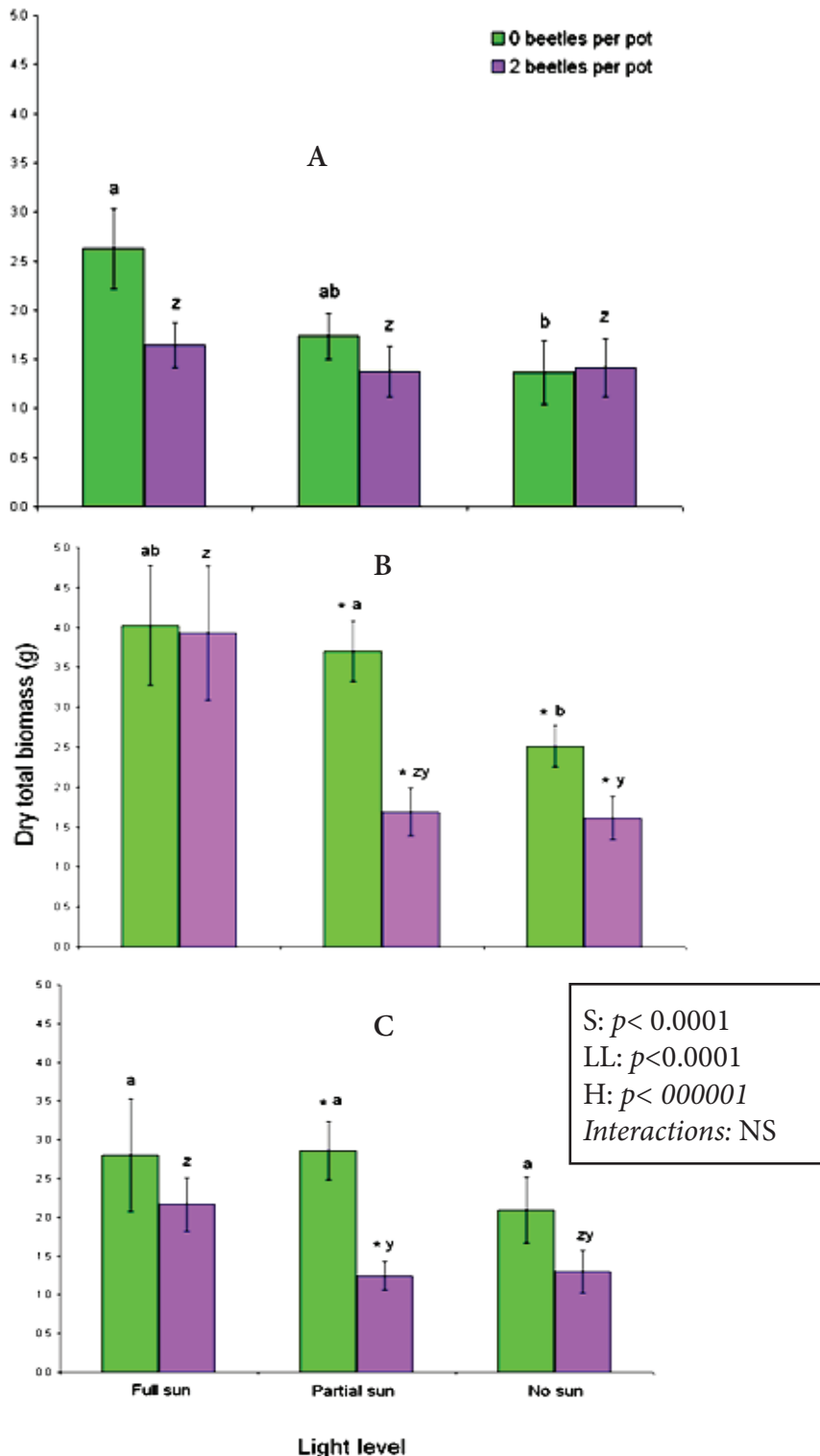


Figure 2. Total dry biomass (g) for A. *Vincetoxicum hirundinaria*, B. *V. nigrum*, and C. *V. rossicum*. Biomass plotted against beetle density and light level for the shading and herbivory experiment conducted outside of Montpellier, France in June and July 2010. Within light level, an asterisk denotes significant difference; between light levels within herbivory treatment, no shared letters denote significant differences (Wilcoxon rank sum test, $p < 0.05$). The legend above the graph reports the results of a Scheirer-Ray-Hare test in R (S = Species; LL = Light Level; H = Herbivory). Bars represent significant error.

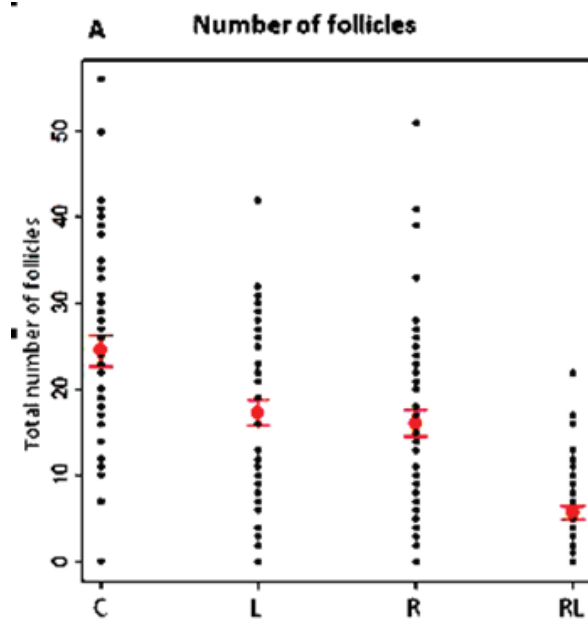


Figure 3. Number of follicles at harvest by treatment. Treatments: C = control; L= simulated herbivory on leaves; R = simulated herbivory on roots; RL = simulated herbivory on leaves and roots.

Evidence of Rapid Evolution from Weed Biological Control Introductions

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Abstract

A capacity to measure and the potential importance of real time rapid evolution in species is starting to revolutionize evolutionary biology turning it from a theoretical to a more empirical or even applied science. An increasing number of examples are being found of species that have been shown to be evolving rapidly and adaptively in response to selection pressures in empirically measurable timeframes. This has been particularly true for insect herbivores and the plants they feed on, most notably seed beetles and soapberry bugs. This potentially questions the argument that biological control agents and their targets have a low risk of evolving to the detriment of either biological control efficacy or increased non-target impacts within management relevant timescales. Biological control releases have been ongoing now for more than 100 years, which based on other observations of rapid evolution, should have provided ample time for rapid evolution in the agents released or their target weeds. It would seem that introducing agents onto target host plants in totally new environments should provide multiple new evolutionary adaptive pressures on each trophic level, through their ongoing “arms race”, via the changed circumstances of their ecological interactions. This paper will review the evidence of post-release rapid evolution in weed biological control agents and their target weeds (particularly where effective biological control may be breaking down), studies that have looked for such post-release rapid evolution, and theoretical studies that suggest when adaptive evolution might be likely. Finally we will discuss evidence found in the context of systems that are showing such evolution, and suggest future science directions for rapid evolution research with practical relevance to weed biological control programs.

Polyploidy and Invasion Success in Spotted Knapweed, *Centaurea stoebe*: Specialist Herbivores as Drivers of Invasions and Effective Control Agents?

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Abstract

Evidence indicates that invasive plants are frequently characterized by natural variation in ploidy level in their native range but only polyploid forms in their invasive range. In addition, cytotypes in the native range of a species can be spatially separated despite occupying similar environmental niches. The mechanisms dictating these patterns are poorly understood but may relate to interactions with specialist herbivores. Selection derived from plant-herbivore interactions has the potential to contribute to the evolution of plants following polyploidisation events by differential selection for defense and life history traits. Escape from such interactions may result in both different population-level responses for cytotypes with different traits and, in the case of introduced populations, lead to rapid evolutionary change. Such evolutionary changes between native and invasive cytotypes may, in turn, lead to increased biological control efficacy. We analyzed in detail the multiple interactions between over 100 populations of spotted knapweed, *Centaurea stoebe* L., and its specialist insect herbivores, some of which have been introduced as biological control agents for the past 30 years. Both diploid and tetraploid cytotypes occur in the native European range, but only tetraploids occur in the introduced range in North America. Our studies include gene expression studies of transcripts related to defense, a series of bioassays with specialist root feeders and a common garden demography experiment with various accessions of *C. stoebe* cytotypes that differ in their life histories, in the presence and absence of these root feeders. We will summarize and discuss our results and that of other groups to come up with a general framework towards a better understanding of the role of specialist herbivores in promoting invasion success of polyploids and to predict their biological control efficacy.

The Roles of Demography and Genetics in the Founding of New Populations

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Abstract

The successful founding of new populations is critical in biological control. Three main issues that influence establishment are: the environment, the number of individuals in the founding group (a fundamental aspect of demography), and the genetic background and diversity of those individuals. We have little understanding of interactions between these factors. In particular, demography and genetics are inherently linked because more individuals typically harbor greater genetic variation. Using two different model study organisms, *Bemisia tabaci* (Gennadius) and *Tribolium castaneum* (Herbst), we show that genetics can play a key role even when demography is held constant, and that the outcome depends upon environment. Importantly, *Bemisia* were able to reproduce in a harsh environment only when outbred. In a benign environment inbred individuals were as successful as outbred individuals. Somewhat similarly, outbred *Tribolium* performed better than inbred individuals when in a harsh environment. In this case, however, inbreeding led to inbreeding depression even in a benign environment. For both model systems, performance increased with the size of the founding group. These data suggest that when conducting releases for biological control, it may be critical to conserve or even enhance diversity of the founding group while maximizing release size. *The authors note that two publications are anticipated from this work, but have not yet been submitted.*

Evolutionary Interactions between the Invasive Tallow Tree and Herbivores: Implications for Biological Control

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Abstract

Understanding interactions between insect agents and host plants is critical for forecasting their impact before the insects are introduced, and for improving our knowledge of the mechanisms driving success or failure in biological weed control. As invasive plants may undergo rapid adaptive evolution during the process of range expansion, the potential evolutionary interactions of insects and plants may influence the effectiveness of biological control. In this presentation we will discuss the biogeographic variation in plant defense to insects in the tallow tree, *Triadica sebifera* (L.) Small, which is native to China but invasive in the United States. Because the U.S. populations showed reduced resistance but increased tolerance to herbivory by specialists, we predict that the invasive tallow tree may support a rapid population build-up of insect agents but the insects' impact may be low if these specialists are introduced. Our chemical analysis shows that the U.S. populations had low concentrations of quantitative defense compounds but high qualitative defense compounds, which suggests that plants from invasive populations have altered chemistry that influences the selection and development of insect biological control agents. We will also discuss our current study on the evolutionary interactions of above-below ground herbivores in tallow tree, which can affect invasion success, herbivore population dynamics and biological control. As invasive plants may employ novel defense strategies to cope with the differing herbivore communities, thus affecting biological control, we conclude that without taking into account the differences in resistance and tolerance to herbivores of plants from the native vs. invasive range, predictions of the ease of establishment of agents and their effectiveness at controlling host plants may both be incorrect.

The Evolutionary Response of *Lythrum salicaria* to Biological Control: Linking Patterns in Plant Evolution and Management Efficacy

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Abstract

Like the introduction of invasive species, management programs can impose novel selective pressures in ecosystems. In some cases sufficient variation may exist in populations of invasive species for them to evolve resistance to management techniques. The evolution of resistance to management by herbicide has been repeatedly documented in weedy invasive species. Biological control is becoming increasingly common, and it is possible that invasive species may evolve resistance to biological control agents ultimately reducing the efficacy of these programs. Purple loosestrife (*Lythrum salicaria* L.) is an invasive wetland plant introduced to the U.S. in the early 1800s. In 1992 a classical biocontrol program was launched introducing leaf feeding beetles from Germany to manage invasive populations. As a result of this program, two beetle species have established in Minnesota. Variable success has been achieved in wetlands throughout the state; biocontrol agents have defoliated 90-100% of some purple loosestrife populations and had little to no observed effect on others. We identified three sites that consistently experienced historically high levels of herbivory by the biocontrol agents as well as three sites experiencing low levels of herbivory. In this study we examined the evolutionary divergence of plant vigor, herbivore defense and traits associated with competitive ability between historically high and low herbivory populations. Purple loosestrife from populations subject to greater selective pressure from the biocontrol agents has evolved higher vigor and produces lower concentrations of herbivore defense compounds. Taken together these results suggest that *L. salicaria* is in the process of evolving tolerance to herbivory from biological control agents. In ongoing work, we will 1) investigate the effect of this evolutionary divergence on herbivore preference in colonization, feeding and egg laying and 2) quantify the heritability of plant variation to model the evolutionary trajectory of these traits under continued biological control.

Regarding the Role of New Host Associations in the Success of *Cactoblastis cactorum* as Both a Biological Control Agent and Invasive Species

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Abstract

A key theoretical basis for using classic biological control against invasive alien species (IAS) has been the enemy release hypothesis (ERH), which suggests that the increased vigor and invasiveness of IAS in the introduced range is strongly influenced by their release from co-evolved natural enemies. Classical biological control aims to reunite IAS with their natural enemies and restore ecological balance and stability. The ERH is supported by the historical example of highly-invasive *Opuntia* (Mill.) spp. in Australia that had been introduced from North America without their natural enemies. It also is supported by the legendary control of these invasive *Opuntia* spp. when the Argentine cactus moth, *Cactoblastis cactorum* (Berg), was introduced from Argentina without its natural enemies. *Cactoblastis cactorum* later was unintentionally introduced into Florida where it has rapidly expanded its geographical range along both the Atlantic and Gulf of Mexico coasts, invaded the Yucatan Peninsula, Mexico, and threatens *Opuntia*-based agriculture and ecosystems in the southwestern USA and Mexico. Although the ERH predicts that classical biological control would be indicated for this invasive pest, we wanted to examine the role of all mortality factors in the native range using life table analysis on both native, co-evolved *Opuntia* host species and on an introduced, non-co-evolved *Opuntia* host species. We found limited egg and larval mortality due to predators or parasitoids, however there was a strong influence of co-evolved host plant resistance by the native *Opuntia* spp. Larval establishment, rate of larval development, and number of generations per year were lower on native *Opuntia* spp. than on introduced *Opuntia* spp. Although the ERH may be a factor in the success of *C. cactorum* as both a biological control agent and an invasive species, results from our studies in its native range suggest that release from co-evolved host plant resistance may exert a greater influence.

Multitrophic Interactions in Biological Control: Evaluating Shifts in the Competitive Ability of *Lagarosiphon major* as Influenced by Herbivory and Parasitism

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Abstract

Lagarosiphon major (Ridley) Moss (Hydrocharitaceae) is a submersed aquatic macrophyte, indigenous to South Africa, and poses a significant threat to water bodies in Europe, New Zealand and Australia. Dense infestations of *L. major* in invaded ranges readily out-compete indigenous submerged species, altering the ecology of freshwater systems. Laboratory studies have also shown *L. major* to be a superior competitor. A recently discovered ephydrid fly from South Africa, *Hydrellia lagarosiphon* (Diptera: Ephydridae), has been investigated as a potential control agent against *L. major*. Often, the subtle effects of herbivory have a significant impact on the competitive ability of invasive plants, therefore the impact of feeding by *H. lagarosiphon* on the competitive interactions between *L. major* and *Myriophyllum spicatum* L., a submerged aquatic plant native to Eurasia, was evaluated using an inverse linear model. The results showed that herbivory by *H. lagarosiphon* reduces the competitive ability of *L. major* in favour of *M. spicatum*, providing further support for *H. lagarosiphon* as an effective biological control agent. Parasitism of *Hydrellia* species by braconid parasitoids is well documented, and could be realized in the field should *H. lagarosiphon* be released. Therefore competitive interaction between the two plant species under the influence of *H. lagarosiphon* and a parasitoid wasp, *Chaenusa* Haliday sp. (Hymenoptera: Braconidae) was also examined. The addition of the parasitoid reduced the impact of herbivory by the fly on *L. major*, thereby shifting the competitive balance in favour of *M. spicatum*. This study highlights the need to evaluate multitrophic interactions in biological control programmes, particularly those where the agents are known to be susceptible to parasitism.

Searching for the Signal of Competition in Plant-Mediated Interactions among Coexisting Gall Insects on Broad-Leaved Paperbark

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Abstract

The central prediction of competition theory is that a reciprocal struggle for resources should manifest itself among coexisting species under conditions of increasing functional similarity (e.g. similar feeding niche), density and spatio-temporal overlap. We investigated whether the signal from such a struggle was evident in a community of phytophagous gall insects on *Melaleuca quinquenervia* (Cav.) S.F. Blake meeting these conditions. Specifically, we examined plant-mediated interactions among three species galling vegetative tips with varying degrees of temporal overlap. The abundance of early gallers (*Sphaerococcus ferrugineus* Froggatt and *Fergusonina turneri* Taylor) did not influence resource availability for the late galler (*Lophodiplosis indentata* Gagné), suggesting the absence of temporally separated exploitative competition. However the abundance of *S. ferrugineus* was positively correlated with the abundance of *L. indentata*, which suggests facilitation or similar responses to the prevailing conditions. Examination of the reciprocal impacts of the late galler on early gallers paradoxically revealed that though galling by *L. indentata* may be reducing the resource availability for early gallers, the abundance of *L. indentata* was positively influenced by the abundance of early gallers (an effect that is stronger for *S. ferrugineus* than *F. turneri*) suggesting facilitation. The strong influence of site on the interactions among the different cecidogenic species indicated that any role for competition/facilitation may be spatially constrained. We explore potential processes that may be driving the patterns that we detected, and also the implications for the use of these species as biological control agents for *M. quinquenervia*.

Acknowledgements

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References

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Biological Control, Prey Subsidies, and Food Webs: One Plant, Two Insects, and Two Outcomes

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Abstract

Introducing insect biological control agents into communities dominated by a single plant species can affect food webs as existing consumers respond to a new resource. The short and long term implications of these new interactions on community ecology are poorly understood and interpreting their influence remains subjective. Two insect biological control agents were introduced to control *Melaleuca quinquenervia* (Cav.) S.F. Blake in Florida wetlands in 1997 and 2001. The first, *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae), has larvae that are chemically defended by a secreted sticky covering of essential oils derived from the plant. The second species, *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae), has no obvious defenses. The defended *O. vitiosa* larvae were predated primarily by heteropterans, especially *Podisus mucronatus* (Say) (Hemiptera: Pentatomidae), while nymphs and adults of the undefended *B. melaleucae* were prey for spiders (10 species), coccinellids, neuropterans, syrphids, and heteropterans. Predator guilds and densities varied in time, space, and scale. In some cases, spiders built webs over branch tips that contained colonies of *B. melaleucae* and those colonies contained 40% fewer adults and nymphs than colonies outside webs. In other situations where spiders were limited and coccinellids were abundant, *B. melaleucae* populations were reduced by 95.2%. Ironically, one of their major predators was an introduced insect biological control agent, *Harmonia axyridis* (Pallus) (Coleoptera: Coccinellidae). Although some predator populations did increase in response to increases in biological control agent populations, intraguild predation and prey switching likely complicated these interactions. For example, coccinellid densities were negatively correlated with spider densities. Despite the presence of persistent and growing populations of *O. vitiosa*, there was no density dependent response by *P. mucronatus*, illustrating that even consistently elevated levels of biological control agents do not inevitably translate into persistent resource opportunities for consumers that may result in unpredictable modifications to food webs.

Who is Controlling Knapweed? A Genetic Investigation of *Larinus* spp. in a Successful Biological Control Program for Knapweed in Canada

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Abstract

Two species of seed feeding weevils, *Larinus minutus* Gyllenhal and *L. obtusus* Gyllenhal, were introduced to Canada in the early 1990s for the biological control of diffuse, *Centaurea diffusa* Lam., and spotted, *Centaurea stoebe* L. ssp. *micranthos*, knapweeds. These two weevil species are very similar morphologically, and this has made their identifications in the field difficult. The original introductions of *L. minutus* were made from 3 collections from *Centaurea arenaria* M. Bieb. ex Willd. at one site in Romania in 1991. *Larinus obtusus* for introduction were collected from *Centaurea phrygia* L. at one site in Romania and from *Centaurea jacea* L. at a second site in Serbia. *Larinus minutus* has successfully controlled diffuse knapweed in many areas of North America and *L. obtusus* has reduced densities of spotted knapweed in some locations. We sequenced the mitochondrial CO₁ locus for individuals in British Columbia and Romania, and have genotyped these individuals at a suite of polymorphic microsatellite loci. We identify the distributions of the *Larinus* lineages in British Columbia, Canada and explore their levels of genetic variation since introduction. The genetic structure of current populations is compared to that of source populations in Romania and Serbia and type specimens preserved at the time of the original introductions. Genetic typing of biological control agents is recommended for all programs to provide a baseline of information on biotypes and genetic evolution of introduced populations.

Hares or Tortoises? How to Choose an Optimally Dispersing Biological Control Agent

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Abstract

For insect-plant interactions, dispersal can be an important process affecting the long-term dynamics of both populations. This is particularly relevant to the field of weed biological control which aims to correctly match a specialist herbivore with a target plant species such that a stable, low-density equilibrium will be achieved. There is, however, little information available to guide decisions with respect to the dispersal abilities of the insect agents chosen. To address this, an individual based simulation model was constructed to explore how the interaction between plant and insect dispersal affect both 1) plant population stability; and 2) average plant density. For plant population stability we found that simulating turning angles and step-lengths that resulted in intermediate rates of insect dispersal produced the most stable dynamics. We also found, somewhat counter-intuitively, that as plant dispersal increased, lower rates of insect dispersal were required to produce stable dynamics. Secondly, when considering average plant density, shorter step-lengths and straighter turning angles for insects tended to result in lower plant densities compared to larger steps and more tortuous paths. This pattern was consistent across the range of simulated plant dispersal strategies. These results suggest that in the absence of other stabilizing mechanisms, the dispersal rate of the agent relative to the dispersal of the target weed is an important factor to consider and that accurate dispersal estimates will enable more reliable predictions of overall efficacy.

The Evolution of Invasiveness: Testing the EICA Hypothesis with Three Weeds of Hawaiian Forests

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Abstract

Invasive plants often appear larger in introduced versus native ranges. This has historically been attributed to better growing conditions in the absence of natural enemies (Enemy Release Hypothesis – ERH), but more recent research suggests invasive populations may evolve rapidly when freed from enemy attack (Evolution of Increased Competitive Abilities – EICA). To test the EICA hypothesis, I compared three species of disruptive weeds of Hawaiian rainforests with their native South American counterparts in a common environment. In quarantine in Hawaii, I raised 1,400 individuals of the tree *Psidium cattleianum* Sabine, the shrub *Clidemia hirta* (L.) D. Don, and the herb *Tibouchina herbacea* (DC.) Cogn. from seeds collected from 24 populations in South America and Hawaii. As predicted, I found superior relative growth rate, height, and biomass of individuals descended from Hawaiian populations, where these plants are invasive. However, contrary to EICA predictions, I found no reduction in defense including resistance to specialist enemies among the invasive populations. I conclude that the growth differences between Hawaiian and South American populations represent an evolutionary shift favoring invasion, though this shift appears more likely driven by founder effects rather than natural enemy release. The growth and defense differences observed also suggest that co-evolved specialist enemies may exert lower population-level control on these invasive plants, and that a suite of biological control agents may be necessary to maintain these species at ecologically acceptable levels across landscapes. These findings support further research to identify the founder populations of invasive plants. This research could lead to the discovery of additional co-evolved natural enemies and potential biological control agents.

How Will Predicted Climate Change Affect Weed Biological Control in New Zealand?

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Abstract

By 2090, New Zealand (NZ) is predicted to be around 2°C warmer, on average, than in 1990. Rainfall is expected to increase in the west and decline in the east, and extreme weather events may be more common. We report on a recent assessment of the potential effects of these predicted climate changes on weed biological control systems in NZ. We conclude that “sleeper weeds” are likely to become problems under future climate change scenarios, and pre-emptive action could be taken, particularly if biological control has already been successful overseas. Existing weeds are likely to expand or shrink their geographic ranges under predicted climate change, but we consider that existing biological control agents will mostly track the changing distributions of their host plants. Exceptions could occur with existing weed biological control systems being affected positively or negatively. For example, the released biological control agents for *Pilosella officinarum* F. W. Schultz & Sch. Bip. do not do well in summer droughts in NZ, so the increased rainfall predicted for inland South Island areas might improve biological control of this weed. Conversely, increases in flooding may decrease the effectiveness of biological control of alligator weed. Other issues of potential concern include losses of synchrony between weeds and their biological control agents, changes in host plant nutrition, and possible increases in non-target effects, but almost all are speculative as we lack data. However, recent studies show that suppression of ragwort by flea beetle, *Longitarsus jacobaeae* (Waterhouse, 1858), is likely to fail when mean annual rainfall exceeds 1670mm. A preliminary GIS analysis showed increased annual rainfall could cause ragwort biological control to fail in some western regions, while suppression of ragwort through biological control could be attained in parts of North Island as rainfall decreases.

Modeling Current and Future Climate to Predict the Spread of Invasive Knotweeds and their Biological Control Agent in Western North America

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Abstract

Invasive knotweeds, including Japanese knotweed, *Fallopia japonica* (Houttuyn) Ronse Decraene), giant knotweed, *Fallopia sachalinensis* (F. Schmidt) Ronse Decraene, and their hybrid cross, *Fallopia x bohemica* (Chrtek & Chrtková) J. P. Bailey, pose a serious threat to North American habitats. Examination of existing point data for knotweed distribution in the Pacific Northwest indicates sampling gaps and potential areas of spread within jurisdictions and across borders. Climatic conditions including annual degree days, mean-annual minimum temperatures and precipitation at knotweed sites in British Columbia, Washington and Oregon were modeled in BioSIM, using weather normals and long-term daily-weather data. These conditions were compared to published biotic thresholds for knotweed (degree day = 2505 DD, minimum temperature = -30.2 °C, base temperature 0 °C, precipitation=735mm) to estimate its potential range. The key limiting climatic thresholds varied between and within the three jurisdictions; in British Columbia the degree-day threshold was most limiting for Japanese knotweed with 12.3% of province habitat suitable whereas in Washington and Oregon precipitation was the most important single factor. There are still significant areas for new invasion in all jurisdictions associated with climate thresholds. Habitat suitability maps generated using local parameters will enable better targeting of knotweed surveys based on the risk of knotweed establishment. Consideration of shifts in temperature associated with climate change models suggest an even larger potential for spread that also varies by jurisdiction. The psyllid, *Aphalara itadori* (Shinji), is a promising biological control agent for knotweeds in North America. Knotweed genotypes have been shown to vary significantly in North America and Europe. In pre-release screening experiments, the performance of *A. itadori* differed significantly among knotweed clones collected from British Columbia. Variable climatic thresholds across the invaded region of the Pacific Northwest may result in selection for differing knotweed genotypes locally. This may affect the overall impact of the biological control agent, depending on the host-suitability of the available knotweed genotypes in a region.

Mapping Giant Reed along the Rio Grande Using Airborne and Satellite Imagery

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Abstract

Giant reed (*Arundo donax* L.) is a perennial invasive weed that presents a severe threat to agroecosystems and riparian areas in the Texas and Mexican portions of the Rio Grande Basin. The objective of this poster is to give an overview on the use of aerial photography, airborne multispectral and hyperspectral imagery, and high resolution satellite imagery to detect and map giant reed infestations along the Texas-Mexico portion of the Rio Grande. Aerial color-infrared photographs were taken along the Rio Grande between Brownsville and El Paso, Texas in 2002 and 2008. QuickBird and SPOT 5 satellite imagery was acquired along a section of the river near Del Rio, Texas in 2005. More recently, airborne multispectral and hyperspectral imagery was also acquired along the river near Quemado, Texas in 2009 and 2010. Methods and procedures for image acquisition, processing and classification of different types of imagery as well as accuracy assessment are briefly discussed. Examples are given to illustrate how the different types of imagery have been used to map giant reed. Results from these studies indicate that all the types of imagery can be successfully used to map giant reed. Analysis of the aerial photographs along the river showed that a total of 6000 ha of giant reed infested both sides of the Rio Grande over 900 river kilometers between Lajitas and San Ygnacio. The methodologies and techniques presented in this poster can be used for monitoring and mapping giant reed in the Rio Grande Basin and other giant reed-infested areas. The area estimates are useful for both land owners and government agencies for the estimation of water usage and economic loss and for the management and control of giant reed.

Effects of Drought on the Biological Control of Spotted Knapweed

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Abstract

Biological control provides a powerful tool for suppression of invasive plants, but the success of this tool can depend on abiotic conditions which are sensitive to climate change. Spotted knapweed (*Centaurea stoebe* L.) is one of the worst weeds in western North America, but the root weevil *Cyphocleonus achates* (Fahraeus) has shown promise as a biocontrol agent. Recent declines in knapweed documented in Montana coincided with both prolonged drought and increased distribution of *C. achates*, suggesting that the effectiveness of this biocontrol agent may depend on precipitation. We conducted a microcosm experiment to determine the effect of *C. achates* on knapweed and competition with native grasses under drought relative to normal precipitation levels. We found that both the *C. achates* and drought treatments reduced survival of adult knapweed plants. However, the negative effect of the biocontrol agent on knapweed survival was particularly evident under normal compared to drought conditions. Seed production per knapweed did not differ between plots including or excluding the agent, nor did densities of knapweed seedlings or juveniles. However, recruitment of new knapweed adults was significantly higher in the presence versus absence of *C. achates*, specifically in plots receiving normal precipitation where adult mortality was also higher. Even so, there were trends towards increased reproduction and recruitment of the native bunchgrass, *Pseudoroegneria spicata* (Pursh) A. Löve ssp. *spicata*, in the presence versus absence of *C. achates*, though these differences were not significant as of two years following release of the agent. We will continue the experiment to determine the ultimate fate of knapweed populations and the degree to which this native grass may recover under the differing abiotic scenarios. At this point, our results demonstrate that precipitation levels can influence the efficacy of knapweed biological control, suggesting that prolonged droughts triggered by climate change may alter the effectiveness of this tool.

***Solanum elaeagnifolium* (Solanaceae), an Alien Invasive Weed for Greece and Southern Europe, and its Newly Discovered Endemic Natural Enemies**

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Abstract

Solanum elaeagnifolium Cavanille (Solanaceae) is an alien invasive weed to Greece and many North African countries that have a Mediterranean climate. Since its establishment in Greece in the early 1900s, it has become rapidly one the most dangerous and aggressive weeds in the country, especially in the north and in the central area. Climate change, reduction in precipitation and increasing temperatures in the Mediterranean Basin increases the possibility of spread and establishment of the weed in new regions because of its high level of drought resistance. In 2010, four new insect natural enemies of silverleaf nightshade were discovered in Greece. These attack the reproductive parts of the weed, and the damage to seed production appears to be substantial. *Solanum elaeagnifolium* reproduces mainly using its extensive rootstocks but further studies are needed to determine the impact of its native natural enemies on the weed's suppression and population. The use of endemic natural enemies of the invasive, alien weeds in Europe brings the challenge of their control to a new level in the beginning of 21st century.

Microsatellites Uncover Multiple Introductions of Clonal Giant Reed (*Arundo donax*) in the New World

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Abstract

Giant reed, *Arundo donax* L., is a clonal, invasive weed that is native to the Mediterranean region. Tens of thousands of hectares of riparian habitat in the Rio Grande Basin (RGB) in Texas and Mexico have been heavily impacted by invasions of *Arundo*. In addition, many other watersheds across the southwestern United States have also been affected. Giant reed is being targeted for biological control because it displaces native vegetation and consumes water that could potentially be used for agricultural purposes, especially in areas with limited access to water, like the RGB. Finding the best-adapted insects for biological control involves locating the origin(s) of this plant. To locate the source(s) and trace the invasion of giant reed in the RGB, ten microsatellite markers were developed. An analysis of 203 Old World and 159 North American plants, with an emphasis on the RGB, indicate a reduction in the allelic diversity in the introduced range compared to the native range. The results also indicate that there were as many as seven or more introductions in North America, with one lineage responsible for the invasion of the RGB, Argentina, and Northern Mexico and other parts of the Southwestern United States. While no identical matches with the RGB lineage were found in the native range, several close matches were found on the Mediterranean coast of Spain.

Utility of Microsatellite Markers from the Wheat Genetic Map in the Genome of Medusahead Rye (*Taeniatherum caput-medusae*)

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Abstract

Medusahead rye (*Taeniatherum caput-medusae* (L.) Nevski) is a winter annual grass that is native to Eurasia and invasive in western North America. DNA markers were desired to facilitate the study of medusahead population genetics as well as for analysis of the inheritance of key traits. In this study we demonstrate the utility of PCR-based microsatellite markers (SSRs) from the wheat genome as polymorphic genetic markers in the medusahead genome. In a preliminary screen of 37 wheat SSRs taken from across the three wheat genomes (A, B, and D), 19 wheat SSR primer pairs (51%) successfully amplified bands from medusahead template DNA. From these 19 markers, 12 of which produced multiple bands, 40 polymorphic bands have been scored among a group of six medusahead populations from NW Nevada and NE California. Wheat SSRs from the A genome, which is more closely related to medusahead phylogenetically than the B or D genomes, were no more likely to amplify than SSRs from the other wheat genomes. This study shows that intergeneric use of existing PCR-based genetic markers can provide an inexpensive source of molecular genetic tools for study of invasive weed species, particularly those that are closely related to economically important plant species with established genomic resources.

The Interaction between Drought and Herbivory by a Biological Control Agent on Populations of the Invasive Shrub *Tamarix* sp.

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Abstract

Having a clear picture of how herbivory and environmental stress affect plant performance can contribute to more effective weed biological control programs. However, the ways these two factors interact are often hard to predict. We aimed to test two popular hypotheses regarding the outcome of plant environmental stress and herbivory: the Compensatory Continuum Hypotheses (CCH) and the Limited Resource Model (LRM). The CCH predicts that a plant's tolerance to herbivory should be greater in high-resource environment whereas the LRM predicts that plant fitness is dependent upon the particular types of stressors and herbivores and will not be necessarily favored in a high-resource environment. In a common garden, we subjected potted individuals of *Tamarix* L. sp. to a 2x2 factorial treatment design of drought and herbivory by biological control agent, *Diorhabda carinulata* (Desbrochers, 1870) (Coleoptera: Chrysomelidae). We designed our experiment to detect differences in responses to herbivory and drought among six North American populations of *Tamarix* sp. representing a latitudinal gradient. The size, growth rate and vigor of 120 plants were measured over a 17-week period. Plants grown in drought conditions had higher tolerances to herbivory than those grown under well-watered conditions, providing support for the LRM. For the population effect, plants from southern latitudes appeared to be more negatively affected by drought or herbivory than were plants from northern latitudes. Our hope is that a greater understanding of interactions among resource availability, herbivory, and populations will lead to more successful weed biological control programs, particularly in this system.

Post-Introduction Evolution in the Biological Control Agent *Longitarsus jacobaeae*

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Abstract

Biocontrol introductions provide excellent opportunities to study microevolutionary processes since the time and source of release of an exotic organism to a new environment is usually precisely known. The tansy ragwort flea beetle, *Longitarsus jacobaeae* (Waterhouse), was introduced in 1969 from a Mediterranean climate in Italy to California to control the invasive tansy ragwort, *Jacobaea vulgaris* L. During the past 40 years, these beetles have established in a wide range of environments, from sea level to 1300 m elevations, and from the Pacific coast to eastern Montana. We tested whether rapid evolution has taken place, leading to adaptation of the ragwort flea beetle to a high-elevation environment at Mt. Hood, Oregon. At this site, temperatures are much cooler and the growing season is about six months shorter than in Italy. The life history of Mt. Hood beetles was compared to two low-elevation Italian beetle populations in Oregon, and to a cold-adapted Swiss population, using common garden and reciprocal transplant experiments. The results indicate that the Mt. Hood population of the beetle underwent rapid evolution and adapted to the cooler conditions in less than 30 years with shifts in life history (and morphological) traits that conform to predictions based on models, empirical studies and the phenology of the known cold-adapted Swiss beetles.

Eurasian Watermilfoil Phenology and Endophyte Abundance and Diversity

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Abstract

Eurasian watermilfoil (*Myriophyllum spicatum* L.) plants were collected monthly from late May through late October 2007 from a culture pond located at a United States Army Corps of Engineers research facility in Lewisville, Texas and examined for endophytes in the roots, root crowns, stems, and leaves. At each collection period, ten randomly selected plants were partitioned into 2 cm segments and sequentially plated onto Martin's agar starting from the root tip and progressing to the plant apex. A total of 1479 endophytic fungi comprising 59 species in 36 genera were isolated over the six month period. Of the 12 most frequently isolated genera (i.e. those isolated greater than 30 times), *Mycrocyclus* had the highest relative frequency (18 %) in milfoil tissues followed by *Penicillium*, *Plectosphaerella*, *Aspergillus*, and *Trichoderma*. The 12 most frequently isolated genera were all found in roots, stems, and leaves but were often absent from root crowns. In general, the number of isolates and species steadily decreased from stem base to plant apex. The Jaccard coefficient (J_c) was used to determine similarities between endophyte communities from month to month and between tissue types. The highest monthly overlap ($J_c = 0.439$) was observed for the fungal communities from the June/July collections. The similarity of the June collections compared to other months was much lower ($J_c = 0.225$ to 0.289). The highest similarities ($J_c = 0.707$) were observed for the endophyte communities in stems and leaves indicating that their close proximity on the host apparently resulted in a higher number of shared common endophyte species. This value was almost 20% higher when compared to either of the root tissues. The study of endophytes offers great potential to find new biological control agents. Agents that can colonize and impact all plant tissues would be more effective than those that can only attack specific tissues.

Herbivore-Induced Plant Defenses and Biological Control of Invasive Plants

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Abstract

Biological control is one of the few tools capable of managing widespread exotic plant invasions, which, at its most successful, can offer long-term solutions to weed problems. However, some biological control agents obtain approval and are released, but fail to impact weed populations. This is troublesome because exploration, testing, and approval for each agent take many years and is estimated to cost several millions of dollars to complete. Moreover, ineffective agents can persist and cause unwanted ecological changes in the communities in which they occur. A better understanding of the interactions between biocontrol agents and their invasive host plants is needed to identify the factors which promote or limit successful biocontrol. Our approach is to apply the chemical ecology of plant-herbivore interactions to classical biological control of weeds – two fields which have largely progressed independently to date. Chemistry plays a central role in determining ecological outcomes between plants and insects, and should provide information that can be used to better predict which potential agents are most likely to be effective. Here we focus on induced plant responses – defenses activated in response to insect feeding – and the potential role these costly chemicals play in determining the success or failure of biocontrol.

Comparison of Native and Invasive Populations of *Taeniatherum caput-medusae* ssp. *asperum* (medusahead): Evidence for Multiple Introductions, Source Populations and Founder Effects

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Abstract

The native range of *Taeniatherum caput-medusae* (L.) Nevski includes much of Eurasia, where three distinct subspecies have been recognized, but only *T. caput medusae* ssp. *asperum* (hereafter referred to as medusahead) is believed to occur in the United States (U.S.). Medusahead, a primarily self-pollinating annual grass, was introduced into western U.S. in the late 1800s. The results of an earlier allozyme analysis were consistent with the genetic signature associated with multiple introductions, although this finding can only be confirmed with the analysis of native populations. In the current study we compared allozyme diversity in native and invasive populations of medusahead to test the multiple introduction hypothesis, identify source populations for the U.S. invasion, and determine the genetic consequences of these events. Five of the seven homozygous multilocus genotypes previously observed in the western U.S. have been detected in native populations, thereby providing support for the multiple introduction hypothesis. Source populations for these introductions appear to have been drawn from France, Sardinia, Greece and Turkey, although additional analyses are ongoing. Across native populations, 17 of 23 loci were polymorphic and a total of 48 alleles were detected, while only five polymorphic loci and 28 alleles were found among invasive populations. On average, invasive populations possess reduced within-population genetic diversity, compared with those from the native range. While U.S. populations have experienced founder effects, 38% (17 of 45) these populations appear to be genetic admixtures (consisting of two or more native genotypes). Results of this study have implications for the biological control of medusahead: i) the search for effective and specific biological control agents will have to occur broadly across the species' native range, ii) multiple agents may be required to control invasive populations that are admixtures, and iii) because many invasive populations are genetically depauperate, highly adapted biological control agents are likely to be quite effective.

Morphological and Genetic Differentiation among Subspecies of *Taeniatherum caput-medusae*: Disentangling Taxonomic Complexity in the Native Range

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Abstract

Across its native range, *Taeniatherum caput-medusae* (L.) Nevski (medusahead) exhibits taxonomic complexity. Three subspecies have been recognized: *T. caput-medusae* ssp. *caput-medusae*, *T. caput-medusae* ssp. *asperum*, and *T. caput-medusae* ssp. *crinitum*. While subspecies *caput-medusae* is found in the western Mediterranean and subspecies *crinitum* occurs from eastern Europe to Central Asia, subspecies *asperum* is found throughout the entire geographic distribution of the species. Only subspecies *asperum* is believed to occur in the United States, where it is now invasive in portions of California, Idaho, Nevada, Oregon, Utah and Washington. As part of our ongoing research to better understand and manage this invasion, we are conducting genetic analyses of both native and invasive populations of medusahead. An important prerequisite to these analyses is the proper identification of the three subspecies. In the current study, plants from each native population were grown in a greenhouse common garden, harvested at maturity, and measured using previously described morphological characters. After Bonferroni correction, three characters: glume length, glume angle and palea length, were found to be statistically significant. Thus, these three characters were quite useful in assigning plants to each of the three subspecies. We found that two other characters, lemma hairs and conical cells, were less informative. Differentiation among native populations of medusahead was further assessed using a molecular genetic marker. The results of a UPGMA cluster diagram based on allozyme data, indicates that subspecies *crinitum* is genetically differentiated from the other two subspecies; some populations of subspecies *caput-medusae* and *asperum* co-occur within different clusters; and subspecies *asperum* is the most variable. Results of the analysis of multilocus genotypes are generally consistent with the UPGMA diagram (e.g., subspecies *caput-medusae* and *asperum* share six multilocus genotypes). Our findings confirm the need of such studies to disentangle the taxonomic complexity that can be found in the native range of invasive species.

Biological Control of *Ambrosia artemisiifolia*: Learning from the Past

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Abstract

Common ragweed, *Ambrosia artemisiifolia* L., has uniquely raised the awareness of invasive plants. The main concern is its particularly large production of highly allergenic pollen that causes allergic rhinitis and severe asthma in over 20% of the population of affected areas. Furthermore, ragweed is presently the worst weed of major crops in North America and several countries in Eastern Europe. Its range is still expanding in Europe and is likely to accelerate under a changing climate. We plan to initiate and coordinate long-term management options such as biological control and vegetation management, as sustainable control measures are lacking in Europe. Ragweed is an excellent target for biological control and up to now *Ambrosia* has been subjected to classical biological control programs in Russia, Australia, and eastern Asia with variable success, as Australia alone has implemented a successful biological control program, resulting in a benefit to cost ratio of >100. Recently the first successes have also been documented for China. Building on the extensive studies on antagonists of ragweed in its native range in North America and on the biological control activities conducted worldwide, we recently proposed a set of seven prime candidate agents for a classical or inundative biological control of *Ambrosia* in Europe. Of special interest are agents with a very narrow host-range that reduce pollen and seed production, the stage most sensitive for long-term population management of this winter annual. Integration of biological control and of habitat management into existing short-term control measures may then lead to a sustainable management strategy of *Ambrosia* in Europe.

See our recent publication: Gerber, E., Schaffner U., Gassmann A., Hinz, H.L., Seier M. and Müller-Schärer H. (2011) Prospects for biological control of *Ambrosia artemisiifolia* in Europe: learning from the past. *Weed Research* **51**, 559-573.

Effect of Nitrogen Addition on Population Establishment of the Arundo Armored Scale *Rhizaspidotus donacis*

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Abstract

Plant nitrogen content influences suitability for herbivorous insects through direct impacts on insect nutrient uptake, and physiological effects on plant tissue toughness, growth, and production of chemical defenses or feeding stimulants. Specialist insects released for biological weed control, especially agents that create or manipulate nutrient sinks, may benefit from positive interactions between nitrogen fertilization and biocontrol agent development, survival, and/or reproduction, which could enhance mass-production or improve field establishment. We examined the influence of urea addition on population establishment and reproduction of the arundo armored scale *Rhizaspidotus donacis* (Leonardi) (Hemiptera: Diaspididae), released in 2011 in the Lower Rio Grande Basin of southern Texas (USA) and northern Mexico to control giant reed (*Arundo donax* L.), an exotic, invasive giant grass in North and South America, South Africa and Australia. Rhizomes of *A. donax* were fertilized with urea solution once immediately before and again one month after release of neonate crawlers (rate = 67 kg ha⁻¹ urea). Six months after crawler release in a quarantine lab study, mature adult females and combined females and adult males (empty male scale covers) were 40% more abundant on fertilized than unfertilized rhizomes (ANOVA, $F = 71.9$, $df = 1,6$, $P = 0.0001$), and females reared on fertilized rhizomes produced 33% more crawlers ($F = 9.4$, $df = 1,5$, $P = 0.038$). However, settling of second-generation crawlers was 40% lower on fertilized rhizomes ($F = 83.1$, $df = 1,3$, $P = 0.003$), due likely to limited availability of settling locations. In large greenhouse tubs after six months, adult counts and female reproduction did not differ on the basis of fertilization, but second-generation settling was 1.5- to 3.5-fold higher on rhizomes in fertilized tubs ($F = 583$, $df = 1,5$, $P < 0.001$), as crawler settling locations were not limited. Inconsistent lab and greenhouse results likely reflect different growth conditions, but in no case did fertilization have a directly negative effect on population development of the arundo armored scale. Field studies are underway to further examine interactions between tissue nitrogen in *A. donax* and establishment of this novel biological control agent.

***Stenopelmus rufinasus* Proves to be an Excellent *Azolla* Taxonomist**

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Abstract

The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal continues to provide excellent control of *Azolla filiculoides* Lamarck in South Africa. During the host specificity testing of this agent, some feeding and development was recorded on *Azolla pinnata africana* (Desv.) R. M. K. Saunders & K. Fowler, which was sourced from Malawi, but it was argued that this was not significant and most probably a laboratory artifact (Hill, 1998), and the weevil was cleared for release. Subsequent host specificity testing, post-release of the agent by McConnachie (2004) showed significantly higher levels of feeding and development on *Azolla pinnata africana* collected in Mpumalanga, South Africa. A ten year post-release evaluation showed that some established has occurred on *A. pinnata africana* in the field, but that this is far less than on *A. filiculoides*, and the weevil did not appear to impact *A. pinnata africana* populations. Recently molecular analysis has shown that what we have been referring to as *A. pinnata africana* in South Africa is in fact *Azolla microphylla* Kaulf., an introduced species. Extensive surveys in South Africa and Mozambique have failed to find *A. pinnata africana* and we now believe that the species used by McConnachie (2004) was *A. microphylla* which is in the same section of the genus as *A. filiculoides* and explains the discrepancy in the host specificity studies.

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What do Chloroplast Sequences Tell us about the Identity of Guinea Grass, an Invasive Poaceae in the Southern United States?

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Abstract

The commonly named Guinea grass of the Poaceae family is a native African grass that has been extensively and successfully introduced as a source of animal fodder to other tropical areas of both hemispheres. On a global scale but particularly in the southern United States, the Caribbean and Hawaii, it is becoming a serious threat to biodiversity not only due to its invasiveness but also because it produces high fuel loads for fires. For the first time, a biological control program is being attempted in Texas. Source populations of the Texan invasion have to be identified in the native range in order to facilitate the search for potential biological control agents. This raises the critical issue of a proper taxonomic identification for this taxon with a history of taxonomic revisions, multiple scenarios of massive introductions and hybridization and polyploidisation events. Guinea grass in the strict sense should refer to *Megathyrsus maximus* (Jacq.), also known as *Panicum maximum* and *Urochloa maxima*. To unravel the taxonomic identification and the evolutionary history of this controversial taxon, we have begun to analyze sequences of two chloroplast regions in modern African and Texan samples as well as historical specimens in the CIRAD collection, some dating back to 1944, prior all extensive improvement programs in Africa. None of the sequences matched the sequence of a voucher specimen of *Megathyrsus maximus* (Jacq.). Results provided evidence of two different maternal lineages, one distributed from eastern Africa to southeastern Africa and Texas that fully matched the sequence of a voucher specimen of *Megathyrsus infestus* (Andersson) and one distributed across western/central Africa and French Guiana that do not belong to *Megathyrsus* genus. Future programs of exploration and collection of natural enemies are to be reviewed in light of these findings.

Evolutionary Insights from the Invasion of Greece by *Solanum elaeagnifolium* (Solanaceae): Implications for Biological Control

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Abstract

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is considered to be not only a noxious Solanaceae in its subtropical American native range but also an invasive neophyte in many regions across the world, including the Mediterranean Basin. Its invasiveness in cultivated lands and disturbed areas is aggravated by high seed output and an extensive creeping root system, both attributes that render conventional chemical and mechanical control methods very difficult. Through collaboration between USDA-ARS, the Benaki Phytopathological Institute, and the Universities of Athens and Thessaloniki in Greece, a biological control program including mapping against this weed is being attempted for the first time in the Mediterranean Basin. Notwithstanding that biological control of this target has already been successful in South Africa, conducting rigorous specificity testing of candidate biological control agents in the Mediterranean region still boils down to i) the weed is in the same *Leptostemonum* clade as Old World eggplants, all major crop plants in this region ii) the origin of invasions in Greece, and iii) the levels of genetic and phenotypic variations relative to their native range. In Greece, we identified the source populations across the native range (Texas, Mexico) using Cp sequencing and multilocus genotyping approaches. Molecular data suggested a taxonomic revision of the Argentinean silverleaf nightshade. They showed that invasion in Greece resulted from several introduction events with some populations composed of admixture of introduced genotypes. No change in ploidy levels occurred following invasion. Based on the links between some life history traits and reproductive

strategies, first hypotheses related to its invasion success can be drawn and be presented here. From preliminary molecular and cytogenetic data in Moroccan populations, it is clear that patterns of invasion in Greece and Morocco share striking similarities, creating a situation more conducive for future global biological control programs in the Mediterranean Basin.

Ploidy Level and Genome Size of *Vincetoxicum nigrum* and *V. rossicum* (Apocynaceae), Two Invasive Vines in North America

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Abstract

Vincetoxicum nigrum (L.) Moench (black swallow-wort) and *V. rossicum* (Kleopow) Barbarich (pale swallow-wort) (Apocynaceae) are perennial vines that are targeted for classical biological control as a result of their massive invasion in natural areas and horticultural nurseries in the U.S. and Canada. Native ranges of *V. nigrum* and *V. rossicum* are limited to southwestern Europe and to Ukraine-southwestern Russia, respectively. The evolutionary mechanisms that have facilitated the range expansion since their introduction 150 years ago into North America have yet to be understood. In this study we examine two characteristics of the genome organization, i) the most frequently assessed ploidy level and ii) the variation in genome size, i.e., variation in the amount of DNA per monoploid set of chromosomes through loss or gain of repeated DNA sequences. Both can allow rapid changes in key phenotypic traits that enhance invasive ability. Flow cytometry using propidium iodide for the analysis of genome size variation and chromosome counting using DAPI were conducted on plants sampled from the introduced and native ranges of both species. In *V. nigrum*, accessions from Southern France and North America were all tetraploid ($2n = 4x = 44$). In *V. rossicum*, accessions from Russia and North America were all diploid ($2n = 2x = 22$). The mean 2C value ($\pm$ STD) of *V. nigrum* and *V. rossicum* is 1.44 ± 0.03 pg and 0.71 ± 0.02 pg, respectively. This is the first report of genome size for the genus. At the species level, no evidence for genome size variation was found between the two ranges. Our data indicate that the invasive spread of both species was not triggered by differences in ploidy level or genome size between native and introduced populations. Alternative explanations should be sought.

Interactions between the Biological Control Agents of Diffuse Knapweed in Southern British Columbia, Canada

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Abstract

In weed biocontrol, multiple species of insects (and diseases) are often introduced. With each introduction, there is a risk that competitive interactions between species will result in a reduction in plant damage, compared with when one species occurred alone, for example if the agent with the potential to be a successful biocontrol agent is out-competed by an unsuccessful one. Attempts to control diffuse knapweed (*Centaurea diffusa* Lam.) in British Columbia resulted in the introduction of 12 insect species over 20 years. Successful biocontrol has been achieved with the introduction of the seed-head weevil, *Larinus minutus* Gyllenhal. However, the introductions of *Urophora affinis* Frauenfeld and *Sphenoptera jugoslavica* Obenberger were not associated with a reduction in diffuse knapweed populations, even though these species are very common in southern British Columbia. We were interested in how *S. jugoslavica* and *U. affinis* interact with *L. minutus*, and to determine if these interactions have a negative impact on knapweed control. The two species are likely to act in different ways. *Urophora affinis* and *L. minutus* compete directly for the seed-head whereas the interaction between the root feeding *S. jugoslavica* and *L. minutus* is indirect and plant-mediated. We conducted a range of experiments to test if the presence of one of the unsuccessful biocontrol agents could impede or promote control *L. minutus*, the successful biocontrol agent. Preliminary results suggest that the presence of *U. affinis* in a seed-head does not prevent *L. minutus* from colonizing the seed-head; however, *L. minutus* destroys *U. affinis* wherever they co-occur in the seed-head. Damage by *S. jugoslavica* in the root does not reduce the proportion of seed-heads infested by *L. minutus*. Therefore, the unsuccessful biocontrol agents are unlikely to be impeding control of diffuse knapweed by *L. minutus*.

Endophytes Associated with *Cirsium arvense* and their Influence on its Biological Control

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Abstract

The fungal plant pathogen *Sclerotinia sclerotiorum* (Libert) de Bary was investigated as a potential biocontrol agent for the pasture weed Californian thistle, *Cirsium arvense* (L.) Scop., in New Zealand. However, its biocontrol activity was found to be inconsistent. Three reactions were observed when spores of the pathogen were applied in high numbers: the pathogen (1) killed the plant, (2) killed the aerial tissues, but the plant resprouted, or (3) it had no effect on the plant. Evans (2008) proposed the endophyte-enemy release hypothesis (E-ERH), predicting that the presence or absence of co-evolved host plant resident microbes (endophytes) makes the plant either more resistant or more susceptible to attack by a pathogen. It was therefore hypothesised that the observed inconsistency of *Sclerotinia* to control Californian thistle was attributed to variation in the presence/absence of key endophyte populations. To test this, we first assessed which endophytes were present in Californian thistle plants and how much they varied within a plant and between plants at varying distances. Both culturing methods and the molecular technique DGGE were employed to identify endophyte populations. Results indicate the endophyte populations are not influenced by individual plants or fields, but may be influenced by their location in the plant (i.e. leaf, root, seed and seed pappus). Key endophytes were identified, selected and tested to determine if they had a significant impact on the pathogenic activity of *Sclerotinia* on Californian thistle. Preliminary results indicate endophytes can influence the success/failure of this weed biocontrol agent.

