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Biology and host specificity of *Rhinusa pilosa*, a recommended biological control agent of *Linaria vulgaris*

André Gassmann · Rosemarie De Clerck-Floate · Sharlene Sing · Ivo Toševski · Milana Mitrović · Olivier Krstić

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Abstract *Linaria vulgaris* Mill. (Plantaginaceae), common or yellow toadflax, is a Eurasian short-lived perennial forb invasive throughout temperate North America. *Rhinusa pilosa* (Gyllenhal) (Coleoptera, Curculionidae) is a univoltine shoot-galling weevil found exclusively on *L. vulgaris* in Europe. Under no-choice test conditions, 13 non-native *Linaria* species exposed to *R. pilosa* were accepted for oviposition and most were found to be suitable, to varying degrees, for gall and larval development. Adult feeding and survival was minimal on native North American species in the plant tribe Antirrhineae which includes the target plant. In no-choice tests with 63 native North American species and 24 other non-target species outside *Linaria*, oviposition was limited to four native North American species. Only three larvae developed to the adult stage on *Sairocarpus virga* (A. Gray) D.A. Sutton, with no negative impact on plant growth. Risks to native flora from the release of *R. pilosa* are therefore expected to be minimal. The Technical Advisory Group for the Biological Control of Weeds (TAG—BCW) has recommended release of *R. pilosa* in September 2013.

Keywords Common toadflax · Yellow toadflax · Curculionidae · Host range tests · Pre-release studies · Biological control of weeds

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A. Gassmann (✉) · I. Toševski
CABI, Rue des Grillons 1, 2800 Delémont, Switzerland
e-mail: a.gassmann@cabi.org

I. Toševski
e-mail: tosevski_ivo@yahoo.com

R. De Clerck-Floate
Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403 1st Avenue South, Lethbridge, AB T1J 4B1, Canada
e-mail: rosemarie.declerck-floate@agr.gc.ca

S. Sing
USDA Forest Service – Rocky Mountain Research Station, 1648 South 7th Avenue – MSU Campus, Bozeman, MT 59717-2780, USA
e-mail: ssing@fs.fed.us

I. Toševski · M. Mitrović · O. Krstić
Department of Plant Pests, Institute for Plant Protection and Environment, Banatska, Zemun, Serbia
e-mail: milanadesancic@yahoo.co.uk

O. Krstić
e-mail: oliverk13@yahoo.com

Introduction

Linaria vulgaris Mill. (Plantaginaceae), common or yellow toadflax, a short-lived perennial forb native to most of Europe and northern Asia (Chater et al. 1972;

Sutton 1988), rapidly and comprehensively colonized temperate North America after its surmised seventeenth century eastern USA introduction (Mack 2003). This weed now occurs in all mainland states of the USA and most provinces and territories of Canada (USDA, NRCS 2013).

Several exotic toadflax-feeding insect species can be found in North America today. Their presence is due both to unintentional and intentional introductions from Europe, along with their hosts *L. vulgaris* or the congeneric weedy species, *Linaria dalmatica* (L.) Miller and *L. genistifolia* (L.) Mill. Three adventitiously introduced seed-feeding beetles, *Brachypterolus pulicarius* (Linnaeus, 1758) (Coleoptera: Nitidulidae), *Rhinusa antirrhini* (Paykull, 1800) (formerly *Gymnetron antirrhini*), and *Rhinusa neta* (Germar, 1821) (formerly *Gymnetron netum*) (Coleoptera: Curculionidae), are now ubiquitous on North American *L. vulgaris*, either self-dispersed or intentionally spread as part of the North American toadflax biological control program (De Clerck-Floate and McClay 2013; De Clerck-Floate and Turner 2013; Sing et al. 2005; Wilson et al. 2005).

Five additional insect species have been approved and intentionally released in North America (De Clerck-Floate and Harris 2002; McClay and De Clerck-Floate 2002; Harris 1963). These are: the defoliating moth *Calophasia lunula* (Hufnagel, 1766) (Lepidoptera: Noctuidae), two congeneric root-boring moths, *Eteobalea intermediella* (Riedl, 1966) and *E. serratella* (Treitschke, 1833) (Lepidoptera: Cosmopterigidae), the root-galling weevil *Rhinusa linariae* (Panzer, 1792) (formerly *Gymnetron linariae*) (Coleoptera: Curculionidae), and the stem-mining weevil, *Mecinus janthinus* Germar (Coleoptera: Curculionidae) (De Clerck-Floate and McClay 2013; De Clerck-Floate and Turner 2013).