Dispersal and Impact of *Larinus minutus* among *Centaurea diffusa* Patches in Alberta, Canada

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Abstract

Although *Larinus minutus* Gyllenhal has been implicated in the successful control of diffuse knapweed (*Centaurea diffusa* L.) in western North America, most studies have been confounded to some extent by the presence of root feeders. Furthermore, relatively little is known regarding its rate of dispersal and its impact on its host plant in the first few years post release. To address these knowledge gaps we initiated a study in 2005 in Alberta, Canada, to measure the spread and impact of this agent in an area that had no previous history of intentional biocontrol releases against diffuse knapweed. In 2005, 300 *L. minutus* were released along the Oldman River at a riparian site that was infested with diffuse knapweed. Three additional knapweed patches at 2, 7, and 9 km downstream were identified at that time. For five years, beginning in 2006, the density of knapweed stems and rosettes as well as the density of *L. minutus* was measured at each patch. We found that after five years post-release, *L. minutus* had colonized the downstream patches at a rate of approximately 1.9 km/yr. At the release patch, *L. minutus* numbers grew quickly reaching densities of 400 beetles/m² after three years. At the patch 2 km downstream, populations grew more slowly but reached a density of 166 beetles/m² five years after the initial release. The patches 7 and 9 km downstream were colonized 3 and 4 years after release respectively and populations there are still growing quickly. At all of the sites, both knapweed stem and rosette densities are significantly higher five years post-release than they were in 2006. These results suggest that while *L. minutus* populations can grow and spread quickly, impact on diffuse knapweed densities, at least at some sites, may take more than five years.

Hybrid Weeds! Agent Biotypes!: Montana's Ever-Evolving Toadflax Biological Control Soap Opera

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Abstract

An exotic toadflax stem mining weevil conventionally identified as *Mecinus janthinus* Germar has become widely established on Dalmatian toadflax [*Linaria dalmatica* (Linnaeus) Miller] in western North America, although agent density and control efficacy are highly variable across release sites (De Clerck-Floate & Miller, 2002; McClay & Hughes, 2007; Van Hezewijk et al., 2010). Naturally-occurring and fertile hybrid toadflax (HT) populations resulting from the cross-pollination of Dalmatian toadflax (DT) and a sister species, yellow toadflax (*Linaria vulgaris* Miller) (YT), have been discovered in Montana (Ward et al., 2009). Genetically distinct, host-specific *Mecinus* species have been confirmed from native range populations (Toševski et al., 2011). In Montana, the DT-associated *M. janthiniformis* Toševski & Caldara sp.n. appears to be abundant and widespread, while the YT-associated weevil confirmed to be *Mecinus janthinus* Germar, 1821 appears to occur much less frequently (Toševski, pers. comm.). Naturally-occurring hybridization of DT and YT coupled with the discovery that the host associated *Mecinus* biotypes were in fact separate species has at the very least increased the complexity of toadflax biocontrol. Strategic implementation of biological control for forests and rangelands affected by widespread, trenchant infestations of both toadflax species in particular seems less straight-forward. Our research results seek to address a range of questions regarding the optimal deployment of the two recently confirmed and host specific *Mecinus* species, and strategies for effective biological control of hybrid toadflax.

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SESSION 8: SOCIAL AND ECONOMIC ASSESSMENTS OF BIOLOGICAL CONTROL

The Garlic Mustard (*Alliaria petiolata*) Case, What Makes a Good Biological Control Target: The Intersection of Science, Perspectives, Policy and Regulation

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Abstract

In this paper, we present an overview of our shared experiences from a thirteen-year discovery and testing period in search of effective biological control agents for garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande). Our experiences during this time reflect much of the dialog, debate, dilemmas, and policy discussions occurring in biological control of weeds today. For example, in the last decade, the values that underpin biological control, as well as standard requirements and stakeholder perspectives have been in a state of flux. Many research programs fail to sustain funding for such long pre-release periods. Policy goals and acceptable safety criteria have changed. Moreover, the fundamental perception of garlic mustard as a pest is shifting, leading some to question whether garlic mustard is a driver of change in invaded habitats or rather a symptom of habitat disruption. If it is a symptom, this can shift the perception of the risks of biocontrol. In this shifting scientific and social milieu, land managers are still challenged by stakeholder demands for management of garlic mustard. Land managers have a responsibility to manage their sites for the purposes for which the land is preserved and have limited control, or no control over potential higher-level drivers such as earthworms, deer, climate change, and human population pressures. The intent of this presentation is to discuss these and other issues common to many who work in biological control, framing the discussion within our garlic mustard experience as the basis for dialog.

Background

Awareness and public interest in garlic mustard invasion of hardwood forests in the Midwest and Northeastern USA gained significant momentum in the 1980s with a series of key publications by Nuzzo (1991; 1996) and Nuzzo et al. (1996), culminating in the effort to develop a biological control program that started in 1998 with a broad base of support. At that time, available assessments 'indicated that the only viable long-term option for successful management of garlic mustard is classical biological control' (Blossey et al., 2001a). As part of the biological control project, a test plant list was developed, and CABI in Delémont, Switzerland contracted to conduct surveys and host specificity testing of candidate agents. Additional host specificity testing began in 2003 at the University of Minnesota, USA on plant species that were difficult to obtain or grow in Switzerland. Throughout this process stakeholders were engaged through various workshops (e.g. Skinner, 2005) and in the development (Blossey, 1999) and implementation of a long-term monitoring protocol for garlic mustard. The availability of pre-release data would allow us to gauge the impacts of anticipated biological control agent release(s) (Evans and Landis, 2007; Van Riper et al., 2010).

Gerber et al. (2009) summarized the biology and host-specificity results for the root-crown mining weevil (*Ceutorhynchus scrobicollis* Nerensheimer and Wagner) based on which, a petition for field release of the species was submitted in 2008 to the USDA APHIS TAG (United States Department of Agriculture, Animal and Plant Health Inspection Service, Technical Advisory Group). Based on the comments of reviewers, additional host testing was conducted from 2009 through 2011. Responses to reviewer comments to the 2008 petition and the results of additional host specificity testing were re-submitted to TAG in September of 2011.

Discussion

How safe is safe enough?

Typical of many weed biocontrol endeavors, the effort to release a biological control insect for

garlic mustard in North America has been long and arduous. Thirteen years after officially initiating the research, we are awaiting TAG review of our latest submission. Much has changed during this time. For example, phylogenetic relationships among tribes within the Brassicaceae were redefined (Al-Shehbaz et al., 2006), necessitating continuous adaptation of our test plant list. Also, during this lengthy testing period, the concept of acceptable risk has changed. As common in risk assessments, "safe enough" is rarely achieved to the satisfaction of all stakeholders. One might conclude that agreement is rarely achieved now compared to biocontrol programs in decades past, as seen in papers presented at this conference. Lincoln Smith (*in press*) discussed an insect which has broad support for yellow starthistle (*Centaurea solstitialis* L.) control, but ultimately was not approved for release. A retrospective review of past agents approved for release was presented by Hinz et al. (*in press*), exploring the possibility that most of these agents would not be approved in today's regulatory climate in the USA.

In our case, *C. scrobicollis* did develop on the commercially grown watercress (*Nasturtium officinale* Ait. f.). Adult development on watercress was not consistent throughout tests conducted in different years. Moreover, *C. scrobicollis* development was only found when watercress was grown in artificial dryland mesocosms. Cultivated watercress is grown under water-saturated conditions (e.g., in running water). In refined host-specificity tests altered to simulate these growing conditions, *C. scrobicollis* was not able to complete its development on watercress. Additionally, *C. scrobicollis* has not been recorded as an economic pest, nor even in association with watercress in its native range where both co-exist, arguably the most comprehensive specificity testing possible.

While the overall host specificity package for *C. scrobicollis* on garlic mustard in North America suggests the ecological host range will be narrower than the physiological host range with the latter defined as development under highly artificial laboratory conditions, such data points could prove troublesome for the approval process. 'Troublesome' data points refer to data generated under circumstances which render the data suspect upon further scientific scrutiny. Once generated, however, these 'troublesome' data points do not go

away. Despite subsequent work that more accurately reflects scientifically valid outcomes, the initial data remains in the body of evidence submitted for approval and often result in lingering concerns, particularly at the policy level, rather than at the scientific review process for biological control agents within USDA APHIS.

Is garlic mustard really that bad?

Another phenomenon that has evolved during our lengthy testing period is the notion that yesterday's demonized pest may become today's ecosystem services star. Apropos the papers presented at this symposium by Dudley et al. (*in press*) and Norton et al. (*in press*) discussed litigation over biological control of saltcedar (*Tamarix* spp.) impacting the southwestern willow flycatcher (*Empidonax traillii eximius* A.R. Phillips). Campaigns to disparage target invasives are common, prompting critical reviews reflecting on the fear-based language used with the public to generate support for control efforts (Gobster, 2005). Indeed, we used the Good, the Bad, and the Ugly campaign effectively in Minnesota to generate support for the biological control of purple loosestrife (*Lythrum salicaria* L.). Paradoxically, this terminology is now being used by opponents of biological control to describe the biological control agents. Warner & Kinslow (2011) explored this phenomenon more broadly in the context of manipulating risk communication to the public in the case of biological control of the strawberry guava tree (*Psidium cattleianum* Sabine) in Hawaii, resulting in an outcome different than intended by the scientific and conservation communities.

In the thirteen years since our effort began on garlic mustard, views of how we view this plant are evolving. Some studies have shown negative impacts of garlic mustard in invaded ecosystems while others found no impacts. Is garlic mustard a principal driver of detrimental impacts? Research showed that garlic mustard competition for light negatively impacted tree seedlings and annual herbaceous species (Anderson et al., 1996; Cipollini and Enright, 2009; Meekins and McCarthy, 1999), altered nutrient levels (Rodgers et al., 2008), and was toxic to arbuscular mycorrhizal fungi which could result in altered nutrient and water acquisition by many native species (Callaway et al., 2008; Cipollini

and Gruner, 2007; Roberts & Anderson, 2001). Of concern to the forest industry, research suggested garlic mustard negatively impacted desirable tree seedlings (Stinson et al., 2006).

Alternatively, is the presence of garlic mustard merely a symptom of a response to higher-level changes? Indeed, garlic mustard often is observed in disturbed areas that lack native cover (Trimbur, 1973; Nuzzo, 1991; Van Riper et al., 2010). Recently it has been proposed that the action of deer and earthworms facilitate garlic mustard invasion (Blossey et al., 2005; Knight et al., 2009; Nuzzo et al., 2009). Deer herbivory on natives can create disturbed microsites that promote dispersal of garlic mustard seeds (Anderson et al., 1996). Loss of native plants may create suitable conditions for garlic mustard invasion through increased light levels, moisture, and nutrient availability (Anderson et al., 1996) and decreased litter levels (Trimbur, 1973), as well as through anthropogenic effects such as erosion.

Who is the driver?

If garlic mustard is not the principal driver of negative impacts, some on our team propose that we should focus efforts on the higher-level drivers (e.g., deer and earthworms), not the symptoms (e.g., garlic mustard). Such ideas are gaining support in the ecological literature where for example, Davis (2011) argued that species such as garlic mustard do not pose as big a threat as scientists think. Some are finding evidence that native insects impacted by garlic mustard may be adapting to it (Keeler and Chew, 2008). As a result, after a decade of testing, we have reached the juncture where our group is discussing whether we should release *C. scrobicollis* even if approved by TAG.

Exotic earthworms are widely discussed relevant to invasion in forest ecosystems (Nuzzo et al., 2009) and once established, few, if any management options exist to remove them. There has long been evidence about the negative impacts of deer on native plants (e.g., Hough, 1965; Tilghman, 1989; Diamond, 1992). However, limiting deer populations is difficult. State natural resource agencies both promote deer for hunting and as an income generator via hunting permits, while concomitantly expending resources to remove deer or to install exclusion devices to promote regeneration of tree species impacted

by deer browse. Neither of these factors is likely to change significantly in the near term. Also, it is not clear how the public would react to deer herd reductions to the low level required to reduce disturbance to a degree that may stop the invasion of plants like garlic mustard.

What is involved if land managers were to shift from managing garlic mustard to instead managing higher-level drivers? Figure 1 shows the relative geographic scale and management difficulty of several drivers that impact invasive species. This concept was adapted from a CABI Biosciences schematic depicting the centrifugal phylogenetic method. This driver schematic assumes garlic mustard as a symptom, not a driver. As we move out from the center, the geographic scale of the potential negative impact of the driver, and concomitantly, the difficulty in altering that impact increases. Earthworms are problematic, but at present are less widely distributed in the Midwest USA compared to deer. As we move to a wider geographic scale, anthropogenic effects such as pollution (e.g., nutrient loading, sediment runoff, etc.) and more broadly, climate change are clearly drivers of

negative environmental change. Managing drivers such as climate change is distinctly long-term and the outcome uncertain. Ultimately, it is people and the resultant impact of our lifestyles and actions that is the overarching driver. Changing any of these on a scale to reduce negative impacts to ecosystems is a daunting endeavor, especially for a land manager.

Will garlic mustard go away?

During the time invested to find a biological control agent for garlic mustard, some members of our team have observed a decline in long-standing populations of garlic mustard absent the introduction of a biological control agent (Blossey and Nuzzo, *in press*). Perhaps we are just seeing the beginning of a decline in garlic mustard populations in North America, or are these population density fluctuations, related to climate cycles reflecting the natural ebb and flow of invasive species? If populations do significantly decline, will they resurge and expand to a point where we have populations of garlic mustard that are even more widely dispersed?

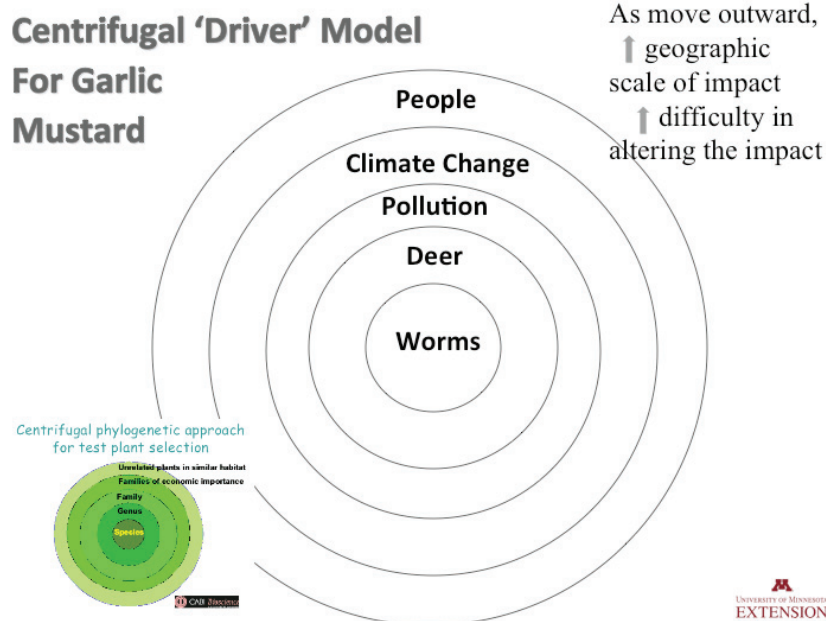


Figure 1. The centrifugal driver model. An adaptation of a CABI diagram depicting the centrifugal phylogenetic method of Wapsphere (1974).

Additionally, would there be a benefit to uninvaded communities if a biocontrol agent could avoid a boom and bust cycle of garlic mustard?

More broadly, the field of ecology is exploring fluctuations in population densities of invasive species (Simberloff and Gibbons, 2004, Ahern et al., 2010). In the experiences of an Extension State Weed Scientist at the University of Minnesota (Becker), it is well known that species shift, population densities ebb and flow, and weed patches move around on the landscape. Landscape-scale changes in problem species in agricultural systems are driven by what weed scientists call the 'big hammers'; typically system-wide shifts in tillage, fertility, periodicity of operations, or the periodic dominance of one herbicide mode of action in the marketplace. An example of dramatic population fluctuations that may inform invasions that dominate the landscape and then moderate, was the effort in the USA to domesticate the native common milkweed (*Asclepias syriaca* L.) during World War II to produce floss (pappi) to fill life jackets when imports of goose down were blocked. Common milkweed in North America can be found throughout a broad habitat range. As it naturally occurs, common milkweed remains at low population densities and scattered across the landscape. In attempts at domestic production, when planted in monocultures in fields, density-dependant diseases quickly became an impediment to successfully growing the crop in many locales, and in many cases resulted in abandonment of fields. Experienced weed scientists often recount such phenomena, but as is often the case with experiential knowledge, it is seldom documented in peer-reviewed journal articles. Similar to the disease limiting phenomena seen in milkweed, we have observed that Canada thistle populations approaching monotypic stands decline after six to seven years due to generalist pathogens *Fusarium* and *Pythium* resulting in reduced population densities that are relatively dispersed.

Herbaceous perennial or biennial weeds in the Upper Midwest USA are dynamic in population density, population size, and location in response to climate. Minnesota is at the intersection of the hardwood forest, boreal forest, and the tall grass prairie regions of the USA. Here, herbaceous invasive plants respond to temperature and moisture cycles. Historically, these occurred in 20-year cycles

in records since the 1800s, but with climate change, the cycles are lengthening and becoming more local with drought and flood cycles occurring in the same season within the same county (Minnesota Climatology Working Group, 2011). These climate changes can be tracked by shifts in the species that become problematic for land managers. During wet cycles in Minnesota it is common to see Canada thistle (*Cirsium arvense* (L.) Scop.) and buttercup (*Ranunculus* spp.) thrive and expand geographically. Conversely, during dry cycles hoary alyssum (*Berteroa incana* (L.) DC.), wormwoods (*Artemisia absinthium* L.), and leafy spurge (*Euphorbia esula* L.) thrive and expand. This increase in localized variability due to climate change will accentuate changes in population dynamics of many of the invasive weeds with which we work.

Garlic mustard is a biennial species cycling in a perennial system. Sustaining a population is wholly dependent on constant regeneration of rosettes from seedlings. Seedling regeneration depends on disturbance and is subject to episodic widespread seedling mortality. At some of our garlic mustard monitoring sites in Minnesota, we see cycles where either the seedling/rosette or the flowering second-year growth stage dominate in a given year, while at other sites they occur simultaneously (Van Riper et al., 2010). By its biennial nature, garlic mustard populations will fluctuate dramatically, and in extreme climatic events, may even skip population cycles altogether, only to resurface in the future. Thus, multiple forces are at work resulting in garlic mustard populations that are very dynamic. Our challenge is to determine the long-term trends, and what that means within the construct of our original justification for biological control of garlic mustard.

Conclusions

Many on our team were also part of the biological control effort of purple loosestrife in North America, informing our approach to biological control of garlic mustard. Many of the same stakeholders and funding sources were used in both efforts, and the perceived success of purple loosestrife biocontrol resulted in built-in enthusiasm for the garlic mustard effort. For example, the network of pre-release garlic mustard monitoring sites included many managers who were

cooperators on the purple loosestrife effort. Blossey et al. (2001b) was in part a response to criticisms of the purple loosestrife biological control effort. Yet almost two decades after the release of *Galerucella* spp. for biological control of purple loosestrife, science has not settled the debate surrounding biological control of that invasive species (Lavoie, 2010). More studies are being proposed to answer the next set of garlic mustard research questions. We are on the cusp of gaining approval for release of *C. scrobicollis*, but are debating similar questions that are still debated for purple loosestrife. Experience indicates that scientific discourse will be unable to expeditiously address the complex interactions to manage higher-level drivers, nor quickly settle the more direct question of whether invasion by garlic mustard negatively impacts native ecosystems.

So, considering the debate over whether garlic mustard negatively impacts forest ecosystems and whether it is only present because of higher-level drivers, what can land managers do in response to public demands for action? As is the case for many pest problems, the default action is to treat the symptoms – in this case an invasive weed that has become abundant. This option is something we can do and can measure the success of in terms of cost and effectiveness, providing justification to those who fund such programs. Control of invasive, noxious weeds is often required via regulated weed laws in the USA. Managing higher-level drivers arguably might be the most efficacious and efficient approach; however, it would involve a higher degree of complexity, is more difficult to implement, and is an approach that takes a long time to provide results, thus, making it more challenging to garner and maintain support.

One of our team members summed it up this way: We should address the symptoms, i.e., control garlic mustard if it: 1) provides additional time to address root causes, 2) prevents degradation in the meantime, 3) poses minimal risks, and 4) does not clearly jeopardize a long term solution. Doing so may spare uninvaded and minimally invaded habitat in the Midwest the upheaval of a garlic mustard invasion. This may not be true in parts of the northeast. Midwest ecosystems could benefit from delay or reduction of garlic mustard invasion considering our host specificity data suggest minimal risk.

We are left with a dilemma. On one hand, we must consider the implications of releasing an organism against a pest that may not be the root cause of detrimental changes. This would be an especially egregious error if the biological control agent caused unintended nontarget damage in the future. On the other hand, we must also consider the implications of not releasing a biological agent deemed safe for a target that many stakeholders feel has significant negative impacts. Managers may not be able to eliminate earthworms and deer, but biocontrol could give them a tool to reduce one stressor to the system: garlic mustard. Not releasing a biocontrol agent is particularly problematic if future work confirms significant impacts on forest ecosystems, and populations do not undergo a natural decline but rather persist across the landscape. Considering the ongoing controversies regarding biological control of weeds, we must also reflect on the implications these two scenarios may have for the future of biological control of weeds, both from a policy and funding viewpoint.

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Public Engagement with Biological Control of Invasive Plants: The State of the Question

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Abstract

The practice of biocontrol has been impacted by in the evolution of environmental values in societies, and difficulties in obtaining release permits. These challenge biocontrol stakeholders, researchers and regulators to foster more effective public engagement with invasive species management. To succeed, public engagement requires the disambiguation of research activities from public agency decision making. This requires greater up-front investment in public communication and consultation, and more transparency by agencies in the application of their decision making criteria. However, these additional costs can be offset if the result is attenuated surrounding controversies and amplified public support for invasive plant control. This article draws from a five year comparative study of biocontrol practice, policy & public engagement in the U.S., South Africa, New Zealand, and Australia. It presents key findings to guide public engagement with biocontrol of invasive plants.

Introduction

The social context of weed biocontrol has changed dramatically since the first International Symposium on Biological Control of Weeds (ISBCW). Formerly, biocontrol researchers labored in autonomy from society, but now they are increasingly expected to communicate their work to non-expert public officials and members of the public. With the rise of environmental values and legislation, environmental scientists and agencies funded with public monies were increasingly asked to justify their activities to the public (Speth, 2004). This gave rise to early efforts to cultivate public support for biocontrol of invasive plants, using the tools of public outreach and public consultation. These early efforts push information out to the public, or gather comments from the public. In the 21st century social context, unidirectional communication to or from the public regarding science is not sufficient to garner public monies, nor public support, for any type of scientific activity.

By studying cases where there is greater public support for the application of science and technology in addressing social needs, social scientists have articulated a new model for relating scientists and their institutions to society: public engagement (McCallie et al., 2009). Unlike the unidirectional communication implicit in public communication and comment, participatory public engagement with science and technology (shortened to “public engagement”) facilitates mutual learning among publics, scientists, and others with respect to the development and application of science and technology in modern society (Rowe and Frewer, 2005; Mooney, 2010). Public engagement is more costly in terms of time and resources. However, members of the public are challenging publicly-funded researchers and regulatory agencies to be more transparent in their decision making, and the early models of public communication do not support effective responses. Public engagement has the potential to cultivate greater public support for biocontrol.

Invasive plant control in the future will require more than passive public support. It will require active and sustained engagement by citizens and stakeholders, who reasonably expect public agencies to demonstrate how this practice addresses economic and conservation goals. Public engagement fulfills democratic values, which is especially important for any public interest science (Warner et al., 2011), but also good practice to minimize social conflicts over biocontrol agent releases (Warner and Kinslow, *in press*). Since the public funds most invasive species control programs, it is reasonable to educate and engage them on a continuing basis. Controversies surrounding the introduction of biocontrol agents have dogged high profile and costly restoration projects, and the future of classical biocontrol as an invasive species management practice is threatened by persistent unresolved controversies (Strong and Pemberton, 2000; Warner, *in press*). To be effective, public engagement must:

1. Construct greater social understanding of the problems of invasive plants;
2. Create greater social consensus on the need to control invasive plants and the conditions under which biocontrol is a socially preferable approach; and
3. Incrementally increase the public's trust that government agencies are upholding the public's interest through appropriate regulatory review.

Here is the state of the question: "could greater public engagement with biocontrol of invasive plants foster greater stakeholder support without hindering research?" Public engagement challenges scientists and their institutions to develop skills in public communication, and challenges public regulatory institutions to facilitate appropriate public review with biocontrol release decisions. Researchers and public agencies need forms of public engagement that do not:

1. Interfere with scientific research and practice;
2. Impose significant additional burdens on their own time;
3. Delay regulatory review.

To avoid these problems, public engagement should disambiguate scientific research activities from public agency decision making. This requires greater up-front investment in public communication and consultation, and more transparency by agencies in the application of their decision making criteria. However, these additional costs can be offset if the result is attenuated surrounding controversies and amplified public support for invasive plant control. The balance of this paper introduces the material and methods supporting this study; explains how public engagement differs from early forms of public communication; and summarizes conclusions from this study.

Methods and Materials

Social science field work was conducted in the U.S., South Africa, Australia and New Zealand. Between 2007 and 2009, 183 semi-structured interviews were conducted with 178 research scientists, laboratory directors, regulators, communication officers, critics, and clients of the practice of classical biocontrol in all four countries. Interviews addressed the following topics: the history of invasive species control, and biocontrol practice and their institutions; the impact of rising concern about nontarget effects of biocontrol agents; and how legislation and regulatory institutions have responded to risk concerns regarding biocontrol agent introductions. Several of these interviewees provided extensive documentation on the policymaking and regulatory processes in these countries.

Results

The introduction of a novel biocontrol agent is a socio-political decision as well as a biological and environmental action. At the time of the first ISBCW in 1969, virtually all biocontrol decisions could be made within one public agency. Target selection, agent selection, testing criteria, and release permitting were all internal to one institution, often a department of agriculture. Now these decisions are generally distributed between multiple public agencies and are at times more contested, reflecting broader social unease about environmental issues.

Public communication by public agencies has improved over the past 20 years, but the new media environment is remaking social context for mass communication faster than public agencies can respond (Press and Williams, 2010). The new media environment highlights trust-destroying events: facts and science are disputed, the public doubts the existence of the problem, and public skepticism of proposed remedies grows. This results in fraying of the relationship between scientists and society at large, and undermines the ability of scientists to address social needs. Public mistrust in scientists and government agencies (and what they do) is generally on the rise. Studies have demonstrated that the public does not evaluate novel risks on the basis of data, but on the basis of trust based on the trustworthiness of messengers. This finding has been repeatedly confirmed across scientific applications and novel technologies (Slovic, 2001). This mistrust—and the potential of public engagement to foster trust -- is an issue that has implications for all stakeholders in the biocontrol of invasive plants: researchers, regulators, conservationists, and beneficiaries.

Public engagement is a semi-structured transparent deliberative process that establishes consensus views on evidence, method, interpretation, and social values frameworks as the basis for making a scientifically-informed decision (Rowe and Frewer, 2005). Public engagement differs from public outreach or consultation in that it requires bidirectional communication between scientists, decision makers, and lay publics (McCallie et al., 2009). It is a deliberative “dialogue” in which publics and scientists both benefit from listening to and learning from one another, which can be described as mutual learning (McCallie et al., 2009). Public

engagement includes members of the public doing more than merely asking questions of experts. It requires scientists to do more than merely present their knowledge and perspectives. Public engagement requires lay publics to learn about science and policy, and scientists to learn what members of the lay public know and don't know about science, but also about social values. Thus, “engagement” in this sense includes both political engagement and educational engagement. Participants from a variety of perspectives participate over a sustained period of time, guided by shared goals and a code of conduct. It has the ability to actually foster trust and consensus (McCallie et al., 2009).

The U.S. was a pioneer in early models of public participation; however, 1970s era legislation required only public communication and gathering public comments. This model is now unable to support social expectations of transparency and the need to cultivate active public participation these decisions. In the U.S., biocontrol agent review and permitting are functionally inaccessible to the public, and have remained so despite calls for greater transparency, peer review and public input (Strong and Pemberton, 2000). In contrast, New Zealand has created participatory public processes for identifying targets and cultivating support for biocontrol projects, and has created a new agency to review proposed introductions of all novel organisms, including biocontrol agents. New Zealand has a national extension system for the biological control of weeds (Hayes, 1999). Although described as a technology transfer program, in reality it is much more sophisticated, for it trains local land managers in the ecology of weeds, the management of released control agents, and public outreach. This has the potential to prompt public interest and demand for invasive species control. In 1996, New Zealand passed legislation to require transparency in decision-making processes regarding proposed novel organism introductions. It also requires the applicant to provide evidence of anticipated benefits exceeding risks (Campbell, 2010). This has created the world's most sophisticated decision-making process for evaluating novel organism introductions, with explicit reference to biocontrol agent introductions. It lays out clear decision-making criteria based on transparent and replicable ecologically-based risk-cost-benefit analysis, fixed time periods for

decisions, and participatory public engagement (Campbell, 2010). New Zealand has developed biocontrol decision making structures that best reflect the goals and methods of participatory public engagement. Australia and South Africa have also undertaken efforts to enhance public engagement activities surrounding biocontrol.

Discussion

This section summarizes key findings emerging from this study.

Most members of the public are not interested in invasive plants

There is little return on efforts to reach out to generic publics. Instead, public engagement strategies suggest public agencies should identify, reach out to, and convene all possible stakeholders, especially including potential critics. For public engagement to succeed, it is essential to begin by identifying stakeholders with strongly-held opinions, pro or con, and to convene them in a dialogical process. Stakeholders with strongly-held opinions -- but are unknown to those leading biocontrol projects -- are those most likely to contest and delay biocontrol projects. Identifying these stakeholders is a task proper to public agencies and the stakeholders themselves. For example, Australia has an on-line stakeholder registry, and New Zealand actively encourages public comments on proposed introductions. However, these need to be designed so as to not amplify risk concerns (Slovic, 2001).

A public process should enhance the capacity of stakeholders to understand science and agency decision making processes

For public engagement to succeed, it must convene a structured co-learning process in which everyone, from critics to supporters, participates over time in establishing the same scientific information about the invasive species and possible control methods. Public engagement fails if parties have divergent information about the problem and possible remedies. Most public concerns about biocontrol are founded, at least loosely, on conservation values,

such as: is the invasive plant really a problem?; why introduce another organism?; what other organisms will the agent attack?; and what will the agent do when it consumes all its hosts? These have a scientific but a democratic dimension as well, because concerned citizens want to be heard and have their views respected. Few stakeholders are able to play any kind of constructive role with the knowledge that they bring to such a process, therefore, education of stakeholders is integral to any kind of engagement. For example, in South Africa, Rhodes University offers a two week short course which enhances the capacity of anyone to understand the basics of biocontrol, and a wide range of stakeholders are invited to attend it (Gillespie et al., 2003). In New Zealand, efforts to engage indigenous Maori communities have dealt with biocontrol issues chiefly from the perspective of cultural and ethical values, and not biology, however, they have been successful because everyone's opinion is dealt with respectfully (Hayes et al., 2008).

The beneficiaries (stakeholders, not researchers) are the most appropriate parties to explain why control of the invasive plant is in the public's interest

Creating greater consensus on the need to take action is a critical first step that is fundamental to success. For example, Australia has a national weeds strategy that justifies action (Natural Resource Management Ministerial Council of Australia, 2006). In New Zealand, regional councils serve as critical intermediaries between tax payers (or rate payers) as stakeholders with research institutions (Hayes, 1999). This insulates researchers from public suspicions of conflict of interest, in other words, that the researcher loses objectivity by promoting a project that advances their career.

The beneficiaries should present a risk/cost/benefit analysis that justifies a biocontrol strategy

In New Zealand, regional councils articulate an economic justification that makes clear the advantages of biocontrol over other forms of control to tax payers. In the New Zealand regulatory

system, these regional councils are generally those who petition for invasive plant biocontrol release permits, and they are better positioned to articulate these advantages, and to engage in discussions over conflicts of interest. These regional councils represent the public better than a scientist can, so the scientist serves as scientific expert advisor, and never the advocate for controlling a pest (Campbell, 2010). Legislation imposes the burden of public consultation and engagement on the petitioner for a permit. Although this appears costly, in practice it appears that this is more than offset by decreased costs and conflicts associated with the actual regulatory decision (Campbell, 2010). Other countries could benefit from this approach, although in the U.S., it would require going beyond what is required by law.

Public agencies should articulate their decision criteria clearly and gather stakeholder input of how their criteria apply to a specific permit application

The New Zealand permitting system is efficient because any decision to release a biocontrol agent is made on a very narrow basis. It presumes that there has been prior public engagement with the desirability of targeting the invasive plant and the suitability of the biocontrol agent. Then, the question upon which the decision is made is simple (as in straightforward): are the anticipated benefits greater than the costs and risks? In New Zealand, this has frontloaded costs and public engagement efforts, but has made release decisions less contested.

Conclusion

Biocontrol of invasive plants is a public interest science. It is chiefly funded by governments and is done on behalf of the public. Some form of public consent is necessary in a democratic society. To foster sustained public engagement over time, the problem definition of invasive plants should be disambiguated from the solution of biocontrol.

Public engagement can be structured so that it enhances public stakeholder support for biocontrol of invasive plants without imposing burdens upon researchers. However, lessons of prior public engagement suggest that scientific research activity

should not be confounded with advocacy for invasive plant management using biocontrol. Fostering social consensus on the need to control the invasive plant is a pre-requisite. Public engagement requires careful attention to devising appropriate roles for stakeholders, and nodes for public input in decision making processes. Greater public engagement with biocontrol of invasive plants can be achieved by disambiguation of problem definition from solution options, and research activities from stakeholder advocacy.

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Outreach Challenges for Biological Control in Hawaii

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Abstract

Public understanding of Hawaii's use of biocontrol is limited. This can create problems when support for releases is sought. Release of a strawberry guava (*Psidium cattleianum* Sabine) enemy was delayed by public opposition. Raising awareness about invasive species in Hawaii is the purpose of the Hawaii Invasive Species Council Public Outreach Working Group (POWG). POWG organized statewide biocontrol educational activities. For Big (Hawaii) Island Invasive Species Committee (BIISC) outreach staff, biocontrol issues became particularly important with the strawberry guava proposal. One vocal Big Island activist raised public concern against biocontrol using a variety of tactics (described in Warner and Kinslow, 2011). BIISC outreach strategy focused on responding to issues that resonated with many members of the population. Key issues raised by the public to outreach staff revealed: the lack of agreement that strawberry guava is a problem that needs biocontrol (the tree has food value and natural area impacts are unseen); the public is primarily aware of examples of disastrous introductions and unaware of the extent and successes of biocontrol releases in Hawaii; the fear of rapid evolution of biocontrol agents to new hosts is pervasive; the lack of understanding of insect biology and genetics contributes to fear of rapid evolution; and, the public does not understand the selection process, research and testing protocols, and the regulatory process involved in classical biological control. A long-term education program with basic curricula plus materials on each species released would help agencies build public support for future releases.

Introduction

Biocontrol has a long history in Hawaii, with almost 800 species introduced, 300 established, complete control of approximately 40 insect species and substantial control of approximately 150 insect species, and successful control of approximately 10 weed species (Funasaki et al., 1988; Culliney and Nagamine, 2000; Culliney et al., 2003).

However, many people are familiar only with the famous mistakes (mongoose, cane toad) and not at all familiar with the extent or successes of other biocontrol releases. Biocontrol history in Hawaii commenced under the leadership of King Kalakaua. This last king, revered for his leadership

in preserving Hawaiian culture, also passed laws (1890) to prevent immigrant insect pests from entering Hawaii. The first biocontrol release (1890) was the vedalia beetle (*Rodolia cardinalis* Mulsant), which successfully controlled the cottony cushion scale (*Icerya purchasi* Maskell). After the reign of Queen Liliuokalani, Albert Koebele was hired as entomologist and biological control expert for the Republic of Hawaii. In the early period, attention was focused on agricultural pests and the general public had little knowledge of biocontrol.

One might characterize the 20th century in "biocontrol eras", beginning with a long period of introductions to address agricultural pests with little review, then an era euphoric about pesticide efficacy,

and next an era impressed with biocontrol. With the greater ecological consciousness of the 1970's and entomological research, awareness of non-target impacts began to increase. However, there was also developing interest in the idea of using natural enemy introductions to slow the spread of weeds in conservation areas. Concurrently, the regulatory review process became increasingly strict, with committees of specialists reviewing proposals, and requirements for NEPA documents.

Still, most biocontrol proposals were not widely noticed by the public until an activist became concerned about proposals to introduce a scale insect to control strawberry guava (*Psidium cattleianum* Sabine). This vocal Big Island resident activist raised public concern against biocontrol using a variety of tactics. His tactics have been described in Warner and Kinslow (2011) and were familiar to BIISC, as he has opposed numerous other projects to control coqui, mangrove, and invasive species work in general.

Methods

Raising awareness about invasive species in Hawaii is the primary purpose of the Hawaii Invasive Species Council Public Outreach Working Group (POWG). Core members of the group include the outreach staff of the invasive species committees (ISC) on each island. In 2009 four focal topics were identified as outreach priorities, one of which was biocontrol. POWG organized several biocontrol educational activities, including a documentary video, a biocontrol communications conference held March 2010, and a general brochure (produced collaboratively with the Hawaii Department of Agriculture) for public and legislator education (distributed at Ag Day at the Capital). Several video segments about biocontrol were shown on Outside Hawaii (an audience of 20,000 every week on TV alone, plus viewers at the website). The video focused on the recovery of the native wiliwili tree after a successful biocontrol effort. There were also some interviews about the impacts of strawberry guava and the need for biocontrol as a separate segment (<http://www.oc16.tv/shows/32>) A website was posted about strawberry guava biocontrol specifically to assist with the EIS public review process (<http://www.hear.org/strawberryguavabiocontrol/>).

The biocontrol communications workshop brought agency staff, researchers, land managers and outreach specialists together to talk about challenges and approaches to communicating about biocontrol. Since then, the biocontrol working group was convened for one meeting. The Maui Invasive Species Committee (MISC) worked with their county council to pass a resolution supporting the use of biocontrol. The Big Island County Council, in response to the strawberry guava controversy, passed a resolution against biocontrol. A site visit to a public forest infested by dense strawberry guava convinced the participating council members of the need for biocontrol, but not all council members chose to or were able to attend.

BIISC outreach strategy, particularly with regards to the strawberry guava proposal, focused on responding to biocontrol issues that resonated with many members of the public. The BIISC program participates in an average of one public outreach event per week, often in the form of information booths at varied festivals, plant sales, farmers markets, or spoken presentations to public or school groups. The BIISC outreach specialist presented an oral presentation on the history and successes of biocontrol in Hawaii at the 2009 Hawaii Conservation Conference. Presentations were also developed to educate and intrigue the public on the biology and importance of insects. Better understanding of insects will help the public to assess risk.

Results

Key issues raised by members of the public to outreach staff revealed that: the public generally is aware of one or two examples of disastrous failed introductions and is totally unaware of the extent and successes of biocontrol in Hawaii; fear of rapid evolution of the host to new targets is pervasive; a lack of understanding of insect biology and genetics contributes to the fear of rapid evolution; and the public does not understand the quarantine testing, regulatory process and limits on biocontrol releases. Through discussions and exhibits, many individuals expressed relief that biocontrol introductions were not as haphazard and uncontrolled as they had thought them to be. Most significantly, other

biocontrol releases have not been met with much opposition, before, during, or since the strawberry guava biocontrol issue came to a head.

The public's view of the invasive species considered for biocontrol affects whether or not a project receives support. For example, in the case of the wiliwili tree decimated by an accidentally introduced wasp, people saw the trees die and understood the gall wasp was a problem. Biocontrol of the gall wasp was not opposed. People do not see the watershed, do not see the full extent of the strawberry guava invasion, and therefore, they do not understand the impact (on groundwater, on cultural values, and on native species). Strawberry guava is not a recently introduced species and so has social familiarity and is perceived as a useful tree. Because it has some food value, attempts to control strawberry guava were portrayed as attempts by government to control the food supply, which is linked to fears of genetically modified foods. The relationship of strawberry guava fruit in promoting damaging fruit flies is not well understood by the public.

Discussion

Agencies may give undue weight to public opposition to biocontrol projects if that opposition is based on misinformation which can be corrected. Public opinions can change rapidly when a broader context of history, methods, successes, and regulation is described. Biocontrol is an important management tool for the threats facing Hawai'i. For biocontrol to be successful, agencies must be committed to and have the resources necessary for the research, development and education necessary before a release. This strong agency support and education will help the public in supporting this tool. Limited support runs the risk of achieving neither conservation goals nor reducing public concern with risk.

It is recommended that agencies and resource managers in Hawaii devote significant resources to produce educational materials to publicize biocontrol methodology and successes in Hawaii. Basic curricula should educate and intrigue the public on the biology and importance of insects.

A discussion of genetics and reasons for host specialization is also important. Good guy and bad guy cards, identification cards, and the current fascination with forensic anthropology may be useful lures. Another interesting possibility would be to engage citizen groups in rearing of approved biocontrol agents, as has been done elsewhere in the world. Future biocontrol projects should evaluate public attitudes towards the particular species, and plan outreach accordingly, while building general awareness and support.

Other current limitations for the state are the shortage of adequate quarantine facilities for testing. Public support for biocontrol proposals would help convince policy makers that these facilities should be funded.

Acknowledgements

Interviews with biocontrol staff from the Hawaii Department of Agriculture, USFS, and biocontrol researchers present at the second Miconia conference (Hana, Maui, 2009). Members of the Public Outreach Working Group.

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The Role of Implementation in Weed Biological Control in South Africa

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Abstract

Biological control of weeds was initiated in South Africa in 1913 with the introduction of the cochineal insect *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) on the invasive cactus *Opuntia monacantha* Haw. (Cactaceae) (Moran et al., 2011). Since that time some 113 agent species have been released against 48 weed species with varying levels of success (Klein 2011). The implementation of weed biological control agents has historically been neglected and there is very little research on this topic (Grevstad 1999, Memmott et al., 1998). In South Africa, initially agents were mass-reared and released by the researchers and a few landowners. In 1996 with the advent of the Working for Water Programme biological control implementation officers were appointed in each province of the country to serve as a conduit between the research scientists and the landowners (Gillespie et al., 2004). The role of the implementation officers was to mass-rear, release and monitor for establishment of the agents and redistribute, where necessary. Key to the success of this programme was record-keeping and the ensuring that information regarding releases and establishment of agents was provided to the researchers. This was achieved through the establishment of biannual technical liaison committee meetings and annual weed biological control workshops. More recently the task of mass-rearing has been outsourced to a commercial facility, which has greatly improved the quantity and more importantly the quality of agents being released. In the last five years weed biological control implementation has been rolled out to a number of schools and this has facilitated the incorporation of weed biological control into the National School Curriculum. Further, a programme that trains physically challenged individuals to mass-rear and distribute weed biological control agents around South Africa has been highly successful.

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“Of Miconia and Men”: The Story of a Scientifically and Socially Successful Biological Control Program in Tahiti, French Polynesia

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Abstract

Many biological control programs against invasive plants have failed or have been abandoned because of negative human perceptions or strong conflicts of interests, *e.g.*, the fear of introducing alien predators or pathogens (the so-called “pathophobia”, Warner, in press), the potential threats for related species of economic or conservation value, and the uncertainty of successful control (see *e.g.* Louda & Stiling, 2004). In this regard, biological control scientists often appear as sorcerer’s apprentices. This talk describes how a biological control program against the invasive tree *Miconia calvenscens* (Melastomataceae), a formerly popular ornamental plant species, was successfully conducted (1997-2010) on the island of Tahiti (French Polynesia, South Pacific) using a fungal pathogen (Meyer et al., 2008; Meyer et al., in press), despite the very bad reputation of past “biological control experiments” in the region (carnivorous snails introduced to control the Giant African snail, myna birds for wasps, raptors for rats, *etc.*). This case-study tries to demonstrate that rigorous scientific (pre- and post-release) studies are necessary but not sufficient for the acceptance of biological control by human society. Information and education at all levels (from public to politicians), consultation process including all stakeholders, and communication involving different media are equally important to avoid that “*The best laid schemes of mice and men go often askew*” (inspired by Robert Burns’ famous poem written in 1785). Paradoxically, biological control projects provide excellent opportunities to explain basic ecological processes and the methodology of science to the general public and schoolchildren in particular.

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Russian Olive – a Suitable Target for Classical Biological Control in North America?

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Abstract

Projects to develop biological control solutions against invasive plants are mid- to long-term endeavors that require considerable financial support over several years. Discussions of concerns and potential conflicts of interests often occur when biological control agents are first being proposed for release into the environment. Such late discussion, which in some cases results in delays or in the halt of ongoing biological control programs, has led to uncertainty, confusion and frustration among the various stakeholder groups, including the biological control practitioners. Russian olive (*Elaeagnus angustifolia* L.), a small tree or multi-stemmed shrub native to south-eastern Europe and Asia, was introduced to North America in the late 19th century as a horticultural plant. It has since spread into the environment, particularly along river courses where it now occupies similar habitats as tamarisk. To date, Russian olive has become a declared noxious weed in four US states. Because of the perceived benefits of planting Russian olive in some regions, developing a classical biological control program against Russian olive could give rise to a conflict of interests. To address and discuss potential conflicts of interests right at the onset of this new biological control initiative, we recently created a platform to collect, analyze and disseminate science-based information on Russian olive. Particular emphasis is being put on the following questions: 1) what are the economic, environmental or social impacts caused by Russian olive in North America or in other parts of the invaded range, 2) what are the goals of Russian olive management, and 3) is classical biological control a useful and feasible way to achieve these management goals? We will present first results of our data analysis and propose a way forward to reach common ground among key stakeholders regarding under which conditions Russian olive is a suitable target for biological control.

The Economics of Classical Biological Control: A Meta-Analysis of Historic Literature and Suggested Framework for Future Studies

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Abstract

Classical biological control (CBC) programs are initiated to protect natural resources, agricultural and other human interests. CBC programs typically involve an investment of public funds and their success is often determined by welfare measures such as benefit/cost analyses. An initial review of the literature shows previous efforts at measuring program benefits in monetary terms have often been incomplete and/or misguided. This review reveals that the basic analytical challenge can be broadly traced to two areas; project benefits lacking marketable measures and confusing or under reporting of project costs and benefits. The economics of CBC projects should be analyzed within the neo-classical economic view of supply and demand. On the supply side, costs are expenses directly related to project development and implementation. These include all direct expenditures necessary to locate and test the control agent and affect its release. These costs are typically covered by public funds and justified by the public nature of the anticipated project benefits. However, cost should also include any value lost to agents as a result of the project's success. On the demand side, agents with marketable goods and services that benefit from the project will provide a direct measure of the economic gain. Furthermore, their gains will lead to an indirect benefit or ripple effect through the economy. However, there are also benefits that lack market value and include items such as improved ecological services and other non-market activities such as improved fishing, hunting, *etc.* CBC projects would benefit from a strategic approach to assessing their economic efficiency. A meta-analysis of the use of economics in historic CBC literature is conducted and an analytical framework introduced to guide future benefit/cost studies for CBC projects. The framework will help generate support for CBC programs by providing a clear guideline for their effective economic evaluation.

Biological Control of Strawberry Guava in Hawaiian Forests

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Abstract

Over the last two decades, scientists in Hawaii, Florida and Brazil have researched biological control as a new tool for managing strawberry guava, an invasive tree in Hawaiian forests. A leaf galling scale insect from Brazil, *Tectococcus ovatus* (Hemiptera: Eriococcidae), was found to be highly target-specific and has been proposed for release in Hawaii. This natural enemy is expected to slow the spread of strawberry guava into native forests by reducing growth rates and seed and fruit production over time. A State of Hawaii environmental assessment of the proposed biocontrol release included detailed data from researchers as well as inputs from stakeholders and the public in recent years. Although this project has been strongly supported by partner agencies and conservation workers in Hawaii, it has encountered substantial opposition from some quarters of the public who value strawberry guava for a variety of reasons. As a prominent and provocative target for biocontrol, the case of strawberry guava offers some important lessons on the challenges and opportunities facing biocontrol as a management tool for conservation and restoration of Hawaiian forests.

The Economic Benefits of TSA Biological Control

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Abstract

Tropical Soda Apple (*Solanum viarum* Dunal) (TSA) is an invasive exotic plant from South America that has become a weedy pest, choking pastures and afflicting Florida's beef producers. In 2007, state-wide economic losses were documented to range from \$6.5 million to \$16 million annually. In 2008, efforts to control TSA resulted in the release of the green tortoise beetle (*Gratiana boliviana* Spaeth) (GTB) across central and southern portions of the state. Also a native of South America, the GTB is particularly fond of TSA foliage with no alternative native hosts. Initial results indicate the beetle is spreading rapidly and significantly reducing TSA density in many areas of the state. During the summer of 2010, a survey of Florida's cattle producers was conducted to evaluate the impact of the recent TSA biological control efforts (*Gratiana boliviana* Spaeth) in central and southern Florida. A survey was mailed statewide to 3,500 members of the Florida Cattleman's Association. The survey asked participants to identify their type of cattle operation, the distribution of TSA in their pastures and their assessment of TSA density and the effort required to control this plant. Slightly more than 30% of those surveyed responded. When compared to 2007, preliminary results indicate significant declines in both TSA density and control efforts across central and southern Florida. On the other hand, northern Florida has experienced an increase in TSA density and control effort. These preliminary results support the hypothesis that the GTB has reduced TSA density and lowered control costs to cattle producers.

Is Post Hoc Development of Risk Management in Weed Biological Control Too Late? Lessons Learned from *Cactoblastis cactorum*

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Abstract

The Argentine cactus moth, *Cactoblastis cactorum* (Berg), is renowned for its success as a biological control agent against exotic *Opuntia* spp. in many locations including Australia, South Africa and Hawaii. However, in 1957, its introduction into the Caribbean to control native *Opuntia* spp. ultimately resulted in its arrival to southern Florida where it became an invasive pest of native and rare *Opuntia* species and a threat to the *Opuntia*-rich areas of the western U.S. and Mexico. To mitigate this risk, survey and control tactics were developed in the U.S. and an awareness campaign was initiated in Mexico. A Bi-National Cactus Moth Control Program was established to facilitate risk management, which involved identifying, evaluating, selecting and implementing actions to prevent, reduce or control adverse effects of *C. cactorum*. The risk management process included comparing the risks of taking no action with the risks associated with each remedial alternative, while taking into account social, cultural, ethical, economic, political, and legal considerations. Although these risk management activities were undertaken after the initial release of *C. cactorum*, management tactics were available and used successfully to eradicate this pest when there was an incursion in Mexico. Efforts remain ongoing in the U.S. where the westward expansion of *C. cactorum* has been mitigated through regulatory and control actions. The lessons learned from *C. cactorum* in North America underscore the need to have regional involvement in the risk analysis process and in the development of risk management prior to the release of a weed biocontrol agent.

Biological Control as a Tool to Mitigate Economic Impacts of Facilitative Ecological Interactions between the Giant Reed and Cattle Fever Ticks

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Abstract

Annual domestic impacts associated with introduced weeds are conservatively estimated at \$27 billion, which incorporates costs of weed management, crop losses and displacement of productive rangeland, and displacement of some environmental services. Estimating the total economic damage of invasive weeds can be difficult, especially when they impact non-market services, or when impacts are indirect. The giant reed, *Arundo donax* L., is an invasive grass infesting riparian corridors and waterways in the southwestern U.S. and northern Mexico. In addition to the economic implications of water loss in this arid agricultural area, deleterious non-market effects ascribed to giant reed invasion include riparian habitat fragmentation, biodiversity loss, stream-bank erosion, and physical and logistical obstruction for border security and enforcement. These thick swaths of giant reed are also a highly suitable habitat for the cattle fever tick, *Rhipicephalus microplus* (Say), an important vector of the protozoa causing bovine babesiosis. Survival rates, fecundity, and fertility of engorged adult female cattle fever ticks were tested in tick cohorts placed in pastures, mixed brush, and arundo stands. Ticks were more likely to lay eggs and larger egg masses in giant reed and mixed brush when compared to ticks in mixed-grass pastures where microclimatic conditions are less favorable. Animals such as cattle, horse, and white-tailed-deer traversing through nearly-impenetrable stands of giant reed create common-use corridors that in effect facilitates parasitism of suitable hosts by cattle fever ticks thriving in that habitat. Our findings document the economically significant indirect impact by giant reed as a complicating factor to keep the U.S. free of cattle fever ticks and bovine babesiosis. Such considerations should be incorporated when modeling the total economic costs associated with an invasive plant. The use of biological control agents against giant reed stands represents a sustainable strategy to mitigate the indirect economic impacts of giant reed and disrupt facilitative ecological interactions between invasive species like cattle fever ticks and giant reed in south Texas.

SESSION 9: POST-RELEASE EVALUATION AND MANAGEMENT

One Hundred Years of Biological Control of Weeds in Australia

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Abstract

In the first comprehensive review of biological control of weeds in Australia since 1960, 73 different programs have been reviewed, including 69 on weeds exotic to Australia. This account summarizes some findings from this review. The programs are categorized according to the scale of activity, type of problem caused, plant growth form of the weed and overall success rate. Trends in the last 50 years are examined, including the use of pathogens, emphasis on risks to native species, and environmental weeds as targets. Advances in defining the native range and in host specificity risk analysis are reviewed and questions posed on climate matching. The extent of evaluation and integration of biological control with other management options is considered and emphasis placed on the enormous economic benefit demonstrated in benefit: cost analyses. Biological control of weeds has been an extremely active field in Australia with many successes and has yielded considerable economic, environmental and scientific benefits.

Introduction

Biological control of weeds in Australia was reviewed by Wilson (1960) who devoted 18 pages to work on 12 weed species (or groups of related species). Around this time, in the early 1960s, work was fairly limited, until support was obtained for a program aimed at the possible biological control of skeleton weed, *Chondrilla juncea* L., considered the worst weed in Australia at the time. As a result of studies in the native range of this weed, in Mediterranean Europe, Wapshere made the revolutionary proposal to introduce the rust fungus *Puccinia chondrillina* Bubak & Syd. (Cullen, 2012). After considerable discussion, debate among plant pathologists and inspection of testing procedures and protocols, introduction into quarantine and subsequent release was approved in 1971. The spectacular success of this introduction (Cullen et al., 1973, Cullen, 1978) reignited interest in the field and there was enormous expansion of projects during the 1970s

and 1980s, with several notable successes. After 50 years of intense activity, an updated review was necessary and Julien et al. (2012) have coordinated an extensive review of work up to late 2010, covering 73 weed species or species groups, each reviewed by researchers involved with the relevant programs. This paper summarizes some of the principal trends and lessons learned from this review.

Overall Statistics

Of the 73 programs reviewed, 69 deal with exotic species, primarily of European, South African or Central and South American origin, which are weeds in Australia. Three programs cover attempts to control Australian species weedy in Australia, using native natural enemies, while one chapter deals with exploration in Australia for biological control agents for Australian plants weedy in South Africa and the USA. The 69 “classical” programs can be categorized in a number of different ways. Table 1a shows the

number of “full” programs with exploration carried out specifically for the target weed in question, “serendipitous” programs with exploration often an opportunistic “add on” to work being carried out primarily on another species, programs capitalizing on work carried out by other agencies in other countries with the same weed problem (therefore introducing agents already established as safe and effective), and programs that did not progress beyond a preliminary stage, possibly involving some minor exploration and testing, but not involving any releases. Table 1b gives a breakdown according to the broad category of problems caused by the weed and illustrates the preponderance of programs on weeds of agricultural importance, reflecting the dominant source of funding. However, a significant number were also of environmental concern and five programs were on weeds solely of environmental concern.

The overall success rate of the 58 developed programs is shown in Table 2a and their classification according to plant growth form, with the relevant success rate, in Table 2b. A distinction is made in Table 2a between very successful, where control is extensive, and where success varies according to the region or season. In Table 2b these categories are combined. While some differences in the success rate for different growth forms can be observed, the only significant point is that all forms have been targeted (two programs on grasses are included in the perennial herbs) and that successes have been obtained against all forms. Tables 1 and 2 illustrate a number of overall characteristics and success rates for programs, but it is also valuable to consider how programs have been pursued over the last 50 years and the ways in which this has changed from the earlier half-century.

Table 1a. Types of programs

Full	45
Serendipitous	8
Other agency's agents	5
Preliminary only	11

58 developed programs

Table 1b. Importance of the weed

Agricultural production	63
Environment	36
Recreation, amenity, health	17

(Five solely of environmental importance)

Table 2a. Overall success rate of programs

Very successful	14
Seasonally/regionally successful	11
Too early, still ongoing	22
Unsuccessful	11

Table 2b. Numbers of each plant growth form targeted and number successful.

Shrubs/trees	17	7
Perennial herbs	21	5
Annual/biennial herbs	21	7
Climbers/creepers	5	2
Aquatics	5	4

Trends

Use of plant pathogens

Following the success of the introduction of *P. chondrillina*, interest in the use of plant pathogens as well as arthropods increased rapidly, though it was 20 years before the next legal introduction and release, of *P. abrupta* Diet. & Holw. var. *parthenicola* (Jackson) Parmelee for control of parthenium *Parthenium hysterophorus* L. (Dhileepan and Mcfadyen, 2012), due largely to the longer testing programs required and lingering concerns over the safety of such introductions. In all, 31 of the 58 developed programs have considered plant pathogens, principally rusts, with another six programs recognizing the potential for investigation. Significantly, pathogens have been the main agents of success in nine programs, those against *Ageratina adenophora* (Spreng.) King & Robinson, *Ageratina riparia* (Regel) K & R, *Asparagus asparagoides* (L.) Druce, *C. juncea*, *Cryptostegia grandiflora* (Roxb.) R.Br., *P. hysterophorus*, *Rubus fruticosus* L. agg. and *Xanthium occidentale* Bertol. While the emphasis has been on rusts, other pathogens have been considered, though considerable concern has been expressed over the use of powdery mildews. The use of plant pathogens as mycoherbicides was considered in ten programs, but with no viable product to date. Plant pathogens have been notable for accidental and/or illegal introductions, e.g. on *A. adenophorum*, the first strain of *Phragmidium violaceum* (Schulz) Winter on *R. fruticosus*, *Puccinia xanthii* Schw. on *X. occidentale* and most recently, the smut *Entyloma ageratinae* sp. nov. Barreto & Evans on *A. riparia*.

Risks to native non target species

The interest in the use of plant pathogens also raised the importance of risks to the native flora, starting with *P. chondrillina*. These risks were considered from the late 1960s for plant pathogens, but it took until the mid to late 1970s for this to become an issue for arthropod introductions, *Longitarsus flavicornis* (Stephens) being the first arthropod specifically tested against native flora in 1977-78 prior to consideration for release against ragwort, *Senecio jacobaea* L. (Ireson and McLaren, 2012)

Environmental weed as targets

The occurrence of weeds of environmental concern among the list of programs has already been noted (Table 1b), but this only evolved as research funds became available for such programs in the 1980s. The 1998 declaration of “Weeds of National Significance”, which included several species of purely environmental concern, has assisted the provision of funds for such programs since the 1990s.

Use of Guiding Principles

Overlapping some of these trends has been an increasing interest in applying some general ecological and evolutionary principles to the conduct of biological control programs against weeds.

Refining the native range

Determining the native range of a target weed has increasingly become a research area in itself, with detailed taxonomy necessary to determine the origin of a weed in a third of the programs. With the advent of modern genetic technologies, genetic typing was mentioned in 19 of the 58 developed programs. This was considered critical when determining the origin of a precise form of a variable species has been necessary, particularly where potential agents with a closely coevolved association have been involved, e.g. plant pathogens, eriophyid mites and a few insect species. *Chrysanthemoides monilifera* (L.) T. Norl. (Adair et al., 2012) and *A. asparagoides* (Morin and Scott, 2012) are good examples of the value of this approach.

Area of speciation of the target weed

The principle that the locality where a plant species evolved should be the best source of specific coevolved natural enemies was well illustrated in the *C. juncea* program. Wapshere (1974) found increasing numbers of specific natural enemies the further east exploration was carried out, from the western Mediterranean to Iran. However, even where geography, politics and sufficient taxonomic knowledge permit, this approach has not been followed significantly, with only one other program mentioning the possibility.

New associations

Hokkanen and Pimentel (1984) in their classic paper suggested the value of new host-agent associations as well as the long coevolved associations usually sought. This was referred to in only three programs, *C. monilifera*, *Cylindropuntia rosea* (DC.) Backeb. and *Prosopis* spp., with the authors of the account on *C. monilifera* (Adair et al., 2012) suggesting that resources might have been better spent determining the precise form and its origin rather than investigating possible new associations.

Climate matching

Where the native range includes different climatic zones, trying to match the climate of the source of agents with the climate of the weed has been common for some time (cited in 29 of the 58 developed programs). The failure of agents has sometimes been attributed to inappropriate climatic adaptation, while the aim of trying to source new agents from more appropriate climatic regions is cited some programs e.g. *Emex* spp. (Yeoh et al., 2012)

However results from some programs suggest that the situation is not always straightforward. *Acacia nilotica* subsp. *indica* (Benth.) Brenan occurs in two regions in Australia, the more humid coastal Queensland and the drier interior grasslands, but is only a serious problem in the drier inland. Climatic matching suggests that the weed is in fact more suited to the coastal climate, which in itself not necessarily novel, as many species have expanded ranges in their country of introduction. However, the two agents considered established at this stage were both collected deliberately from drier regions of the native range, but have also been shown to be better adapted to the more humid coastal region (Palmer et al., 2012).

In the only experimental approach to testing this principle, Cullen and Sheppard (2012) report the introduction of three separate populations of *Rhinocyllus conicus* Froehlich for control of *Carduus nutans* L.: one from southern France, climatically adapted to the southern part of the Australian distribution of *C. nutans*; one from northern Italy, adapted to the northern part; and a third population originally from central Europe, via New Zealand, not climatically adapted to any part of the Australian *C. nutans* distribution. All three populations were

released in separate sites in the northern, central and southern part of the *C. nutans* distribution. The third population established and flourished at all three sites, while the other two, apparently better adapted populations, did less well. The authors speculate as to why this might have occurred, but climatic adaptation was clearly not the major influence.

Equally, where attempts were made to obtain suitable rust strains using trap gardens in the region of origin, e.g. *C. juncea* and *R. fruticosus*, the conclusion was that “Compatible host-pathogen interactions.....were more critical than climate matching.....” (quote from Morin and Evans (2012) referring to the *R. fruticosus* program).

Host specificity

Fuelled to some extent by the increased emphasis on possible risks to native species, several targets having close relatives, the study of what constitutes “natural behavior” and the process of risk analysis have become more critical over the last 50 years. Ten programs mention specific cases of more intensive examination of host specificity issues. The use of open field testing is reported in the accounts of the programs on *Heliotropium europaeum* L. (Sheppard et al., 2012), *Heliotropium amplexicaule* Vahl (Briese, 2012) and *C. monilifera* (Adair et al., 2012). The first of these required critical Australian species to be grown under quarantine in Greece and placed in the field under natural, but strictly controlled, conditions to assess the behavior of the weevil *Pachycerus segnis* Germar (Sheppard et al., 2012).

A number of programs describe examples where risks have been carefully weighed before release was authorized. Possible damage to non targets by the moth *Platphalonidia mystica* (Razowski & Becker) was eventually discounted as unlikely and unimportant in the program against *P. hysterophorus* (Dhileepan and McFadyen, 2012), and the possible damage to native species from the moth *Euclasta whalleyi* Popescu-Gorj & Constantinescu was weighed carefully against the risk to native species posed by the target weed *C. grandiflora* (Palmer and Vogler, 2012). In the case of *Longitarsus flavicornis* (Stephens), the very low number of adults developing indicated an extremely low probability of populations persisting on native species of *Senecio*, while for *Maravalia cryptostegiae* (Cummins) on *C. grandiflora* and *Uromyces heliotropii* Sred. on *H.*

europaeum, geographic and climatic separation from native species showing some slight susceptibility was considered sufficient for the risk to be discounted (Palmer and Vogler, 2012, Sheppard et al., 2012).

Choice of Agents

While the default position for choosing which agents to introduce has always been the observation of obvious damage to the weed in its native range, a number of programs attempted to assess the options more critically. In particular, attempts have been made to examine agent interactions and to compare impacts on the weed's population dynamics in the region of origin in order to determine the most effective agents and the best order for introduction, to avoid negative interspecific competition and to predict overall impact.

Order of introduction

In the more recent phases of the program against *Hypericum perforatum* L., careful examination of impacts suggested that control might have been more stable and effective if the buprestid beetle *Agrilus hyperici* (Creutzer) and the eriophyid mite *Aculus hyperici* (Liro) had been introduced before the two *Chrysolina* spp. *C. hyperici* (Forst.) and *C. quadrigemina* (Suffr.), introduced in the 1930s. In the native range, the buprestid and the eriophyid are the most important mortality factors, but attempts to establish them have been continually hampered by the unstable populations of the *Chrysolina* spp. (Briese and Cullen, 2012).

Interspecific competition

The common practice of choosing agents attacking different parts of a weed's biology or phenology has aimed to try to avoid interspecific competition, but few attempts to assess this probability, even for agents attacking the same part of the plant, have been made prior to introduction. In the program against *C. nutans*, reducing the production of seed was the main aim and, given two apparently effective seed destroying agents, *R. conicus* and the seed fly *Urophora solstitialis* (L.), studies were made to assess the likely impact of interspecific competition between them. This seemed to be avoided in the

country of origin, but did occur when introduced into Australia, the authors noting the different weed population levels, different phenology and lack of parasites as being important (Cullen and Sheppard, 2012). This demonstrated the difficulty of extrapolating from one environment to another without a high level of detail and sufficient knowledge to judge the most important factors.

Impact on weed population dynamics

While studies of agents' impacts in the country of origin were too late to determine the best order of agent introduction for *H. perforatum* and failed to predict the extent of interspecific competition between *R. conicus* and *U. solstitialis* in Australia, they were instructive in determining the agents most likely to be effective and in subsequent follow up and choice of further agents. In the case of the *C. nutans* program, it became clearer which subtle differences in the environment of the weed were important, and the studies allowed projections of impact and (different) orders of introduction of agents for Australia and New Zealand (Cullen and Sheppard, 2012). It is also important to remember that it was Wapshere's demonstration of the impact of *P. chondrillina* in the field in Europe that led to, and supported, this critical introduction (Wapshere et al., 1974).

Evaluation

Twenty eight of the 58 developed programs included some degree of detailed follow up and, in 20 cases, the follow up involved significant ecological analysis. While evaluation of programs has seldom been adequately funded, its value has been widely accepted though not always by funding agencies. Objective evidence of decline is immensely valuable and can also be vital for subsequent benefit/cost analysis, itself a powerful accounting and reporting tool. Knowledge of the weed - agent system, combined with accurate followup work, can provide pointers for improving control, either by the introduction of further agents or by developing programs of integrated control.

Weed decline

Evaluation received considerable emphasis in the *C. juncea* program and the detailed record of

decline, coupled with experimental evidence, helped establish not only the value of the program, but the success of the controversial introduction of *P. chondrillina* and the potential benefits of biological control of weeds in Australia generally (Cullen, 2012). Similar studies were invaluable in charting progress in the *A. asparagoides* program (Morin and Scott, 2012), in ensuring the success against *Salvinia molesta* D.S.Mitchell (Julien, 2012) and in disentangling different agent impacts in the *Echium plantagineum* L. program (Sheppard and Smyth, 2012).

Benefit-cost analysis

The detailed data on *C. juncea* allowed a comprehensive benefit-cost analysis to be undertaken in 1976, demonstrating the enormous benefits to Australian cereal growing. This analysis was updated in 2006, when benefit-cost analyses were carried out on 35 other programs (Page and Lacey, 2006). This allowed an invaluable assessment to be made of the economic benefits of many Australian programs. Only nine programs yielded few or no economic benefits and the overall benefit-cost ratio for the 28 programs for which there was reasonable data was 23:1 (McFadyen, 2011). Even programs not considered successful at the time of the analysis in fact showed significant benefits due to the measure of control obtained so far, e.g. benefit-cost of 6:1 for *Lantana camara* L. and 2.5:1 for *R. fruticosus*. Table 3 shows the five highest benefit-cost ratios, with the prickly pear program still leading the way. It is worth noting that the *Ambrosia artemesiifolia* L. program was a very small and therefore very cheap program, hence the high ratio, and that the benefits were in

human health. Otherwise, the benefits were mainly in increased agricultural production and decreased control costs. Environmental and amenity benefits could not be costed and were not included.

Integration

Integrated management was considered a viable option in 13 of the 58 programs, and requires sufficient knowledge of the weed-agent system to allow its manipulation. The classic example has been salvinia *S. molesta* where control has been spectacularly successful in many tropical and subtropical systems in Australia and around the world. However, in the billabongs of Kakadu National Park in northern Australia control is intermittent, depending on rainfall and flushing of the system with its differential effect on weed and weevil populations. However, with a good knowledge of plant growth and weevil growth and their relationships with temperature and nutrients, it has been possible to develop a management program using carefully timed herbicide applications to allow the system to switch back into balance (Storrs and Julien, 1996).

Conclusion

Biological control of weeds has had a high profile in Australia ever since the prickly pear program, but activity declined towards the end of the first 50 years. The revamping of activity during the 1970s and 1980s has resulted in many successes. The investment in this field has been enormously beneficial; economically, environmentally and scientifically. The contributions in the review by Julien et al. (2012) help demonstrate why and how.

Table 3. Benefit:cost ratios for top five biological control programs.

Weed	Benefit:cost ratio
<i>Opuntia</i> spp.	312:1
<i>C. juncea</i>	112:1
<i>C. grandiflora</i>	109:1
<i>A. artemesiifolia</i>	104:1
<i>S. molesta</i>	53:1*

* Figure for Sri Lanka project. Australian analysis combined it with other aquatic weeds.

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Revisiting Release Strategies in Biological Control of Weeds: Are We Using Enough Releases?

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Abstract

The initial establishment of biocontrol agent populations is a critical step toward successful biocontrol. Theoretical approaches have revealed factors that determine the optimal release strategy (the combination of release size and number of releases), including the presence or absence of Allee effects in the agent population and its susceptibility to environmental variability. In this study, we take a more generalized empirical approach that may be more useful for guiding future releases. We analyzed release and establishment records for 74 species of biocontrol agents introduced against 31 weeds in the state of Oregon, U.S.A. Our main findings were that (1) establishment was not affected by release size over the range of release sizes typically used; (2) biocontrol agent species vary in how readily individual releases lead to establishment; and (3) biocontrol programs often use fewer initial releases than is optimal to obtain a high probability of overall establishment. The case of *Prokelisia marginata* (van Duzee), a planthopper introduced as a biocontrol agent for *Spartina alterniflora* Loisel (smooth cordgrass), is presented as an illustrative example of the benefits of using more releases.

Introduction

Roughly one-third of biological control agents introduced against weeds worldwide fail to establish permanent populations in the introduced range (Lawton, 1990; Syrett et al., 2000). Among the many factors that can influence whether or not biocontrol agents establish, release strategies are one of the few that practitioners have control over (Coombs, 2004). Thus, it is important to understand whether and how release strategies can improve establishment success. In this paper, “release strategy” is considered to be the combination of number and size of releases used in the initial effort to establish agent populations.

Assuming that a limited number of insects are available to release, which is most often the case with initial introductions, there is a potential tradeoff in the use of many (smaller) releases that provide more chances to establish and the use of larger (fewer) releases that may have a higher chance of establishment per release.

Prior theoretical models have revealed that the optimal release strategy depends on the shape of the relationship between the number of individuals released and the probability of establishment, and that this relationship depends on the relative influences that Allee effects and environmental variability have on the colonizing populations

(Grevstad, 1996, 1999a; Fig. 1). An Allee effect is defined as a reduction in population growth rate that occurs at low population densities. For example, a reduced population growth rate may occur if individuals have difficulty finding mates at low densities. When an Allee effect is operating, there will be a strong dependence of establishment on release size and the optimal release strategy is to make fewer large releases. On the other hand, when environmental variability has a large influence, then establishment probability will be weakly dependent on release size and a strategy of many small releases will be optimal (Grevstad, 1996, 1999a; Fig. 1).

In this study, we revisit the concept of release strategies using an empirical approach of analyzing the known outcomes of past releases. Using a vast database of release and establishment records maintained by the Oregon Department of Agriculture, we determine whether release size and number of releases are likely to affect establishment. Moreover, we ask whether biocontrol programs

typically use effective release strategies or if improvements could be made. We illustrate our main points with a revealing case study of the biocontrol agent *Prokelisia marginata* (van Duzee), a planthopper introduced as a biocontrol agent for *Spartina alterniflora* Loisel in Washington State.

Methods and Materials

Analysis of ODA data

The Oregon Department of Agriculture (ODA) has been involved in biological control of weeds since the earliest program against St. Johnswort (*Hypericum perforatum* L.) in 1947. Since that time, ODA has made and recorded more than 12,000 releases of 74 agent species against 31 noxious weed species. The records include the target weed, agent species, release date, number of individuals released, and location of the release. For a subset of 611 cases,

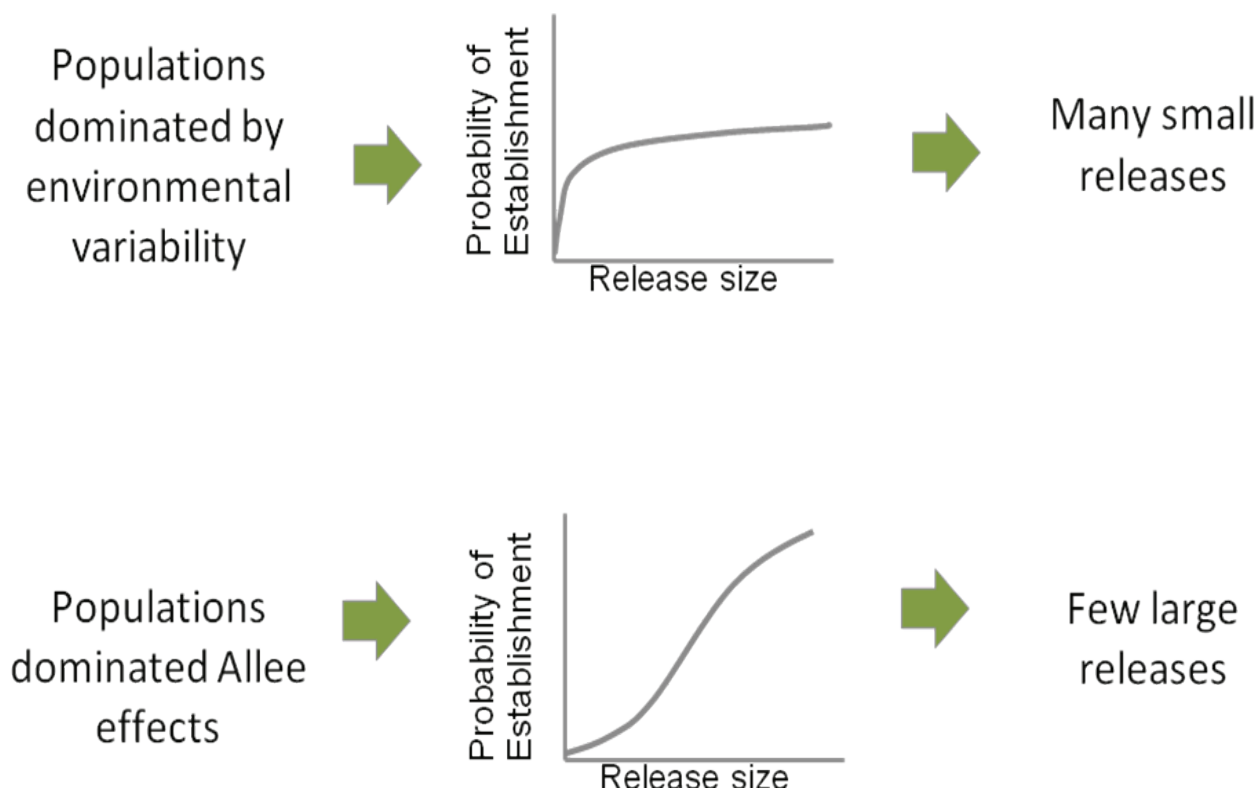


Figure 1. Theoretical predictions from stochastic simulation model (adapted from Grevstad 1999a). Establishment of populations that are strongly influenced by environmental variability will have weak dependence on release size and the optimal strategy is to make many small releases. In contrast, establishment of populations influenced by Allee effects have a strong dependence on release size and the optimal release strategy is fewer large releases.

there is also definitive record of whether or not the released population established at that site (defined as present after the 3rd year).

To test whether release size was correlated with establishment, we carried out logistic regression using all early releases for which there was a known outcome. We excluded releases of a nematode and two pathogens because the numbers of eggs and spores were vastly out of range compared to release numbers of insects and mites. The number of individuals released was log-transformed prior to analysis and we included agent species as a co-factor. Additionally, we summarize the frequency with which different agent species establish following release and use this information to quantify the expected benefit of using more independent releases.

***Prokelisia marginata* case study**

For illustration of the importance of using a greater number of releases, we present the case of *P. marginata*, a delphacid planthopper that was introduced as a biocontrol agent against *S. alterniflora*, a cordgrass native to the Atlantic Coast of North America and invasive in Pacific Coast estuaries. This insect was initially released into Willapa Bay, Washington in 2001 using a strategy of three very large releases of 65,000 (Grevstad et al., 2003). This strategy was chosen in order to avoid an Allee effect, obtain a more rapid impact on the plant, and facilitate the monitoring population growth and spatial spread. In 2002, an additional 12 releases of 9,000 were made. Site characteristics such as the percentage of intact thatch, nitrogen content of plants, and spider density were measured as possible influences on establishment (see Grevstad et al., 2004). In the final set of releases made in 2004, even smaller releases were used (5,000 each), but sites were selected based on habitat characteristics found to be correlated with higher success. The population densities of all released populations were sampled in spring and fall using an insect vacuum.

Results

Effect of release size on establishment

For the 611 releases made into Oregon that have known outcomes, there was no significant

effect of release size on whether or not populations established (SPSS binary logistic regression: Wald statistic = 0.683, df = 1, P = 0.408; Fig. 2). In fact, we did not find a single species of agent for which there was a significant effect of release size. It may be that release programs typically use release sizes that are large enough to avoid Allee effects, that Allee effects are not present in these populations, or that environmental variability is the dominant influence on release outcomes. Release sizes varied widely within and between species. The smallest release size used was five individuals (*Pterolonche inspersa* (Staudinger) on diffuse knapweed) and the largest was one million (*Aceria malherbae* Nuzzaci on field bindweed). The mean release size (excluding nematodes and pathogens) was 3197.

Rates of establishment among agent species

Different agent species were found to establish at different rates (Fig. 3). Approximately 35% of agents established all of the time, 24% establish none of the time, and 40% establish some portion of the time. Those that established a portion of the time fell uniformly along a continuum from very easy to very difficult to establish. The mean rate of establishment was found to be 0.55. Table 1 lists examples of agent species that fall into different establishment categories.

Numbers of releases used

The number of initial releases used (first 2 years) for agent introductions made in Oregon ranged from 1 to 68 with a mean of 6.39 ± 1.20 and a median of 3 (Fig. 4). Surprisingly, a large proportion of the projects (48%) used only one or two initial releases. Eighty-seven percent of projects used 10 or fewer initial releases. Most of the agents released into Oregon were also introduced into other states at the same time, so the numbers of releases we report do not represent release strategies used for the country as a whole.

Implications for release strategies

Given that establishment was independent of release size for the range of release sizes typically used, we can calculate the probability of overall

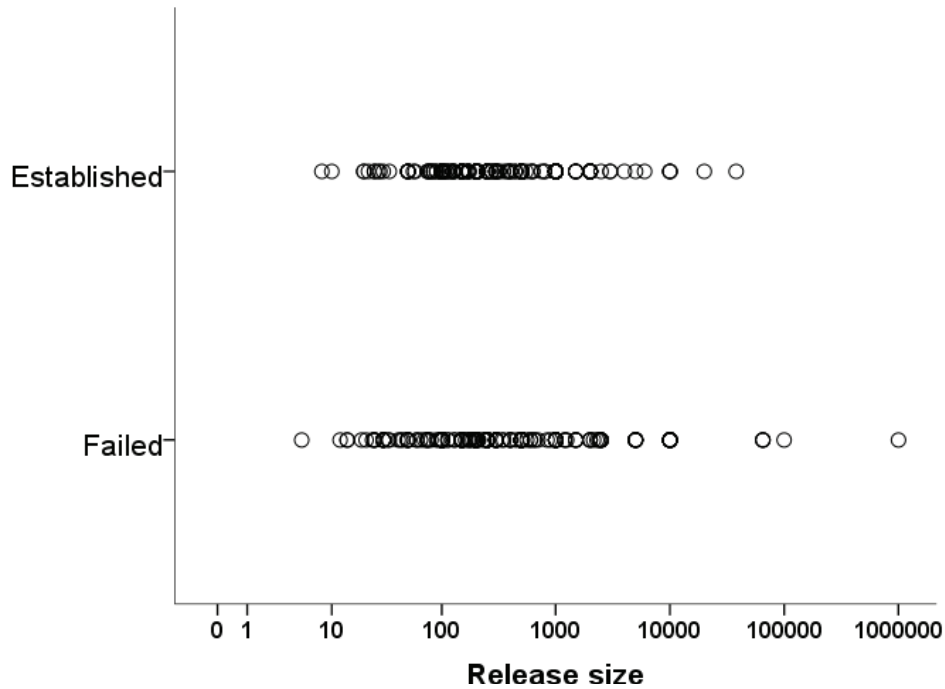


Figure 2. Relationship between release size and establishment for all weed biocontrol agent species introduced into Oregon. Each circle represents a different release for which the outcome (established or failed) is known. Logistic regression analysis did not detect a significant effect of release size on establishment.

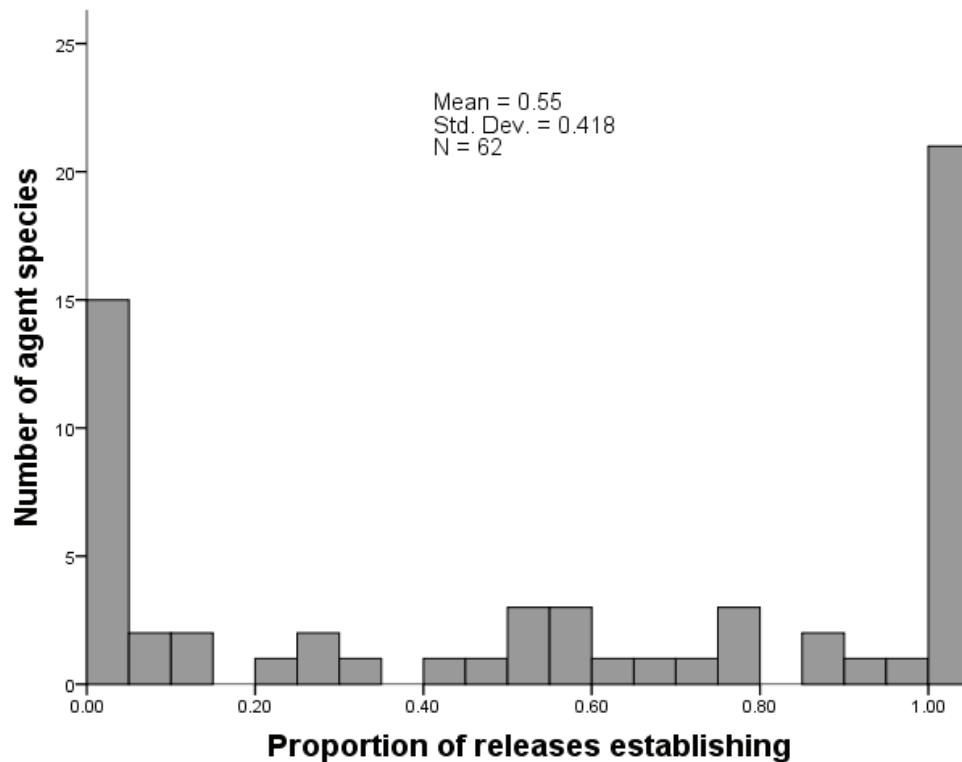


Figure 3. Number of weed biocontrol agent species establishing at different rates when released into the State of Oregon.

Table 1. Examples of biocontrol agent species falling into different categories of ease of establishment following release into Oregon or nearby counties in Idaho and Washington. Results may vary in other regions. Species marked with an asterisk were difficult to establish during the initial release period, but became easy to establish later.

Impossibles	Difficult	Easy
<i>Apthona abdominalis</i>	<i>Aceria malherbae</i> *	<i>Bangasternus fausti</i>
<i>Chamaesphecia crassicornis</i>	<i>Aplocera plagiata</i> *	<i>Chrysolina quadrigemina</i>
<i>Hyles euphorbiae</i>	<i>Bradyrrhoa gilveolella</i>	<i>Cystiphora schmidtii</i>
<i>Microlarinus lypriformis</i>	<i>Pterolonche inspersa</i>	<i>Eriophyes chondrillae</i>
<i>Pelochrista medullana</i>	<i>Calophasia lunula</i>	<i>Galerucella</i> spp.
<i>Phrydiuchus spilmani</i>	<i>Diorhabda elongata</i>	<i>Larinus minutus</i>
<i>Spurgia esula</i>	<i>Larinus obtusus</i> *	<i>Metzneria paucipunctella</i>
<i>Urophora solstitialis</i>	<i>Prokelisia marginata</i>	<i>Mecinus janthinus</i>
<i>Zeuxidiplosis giardi</i>	<i>Tyta luctuosa</i>	<i>Nanophyes marmoratus</i>
		<i>Urophora affinis</i>
		<i>Urophora quadrifasciata</i>

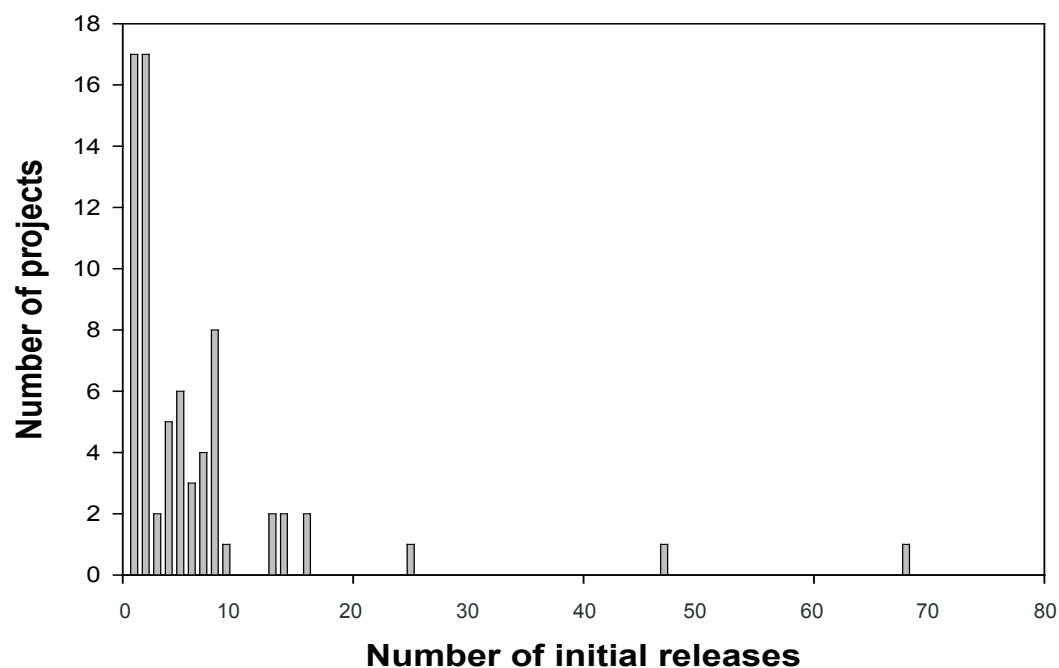


Figure 4. Number of weed biocontrol projects in Oregon that used different numbers of initial releases. Initial releases are considered to be those made during the first two years of a release program.

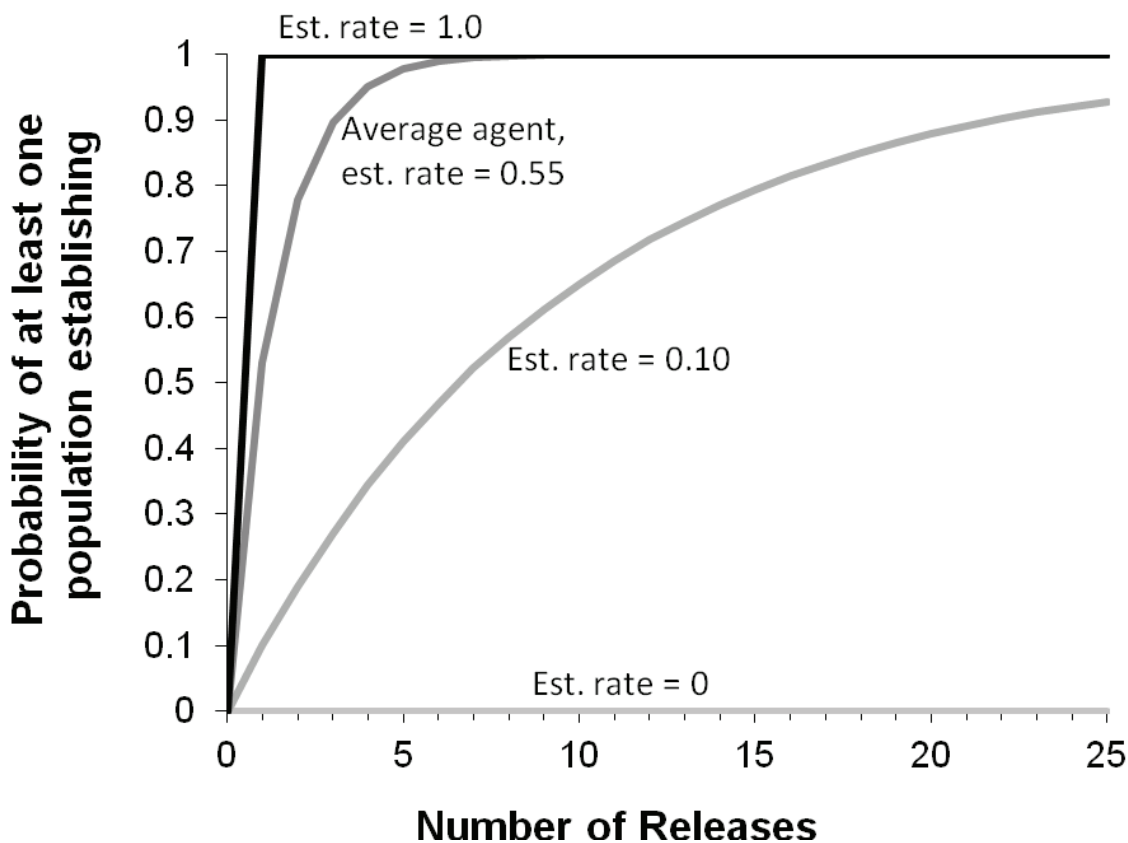


Figure 5. The probability of establishing at least one population as a function of the number of independent releases made for different single release establishment rates.

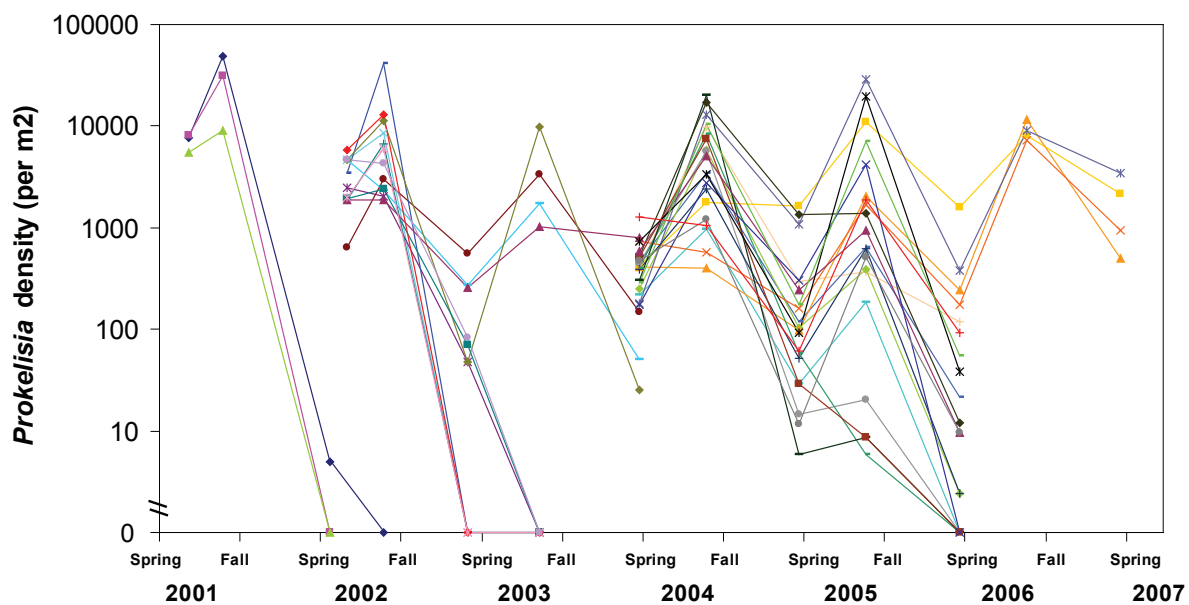


Figure 6. Population densities through time for 3 periods of releases of *Prokelisia marginata*, a biocontrol agent introduced against *Spartina alterniflora* in Willapa Bay, Washington State. Three releases were made in 2001, 12 in 2002, and 25 in 2004.

establishment (i.e. the probability of at least one release succeeding) based only on the number of releases made and the expected per-release establishment rate. This approach assumes that the releases are independently distributed across the pool of environmental conditions for which the per-release rate of establishment is valid. The probability of overall establishment (P_{all}) is the same as the probability that not all releases fail, which can be expressed in terms of the per-release establishment rate (P_1) and the number of releases made (N):

$$P_{\text{all}} = 1 - (1 - P_1)^N$$

Figure 5 shows the relationship between P_{all} and the number of releases made for hypothetical species with different values of P_1 . Providing that P_1 is greater than zero and not too close to one, there is a clear benefit to using more releases in order to increase the probability that at least one population establishes. Using this formula, an average biocontrol agent ($P_1 = 0.55$) that was introduced using the median number of releases (three) would establish 91% of the time. Although the average biocontrol agent is likely to establish with relatively few release attempts, it is not possible to know ahead of time whether a new agent being released is an average agent. Instead, it would be prudent to assume that the agent will be more difficult to establish. A difficult agent that establishes only 10% of the time would require 25 to 30 initial releases to be sure that it is given a fair chance to establish.

Prokelisia marginata case study

The outcomes of releases of *P. marginata* serve to illustrate the advantages of using a strategy of more (smaller) releases. The initial release strategy employing 3 very large releases of 65,000 individuals each was not successful (Fig. 6). Although the populations reproduced well during the first summer, survival was very low during the winter and all three populations were extinct by the following summer. From the second set of 12 releases of 10,000, four populations successfully established. Thus the rate of establishment for the initial two-year effort to establish *P. marginata* was 4 out of 15 attempts, or 26.6%. Given this rate of establishment, it is not surprising that the initial 3 releases failed. The likelihood of this happening would have been the probability of failure (0.734) raised to the power of 3 (the number of attempts), or 0.395. In retrospect,

we can say that the number of releases that should have been used to ensure a high probability of establishment (say 95%) would have been 10.

The *P. marginata* example illustrates another advantage to using many initial releases, which is that we can learn from these initial releases to achieve better results in the future. From the second set of 12 releases, a key habitat requirement was found that correlated with successful establishment. Sites where the senesced *Spartina* culms (thatch) remained intact over the winter supported much higher overwintering survival and population persistence than sites in which the senesced *Spartina* broke off and drifted away (Grevstad et al., 2004). Subsequent releases made in 2004 into sites with thatch intact had a much higher establishment rate of 80%.

Discussion

In our analysis of past biocontrol introductions, we found no evidence for an effect of release size on establishment, at least for release sizes typically used. This contrasts with small number of experimental tests of release size where establishment frequency increased with release size (Memmott et al., 1998; Grevstad, 1999b; Memmott et al., 2005). Indeed there are also examples of release experiments where there was no effect of release size on establishment (Fauvergue et al., 2007; De Clerck-Floate and Wikeem, 2009). There are several possible explanations for a lack of effect of release size in practice. First, release sizes typically used in biocontrol practice may be sufficiently large to avoid such effects (experimental demonstrations tended to use much smaller releases). Second, Allee effects may be uncommon in insect populations. In fact, a majority of experiments designed to detect Allee effects in insects have failed to find them (Ôtake and Oyama, 1973; Kindvall et al., 1998; Fauvergue et al., 2007). Third, it may be that Allee effects are largely avoided in biocontrol releases because releases most often use adults that have already mated. Finally, it may be that density-independent effects of environmental variability (site to site and year to year) are more influential in determining population establishment than Allee effects, resulting in weak dependence of establishment on initial release size. Site to site variability is evident in wide variation in population growth of biocontrol agents among sites,

as in the case of *P. marginata*.

The absence of a release size effect is important because it suggests that release programs might benefit from making more and smaller releases. The primary benefit of using more releases is that it increases the number of chances to obtain establishment, much like buying more raffle tickets increases the chances of winning a prize. Using more releases is particularly important when the agent has a low per-release establishment rate. In the case of *P. marginata*, the initial three releases by chance resulted in failure. However, the use of more releases eventually allowed this agent to establish. Other notable examples of agents in Oregon that required many releases are *A. malherbae* on field bindweed, for which 17 failed releases were made over a four year period before one establishment occurred, and *Diorhabda elongata* Bullé for which 18 failed releases occurred over four years before one finally succeeded.

In addition to increasing the likelihood of establishment, the use of more releases can have other benefits. Initial release can be used to identify habitat characteristics that influence performance such that future releases can be more successful. Identifying these patterns, either observationally or experimentally, requires the use of many replicate releases. In the case of *P. marginata*, identifying a key habitat requirement increased the rate of successful establishment from 26 to 80%. Similar improvements in establishment rate have been seen in *A. malherbae* on bindweed mentioned above and other agents in Oregon. Experience gained by making more releases may help implementation specialists better understand methods of agent handling, by identifying factors in past projects that may have led to failure (Coombs, 2004). Another advantage of the use of more releases is that it increases the chances that at least one population will end up in a site where explosive population growth is supported. This is important in providing for the collection and redistribution of agents to other sites, which can help to achieve a more rapid impact on the weed.

In summary, this practical approach to assessing biocontrol release strategies has found no evidence that the use of larger releases will lead to improved establishment success. In contrast, the benefits of using more releases are clear, particularly when the agent is one that is difficult to establish.

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Factors Contributing to the Failure of the Biological Control Agent, *Falconia intermedia* (Miridae: Hemiptera), on *Lantana camara* (Verbenaceae) in South Africa

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Abstract

Countrywide releases of an estimated twenty million *Falconia intermedia* Distant (Miridae: Heteroptera) individuals have not enhanced the biological control of the invasive *Lantana camara* L. (Verbenaceae) in South Africa. Although the leaf-feeder initially colonized plants at many sites, it only established at two coastal sites found in the Eastern Cape Province. Recent studies have identified additional factors that may have contributed to the failure of this agent to establish. Firstly, the mirid is incompatible with some of the hairier and tougher leafed varieties of *L. camara* with significantly less oviposition. Secondly, feeding by *F. intermedia* has been shown to induce an increase in systemic plant resistance (leaf toughness, leaf hairiness) in new leaves, leading to a significant reduction in the agent's reproduction and impact. Thirdly, there is evidence of ant predation by two *Crematogaster* spp. of ants on *F. intermedia*. The ants removed 50% of the mobile *F. intermedia* nymphs while survival on ant excluded shrubs was significantly higher. Each of these factors has severely hampered the establishment and performance of this agent.

Introduction

Attempts at the biological control of the highly invasive shrub, *Lantana camara* L. *sensu lato* (Verbenaceae), have been on-going in South Africa since 1960. In what is considered a moderately successful biological control program, only three of a total 26 insect and fungal biological control agents released against *L. camara* are considered relatively effective (Baars and Naser, 1999; Klein, 2011). One of these agents, *Falconia intermedia* (Distant) (Hemiptera: Miridae), was released on *L. camara* infestations in South Africa in 1999 to intensify the feeding pressure exerted on *L. camara*. The mirid is endemic to Mexico, Central America and the Caribbean Islands and its adults and nymphs are highly active and mobile, especially when disturbed (Palmer and Pullen, 1998). The adult is about 2 mm long and 0.9 mm wide, with a dark brown body and

pale legs. The translucent pale green eggs are laid on the under-surface of leaves, with the emergent green nymphs going through five instars (Baars et al., 2003). The adults and nymphs feed on the intercellular tissue of lantana leaves causing entire shrubs to appear silvery white and leaves to abscise.

Releases were made in a number of sites in the Mpumalanga, KwaZulu-Natal and Limpopo provinces where the bug initially established and built up large populations causing considerable damage. However, the large populations of *F. intermedia* declined and eventually disappeared at most sites with only minimal dispersal (Heystek, 2006). In further field releases in 2001 in the Eastern Cape Province, *F. intermedia* established at two of five sites found along the coast (Whitney Farm and East London) (Heshula, pers. obs.; Heshula, 2005). It is only at these sites where *F. intermedia* may still be found.

The failure of biological control agents released against lantana in South Africa has mainly been attributed to climatic incompatibility, genetic variability of native *Lantana* versus naturalized *L. camara* varieties, the varied performance of agents on *L. camara* varieties, and predation and parasitism (Day and Naser, 2000; Day and Zalucki, 2009). In one set of studies *F. intermedia* showed varied reproductive performance and higher preference for South African *L. camara* varieties over Australian in no-choice tests (Urban and Simelane, 1999; Urban et al., 2004). Baars (2002) found however that *F. intermedia* did not show difference in performance on some major varieties tested in South Africa, and consequently suggested that varietal preference and performance had been overestimated as a reason for failure of this biological control agent.

None of the studies has ever investigated the mechanism by which the plants resist the agents however, despite the fact that it is known that insect feeding may elicit responses in plants (Karban and Baldwin, 1997). Related to that was the lack of knowledge into whether some varieties induced responses after feeding that may affect the performance of *F. intermedia*. Further, the role of ant predation on this agent had never been studied.

The aim of this article was thus to provide a brief report on recent post release studies conducted in South Africa in an effort to further understand the matrix of factors contributing to the failure of *F. intermedia*. Three factors in particular have emerged as important: the effect of previous feeding on performance, incompatibility with feeding induced plant quality factors, and the effect of ant predation.

The effect of previous feeding on performance

The observed release, colonization, population increase, and population crash pattern in *F. intermedia* releases provided the rationale for an investigation into whether feeding has an effect on the subsequent feeding and performance of *F. intermedia* on *L. camara*. Plants from two of the Eastern Cape sites, Lyndhurst Farm where the agent failed to establish, and Whitney Farm where a small population of the agent persisted for a number of years before being chemically excluded by the landowner, were tested in no choice trials (Heshula, 2009). The first phase of the trial (induction) initially consisted

of two treatments, un-induced test treatment (T) on which *F. intermedia* adults were allowed to feed, and control (C) with no feeding. After eight weeks of feeding, and a recovery period in which plant and insects parameters were measured, the mirids were re-released onto the test plants, now named test induced (Ti), and the control plant (C), now named control induced (Ci). The populations of *Falconia intermedia* adults (Figure 1), nymphs (Figure 2), and oviposition (Figure 3) on Whitney Farm *L. camara* plants was higher than on Lyndhurst Farm *L. camara*. This suggested a superior suitability of Whitney Farm plants for *F. intermedia* performance. The un-induced plants (Ci) had significantly higher *F. intermedia* feeding damage (21.4% higher), number of adults (increases of 187.5%), number of nymphs (110% more), and oviposition (99.8% more) than plants previously fed on (Figures 1-3). The reduction in the performance parameters of *F. intermedia* on plants previously fed on from Lyndhurst Farm show that this *L. camara* variety possesses factors that enable it to induce resistance to feeding. The different varieties of lantana have different physiological and morphological features that may contribute towards the observed responses, such as leaf toughness, leaf hairs/trichomes. Varietal differences in responses by other plant species, such as soybean *Glycine max* (L.) Merrill, have also been shown to affect plant responses due to previous feeding by the same herbivore, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) (Endo et al., 2007).

Incompatibility with feeding induced plant quality factors

Plants may effectively respond to herbivory by increasing or decreasing the quality of their leaves (Karban and Baldwin, 1997). In this way an increase or decrease in herbivore performance may be achieved (Karban and Baldwin, 1997). This is the alternative to responses that are directed towards decreasing the impact of feeding damage by increasing plant tolerance, resulting in the plant initiating some level of compensatory growth (Strauss and Agrawal, 1999). The nature of the response by Lyndhurst Farm *L. camara* variety observed in the study summarized above, was determined in eight week long bioassays (Heshula and Hill, 2011). The adults of *F. intermedia* were allowed to feed on the plants from Lyndhurst and Whitney Farm varieties

in no choice caged trials. *Falconia intermedia* inoculated plants from Lyndhurst Farm showed a significant increase in the toughness of its new leaves compared to control plants with no feeding. Feeding did not have a similar effect for plants of Whitney Farm variety (Figure 4). Additionally, plants from Lyndhurst Farm significantly increased leaf trichome density on new leaves after prolonged feeding by *F. intermedia* (Figure 5). This increase was significantly correlated to a decrease in *F. intermedia* survival (Figure 6). The defensive responses were systemic and rapidly induced about eight weeks after insect feeding. Although more work needs to be conducted on more varieties in South Africa, these results suggest that there may be a role that leaf quality responses play in the poor performance of *F. intermedia* in South Africa.

Effect of ant predation

Similar to other agents, predation by ants and spiders has been reported to occur in the field and under laboratory conditions (Heystek and Olckers, 2003; Heshula, 2009). In mass rearing facilities, ant predation of eggs and adults has been reported, and is likely linked to the availability of the agent as a food resource. These reports have been based on individual observations however, and until recently no studies have sought to quantify the effect of predation on *F. intermedia*. The effect of ant predation by two *Crematogaster* species on *F. intermedia* and two biological control agents, *Hypena laceratalis* (Walker) (Lepidoptera: Noctuidae) and *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), was recently investigated on *L. camara* varieties in the Eastern Cape Province (Tourle, 2010). *Crematogaster* sp. 1 and 2 colonies were offered the immature stages of the three agents in choice and no-choice trials. *Crematogaster* sp.1 foragers removed 50% of *F. intermedia* nymphs, followed by 45% of *H. laceratalis* larvae and only 9% of *T. scrupulosa* nymphs (Figure 7). Density dependent predation on *F. intermedia* was also observed with more predation at high

nymph and/or forager densities. This represents a large proportion of the population removed by predation activity, suggesting that ant species would put agent populations under immediate pressure and affect establishment. Survival of *F. intermedia* and *T. scrupulosa* nymphs in particular was low on ant-accessed shrubs in choice experiments and high on ant-excluded shrubs. Tourle (2010) suggested that ants were likely to significantly depress *F. intermedia* populations in the field, aided by *Falconia intermedia*'s fast movement that triggered a predatory response by ant species. In contrast, the relatively immobile behaviour of nymphs of the most successful agent in South Africa, *T. scrupulosa*, was identified as a highly effective predator avoidance strategy.

Conclusion

The biological control agent *F. intermedia* has had negligible benefit to the biological control programme of *L. camara* in South Africa. The studies on induced resistance above have been conducted using only two varieties, and there is a need to extend the work to cover more varieties in South Africa to ascertain the extent to which they are important. Certainly under field conditions there is a negative relationship between *F. intermedia* abundance and leaf hairiness of *L. camara* (Heystek, 2006). It is more likely however that these factors in concert with any of the other factors, such as varietal incompatibility and inclement climatic conditions, have been responsible for the current status. Localized outbreaks of the agent have been reported in the Eastern Cape Province in recent summers, and it appears that this species is likely to be a boom and bust species. This is similar to its status in Australia, where *F. intermedia* has established only in a few sites in north Queensland (Day and Zalucki, 2009).