Despite the use of the mentioned insect species against invasive toadflaxes beginning in the 1960s (McClay and De Clerck-Floate 2002; Sing et al. 2005; Wilson et al. 2005; De Clerck-Floate and McClay 2013; De Clerck-Floate and Turner 2013), the reported efficacy of the majority of these on *L. vulgaris* has been minimal, with only *M. janthinus* recently showing some promise since its first releases in the 1990s in both the USA and Canada (Sing et al. 2005; De Clerck-Floate and McClay 2013). Since 2000, several other European insects have been investigated for potential use against *L. vulgaris*, and the first of these, the shoot-galling weevil, *Rhinusa pilosa* (Gyllenhal) (Coleoptera: Curculionidae), was petitioned for release in the USA and Canada in 2012 (De Clerck-Floate and McClay 2013). It was chosen as a candidate for testing on the basis of both native range host records suggesting that it is highly host-specific, and on field observations indicating that gall formation by the weevil could severely stunt shoots and prevent flowering of *L. vulgaris* (Toševski, unpublished data). The current paper presents the results of a combination of field records, laboratory, greenhouse and garden experiments used to complete an in depth study of the biology, ecology and host range of *R. pilosa* in order to gauge its safety and efficacy before seeking regulatory approval for its field release on *L. vulgaris* in North America. *R. pilosa* was recommended for release in September 2013.

Target plant systematics and biological information

The genus *Linaria* Mill. was traditionally placed in the Scrophulariaceae (figwort) family (Sutton 1988). Revisions based on molecular phylogenetic analyses indicated that *Linaria* would be more appropriately included within the expanded Plantaginaceae (plantain) family (Albach et al. 2005; Ghebrehiwet et al. 2000; Olmstead et al. 2001). *L. vulgaris* belongs to the Antirrhineae tribe, one of eleven currently circumscribed Plantaginaceae tribes (Estes and Small 2008; Ghebrehiwet et al. 2000; Tank et al. 2006). The Antirrhineae tribe consists of six well-supported clades (*Cymbalaria*, *Anarrhinum*, *Chaenorhinum*, *Antirrhinum*, *Galvezia*, and *Linaria*) (Fernandez-Mazuecos et al. 2013; Vargas et al. 2004). In North America, members of the monophyletic *Linaria* clade are limited to fourteen established non-native species, including *L. vulgaris, L. dalmatica* and *L. genistifolia* (L.) (USDA, NRCS 2013). Several of the non-native *Linaria* spp. that currently occur in North America have found limited use in ethnobotany and/or gardening (e.g., *L. purpurea* (L.) Mill.; see URL Missouri Botanical Garden 2013; also see Saner et al. 1995, and Vujnovic and Wein 1997 for beneficial economic uses of *L. vulgaris* and *L. dalmatica*, respectively). With respect to their ecological value in North America, it is suspected to be minimal, although there are reports of *Linaria* pollination by native bumble bees (De Clerck-Floate and Richards 1997). Three native North

American taxa conventionally placed within *Linaria* were reclassified in 1988, remaining in the tribe Antirrhineae but moved to a new genus, *Nuttallanthus* D.A. Sutton (Sutton 1988). Fernández-Mazuecos et al. (2013) suggests however that *Nuttallanthus* may, pending further investigation, be appropriately circumscribed as a section of *Linaria*. The tribe Antirrhineae includes 11 native North American genera and 33 native North American species (Vargas et al. 2004; USDA, NRCS 2013).

Linaria vulgaris is an erect perennial forb with plants consisting of single to numerous shoots attaining heights of 25–120 cm (Sutton 1988). Dense, persistent patches form primarily through vegetative reproduction from adventitious shoots that arise from tap and lateral roots (Bakshi and Coupland 1960). Sexual reproduction of this obligate out-crossing species is generally less successful (Nadeau and King 1991). Molecular diagnostic techniques have confirmed the occurrence of hybridization between yellow and Dalmatian toadflax from samples field collected at sites in Montana, Idaho and Colorado, USA (Boswell 2013; Turner 2012; Ward et al. 2009).

Biological control agent taxonomic information and biology

The candidate agent was known as *Gymnetron hispidum* Brullé, 1832 at the inception of this project. The revised identity of this taxon, presently placed in the genus *Rhinusa* (Caldara 2001), was taxonomically uncontroversial (Caldara et al. 2008 and references therein) because of its clear differentiation from all other species within the genus, i.e. by the ground color of the body, black with soft dark grey pubescence, densely covered with bristled blackish hairs. However, it was subsequently determined through a study of the type specimens that *G. hispidum* is a younger synonym of *Rhinusa tetra* (Fabricius, 1792), while the two closely related species *R. pilosa* and *R. brondelii* (Brisout, 1862) were ascribed full species status (Caldara et al. 2008; Caldara et al. 2010). *R. pilosa* is a univoltine shoot-galling species that overwinters as an adult. The species was described as a gall-inducer associated with *L. vulgaris*, while *R. brondelii* (also a galler) was primarily associated with *L. purpurea* in southern Italy, *L. genistifolia* in Serbia, *L. reflexa* (L.) Desf. in Tunisia and *L. gharbensis* Bat. & Pit. in Morocco (Caldara et al. 2010). A detailed description of *R. pilosa* and morphological differences between *R. pilosa* and *R. brondelii* are given by Caldara et al. (2008). Initiated by Lorenza Legarreta and Brent Emerson, University of East Anglia, Norwich, UK (Caldara et al. 2008), a recent population genetic study of weevils from the *R. pilosa–R. brondelii* complex yielded strong molecular evidence of the highly specific host association of these weevils with their confirmed field host plants (Toševski unpublished data).

All known species in the genus *Rhinusa* utilize Plantaginaceae (*Linaria*, *Antirrhinum*, *Mesopates*, *Chaenorhinum* and *Kickxia*) and Scrophulariaceae (*Verbascum*, *Scrophularia*) host plants, while *Gymnetron* from the Palaearctic region are associated only with plants from the genus *Veronica* (Scrophulariaceae). Species-specific host records from the literature are questionable since they do not adequately distinguish *R. pilosa* from *R. brondelii*. According to Hoffmann (1958), *R. pilosa* induces galls on several *Linaria* species, but in central and southeastern Europe it mainly occurs on *L. vulgaris*. The reported alternative hosts are *Chaenorrhinum minus* (L.) Lange, *L. repens* (L.) Mill., *L. purpurea*, *L. simplex* (Willd.) DC., *L. reflexa* (Hoffman 1958; Lohse and Tischler 1983) and *L. gharbensis* in North Africa (Mimeur 1949). A detailed account of the histology of gall induction and gall development by the weevil has been provided by Barnewall (2011) and Barnewall and De Clerck-Floate (2012) using the same insect population.

Materials and methods

Surveys and agent biology

The specimens in the current study were identified by Roberto Caldara, Milan, Italy. Spring surveys were conducted mainly in Serbia where *R. pilosa* was found most commonly, as well as in Romania and Hungary between 2003 and 2011. Galled plants were field collected with surrounding soil to keep roots intact, then individually transplanted into plastic pots. Pots were enclosed with a plastic cylinder (10 × 35 cm) fitted with ventilated (gauze) lids to maintain moisture and retain insects emerging from plants. Newly emerged adult weevils were overwintered in mesh field cages (210 × 210 × 190 cm), in plexiglass-

walled outdoor cages (70 × 70 × 70 cm) or in an outdoor insectarium in mobile framed cages (30 × 30 × 45 cm). The latter contained naturally growing *L. vulgaris* plants and wood blocks configured with grids of drilled 8 mm-diameter holes to serve as overwintering shelters for the weevils.

Adult weevils appearing on the walls of overwintering cages early in spring were collected and used in life history and host specificity studies. Adult survival was found to be on average higher in the outdoor insectarium than in either type of field cage, so from 2009 on, all adults were overwintered in the former. Field collected adults were initially used to establish a rearing colony, and from 2008 on, only adult progeny from this rearing colony were used in host specificity tests. Mass-rearing was conducted in field cages (210 × 210 × 190 cm) containing approximately 100 *L. vulgaris* plants planted directly into the soil. Galled plants were collected in late May and transplanted into pots (as described above) to secure emerging adults. Embryogenesis of *R. pilosa* and *R. eversmanni* (Rosenschöld) was studied at 20 °C.

Host range

Rhinusa pilosa originating from *L. vulgaris* sites near Dobanovci (Srem district), approximately 25 km west from Belgrade (N44° 50.332′, E20° 09.396′, elevation 72 m), were used in the host specificity tests. The majority of tests were conducted in the greenhouse or garden at the Institute for Plant Protection and Environment (IPPE), Zemun (Belgrade), Serbia. Additional tests were conducted in the Agriculture and Agri-Food Canada quarantine facility at the Lethbridge Research Centre in Lethbridge, AB, Canada.