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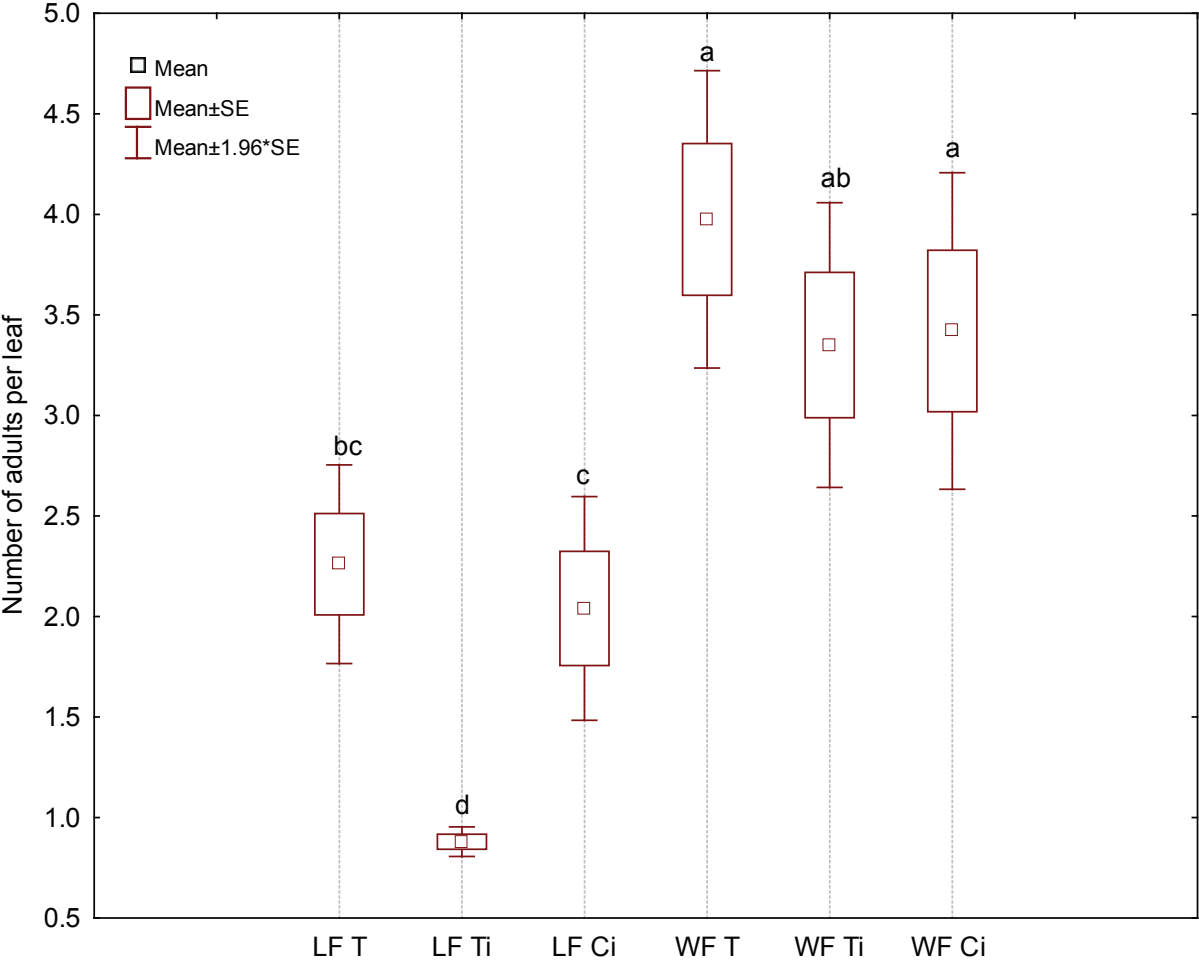


Figure 1. Number of adults of *Falconia intermedia* on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letters are not significantly different ($p > 0.05$, T - tests) (Heshula 2009).

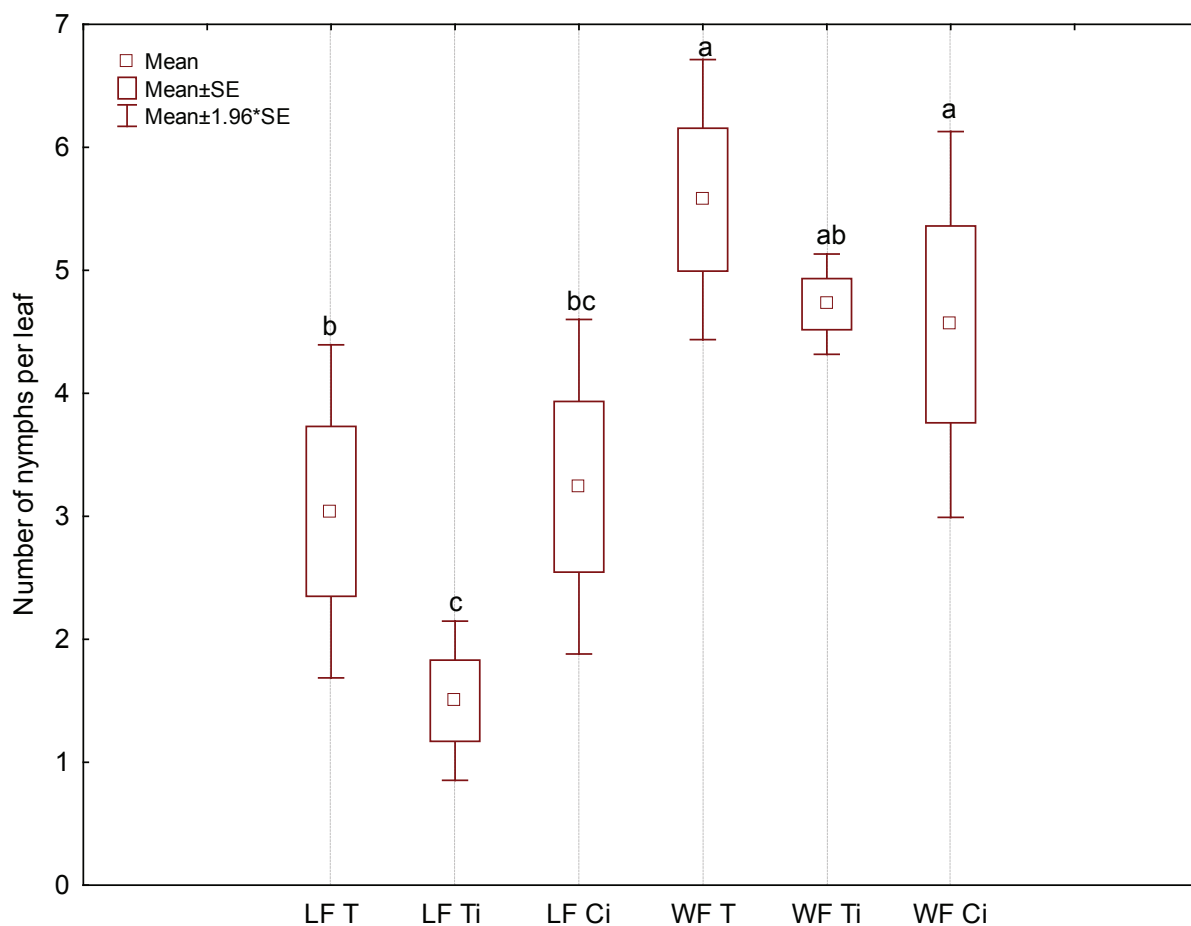


Figure 2. Number of nymphs of *Falconia intermedia* on two *Lantana camara* varieties (Lyndhurst Farm –LF, Whitney Farm – WF) subjected to three treatments (T – un-induced, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different ($p > 0.05$, T - tests) (Heshula 2009).

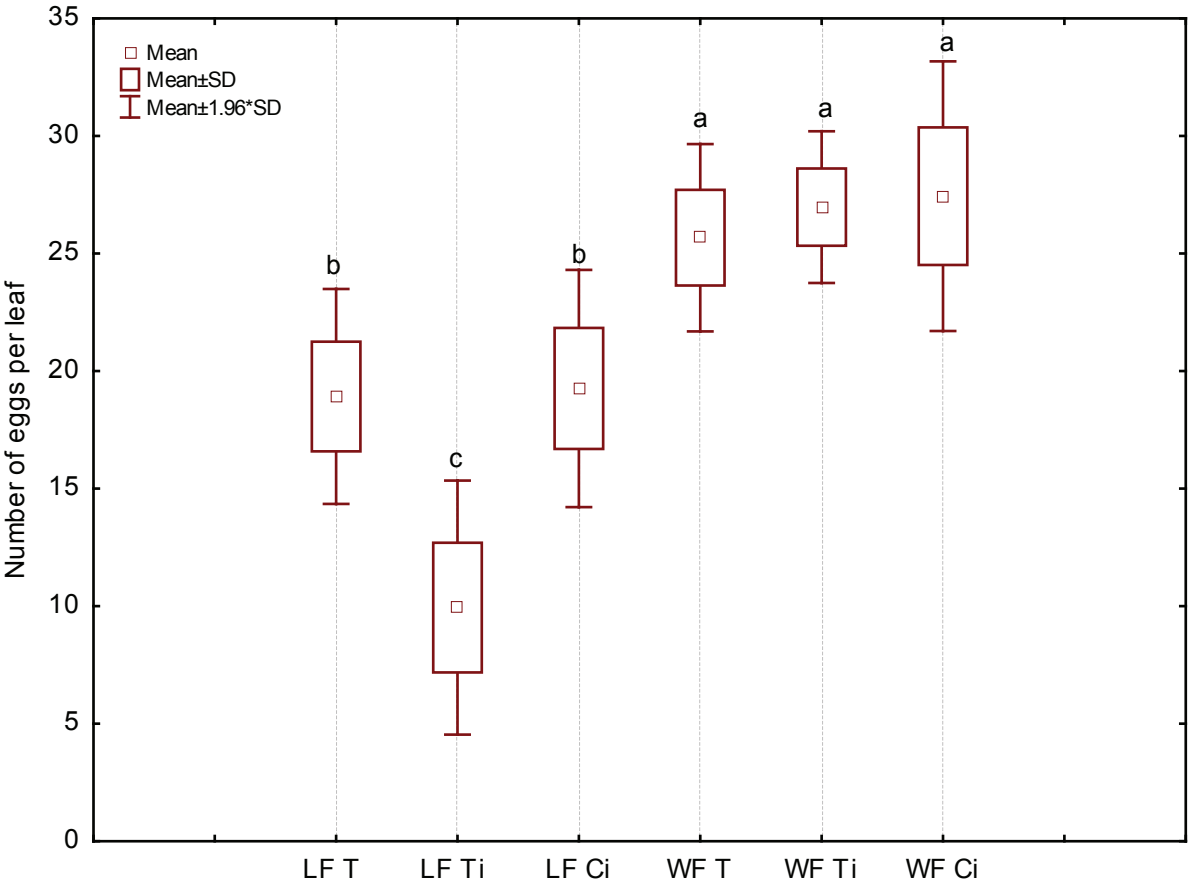


Figure 3. Numbers of eggs laid by *Falconia intermedia* on two *Lantana camara* varieties (Lyndhurst Farm – LF, Whitney Farm – WF) subjected to three treatments (T – un-infested, Ti – induced, Ci – control induced). Plots with the same letter are not significantly different ($p > 0.05$, T - tests) (Heshula 2009).

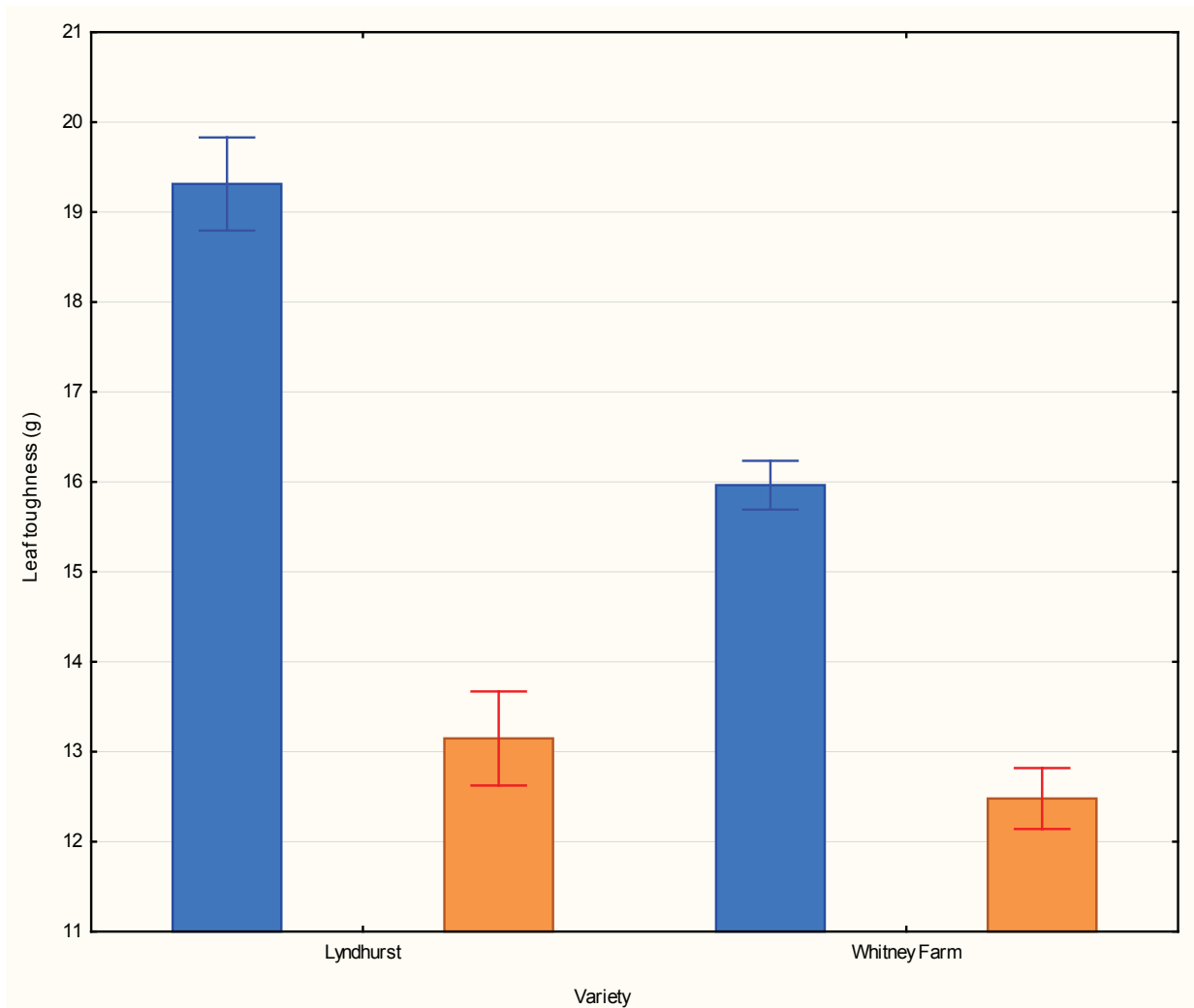


Figure 4. Mean leaf toughness for *Falconia intermedia* infested test (dark bars) and un-infested control treatments (light bars) for *Lantana camara* varieties. Means within one variety followed by different letters are significantly different ($p < 0.05$, Mann-Whitney U Test). Whiskers denote standard errors (Heshula and Hill 2011).

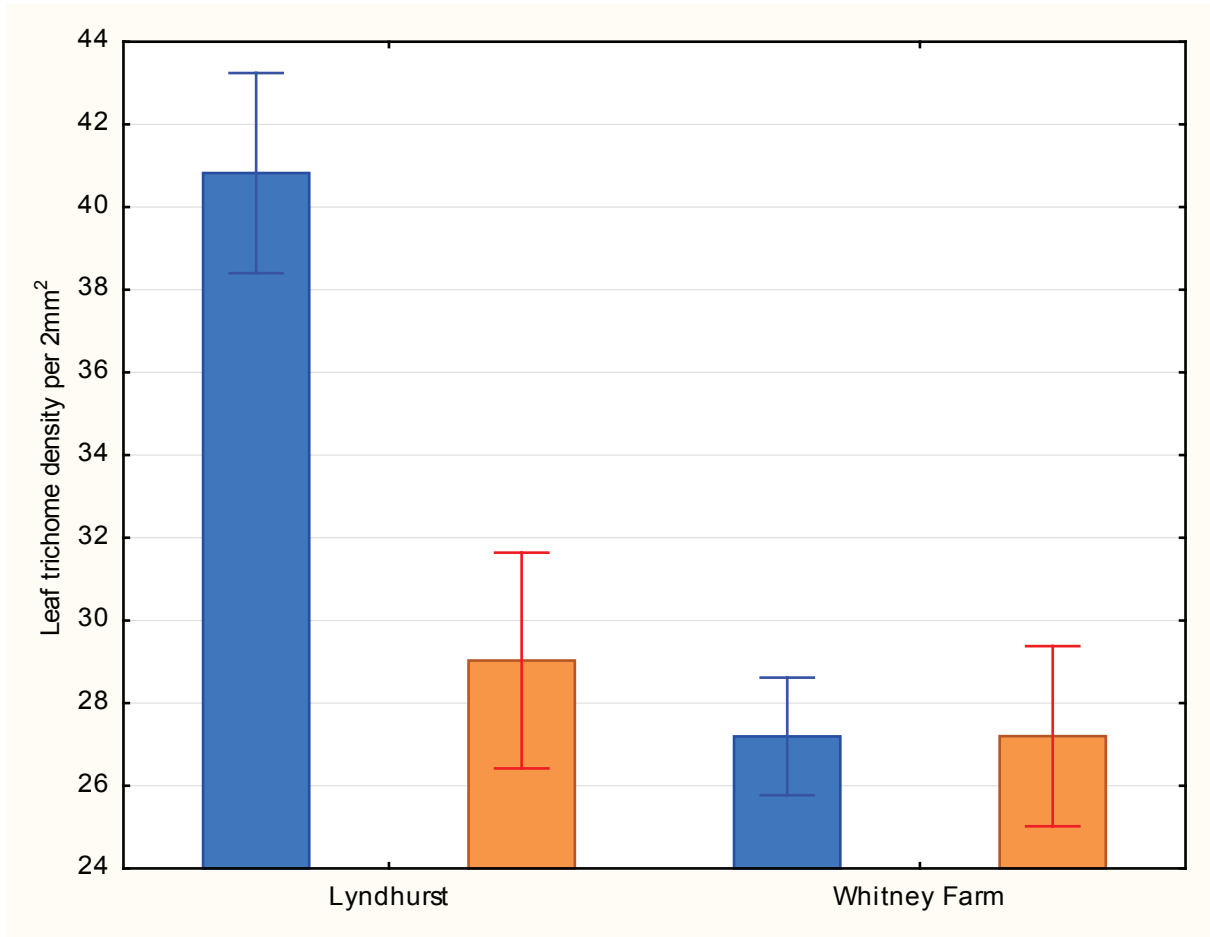


Figure 5. Mean number of leaf trichomes in 2 mm² for *Falconia intermedia* infested test (dark bars) and un-infested control treatments (lighter bars) for *Lantana camara* varieties. Means in one variety followed by different letters are significantly different ($p < 0.05$, Mann-Whitney U Test). Whiskers denote 95% confidence intervals (Heshula and Hill 2011).

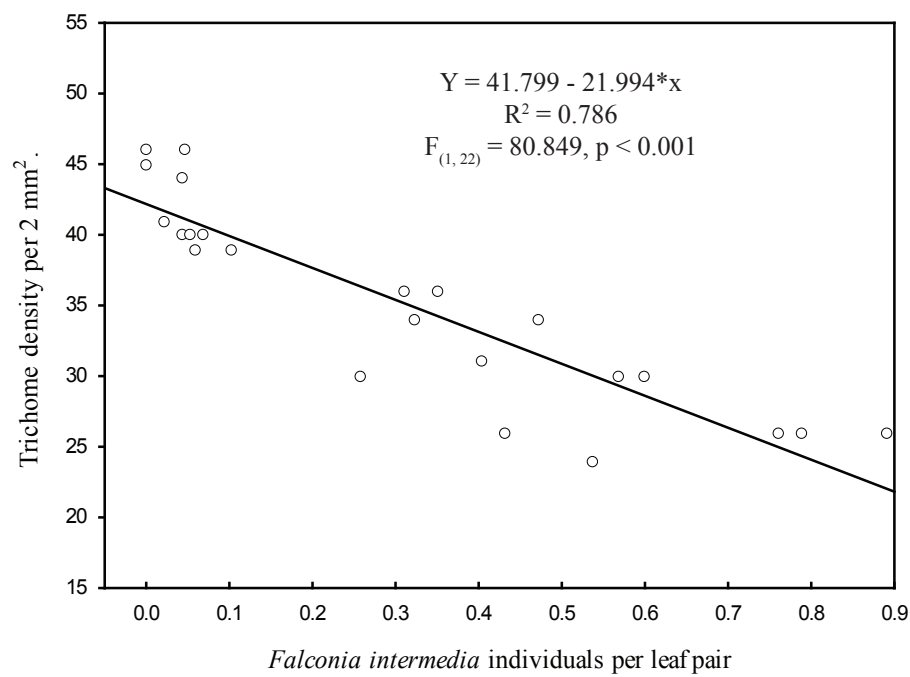


Figure 6. Relationship between *Falconia intermedia* population numbers and trichome density (Heshula and Hill 2011).

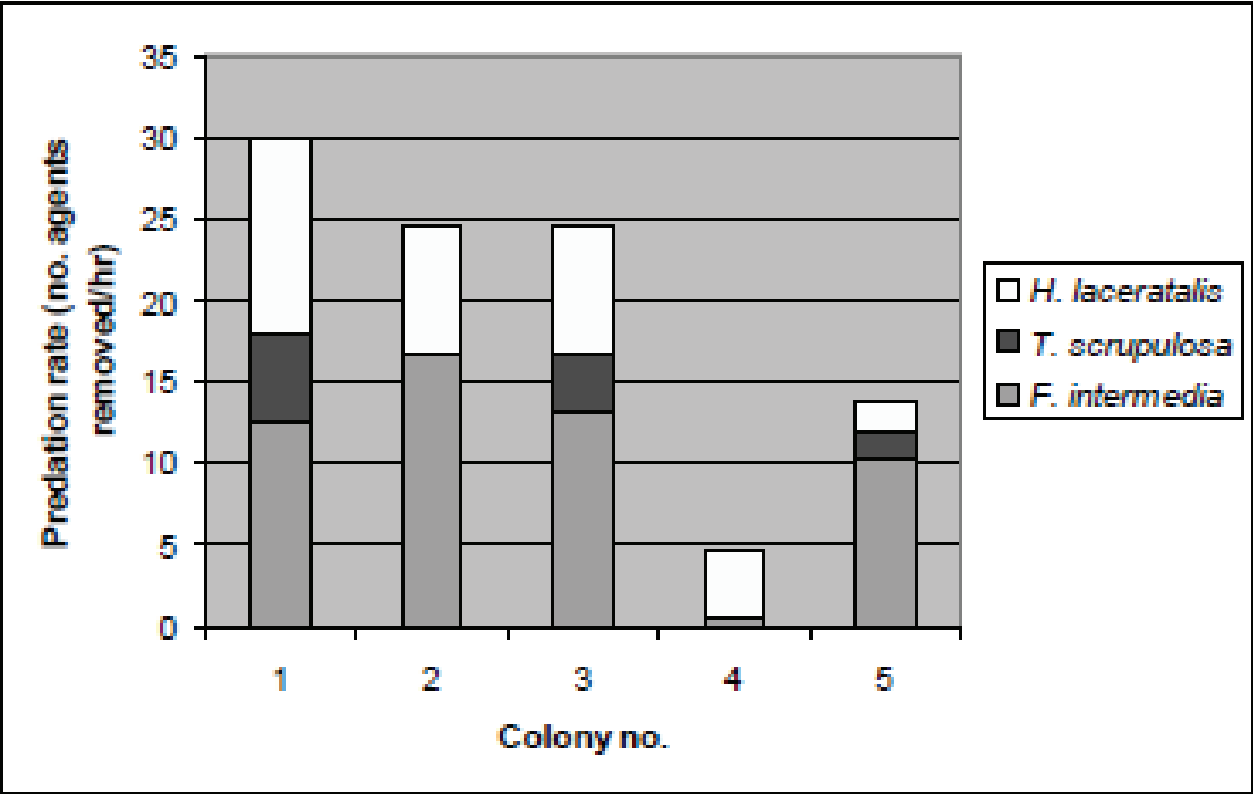


Figure 7. Predation rates (No. agents removed/hour) on *Falconia intermedia*, *Hypera laceratalis* and *Teleonemia scrupulosa* by *Crematogaster* sp.1 colonies (Tourle, 2010).

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Host Specificity and Impacts of *Platyptilia isodactyla* (Lepidoptera: Pterophoridae), a Biological Control Agent for *Jacobaea vulgaris* (Asteraceae) in Australia and New Zealand

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Abstract

Jacobaea vulgaris Gaert. (ragwort) is a serious noxious weed of high fertility pastures in high rainfall regions of southern Victoria and Tasmania in Australia. Biological control of *J. vulgaris* in Australia has been underway since the 1930s. Overseas explorations in Europe identified the ragwort plume moth, *Platyptilia isodactyla* Zeller as a potential biological agent. The host specificity of *P. isodactyla* was tested to determine its safety. Seventy-three plant taxa were screened for *P. isodactyla* phytophagy and survival. *P. isodactyla* development and survival was restricted to a few taxa in the tribes Senecioneae and Asterae, but was negligible on species other than *J. vulgaris*. *P. isodactyla* showed only weak oviposition preference for *J. vulgaris* but this behavior may have been affected by confined test conditions. Only *J. vulgaris* was able to support continued *P. isodactyla* population growth. *P. isodactyla* was released for biological control of *J. vulgaris* in Australia during 1999 and in New Zealand during 2005. Field site damage assessments have shown that *P. isodactyla* can have substantial impact on *J. vulgaris* flowering and survival. A survey of *Senecio* species in close proximity with *J. vulgaris* attacked by *P. isodactyla* during 2004 showed no off-target impacts. *P. isodactyla* is well established in both Australia and New Zealand. Its ability to survive on *J. vulgaris* in wetter habitats is expected to complement other biological control agents enabling it to make a significant contribution to *J. vulgaris* suppression in Australia and New Zealand.

Introduction

Ragwort, *Jacobaea vulgaris* Gaertn. (Asteraceae) is a biennial, perennial or occasionally annual herb that is native to Europe and western Asia (Schmidl, 1972) and has become a serious noxious weed of high fertility pastures in Victoria and Tasmania (Parsons and Cuthbertson, 1992). Annual costs

of *J. vulgaris* control have been estimated at more than \$4 million per year to Australia (McLaren and Micken, 1997). *Jacobaea vulgaris* is also naturalised in New Zealand, Canada, South Africa and the Americas (Walsh, 1999). Several biological control agents have been introduced and released in Australia in an attempt to control *J. vulgaris*. These include the cinnabar moth, *Tyria jacobaeae* L., a seed fly, *Botanophila jacobaeae* (Hardy), two

flea beetle species *Longitarsus flavicornis* Stephens and *L. jacobaeae* Waterhouse and the crown-boring moth *Cochylis atricapitana* (Stephens) (McLaren et al., 1999). This paper describes host specificity testing of the most recently introduced species *Platyptilia isodactyla* Zeller that was originally collected from near Lugo in Spain (43.07°N, -7.27°W). It also documents the damage caused by *P. isodactyla* to *J. vulgaris* and its establishment and impacts in Australia and New Zealand.

Materials and Methods

No-Choice Tests

Overseas exploration by CSIRO had previously identified *P. isodactyla* as a potential biological control agent for *J. vulgaris* in Australia as it was host specific (Cullen et al., 1985) and its larvae caused considerable damage to *J. vulgaris* (Vayssières and Rahola, 1985). *P. isodactyla* larvae could be easily reared using cut leaves and petiole plant samples in Petri-dishes kept hydrated with moistened filter paper in an insectary with temperatures fluctuating between 15°C-25°C and a photoperiod of 12L: 12D. Plants selected for host specificity testing were either purchased from commercial nurseries or grown from seeds, cuttings or whole plants collected from the field. In no-choice feeding tests, neonate larvae that had hatched from eggs were collected and stored in Petri-dishes on moist filter paper. Larvae were placed onto an individual test plant leaf and petiole in a separate Petri-dish and their survival was monitored at five-day intervals. All observations on final larval development were made after five to six weeks. Fresh plant segments were made available to the larvae at all times. In total forty-two plant species were tested using Petri-dish treatments (Table 1).

Whole plant no-choice host specificity tests were conducted in a quarantine facility at the Victorian Department of Primary Industries Frankston, Australia. All tests were carried out on comparably sized whole plants growing in 15 cm pots. Tests were conducted by placing 10 unfed neonate *P. isodactyla* larvae on each test taxon and the plants were enclosed separately in nylon gauze cages maintained in a CT room kept at a constant photoperiod of 16L: 8D and temperature of 26:18°C respectively. *P. isodactyla* adults were collected upon emergence from cages and their emergence times

recorded. Test plants were examined for *P. isodactyla* one week after emergence from the control test plant (*J. vulgaris*) had ended or had become irregular.

Choice Tests

Taxa on which feeding and subsequent emergence of moths occurred during no-choice tests were included in oviposition choice tests (Figure 1). *Senecio linearifolius* A. Rich. and *S. quadridentatus* Labill. were also included in choice tests because of their sympatric distribution with *J. vulgaris* in Australia and the development of *P. isodactyla* larvae on them in no-choice tests. Thirteen choice tests were conducted. Each test comprised one plant of four different test taxa plus the target species, *J. vulgaris*. Choice tests were conducted in a large insect screen cage (2m x 2m x 1.5m) within a quarantine glasshouse receiving natural summer daylight. *P. isodactyla* was being routinely reared on ragwort plants in smaller cages in the same quarantine glasshouse while these tests were being conducted. Two unmated pairs of adult *P. isodactyla* were released into the cage for each test. Test plants were assigned random positions using a Latin square design within the cage and care was taken to ensure test plants did not come in contact with the sides of the cage or each other. Plants were monitored daily for oviposition until both female *P. isodactyla* had died. Plants were then removed and individually caged in a CT room maintained at 16L: 8D photoperiod, and 26°C-18°C temperature to monitor *P. isodactyla* development. Adult emergence was recorded and plants carefully dissected and examined for attack by *P. isodactyla* 60 days after the trial began. To compare the relative development of *P. isodactyla* in this experiment, an arbitrary index of development on each taxon was calculated by scoring a 1 for each first instar found through to a score of 6 for emergence of each adult (Figure 1).

Generation Trials

A generation trial was undertaken to determine whether large populations of the host specificity test species *Senecio lautus lanceolatus* (Benth.) Ali (66 plants), *S. linearifolius* (20 plants) and *Arrhenechthites mixta* (A. Rich.) Belcher (20 plants) and the target species *J. vulgaris* (20 plants) could

sustain two or more generations of *P. isodactyla*. Large screen cages (2m x 2m x 1.5m) were used to test each taxon separately in a quarantine glasshouse at temperatures ranging from 18-25°C with *J. vulgaris*, *S. lautus lanceolatus*, *S. linearifolius* and *A. mixta* being inoculated with 34, 141, 34 and 40 *P. isodactyla* respectively (Table 2). First generation moths emerging from the *S. lautus lanceolatus* treatment were then used to inoculate taxa of *J. vulgaris*, *S. lautus lanceolatus*, *S. lautus maritimus* Ali using eight plants in separate smaller screen cages (inoculation rate of 20, 20 and 18 *P. isodactyla* respectively). The number of *P. isodactyla* that emerged and plant survival were recorded (Table 2).

Field Non-Target Impacts

Three sites (Travers - 38.57°S, 146.38°E, Beech Forest - 38.14°S, 143.34°E, Kemps - 38.36°S, 146.62°E) where *P. isodactyla* had been released three years previously were assessed to determine post-release host specificity of *P. isodactyla*. Whole plants of 30 *J. vulgaris* and 30 native *Senecio* plants were collected at each site during late summer 2003 (all sites) and 2004 (Kemps). At each site, plants were selected at random using two 30m linear transects to sample plants at two meter intervals. In total, 120 native *Senecio* species and 90 *J. vulgaris* were collected, returned to a laboratory, dissected and examined in detail for *P. isodactyla* impacts. Specimens of native *Senecio* species were sent to the National Herbarium for identification.

Insecticide exclusion trial

A field site at Foster North, in Australia (38.59°S, 146.19°E) where *P. isodactyla* had been released three years previously was used to assess *P. isodactyla* impacts on *J. vulgaris*. Vegetation comprised grasses and forbs, including *J. vulgaris*. *P. isodactyla* was well established at the site with approximately 40% of *J. vulgaris* plants showing signs of *P. isodactyla* attack (n=100). No other biological control agent of *J. vulgaris* was present at the site.

In November 2003, 90 *J. vulgaris* rosettes were paired by leaf number and size of the smallest ellipse that could encircle the rosette. Leaf numbers ranged from 4 to 14 and ellipse areas ranged from 19 to 636 cm². Plants showing *P. isodactyla* damage were

not used. Forty-five pairs were designated. Each plant size index (S_w) was calculated by multiplying its rosette ellipse area by leaf number. The 45 pairs of *J. vulgaris* rosettes were ranked by mean S_w . The smallest plant in each successively larger pair was alternately assigned as either treatment or control. S_w ranged from 113 to 6616 for treatment plants and from 110 to 6361 for control plants. A matched pairs t-test (StataCorp 2009) did not suggest that the two sets were from different S_w populations ($p>0.3$).

The insecticide treatment was applied to the treated *J. vulgaris* rosettes using 0.22 g/L a.i. thiacloprid insecticide plus 0.2 mL/L alcohol alkoxylate surfactant sprayed to runoff using a hand-pressurised sprayer. Applications were made every 3-4 weeks from spring to autumn during 2003-4 and 2004-5. These applications prevented *P. isodactyla* damage on treated plants. The control plants were treated with surfactant only and this gave no protection against *P. isodactyla* damage. Assessments of *J. vulgaris* survival and capitula number were made during February 2005. Treatment effects on survival and flowering were assessed using McNemar's exact test (StataCorp 2009). Pairs were excluded from the analysis if either member flowered during 2004 (could affect survival for following season) or was destroyed by wildlife. Thirty-eight pairs were included in the analysis.

Dispersal and distribution of *P. isodactyla*

The distribution of *J. vulgaris* and establishment of *P. isodactyla* in Australia is shown in Figure 2. Mapped *P. isodactyla* distributions include those from release sites at least two years after release and from sightings of *P. isodactyla* that have spread more than 500 m away from release sites.

Results

No-Choice Tests

No choice host specificity trials showed that survival and development of *P. isodactyla* was greatest on the target species, *J. vulgaris* (55%) and there was no survival of *P. isodactyla* on any species from plant families outside the Asteraceae (Table 1). Within the Asteraceae, several species

within the tribe Senecioneae supported relatively low survival percentages of *P. isodactyla* including *S. madagascariensis* Poir., *S. lautus maritimus*, *S. lautus lanceolatus*, *S. lautus dissectifolius* Ali, *S. lautus alpinus* Ali, *Jacobaea maritima* (L.) Pelser & Meijden, *A. mixta* and *Emilia sonchifolia* (L.) DC. ranging from 5.8% to 1.3%. (Table 1). *Callistephus chinensis* (L.) Nees (Astereae) was the only species to support survival (0.5%) of *P. isodactyla* outside of the Senecioneae tribe. Some *P. isodactyla* larval development was recorded from species within the Tageteae and Cynareae tribes, but no survival was recorded. Even when feeding was evident, the narrow woody nature of the stems of most of the Australian native *Senecio* species tested precluded survival of the *P. isodactyla* larvae. Larvae would bore in and feed initially, but would then exit the plant and not re-enter it.

Choice tests

A summary of results of oviposition choice tests and development index are presented in Figure 1. Though there was substantially more *P. isodactyla* eggs laid on *J. vulgaris*, oviposition preferences were inconclusive. In one of five choice tests conducted for both *S. lautus maritimus* and *S. lautus lanceolatus*, *P. isodactyla* laid more eggs on these species than on the target species, *J. vulgaris*. *P. isodactyla* are sexually mature at emergence and are active at night when mating occurs with moths laying on average 101 eggs (Masri, 1995). In this experiment, only 6% of the expected oviposition occurred on plant foliage suggesting that cage size and/or experimental conditions may not have been conducive to natural *P. isodactyla* oviposition. Pre-alighting cues used in host location often rely heavily on the sensory modality of olfaction (Heard 2000). These trials were undertaken in quarantine glasshouse without air movement which may have restricted *P. isodactyla* searching capacity and partially explain low oviposition rates on test species.

The development index for *P. isodactyla* on *J. vulgaris* (46.9) was more than six times greater than on any other taxon (Figure 1). Other than *J. vulgaris*, the taxa with the next highest *P. isodactyla* development indices were *S. lautus lanceolatus*, *S. lautus maritimus* and *S. linearifolius* (6.6, 2.8 and 2.5 respectively). *P. isodactyla* development was observed on *S. linearifolius* and *J. maritimus* when

no oviposition was observed on the leaves of these species suggesting that eggs may have been laid elsewhere within the cage and larvae have then made their way onto these plants subsequently.

Generation trials

A high inoculation rate of *P. isodactyla* on 66 *S. lautus lanceolatus* plants only resulted in emergence of 58 adults (Table 2). These moths were used to inoculate *J. vulgaris*, *S. lautus lanceolatus* and *S. lautus maritimus* in an attempt to generate a second generation. Only *P. isodactyla* on *J. vulgaris* produced a second generation showing that only *J. vulgaris* will sustain populations of *P. isodactyla*.

Field Non-Target Impacts

None of the 120 native *Senecio* plants (*S. lautus lanceolatus* 23%, *S. linearifolius* 18%, *S. glomeratus* Desf. Ex Poir. 3%, *S. minimus* Poir. 43%, unidentified 3%) examined in detail for *P. isodactyla* collected from three sites where *P. isodactyla* had been previously released in Australia were attacked. *P. isodactyla* was recorded attacking 7% of the 60 *J. vulgaris* plants assessed from the Kemp and Beech Forest sites. No *J. vulgaris* were found at the Travers site.

Insecticide exclusion trial

Field chemical exclusion of *P. isodactyla* from *J. vulgaris* resulted in a greater proportion of treated plants (30) surviving than control plants (23) ($p=0.065$) and more plants flowering (19) than the control plants (9) ($p=0.041$). Capitula production was 262% greater for the treated *J. vulgaris* (68.3 capitula/plant) than the untreated controls attacked by *P. isodactyla* (26.2 capitula/plant) ($p<0.017$).

Dispersal and distribution

Platyptilia isodactyla has been recorded surviving on *J. vulgaris* at more than 40 sites across south-eastern Australia where it is now impacting on *J. vulgaris* populations (Figure 2). The greatest detected dispersal from a release site has been 5.9 km.

In New Zealand, over 200 releases of *P. isodactyla* have been made primarily on the west coast of the South Island, but also throughout the rest of the

country since 2006. Approximately 70% of these releases have established. Monitoring of release sites for establishment and efficacy has shown that within 3 years *J. vulgaris* has been removed from sites where the moth had established (Caryl Coates pers comm.).

Discussion

P. isodactyla was selected as a potential biological control agent for *J. vulgaris* in Australia as *J. vulgaris* was believed to be its primary host. After host specificity trials were well under way it was discovered that *Jacobaea aquaticus* (Hill) Bonnier & Layens (marsh ragwort) was actually the primary host of *P. isodactyla* in Europe (Emmet and Heath 1989), but it also feeds and reproduces readily on *J. vulgaris* (Clifton 2008). *J. aquaticus* is very closely related to *J. vulgaris* as they hybridize and the F1 hybrids are fertile (Kirk et al., 2004). Given the extremely close taxonomic relationships of *J. vulgaris* and *J. aquaticus*, the positive results from host specificity testing and no evidence of *P. isodactyla* feeding on any other asteraceous species in Europe, host specificity testing of *P. isodactyla* was completed. Had *P. isodactyla* oligophagy been known prior to beginning these trials it is unlikely that *P. isodactyla* would have been selected as a biological control agent.

In the present study, some plants of economic importance, others related to the weed as suggested by Wapshere (1974), and representatives of Australian native flora were exposed to larvae of *P. isodactyla* in no choice tests. This was followed by choice tests of species showing some survival in no-choice tests and a generation trial to determine whether *P. isodactyla* could sustain a population on species other than *J. vulgaris*. To be at significant risk from *P. isodactyla*, a taxon needs to attract oviposition, support unretarded development of numerous larvae, sustain damage that impairs plant health and be able to support the growth of a *P. isodactyla* population. The host specificity testing conducted in this study show that *J. vulgaris* was the only species to exhibit all these factors for *P. isodactyla*. There was some survival of *P. isodactyla* in no choice tests but this was generally below 5% compared to survival

on *J. vulgaris* of 55%. It would be highly unlikely that any species could sustain a population with survival rates below 5%.

Following approval by Environment Australia and the Australian Quarantine and Inspection Service, *P. isodactyla* was first released as a biological control agent for *J. vulgaris* in Australia in December 1999. *P. isodactyla* was subsequently introduced and released in New Zealand during 2005. Field assessment of possible *P. isodactyla* off target impacts shows no attack on native Australian *Senecio* species, supporting the results obtained from the host specificity testing. *P. isodactyla* ability to survive on *J. vulgaris* in moist habitats (as with marsh ragwort), may well complement other biological control agents such as *Longitarsus* species that are susceptible to flooding events (Potter et al., 2007) and fill an important biological control ecological niche in the control of *J. vulgaris* in Australia and New Zealand. This study shows that *P. isodactyla* can significantly reduce *J. vulgaris* field populations and reproductive capacity that should result in ongoing agricultural benefits through increased production and reduced reliance on chemical control methods. It should also result in environmental benefits through reduced competition to indigenous forb species that currently overlap distributions with *J. vulgaris*. *P. isodactyla* is now widely established in both Australia and New Zealand and beginning to produce substantial impacts on *J. vulgaris* populations.

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Table 1. No Choice Tests - Host Specificity Results for *P. isodactyla*

Scientific Name	Common Name	No. of larvae (whole plants 10/pot)	No of larvae (Petri-dish 1/Petri-dish)	% Survival to adult instar *
ASTERACEAE				
Senecioneae				
<i>Jacobaea vulgaris</i>	Ragwort	550	110	55
<i>Jacobaea maritima</i>	Dusty Miller	80	60	1.3
<i>Senecio madagascariensis</i>	Fireweed	120		5.8
<i>Senecio lautus maritimus</i>	Coastal Groundsel	80		3.8
<i>Senecio lautus lanceolatus</i>	Fireweed	80		1.3
<i>Senecio lautus dissectifolius</i>		80		1.3
<i>Senecio lautus alpinus</i>	Alpine Groundsel	80		1.3
<i>Arrhenechthites mixta</i>	Purple fireweed	80		1.3
<i>Emilia sonchifolia</i>	Purple Sow Thistle	80		1.3
<i>Senecio hispidulus</i>	Hill Fireweed	120	50	4th
<i>Senecio odoratus</i>	Scented Groundsel	80		4th
<i>Senecio macrocarpus</i>	Fluffy Groundsel	180	50	3rd
<i>Senecio linearifolius</i>	Fireweed	80	24	3rd
<i>Senecio quadridentatus</i>	Cotton Groundsel	180	160	2nd
<i>Senecio squarrosus</i>	Leafy Groundsel	80		2nd
<i>Senecio vellioides</i>	Squarrose Fireweed	80		None
<i>Senecio pterophorus</i>	African Daisy	45	50	None
<i>Senecio glomeratus</i>	Annual Fireweed	80	60	None
<i>Senecio biserratus</i>	Jagged Fireweed	80		None
<i>Senecio vulgaris</i>	Common Groundsel	80		None
<i>Senecio vagus</i>	Saw Groundsel	80		None
<i>Euryops pectinatus</i>	Golden Euryops	80		None
<i>Euryops abrotanifolius</i>	Paris Daisy	80		None
<i>Bedfordia arborescens</i>	Blanket Leaf	80		None
Astereae				
<i>Callistephus chinensis</i>	Chinese Aster	190		0.5
<i>Aster alpinus</i>	Alpine Aster		60	None
<i>Olearia lirata</i>	Snow Daisy Bush	80		None
<i>Bellis</i> sp.	Daisy		20	None
Tageteae				
<i>Flaveria australasica</i>	Speedyweed	80		2nd
<i>Tagetes</i> sp.	French Marigold		60	None
Cynareae				
<i>Cynara scolymus</i>	Globe artichoke	80		3rd
<i>Carthamus tinctorius</i>	Safflower		50	None
Gnaphalieae				
<i>Cassinia aculeata</i>	Dogwood	80		None

Scientific Name	Common Name	No. of larvae (whole plants 10/pot)	No of larvae (Petri-dish 1/Petri-dish)	% Survival to adult instar *
<i>Gnaphalium pensylvanicum</i>	Wandering Cudweed	80		None
<i>Helichrysum luteoalbum</i>	Jersey Cudweed		50	None
<i>Ozothamnus ferrugineus</i>	Tree Everlasting	80		None
Calenduleae				
<i>Calendula</i> sp.	Marigold		60	None
Anthemideae				
<i>Chrysanthemum</i> sp.	Chrysanthemum		60	None
Cichorieae				
<i>Hieracium</i> sp.	Hawkweed		60	None
<i>Lactuca sativa</i>	Lettuce		60	None
<i>Cichorium intybus</i>	Common Chicory		60	None
Coreopsidaeae				
<i>Dahlia</i> sp.	Dahlia		60	None
Heliantheae				
<i>Helianthus annuus</i>	Sunflower		60	None
<i>Zinnia</i> sp.	Zinnia		60	None
ARALIACEAE				
<i>Astrotricha ledifolia</i>	Common Star-hair	80		None
GENTIANACEAE				
<i>Pelargonium australe</i>	Austral Stork's bill	80		None
<i>Gentianella diemensis</i>	Ben Lomond	80		None
SCROPHULARIACEAE				
<i>Derwentia perfoliata</i>	Austral Storksbill	80		None
<i>Antirrhinum majus</i>	Snapdragon		60	None
CARICACEAE				
<i>Carica papaya</i>	Pawpaw		60	None
FABACEAE				
<i>Acacia melanoxylon</i>	Black Wattle		60	None
<i>Acacia molissima</i>	Sydney Wattle		60	None
<i>Glycine hispida</i>	Soybean		60	None
<i>Medicago littoralis</i>	Coastal Medick		60	none
<i>Trifolium subterraneum</i>	Subterranean Clover		60	None
<i>Arachis hypogaea</i>	Peanut		60	None
GERANIACEAE				
<i>Geranium</i> sp.	Geranium		60	None
LAMIACEAE				
<i>Mentha</i> sp	Mint		60	None
<i>Salvia officinalis</i>	Common Sage		60	None

Scientific Name	Common Name	No. of larvae (whole plants 10/pot)	No of larvae (Petri-dish 1/Petri-dish)	% Survival to adult instar *
MALVACEAE				
<i>Gossypium hirsutum</i>	Upland Cotton		60	None
MUSACEAE				
<i>Musa sapientum</i>	Banana		60	None
MYRTACEAE				
<i>Eucalyptus grandis</i>	Flooded Gum		60	None
<i>Eucalyptus globulus</i>	Blue Gum		60	None
PINACEAE				
<i>Pinus radiata</i>	Radiata Pine		60	None
PROTEACEAE				
<i>Macadamia tetraphylla</i>	Macadamia		60	None
ROSACEAE				
<i>Rosa</i> sp.	Rose		60	None
<i>Rubus</i> L. sp	Berries		60	None
VITACEAE				
<i>Vitis vinifera</i>	Grape vine	80		None
POACEAE				
<i>Oryza sativa</i>	Rice		60	None
<i>Phalaris aquatica</i>	Phalaris		60	None
<i>Saccharum officinarum</i>	Sugar		60	None
<i>Sorghum bicolor</i>	Sorghum		60	None

* Last instar stage observed

Table 2. Generation trial. Survival and impacts of *P. isodactyla* on Australian native *Senecio* species and *J. vulgaris*.

Taxon	No. of Plants	<i>P. isodactyla</i> inoculationrate	<i>P. isodactyla</i> emergence		% Plant survival
			Total	Per Plant	
First Generation (F1)					
<i>J. vulgaris</i>	20	34	173	8.65	0
<i>S. lautus lanceolatus</i>	66	141	58*	0.88	27.3
<i>S. linearfolius</i>	20	34	0	0	100
<i>A. mixta</i>	20	40	2	0.1	100
Second Generation (F2)					
<i>J. vulgaris</i>	8	12*	28	3.5	0
<i>S. lautus lanceolatus</i>	8	21*	0	0	87.5
<i>S. lautus maritimus</i>	8	20*	0	0	37.5**

* All 2nd generation trials used moths from *S. laetus lanceolatus* F1 generation.
 ** Also found infested with the native moth, *Nyctemera amica* (White).

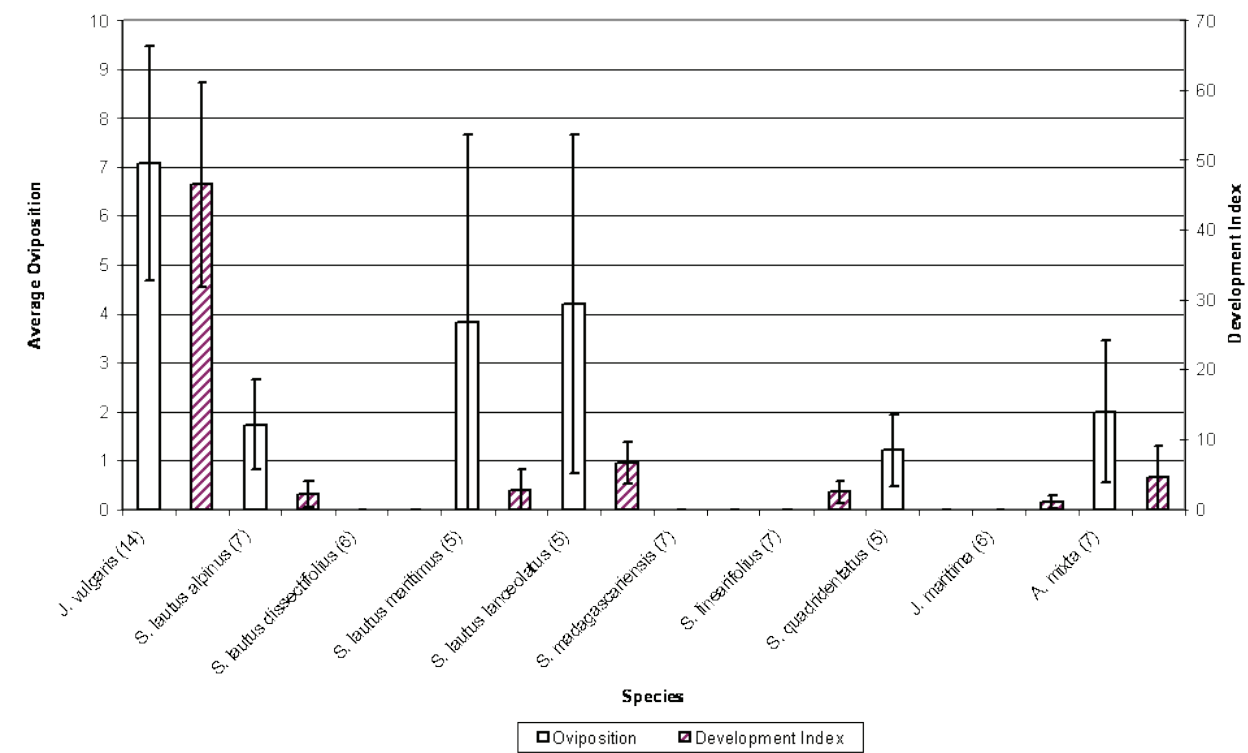


Figure 1. Average oviposition and development index for host specificity latin square choice tests for *P. isodactyla*. Numbers in parenthesis represent number of replications.
 Development Index = ((I1x1)+(I2x2)+(I3x3)+(I4x4)+(Px5)+(Ax6))/n Where:
 DI = development index: I1, I2, I3, I4, P, A equal number of first, second, third and fourth Instar larvae, pupae (not including emerged cases) and adults respectively found in dissected plants of each separate test taxon in all tests.
 n=number of test plants.

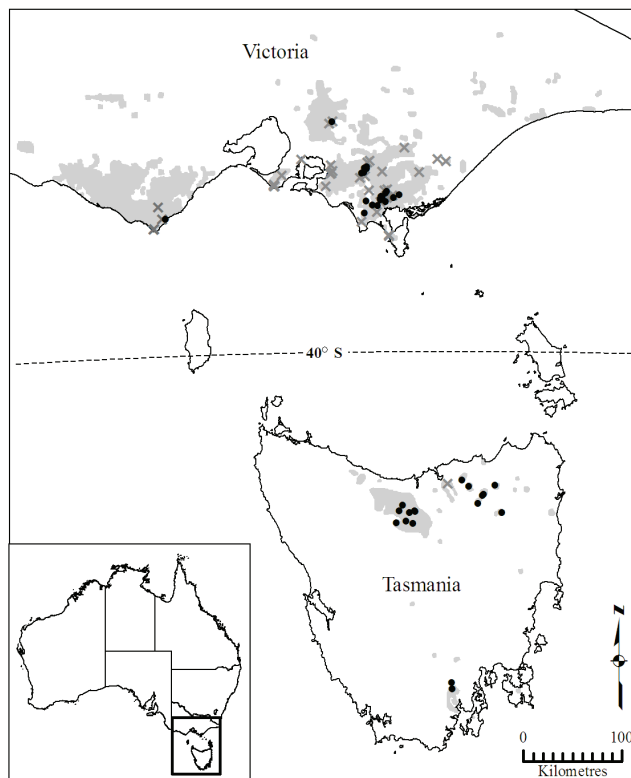


Figure 2. Distribution of *J. vulgaris* in Australia (grey).

• *P. isodactyla* found, X *P. isodactyla* not found.

Successful Biological Control of *Chromolaena odorata* (Asteraceae) by the Gall Fly *Cecidochares connexa* (Diptera: Tephritidae) in Papua New Guinea

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Abstract

The impact of the stem-galling fly *Cecidochares connexa* (Macquart) introduced into Papua New Guinea to control *Chromolaena odorata* (L.) King and Robinson was assessed. Field plots were established to determine the impact of the agents on chromolaena and a questionnaire was developed to determine any benefits to landholders. Over 115,000 galls were released in the 13 provinces infested with chromolaena and establishment was readily achieved. Populations increased quickly and the gall fly spread up to 100 km from some release sites. The gall fly caused a decrease in cover, height and density of chromolaena. Chromolaena is now considered under control in nine provinces, resulting in the re-establishment of food gardens and the regeneration of natural vegetation. In socio-economic surveys, over 80% of respondents believed that there is substantially less chromolaena now than before the gall fly was introduced. There has been a significant reduction in the time spent weeding chromolaena and an increase in the size of food gardens, thus increasing productivity and income for landowners. Indirect benefits due to the control of chromolaena include reduced harbor for snakes and wild pigs, reduced need to erect fences around food gardens to exclude pigs, and fewer lacerations resulting from the need to slash chromolaena. It is anticipated that the gall fly will continue to spread and reduce the impact of chromolaena in PNG.

Introduction

Chromolaena odorata (L.) King and Robinson (Asteraceae) (chromolaena) is a woody shrub native to tropical America and the Caribbean. It was first reported in Papua New Guinea (PNG) in East New Britain Province in the 1960s (Henty and Pritchard, 1973), presumably spread from South-East Asia, during or soon after World War II. It subsequently spread to other provinces, through the movement of people and machinery, particularly logging equipment (Day and Bofeng, 2007). Chromolaena

affects agricultural production, particularly small subsistence farms and natural ecosystems. It can quickly invade cleared lands and smother crops such as taro, yams, papaw and bananas. Its presence increases the time spent in weeding farms, thus causing some landholders to farm smaller plots (Orapa, 1998; Day and Bofeng, 2007). As a result, yield and income is reduced and clearing new areas for gardening becomes increasingly difficult. In plantations, it can form a complete understory, impeding landholders from collecting coconuts and oil palm nuts. Chromolaena also infests grazing lands, displacing preferred pasture species,

and reducing productivity. Regeneration of plant species in logged areas and natural succession is also adversely affected by chromolaena (Orapa et al., 2002).

In 1991, a biocontrol program for chromolaena, funded by the Australian Government and managed by the Queensland Government, was initiated in Indonesia and the Philippines. The moth *Pareuchaetes pseudoinculata* Rego Barros (Lepidoptera: Arctiidae) and the stem-galling fly *Cecidochara connexa* (Macquart) (Diptera: Tephritidae) were introduced. In 1998, the project was extended to PNG. *P. pseudoinculata* was introduced into PNG from Guam where it had successfully established (Muniappan et al., 2007) and *C. connexa* was introduced in 2001 from the Philippines, with both agents establishing (Bofeng et al., 2004). The leaf-mining fly *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) was introduced unsuccessfully in 2004 from South Africa, where it had established (Day et al., unpubl. data).

C. connexa spread rapidly from all sites and exerted control in many parts of the country. *P. pseudoinculata*, however, was more limited in its distribution and impact (Day and Bofeng, 2007). Field monitoring was conducted at several sites and a survey was conducted to gauge potential benefits to landholders from the introduction of *C. connexa*. This paper reports on the impact of *C. connexa* on chromolaena in PNG and its subsequent benefits to landholders.

Materials and Methods

Mass-rearing, field release and monitoring

C. connexa was initially mass-reared in cages (90 cm x 56 cm x 88 cm) by placing 5-10 pairs of adults into cages containing pots (250 mm dia) of large chromolaena plants. Cages were sprayed with water to allow the flies to drink. Plants were removed after three days and held for gall development. New plants were then added to the same cage and the process was repeated until the adults had died after about 11 days (Orapa and Bofeng, 2004).

Stems with mature galls were cut and released in the field, in batches in plastic cups filled with water, placed under clumps of chromolaena. Early in the

release program releases of 500 galls were conducted but the number per release was reduced subsequently to 100, with little change in establishment success.

Once *C. connexa* had established in the field and populations increased sufficiently, it was more efficient to collect galls from the field. Over 2,000 galls could be collected in a few hours compared to a few weeks if being mass-reared. Release sites were checked after three months following releases, by which time galls should be present in the field.

Intensive field monitoring was conducted at five sites in Morobe Province where the project was based and one site in East New Britain Province. A 100 m transect line was run through each study site and the percent chromolaena covering the line was calculated. At each site, five fixed 1 m² quadrats were established and the number of stems and their height recorded. The number of galls per stem was also recorded. Monitoring was conducted approximately every two months until there were no plants remaining in the quadrats.

Socio-economic assessment

To gauge the impact of the project and specifically the introduction of the gall fly on landholders, a survey form of 22 questions was developed. The form gathered information on province and land use of each respondent and estimated the impacts of the gall fly in terms of changes in abundance of chromolaena post release of the gall fly, as well as changes in time spent weeding, cost of control, yield and income. The final question attempted to gain an overall view of the project and the introduction of the gall fly.

Surveys were conducted only in provinces where the gall fly had established and respondents were chosen randomly at roadside markets to minimize bias towards particular land uses or weed control status.

Results

Mass-rearing, field release and monitoring

Over 115,000 galls were released at over 350 sites in all 13 provinces infested with chromolaena. The establishment rate was 99% and the few sites where the gall fly failed to establish were sites that were

slashed or burned soon after releases. There are still 22 sites where establishment is yet to be confirmed. The gall fly spread rapidly, up to 100 km in seven years from some sites. Spread was assisted by landholders who readily moved the gall fly around. By mid 2011, the gall fly was present at 89% of all known sites with chromolaena, covering 12 provinces, with the only chromolaena site in Western Province still to be checked. There are still about 50 sites, mainly in remote areas, where the gall fly has not yet been released or is not present (Fig.1).

Chromolaena is considered under control at over 200 sites in nine provinces, namely Bougainville, East New Britain, Eastern Highlands, Madang, Manus, Morobe, New Ireland, Oro and Sandaun. Stem or branch dieback was noticeable where the number of galls per plant exceeded 20.

At the six monitoring sites, chromolaena cover, height and density decreased with the presence of the gall fly. Complete control of chromolaena, where plants disappeared from all quadrats and about 80% of transect lines occurred at three sites, namely Kasuka (Fig. 2), Trukai Farm (Fig. 3) and Wantoat Road (data not shown), all in Morobe Province. At the remaining study sites, fires and slashing also contributed to the control of chromolaena.

Socio-economic assessment

Over 190 interviews with landholders from over 100 villages in eight provinces were conducted. A large proportion of respondents (44%) were from East New Britain, and most (67%) were mixed cropping subsistence farmers. Approximately 83% of all respondents reported less chromolaena after the gall fly was released, irrespective of province and land use. However, in East Sepik Province, where releases were made later and the full effect of the agent may not yet have been achieved, only 60% of respondents thought there was less chromolaena now than before the gall fly was introduced.

Over 50% of respondents stated that there was about half the chromolaena present following the introduction of the gall fly. Interestingly, about 12% thought that chromolaena was still increasing. Most of these people were in areas where the gall fly had been released more recently and may not yet had time to have much impact on chromolaena (Fig. 4).

There was an overall decrease in the time spent controlling chromolaena. About 33% of

respondents stated that they spend less than half the time controlling the weed than before the gall fly was released, including over 7% who no longer use any control methods (Fig. 5). Similar trends were observed for the cost of controlling chromolaena after the release of the gall fly, with 26% of respondents reporting that control costs had been reduced by 50% since the introduction of the gall fly (Fig. 6).

There was a subsequent increase in yield and income after the introduction of the gall fly, with about 60% of respondents reporting an increase in yield and income (Fig. 7 and 8). About 36% of the landholders reported moderate to substantial benefits of the project and thought the introduction of the gall fly was most useful. About 31% of respondents reported minor benefits from the project (Fig. 9).

In addition, there were many anecdotal benefits reported; from a decrease in knife wounds resulting from the reduction in the need to slash chromolaena to fewer snakes or wild pigs hiding in chromolaena infestations. Villagers also reported that roadsides did not have to be slashed so often to maintain adequate visibility and access.

Discussion

C. connexa is the most successful of the three biocontrol agents introduced into PNG to control chromolaena. Not only was it easy to mass-rear, field-release and establish, it spread quickly from the point of release, moving up to 100 km in seven years. *C. connexa* is now present at 89% of known chromolaena sites throughout PNG and is expected to keep dispersing as chromolaena also spreads. However, it is also expected that the fly will reduce the rate of weed spread.

Complete control of chromolaena was observed at three of the monitoring sites, while at the other sites, there has been a general decrease in the size of chromolaena infestations. While formal monitoring was not undertaken in other provinces, similar trends were observed.

In most provinces, chromolaena is considered under control by the gall fly in at least some areas. Control was generally achieved more quickly in some provinces, particularly East New Britain, New Ireland and Bougainville, than in others such

as Morobe and Madang where it is drier (Day and Bofeng, 2007). Control in West New Britain has been slower and less complete, possibly due to the cloudy conditions affecting mating and oviposition of *C. connexa* (R. McFadyen pers. comm., 2010; Day et al., unpubl. data).

The introduction of the gall fly into PNG has been well received, with landholders stating that there is significantly less chromolaena present now than before the gall fly was introduced. Consequently, there is a substantial decrease in weeding times and costs to control chromolaena. In addition, there has been a substantial increase in yield and income and part of this has been due to an increase in the size of farmed lands, due to the reduced effort to weed and manage the land.

Thus controlling chromolaena in PNG has increased food security and income of landholders, as well as increasing general health and well-being through decreased knife wounds and snake bites. The decrease in weeding times has also allowed landholders the opportunity to undertake other activities, such as maintenance of houses, fences and fishing nets.

C. connexa has also been released in Indonesia, Guam, Federated States of Micronesia and Palau where it controls chromolaena (Muniappan et al., 2007; Zachariades et al., 2009). More recently, *C. connexa* was re-introduced into Thailand and is being considered for introduction into Australia, China and Taiwan.

Acknowledgements

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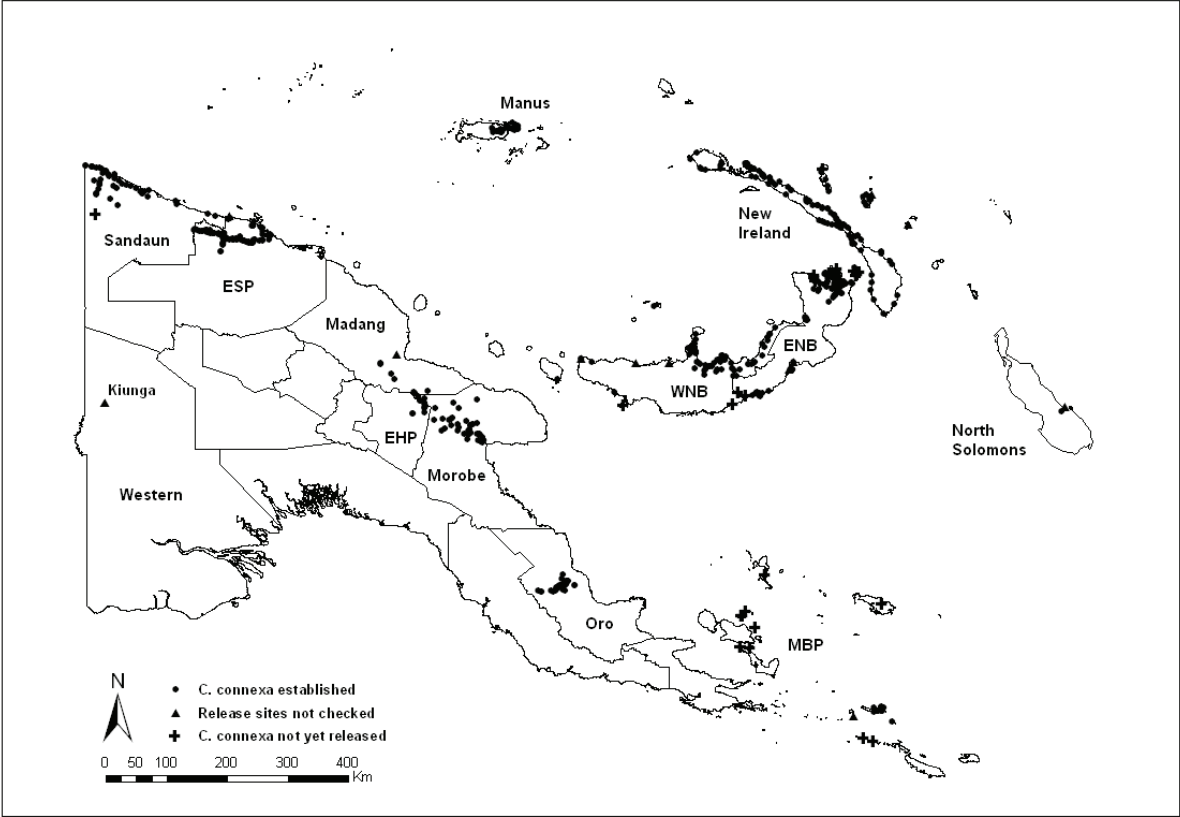


Figure 1. The distribution of *C. odorata* in Papua New Guinea (all dots), showing where *C. connexa* has established, been released but not checked and where it is yet to be released. EHP = Eastern Highlands Province; ENB = East New Britain; ESP = East Sepik Province; MBP = Milne Bay Province; WNB = West New Britain.

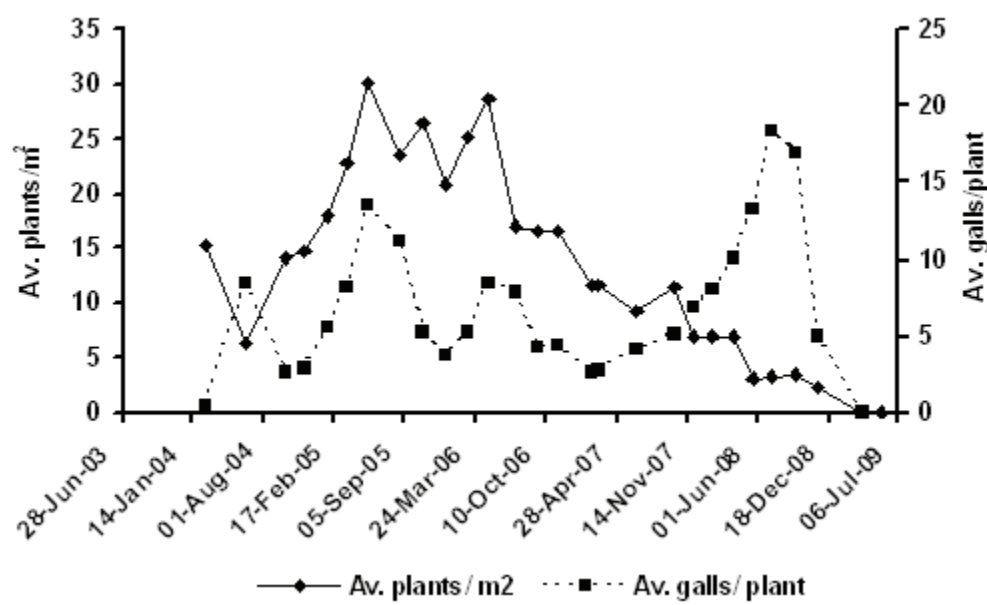


Figure 2. Mean number of plants/m² and the mean number of galls/plant over time at Kasuka, Morobe Province.

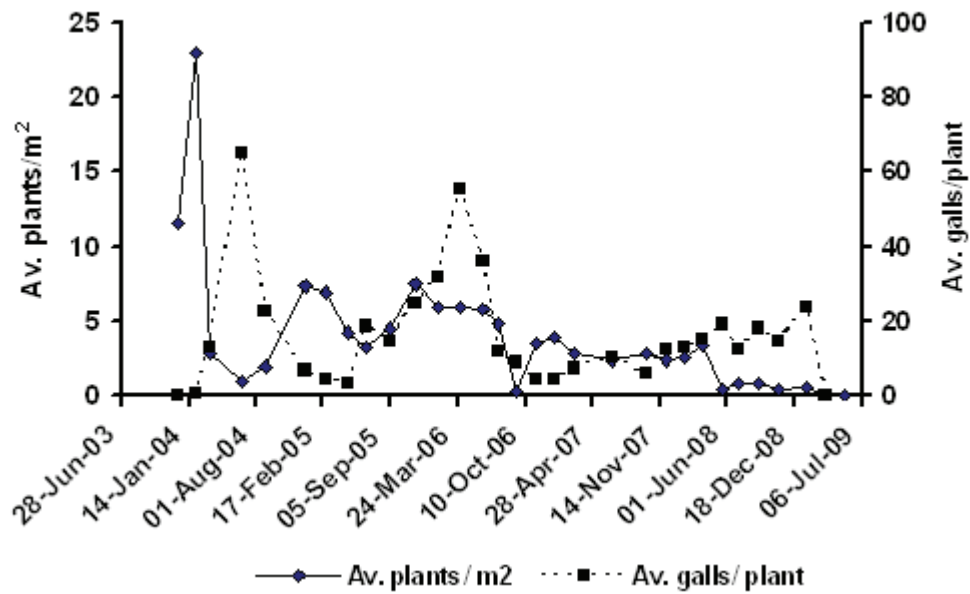


Figure 3. Mean number of plants/m² and the mean number of galls/plant over time at Trukai Farm, Morobe Province.

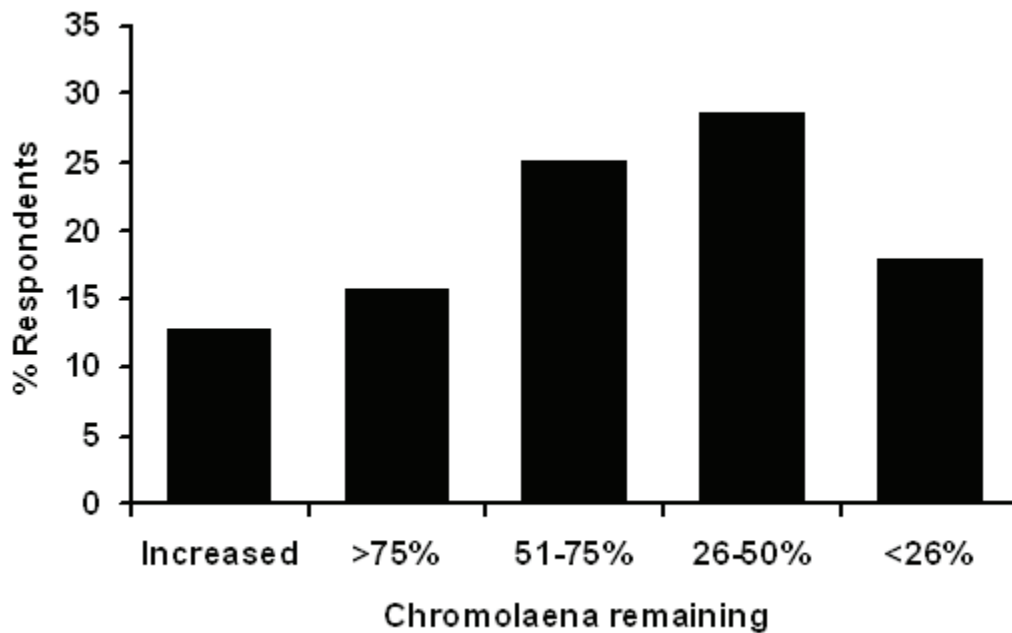


Figure 4. Responses when landholders were asked how much chromolaena remained after the gall fly was introduced.

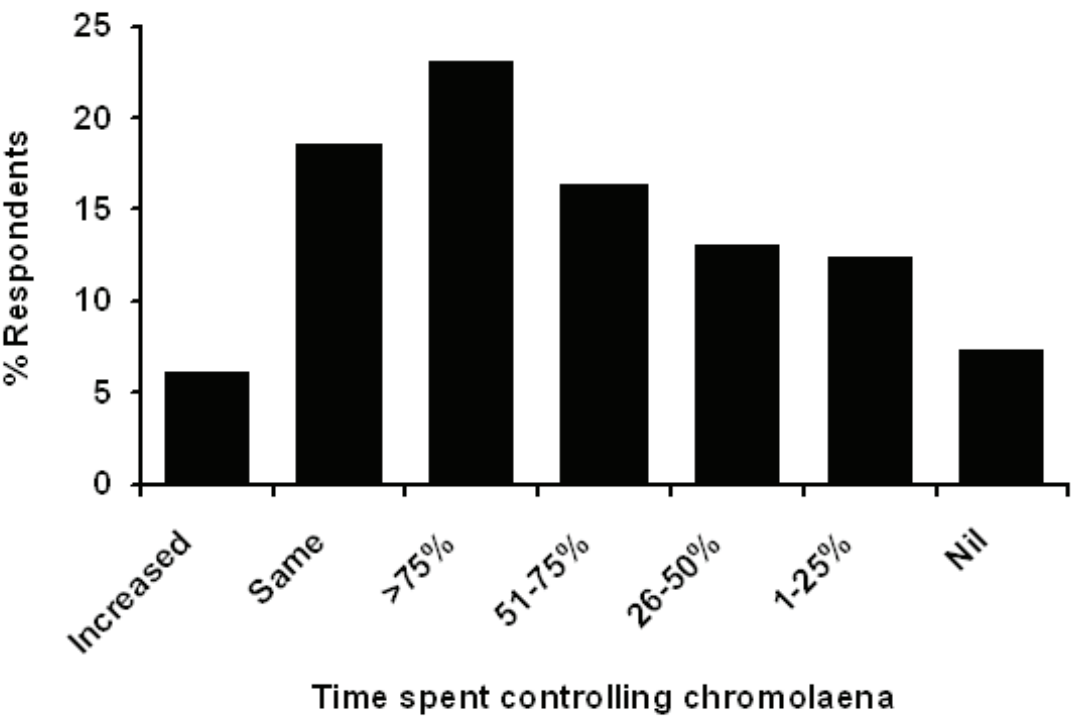


Figure 5. Responses when landholders were asked how much time is now spent on controlling *Chromolaena* compared to before the gall fly was introduced.

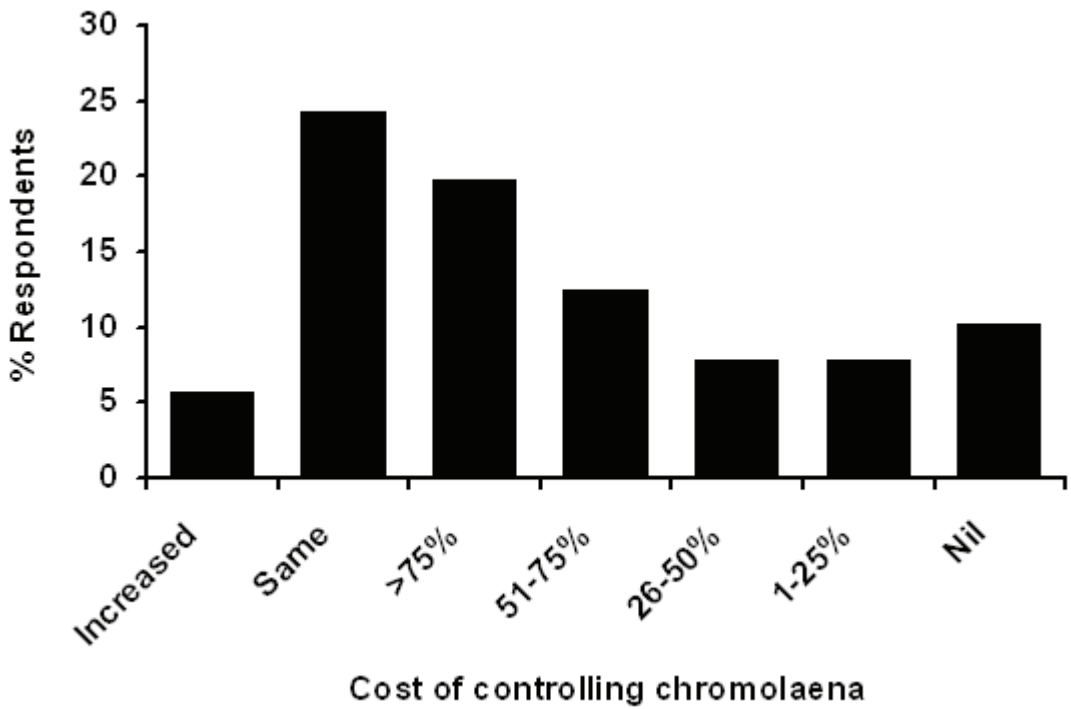


Figure 6. Responses when landholders were asked how much the cost of controlling *Chromolaena* has changed compared to before the gall fly was introduced.

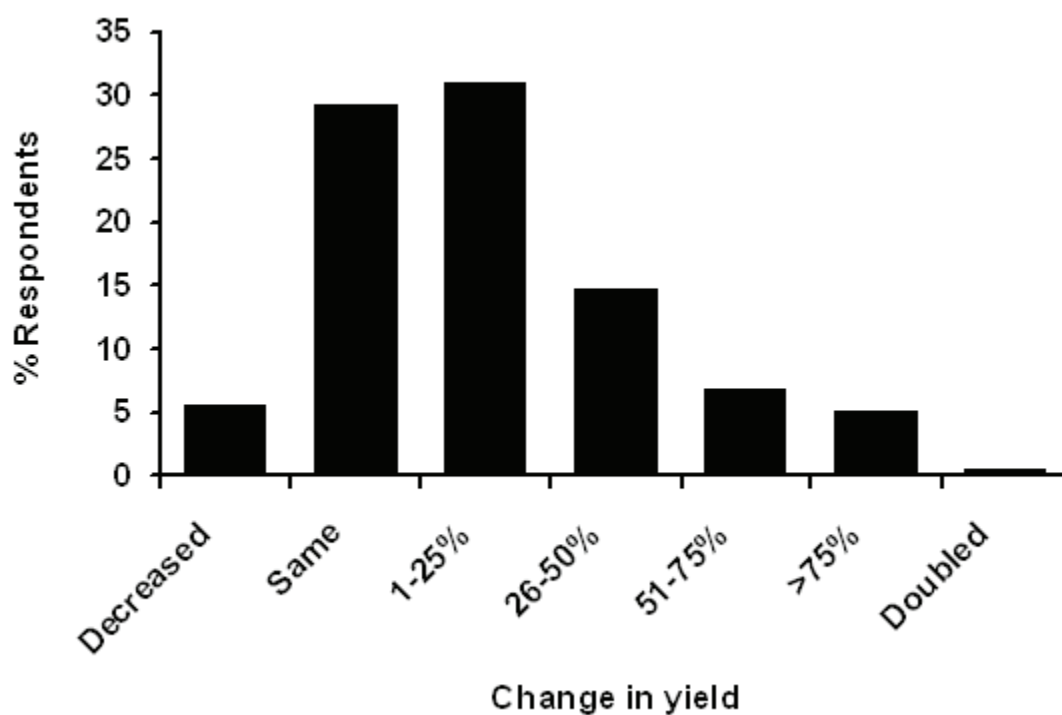


Figure 7. Responses when landholders were asked how much yield has increased compared to before the gall fly was introduced.

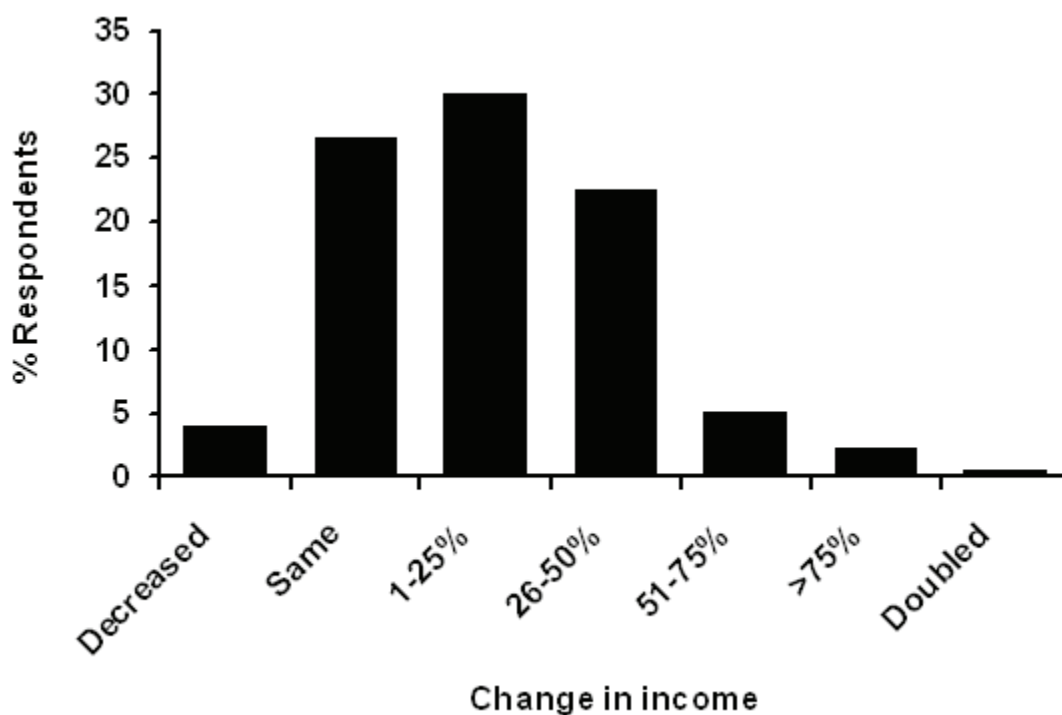


Figure 8. Responses when landholders were asked how much income has increased compared to before the gall fly was introduced.

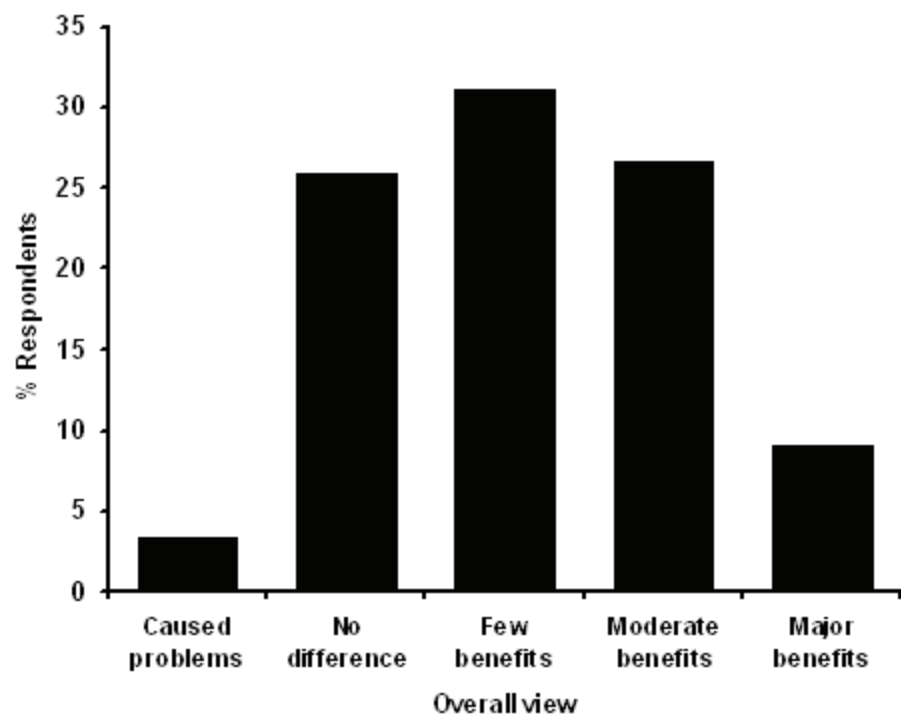


Figure 9. Responses when landholders were asked their overall view of the biocontrol project and the introduction of the gall fly.

Host Specificity Testing, Release and Successful Establishment of the Broom Gall Mite (*Aceria genistae*) in Australia and New Zealand for the Biological Control of Broom (*Cytisus scoparius*)

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Summary

A form of the eriophyid mite, *Aceria genistae* (Nalepa) was tested between 1999 and 2001 against 34 test plant taxa and cultivars from 12 tribes for its specificity towards the invasive shrub Scotch broom, *Cytisus scoparius* (L.) Link, and was shown to be highly specific. The mite was approved for release in Australia and New Zealand where redistribution and monitoring programs have been put in place. After three years, 106 releases of the mite have been conducted in Australia with a 32% establishment rate. In New Zealand, 40 releases have been made with 50% establishment. Both countries are continuing releasing this mite and are monitoring its establishment.

Introduction

The leguminous shrub Scotch broom, *Cytisus scoparius* (L.) Link (Fabaceae) is native to the UK, western, southern and central Europe. There it is considered moderately weedy and it occasionally colonises forest areas and pastures. Elsewhere, it has become a serious invader in several countries: eastern and western USA including Hawaii, British Columbia, Australia, New Zealand, Chile and India. Mechanical and chemical control methods of broom in invaded natural ecosystems are difficult to

implement, expensive, have negative environmental impacts and require follow-up due to large seed banks and reinvasion by seedlings and young plants (Downey and Smith 2000; Paynter et al 1998).

A number of demography studies have focused on patterns and processes of broom invasion in its native range (France) (Paynter et al. 2003; Paynter et al 1998) and its introduced range in Australia (Downey and Smith 2000; Paynter et al 2003; Sheppard et al 2002; Waterhouse 1988) and New Zealand (Paynter et al. 2003; Williams 1981). In addition, studies in the weed's native range have highlighted the role of natural enemies in limiting

broom performance (Waloff and Richards 1977), especially the potential of arthropods to reduce seed production and broom longevity (Rees and Paynter 1997). Broom plants in Australia and New Zealand are largely devoid of specialist insect herbivores (Memmott et al 2000), indicating that biological control may have some potential to control broom in these countries. New Zealand (NZ) began a broom biological control program by releasing the seed feeding beetle, *Bruchidius villosus* (Fabricius) and the sap-sucking psyllid, *Arytainilla spartiophila* (Förster) in 1987 and 1993 respectively. Meanwhile, an accidental introduction of the broom twig-mining moth, *Leucoptera spartifoliella* Hübner resulted in extensive damage to broom in NZ south island (Syrett et al 1999). Building on New Zealand's experience, CSIRO (The Commonwealth Scientific and Industrial Research Organisation) and the New South Wales Department of Agriculture imported *L. spartifoliella* from NZ and released it in 1993 (Wapshere and Hosking 1993), followed by releases of *A. spartiophila* in 1994 and *B. villosus* in 1995 (Syrett et al 1999). A fourth agent, the eriophyid gall, *Aceria genistae* (Nalepa) was identified as a potential biological control agent during European field surveys (Hosking 1990; Syrett et al 1999; Wapshere and Hosking 1993). *A. genistae* was originally described by Nalepa from galls developing on Scotch broom in eastern France. The known native range of the mite includes the UK, Italy, Spain and central Europe. Colonies of *A. genistae* start at the inner base of stem buds and cause growth deformities on bud burst becoming round, pubescent galls. Several overlapping generations develop in galls during spring and summer. Non-woody galls wither in late summer and autumn, forcing mites to crawl into dormant stem buds where they overwinter. Gravid females are also wind dispersed in spring and there is a sex ratio of about 1:20 male: females (J-L Sagliocco pers. obs.). '*Aceria genistae*' is probably a complex of specific forms or sibling species. It has been recorded on a number of Genisteae species including *Cytisus* spp., *Ulex europaeus* L., *Genista* spp. and *Spartium junceum* L. (Castagnoli 1978). However, Cromroy (1979) (Cromroy 1979) noted that eriophyids are often highly host-specific, even to the level of plant form. Castagnoli (1978) has shown that *A. genistae* developing on broom did not develop on *S. junceum*, while the mites found on *Spartium* are a separate species (redescribed

as *Aceria spartii*). Mites identified as *A. Genistae* discovered on stunted shoot tips of gorse and French broom, *Genista monspessulana* (L.) L.A.S. Johnson in the USA caused limited damage and did not develop on any other species (Chan and Turner 1998). Similarly, mites identified as *A. genistae* were found infesting gorse, *U. europaeus* but not broom, in New Zealand (Manson 1989).

Methods

Host specificity testing

Initial testing of *A. genistae* by CABI (the Centre for Agriculture and Biosciences International) and CSIRO was conducted with a small number of broom's close relatives at CSIRO European laboratory in Montferrier (France). Field tests were carried out under natural conditions in a native broom infestation where *Cytisus striatus* (Hill) Rothm., *Chamaecytisus palmensis* (H.Christ) F.A.Bisby & K.W.Nicholls, *Spartium junceum* L., *Genista tinctoria* L., *Medicago arborea* L., *Laburnum anagyroides* Medik. and *C. scoparius* were planted as test plants. After two years, *A. genistae* galls were only on *C. scoparius* and no attack on any of the species tested had been observed. Additional tests were conducted in a glasshouse where *A. genistae* galls were tied onto *C. palmensis* and *U. europaeus* with *C. scoparius* as a control. Gall development occurred on *C. scoparius* only. The high specificity of the mite towards *C. scoparius* resulted in comprehensive host specificity study being conducted in Australia. *A. genistae* galls collected in the Cevennes mountains range, north of Montpellier (France) were shipped to the CSIRO quarantine facility in Canberra for rearing and further evaluation against a number of Australian and New Zealand native plant species (Table 1). In quarantine, mites were inoculated onto broom plants where they developed galls, providing a large colony for host specificity tests.

There are no Australian native plant species in the tribe Genisteae. Therefore, the focus of the testing was on species of economic importance in the Genisteae and other related tribes in the Faboideae, plus native species in related tribes, with less intensive testing of representative Australian natives in other subfamilies of the Fabaceae. Plants tested for New Zealand included local cultivars of two *Lupinus*

species and seven species representing all native genera with the exception of the monophyletic genus *Montigena*.

Between 1999 and 2001, 34 taxa and cultivars from 12 tribes were tested as part of the risk assessment required to obtain release permits for the mite in Australia and in New Zealand (Table 1). Each test consisted of five replicates of one taxon paired with five *C. scoparius* of similar size as controls. Each plant tested had dormant buds required for mite development. *A. genistae* galls were harvested from broom gall-producing plants kept in quarantine, and each gall was scored for number of mites present. Five to ten galls with comparable numbers of mites were then tied onto the foliage of each test plant and control and covered with a plastic bag for 72 hours to prevent galls drying out too quickly and to encourage mites to migrate out of the galls. Test and control plants were kept at temperatures of 18° C (day) and 12° C (night) (10 hrs light: 14 hrs dark) under high intensity artificial light for one month to help mites colonise plants while there was no bud development. After one month temperatures were increased to 20° C (day) and 15° C (night) (12 hrs light: 12 hrs dark) to initiate bud growth resulting in gall formation caused by mite feeding activity. After three to five months, and once several galls had developed on control plants, all fresh buds on the test plants were dissected for mite presence. When test plants were too large, an area equivalent to the smallest test plants was sub-sampled for mite presence.

Results

Host specificity testing

Among the species tested, some initial gall development was observed on *S. junceum* and *C. palmensis*. However mites did not survive and no further development was observed. Initial gall formation was also observed on *Cytisus* 'Crimson King', an ornamental with *C. scoparius* parentage, but mite survival did not occur. No gall development or mite presence was observed on any other species including all Australian and New Zealand natives.

Approval for release in Australia

In 2002, CSIRO submitted an application to federal agencies to obtain approval for release of *A. genistae* into the Australian environment. After examining the submission, AQIS (the Australian Quarantine and Inspection Service) Plant Biosecurity Australia and the Department of Environment approved release of the mite in the environment. In 2003, before the mite was released, a broom fungus that had been ruled out as a broom potential biological control agent for Australia due to lack of specificity (Morin et al 1999) was discovered in the Canberra mite culture (Morin et al 2006). Consequently, the mite culture had to be destroyed and its release was postponed. A lack of funding caused further delays. In 2006, with joint funding from the Australian Government and the Department of Primary Industries (DPI), Frankston, Victoria, Australia imported the mite into quarantine in Frankston and a clean colony protocol was developed using the transfer of individual mites. Mite populations were increased by transferring mites onto new plants for several generations until approval for release was granted. Outside quarantine, broom plants were inoculated with mites to provide material for releases. In October 2008, the first releases were conducted in the Australian Alps in eastern Victoria, and a rearing colony was established in Tasmania. Subsequently, Tasmania provided galls to South Australia and New South Wales (NSW) for releases.

Approval for release in New Zealand

As *A. genistae* was already present in New Zealand on gorse (Manson 1989), Environmental Risk Management Authority approval to release the broom form was not required. Nevertheless, testing of the broom form from Europe was conducted as if it was a new organism to New Zealand. Ministry of Agriculture and Fisheries approval to release *A. genistae* from containment was granted in November 2007.

Releases and establishment in Australia

In Victoria a number of nursery sites intended for detailed monitoring of mite survival and dispersal were established using two release methods. In autumn, two to four broom plants bearing galls were planted in the ground at the edge

of an infestation, and were watered and fertilised to allow their establishment and mite migration. A direct release technique was also used. It consisted of attaching broom branches with galls on 20 wild plants at the edge of the infestation and marking each plant with surveyor tape for later monitoring. At release sites to be assessed for mite establishment, only the later method was used. Monitoring of transferred plants and gall numbers, mite presence and gall formation was conducted at all sites twice a year, in spring and autumn, mostly because of the difficulties to access most of the remote release sites in the high country. In the other states, the second release method was mostly used and monitoring was conducted whenever possible. During the period 2008-2011, at total of 106 releases of *A. genistae* were conducted in four states. Releases were conducted in the Australian Alps (Victoria), throughout the state of Tasmania, around Adelaide (South Australia), near Canberra (southern NSW) and in the Northern Tablelands (NSW). The altitude of most of the release sites ranged between 220 and 670 m, the highest sites being at 1,500 m altitude at Barrington Tops (southern end of the Northern Tablelands in NSW). In 2011, establishment of the mite had been confirmed at 34 of the sites (32 %) (Table 2). Releases of the mite and monitoring of its establishment and dispersal are continuing. In the field, mites were observed to induce gall formation after 6-12 months. Although the program still being in its early stage, mites were observed to establish and cause gall formation faster in Tasmania than in Victoria, both under semi-natural conditions and in the field.

Releases and establishment in New Zealand

Since 2008, 40 releases of the mite have been made in New Zealand. Establishment has been confirmed at approximately 50% of these sites. Gall formation at many sites has been extensive, covering much of infested broom plants with some mature plants bearing hundreds of galls. Significant damage has been recorded in some areas where whole branches of broom plants have been observed to suffer dieback and in some cases whole plants have died. Gall formation has also been found on broom plants in the absence of gall mites although these galls have a different architecture to those caused by *A. genistae*. Initial observations suggest that a

disease (unidentified *Fusarium*) is associated with gall formation and mite activity (Daniel Than pers. comm.). It is not yet known if the mite may enhance disease occurrence and if it plays a role in plant dieback.

Discussion

The *C. scoparius* form of *A. genistae* was shown to be restricted to this weed and during host specificity studies did not survive on different taxa tested. The mite was released in 2008 both in Australia and New Zealand and since then each country has implemented release and monitoring programs. In Australia, the mite has so far established at a third of the release sites, while in New Zealand the establishment success rate is currently at about 50%. Releases are on-going in both countries. In Australia, differences in the time required for mites to develop galls in the field have been observed between Victoria and Tasmania as well as with establishment success rate. It is not known if this is due to climatic reasons or the occurrence of different broom plant forms in these states. Mite activity results in severe stunting of growth and reduction of plant biomass, reduced seed production and very occasionally reported plant death in its native range. Plant death has been observed in New Zealand following release, although this has not been observed in Australia yet. In Victoria, a field study is currently underway to quantify the impacts of the mite and other natural enemies.

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Table 1. Results of host specificity tests conducted by CSIRO with *Aceria genistae*

Plant species tested	No. of replicates	Mean no. galls attached/plant	Test duration (weeks)	Live mites on test plants	Galls on control plants
Tribe Genisteae, Subtribe Genistineae					
<i>Cytisus alba</i> §†	5	10	28	No	Yes
<i>Cytisus</i> 'Crimson King'	5	10	28	Yes†	Yes
<i>Chamaecytisus palmensis</i> (H.Christ) F.A.Bisby & K.W.Nicholls	9	7.6	27, 34	No	Yes
<i>Spartium junceum</i> L.‡	5	5.4	66	No	Yes
<i>Genista monspessulana</i> (L.) L.A.S.Johnson	5	10	23	No	Yes
<i>Laburnum x watereri</i> (G.Kirchn.) Dippel	6	6.7	14	No	Yes
<i>Ulex europaeus</i> L.	5	10	29	No	Yes
<i>Lupinus angustifolius</i> L. cv Yandee	5	6.8	13	No	Yes
<i>Lupinus angustifolius</i> cv Merritt	5	6.2	13	No	Yes
<i>Lupinus angustifolius</i> L. * NZ origin	5	6.2	13	No	Yes
<i>Lupinus polyphyllus</i> Lindl*	4	5.8	20	No	Yes
Subfamily Faboideae, Tribe Bossiadeae					
<i>Bossiaea buxifolia</i> A.Cunn.	5	10	25	No	Yes
<i>Goodia lotifolia</i> Salisb.	4	8.3	22	No	Yes
<i>Hovea acutifolia</i> A.Cunn. ex G.Don	5	6.6	19	No	Yes
<i>Hovea montana</i> (Hook.f.) J.H.Ross	4	10	26	No	Yes
Tribe Crotalariaeae					
<i>Crotalaria cunninghamii</i> R.Br.	5	5.2	22	No	Yes
Tribe Sophoreae					
<i>Sophora microphylla</i> Aiton *	5	6.1	16	No	Yes
Tribe Indigofereae					
<i>Indigofera australis</i> Willd.	5	8	27, 19	No	Yes
Tribe Loteae					

Plant species tested	No. of replicates	Mean no. galls attached/plant	Test duration (weeks)	Live mites on test plants	Galls on control plants
<i>Lotus australis</i> Andrews	5	10	27	No	Yes
Tribe Mirbelieae					
<i>Davestia mimosoides</i> R.Br.	5	5.4	16	No	Yes
<i>Dilwynia juniperina</i> Lodd., G.Lodd. & W.Lodd.	5	5.4	17	No	Yes
<i>Dillwynia prostrata</i> Blakely	5	7	18	No	Yes
<i>Eutaxia baxteri</i> Knowles & Westc.	5	6.2	21	No	Yes
<i>Oxylobium ellipticum</i> (Vent.) R.Br.	5	10	25	No	Yes
<i>Podolobium ilicifolium</i> (Andrews) Crisp & P.H. Weston	5	6.2	20	No	Yes
<i>Pultenaea microphylla</i> Sieber ex DC.	4	10	25	No	Yes
<i>Pultenaea juniperina</i> Labill.	5	6.2	20	No	Yes
<i>Viminaria juncea</i> (Schrad.) Hoffmanns.	5	5.4	25	No	Yes
Tribe Galegeae					
<i>Clanthus puniceus</i> (G.Don) Sol. ex Lindl.*	5	6.2	15	No	Yes
<i>Carmichaelia arborea</i> (G.Forst.) Druce*	5	5	21	No	Yes
<i>Carmichaelia kirki</i> Hook.f.*	5	5	21	No	Yes
<i>Carmichaelia monroi</i> Hook.f.*	5	5	15	No	Yes
<i>Carmichaelia stevensonii</i> (Cheeseman) Heenan*	5	6.4	15	No	Yes
<i>Carmichaelia torulosa</i> (Kirk) Heenan*	5	5.4	24	No	Yes
Tribe Phaseoleae, Subtribe Glycininae					
<i>Glycine clandestina</i> J.C.Wendl.	5	5.8	15	No	Yes
Subtribe Kennediinae					
<i>Hardenbergia violacea</i> (Schneev.) Stearn	4	10	27	No	Yes

Plant species tested	No. replicates	Mean no. galls attached/plant	Test duration (weeks)	Live mites on test plants	Galls on control plants
Tribe Trifolieae					
<i>Medicago polymorpha</i> L.	5	6.8	18	No	Yes
<i>Medicago sativa</i> L.	5	7	18	No	Yes
<i>Trifolium repens</i> L.	5	5.8	19	No	Yes
<i>Trifolium subterraneum</i> L.	5	6.4	18	No	Yes
Subfamily Mimosoideae, Tribe Acacieae					
<i>Acacia dealbata</i> Link	5	10	25	No	Yes
<i>Acacia melanoxylon</i> R.Br.	5	10	26	No	Yes

* species tested for approval assessment in New Zealand

† Some gall development occurred

‡ Some gall development occurred but mites did not survive

§ The taxonomy of ornamental broom cultivars is poorly defined in Australia. We chose to select one with and one without *C. scoparius* as a likely parent. We have selected *Cytisus praecox* 'Crimson King' which is considered a cultivar of *Cytisus scoparius* 'Andreanus' crossed with *Cytisus multiflorus* (Rowell 1991) and a cultivar named 'Cytisus alba Snow Queen'. There is no species called *Cytisus alba*, but *Cytisus albus* (Lam.) Link is now considered to be *Cytisus multiflorus* (L'Hér.) Sweet while *Cytisus albus* Hacq. is now considered to be *Chamaecytisus albus* (Hacq.) Rothm. The main parent of the selected hybrid *Cytisus alba* 'Snow Queen' is probably *Cytisus multiflorus* as this species is the parent of a number of ornamental broom hybrids (Rowell 1991).

Table 2. Releases and establishment of *A. genistae* in Australia for the period 2008-2011

State	No. sites where releases conducted	No. sites with establishment confirmed	Proportion established
Victoria	37	8	0.21
Tasmania	37	17	0.46
South Australia	26	9	0.34
NSW	6	1	0.17
Total	106	34	0.32

Observational Monitoring of Biological Control vs. Herbicide to Suppress Leafy Spurge (*Euphorbia esula*) for Eight Years

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Abstract

The effectiveness of *Aphthona* flea beetles (87 percent *A. lacertosa* Rosenhauer and *A. czwalinae* Weise, and 13 percent *A. nigriscutis* Foudras) as biological control agents of leafy spurge, *Euphorbia esula* L. was compared with a single application of herbicide (picloram) and untreated plots for a period of 8 years. Percentage of cover of leafy spurge, grasses; and flea beetle numbers were measured each year from 2000 through 2007. Cover of leafy spurge on *Aphthona* biological control plots exhibited annual declines until 2005. In 2006, these plots showed a temporary rebound in leafy spurge coverage followed by a decline in 2007. Spurge cover increased on the herbicide-treated plots and remained unchanged on the untreated check plots from 2000 through 2003. In 2003, the flea beetles began to emigrate from the release points within the biological control plots and dispersed throughout much of the surrounding leafy spurge infested area including the herbicide treated and check plots. This dispersal and colonization caused a subsequent decline in spurge cover on the herbicide-treated and control plots from 2004 through 2007.

Keywords: Biological control; *Aphthona*; Leafy spurge; Weed management; Invasive weeds

Introduction

Leafy spurge, *Euphorbia esula* L. is a deep-rooted perennial weed with erect stems 40 to 80 cm tall (Hansen 2004, Stevens 1963). The weed reproduces by vegetative buds and seeds. A native of Europe and Asia, leafy spurge was first reported in North America in Massachusetts in 1827 (Noble et al. 1979). The plant is now well established on 2 million ha throughout 35 states and in the southern edges of most Canadian provinces. Leafy spurge is primarily a grasslands problem for ranchers and public land managers (Anderson et al. 2003).

Chemical controls are often prohibitively expensive to apply over extensive grazing lands characterized by low productivity (Bangsund et al. 1996). Leafy spurge cannot be eradicated with a single chemical treatment. The seed bank of leafy spurge remains viable in the soil for more than 8 years (Bowes and Thomas 1978). Eradication with herbicides may be possible, but the land manager must be committed to being more persistent than the weed. Unfortunately, it would probably require 5 to 10 consecutive years of herbicide treatment to eradicate an established stand (Lym and Messersmith 1985). As a result, a strong emphasis has been placed on developing biological control agents for managing

leafy spurge.

It is estimated that eventually 60 to 70 percent of all leafy spurge infestations could be managed with biological control agents (Bangsund et al. 1999) such as *Aphthona* spp. (Coleoptera: Chrysomelidae) flea beetles, currently the most successful insects on leafy spurge. Six root-feeding flea beetles, *Aphthona cyparissiae* Koch, *Aphthona flava* Guill, *Aphthona czwalinae* Weise, *Aphthona lacertosa* Rosenhauer, *Aphthona abdominalis* Duftschmid, and *Aphthona nigriscutis* Foudras, have been introduced into North America (Hansen et al. 1997, Lym and Carlson 2002). However, there is limited information on the impact of *Aphthona* spp. on leafy spurge and the associated plant community (Bangsund et al. 1999), and even less information comparing the efficiency of flea beetles with herbicide control. The objective of this study was a long term comparison of the efficacy of *Aphthona* flea beetles with an herbicide treatment for control of leafy spurge and their effect on grass and forb coverage.

Methods and Materials

The study was conducted from 2000 to 2007 on an area of approximately 50 ha of contiguous leafy spurge infestation in south central Idaho along the South Fork of the Boise River in the Sawtooth National Forest (elev. 1,524 m). The study area has a south-facing aspect, ranges between 5 to 10 percent slope and received an average of 55.80 cm of precipitation per year. For the purpose of this study the infestation was divided into three equally sized blocks and each assigned one of three treatments: release of *Aphthona* flea beetles, herbicide treatment, or untreated control.

In 2000, we established six replicated plots in each of the three treatments. Eighteen, 30.5 m radius study plots were established by erecting a steel fence post in the center of each. Treatments were established as follows: (1) Six plots in an area treated with picloram (Tordon 22K liquid concentrate) at the rate of 3.2 kg/ha in 1999, (2) six plots in an area where flea beetles (approximately 1,000 beetles each, comprising 87 percent *A. lacertosa* and *A. czwalinae* and 13 percent *A. nigriscutis*) were released in 2000, and (3) six plots in an area without herbicide application or biological control agent treatments to be used for comparison. Individual plots were separated by at least 100 m from each other and the perimeter of the treatment

areas. Plots were surveyed by establishing 30.5m line transects in each cardinal direction from the steel fence post. At 3m intervals along each transect using 0.10 m² sampling frames (quadrats) (Daubenmire 1959), leafy spurge canopy cover and grass cover were ocularly measured to the nearest 5 percent. We also completed five sweeps with a standard 38cm diameter sweep net along the transect adjacent to each quadrat sample (Southwood 1978) to survey for *Aphthona* flea beetles. Sampling was usually conducted in late June or early July during peak *Aphthona* activity.

The herbicide treatment was applied operationally in 1999 the year before sampling began, with a truck-mounted-boom sprayer. The herbicide was applied to the entire treatment block and not randomly to the individual plots, therefore the plots could not be randomized and the data were not analyzed statistically. The mean values for each parameter for all quadrats within the plots were calculated and averaged for each treatment. Raw cover class data (Daubenmire 1959, Southwood 1978) were converted to percentage of cover by averaging the midpoint for each category over the six replicates. The plot means (n = 6) and standard errors for each variable were calculated by year and treatment (SAS 1989) and presented graphically.

Results

At the beginning of the study in 2000, one year after the herbicide treatment, leafy spurge canopy cover averaged 6 percent on the herbicide treated plots, 38 percent on the control plots, and 65 percent on the plots where *Aphthona* biological control agents were released (Figure 1A). From 2000 to 2003, leafy spurge cover increased on the herbicide treated plots to 37 percent but declined to 39 percent on the plots where *Aphthona* were released. On plots treated with *Aphthona* beetles, leafy spurge cover declined further to a low of 5 percent in 2005, after which it resurged to 21 percent in 2006 then decreased to 17 percent in 2007. In 2003, a few *Aphthona* beetles were beginning to be caught in sweep net samples conducted on the check and herbicide treated plots indicating emigration from the biological control plots had begun. By 2005 the beetles had dispersed throughout the study area and were pervasive throughout most of our plots (Figure 2). In 2004, leafy spurge coverage began to decline on both the check plots and on the herbicide-treated plots. This trend continued through

2007. Coverage of leafy spurge was similar among the three treatments in 2006 and 2007.

At the start of this study in 2000, grass cover was similar on the check (14 percent), herbicide treated plots (15 percent), and plots containing flea beetles (10 percent) (Figure 1B). In 2003, grass cover was 11 percent on the check plots, 13 percent on the herbicide plots and 21 percent on the plots treated with flea beetles. In 2007, grass cover was similar among treatments: flea beetles (23 percent); check plots (20 percent); herbicide plots (21 percent).