Plant species tested

The *Linaria* test plant list used in the current investigation applied the conventional phylogenetic approach (Wapshere 1974; Briese 2005). The inclusion of closely related plant species identified as economically (=crop or ornamental), culturally or ecologically important (especially threatened, endangered or species of concern) was prioritized in this research. The final list included 87 Plantaginaceae species/subspecies and 35 species from 14 additional plant families. 66 species, across 34 plant genera, were native to North America. The so-called critical plants included species in the genus *Nuttallanthus* (colloquially known as the North American toadflaxes) and other native North American species from the same tribe (Antirrhineae) as the target weed.

Host specificity tests

No-choice adult feeding and survival

Feeding was assessed in early spring with reproductive adults that had overwintered in field cages ("post-hibernated adults") in the garden at IPPE, Zemun (Belgrade), Serbia. The upper part of vegetative 3–6 months old cut stems of 12 different test plant species were each exposed at room temperature in petri dishes (10 cm diameter) to four adult weevils in unreplicated tests for four weeks. Cut stems were changed every day. Feeding (mm^2 of plant surface consumed) and the number of living adults by day of study (i.e., daily census on Day 1 through Day 28) were recorded.

No-choice oviposition, gall induction and larval development

Individually potted plants with actively young growing stems were used in all oviposition and larval development no-choice tests to ensure that conditions were optimal for gall initiation and formation. Each potted plant was caged with a capped, ventilated plastic cylinder (10 × 35 cm) and the soil surface was covered with coarse sand to reduce buildup of excessive moisture inside the cage and to make the weevils more visible. Plants were exposed for 1–4 days, depending on plant size and weevil oviposition activity, to one newly emerged, post-hibernated and mated female. Only actively egg-laying females were used in this study, which was confirmed by observing each female weevil ovipositing on a *L. vulgaris* plant prior to being added to caged test plants.

All study plants were kept at 21–24 °C with a 16:8 L:D photoperiod and 60 % humidity to allow normal gall development. Each potted plant was covered with a gauze bag in early June, as dictated by weevil phenology, to ensure retention of emerging F1 adults. All plants with galls were examined under a stereo microscope and the number of oviposition marks (those still visible on the surface of galls) was

recorded. All galls or stems with oviposition marks from which no adults had emerged were dissected and gall contents inventoried for dead or alive adults. Each plant with its separate female *R. pilosa* was considered a replicate within each test, and replicate number per plant species per test was 5–10. A one-way ANOVA followed by a Tukey's HSD test was used to compare means for the number of adults per replicate and the number of adults per gall.

Sequential no-choice oviposition, gall induction and larval development

Single female–male *R. pilosa* pairs retained within a cylinder cage (10 × 35 cm) were placed for 24 h on each of ten replicate potted *Sairocarpus virga* (A. Gray) D.A. Sutton plants (i.e., one weevil pair per plant). The same single caged pairs of *R. pilosa* were then transferred and retained for 24 h on ten replicate potted *L. vulgaris* plants. Caged weevils were sequentially transferred between *S. virga* and *L. vulgaris* where they were retained for a 24 h period. Test plants were exposed only once to a specific *R. pilosa* pair. The test was continued until all the female weevils had died. The number of oviposition marks visible on induced galls was recorded approximately seven weeks after set-up.

Single-choice gall induction and larval development

L. vulgaris versus L. genistifolia A variety of single-choice tests were set-up that differed in the ratio of the number of *R. pilosa* pairs per plants used, and/or the size of the test arena. Five *R. pilosa* pairs were released into a field cage (210 × 210 × 190 cm) that contained 40 potted plants each of *L. vulgaris* and *L. genistifolia*. The plants within the cage were thereafter regularly inspected and any instances of gall development were recorded. All potted, galled plants were removed from the cage approximately 2.5 months after set-up and transferred to a greenhouse to follow gall and larval development. Each pot was covered with a gauze bag so that adult emergence could be recorded separately. In a second experiment using lower host plant densities, six *R. pilosa* pairs were released into a field cage (210 × 210 × 190 cm) that contained eight potted plants each of *L. vulgaris* and *L. genistifolia*. Gall development and adult emergence were recorded.