Discussion

Over the long-term, the coverage of leafy spurge on the biological control plots declined from 65% to 17%; beetles emigrating from the biological control plots also most likely reduced the cover of leafy spurge on the adjacent untreated check and herbicide treated plots. Few studies have compared the efficiency of biological control agents and chemical herbicides on leafy spurge in the same design. In our study, one year after its application the herbicide (picloram) reduced foliar cover of leafy spurge to less than 6 percent; however, leafy spurge cover rebounded, increasing each year for the next 3 years, then declined in each subsequent year through 2007. The check plots exhibited a similar pattern of decline in leafy spurge cover from 2003 through 2007 (Figure 1A).

Aphthona flea beetles were found almost exclusively on plots where they were released from 2000 through 2004 (Figure 2). In 2003, the beetles began to disperse from the biological control plots and by 2005 were found in the surrounding areas where no *Aphthona* were originally released, containing the check and herbicide plots (Figure 2). This emigration most likely caused the observed continual decline in the coverage of leafy spurge in these areas in the following years. In the *Aphthona* treated plots, it is likely that leafy spurge coverage was reduced by beetle feeding to the extent that they were forced to disperse into the surrounding area. This reduction of the coverage of leafy spurge to 5 percent in 2005 followed by a rebound in the coverage of leafy spurge to 21 percent in 2006 supports the hypothesis that the beetle emigrated in search of more sustenance. Butler et al. (2006) also noted an increase in the cover and density of

leafy spurge the year following peak control by flea beetles while beetle abundance declined. They hypothesized that the resurgence in leafy spurge cover may have been from developing seedlings or young plants that lack a sufficiently developed root system to support *Aphthona* larva (Fornasari 1996).

After leafy spurge, graminoids (grass and grass-like plants) were the dominant cover type on all study plots. Graminoids increased in coverage each year following treatment on the *Aphthona* treated plots from 10 percent in 2000 to more than 30 percent in 2005 and 23 percent in 2007 (Figure 1B). Butler et al. (2006) also showed a more rapid and larger response in graminoids following the decline of leafy spurge.

In summary, we began with three disparate covers of leafy spurge among our treatments. The plots where *Aphthona* beetles were released had the highest coverage of leafy spurge at the beginning of the study, followed by the untreated check plots, and finally the herbicide (picloram) plots. Through the following 8 year period the *Aphthona* biological control agents caused a decline in the cover of leafy spurge from 65 percent to less than 5 percent on the treated plots. With insufficient leafy spurge to adequately support the flea beetle population on biological control plots, *Aphthona* emigrated throughout the area occupied by leafy spurge including the plots containing the check and herbicide treatments. Interestingly, as beetles dispersed, the cover of leafy spurge on the biological control plots rebounded from 5 percent to 21 percent from 2005 to 2006. This may be the initiation of a cycle of increasing leafy spurge cover followed by an increase in flea beetles causing a subsequent decline in leafy spurge. As leafy spurge becomes naturalized into the indigenous plant population, this cycle will most likely decline in amplitude.

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and Idaho (Gunderson-Izurieta et al., 2009). Local junior high and high schools received grants from the state of Idaho, the USDA Forest Service (State and Private Forest Health Protection), and the Bureau of Land Management to establish student crews that monitor, collect, and redistribute biological control agents in their home counties during the summer. We particularly would like to thank Dan Reedy and Becky Frieberg for providing and supervising the student crews from the Southern Idaho Biological Control Program who assisted with plot establishment and conducted the vegetation monitoring for this project. For doing the herbicide application and local support of the program we thank John Shelly and his weed control staff of the USDA Forest Service, Fairfield Ranger District. This project was funded in part by the USDA Forest Service Forest Health Protection Office.

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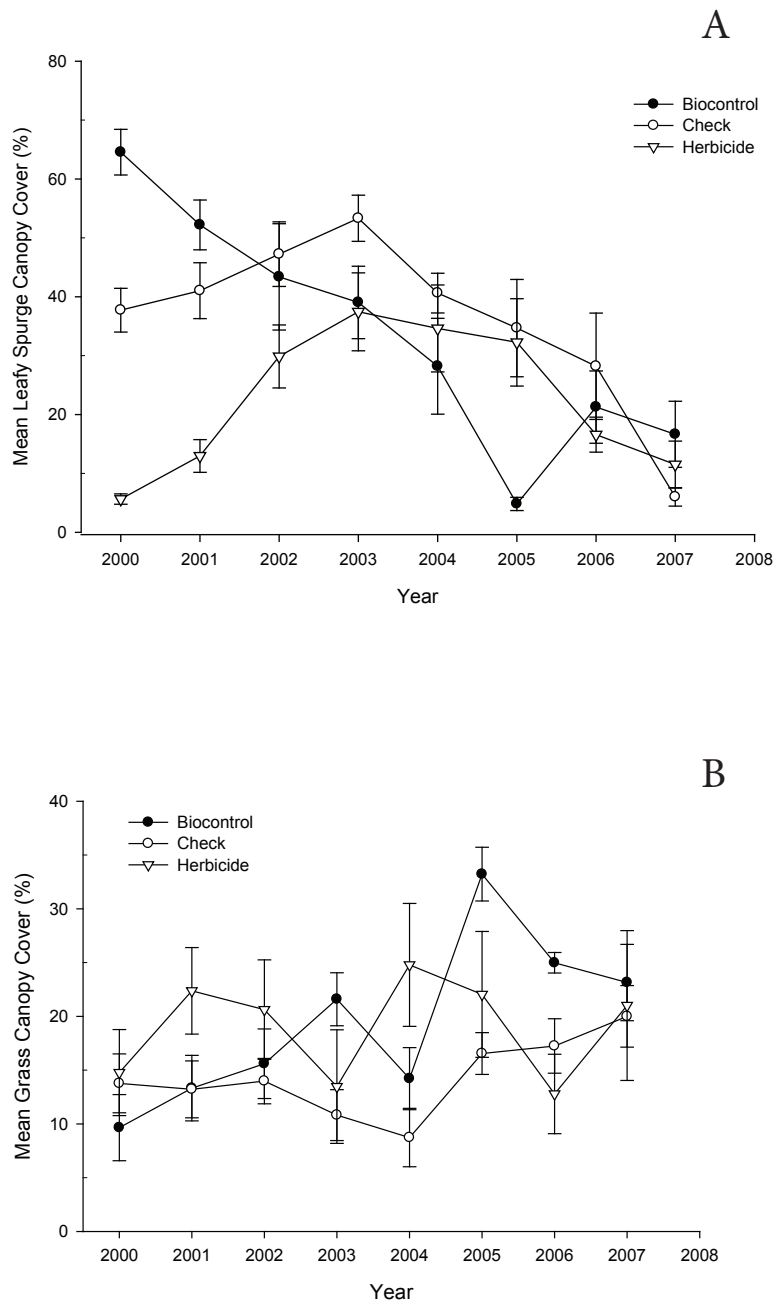


Figure 1. Change in canopy cover from 2000 to 2007. (A) Leafy spurge, (B) Grasses.

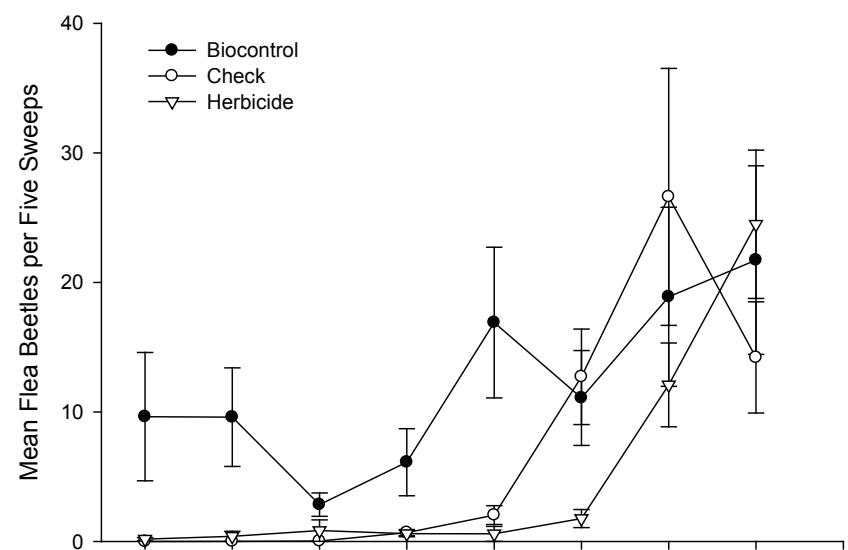


Figure 2. The average number of *Aphthona* flea beetles caught in plots for each of the three treatments.

Effective Landscape Scale Management of *Cirsium arvense* (Canada Thistle) Utilizing Biological Control

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Abstract

The stem mining weevil, *Ceutorhynchus litura* Fabricius, the gall forming fly, *Urophora cardui* L., and the seedhead weevil, *Larinus planus* Fabricius, were established as biological control agents on an 1800 hectare multiple-habitat wildlife refuge in northwestern Oregon in the mid-1990s. At the time, Canada thistle was the most wide spread, aggressive, and difficult weed to control and was being contained only by an extensive herbicide control program. A ten-year monitoring program for these natural enemies (1997 through 2007) showed a significant decline in Canada thistle (*Cirsium arvense* (L.) Scop.) plant abundance along with a measurable decrease in individual plant size and flowering. A few small stands of Canada thistle still remain that seem resistant to biological control but the need for direct control using herbicide has been discontinued as it is felt that the few scattered remaining populations are no longer an ecological threat and instead serve an important role in acting as a permanent reservoir for the biocontrol agents.

Introduction

The spiny, perennial weed *Cirsium arvense* (L.) Scop., commonly known as Canada thistle, California thistle, creeping thistle and European thistle, is of Eurasian origin and is now established in over 39 countries (Holm et al., 1979). In North America, Canada thistle can be found in a broad band across the entire northern half of the United States and adjacent southern Canada and is the most frequently cited problem weed in surveys and weed lists for North America (Skinner et al., 2000). The plant spreads readily, both by seeds and through root fragments during cultivation. It is clonal and most patches, therefore, are formed by a single individual plant with many aerial shoots, but are interconnected by an extensive lateral root system that make it very difficult to control with herbicides (Donald, 1994; Moore, 1975; Nadeau and Born, 1989).

Up to 1995, it was generally considered that

the impact of the approved agents for Canada thistle was minimal (Julien and Griffiths, 1998) and they were providing little or no effective control (Piper and Andreas, 1995; McClay et al., 2002). However, a review of the earlier studies indicate that these conclusions were mostly based on either controlled studies using potted plants or small research plots in which only the direct impact of the insect feeding was monitored for such short durations that they would not have detected long-term, cumulative stress that might compromise the extensive root system. A long-term biological control program under field conditions was needed where the combined attack of these agents over a long period of time might stress the Canada thistle population enough that desirable plants could replace them.

In 1995, the opportunity for a long-term study presented itself in the Ladd Marsh Wildlife Management Area and Refuge (= Refuge) near La Grande, Oregon, USA. The Refuge provided a stable landscape scale area for a long-term study where land

uses, management practices, and ownerships would not change within the ten years or longer required for population changes to become obvious. In 1995, a cooperative effort was undertaken between the Oregon Department of Fish and Wildlife, Ladd Marsh Wildlife Management Area and Refuge, the Oregon Department of Agriculture, and the US Forest Service's Rocky Mountain Research Station's Bozeman, Montana biological control program, to study a complex of biocontrol agents of Canada thistle on the Refuge, and conduct a long-term monitoring program to determine their effectiveness.

Target area

Ladd Marsh Wildlife Management Area and Refuge lies in Union County in northwestern Oregon at an elevation of approximately 780 meters (8 kilometers) east of the community of La Grande. In 1995, the Refuge consisted of approximately 1800 hectares of land (it has since expanded considerably) that had originally been a natural marsh, but extensive draining during historic times had converted much of it to agriculture. When cultivation stopped and extensive earth moving began for dam and reservoir construction for waterfowl a major surge of Canada thistle occurred. For the first ten years after its founding, Refuge managers considered Canada thistle the most severe threat to their goal of returning the land to a natural environment suitable for sustaining wildlife and conducted an extensive herbicide spray program in an attempt at its control.

Agents utilized

Urophora cardui L. [Diptera: Tephritidae].

This gall-forming fly had been released in 1993 at two locations in the Refuge using galls obtained from the Willamette Valley in western Oregon. Two years later, in 1995, it was confirmed that the fly had established and naturally dispersed 100 to 200 meters. Two more redistributions using galls from this local population were made in 1996 to far sides of the Refuge.

Ceutorhynchus litura (= *Hadroplontus litura*) (Fabricius) [Coleoptera: Curculionidae].

In 1995, this stem mining weevil was well established (Julien and Griffiths, 1998) in the lower elevation, warmer coastal environment of western Oregon, but despite earlier releases, was not found established at the start of this study in Ladd Marsh in eastern Oregon. We therefore used a cold-adapted strain established in Gallatin Valley, Montana (Rees, 1990). Two collections of newly emerged adults were obtained from the Gallatin Valley in May 1995 and released at two sites in Ladd Marsh. By 1996, both populations established. Redistributions to two other points within the Refuge made in 1997 and three additional redistributions in 1998.

Larinus planus Fabricius [Coleoptera: Curculionidae].

A seedhead feeding weevil was found already established at the beginning of the study. Initially we believed it to be *Rhinocyllus conicus* Frölich, an agent of musk thistle known to be established in the area, however, on rearing we found it to be a similar seedhead attacking weevil, *Larinus planus*. *L. planus* is not an approved biological control agent in North America, i.e. one that has been deliberately introduced after extensive testing, but since it was already present we included it in our monitoring. It has a single generation per year with adults emerging in spring to feed on plant foliage. When flower buds are forming, the female inserts an egg into the bud and the growing larva mines into the flower (Wheeler and Whitehead, 1985).

Methods and Materials

Permanent monitoring plots

A grid was laid over an aerial photograph of the Refuge and 50 points randomly located. After on-the-ground visits, 10 locations were discarded because they fell in open water or cultivated land.

At the remaining 40 locations, an open area of land containing a representative population of Canada thistle was marked with a white fiber glass fence post to use as a permanent vegetative sample site. After establishment, the plots were visited in 1995 and 1996 but limited monitoring conducted while we concentrated on releasing and redistributing the insects. Intensive monitoring began in 1997 and the plots were then visited regularly through 2007.

Sampling Canada thistle

Sampling was conducted in late fall after plant growth and insect activity had terminated, using four quadrats encompassed by a one-fourth meter square metal frame dropped randomly onto the ground within a three meter radius of the central stake.

Density

The total number of all Canada thistle plants in each quadrat was counted and the four samples combined together to give a total number of plants in one square meter at each sample site.

Height

The mean canopy top height was determined by selecting the five tallest plants in each quadrat and measuring their height. The canopy top height for each sample site was therefore based on the 20 tallest plants.

Insect sampling

We did not measure the actual number of gall flies but used the number of galls as an indication of their population. The galls in each quadrat were recorded as either being terminal galls that had formed at the growing tip of the elongating shoot, or as lateral galls forming along the sides of the shoot on the leaves' petioles. Five random plants in each quadrat were selected and dissected for evidence of attack by *C. litura*. The frass-filled, and discolored empty mines in the plants were still clearly visible in the dead plant stalks in late fall and the lengths of these mines were used as an indicator of the relative abundance of *C. litura* larvae that had formed

them. Finally, at each quadrat, a sample of 25 to 50 seedheads were collected at each sample site, returned to the laboratory, and opened to determine if they had been attacked by *L. planus*.

Results and Discussion

Urophora cardui

The gall fly had been introduced earlier and was well established when this study began. When monitoring began in 1996, it was found that the gall fly had already reached 12 of the studies in 40 locations, and by 2000 had dispersed throughout the entire Refuge. A steady build up in abundance of galls was found through the remainder of this study and seemed to still be increasing when the sampling was terminated in 2007 and averaged 1.2 galls per plant.

Ceutorhynchus litura

C. litura was first released at two locations in 1995, establishment confirmed by 1996 and redistributed to five locations in the Refuge in 1997 and by 2002 the *C. litura* had dispersed to all parts of the Refuge. During this time, percent of the plants attacked by *C. litura* steadily increased until 2003, but since then has leveled off at between 70 and 80%. By the end of this study in 2007, the plant in the Refuge had 33% of its stem length (height of the plant) mined; since a single larva only mines two to three cm of stem, an unusually high population of larvae must have been present.

Larinus planus

The seedhead weevil, *L. planus*, was already well established in the Refuge when the sampling began. However, sampling of seedheads to determine possible impact and whether there was a trend in its population levels was difficult. When we sampled in late fall, the plants had often lost many of their seedheads. However, we obtained good samples on four separate years to obtain an estimate of *L. planus* attack that ranged from 28 to 43%.

Impact on Canada thistle population

Plant density (plants per m²) showed an increase for the first few years until the Canada thistle populations reached 50 plants per square meter. However, a significant decline began after 1999 and the population in 2007 seemed to have stabilized at approximately 8/m² (Fig. 1).

Besides the obvious change in Canada thistle density, we were also able to observe and measure several changes in the plant morphology. Over the period that the Canada thistle population was declining, we noticed a slow decrease in the height of the canopy top (Fig. 2). This decline was caused in part by an increase in the frequency of plants with *U. cardui* terminal galls. When the fly attacked the apical tip of the growing shoot, this formed a large gall which generally stopped the further growth of the plant (Fig. 2). A second cause in stunting was when the *C. litura* larval populations were great enough that their mines inside the stem expanded faster than the rate of shoot elongation. When the mine reached the apical tip, it killed it. Usually the plant then sprouted a number of vertical shoots from lateral buds below the dead tip that formed a shorter plant. The increase in abundance of these shorter, "branched" plants contributed to the overall decline in plant height observed.

Conclusion

The sampling methods used to measure Canada thistle plant density indicated that during the ten years of the study, a progressive decline in Canada thistle occurred. But was the decline real or an artifact of the sampling design? Other personal observations made of the overall population along with a yearly photo record indicate that this decline was real. Other supportive evidence that a major population change took place is the herbicide spray records maintained by the Refuge. Founded in 1985 until we began work there in 1995, the annual Canada thistle spray program required 80 to 100 gallons of herbicide. However, by the end of our study, the thistle population had declined to the point where Canada thistle was no longer a problem but only a minor component of the ecosystem that no longer need to be controlled. After 2004, spraying for Canada thistle was discontinued since, in the

opinion of the Refuge management staff (junior author), the main objective of our biological control program to render the plant no longer ecologically damaging had been met.

We have concluded that the combined impact of the biological control agents, both through direct impact on the plants themselves and through the reduction of over wintering root reserves probably stressed the Canada thistle population enough that the existing complex of grass was able to out-compete the stressed plants. Presently, the Refuge management staff is satisfied with the control obtained and the few small remaining patches or scattered plants of Canada thistle are no longer being sprayed, but accepted as a component of the ecosystem that will form a permanent reservoir for our biological control agents. These few scattered plants and patches are therefore now a key component of a long-term stable plant community.

Acknowledgements

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Figure A

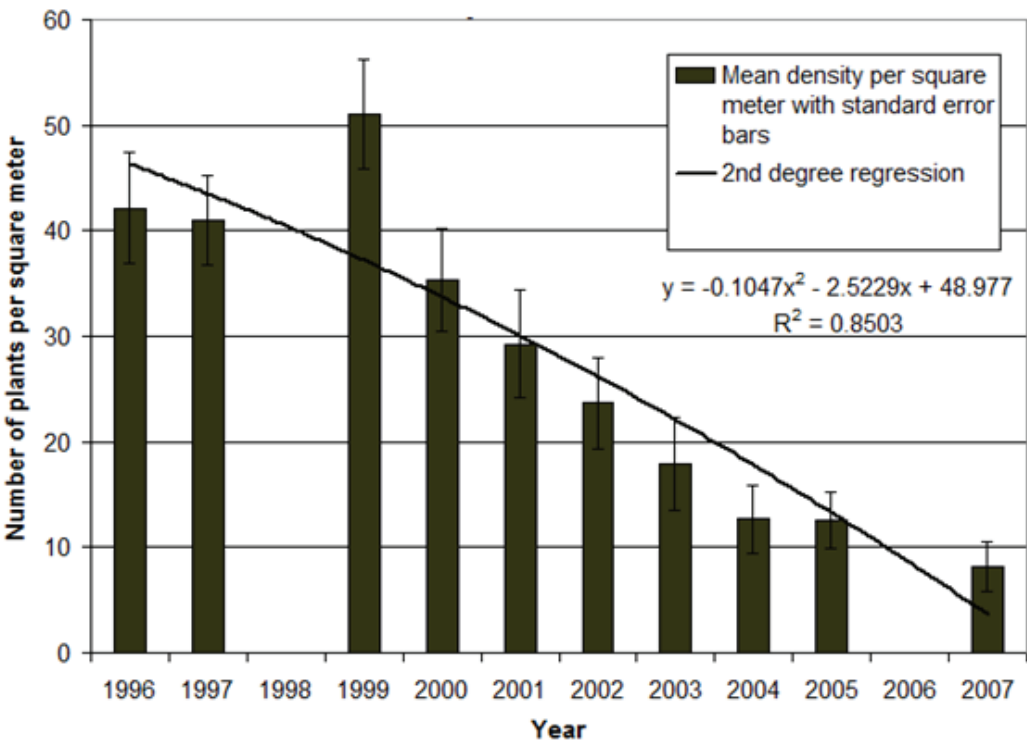


Figure B

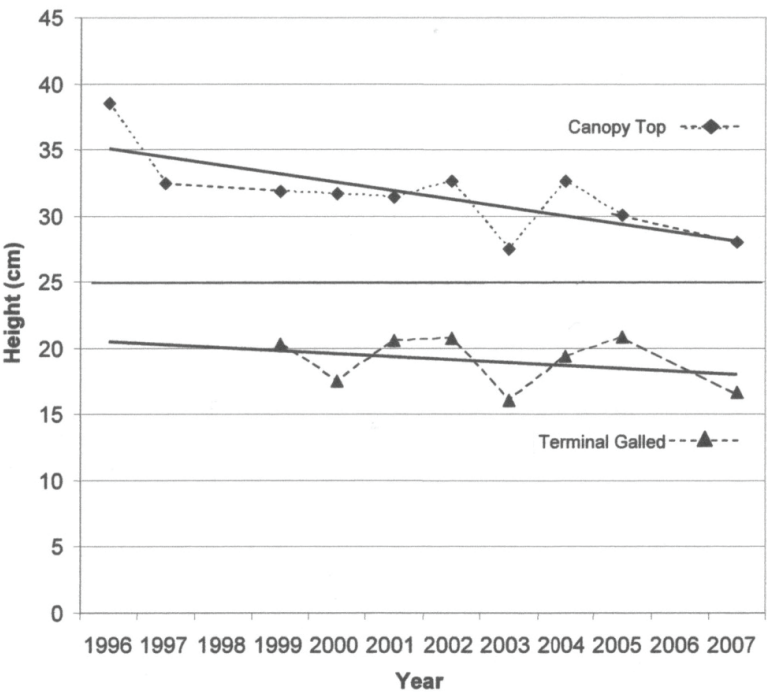


Figure 1: Impact on Canada thistle. (A) Density of Canada thistle plants (#/m²) at Ladd Marsh Refuge over the 12-year duration of study. (B) Mean height of Canada thistle plants sampled at Ladd Marsh Refuge over 12-year duration of study.

Status of Biological Control of the Shrub Gorse (*Ulex europaeus*) on the Island of Hawaii

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Abstract

On the island of Hawaii, gorse (*Ulex europaeus* L.) is limited to an isolated core infestation of approximately 2000 hectares with scattered plants and small patches in the surrounding 10,000 hectares. Between 1985 and 2000, seven biological control agents were introduced, five of which successfully established. By 2000, their combined impact had reduced the yearly growth of new shoots by over 50%. In 2001/2002, the ranch leasing the area decided to solve the gorse problem with an area wide program of aerial spraying and burning that destroyed over 95% of the gorse in the core area. With no follow up treatment, within three years the gorse had regenerated and was as abundant as before the control program. The effect on the complex of biocontrol agents, however, was devastating. One agent disappeared; the distribution and relative abundance of the other four was permanently altered, and their combined impact now appears to be significantly less than before the control effort.

Introduction

Gorse (*Ulex europaeus* L.) [Fabaceae], a spiny, multi-branched shrub of western European origin, at one time was considered useful as hedge rows and for grazing by sheep. As a desirable plant, European colonists spread it around the world, but in almost all new locations, it soon escaped from cultivation. Gorse has become a major weed in over 22 locations (Holmes et al., 1979) and since 1927 has been the target of numerous attempts at biological control (Coombs et al., 2004; Hill et al., 2008). In the state of Hawaii (USA) it was introduced for use as hedge rows to the island of Maui in the late 1800s, but soon escaped cultivation to become a major weed. However, in Maui a major cooperative management program has kept the original infestation suppressed to an acceptable level in the original area. A much

larger population exists on the adjacent island of Hawaii (Markin et al., 1988).

As a major weed problem in the state of Hawaii, gorse has been repeatedly targeted for biological control. The first program in the mid-1920s targeted gorse on the island of Maui. A second program in the late 1950s and early 1960s resulted in the release of three potential agents. The third and most extensive program was a cooperative international effort between the state of Hawaii, the state of Oregon, and New Zealand between 1985 and 2000 (Markin and Yoshioka, 1988; Markin et al., 1996; Markin et al., 2002). This recent program resulted in a total of seven agents being introduced, primarily to the island of Hawaii, of which four are now well established. This report summarizes the eight known agents that have been introduced to the state of Hawaii, their present status, and their impact on gorse.

Agents Released

Exapion (Apion) ulicis (Forster) [Coleoptera: Apionidae]

The biology of this small (2 to 3 mm long) seed-feeding weevil is tightly synchronized with the phenology of gorse. During flowering the adult female chews a hole in the developing green gorse pod and lays her eggs inside where the larvae develop (Crowel, 1983). In temperate England and New Zealand, gorse flowering occurs in late spring or early summer. However, in Hawaii flowering occurs in mid-winter (Markin and Yoshioka, 1996). This weevil was first released on the island of Hawaii in 1926 from a colony collected in Europe but failed to establish. A repeated attempt in 1949 using insects from New Zealand (but originally from Europe) also failed. Realizing the problem was poor synchronization between the weevil and flowering of gorse, the third effort in 1958 succeeded using weevils collected from southern France where the gorse flowered earlier – at the same time as in Hawaii. After establishment on the island of Maui, the weevil was introduced to the island of Hawaii and declared established a few years later. However, in 1983, in a survey of gorse on the island of Hawaii, no evidence of this weevil could be found. Subsequent talks with the entomologist involved in the release (Clifford J. Davis, Hawaii Department of Agriculture) confirmed that the weevil had been established but only on a small isolated pocket of gorse. Apparently a subsequent chemical control program destroyed this pocket before the weevil had had a chance to spread. Accordingly, in 1986 the weevil was reintroduced from the island of Maui (Markin and Yoshioka, 1998). The population, as indicated by the percent of pods being attacked, steadily built up through 1995 when a total of over 70% were attacked. The attack rate remained stable until 2000. A major control program in 2001/2002 destroyed the gorse bringing about a collapse of the weevil population. Gorse within this core area has rapidly regenerated from the coppicing of the burnt stumps and the soil seedbank. However, because of the large size of the area treated, *E. ulicis* has been slow to reinvade and as of 2010 was attacking only 54% of the pods.

Apion sp. (possibly A. uliciperda Pandellé) [Coleoptera: Apionidae]

During the collection of *E. ulicis* in southern France, a second slightly larger seed pod attacking weevil was found as a contaminant in the shipments made to Hawaii and accidentally introduced. In the 1985 to 2000 program, while tens of thousands of pods were opened to monitor *E. ulicis* (Markin and Yoshioka, 1998), no evidence of this second weevil was found.

Apion scutellare Kirby [Coleoptera: Brentidae, formerly Apionidae], the gorse gall weevil

This weevil is considerably larger and darker than *E. ulicis*. In spring when the new flush of foliage has begun to elongate, the female oviposits in the growing shoot tip. The shoot continues to elongate, but within a month, a 1 cm gall forms in which the larva develops. While the gall does not kill the attached shoot, its growth is halted or significantly reduced. In the late 1950s – early 1960s biocontrol program, adult weevils collected in Portugal were released on the island of Maui, but did not establish. In the recent effort, adults collected in late winter in northeastern Spain were shipped to Hawaii and held on potted gorse plants in quarantine until oviposition was observed, then directly released in the field. A total of nearly 800 adults were released 1989-1991 (Markin et al., 1996), but an effort to establish a laboratory colony failed and no further releases were attempted. To date, an intensive monitoring of shoots for the presence of other agents has failed to detect any sign of galls and we presume this weevil did not become established.

Agonopterix ulicetella (Stainton) [Lepidoptera: Oecophoridae]

In the recent program, New Zealand had already begun testing this moth and provided a colony to Hawaii which was tested and released in 1988. The larvae spin a silk feeding shelter on the elongating shoot in early summer from which they emerge to feed on the adjacent gorse spines. The feeding strips the spines from several inches of the shoot and

attack by multiple larvae can totally defoliate a shoot and kill the developing tip. On the island of Hawaii, *A. ulicetella* readily established and rapidly spread through the gorse area. Populations estimates based on the number of silk feeding shelters per square meter of gorse bush surface showed a continued population build up through 2000. An aggressive area-wide chemical/burning control program in 2001/2002 devastated the moth's population, but being a strong flier it readily reinvaded the treated area after the gorse resprouted. Its population now seems to be increasing and each year we find numerous pockets where larvae are abundant enough they totally defoliate the new flush of foliage. Unfortunately larval feeding is over by mid-July, but the plants continue to put out new vegetative growth during the remainder of the summer which soon hides the feeding damage.

***Sericothrips staphylinus* Haliday**
[Thysanoptera: Thripidae]

This small, normally wingless thrip (1 to 1.5 mm) was selected as a potential biocontrol agent because of its rapid life cycle (a generation per month during the summer) and also the advantage that occasionally alate forms are produced, facilitating dispersal. We had high hopes for the thrips when released in 1991-1992 using colonies obtained in southern England, western France, and central coastal Portugal. The colonies all readily established and, through a redistribution program, by 1995 had spread throughout the gorse infestation. Unfortunately after an early and rapid buildup, the population leveled off at only two to five thrips per branch when beaten into a plastic pan. During beatings the most obvious predator were two species of anthochorid, which in the laboratory readily attacked and fed on the thrips and are suspected of being the primary predators suppressing them.

***Tetranychus lintearius* Dufour**
[Acari: Tetranychidae]

After host testing in Silwood Park, England, the mite was released in New Zealand in 1989. Colonies from New Zealand were sent to the USDA quarantine in Albany, California in 1994 and released in Oregon by the Oregon Department of Agriculture. In the

spring of 1995, an Oregon colony was sent to the Hawaii Department of Agriculture in Honolulu where after two generations in quarantine, they were shipped to the island of Hawaii and released in the summer of 1995. The mite readily established and by 1996, a population explosion was underway. The small (0.4 mm) bright red females feed gregariously in bright red clumps of several hundred that migrate up the gorse shoot, spinning a protective web that covers the colony as it moves. A similar explosion had first been noted in New Zealand but after a few years, attack by a coccinellid beetle, *Stethorus punctillum* Weise, brought about a collapse. In Oregon after four years, the combined attack of this beetle and a predatory mite, *Phytoseiulus persimilis* Athias-Henriot, also devastated the population (Coombs et al., 2004). In Hawaii through 2001, we hoped our isolated population would escape these predators. Unfortunately, in 2001/2002, a major control effort temporarily eliminated gorse over most of the core gorse area and coincided with a collapse of the mite population. While numerous small colonies can still be found, particularly during mid and late summer, the massive sheets of webbing formed by the huge colonies that we had seen previously are no longer present. No evidence of *S. punctillum* has been found but the predatory mite *P. persimilis* is present and is probably the suppressing factor.

***Pempelia genistella* Duponchel**
[Lepidoptera: Pyralidae]

Larvae of the first moth, *A. ulicetella*, are active only during the first half of the flush of new foliage in early summer. It was therefore our desire to find a biocontrol agent which would be active later in the summer and attack the remaining new foliage. The potential agent selected was another moth, *P. genistella*, from the coast of Portugal. Its adults emerge in early summer and lay eggs on the new gorse shoots. The larvae develop slowly and do not begin actively feeding until early fall after the new foliage has hardened. The gregarious larvae form a large silken, trash-laced feeding shelter at the base of the shoot, girdling and usually killing it. Testing began in quarantine in mid 1990s and had just been completed when the project was terminated in 1995. Rather than see this agent dropped after a permit had finally been approved for its release in 1996,

arrangements were made for pupae from Portugal to be sent to the Montana State University quarantine in Bozeman, Montana. Here the adults were allowed to emerge and lay eggs on gorse shoots which were surface sterilized, sent to Hawaii, and tied to gorse plants in the field. In 1996, approximately 1000 *P. genistella* eggs were placed in the field, and this effort was repeated in 1997. By 1998 and 1999, *P. genistella* had become established at two of five sites. These populations remained small and localized through 2000. However, in 2001/2002 all the *P. genistella* sites were sprayed and then burned as part of an area wide control program. Since then, despite intensive sampling, we have failed to find its feeding shelter or distinctive feeding damage and presume the population was eradicated during the control program.

***Uromyces pisi* f. sp. *europaei* Wilson and Henderson [Uredinales]**

Late in the last biocontrol program, this fungus was selected as a potential agent and underwent host testing in the Hawaii Department of Agriculture plant pathogen containment facility in Honolulu. Testing continued even after the insect host testing was terminated in 1995, but was not completed and a permit for its release issued until 2000. In 2000, potted gorse plants infested with the fungus were sent to the island of Hawaii and placed in the field adjacent to healthy gorse plants. Later in the season, what were believed to be the fruiting bodies and symptoms of this disease were seen on several adjacent plants. However, since then, no sign of this disease has been observed in repeated searches.

Results and Discussion

As each new biocontrol agent in Hawaii was established, a method of monitoring its population was developed. However, by 1993 when we knew that three agents were feeding on gorse, a method was needed to measure their cumulative impact. Measuring the annual growth of the new terminal shoots was selected for monitoring overall growth and health of the gorse plants.

Results of this survey from 1994 through 2010 are shown in Figure 1. The decline in growth of the new terminal shoots from 1997 through 2000

corresponds to the steady buildup of the two most common agents, *A. ulicetella* and the gorse mite, although we suspect the spectacular build-up of the mite was the most important factor. The gap for 2001 indicates when a wide scale control program destroyed the gorse at all our sampling sites. The spectacular elongation identified in 2002 and 2003 probably resulted from a flush of nutrients released by the burning. By 2005 four insect agents had begun to re-invade from the outlying, untreated pockets of gorse. The decline in growth of the new terminal shoots from 2006 through 2010 is probably a combination of the exhaustion of the minerals released by the burning and a steady buildup of the agents as their populations recover. Unfortunately without the high populations of the gorse mite, which we feel was having a major impact before the treatment, a new level of yearly growth seems to have been reached but is still greater than before the control event.

The major visible impact we now observe is feeding by *A. ulicetella*. However, the impact of the other two agents, feeding by adult seed weevils and the gorse thrips, cannot be discounted. While their populations are low at any one time, they both are active all year long so their cumulative feeding damage is probably considerably more than would be measured at any one time during the year. In conclusion, there is no evidence that the complex of established biocontrol agents will stop the further growth of gorse, but we are observing an impact on its growth and a reduction in seed production.

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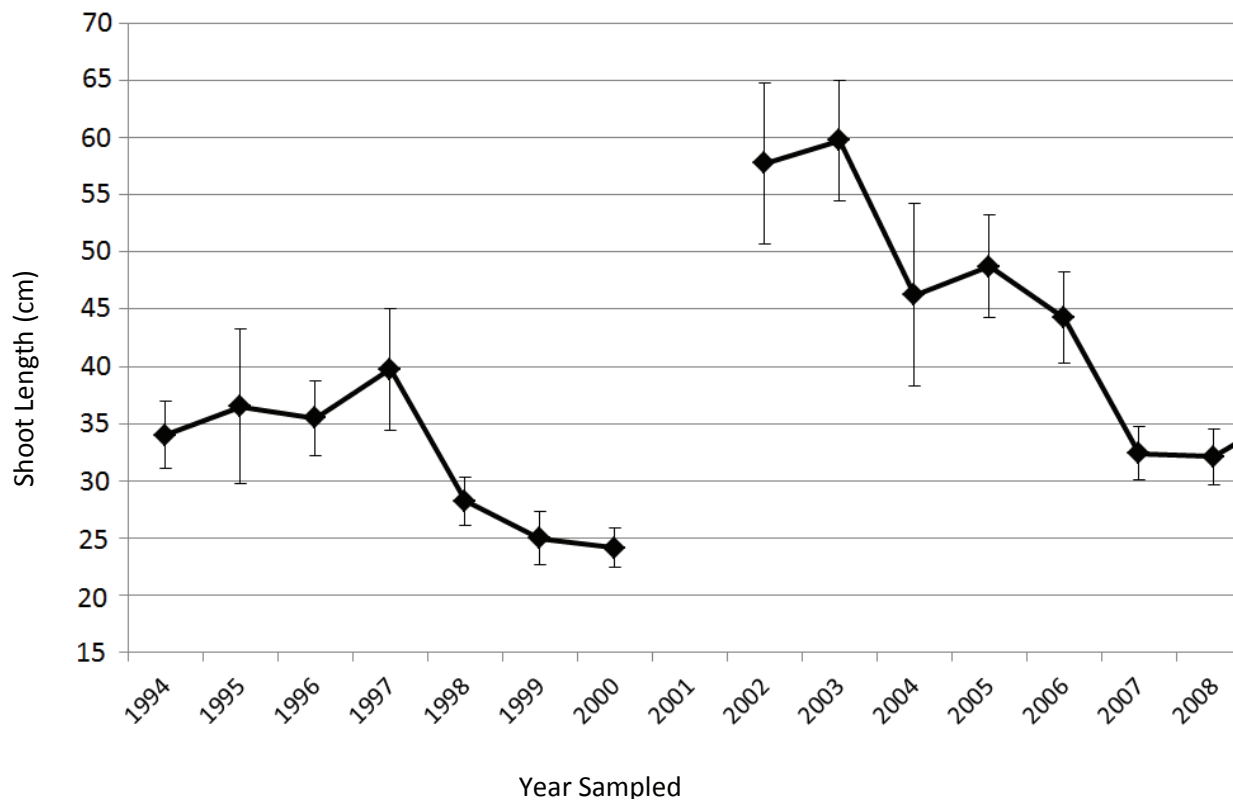


Figure 1. Mean yearly growth in centimeters (and SE) of 300 terminal gorse shoots on the island of Hawaii measured in fall or winter after the summer growth was completed. No sample taken in 2001 because a control program destroyed most of the plants.

An Overview of Biological Control of Weeds in Tasmania

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Summary

Thirty-five agents have been deliberately released for the biological control of 16 weed species in Tasmania, Australia, with 31 of these released during the last twenty years. The agents include three fungal pathogens and 32 species of invertebrates of which 29 are insects and three are mites. Of these, 24 have established, seven have failed to establish and the establishment of four is still to be confirmed. Four of the seven agents that failed to establish were foliage feeders on boneseed (*Chrysanthemoides monilifera* ssp. *monilifera* (L.) T. Norl.). Only the ragwort (*Jacobaea vulgaris* Gaertn.) program has been completed, with the establishment of a root feeder and two stem and crown borers now providing substantial to complete control. The benefit:cost ratio of the ragwort program has been estimated at 32:1 through annual multimillion dollar savings in lost production to pastoral industries, with benefits expected to increase through the continuing dispersal of established agents. Work on continuing programs involves inputs into the host testing, rearing and release of additional agents, the redistribution of established agents from nursery sites and agent efficacy assessments. Completion will continue to rely heavily on long term funding from state and national governments if the potential public benefits that these and future programs offer are to be achieved.

Introduction

In 2006, the annual cost of weeds to Tasmanian pastures and field crops was conservatively estimated at *ca.* \$58 million (Ireson et al., 2007). This figure consisted of approximately \$49.2 million in production losses and \$8.8 million in herbicide costs. Labour costs and other associated weed control activities were not included in the estimate of \$857 million which was about 7% of the then gross annual value of agricultural production in Tasmania. There has been no attempt to calculate the cost of environmental weeds to Tasmania due to the lack of quantitative data, although a system for rating weed impact in Tasmanian natural ecosystems has been described (Rudman 2006).

Biological control programs in Tasmania are

currently targeting 11 species of pasture weeds, and five predominantly environmental weeds including blackberry (*Rubus fruticosus* L. aggregate) which is also a significant pasture weed. The majority (89%) of the biological control agent releases have been conducted over the last two decades. Only four agents were released in Tasmania prior to 1990 with the gorse seed weevil, *Exapion ulicis* (Forster), being the first in 1939. The ragwort seed fly, *Botanophila jacobaeae* (Hardy), was released 24 years later in 1963, but failed to establish. These were followed by the initial releases of two species of ragwort flea beetle, *Longitarsus flavicornis* (Stephens) and *Longitarsus jacobaeae* (Waterhouse), in 1979 and 1988 respectively. This paper lists the agents that have been deliberately released for the biological control of weeds in Tasmania and summarises the current status of each program.

Method of agent introductions

Many of Tasmania's most important pasture and environmental weeds are also problems in other parts of south-eastern mainland Australia especially Victoria and to a lesser extent South Australia and south-eastern New South Wales. Therefore, in most cases, Tasmania has been the recipient of biological control agents from programs initiated either by the Commonwealth Scientific and Industrial and Research Organisation (CSIRO) or jointly by CSIRO and State Government Departments of Primary Industries in other states, particularly Victoria. An exception was the biological control program for gorse (*Ulex europaeus* L.). Gorse, a Weed of National Significance (Thorp and Lynch, 2000) and a serious problem in Tasmania, was declared a target for biological control in 1995 by the Standing Committee of Agriculture and Resource Management after nomination by the then Tasmanian Department of Primary Industry and Fisheries (Ireson et al., 1999a). The Tasmanian Institute of Agricultural Research (TIAR) contracted Landcare Research New Zealand Ltd. to conduct host specificity tests on gorse agents established in New Zealand with funding support from the Commonwealth Government's Natural Heritage Trust. Agents were introduced to Australia through the Department of Primary Industries (DPI) Victoria, using the quarantine facility at Frankston. Agents for boneseed (*Chrysanthemoides monilifera* ssp. *monilifera* (L.) T. Norl.), English broom (*Cytisus scoparius* (L.) Link), Cape broom (*Genista mospessulana* (L.) L.A.S. Johnson) and ragwort (*Jacobaea vulgaris* Gaertn.) were also introduced to Tasmania through this quarantine facility or through collections from established mainland field sites. The agents have either been released directly into the field or used to start mass rearing cultures to provide stock for ongoing release programs. All weed biological control programs in Tasmania are now conducted through TIAR.

Results

By the end of 2011, 35 agents had been deliberately released for the control of 16 weed species. The agents include three fungal pathogens and 32 species of invertebrates of which 29 are insects and three are mites (Table 1). Of these, 24 (68.6%) have established,

seven (20.0%) have failed to establish and the establishment of four (11.4%) is still to be confirmed. The guild of invertebrate agents released includes 13 foliage feeders, eight root and crown feeders, three stem and/or crown feeders, three seed feeders, two bud feeders, one branch borer and one inflorescence feeder (Table 1). Four of the seven agents that have failed to establish were foliage feeders on boneseed (Table 1). Failure of these agents to establish was attributed to high levels of predation by a complex of mainly generalist predators (Ireson et al., 2002).

Of the programs currently underway (Table 1), only the ragwort project is close to completion. The root feeding effects of the ragwort flea beetle, *Longitarsus flavicornis* (Stephens), have provided effective control at many sites around the state. In areas where the impact of this agent has been restricted by site conditions (Potter et al., 2007), there is now anecdotal evidence that the complementary effects of the ragwort stem and crown boring moth, *Cochylis atricapitana* (Stephens) and the ragwort plume moth, *Platyptilia isodactyla* (Zeller) are now resulting in effective control as they continue to spread naturally. Work being conducted on the other programs includes the host testing, rearing and release of additional agents, the widespread redistribution of established agents from localised nursery sites and assessment of agent efficacy (Table 1).

Discussion

Using the criteria of Hoffmann (1995) the biological control of ragwort can be classified as ranging from substantial to complete in many of parts of Tasmania where the weed has been a major problem. Evidence that *L. flavicornis* has been a key factor in the control of ragwort comes from long term efficacy studies, the widespread establishment of *L. flavicornis* and photographic records (Ireson et al., 1991; Ireson et al., 2007). The control achieved by *L. flavicornis* is now producing significant economic benefits (Page and Lacey, 2006); however, on some properties unfavourable site conditions and incompatible management strategies have restricted its impact. Two other agents, the ragwort stem and crown boring moth, *C. atricapitana*, and the ragwort plume moth, *P. isodactyla*, are also now well established in Tasmania. In Victoria, McLaren et al. (2000) and Morley and Bonilla (2008) showed that

these agents are capable of significantly reducing plant vigour and reproductive output. Both species have been observed causing considerable damage to crowns and stems at field sites in Tasmania (Ireson, unpubl. data) and are expected to provide significant control in areas where *L. flavicornis* impact is restricted.

Apart from ragwort, the other Tasmanian weed biological control programs vary in their stage of development and the amount of resources available for their continuation. Of the other weeds targeted, gorse, blackberry, spear thistle (*Cirsium vulgare* (Savi) Tenore), slender thistle species (*Carduus* spp.), horehound (*Marrubium vulgare* L.) and dock species (*Rumex* spp.), were all listed among the top twenty weeds either across the state or regionally following landholder surveys (Ireson et al., 2007). Agent releases have also been conducted for Paterson's curse (*Echium plantagineum* (L.)) and cotton thistle (*Onopordium acanthium* L.). Although causing problems in localised areas, these two pasture weeds are not serious problems in Tasmania compared to other states. The small, scattered infestations of these weeds in Tasmania are being contained through spray programs and, if persistent enough, could enable eradication from the localised areas in which they occur. However, as biological control is being used against these weeds in mainland states, agents have been introduced to Tasmania and selectively released at sites which have been difficult to access by conventional control methods. In the long term it is hoped that these sites can act as nursery sites from which the agents can either disperse naturally or can be collected and distributed to other sites in areas and suppress these weeds where control has been found difficult.

More work is required on the establishment and redistribution of agents for spear thistle, slender thistles, nodding thistle and cotton thistle in Tasmania. Host specific strains of the thistle receptacle weevil, *Rhinocyllus conicus* (Frolich), adapted to the life cycle of spear thistle, slender thistles and nodding thistle have been released in Victoria and New South Wales, but field surveys to determine their distribution in these states are yet to be conducted. As no previous releases have been conducted in Tasmania, transfer could be undertaken if the agents are found to be well established at any mainland release sites. The spear thistle crown weevil, *Trichosiocalus horridus* (Panzer), the nodding

thistle gall fly, *Urophora solstitialis* L., and two cotton thistle agents, the crown weevil, *Trichosiocalus briesei* (Alonso-Zarazaga & Sánchez-Ruiz) and the noctuid moth, *Eublemma amoena* (Hübner), have also been released at mainland sites so these agents could also be considered for transfer to Tasmania once their establishment status has been assessed. A continuing redistribution programme for the thistle crown weevil, *Trichosiocalus mortadello* (Alonso-Zarazaga & Sánchez-Ruiz) (Alonso-Zarazaga and Sánchez-Ruiz 2002), on species of slender thistle is also required from the one established Tasmanian site where it was released in 1998 (Table 1).

Boneseed, English broom and Cape broom are currently the main focus of biological control programmes on environmental weeds in Tasmania. Bridal creeper (*Asparagus asparagoides* (L.) Druce) infestations are relatively small and localised in Tasmania, but the relevance of biological control will now depend on the outcome of attempted eradication programs.

Host specificity testing to enable the introduction of new agents is either underway or planned for several weed species. For instance, three folivores and one seed feeder have already been released for gorse but it is evident that an additional agent or agents will still be required to significantly reduce plant vigour (Ireson et al., 2006; Hill et al., 2008). The seed-feeding gorse pod moth, *Cydia succedana* (Denis and Schiffermüller) is widely established in New Zealand and, in combination with the gorse seed weevil, *Exapion ulicis* (Forster) has resulted in seed destruction levels as high as 92% (T.R. Partridge unpubl. data cited by Hill and Gourlay, 2002). Modelling studies (Rees and Hill, 2001) have indicated this should be high enough to reduce the recruitment of gorse below replacement levels. The gorse pod moth therefore has the potential to play a significant role in the biological control of gorse and host specificity studies are underway to determine whether the agent is safe to release in Australia. The host specificity of the broom leaf beetle, *Gonioctena olivacea* Förster, the broom shoot moth, *Agonopterix assimilella* Treitschke, on English broom (Sagliocco, 2009; Sagliocco, pers. comm.) and the boneseed rust, *Puccinia mysirphylli* G. Winter, are also currently under investigation.

Weeds that may be the target of future programs include sea spurge (*Euphorbia paralias* L.), a major environmental weed along the coast of southern

Australia and a significant problem along the Tasmanian coastline. The weed was declared a target for biological control by the Australian Weeds Committee in 2010 following nomination by CSIRO and Department of Primary Industries and Water, Tasmania (Scott et al., 2010b). Potential agents were reviewed by Scott et al. (2010a). Investigations into the biological control of serrated tussock (*Nassella trichotoma* (Nees) Arech.) (Casonato et al., 2004) may also eventually result in agents being made available for control of this weed in Tasmania.

The economic benefits of successful Australian biological control programs have been demonstrated (Page and Lacey, 2006), but as many programs may take longer than twenty years it can be difficult to justify continued funding over such a long period. Consequently, many biological control programs are often poorly resourced and not fully evaluated once the agents are released (McFadyen, 2000). Therefore, it will be important that evidence of any long-term successes are recorded in order to justify further investment in weed biological control (McFadyen, 2000; Briese et al., 2003).

In recent years, a combination of factors including new regulatory procedures, an overall decline in funding as well as the retiring of experienced practitioners or loss of experienced staff due to funding cuts has contributed either to a declining trend in the number of agents released in Australia or lack of project continuity during the last decade. In Tasmania as in other Australian states, the maintenance of weed biological control programs faces an uncertain future.