L. vulgaris versus S. virga Five *R. pilosa* pairs were released into a field cage (210 × 210 × 190 cm) that contained 12 potted plants each of *L. vulgaris* and *S. virga*, and left for approximately 2.5 months. Gall development and adult emergence were recorded. A second experiment evaluated host preference at a finer spatial scale. One *R. pilosa* female-male pair was exposed to two *L. vulgaris* and two *S. virga* plants growing together in a large 15 cm diameter pot covered with a gauze sleeve. Weevil pairs were transferred to new pots (same plant species and densities) every 2–3 days until all the females died. The weevils were always placed on the soil surface to allow them to freely orient toward any plant within the cage. Gall development and adult emergence were recorded.

L. vulgaris versus Sairocarpus nuttallianus Five *R. pilosa* pairs were released into a field cage (210 × 210 × 190 cm) that contained eight potted plants each of *L. vulgaris* and *S. nuttallianus*, and left for approximately 2.5 months. Gall development and adult emergence were recorded.

Multiple-choice gall induction and larval development

Six pairs of *R. pilosa* were released into each of two field cages (210 × 210 × 190 cm), both set up with six potted *S. virga*, six potted *S. nuttallianus* and 24 potted *L. vulgaris*. Plants were left for approximately two months. The design of the experiment was meant to simulate field conditions in which target hosts are typically more abundant than non-target hosts, thus allowing the females to express their preference for ovipositing on unattacked *L. vulgaris* shoots versus *S. nuttallianus*. Gall development and adult emergence were recorded.

Gall impact on host plant fitness

Sixty field-collected, single shoot *L. vulgaris* plants were transplanted into plastic pots with the root system of each plant cut to 3 cm in length to standardize for pre-treatment growth. Half of the plants were randomly selected to be individually caged and exposed to one mated female for 24 h. All plants were harvested two months later and plant height, number

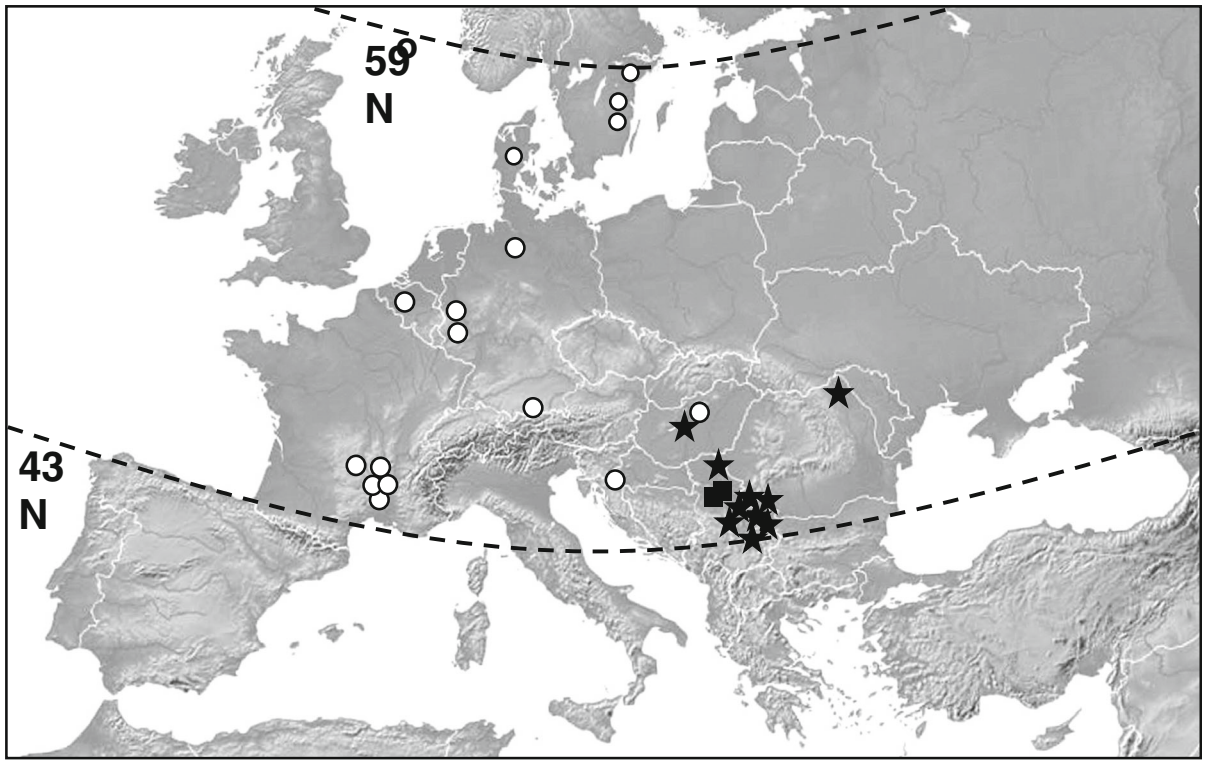

Fig. 1 Confirmed localities for *R. pilosa* (Gyllenhal, 1838) according to archived material (white dots) and newly recorded populations (black stars). Populations used for screening test (black squares)

of shoots and dry biomass were recorded. A one-way ANOVA was used to compare means.

Gall impact on the fitness of *S. virga*

To determine the impact of *R. pilosa* on *S. virga* plants, sixty same-sized *S. virga* plants were paired and one of the pair was randomly assigned to receive one mated and ovipositing *R. pilosa* female for 24 h, while the other was designated as a control. All plants were harvested three months later and plant height, number of shoots and dry biomass were recorded. A one-way ANOVA was used to compare means.

Results

Geographical distribution

In total, 20 *Linaria* species and subspecies from 290 populations were surveyed for *R. pilosa* galls in 12 European countries (Fig. 1). *R. pilosa* stem galls were found on *L. vulgaris* in Serbia (12 populations), Hungary (one population) and in Romania (one population) (Table S1). All galls recorded on *L. genistifolia* and *L. dalmatica* were confirmed as being induced by the closely related species, *R. brondelii*. *R. pilosa* has been recorded exclusively on *L. vulgaris* at three sympatric stands of *L. genistifolia* and *L. vulgaris* in Serbia. To date, all confirmed localities for *R. pilosa* are located between latitudes 43°N and 59°N of the Western Palearctic and *L. vulgaris* is the only confirmed host plant.

Biology

Life history

Activation of adults occured early in the growing season (i.e., March–May) coinciding with the spring burst of shoot growth from host perennating tap roots. Post-hibernated adults fed 3–5 days on *L. vulgaris* shoots and foliage before mating began. Oviposition followed approximately ten days later, loosely timed to occur from April to May, depending on local environmental conditions. Gall development was

Table 1 No-choice adult feeding tests with post-hibernated adults of *R. pilosa*

Plant species	Feeding (mm^2) surface area	Day when feeding ceased	Adult survival when feeding ceased (%)
L. vulgaris EU (control)[a]	367	>27	100
L. vulgaris NA[a]	235	>27	100
L. dalmatica NA[a]	240	>27	100
Species from the same tribe (Antirrhineae) as the target weed species			
Anarrhinum bellidifolium EU[b]	0	15	0
Maurandella antirrhiniflora[c]	0	15	0
Neogaerrhinum strictum[c]	17	>27	50
Nuttallanthus canadensis[c]	6	>27	25
Sairocarpus nuttallianus[c]	35	>27	50
S. virga[c]	41	23	0
Species from tribes other than that of the target weed species			
Collinsia heterophylla[c]	0	15	0
Penstemon procerus[c]	0	14	0

EU European population, *NA* North American population

[a] Geographic origin of the *L. vulgaris* and *L. dalmatica* populations tested

[b] Native European species

[c] Native North American species

complete approximately 8–10 days after oviposition under laboratory conditions, corresponding with emergence of the first larval instar. *R. pilosa* had a total of three larval instars that fed and continued development on host tissues within the developed galls. Pupation was also completed within the gall. Enclosed adults remained within the natal gall for 10–15 days, feeding on remnant host tissues before escaping via holes chewed through the gall's outer surface.

Newly emerged adults fed externally on host shoots for approximately ten days. Thereafter, adult weevils reposed in litter or cracks in the soil during the day. Summer aestivation was interrupted by occasional feeding, mainly in the evening and at night. In late autumn, adults fed shortly before entering dormancy within soil or leaf litter.

Galls induced by *R. pilosa* ovipositing into the stems of *L. vulgaris* were globular, round or oblong green structures that were positioned between the middle and tip of the host stem. On average each gall produced 2.9 adults during host specificity studies. The highest density of *R. pilosa* adults to emerge from a single gall was 17.