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Table 1. Status of weed biocontrol agents deliberately released in Tasmania (E = established, EU = establishment uncertain, N = not established)

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Asparagus asparagoides</i> (L.) Druce (Bridal creeper)	<i>Zygina</i> sp. Hemiptera: Cicadellidae) (Bridal creeper leafhopper)	Foliage, stem	1999 2000	E	Ex South Africa (Bachelor and Woodburn, 2002). Established at the only release site in northern Tasmania and on Flinders Island. As bridal creeper infestations in Tasmania are relatively small and localised, attempts have been made to eradicate them using herbicide.
	<i>Puccinia myrsiphylli</i> G. Winter (Uredinales: Phragmidiaceae) (Bridal creeper rust fungus)	Foliage	2000	E	Ex South Africa (Morin et al., 2002). Established after release at site in northern Tasmania and on Flinders Island where it is still surviving with <i>Zygina</i> sp. despite the above-mentioned eradication program.
<i>Chrysanthemoides monilifera</i> ssp. <i>monilifera</i> (L.) T. Norl. (Boneseed)	<i>Chrysolina scotti</i> Daccordi (Coleoptera: Chrysomelidae) (Black boneseed beetle)	Foliage	1991-1993 1995-1996	N	Ex South Africa (Downey et al., 2007). Failed to establish after multiple releases. Biotic resistance by invertebrate predators is suspected as a key factor in preventing establishment (Meggs, 1995; Ireson et al., 2002).
	<i>Comostolopsis germana</i> Prout (Lepidoptera: Geometridae) (Bitou tip moth)	Foliage	1993-1995 1996-1997	N	As above.
	<i>Chrysolina</i> sp. B (Coleoptera: Chrysomelidae) (Painted boneseed beetle)	Foliage	1995	N	As above.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
	<i>Tortrix</i> sp. (Lepidoptera: Tortricidae) (<i>Chrysanthemoides</i> leaf roller moth)	Foliage	2000-2004	N	As above.
	<i>Aceria</i> sp. (Acari: Eriophyidae) (Boneseed leaf buckle mite)	Foliage	2008-2011	EU	Ex South Africa. Surviving only at release point at five sites and starting to disperse from one site, up to three years after release. Establishment still uncertain.
<i>Carduus</i> spp. (Slender thistles, nodding thistle) <i>Carduus pycnocephalus</i> (L.) (Slender thistle), <i>Carduus tenuiflorus</i> Curtis (Winged slender thistle), <i>Carduus nutans</i> L. (Nodding thistle)	<i>Puccinia cardui-pycnocephali</i> Sydow (Uredinales: Phragmidaceae) Slender thistle rust fungus)	Foliage	1993, 1994, 1997	E	Ex France and Italy. Now widespread. Releases of two aggressive Mediterranean isolates were made in Tasmania in 1993 and again in 1994 and 1997 on <i>C. pycnocephalus</i> . <i>C. tenuiflorus</i> was also present at some release sites. Tasmanian studies showed that the rust could reduce plant size and flower production (Burdon et al., 2000) but the impact of the pathogen alone was insufficient to reduce thistle densities.
	<i>Trichosirocalus mortadelo</i> Alonso-Zarazaga & Sanches-Ruiz (Coleoptera: Curculionidae) (Rosette weevil)	Root crown	1998	E	Ex Germany via Canada via New Zealand (Woodburn, 1997). Originally established at one site at Westbury in northern Tasmania on slender thistle. The site has been used as a nursery to collect and transfer adults to other sites. Agent also uses spear thistle (<i>Cirsium vulgare</i>) (present at Westbury site) as marginal host (Woodburn and Swirepick, 2002). Dispersal and impact in Tasmania has not been assessed.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Cirsium vulgare</i> (Savi) Tenore (Spear thistle)	<i>Urophora stylata</i> (Fabricius) (Diptera: Tephritidae) (Spear thistle gall fly)	Seed head	1997 2010	E	Ex France. Not established from release in 1997, but now established at two sites following re-releases in 2010 following transfer from site in Victoria.
<i>Cytisus scoparius</i> (L.) Link (English broom)	<i>Leucophaea spartifoliella</i> (Hübner) (Lepidoptera: Lyonetiidae) (Broom twig mining moth)	Branches	1996 1998 2004-2008	E	Ex Europe via New Zealand. Failed to establish from releases of material imported from New South Wales in 1996 and again in 1998 at seven sites. Recovered and spreading from one site in the Tasmanian midlands in 2005 following release in 2004 of material collected from New South Wales site. Releases have been conducted at four other sites since 2004 from a shade-house culture but establishment surveys at these sites are yet to be conducted.
	<i>Arytainilla spartiophila</i> (Forster) (Hemiptera: Psyllidae) (Broom psyllid)	Buds, new growth	1996	EU	Ex United Kingdom via New Zealand. Released at three sites at the same time as <i>L. spartifoliella</i> (above) but not recovered. Establishment still possible from planned additional releases of field collected stock from mainland.
	<i>Aceria genistae</i> (Nalepa) (Acarina: Eriophyidae) (Broom gall mite)	Buds	2009-2011	E	Ex France (Sagliocco, pers. com.). Released at 37 sites. Agent has been recovered and is dispersing.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Genista monspessulana</i> (L.) L.A.S. Johnson (Cape broom, Montpellier broom)	<i>Arytinnis hakani</i> (Loginova) (Hemiptera: Psyllidae) (Cape Broom Psyllid)	Foliage, stems, branches	2009-2011	E	Ex France (Henry et al., 2009). Released at 40 sites. Agent is becoming widespread and causing severe damage.
<i>Echium plantagineum</i> (L.) (Paterson's curse)	<i>Dialectica scalariella</i> (Zeller) (Lepidoptera: Gracillariidae) (Echium leaf miner)	Foliage	1990	N	Ex France and Portugal. Reared then released at 14 sites in Tasmania between July 1990 and April 1992 (Ireson, unpubl. data). Initially recovered and dispersed rapidly from release sites but population subsequently declined possibly due to its inability to survive cold winters.
	<i>Mogulones larvatus</i> (Schultz) (Coleoptera: Curculionidae) (Paterson's curse crown weevil)	Crown, petioles, foliage	2004 2007 2008	E	Ex France. Field collected adults imported from South Australia and released at three sites. Recovered and dispersing from one site.
	<i>Mogulones geographicus</i> (Goeze) (Coleoptera: Curculionidae) (Paterson's curse root weevil)	Taproot, petioles, foliage	2004 2006-2007 2009	E	Ex France. Field collected adults imported from South Australia and released at three sites. Recovered and dispersing from two sites.
	<i>Longitarsus echii</i> (Koch) (Coleoptera: Chrysomelidae) (Paterson's curse flea beetle)	Taproot, crown, foliage	2004 2008-2009	E	Ex France and Spain. Field collected adults imported from South Australia and released at six sites. Established and dispersing from two sites. Establishment assessments at other sites yet to be conducted.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
	<i>Meligethes planiusculus</i> (Heer) (Coleoptera: Nitidulidae) (Paterson's curse pollen beetle)	Flower buds, pollen, ovules, immature seed	2008	EU	Ex France. Field collected adults imported from South Australia and released at three sites. Establishment assessments yet to be conducted.
<i>Onopordum acanthium</i> L. (Cotton thistle)	<i>Lixus cardui</i> Olivier (Coleoptera: (Curculionidae) (Stem-boring weevil)	Stem, foliage	1997 2009	E	Ex France. Field collected stock imported from New South Wales. Release at one site in northern Tasmania in 1997 and at site in Tasmanian midlands in 2009. Agent has been recovered and is dispersing.
	<i>Larinus latus</i> Herbst (Coleoptera: Curculionidae) (Seedhead weevil)	Seed	1999 2009	E	Ex Greece. As for <i>L. cardui</i> (above).
<i>Rubus fruticosus</i> L. aggregate (Blackberry)	<i>Phragmidium violaceum</i> (Schultz) (Uredinales) Phragmidiaceae (Blackberry rust)	Foliage, buds, fruit, canes	2009	E	Ex Europe. Three different strains released at three separate sites are established and spreading. It is hoped these new strains will hybridise with existing rust populations and produce genotypes with a greater impact on the growth and vigour of blackberry than an illegally introduced strain first identified in Tasmania in 1985.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Rumex</i> spp. (Dock) Common Tasmanian pasture species are: <i>Rumex crispus</i> (L.) (Curled dock) & <i>Rumex obtusifolius</i> (L.) (Broadleaf dock)	<i>Pyropteron doryliformis</i> (Ochsenheimer) (Lepidoptera: Sesiidae) (Dock moth)	Roots, root crown	1997	E	Ex Morocco (Scott and Saggiocco, 1991). Released at three sites in northern Tasmania and recovered during surveys in 2006. Although no efficacy study has been conducted, anecdotal and visual evidence indicates <i>P. doryliformis</i> has had a significant impact on <i>Rumex</i> spp.
<i>Jacobaea vulgaris</i> Gaertn. (Ragwort)	<i>Botanophila jacobaeae</i> (Hardy) [formerly <i>Pegohylemia jacobaeae</i> (Hardy), also referred to as <i>Hylemia jacobaeae</i> Meade and incorrectly as <i>Hylemia seneciella</i> (Meade)] ³ (Diptera: Anthomyiidae) (Ragwort seed fly)	Seed	1963	N	Ex England via New Zealand.
	<i>Longitarsus flavicornis</i> (Stephens) (Coleoptera: Chrysomelidae) (Ragwort flea beetle)	Roots, foliage, root crown	1979-1985	E	Ex France. Now widespread. High level of control in most areas with reductions in plant densities in excess of 90% (Ireson et al., 1991; Ireson et al., 2000b).

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
			1986-1989		Ex Spain. Established at six sites but dispersal unknown due to difficulties in distinguishing field populations from the dominant French biotype (Ireson et al., 1999b). Efficacy probably similar.
	<i>Longitarsus jacobaeae</i> (Waterhouse) (Coleoptera: Chrysomelidae) (Ragwort flea beetle)	Roots, foliage, root crown	1988-1992	E	Ex Italy via Oregon USA via New Zealand. Established at six sites but dispersal unknown due to difficulties in distinguishing field populations from <i>L. flavicornis</i> (Ireson et al., 1999b).
	<i>Tyria jacobaeae</i> (L.) (Lepidoptera: Arctiidae) (Cinnabar moth)	Foliage, flowers	1993-1999	N	Ex England via New Zealand (Miller, 1929). Not established probably because of high levels of predation and lack of suitable pupation sites (Ireson et al., 1999b).
	<i>Cochylis atricapitana</i> (Stephens) (Lepidoptera: Cochylidae) (Ragwort stem and crown boring moth)	Stem, root crown	1995-2000 2004-2005 2009-2010	E	Ex Spain (McLaren, 1992). Dispersing from 22 release sites. No Tasmanian efficacy studies conducted but surveys indicate larval feeding stunting growth of flowering plants and contributing to a decline in rosette density as in Victoria (McLaren et al., 2000).
	<i>Platyptilia isodactyla</i> (Zeller) (Lepidoptera: Pterophoridae) (Ragwort plume moth)	Stem, root crown	2000-2007	E	Ex Spain (McLaren et al., 2000). Dispersing from 17 release sites. Significant larval damage to plants observed. No Tasmanian efficacy studies conducted but impact on plant vigour and reproductive output probably similar to that recorded in Victoria (Morley and Bonilla, 2008).

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Ulex europaeus</i> L. (Gorse)	<i>Exapion ulicis</i> (Forster) (Coleoptera: Brentidae) (Gorse seed weevil)	Seed	1939	E	Ex England via New Zealand (Evans, 1942). Now widespread (Ireson et al., 2006). Destruction to mature seed ranges from 12.4-55.4% annually but is below that required to have impact on gorse (Davies et al., 2008).
	<i>Tetranychus lintearius</i> Dufour (Acari: Tetranychidae) (Gorse spider mite)	Foliage	1998-2001	E	Ex England, Portugal and Spain via New Zealand (Ireson et al., 1999a). Now widespread (Ireson et al., 2003). Reductions in foliage dry weight of ca. 36% over 2.5 years from the time of release have been measured (Davies et al., 2007). Surveys indicate predation by Chilean predatory mite (<i>Phytoseiulus persimilis</i>) and predatory ladybird beetles (<i>Stethorus</i> spp.) restrict impact (Ireson et al., 2003).
	<i>Sericothrips staphylinus</i> Haliday (Thysanoptera: Thripidae) (Gorse thrips)	Foliage	2001-2009	E	Ex England via New Zealand (Ireson et al., 2008). Now widespread throughout the state but populations have been slow to increase and no visible foliar damage has yet been recorded.
			2003-2009		Ex Portugal via Hawaii via New Zealand (Ireson et al., 2008). Now widespread. Populations now mixed with populations ex England via New Zealand.
	<i>Agonopterix umbellana</i> (Fabricius) (Lepidoptera: Oecophoridae) (Gorse soft shoot moth)	Foliage	2007-2010	E	Ex England via New Zealand (Ireson, unpublished data). Dispersing from two sites and recovered from another four. Releases and monitoring are continuing.

Target weed	Agent	Part of plant affected	Year(s) released ¹	Status	Comments ²
<i>Marrubium vulgare</i> L. (Horehound)	<i>Wheeleria spilodactylus</i> (Curtis) (Lepidoptera: Pterophoridae) (Horehound plume moth)	Foliage	1997-1999	E	Ex France (Sagliocco, 2000). Widespread (Ireson et al., 2000a; Ireson unpubl. data). No efficacy studies conducted in Tasmania, but severe defoliation by larvae during spring probably reducing plant vigour and seed output.
	<i>Chamaesphecia mysiniiformis</i> (Boisduval) (Lepidoptera: Sesiidae) (Horehound clearwing moth)	Roots	2008-2011	EU	Ex France (Sagliocco and Weiss, 2004). Releases at one trial site following importation from South Australia. Releases and monitoring at site continuing.

¹This refers not only to the year the first release of the agent was conducted but also the periods over which the agent has been released from cultures if there has been an ongoing mass rearing program. Periods over which field redistribution programs have been conducted for some agents are not included. Mass rearing and field release programs have continued in Tasmania over several years for some agents difficult to establish or slow to disperse.

²Agent origin as listed by Julien and Griffiths (1998) unless otherwise indicated.

Spatial Monitoring of the Dispersal, Target and Non-Target Impact of the Unintentionally Introduced Biological Control Agent *Mogulones cruciger* in the Northwestern USA

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Abstract

The root-mining weevil *Mogulones cruciger* Herbst. was released for the control of the rangeland weed *Cynoglossum officinale* L. in Canada in 1997. In the USA, concerns about the risk of non-target plant feeding on rare and endangered confamilials of *C. officinale* were raised and the permission for field release was ultimately denied by regulatory authorities in 2002. Differences in the environmental safety assessment of *M. cruciger* between the two countries may be attributed to the much larger number of native confamilials of *C. officinale* in the USA, especially the number of federally protected species (9 in the USA), and the weevil's fundamental host range, which does include some of the federally protected species. The weevil has successfully controlled *C. officinale* in south-central British Columbia but its widespread distribution has led to its inadvertent introduction in the USA most likely during 2008. In 2010, regulatory authorities issued a pest alert for the insect in the USA because of the non-target plant feeding risks and to avert any anthropogenic distribution of *M. cruciger*. The weevil occurs less than 100 km distant from the federally protected confamilial *Hackelia venusta*, that it is able to feed on. We began a project in 2010 to monitor weevil abundance and attack on *C. officinale* and all sympatric confamilials (currently 4 native and 6 exotic species) in a 50 km by 100 km area in northern Washington and a 20 km by 50 km area in northern Idaho where the weevil occurs. We use Global Positioning System (GPS) and Geographical Information System (GIS) tools to track abundance and weevil attack with regard to *C. officinale* and non-target plant densities. Currently weevils can be found up to 40 km south of the border. *M. cruciger* does disperse faster than previously assumed. Non-target feeding thus far is rare and occurred on 2 native confamilials.

Temporary Spillover? Patch-Level Nontarget Attack by the Biological Control Weevil *Mogulones crucifer*

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Abstract

Nontarget attack in weed biological control can be classified as persistent or temporary (spillover). A primary question in assessing nontarget risk is whether an agent is threatening the persistence of nontarget plant populations. Insect damage to individual plants does not necessarily translate to population-level effects, but it can be argued that persistent attack may be more damaging than temporary spillover. *Mogulones crucifer* Herbst. (Coleoptera: Curculionidae) is a root-feeding weevil that was approved for release in Canada in 1997 to control houndstongue (*Cynoglossum officinale* L., Boraginaceae). Since its release, *M. crucifer* has frequently been successful in suppressing houndstongue, but it also has been observed attacking native, nontarget Boraginaceae in western Canada. In 2009, groups of 300 *M. crucifer* were released at nine rangeland sites containing the native nontarget borage, blue stickseed (*Hackelia micrantha* (Eastw.) J.L. Gentry), either growing without houndstongue or interspersed with the weed. Release sites were revisited 4-7 weeks later and indications of *M. crucifer* attack were observed on both plant species within a 10-meter radius of release, but significantly more frequently on houndstongue. When plants from three sites were harvested and dissected 11 weeks after release, *M. crucifer* larvae were found in both species, but were significantly more abundant in houndstongue (Wilcoxon Rank Sum test, $p=0.0425$). Release sites were revisited in 2010, when attack on houndstongue continued, but indications of nontarget attack were rare. To determine whether nontarget attack observed in 2009 was temporary spillover, or the initial establishment of weevils on nontargets, plants of both species were harvested and dissected in 2011 to quantify the level of target and nontarget attack 2 years post release. *M. crucifer* eggs and larvae were found in houndstongue, but not in *H. micrantha*, suggesting the attack observed in 2009 was temporary spillover.

Avoid Rejecting Safe Agents – What More Do We Need to Know? St. John's Wort in New Zealand as a Case Study

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Abstract

St. John's wort beetles (*Chrysolina hyperici* Forster and *C. quadrigemina* Suffrian), were introduced to New Zealand (NZ) in 1943 and 1965 (respectively) to control St. John's wort (*Hypericum perforatum* L.), without any NZ-focused host range testing. The beetles produced one of NZ's greatest classical weed biocontrol successes. In a recent retrospective host range testing study we found that, under current safety standards, these beetle species would almost certainly have not been introduced into NZ. This is due to successful oviposition, feeding and development on indigenous *Hypericum* species. However, field surveys portray a more complex picture, with the indigenous *Hypericum* species possibly declining, but suffering little to none non target feeding by the biocontrol agents. This raises the question – what more do we need to know in order to better interpret risk apparent in artificial arenas in containment to improve risk assessment? Unusually, the response of some *Chrysolina* spp. to the secondary plant chemical hypericin in *Hypericum* spp. has been well-studied, but we show that this information would not have helped in *a priori* risk assessment. More positively, we discuss how knowledge of the seasonal phenology of the herbivores and plants, and the potential for direct and apparent competition between the target weed and indigenous congeners, could be used to improve agent risk assessment and perhaps avoid rejecting excellent and safe weed biocontrol agents in the future.

This study is now published and the full reference is:

Groenteman, R., Fowler, S. V., & Sullivan, J. J. (2011) St. John's wort beetles would not have been introduced to New Zealand now: A retrospective host range test of New Zealand's most successful weed biocontrol agents. *Biological Control* 57, 50-58.

Predicting Success? A Tale of Two Midges

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Abstract

Two closely related midges, *Dasineura dielsi* Rübsaamen and *Dasineura rubiformis* Kolesik, are successfully established biological control agents in South Africa against *Acacia cyclops* A. Cunn. Ex. G. Don and *Acacia mearnsii* de Wild respectively. Initial establishment for both species occurred at the same locality and at the same time but their subsequent performance varied considerably. Both species oviposit within the flower heads of their hosts and the developing larvae induce clusters of galls that preclude pod development. However, the two species differ in several respects enabling comparison of the biological characteristics underlying their performance. *D. dielsi* develops entirely within the gall structure, is multivoltine and disperses rapidly, whilst *D. rubiformis* spends part of its life cycle in the gall and the remainder in the soil, is univoltine and disperses less readily. Mortality factors for each of the midge species are quite different, and impact different life stages, but overall mortality for both species can be extremely high. Despite this, both species are proving to be successful in their own right which highlights the difficulties of predicting a biological control “winner”.

Biological Control of Musk Thistle in the Southeastern United States: A 20-year Assessment of Benefits and Risks

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Abstract

A state-wide, multi-agency project was initiated in 1989 in Tennessee to implement an integrated pest management (IPM) program, emphasizing classical biological control, against a widespread and problematic exotic weed, musk thistle (*Carduus nutans* L.). The biological control component included releases of two herbivorous weevils, *Rhinocyllus conicus* (Froelich) and *Trichosiocalus horridus* (Panzer), at numerous locations throughout Tennessee. This program was widely embraced by farmers, landowners, urban dwellers, state agencies, federal agencies, extension personnel, and others. Funding was provided by state and federal agencies, including the Tennessee Department of Transportation (TDOT). In 1991, this program was expanded regionally into the southeastern U.S. (Georgia, North Carolina, Tennessee, and Virginia [where releases began in 1970s]). Program benefits to farmers and the citizenry of Tennessee are tremendous. Densities of musk thistle have been reduced by approximately 90% in areas where introduced herbivorous insects have become established. This reduction in weed densities has contributed to a significant economic savings to farmers and landowners, as well as state and federal agencies. For example, TDOT saves about one million dollars annually in reduced costs for labor and materials for herbicide applications and mowing of thistle-infested areas. Although program benefits are extensive, potential risks, especially environmental concerns and impacts on non-target native thistle species, do exist. In studies conducted in other areas of the continental U.S., feeding of *R. conicus* and *T. horridus* on non-target, native *Cirsium* species has been documented. Research conducted in Tennessee did not document *R. conicus* on naturally-occurring populations of native thistles; however, field-cage studies demonstrated the potential for *R. conicus* to use several native thistle species as hosts. Several non-target native thistle species also supported populations of *T. horridus* in Tennessee. This paper addresses the benefits and risks of using biological control as a component of an IPM program directed against musk thistle in the southeastern U.S.

Differences in Growth and Herbivore Resistance in Hybrid Populations of the Invasive Tree Tamarisk (*Tamarix* sp.) in the Western United States

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Abstract

The biological control agent *Diorhabda carinulata* (Desbrochers) (Coleoptera: Chrysomelidae) sometimes fails to establish or repeatedly avoids particular hybrid genotypes of the invasive plant tamarisk (*Tamarix* sp.). While the failure of *D. carinulata* to establish can sometimes be attributed to predation, the exact role that plant hybridization plays in resistance to herbivory is unknown. We designed field and common garden experiments to examine how hybrid genotypes from various populations affect *D. carinulata* feeding. Hybrid tamarisk plants representing 14 North American populations and two Chinese populations of *Tamarix chinensis* Lour. were collected and propagated in a common garden in Colorado. The level of species introgression for each plant in the study was determined using amplified fragment length polymorphisms (AFLPs). Plants were measured for growth rates, height, canopy size, and biomass. We subjected living plants to herbivory by *D. carinulata* confined within cages. Additionally, we used a novel bioassay consisting of dried plant material, agar, and water to test resistance of hybrid genotypes to herbivory by *D. carinulata*. Plants with high levels of *T. chinensis* introgression were fed upon less than plants with high levels of *Tamarix ramosissima* Ledeb. introgression. Also, plants from southern latitudes (high levels of *T. chinensis* introgression) grew faster and larger than plants from both northern USA latitudes and China, suggesting possible evolution of competitive ability. These differences in growth and resistance to herbivory help explain why some populations and individual plants are not attacked by *D. carinulata*. Future host choice experiments may reveal that other species of *Diorhabda* are more successful at controlling these particular individuals.

Estimating Target and Non-Target Effects of *Diorhabda carinulata*, a Biological Control Agent of *Tamarix* in North America

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Abstract

In 2005, *Diorhabda carinulata* (Desbrochers) was approved for field implementation release onto *Tamarix* spp. in 11 states in the US. Since its release, the beetle has spread and is now common and abundant in several Western states. When abundant, beetles will partially or completely defoliate the plant. Starting in 2006, we have been evaluating the effect of the biological control agent on *Tamarix* foliage quantity, quality and tree size. Additionally, we have been collecting data on the density and composition of the surrounding plant community and on the density and species composition of the arthropod community found on *Tamarix* and two other common riparian species, *Populus deltoides* Bartram ex Marsh and *Salix exigua* Nutt. *D. carinulata* is now extremely abundant at 2 of 5 initial release sites. At sites where establishment has occurred, *Tamarix* size is now 40 – 60% smaller than at the time of release. Tree size has increased over the same time period at the sites where the beetle did not establish. Non-target vegetation response: At sites with extensive defoliation, species richness and percent cover have increased over the last 4 years. However, native plant species are responding in the same manner and magnitude as exotic species. These data suggest that vegetation response following *Tamarix* defoliation is dependent upon initial conditions, and that exotic or weedy species are not differentially replacing *Tamarix* at the sites. Non-target arthropod response: On average we found more arthropods per sample on *Tamarix* (9.9) than on willow (2.6) or cottonwood (1.4). This was also true when examining only plant-feeding species (average per sample = 9.2, 2.1 and 1.0 for *Tamarix*, willow and cottonwood, respectively). However, the majority of the difference in herbivore abundance per sample between *Tamarix* and the other tree species is due to high densities of two arthropod species found only on *Tamarix*: The biological control agent *D. carinulata* and the exotic *Tamarix* specialist *Opsius stactogalus* Fieber. If these two non-native herbivore species are removed from the analysis, willow holds more herbivore individuals per sample on average (2.1) than cottonwood (1.0) or *Tamarix* (0.6). The number of predatory individuals was greatest on *Tamarix* (0.5 per sample) followed by willow (0.4) and cottonwood (0.2). The average number of species recorded per site per tree species per year is greatest on willow (38.0). *Tamarix* averaged 33.6 species and cottonwood averaged 21.3 species. Taken together, these data show that the non-native *Tamarix* holds fewer species of arthropods than the native willow but more than native cottonwood. However, due to the presence of two introduced insects, one a biological control agent, *Tamarix* now holds a much greater overall abundance of arthropods than either native tree.

Impact of the Heather Beetle (*Lochmaea suturalis*), a Biological Control Agent for Heather (*Calluna vulgaris*), in New Zealand

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Abstract

The heather beetle, *Lochmaea suturalis* (Thomson), was released into Tongariro National Park, North Island, New Zealand, as a biological control agent for heather, *Calluna vulgaris* (L.) Hull, in 1996. Populations have slowly established and started to damage or kill heather. Heather was planted in Tongariro National Park in 1912 to re-create UK grouse moors, but the grouse failed to establish and heather quickly became an invasive weed. It has now infested more than 50 000 ha of the North Island's Central Plateau including Tongariro National Park and the adjacent Waiouru Military Training Area. Between 2007 and February 2011 beetle populations have grown exponentially at three release sites and severely damaged or killed approximately 100 ha of heather. Prior to successful biological control, the herbicide Pasture Kleen® (2,4-D ester) was applied aerially to manage heather within the Waiouru Military Training Area. Impact assessment plots were set up in 2008 to compare and contrast herbicide application with biological control for the control of heather and the associated responses of native and exotic plant species. After two years heather cover has reduced by 90% after herbicide application, by 99% after heather beetle attack, and by 99.9% following a combination of methods. Herbicide application resulted in significant non-target damage to native shrubs and herbaceous plants, and the exotic grass, *Agrostis capillaris* L., is invading plots following a combination of control methods. It is too early to determine the relative impact of each method on grass invasion. No non-target impacts were found as a result of beetle feeding and there is early evidence that native shrub recovery is occurring following biological control.

The Release, Establishment and Impact of Yellow Starthistle Rust in California

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Abstract

The rust fungus, *Puccinia jaceae* (Oth) var. *solstitialis*, was released in California, USA, during 2003 as a biological control for yellow starthistle, *Centaurea solstitialis* L., 25 years after the organism entered containment as a candidate for biological control. It was the first plant pathogen released in the continental United States under the modern permitting system. 62 accessions of starthistle from around the state proved equally susceptible to the rust. From 2003-2006, greenhouse-based production of rust spores supported a state-wide distribution program. Field inoculations produced disease in 93% of 176 releases. Less than 21% of the inoculated sites had disease reemergence after one year. Re-emergence declined each year with only 5% of the sites having disease after three years. In two sites the rust reappeared annually for five years. In one site, the rust spread over 37 acres within two years. All of the known spore stages were detected naturally-occurring in the field. Hot and dry conditions limit repeated infections by urediniospores. However, mature green leaves and stems can support dense pustule development in certain regions of central California. The transition to teliospore production may be the limiting factor for epidemic development. The lifespan of severely attacked leaves was reduced. Chlorophyll levels were reduced in severely attacked leaves. Plants produced new leaves at the same rate regardless of infection level. Infection reduced pre-bolting plant volume and biomass but did not delay bolting initiation or reduce bolting height and had minor impact on bolting stem diameter. Mature plant biomass, total seedheads, and number of seeds per seedhead were not affected. Rust infection did not affect the attack rate or damage by other insect biological control agents or significantly decrease the competitive ability of starthistle. The capacity of yellow starthistle to compensate for early impacts of infection severely limits the ultimate impact of *P. jaceae*.

Factors Affecting the Biological Control of *Leucaena leucocephala* in South Africa

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Abstract

Leucaena leucocephala (Lam.) de Wit (Fabaceae) is a typical 'conflict' species that has become naturalized in several tropical, subtropical and warm temperate countries worldwide, following deliberate introductions for agroforestry. In South Africa, the plant constitutes an 'emerging weed' that is considered to be in the early stages of invasion. Several features, notably the plant's ability to produce excessive numbers of seeds all year round, have facilitated the invasion of forest margins, riparian zones and other disturbed areas. Deliberate biological control efforts have been confined to South Africa, where a seed-feeding beetle, *Acanthoscelides macrophthalmus* (Schaeffer) (Chrysomelidae: Bruchinae), was tested and released to restrict the plant's spread, without negating its benefits. However, the program has been opportunistic and low key with little evaluation of progress. This contribution thus examines some of the factors that could influence the program's outcome namely: (i) whether dehiscent seeds escape 'predation' by the beetle; (ii) whether the beetle is able to inflict significant and consistent damage in the field; and (iii) whether the beetle's immature stages are susceptible to native predators and parasitoids. Results so far have indicated that: loose seeds are less attractive than canopy-held seeds in pods; beetle damage is consistent but variable and; native natural enemies inflict appreciable levels of mortality. The implications of these factors for the biological control of *L. leucocephala* are discussed.

Is a Regional Interagency, Multi-Year, Multi-System Post-Release Impact Assessment Program Possible?

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Abstract

The demand for a comprehensive monitoring solution to assess the impact of biological control on a system-wide, regional level has been a forgotten focal point of many biological control programs throughout the world. Too often, well-intentioned programs see monitoring components left unfunded or listed as too low of a priority, resulting in their dismissal given typical workloads. To combat this, a group of biological control practitioners from Idaho, USA developed a regional, multi-system, interagency post-release assessment program – the Standard Impact Monitoring Protocol (SIMP). SIMP was developed to be citizen science friendly and statistically sound with regard to data analysis. SIMP is used to document the change in vegetation cover, target weed density and biological control agent abundance over time. This provides land managers with a tool to assess the relative impact of the biological control agent and the corresponding change in vegetation post-biological control agent release. To help facilitate the program, two page documents outlining the process have been drafted for each system with a corresponding data sheet. The data are collected with Global Positioning System (GPS) devices or with hard copy data sheets which are compiled at the end of each field season and entered into a Geographical Information System (GIS) database which can then be exported for analysis. Included in this presentation is an analysis of five years of SIMP data for Dalmatian toadflax, *Linaria dalmatica* (L.) Mill., and *Mecinus janthinus* Germar as a case study. The goals of SIMP are to establish a long-term data set, standardize the data collection process, provide baseline data for new agents, and assess what environmental factors contribute to the success or failure of a biological control agent utilizing a vast data set spanning the range of each target weed's infestation.

The Possible Use of Two Endemic Natural Enemies for Canada Thistle (*Cirsium arvense*) Biological Control in the USA

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Abstract

Canada or creeping thistle, *Cirsium arvense* (L.) Scop. (Asteraceae), is a widespread exotic weed in most of the continental USA. Since the 1960s, five classical biological control agents have been introduced into the USA for thistle management; generally, introduced agents have not been reliably effective. We are examining the possible role of native arthropods and pathogens in controlling Canada thistle populations. One is the distinct lace bug, *Corythucha distincta* Osborn & Drake (Hemiptera: Tingidae), a native insect often found on *C. arvense* in Colorado and adjacent states. Field observations indicate that *C. distincta* periodically reaches outbreak populations on Canada thistle, and may kill leaves and shoots. To assess the possible applied biocontrol potential of the distinct lace bug, we documented host specificity using laboratory no-choice and host choice tests and field host choice tests in 2009 and 2010. These experiments employed nine native US *Cirsium* thistles, sunflower (a native crop plant), two introduced crop plants related to thistles (safflower and cardoon), and three introduced weedy thistles (including Canada thistle). *C. distincta* readily utilized all native thistles tested, with feeding levels similar to, and often exceeding, those on *C. arvense*. Other weedy thistles were very rarely fed upon, and plants outside the subtribe Carduinae (i.e. the 'true' thistles) in Asteraceae were not utilized at all. Thus, the host range of the distinct lace bug apparently consists of native *Cirsium* spp., while introduced weedy *Cirsium* thistles (e.g. Canada thistle and bull thistle, *C. vulgare*) are also utilized. A second endemic organism attacking Canada thistle is the pathogenic fungus *Alternaria cirsinoxia* Simmons & Mortensen (Ascomycetes: Pleosporales: Pleosporaceae). *A. cirsinoxia* was first discovered on *C. arvense* in western Canada and has also been reported from Montana, USA; we have identified it for the first time in Colorado. *A. cirsinoxia* causes Canada thistle foliar chlorosis and necrosis, frequently leading to shoot death, but little is known of its biology under field conditions and, thus, its potential as an applied biocontrol agent. Host specificity of the fungus was assessed by inoculating two native *Cirsium* spp., two crop plants (sunflower and safflower), and Canada thistle. All tested plants developed disease symptoms, though severity was greater on *C. arvense*, safflower, and sunflower (>60% necrosis) than on the two native thistles (<30%). Thus, though *C. distincta* and *A. cirsinoxia* may cause significant damage to Canada thistle under field conditions, both have fairly broad host ranges; they present risks to nontarget native and crop plants, and thus should not be developed as Canada thistle biocontrol agents in the USA.

Long-Term Control of Leafy Spurge, *Euphorbia esula*, by the Flea Beetle *Aphthona nigriscutis*

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Abstract

Although the reputation of classical biological control depends on the scientific evidence for a successful suppression of the population density of the target species by the introduced biological control agent, post-release monitoring has been and still is largely understudied in classical biological weed control. Here we report results of a well-replicated post-release monitoring study assessing the impact of the flea beetle *Aphthona nigriscutis* Foudras on the invasive plant leafy spurge, *Euphorbia esula* L. At eight release sites in Fremont County, Wyoming, USA, the vegetation composition was assessed along permanent transects over a period of 18 years. In addition, flea beetle density was estimated during the initial years after release, as well as the area around each release site with damaged leafy spurge plants. The results show that leafy spurge populations collapsed soon after the release of *A. nigriscutis*, and that perennial grasses and forbs increased in cover, despite the presence of cheatgrass, *Bromus tectorum*. Furthermore, the results strongly suggest that the reduction in leafy spurge cover and biomass was due to the release of the biological control agent.

Drought Stress on Two Tamarisk Populations (Wyoming and Montana) in Containment: Effects on *Diorhabda carinulata* Survival and Adult Size

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Abstract

Several *Diorhabda* spp. beetles (Chrysomelidae) have been released and established as biological control agents of salt-cedar plants or tamarisk (*Tamarix* spp.) in the western US, and the defoliation over several years begins to kill tamarisk plants. Although *Diorhabda carinulata* Tracy and Robbins 2009 has established in northern Wyoming (Lovell), limited or no established has resulted at multiple locations in Montana (including a 250K beetle release near Ft. Peck MT Reservoir). Cage studies were conducted in 2007-2008 to examine how drought stress to Tamarisk plants influenced beetle size and survival. Limited survival of *D. carinulata* in field cages made it difficult to examine the influence of tamarisk drought stress treatments on beetles. However, in 2010 the tamarisk-*Diorhabda* related lawsuit forced the drought experiment to be moved into containment since Colorado *Diorhabda* (interstate transport) was provided, and plant population source (Lovell WY vs. Ft. Peck MT) was added as a factor. The survivorship of CO *Diorhabda* (20 first-instar larvae placed onto each plant with 16 replicates of four water treatments with two plant populations) were lower in well watered plants. Although the study in containment makes it more difficult to compare to field conditions and lacked predation pressure, it was a useful environment to isolate water and plant population treatment effects in this experiment, since upwards of 100% adult beetle survival occurred on some treatment plants. Our results will be combined with predation surveys and experiments to try to answer a simple question: why has *D. carinulata* failed to establish strongly in MT, given successful establishment on tamarisk in northern WY? We will continue to seek to explore other biological control agents for tamarisk in Montana, due to a lack of strong *D. carinulata* establishment to date.

Dispersal, Infection and Resistance Factors Affecting Biological Control of Creeping Thistle by *Puccinia punctiformis*

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Abstract

The noxious weed creeping thistle, *Cirsium arvense* (L.) Scop. causes extensive problems in pasture, landscapes and naturalized areas in temperate regions worldwide. Controlling *C. arvense* with conventional management tactics is difficult due to the plant's robust root system, aggressive growth and wind-dispersed seeds. The rust fungus *Puccinia punctiformis* (F. Strauss) Röhl. is a promising biological control agent that reduces *C. arvense* infestations through fatal, systemic infections. Establishing severe, self-sustaining epidemics of *P. punctiformis* in *C. arvense* requires a thorough understanding of the biology of both pathogen and host. To better understand the conditions under which epidemics can develop, we performed a series of experiments to evaluate dispersal characteristics of the various *P. punctiformis* spore types. Dispersal gradients were measured by releasing spores in windy field conditions and capturing spores at varying distances from the source. Terminal velocities of spores were also compared in a particle settling tower. By all measure, aerial movement of the two major types of *P. punctiformis* spores is significantly different. It is hypothesized that *C. arvense* plants can exhibit genetic resistance to some *P. punctiformis* lines. To investigate this possibility, we also compared resistance of *C. arvense* genotypes across Pennsylvania, which showed differential responses. Finally, to assess optimum timing and host tissue infection court, we evaluated the effects of season and placement of inoculum on the plant tissue.

A Tale of Two Strains: a Comparison of Two Populations of *Eccritotarsus catarinensis*, a Biological Control Agent of Water Hyacinth in South Africa

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Abstract

The mirid, *Eccritotarsus catarinensis* (Carvalho), a biological control agent of water hyacinth, *Eichhornia crassipes* (Mart.) Solms, was originally collected from Rio de Janeiro, Brazil, in 1989 and later recollected and imported to South Africa in 1992 from Florianopolis, Brazil. Despite experiencing a significant bottleneck in quarantine which reduced the population to progeny from a single female, it was released in South Africa in 1996 following host specificity testing which found it to be specific to the Pontederiaceae and damaging to water hyacinth. It established around South Africa, largely in the warmer subtropical regions, but struggled to establish permanent populations in high altitude areas characterized by eutrophic waters and cold winters where water hyacinth is most problematic, requiring frequent reintroductions. A second strain of *E. catarinensis* was collected in 1999 from Iquitos, Peru, a higher altitude site thought to be more climatically similar to South Africa than tropical Brazil, in the hope that this strain would be more tolerant of the cooler South African conditions. Studies comparing aspects of the two strains' thermal physiology and host specificity showed the Peruvian strain to have higher thermal requirements than the Brazilian strain, but a narrower host range as it performed significantly worse than the Brazilian strain on pickerelweed, *Pontederia cordata* L., a close relative of water hyacinth. Subsequent sequencing of the CO1 region of the mitochondrial DNA revealed two distinct haplotypes, one Brazilian and one Peruvian, with a 5.2% sequence divergence. This divergence is greater than that often observed in interspecific comparisons in insects, suggesting that these might be two separate species. Inter-Simple Sequence Repeat (ISSR) data supported the mtDNA results. This highlights the need to investigate the effects of inter-breeding or competition between these highly divergent strains before any additional variation is introduced into the current South African population of *E. catarinensis*.

Disease Development Cycle of Canada Thistle Rust

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Abstract

The obligate rust fungus, *Puccinia punctiformis* (F. Strauss) Rohl., is perhaps the first plant pathogen proposed as a biological control agent for Canada thistle, *Cirsium arvense* (L.) Scop., or any other weed. However the disease cycle of the rust has not been completely understood and this has prevented the rust from being successfully used as a biological control agent. The primary misunderstanding has been over the natural infection court, which has been postulated to be shoot buds on underground roots, an oddity among rust fungi. Rather, our research indicates a different scenario. In the spring, systemically diseased shoots arise from shoots on roots infected with the fungus. The first signs of the fungus on these shoots are orange haploid pycnia (spermagonia) that cross fertilize to form haploid dikaryotic aeciospores. These spores infect leaves of nearby plants and give rise to uredinia that produce haploid dikaryotic urediniospores that, in turn, infect other leaves. In the late summer the uredinia transform into telia which undergo nuclear fusion (karyogamy). Through mitotic division the telia give rise to two-celled diploid teliospores. In the late summer and fall, the plants that emerged in the spring senesce and diseased leaves bearing telia dehisce and deposit teliospores onto newly emerging rosettes. Under conditions of adequate dew, that are common in the fall, the teliospores undergo meiosis and germinate into haploid basidiospores that infect the rosettes. The fungus then develops hyphae that grow into the roots of the rosettes where it survives the winter. Systemically diseased shoots emerge from this rootstock the following spring. Repeated inoculations of rosettes with teliospores in the fall is a practical means of producing systemically diseased plants the following spring, initiating a disease epidemic, and possibly affecting successful biological control.

Local Spatial Structure of Dalmatian Toadflax (*Linaria dalmatica*) and its Effect on Attack by the Stem-Mining Weevil (*Mecinus janthinus*) in the Northwestern United States

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Abstract

The stem-mining weevil, *Mecinus janthinus* Germar was introduced into North America in the mid 1990s as a biological control agent of the herbaceous perennial Dalmatian toadflax, *Linaria dalmatica* (L.) P. Mill. Although considerable emphasis has been placed on assessing impact of *M. janthinus* to stem density and reproduction at the local scale, less emphasis has been placed on assessing how the spatial structure of the weed affects weevil attack and persistence. Moreover, it is unclear whether weevil attack affects the spatial distribution and age structure of Dalmatian toadflax. These factors may in turn affect host-finding ability, local population growth, and effectiveness of *M. janthinus*. This study was initiated to evaluate local spatio-temporal dynamics of Dalmatian toadflax biological control. Four sites displaying substantial population-level variation in toadflax and weevil abundance within the northwestern US (Idaho, Washington, and Oregon) were chosen for study. After the first year of sampling it became apparent that despite variation in spatial structure of Dalmatian toadflax and weevil abundance among sites, activity of *M. janthinus* (adult presence, oviposition, and feeding damage) was aggregated in areas of high stem density. Although further sampling is needed, it appears that toadflax populations are less dense, but more spatially aggregated at sites that have been exposed to *M. janthinus* the longest. Sampling is ongoing at these sites to continue evaluating the local spatial population dynamics of *M. janthinus* and its effect on Dalmatian toadflax.

Differences between Plant Traits and Biological Control Agent Resistance in Rush Skeletonweed Genotypes in North America

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Abstract

Rush skeletonweed, *Chondrilla juncea* L., is a perennial apomictic herb native to Eurasia, which has accidentally been introduced and become invasive in Australia, South- and North America. In Australia rush skeletonweed (RSW) biotypes can be distinguished morphologically and show differential resistance/tolerance to some herbicides and classical biological control agents. Anecdotal data for differences in plant phenology and resistance to biological control agents has also been reported for North American RSW biotypes. Only recently, however, a study using highly variable AFLP (Amplified Fragment Length Polymorphism) markers identified seven distinct genotypes in North America. We compared different plant traits (plant size and architecture, time to bolting and reproduction) and resistance/tolerance to three biological control agents, the RSW gall mite, *Eriophyes chondrillae* Canestrini, the RSW root moth, *Bradyrrhoa gilveolella* Treitschke, and different accessions of the RSW rust, *Puccinia chondrillina* Bubak and Sydenham, for the three dominant North American genotypes under standardized conditions. We found significant differences in plant size and architecture but these were so small that it is not possible to distinguish the three genotypes in the field. We also found differences in the resistance/tolerance to *E. chondrillae* and especially to different accessions of *P. chondrillina*: While one genotype was resistant to all four tested rust accessions, the second was susceptible to all rust accession and the third genotype was resistant to two of the rust accessions. We found no resistance to *B. gilveolella*. At least two strains of *P. chondrillina* were released in North America but there is no information when and where each strain was released, on which genotype and whether or not both strains established. Consequently, we do not know whether RSW genotypes acquired resistance post-release or whether our results are indicative for genotype specific *P. chondrillina*. Our data does, however, illustrate the importance of understanding intra-specific diversity of plant invasions to explain control failures and improve biological weed control programs.

Inundative Release of *Aphthona* spp. Flea Beetles (Coleoptera: Chrysomelidae) as a Biological “Herbicide” on Leafy Spurge (*Euphorbia esula*) in Riparian Areas

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Abstract

Inundative releases of beneficial insects are frequently used to suppress pest insects, but not commonly attempted as a method of weed biological control because of the difficulty in obtaining the required large numbers of insects. The successful establishment of a flea beetle complex, mixed *Aphthona lacertosa* Rosenhauer and *A. nigriscutis* Foudras (87% and 13%, respectively), for the control of leafy spurge (*Euphorbia esula* L.) provided an easily collectable source of these natural enemies that enabled us to attempt inundative release as a possible leafy spurge control method in a sensitive riparian ecological zone where chemical control is restricted. Our target weed populations were small isolated patches of leafy spurge along three streams in southwestern, central and northeastern Idaho. This study assessed leafy spurge and associated vegetation responses to inundative releases of 10 and 50 beetles per spurge flowering stem over two consecutive years. Releasing 10 beetles per flowering stem had inconclusive effects on spurge biomass, crown, stem, and seedling density. Alternatively, releasing 50 beetles per flowering stem resulted in a reduction of biomass, crown and stem density in the range of 60 to 80% at all three study sites, and about a 60% reduction of seedling density at one site, compared to untreated plots. In contrast to leafy spurge, associated vegetation did not conclusively respond to beetle release, indicating that it may take more than two years for desired riparian vegetation to respond to reductions in leafy spurge competition. The paper related to this Abstract has been published on the following journal: Journal of Economic Entomology. 103: 242-248.

Population Dynamics and Impacts of the Red-Headed Leafy Spurge Stem Borer on Leafy Spurge

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Abstract

We evaluated the efficacy of the biological control agent, red-headed leafy spurge stem borer (*Oberea erythrocephala* Schrank.) against the nonnative invasive plant leafy spurge (*Euphorbia esula* L.). Our three treatments were release of the biological control agent into uncaged plots, release of the biological control agent into plots caged to prevent agent escape and control plots caged to prevent agent entry. These treatments were replicated three times at six sites in the western U.S. We measured leafy spurge biomass for one or two years following release. We also measured the percentage of leafy spurge stems showing evidence of red-headed leafy spurge stem borer oviposition for either one or two years following agent release, depending on the site. Red-headed leafy spurge stem borer did not demonstrably reduce leafy spurge biomass in our study. Moreover, compared to the release year, evidence of red-headed leafy spurge stem borer oviposition declined with time, suggesting the agent population was diminishing. This suggests the agent is incapable of building large populations capable of controlling leafy spurge at the sites we studied. However, after being released, populations of biological control agents sometimes go through long lag phases and then begin rapid population increases, so we cannot completely dismiss the possibility that red-headed leafy spurge stem borer might become effective given more time. The paper related to this Abstract has been published on the following journal: *Invasive Plant Science and Management* 2011 4: 183-188

Impact of Pre-Dispersal Seed Predation on Seedling Recruitment by Yellow Starthistle in California

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Abstract

A long-term study site provided an opportunity to examine the impact of pre-dispersal seed predation on seedling recruitment by yellow starthistle (*Centaurea solstitialis* L.). Fifty 20 cm by 20 cm plots were established in a grassy field infested with yellow starthistle. Seedling recruitment and seed production was observed for eight years. In years 4-6, 20 of the plots were treated with a foliar insecticide to reduce the activity of introduced seedhead insects and 30 plots were untreated. In years 7 and 8, no plots were treated with insecticides. Results showed no difference in the number of seedlings recruited between treated and untreated plots during years 1-3. However, seedling number diverged in years 4-6 with the untreated plots showed decreasing numbers while the number of seedlings in treated plots slightly increased. In years 7 and 8 when no plots received insecticide treatments, the number of seedlings in the untreated plots continued to decrease and the number of seedlings in the treated plots renewed their downward decline. The results suggest that the attack of seedheads by the exotic insects had a significant effect on yellow starthistle seedling recruitment.

Early Season Aggregation Behavior in Adult *Larinus minutus*, an Introduced Phytophage of *Centaurea* spp. in North America

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Abstract

Larinus minutus Gyllenhal (Coleoptera: Curculionidae) is a Eurasian beetle that was initially released in North America in 1991 for the biological control of several invasive knapweeds (*Centaurea* spp.). Larvae consume developing achenes within the capitula whereas adults feed on foliage and flowers. This weevil preferentially attacks diffuse knapweed, *Centaurea diffusa* Lam. (Asteraceae). Early season feeding on *C. diffusa* by the adults may lead to outright mortality, especially of seedling and rosette stage plants, or, if attacked bolted plants survive beetle depredation, pronounced tissue shredding/dicoloration, stunting, and flower head deformation typically result. From mid-May to mid-June in eastern Washington, large numbers of *L. minutus* adults aggregate beneath the rosettes of certain *C. diffusa* plants, especially those growing in open areas with well-drained sandy or gravelly soils or soils covered with short grasses and mosses. Soil aggregations of up to 800 adults have been recorded within a 40 cm diameter area around a single plant, with beetles being most numerous near the root crown area of the rosette where they often appear to be “stacked” upon one another. This behavior was not reported by European researchers who conducted pre-introduction studies on the weevil. It has not been determined with certainty if an aggregation or sex pheromone is responsible for this phenomenon or if the beetle is attracted to kairomones emitted by healthy or attacked plants. Field investigations have revealed that adults will re-aggregate beneath heavily damaged plants within one to two days following “beetle depopulation”, thus suggesting the possible involvement of a plant kairomone cue.

Predicting How Fast and Invading Weed Biological Control Agent Will Disperse

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Abstract

We reviewed published dispersal data for 66 arthropod and 11 fungal pathogen weed biological control agents and we tested hypotheses regarding agent characteristics that were predicted to affect dispersal and whether agents that dispersed rapidly were more successful than those which dispersed only slowly. Dispersal rates varied by four orders of magnitude: the fastest agents dispersed several hundred kilometers per year and the slowest by only tens of meters per year. Approximately 30% of the arthropod agents and four of the 11 pathogen agents dispersed less than one kilometers per year, indicating that intensive redistribution is often required for rapid widespread establishment. Successful agents were equally likely to be fast or slow dispersers indicating that effort made redistributing slowly dispersing agents can often be beneficial.

Both pathogen and arthropod dispersal rates were positively correlated with voltinism. Arthropod dispersal also significantly varied according to fecundity, dispersal type (crawling or passive wind dispersal versus flight), taxon, life-style, habitat and the diversity of parasitoids attacking the agent in the native range. We conclude that a few parameters, measured prior to introduction of a biological control agent, could be used to predict how fast it is likely to invade a new environment. This should assist optimization of release strategies by determining the geographic scale at which to release agents, according to the agents' ability to rapidly close the gaps by natural dispersal.

The paper related to this Abstract has been published and the relevant citation details are: Paynter, Q. & Bellgard, S. (2011) Understanding dispersal rates of invading weed biocontrol agents. *Journal of Applied Ecology*, 48, 407-414

Determining the Efficacy of *Larinus minutus* (Coleoptera: Curculionidae) in Spotted Knapweed Biological Control: The Silver Bullet?

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Abstract

Spotted knapweed, *Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek, is an exotic, invasive weed that has caused significant damage in the northwestern United States and can reduce forage production by more than 88%. In spite of a successful biological control program for this in the northwest, the weed is expanding rapidly throughout the southeastern United States, where no comprehensive control program exists. One of the insects thought largely responsible for the weed's decline in the northwest is *Larinus minutus* Gyll. Adult *L. minutus* were collected from areas around Colorado Springs, Colorado, from 2007 through 2011. Adult weevils were returned to Arkansas and released at 39 sites at an average of 700 weevils per release. Studies to determine the effect of *L. minutus* on knapweed seed and plant densities were conducted in 2010 and 2011. The only differences in knapweed population variables at release and non-release sites were seed density and plant height. At weevil release sites, the number of seeds produced was significantly lower and the plants were significantly shorter. Other impacts on stands of spotted knapweed are likely to become evident after populations of *L. minutus* have had more time to increase.

Biological Control of *Solanum viarum* in the USA

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Abstract

Solanum viarum Dunal (Solanaceae) is a perennial prickly weed, native to South America that was found in Florida in 1988, and it has invaded at least 400,000 hectares of grasslands and conservation areas in Florida and several other states. The spread in the United States can be partially attributed to a large seed production per plant, an effective seed dispersal by cattle and wildlife that feed on fruits, and introduction of the plant to new areas without its natural enemies (herbivorous and pathogens) that keep weed's population in low numbers in the area of origin. Management practices for *S. viarum* based on herbicides or mowing only provide a temporary solution and are relatively expensive. Cattle ranchers in Florida spend an estimated at \$6.5 to 16 million annually to control *S. viarum*. A biological control project was started in 1997 by the University of Florida in collaboration with Brazilian and Argentinean researchers. The first biological control agent approved for field release in 2003, the leaf beetle *Gratiana boliviana* Spaeth (Chrysomelidae), has been released (approximately 233,000) in 39 counties in Florida, 2 counties in Georgia, 3 counties in Alabama, and 1 County in Texas. Post-release evaluations to determine establishment, dispersal, and feeding effects of the beetles on *S. viarum* plants have been made since 2003 in at least five of the release sites. The beetles got established in south/central Florida. Beetle dispersal (1.6 to 16 km/year) is a function of plant availability. *G. boliviana* is causing significant defoliation (30-100%) and reducing fruit production of *S. viarum*. Management of *S. viarum* infestations at the central and south Florida release sites is now mostly based on the biological control agent with fewer applications of herbicide or mowing. However, new agents are required for northern Florida where *G. boliviana* was not able to establish.

The Life History of *Corythuca distincta*, an Endemic Lace Bug on Canada Thistle in Wyoming

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Abstract

Canada thistle, *Cirsium arvense* L. Scop., is a widespread noxious weed throughout much of North America. The impact of introduction of exotic natural enemies to control this weed has been limited. Several indigenous insects have been considered for their biological control potential. One such insect is the lace bug, *Corythuca distincta* Osborn & Drake (Hemiptera: Tingidae). *Corythuca distincta* is common in much of the western US, and feeds on several *Cirsium* species including Canada thistle. Studies to determine the life history, development, and potential impact and utilization of this lace bug occurred at a field site in central Wyoming, as well as under greenhouse conditions in Montana. The development of *C. distincta* is typical of lace bugs. Eggs were laid singularly (although multiple eggs are laid per leaf) within leaf mesophyll on the underside of leaves. Hatch occurred from 7 to 14 days at 20° C. Nymphs had five instars varying in duration from three to six days. Total duration of the nymphal stage was between 18 to 26 days. Adults were fairly long-lived (up to 129 days with a mean of 58 days) under laboratory conditions. *C. distincta* has two generations per year. Adults emerged from overwintering in early May. By mid-May to early June eggs were evident; nymphs were present from late May to mid-July. The spring population generally appeared to be the smaller of the two. New adults were observed from late June to mid-July and for a period of time there was an overlap of the generations. Newly eclosed nymphs were common in early to mid-August. Few adults were observed late in the summer or early autumn. Adults overwinter in a state of reproductive diapause, presumably in leaf litter or soil near the plant. Feeding by *C. distincta* was confined to the plant leaves. Heavy feeding causes the leaf to turn necrotic and to curl. Both adults and nymphs fed in small groups; frequently feeding together. Although heavy feeding resulted in leaf mortality, vigorous plants sometimes responded by producing additional leaves; often in the form of rosettes of leaf growth. This was particularly evident in mid-summer. Plants that were fed upon were generally shorter than those not infested, although only a small number of plants were measured. The percentage of plants infested varied through the season, but for the study period between 49 and 96% of the plants were infested by late July. Feeding was somewhat contiguous and often resulted in small patches of stem mortality. The potential of *C. distincta* as a biological control agent of Canada thistle is somewhat inconclusive. Although it is bivoltine and capable of damaging Canada thistle, the lace bug is patchy in distribution and is inconsistent in population from year to year, perhaps due to dispersal or unknown mortality factors such as overwintering. Given that the lace bug appears to be associated with plants of several plant families, as well as a number of *Cirsium* species, its utility as a biological control agent may be limited in North America.

The Release and Recovery of *Bradyrrhoa gilveolella* on Rush Skeletonweed in Southern Idaho

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Abstract

Rush skeletonweed (*Chondrilla juncea* L.) is a major noxious weed in Idaho and other areas of the Pacific Northwest. A biological control program was implemented during the late 1970s in an attempt to manage infestations of rush skeletonweed and to limit its spread into new areas. Three agents, *Cystiphora schmidtii* (Rübsaamen) (a gall midge), *Aceria chondrillae* (Canestrini) (a gall mite), and *Puccinia chondrillina* Bubak & Sydenham (a rust fungus) have been established in Idaho and other areas of the western United States. However these agents have provided only limited control of the weed. One additional agent, the root-feeding moth *Bradyrrhoa gilveolella* (Treitschke) (Lepidoptera, Pyralidae), was approved by the USDA-APHIS for release in the United States in 2002. Initial releases were made in southern Idaho in November 2002 and during the summers of 2003 through 2009 (excluding 2005). Releases were made using infested plants (the 2002 initial release) then utilizing first instar larvae and adults from greenhouse colonies. In total we released the moth at eight sites utilizing nine infested plants (est. 27 larvae), 6,095 larvae, and 60 adults. At several sites, releases were made in multiple years. Sites were periodically monitored for evidence of establishment (presence of adults or larval feeding tubes) during the summer or early autumn. At six of the eight releases we observed larvae, empty pupal cases, or feeding tubes the year following the release. However subsequent recoveries were not made; except for one site located near Garden Valley, ID (~ 60 km NW of Boise) where *B. gilveolella* was detected at very low levels at the immediate release until 2010 (not sampled in 2009). In October 2010 we observed infested plants over a much larger area of ~ 7 ha. Although variable, percent infestation at the immediate release approached 70%. On average one larva was observed per root although twice as many empty feeding tubes were observed. In early August 2011, adults were observed over a much wider area (~ 46 ha) although still confined to a small geographic area. We estimated, based on adult counts, that the moth population exceeded 100,000 individuals at the site. We suspect that *B. gilveolella* at this site was founded from a small population of approximately eight adults from the initial release of inoculated plants in 2002; supplemented by an unknown but probably small number adults that resulted from larval transfers (720 total larvae) in 2003 and 2004. The establishment of *B. gilveolella* in Idaho occurred somewhat by happenstance. The site is unremarkable compared with our other release sites in regards to numbers of insects released, release techniques, or habitat/climate. The site is located at 1158 m in elevation, and has a south facing aspect with a variable slope (0-20%). Plant density is high averaging 116 stems/ m²

(range of 0 to 304 stems). The habitat is within the transition zone between *Pinus ponderosa* / *Symphoricarpos albus* and *Purshia tridentata*/*Pseudoroegneria spicata* types; with a granitic based sandy-loam soil. Mean annual temperature at the site is 8.3° C with annual rainfall of 615 mm, which is very similar to that of the collection site at Lake Prespa in northwestern Greece. We are presently examining the phenology of this population, working to develop effective collection and release techniques, and assessing the impact of *B. gilveolella*.