Parasitism

Parasitism of *R. pilosa* in Serbia varied year-to-year. Total parasitism of up to 18.5 and 11.7 % was recorded in 2002 and 2003, respectively. Parasitoids reared from galls included the endoparasitoids *Pteromalus sequester* Walker (Pteromalidae), *Eurytoma curculionum* Mayr (Eurytomidae), *Tetrastichus* spp. (Eulophidae), an unidentified species from the subfamily Entedontinae (Eulophidae), the ectoparasitoids *Scambus nigricans* (Thomson) and *Exeristes roborator* (Fabricius) (Ichneumonidae), *Bracon* (*Glabrobracon*) *pineti* Thomson and *Ascogaster quadridentata* Wesmael (Braconidae). *E. roborator* was the main parasitoid reared from *R. pilosa* galls in Serbia, with *Eurytoma* spp. as the second most abundant.

Predation

Rhinusa eversmanni is an inquiline, or gall intruder, of *R. pilosa*. *R. eversmanni* oviposits in fully developed, ten day-old *R. pilosa* galls, and its larvae directly compete with resident *R. pilosa* larvae. At 20 °C, embryogenesis takes significantly longer ($F_{1,25} = 2055$, $P < 0.001$) for *R. pilosa* (mean ± SE) (11.8 ± 0.06 days, $n = 18$) than for *R. eversmanni* (7.7 ± 0.03 days, $n = 9$). Faster egg development coupled with a significantly ($F_{1,24} = 26$, $P < 0.001$) larger size of *R. eversmanni* first instars (0.26 ± 0.01 mm, $n = 12$) compared to that of *R. pilosa* (0.22 ± 0.003 mm, $n = 14$) reinforce the competitive advantage of *R. eversmanni* over *R. pilosa*. The ratio of *R. pilosa* to *R. eversmanni* adults emerging from *L. vulgaris* decreased from 91–86 to 27–16 % from mid April to mid-May in 2004 and 2005. The decrease in *R.*

pilosa emergence from April to May may be explained by the extended exposure of galls to *R. eversmanni*.

Host specificity tests

No-choice adult feeding and survival

This unreplicated study suggested a greater total amount of feeding on *L. vulgaris* plants collected in Europe than on plants originating from established North American populations of either *L. vulgaris* or *L. dalmatica* (Table 1). *R. pilosa* feeding was found on four of the six species tested in tribe Antirrhineae. Prolonged adult survival was recorded on three native North American Antirrhineae. Feeding was not detected and survival was zero on the two native species tested from other Plantaginaceae tribes.

No-choice oviposition, gall induction and larval development

There was a significant plant effect on the number of adults per replicate ($F_{12,855} = 24.1$, $P < 0.001$) and the number of adults per gall ($F_{12,1934} = 53.3$, $P < 0.001$) (Table S2). However, the number of larvae developing to the adult stage for *L. vulgaris* from European (EU) populations did not significantly differ from *L. vulgaris* from North American (NA) populations. The mean scores for *L. vulgaris* from EU and *L. vulgaris* from NA were significantly different from those of *L. genistifolia* from EU and *L. dalmatica* from NA indicating that the later two species were less suitable *R. pilosa* hosts than *L. vulgaris*, possibly associated with a defensive reaction by plants (i.e., a hypersensitive response) following oviposition (Barnewall and De Clerck-Floate 2012). This reaction can be described as a rejection by the plant of the insect and/or its gall-inducing stimulus, and was characterized by a distinct longitudinal splitting of the stem region where *R. pilosa* oviposition had occurred. The number of adults per replicate and the number of adults per gall for *L. genistifolia* from EU was not significantly different from *L. dalmatica* from NA.

With the exception of *Nuttallanus canadensis*, *S. virga* and *S. nuttallianus*, no gall development was recorded on any native North American species (Table S2). Oviposition marks were recorded on the native North American *Epixiphium wislizeni* (Engelm. ex A. Gray) Munz but did not result in any gall or larval development. The mean number of galls per replicate was 1.1 on *S. virga* and *S. nuttallianus*, but with no larval development reported on the latter species. On *S. virga* only three of the 297 galls resulted in completion of larval development to adult emergence (Table S2). No dead adults have been recorded within a gall or shoot of either field or test plants.

Sequential no-choice gall induction and larval development

Single *R. pilosa* pairs sequentially transferred between host plant species resulted in 40.3 ± 8.1 oviposition marks and 12.0 ± 2.0 galls on *L. vulgaris*, compared to 6.0 ± 2.1 oviposition marks and 1.3 ± 0.4 galls on *S. virga*. A mean number of 31.8 ± 6.1 adults emerged from *L. vulgaris*. No adults emerged from *S. virga*.