Challenges to Establishing *Diorhabda* spp. for Biological Control of Saltcedars, *Tamarix*, in Texas

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Abstract

Saltcedars, *Tamarix* spp., are exotic, invasive shrubs and small trees infesting an estimated 500,000 acres in arid west and southwest Texas. Since 2004, three species of *Diorhabda* spp. (Chrysomelidae) have been released in Texas. In 2006, the Saltcedar Biological Control Implementation Program was initiated to re-distribute leaf beetles throughout west Texas. However, after two years of effort, beetle populations were established at only 1 of 20 sites. To improve establishment success, studies were conducted to identify species adapted to ecological regions of the state and evaluate the impact of ant predation on the pupal stage. Field studies demonstrated that the Mediterranean tamarisk beetle, *Diorhabda elongata* (Brullé), established in the grasslands region of northwest Texas (latitude 32.5-34°N) whereas the larger tamarisk beetle, *Diorhabda carinata* (Faldermann), failed to establish. On the Rio Grande River in southwest Texas (latitude 29°N), the subtropical tamarisk beetle, *Diorhabda sublineata* (Lucas), established and dispersed much faster than *D. elongata*. The impact of ant predation on pupal survival was evaluated using an insecticide bait to exclude ants from field plots. Survival of *Diorhabda* spp. to the adult stage was ten times greater in cages where ants were excluded. Mortality of sentinel pupae after 4 days in the field was 30% when ants were excluded compared to 55% where ants were undisturbed. Sentinel pupae were also observed from sunset to midnight to identify predators attacking exposed pupae. Ants were the most common predator observed feeding on *Diorhabda* pupa and represented 48-87% of all predation events. Unexpectedly, pillbugs (Isopoda) were the second most common predator. These studies confirmed the vulnerability of the pupal stage to predation, primarily by ants. Treating release sites with ant-specific insecticidal bait was incorporated into the release protocol in 2008. During 2009-2010, the Saltcedar Biological Control Implementation Program field collected and released 700,000 adult *Diorhabda* spp. at 17 sites in Texas. The rate of establishing new populations increased from 5% in 2008 to 70% in 2010.

Estimating Non-Target Effects: No Detectable, Short-Term Effect of Feeding by Cinnabar Moth Caterpillars on Growth and Reproduction of *Senecio triangularis*

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Abstract

Studies of the effects of biological control organisms on both target and non-target populations are needed to balance risks, costs, and benefits. The benefits and costs of controlling ragwort have been well quantified both in ecological and economic terms; however, uncertainty remains regarding non-target effects. The 1960 introduction of the cinnabar moth, *Tyria jacobaea* L., to Oregon for control of tansy ragwort, *Jacobaea vulgaris* L., led to colonization of a non-target host arrowleaf ragwort, *Senecio triangularis* Hook. Our 2009-2010 observational study found no detectable effect of defoliation level (0-100%) caused by the cinnabar moth larvae, on rates of plant growth (changes in number, length and thickness of stems) or reproduction (change in number of seed heads) for a population of *S. triangularis* in the wilderness of the Cascade Mountains. Four features of the *S. triangularis* life cycle buffer the plant from potentially adverse effects: perenniality, iteroparity, stored reserves in the roots, and a mismatch of plant and herbivore phenologies. We plan further, long-term studies to estimate (1) mean, variance and covariance in damage by multiple herbivore species, (2) possible delayed effects of defoliation on plant survival, and (3) possible effects of defoliation on life-time reproductive success and plant recruitment from seed. This study highlights (1) the features of life-cycle and environment that buffer plants from adverse effects of herbivory, (2) the difficulty estimating the consequences of herbivory for plant distribution and abundance when plant recruitment and mortality events are rare, growth rates are slow, life is long, and levels of herbivory vary, and (3) the need to weigh target and non-target effects to fully evaluate outcomes of biological control.

Monitoring Biological Control Agents and Leafy Spurge Populations along the Smith River in Montana, USA

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Abstract

The Smith River originates in west central Montana and flows north approximately 100 miles before joining the Missouri River. The central 60 miles of the river flows through a relatively inaccessible, forested, scenic limestone canyon famous for its trout fishing. Because of its popularity, the area was designated Montana's first and only controlled river, with access by a permit system. By 1995, leafy spurge (*Euphorbia esula* L.) infested roughly 639 acres of the canyon, threatening riparian ecosystems and the natural, cultural, agricultural, and recreational resources along the river corridor. Because the riparian environment and mixed ownership make weed control difficult, a cooperative weed management group consisting of multiple agencies along with private landowners was formed and the river corridor was designated a Cooperative Weed Management Area (CWMA). The group selected integrated management with an emphasis on biological control as the only practical long-term management option for leafy spurge. Between 1991 and 2002, over 250 releases of eight biological control agents were made, totaling more than 370,000 insects released. Between 2007 and 2010, the USFS Rocky Mountain Research station monitored 79 of these release sites plus 20 sites with no record of release to determine the status of the biological control agents. We also monitored 62 of the release sites for changes in leafy spurge populations and in vegetative community characteristics. We discuss the status of the biological control agents and the results of integrated management on leafy spurge control along the river corridor.

Implementing EDDMapS for Reporting and Mapping Biological Control Releases

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Abstract

EDDMapS, the Early Detection & Distribution Mapping System launched in 2005 by the Center for Invasive Species and Ecosystem Health at the University of Georgia, is a web-based mapping system for documenting invasive species distribution. Originally designed as a tool for state Exotic Pest Plant Councils to develop more complete distribution data of invasive species, the simple EDDMapS interactive web interface allows users to simply enter specific information about invasive species infestations and images into a standardized on-line data form. Data entered is immediately loaded to the website, allowing real time tracking of species. Being able to see the current data of a species as it moves into a new area helps to facilitate Early Detection and Rapid Response programs (EDRR). The Center for Invasive Species and Ecosystem Health, in collaboration with the Center for Research on Invasive Plants and Small Populations at the University of Idaho, is implementing EDDMapS Biocontrol as a web-accessible reporting and display/mapping system for invasive plant biological control projects. Participants will be able to use internet tools to submit individual records/ observations and enable data visualization with interactive maps or view results through interactive queries with the EDDMapS Biocontrol database. EDDMapS Biocontrol will develop output routines/data exchange mechanisms to enable export of information from EDDMapS to other projects and data systems.

Dramatic Observations of Two Biological Control Agents of *Clidemia hirta* on Kauai

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Abstract

Koster's curse, *Clidemia hirta* (L.) D. Don, is a non-native invasive shrub threatening Hawaiian wet forests and watersheds. Since 1953, it has been subjected to a suite of biological control agents yet remains an important understory weed. In 2009, The Nature Conservancy of Hawaii noticed major defoliation on *C. hirta* from *Colletotrichum gloeosporioides* f. sp. *clidemiae* Trujillo, a leaf fungus. Observations were also made of the recently released seed predator moth *Mompha trithalama* Meyrick, suggesting high infection rates and premature fruit drop. A survey conducted in 2010 found *C. gloeosporioides* and *M. trithalama* ubiquitous across windward Kauai. While the two biological control agents have little effect on plant mortality, there is evidence to suggest possible impacts to seed production and overall leaf cover.

Post Release Monitoring of a 2009 Release of *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) for the Control of Russian Knapweed in Fremont County, Wyoming

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Abstract

Russian knapweed, *Acroptilon repens* (L.) D.C., is well established in Fremont County Wyoming, infesting over 40,000 acres of crop and rangeland. It has been the target of a biological control of weed effort in Wyoming, USA, since 1992 when a nematode, *Subanguina picridus* Kirjanova and Ivanova, was released. The Russian Knapweed Consortium has collected funds primarily from Wyoming Weed and Pest Districts as part of a cooperative effort with USDA/APHIS and CABI Europe-Switzerland to find additional agents. *Jaapiella ivannikovi* Fedotova (Dip., Cecidomyiidae) was approved for release in 2009. It was released north of Riverton, Fremont County, Wyoming on 19 May 2009. By the end of the summer over 50 galls had been located. By the end of 2010, the insect had spread across several hectares of land with over 200 galls being identified. Pre-release data had been collected from permanent transects established at this site for a number of years in anticipation of future releases. Additional transects have been established to monitor population expansion and impact of the agent on the target species. Preliminary data indicates that *J. ivannikovi* reduces Russian knapweed plant size and seed production. Efforts will be made in 2011 to evaluate parasitism, habitat preferences and seasonal phenology of gall formation and adult emergence. Preliminary field data suggests that *J. ivannikovi* is a promising biological control agent for Russian knapweed that has established in Montana, Colorado and Wyoming, has significant impact and is spreading in both density and area.

The Exceptional Lantana Lace Bug, *Teleonemia scrupulosa*

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Abstract

The lantana lace bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), was introduced to Hawaii for biological control of lantana, *Lantana camara* L. (Verbenaceae), in 1902 and again in 1954. Among 43 cases of non-target native plants used as hosts by weed biological control agents in the US and Caribbean in the past century, all appear predictable with the exception of this lace bug. In Hawaii, *T. scrupulosa* is known to develop on one variety of naio, *Myoporum sandwicense* (A. DC.) A. Gray (Myoporaceae), a very distant relative recently placed in the same order as lantana, Lamiales. Biological control efforts using *T. scrupulosa* proliferated around the world following its first use in Hawaii. A few additional instances of non-target host use by this lace bug suggest that its host range may be disjunct but broad within Lamiales, extending to sesame, *Sesamum orientale* L. (Pedaliaceae) and teak, *Tectona grandis* L. f. (Lamiaceae). The possibility of genetic shifts in host specificity among Hawaiian populations of *T. scrupulosa* was investigated in retrospective greenhouse tests. Ovipositional host specificity did not vary among populations, but was consistently disjunct, suggesting a genetic pre-adaptation for selecting a specific subspecies of *M. sandwicense* as a host. However, adaptation for development on *M. sandwicense* also appears to have occurred in the population collected from this plant. Additional studies are needed to determine the basis for host specificity in this insect and whether new protocols for host range evaluation may improve our powers to predict such non-target interactions.

WORKSHOP REPORTS

Workshop Summary: Is Classical Biological Control a 20th Century “Old Science” Paradigm that is Losing its Way?

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Workshop proposal

For years most countries accepted the benefits of biological control as given, leading to facilitated inside lanes through the regulatory maze. “Successes” led to many passionate disciples over science rationalists. Biocontrol targets continue to be selected on assumptions of good value with little direct evidence. Even when successful, biocontrol has rarely delivered environmental benefits that have been measured. Money flow is still healthy, but is arguably being directed against less impactful targets. Lack of science rigour exposes the field to attacks from an increasing number of critics as values change. A global change driven counter-revolution is underway on the dichotomy of hate between natives and aliens. Will climate change undermine even currently successful biocontrol outcomes? Meanwhile negative direct and indirect impacts of biological continue to fuel dissent. Nowhere is this issue hotter than in Hawaii where “invaders” have massively increased biodiversity, make up nearly all the biomass and create whole new ecosystems. This workshop will entertain a panel discussion around the future for classical biological control of weeds. Does it need to change its paradigm in response to changing societal values, if so can it reinvent itself?

Introduction

Scientists engaged in weed biological control are all advocates of the discipline and so what they say about the benefits of biological control in the public arena can be seen as a value judgement and won't be considered as objective or impartial. Historically, biological control has been, at least implicitly, supported by government agencies, because of the recognised accrued benefits against agricultural pests and weeds. The views of

government in developed countries are changing, however, with growing scientific and public concerns about environmental degradation from biological invasions and the increasingly recognised risks from introducing exotic organisms. The recognised historical agricultural benefits compared to the small realised risks (off-target direct and indirect impacts) may be quickly forgotten when biodiversity faces increasing pressures from alien invasive species and as government departments responsible for environmental protection become

increasingly involved in weed biological control implementation. Furthermore, weed biological control is now more a tool for the management of environmental weeds (weeds affecting biodiversity, habitat integrity, ecosystem processes and services) than for agricultural weeds. Use against environmental weeds has grown because the impacts of such weeds are perceived as often even more substantial (e.g. ecosystem “transformers”) than simple economic costs from agricultural weeds. The new problem here however is one of perception, because the impacts of environmental weeds cannot be measured in straight economics. Their impacts can be hard to quantify. When biological control is increasingly used against targets where the impacts might be considered qualitative or subjective, then its use can increasingly be questioned too. It is critically important that a target weed for a biological control program has clear and undisputable negative impacts against which the program can be evaluated in the future.

As an applied science, biological control lives and dies based on its acceptance by its stakeholders and society. It requires good government and community support. There are still many countries in both the developed and developing world where the use of weed biological control has not achieved public acceptance and is not being applied (i.e. most of Europe, Asia and South America). In countries where biological control is both practiced and accepted, scientists may be taking their stakeholders for granted (e.g. seen just as funding sources for their science) when they should be engaging and encouraging them as objective public advocates for the discipline. After a long history, it could be argued that to sustain the discipline, weed biological control is also starting to work on more second tier targets as all the “world’s worst weeds” have already been tackled. Second tier can mean not just weeds where the impacts are hard to quantify, minor or more doubtful, but also weeds for which some sectors of the community perceive benefits from them. Where potential benefits are less explicit, the argument for introducing an exotic potentially beneficial biological control agent may meet greater resistance. Similarly, even if a control program successfully eliminates the environmental weed target, there may be no measureable biodiversity or ecosystem service benefits, only weed replacement. Biological control

may be moving on to targets where the value of the approach will be more vulnerable to criticism, if strong independent advocates are lacking.

Community, government and even scientific perceptions of the harmful impacts of alien invasive plants may also be changing. Firstly, most legislation relating to the importation and release of exotic biological control agents only considers risks not benefits, being largely based on International Plant Protection Convention import risk assessment protocols. Where governments accept the importation and release of biological control agents, this is because of an implicit but not legislative government acceptance of the potential benefits. This allows for a rapid change in attitude in a society that is becoming increasingly risk averse. The rapid decline in recent biological control agent release permit approvals in the USA may be testament to this. Scientific evidence for non-target direct and indirect impacts following the introduction of ineffective agents helps change the risk perception against biological control. Certain scientific and community groups also consider certain elements of an alien flora are beneficial, where, for example they provide habitats or an ecosystem service not provided by the indigenous flora (e.g. forests on islands). These arguments are backed up with the lack of evidence that exotic plants cause extinctions.

Biological control can also be accused of being “an old science paradigm” by science agencies wishing to invest in new state of the art approaches. Biological control can be viewed as a “service from science” discipline and as such should be fully funded by stakeholders. This can lead to declining public investment in infrastructure and programs as public good science. Biological control is also not practiced yet in the context of climate change, in the way other types of natural resource management (NRM) are (e.g. carbon storage). Both target selection and the likely ongoing benefits from successful biological control may change, if future climates are considered in the decision making. Classical biological control won’t be considered as “leading edge” if it fails to adopt new techniques and approaches and consider drivers of global change.

Hawaii was a perfect place to hold a workshop to debate the external perceptions of the discipline. Alien species dominate the biota on these islands and generate new ecosystems and services not

present before their arrival. Many of them are valued for this and are used as food sources by the Hawaiian community. In Hawaii the benefits and risks of biological control have always been strongly debated as there have been contentious historical programs against snails and insect pests. Furthermore, recent proposed releases of biological control agents in Hawaii against strawberry guava (*Psidium cattleianum* Sabine) have raised perhaps the highest level of public contention around any biological control program in recent years, with local opposition on one Hawaiian island having attracted some political support, while other islands in the archipelago remain supportive. Debate at the workshop was encouraged around some proposed options for the future for the biological control scientific community:

Focus on the science – as long as the science is of the highest calibre leading to maximised successful weed control, the community will see and accept the benefits and future funding will flow.

Become our own lobbyists – In the policy arena, abandon an independent scientist or “trusted advisor” role and actively stimulate debate around the benefits of biological control.

Engage the community – Enable communities to take ownership of, and advocate for programs by building and supporting local knowledge about the impacts of weeds and the benefits of biological control.

Change our science – continuously seek to apply modern scientific approaches (e.g., genetics, genomics, and bioinformatics) and considerations, like climate change, to the discipline.

It will be a fine line for scientists to present the scientific evidence of the benefits of their discipline without slipping into an advocacy role.

Panel member comments

Simon Fowler (Landcare Research, NZ): Despite some countries showing reduced commitment, New Zealand is in the golden age for weed biological control. Stakeholder and government support of the projects is the highest it has ever been as is

the number of programs showing signs of success. There is sufficient funding for the science to develop in a number of new ways that are allowing many ecological questions to be addressed about risk assessment, release strategies, impacts of targets and biodiversity benefits of successful control. Evaluation is also underway for a number of projects and has considered non-target impact assessment. New Zealand also has a highly efficient regulatory process in place for assessing and approving biological control agents for release and so the times between permit submission and approval and the rate of agent approval is faster than in other countries. With regards to the effects of climate change, a recent assessment suggests such impacts on future target status and the ongoing success of existing projects appears likely to be unaffected as changes to the New Zealand climate are not expected to be high relative to other regions.

Martin Hill (Rhodes University, South Africa): South Africa too is bucking any apparent trend in skepticism around classical biological control. The Working for Water Programme and the value the leaders of this put in biological control as a cheap and effective approach to long-term weed management in South Africa is inspirational. This provides the ongoing support and development of the discipline in South Africa. While biological control still competes with other NRM activities for funding it holds its own and attracts its justifiable share. Universities and the Plant Protection Research Institute work in effective collaborations across a number of programs and are as good as ever in terms of delivery of agents and research outputs. The regulatory arrangements for obtaining release permits in South Africa are also not prohibitive at this stage. The regulations are under review at the moment and this affords the South Africa biological control fraternity to have significant inputs. The challenge for South Africa weed biological control is the effective implementation and post-release evaluation that shows the environmental benefits of the science.

Keith Warner (Santa Clara University, USA): Recent issues have arisen in Hawaii around a critical reaction by an individual with a capacity to influence debate to the proposed biological control of strawberry guava. This reflects diverging

perspectives between the practitioners and advocates of biological control and the community at large. If, as a result of such high profile opposition, members of the public become more suspicious of biological control, it risks becoming orphaned as a discipline from other NRM strategies and, as such, losing its public licence to operate in some regions. The budget investment by biological control in public engagement and communication is insignificant relative to its importance to the success of the practice. Public engagement and communication will be important in the US, as in other countries, in the long-term. Programs and their stakeholders need to identify and develop public figures in the community as champions and advocates and be prepared to engage in public debate about both the risks and the benefits of this management approach.

Peter McEvoy (Oregon State University, USA): Biological control programs need to target greater interactions with university scientists to sustain the ongoing science rigour of the discipline to make sure the science outputs are of the highest quality, addressing contemporary science priorities and understanding. University collaborators have the freedom, through funding more targeted to addressing fundamental research questions, to more comprehensively address the mechanistic underpinning of successful and unsuccessful biological control systems. Universities keep the ideas flowing and can test non-target direct and indirect and community level impacts and benefits. Universities, and the generations of young scientists they cultivate, provide the knowledge market that will keep the practice of classical biological control as not only a successful tool for managing weeds, but a recognised manipulative approach to push forward ecological understanding about biological invasions, species interactions and evolutionary processes. Furthermore biological control of weeds, arthropods, and other organisms should be more closely allied, because when disciplines become isolated they are less likely to be successful.

Workshop discussion

The proposed issue for discussion that biological control is potentially “losing its way” as

a public supported cutting-edge science discipline, appeared overly pessimistic to the participants based on the response of panel members. Clearly New Zealand and South Africa have ‘never had it so good’ even if resources or public support for classical biological control are more in abeyance in countries like Australia and the USA. However, it was acknowledged that complacency, and lack of focus on the needs of both the scientific and the wider community could put continuation of that public support at risk. The broader discussion at the workshop addressed the following issues.

Biological control and broader NRM activities.

It was suggested that biological control has been poor at integrating its activities into broader NRM efforts and so has failed to gain broad recognition by the wider NRM community. This has become much more significant now biological control is focussed on environmental weeds where the NRM impacts are broader and more complex. For example, purple loosestrife control in the USA was successful, but failed to clearly define a NRM aim beyond weed reduction. There was no clear native community that it had displaced in many areas, and the effects of the changes in loosestrife densities (and increased control agent abundance) on other aspects of the community (e.g. water plants) remains poorly explored. The necessity to more effectively integrate biological control into broader NRM strategies stems from it targeting only one species when many sites suffer from multi-species invasions. Nonetheless the program had been of value for educating the community about biological control (school agent rearing programs), and understanding also has increased around the threats posed by invasive plants.

Community engagement. There was recognition amongst the Hawaiian delegates at the workshop that community acceptance of biological control for recent activities had been assumed, based on historical perceptions, and that the response around the strawberry guava biological control program had been completely unexpected in scale and intensity. This has necessitated a “road show” to try and expose the issues to a wider sector of the community. Future programs will need to be more proactive in investing in public discussions and engagement before programs are initiated and use

historical successes and the benefits obtained from them to attract champions and present convincing arguments. Past successful programs are quickly forgotten in the public psyche. This was particularly true in a state, like Hawaii, where the majority of the biota is exotic and the indigenous community has learnt to adopt and use exotic organisms for the benefit of their community and culture.

Declining relevance of classical biological control of weeds. One of the reasons for the decline of funding for weed biological control in some countries (e.g. Australia), has been that the agencies now responsible for allocating government investments are not agencies that have traditionally managed or understood the historical benefits from, and the need for, sustained investment in weed biological control. As such, weed biological control project proposals, are perhaps judged against less relevant criteria. As funding cycles drop to less than three years, this presents a tighter timeframe for projects to deliver outcomes. Expensive ongoing biological control programs are assessed against a broader array of novel ideas that target a shorter time frame for delivery. It behoves the weed biological control scientific community to work with stakeholder groups to champion the sustainable support of biological control programs. In other jurisdictions (e.g. the USA) problems relate less to investment and more to complex regulatory processes. Decision making for biological control agent releases can become the responsibility of single individuals, who make judgements based

on not just the advice of an independent advisory panel, but also their individual perceived levels of risk aversion. A lack of a collective approach leads to less objective decision making and fewer release permits. Again the solution is greater engagement between stakeholders, the public and the regulators to define the value proposition through understanding the likely benefits relative to the risks.

Conclusions

As is often the case, support for biological control projects waxes and wanes and does so quite independently between countries. At the moment some countries are in the trough while others are at the crest. These positions will change in both directions. Certainly in terms of weed control success, biological control of weeds programs are now probably more successful historically (proportion of targets controlled) than they have ever been, so the benefit arguments for sustained funding should be easy to generate. But the world is changing and along with it societal values. The biological control fraternity cannot lock themselves away and assume that attitudes to their work will remain unchanged. In this workshop the issues around the acceptability and support levels for weed biological control were explored and it was certainly widely recognised that investment in public engagement around the discipline, and continually finding ways to ensure the public are supportive, will forever be as critical to its survival as the need to ensure that this science too is the best it can be.

Workshop report: The Nagoya Protocol on Access to Genetic Resources under the Convention on Biological Diversity

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The Convention on Biological Diversity (CBD)'s access and benefit sharing (ABS) protocol was agreed at the tenth Conference of Parties to the CBD at Nagoya, Japan, in October 2010, and is now known as the Nagoya Protocol (UN 2010). The Nagoya Protocol is an agreement between the countries of the CBD as to how access and benefit sharing of genetic resources (including all biological control agents or BCAs) will be handled in future. Put at its simplest, the protocol provides a framework for the country receiving the genetic resources being required to pay forms of 'royalties' to the exporting country, for example, as a proportion of the financial benefits gained. However, Article 8 'Special Considerations' of the Nagoya Protocol also states:

In the development and implementation of its access and benefit-sharing legislation or regulatory requirements, each Party shall:

(a) Create conditions to promote and encourage research which contributes to the conservation and sustainable use of biological diversity, particularly in developing countries, including through simplified measures on access for non-commercial research purposes, taking into account the need to address a change of intent for such research;

(b) Pay due regard to cases of present or imminent emergencies that threaten or damage human, animal or plant health, as determined nationally or internationally. Parties may take into consideration the need for expeditious access to genetic resources and expeditious fair and equitable sharing of benefits arising out of the use of such genetic resources, including access to affordable treatments by those in need, especially in developing countries;

(c) Consider the importance of genetic

resources for food and agriculture and their special role for food security.

Based on the protocol, each country will prepare its own legislation and regulations. If it is accepted that biological control is non-commercial research, simplified measures for access and benefit sharing should facilitate biological control research. Furthermore, the use of biological control to address emergencies and the needs of food and agriculture should also be facilitated.

However, in practice, a lot will depend on the actual national legislation and regulations put in place by each country, and there is still a risk that biological control is not considered in this process. Some countries may see this as an opportunity to receive substantial payments for biological control agents, while others may inadvertently make it unnecessarily difficult or impossible to access biological control agents. The biological control community in each country has been encouraged to make its inputs into the national legislation and regulation process to encourage the facilitation of biological control along with other non-commercial research activities, e.g., relating to taxonomy, ecology and conservation (Cock *et al.*, 2010; van Lenteren *et al.*, 2011).

Nevertheless, we have already heard of delays of up to four years trying to get permits issued for export of BCAs, while the whole biological control process seems to be indefinitely blocked in some South American countries. What can we do about this and how can we manage this issue?

This workshop was attended by only a few people, mostly from New Zealand and Australia although there were attendees from Chile, Argentina, Hawaii and North America. Questions and comments were made during the workshop about: why is there

a problem, how can we avoid any problems in the future, who has a problem now, what is the reasoning behind ABS? It was stated by more than one attendee that so far no country had refused to issue a permit to collect or export indigenous species. The process for applying and receiving these permits had taken years, in some cases, but a permit had still eventually been issued by the exporting country. Currently agreements and permits with South American countries to collect and export genetic resources as potential biological control agents have been particularly difficult to obtain.

Australia is calling for national comment on the protocol (presumably they have not yet ratified it). It apparently comes into force after 50 countries ratify it and this is expected in 2012. Possibly other countries are also calling for comment and this may be one forum to get any concerns raised. See <http://www.environment.gov.au/biodiversity/science/access/biological-diversity.html>

The NZ government is not planning to ratify at the moment until some issues are clarified. See <http://www.mfat.govt.nz/Foreign-Relations/1-Global-Issues/Environment/7-Species-Conservation/genetics.php>

The Nagoya protocol has the potential to make the present situation worse when it comes into force. The access and benefit sharing scheme will pose major problems for us all if it does not take into consideration the non-commercial beneficial role of biological control in environmental and agricultural systems. It was agreed at the workshop to seek further information from our own countries about the protocol. Secondly we agreed to send out a survey to conference delegates seeking information from them. This was aimed to give us an idea of how many of us know about the protocols and how many of us are affected by them.

Thus, a link to a 12 question anonymous survey was sent out via SurveyMonkey.com to all 204 delegates of the conference and 56 responses were received from people representing 12 countries. Of those who responded almost 60% were unaware of the Nagoya Protocol. Of the remainder, when asked what they thought the impact would be on biocontrol, all believed it to be negative through delay or regulatory controls whilst a quarter mentioned the financial compensation aspect.

When asked if they were worried about the

possible impacts, 47% of respondents were worried and 49% didn't know, but 45% had experienced delays or refusals to make biocontrol agents available on ABS grounds, most often citing trouble with securing survey/collection permits. Seventeen countries were listed as having been difficult to gain access and exporting from, with Argentina dominating (Table 1).

Around a quarter of respondents said that ABS issues had prevented them from starting or continuing biocontrol projects and 32% said that ABS issues would influence their choice of biocontrol project and/or survey country in the future. A further 62% responded that ABS issues may influence their choice.

Regarding the respondents' understanding of their host country's regulations for import and export of BCAs the results to the three questions are summarised below in Table 2. It is interesting to note that with all questions some representatives from the same country contradicted their colleagues so there is a lack of understanding even amongst experts.

When asked about financial recompense 38% said that they thought their countries would pay a share of the anticipated benefits in order to have access to a BCA but when asked whether their country would support shared scientific activities with a BCA source country, this figure rose to 92%.

There were plenty of suggestions about how to overcome any ABS issues, by far the most common was the importance of engaging local co-operators who operate at the appropriate level. Other suggestions included:

- Ensure free flowing communication
- Attempt to get higher level support for negotiations
- Make it worthwhile for the country involved
- Make it clear there are no profits to be made
- Reciprocal cooperation should be encouraged
- Establish treaties to facilitate mutual sharing
- Always go by the book

In conclusion, there are still many involved in weed biological control who are not aware of the

issues, but most anticipate that the export of BCAs will get more difficult. The experiences generated at the workshop were often of long term and sustained frustration at what were seen as unclear, ever changing but unavoidable obstacles. However, in some cases it seemed that the fault lay with the bureaucracy rather than the regulations or interpretation of the CBD. It would also seem that countries may be more willing to provide payments in kind through supporting collaborators' research, training and equipment purchase than any pro-rata payment based on savings through biocontrol successes.

It is clear that biocontrol researchers have a challenge ahead as countries get to grips with the Nagoya protocol and its eventual application to classical biocontrol agents. It can only be hoped that common sense prevails and their use against environmental weeds, for which no direct profit is generated, is not hindered by the application of an instrument that was clearly not designed to prevent such mutually beneficial sharing of biodiversity.

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Table 1. Countries survey respondees listed as difficult to access, or to export from

Country	Number of times cited
Argentina	16
India	5
Brazil	9
Spain, Nepal	2
Canada, Madagascar, Cuba, Ecuador, Kenya, China (concern), Mexico, Thailand, Morocco, Algeria, Libya	1

Table 2. Summary of responses - host country's regulations for import and export of BCAs.

Is there any legislation or guidelines in your country regarding:				
	Yes	No	Don't know	Response Count
The importation and release of BCAs (excluding phytosanitary rules)?	92.0% (all 12 countries)	0.0% (0)	8.0% (4 countries)	50
The export of BCAs from your country?	36.0% (4 countries)	30.0% (7 countries)	34.0% (6 countries)	50
Imported BCAs meeting ABS requirements of the source country?	26.0% (7 countries)	8.0% (3 countries)	66.0% (9 countries)	50

Workshop Report: Wild Gingers (*Hedychium* spp.)

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The *Hedychium* J. Koenig spp. workshop, chaired by Djami Djeddour from CABI-UK, aimed to update interested parties on the biocontrol research carried out on these weed targets to date and highlight opportunities to grow the current consortium ahead of the presentation on the topic.

The three most invasive species, *H. gardnerianum* Shepard ex Ker Gawl. (Kahila garland-lily or Kahili ginger), *H. flavescens* Carey ex Roscoe (awapuhi meleleme; yellow ginger or cream garland-lily) and *H. coronarium* J. Koenig (‘awapuhi-ke’oke’o; gingerlily; white ginger; white garland-lily or butterfly ginger) have been the focus of an ongoing biocontrol initiative since 2008. Working in collaboration with national Indian institutes, permission to export natural enemies from the country of origin (East Indian Himalayas) were only acquired at the end of 2010 after a protracted bureaucratic process. A number of highly promising insect and pathogenic agents, including a highly specific stem mining fly (*Merochlorops dimorphus* Cherian) and a *Puccinia* rust, both on *H. gardnerianum*, have been identified and rearing studies/host range testing has been underway in the UK with a number of species for the current sponsorship consortium, The Nature Conservancy, Hawaii and Landcare Research, New Zealand.

The workshop was also intended to investigate ways in which the project could be tailored to meet the needs of other stakeholders, since the project has been run on minimal funds year to year. Whilst great potential has been identified, it is recognised that in order to progress the research, a greater financial investment needs to be made. *Hedychium gardnerianum* is a major threat to many subtropical forest regions of the world e.g. in South Africa, the Macaronesian Archipelagos (Azores, Madeira and Canary Islands) where it threatens endemic “Laurisilva” forests, La Réunion and Australia. In Hawaii, large areas of native rainforests are invaded and *H. gardnerianum*’s common name, Kahili ginger, named for the flower’s resemblance to the Royal feathered standards, exacerbates the mistaken perception that this is a beautiful, harmless, culturally entrenched Hawaiian plant.

Identifying potential opportunities to grow the consortium was at the heart of discussions and representatives from Hawaii’s Department of Land and Natural Resources, The Nature conservancy, Pacific Cooperative Studies Unit Coordinating Group on Alien Pest Species and Department of Agriculture as well as scientists from Landcare Research New Zealand and Agricultural Research Council in South Africa (PPRI) attended to provide their insight into the spread of the weeds, public perceptions of the problem and offer advice on likely test plant list requirements, particularly in the light of possible conflicts of interest a biological release may face from the public and horticultural trade in Hawaii. The general consensus was that in areas of dense monocultures, chemical and mechanical control are neither feasible nor environmentally sound and that in Hawaii management is largely confined to outlier populations to try to prevent further recruitment and spread. It was agreed that public awareness campaigns and highlighting the negative impacts that these plants have on all the Hawaiian Islands should be a concerted mission and pre-emptive strategy to any biocontrol release. Details of the project have been forwarded to all those in attendance to take forward to potential sponsors.

Please contact d.djeddour@cabi.org if you would like any more information on the project and/or are interested in becoming a partner or funder.

Workshop Report: Best Management Practices for Communication of Weed Biological Control

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Introduction

As we move into the 21st century, biocontrol as a science faces an evolving social landscape. Previously, biocontrol was regulated very little if at all. Biocontrol was lumped with introductions of other organisms, typically vertebrates (mongoose, cane toads, weasels, stoats, etc.) that were not well thought out and often times the work of individuals or small groups without government oversight or regulation. This is no longer the case. Biocontrol as a science is more stringently regulated. Coupled with the digital media increasing the flow of information and awareness, the landscape has changed in which biocontrol practitioners must operate. Environmental awareness within societies, government mistrust, distrust of scientists, and scientists themselves further confound the process of taking a scientific project from the lab to the field. Effective communication strategies are necessary to move the science of weed biocontrol forward. Scientists from 5 continents presented their strategies to engage the public and various stakeholders. A few common themes emerged in the course of the discussion: target audience appropriate messaging with appropriate messengers to deliver the message; pragmatic re-branding of weed targets and even the science of biocontrol to improve shareholder acceptance; early and frequent engagement of shareholders; training of messengers to more ensure more effective communication skills by scientists to shareholder groups or other messengers; and careful use of language to move away from militaristic or aggressive language that can be off-putting to shareholders. This information can be distilled into the following recommendations for an effective communication plan.

The Target

The first step in an effective communication plan is to identify the right target species for control. “Right” can depend on many factors including, but not limited to, the likelihood of success, shareholder buy in and acceptance (which could also translate into short or long-term funding of the project), the availability (or lack thereof) of other control methodologies, and the long-term potential for damage by the target. The target selection process should include some form of shareholder engagement. Shareholder engagement during the planning phases leads to cooperation based on mutual interest. This is the ideal situation from which to work on. Communication early and often is key to long-term project success and if possible should be considered before a project is even launched.

Communication Team and Strategy

Once a target is selected, an inter-disciplinary team should conduct an evaluation of the biological and socio-political issues surrounding the target and, once an agent(s) is selected, the agent itself. The team should include not only scientists, but also outreach and communication specialists to develop a cohesive communications strategy. The strategy should be viewed in part as marketing: the team is selling to shareholders what they need, something they do not already have, creating or increasing the awareness that a problem exists, and presenting solutions to the problem. A key element in defining the strategy is management of expectations—defining what “success” means and how that “success” will be measured. The public cannot be left with the perception that biocontrol will be a silver bullet. Instead, the public must leave with the idea that biocontrol is a tool for long-term, self-sustaining form of control against the target pest.

It is critical for scientists, as part of this program, to be media trained and prepared for speaking about their project. Although communication experts and outreach specialists should handle much of the communication, researchers have an essential role in the communication strategy.

Media training helps to mitigate communication issues typical of scientists. When scientists speak to

the public, they need to communicate with different terms than they use with their scientific peers. When non-experts hear terms such as “likelihood,” “possibility,” “relatively,” and “remote,” they infer that scientists are not confident in their own work or are unwilling to take definitive stands in support of their work. When speaking to the public scientists need to keep in mind the audience, i.e. what is appropriate in a scientific presentation is not appropriate for public engagement. This means simple and direct communication that relies heavily on images to drive the message home. Photos that compare before and after effects or the success of control projects are highly effective. Graphs and statistics are ineffective. Media training is essential help scientists address these common communication issues.

How scientists engage the public is also key. Media tends to “dull” everything and therefore speaking dramatically and enthusiastically increases the effectiveness in communicating a message. Humor not only entertains the audience but also adds a human side to the project. As a cautionary note, the media is always looking for a story and as a consequence is always looking for ways to put spins on stories. It behooves the scientist to be aware of what is happening around them—or to have a media specialist present that can manage the situation and if necessary mitigate any damage done.

Appropriate messengers must be determined for different target audiences. The adage “don’t shoot the messenger” can, in this context, be re-phrased to “pick the wrong messenger and the messenger will shoot himself.” A messenger needs to be able to speak “the language” of the target audience. The approach of one person for all audiences will not work. A team must be defined as a part of the communication strategy to include high impact messengers that will resonate with different target audiences. The scientist must ensure these messengers understand the issues and see the need for the product. Trust must be built between scientist and messenger. If trust does not exist, the team will collapse and by extension so will the communication strategy.

It may be prudent as a part of the communication strategy to do some “rebranding.” Rebranding could include changing the name of the target. In Hawaii many weeds have been given Hawaiian derived or Hawaiian sounding names such as waiwi or waiawi (*Psidium cattleianum* Sabine), kahili ginger

(*Hedychium gardnerianum* Sheppard) and maile pilau (*Paederia foetida* L.). These names create a socio-acceptance issue for control of the target species — an instinctive reaction can be anticipated because the naturalized weed targeted has become associated with the culture or the environment. Substituting skunk vine for maile pilau, for instance could work to good effect. In Brazil, rubber vine (*Cryptostegia grandiflora* R. Br.) is the target for the first classical weed biocontrol project in the country. It has been rebranded as “Devil’s Claw”. This rebranding is based on both the sociological demographics of Brazil and biological tendencies of rubber vine to grow over things, in this case tombstones. Re-branding should be well thought out as a case study of miconia in Tahiti highlights. Miconia was given the name “the green cancer” which was effective on one level but almost too effective as people became afraid of handling miconia for fear they might catch cancer themselves. The rebranding of biocontrol itself might also be considered. The words “control” and “agents” can strike a sinister tone and past associations with biocontrol, no matter how erroneous, can kill a project before it gets off the ground.

Roadblocks to Communication

There are typical roadblocks to secure shareholder support for weed biocontrol that any communication strategy should be prepared to address. These include: vertebrate introductions as examples of biocontrol, discomfort with the deliberate introduction of an alien species, and what happens when the introduced biocontrol agent eliminates the target species. Many of these communication roadblocks are best addressed not by scientists but by other messengers of the communication team. Furthermore, communication to address these misconceptions and public apprehension should be conducted on a consistent, sustained basis irrespective of the target weed or the proposed biocontrol agent.

Biocontrol has become synonymous with the release of vertebrates such as mongoose, cane toads, weasels and stoats. It does not matter to the public that individuals or special interest groups introduced these organisms with no regulatory oversight by the government—the perception is still

there. In Hawaii, the public school system teaches that the mongoose was released as a biocontrol agent for the control of rodents. The logic of biocontrol is called into question as the target (rats) is nocturnal and the control agent (mongoose) is diurnal. To further compound the issue, the mongoose in Hawaii is a predator of bird eggs and is attributed to impacting native bird populations. In effect, the Hawaiian education system perpetuates the idea that biocontrol is bad. While the story of the mongoose’s impact on Hawaii is true, its introduction was not part of an organized biocontrol program but that of an individual. Countering these misconceptions requires consistent messaging that those “releases” were not part of a biological control program. It should, however, be assumed that attempts to dispel these myths and misconceptions will remain a constant battle with the public.

The concept of the releasing of a species to control another, i.e. releasing an alien species to control an alien/invasive species, presents is a mental sticking point with some shareholder groups. Consistent messaging is needed for such issues and again highlights the need for early engagement with shareholder groups before a project even begins. Addressing this issue should focus on value: what would be lost by the target if action is not taken; the safety and long term sustainability of biocontrol; and the track record of weed biocontrol programs. This discomfort is linked in part with misconceptions of what biocontrol is. There may also be some latent cultural sensitivity on the part of introduction of an alien species as indigenous peoples have been displaced by foreign immigrants and subsequently have been disenfranchised from their own land. If speaking to indigenous peoples, cultural sensitivity over the alien issue is needed and again, appropriate messengers must be utilized as part of the communications strategy.

Probably one of the most difficult questions consistently posed regarding biocontrol can be summed up as: “what happens when X runs out of Y weed you’re trying to control?” Invariably this question is followed up with another equally difficult, if not more difficult question to answer, “will it evolve to attack something else?” It is easy to dismiss these questions as those of an uneducated shareholder base but this is not necessarily the case. The questions and concerns are legitimate and need

to be met. Words like “specificity” or “co-evolution” come to mind to address these questions. It is not as simple as that, the question needs to be answered in the context of the target group asking the question and again, the communication strategy must plan ahead for questions such as this.

The Evolution of Communication

As we are thoroughly ensconced in the 21st Century, communication has completed an evolution of sorts. So called “traditional” methods of communication (print media, television and radio) are giving way to digital media consumption and the dissemination of information through social media outlets. Digital and social media has evolved into a powerful tool that is underutilized by communication teams for the biocontrol of weeds. At times faced by agency restraints, scientists are not necessarily in a position to exploit the use of the public’s evolving media consumption base. Meanwhile, these same digital media outlets allow anyone to have a voice and can be effectively used to mobilize people for or against a particular viewpoint to great extent. In light of this, it behooves the communication team to have a digital or social media strategy to promote the program and to engage the public. A well-executed social media campaign can be extremely effective but thus far has been under utilized.

The Language of Communication Strategies

Language in relation to biocontrol has always taken an aggressive slant. Words such as “Control,” “agent,” “defoliator,” all are aggressive and militaristic in nature. It is not unusual to have a phrase such as “a biological control agent to attack the weed reducing its capability...” while the statement is accurate, it does not necessarily engender buy-in with shareholder groups. Phrasing and metaphors that are in line with healing or restoration are much more effective. A biological control program is not aimed to “attack” a weed but to “restore balance,” “mitigate the effects of,” or “manage the impacts of” a weed. The goal of a program is to “manage” or “conserve” the resources we have, to be “good stewards of the land” or to provide a “legacy” for future generations.

Aggressive language creates a greater divide between scientists and the shareholder. Passive or healing language creates bridges and therefore is a more effective communication model to follow.

Conclusions

The science of biological control of weeds has reached a nexus point of sorts. Faced with in some cases more stringent regulations, budgetary constraints and moving projects from in the lab to in the field, communication is a key element each of these dilemmas. Engagement at every level through effective communication strategies can be a project game changer. The strategy must include a team of individuals working early and often with shareholders, scientists trained to work with the media, messengers appropriate to different shareholder groups, preparing for foreseeable obstacles and barriers to obtaining buy-in, utilizing digital and social media while integrating language that bridges gaps between groups.

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Workshop Summary: Biological Control of Fireweed: Past, Present, and Future Directions

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Workshop proposal

Fireweed (common name in Australia and Hawaii) is in the genus *Senecio* (Asteraceae) from KwaZulu-Natal in South Africa. Two very closely related almost indistinguishable species are serious invasive and toxic weeds affecting livestock in grazing lands; *Senecio madagascariensis* Poir. is the weed in Australia, Hawaiian Islands, Argentina, Colombia, Brazil, Venezuela, Uruguay, and Japan, while *Senecio inaequidens* DC. (1837) is the species very widely distributed in Europe. Chemical and mechanical control measures are not effective or economically viable, and in Australia and Hawaii classical biocontrol is thought to be the only long-term solution. The workshop will address the weed status in various countries, review biocontrol efforts, and finding ways to enhance collaboration between researchers.

Workshop summary

This workshop was targeted around the attendance of affected ranchers from Hawaii so they would have an opportunity to understand the state of fireweed biological control in Hawaii and globally. It was attended by ranchers, state pest control officers, scientists from the Hawaiian Department of Agriculture working on fireweed biocontrol, other US biocontrol scientists and International scientists with experience in fireweed management. This included Terry Olckers (University of KwaZulu-Natal) who works on fireweed in its native range in KwaZulu-Natal in South Africa. About 25 people attended.

In the first section of the workshop three ranchers presented the problems that they are having with fireweed on Hawaii. This included the extent of the spread, the difficulties of control and the economic loss to their businesses. They fully supported the

development of biological control for fireweed in Hawaii. A lively debate about the fireweed problem in Hawaii ensued.

In the second section Mohsen Ramadan (HDA) presented the state of his research into potential biological control agents for fireweed in Hawaii. This included an overview of where fireweed comes from, a summary of his various exploratory trips to look for fireweed biological control agents and a presentation of all the species of interest he has found to date. He finished by presenting the risk assessment and host specificity testing work he has recently completed on the arctiid moth *Secusio extensa* (Butler), which has led to an application for release of this agent being submitted to APHIS. The ranchers were very pleased to meet Mohsen and asked him several questions about his work.

The third section was a lively debate about the likelihood of success of biological control of fireweed with many in attendance highly supportive of it as a

target based on past successes against similar weeds. This debate was led by George Markin (retired, ex-USFS) and Rachel McFadyen (retired, ex-DEEDI Australia) as staunch supporters. It finished with some discussion of existing work underway as part of the Australian fireweed biological control program presented by Andy Sheppard and Terry Olckers.

The final section of the workshop considered the process around the approval and release permit

application of *Secusio extensa* in Hawaii. Neil Reimer (HDA) presented where the regulatory process was with APIS and that, while there had been delays, a decision about the release permit was expected soon. Neil and Darcy Oishi (HDA) then led a discussion around how the ranchers might work with HDA and assist in lobbying indirectly USDA should this be necessary if the decision to release be delayed further.

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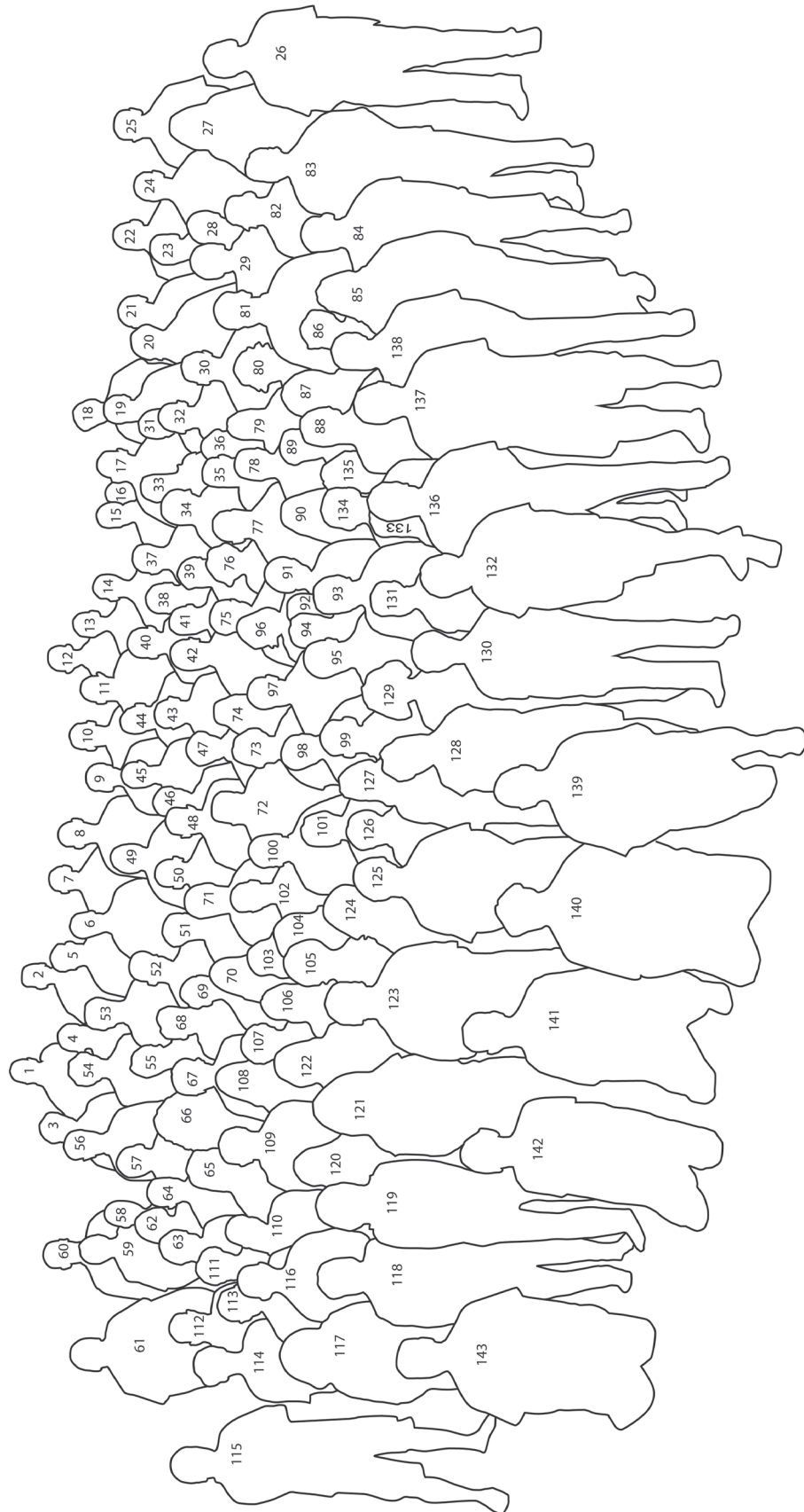
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125	Mann	Ko
126	Puja	Ray
127	Page	Else
128	Natalia	Rector
129	Louise	Morin
130	Yi	Wang
131	Ronny	Groenteman
132	Jialiang	Zhang
133	Rosie	Mangan
134	Alex	Racelis
135	Atanaska	Stoeva
136	Jianqing	Ding
137	Bernd	Blossey
138	Lindsay	Smith
139	Hugh	Gourlay
140	Mohsen	Ramadan
141	Tracy	Johnson
142	Pat	Conant
143	Walter	Nagamine

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82	Urs	Schaffner
83	Michael	Pitcairn
84	Ikju	Park
85	Sharlene	Sing
86	Jennifer	Andreas
87	Vili	Harizanova
88	Jim	Cullen
89	Terry	Ockers
90	Sonal	Varia
91	Carole	Rapo
92	Freda	Anderson
93	Min	Rayamajhi
94	S.	Raghu
95	John	Goolsby
96	Helen	Spafford
97	Quentin	Paynter
98	Don	Sands
99	Joe	Neal
100	Paul	Krushelnicky
101	Judy	Myers
102	Andrew	McConnachie
103	Fiona	Impson
104	Harriet	Hinz
105	Judy	Hough-Goldstein
106	Carey	Minteer
107	Anastasia	Kawi
108	Unknown	Unknown
109	John	Ireson
110	Christy	Martin
111	Jean-Louis	Sagliocco
112	Javid	Kashefi
113	Peter	Toth
114	Link	Smith
115	Massimo	Cristofaro
116	Joshua	Fisher
117	Jennifer	Birdsall
118	Juliana	Yalemar
119	Janis	Garcia
120	Lisa	Tewksbury

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41	Ted	Center
42	Bob	Lalonde
43	Michael	Grodowitz
44	Matthew	Purcell
45	Paul	Pratt
46	Gregory	Wheeler
47	James	Cuda
48	Bill	Palmer
49	Eric	Coombs
50	Al	Cofrancesco
51	Gary	Piper
52	Roger	Becker
53	Dick	Casagrande
54	Rene	Sforza
55	Jeff	Littlefield
56	Marcus	Byrne
57	Don	Davis
58	Julie	Coetsee
59	Michael	Day
60	Paul	Peterson
61	Ludovit	Cagan
62	Ruth	Hufbauer
63	Jenny	Cory
64	Unathi	Heshula
65	Gina	Quiram
66	Djamila	Djedbour
67	Kevin	Delaney
68	Hernan	Norambuena
69	Andrew	Norton
70	Kimberley	Higgs
71	Peter	McEvoy
72	Simon	Fowler
73	Rachel	McFadyen
74	Haley	Catton
75	Rowan	Emberson
76	Pauline	Syrett
77	Alec	McClay
78	Brian	Rector
79	Marion	Seier
80	Lynley	Hayes

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2	Kevin	Floate
3	Lindsey	Milbrath
4	Rob	Bourchier
5	Richard	Hansen
6	Brian	Van Hezewijk
7	Bill	Bruckhart
8	Robert	Barreto
9	Allen	Knutson
10	Davi	Macedo
11	Michael	Thomas
12	Jean-Yves	Meyer
13	Anthony	Boughton
14	Gary	Clewley
15	Esther	Gerber
16	Sonja	Scheffer
17	Nathan	Harms
18	John	Gaskin
19	Iain	Paterson
20	Stephen	Hight
21	Steve	Novak
22	Rieks	van Klinken
23	Phil	Tipping
24	Joey	Milan
25	Jeff	Makinson
26	Unknown	Unknown
27	Angela	Bownes
28	Richard	Hill
29	Grant	Martin
30	Will	Earle
31	Patrick	Moran
32	Richard	Shaw
33	Fritzi	Grevstad
34	Andrew	Sheppard
35	David	McLaren
36	Rosemarie	de Clerck-Floate
37	John	Lydon
38	Martin	Hill
39	Heinz	Mueller-Schaerer
40	Gadi V.P.	Reddy