Single-choice oviposition, gall induction and larval development

L. vulgaris versus L. genistifolia The release of five *R. pilosa* pairs onto 40 potted plants of *L. vulgaris* and 40 potted plants of *L. genistifolia* generated a total of 42 galls only on *L. vulgaris* from which 39 adults emerged. When six *R. pilosa* pairs were released into a field cage set up with eight potted plants of *L. vulgaris* and eight potted plants of *L. genistifolia*, a total of 139 oviposition marks and 32 galls were recorded on *L. vulgaris*, but only 13 oviposition marks and two galls were recorded on *L. genistifolia*. The two galls observed on *L. genistifolia* were induced at the end of April during the peak of gall formation on *L. vulgaris*. 95 adults emerged from the *L. vulgaris* galls, no adults were produced on *L. genistifolia*.

L. vulgaris versus S. virga Five *R. pilosa* pairs released into a field cage set up with 12 potted plants of *L. vulgaris* and 12 potted plants of *S. virga* produced a total of 39 galls on ten of the 12 *L. vulgaris* test plants, and four galls on two of the 12 *S. virga* test plants. The first gall to appear on *S. virga* was recorded one month later than the earliest galls that appear on *L. vulgaris*. No larval development occurred in *S. virga* galls. A total of 83 adult weevils emerged from *L. vulgaris* galls. Single *R. pilosa* female-male pairs exposed to two *L. vulgaris* and two *S. virga* plants growing

together in a large pot covered with a gauze sleeve then transferred to new pots (same plant species and densities) every 2–3 days produced a total of 68 galls and 224 oviposition marks resulting in 189 adults from *L. vulgaris*. In contrast, only two galls, without larval development, were recorded on *S. virga*.

L. vulgaris versus S. nuttallianus Five *R. pilosa* pairs released into a field cage set up with eight potted plants of *L. vulgaris* and eight potted plants of *S. nuttallianus* produced a total of 33 galls on seven of the eight *L. vulgaris* plants and one gall each on two of the eight *S. nuttallianus* plants. No larval development was recorded on *S. nuttallianus* but 99 adults emerged from the *L. vulgaris* galls. As on *S. virga*, gall induction occurred later on *S. nuttallianus* compared to *L. vulgaris*.

Multiple-choice gall induction and larval development

Six pairs of *R. pilosa* released into field cages ($n = 2$) set up with six potted plants of *S. virga*, six potted plants of *S. nuttallianus,* and 24 potted plants of *L. vulgaris* produced a total of 274 galls on *L. vulgaris* from which 622 adult weevils emerged. No galls were recorded on *S. virga* or *S. nuttallianus*.

Gall impact on host plant fitness

Comparison of galled versus control plants showed highly significant reductions in plant height (37.2 ± 3.1 versus 58.4 ± 3.2 cm; $F_{1,\ 58} = 22.1$, $P < 0.001$), dry below-ground biomass (0.4 ± 0.06 versus 1.2 ± 0.1 g; $F_{1,\ 58} = 32.9$, $P < 0.001$), dry above-ground biomass (galls excluded) (4.6 ± 0.7 versus 12.6 ± 1.4 g; $F_{1,\ 58} = 26.3$, $P < 0.001$) and shoots produced (3.5 ± 0.6 versus 13.6 ± 1.3; $F_{1,\ 58} = 10.7$, $P = 0.0018$).

Gall impact on the fitness of *S. virga*

All shoots on *S. virga* plants exposed to *R. pilosa* were atypically galled. Dry below-ground biomass was significantly higher (5.8 ± 0.4 versus 4.6 ± 0.4 g; $F_{1,\ 58} = 5.4$, $P = 0.02$) for attacked plants compared to control plants. Dry above-ground biomass and plant height were similar for attacked and control plants plants (41.9 ± 2.0 versus 39.1 ± 2.8 g; 75.7 ± 2.3 versus 71.1 ± 1.9 cm). Attacked plants produced significantly more shoots than controls ($n = 30$) (12.1 ± 0.6 shoots versus 8.5 ± 0.6 shoots; $F_{1,\ 58} = 20.8$, $P < 0.001$).

Discussion

Rhinusa pilosa is a univoltine shoot-galling weevil found exclusively on *L. vulgaris* in Europe. In a survey of 20 *Linaria* species and subspecies from 290 populations, including three stands where *L. genistifolia* and *L. vulgaris* occur sympatrically, *R. pilosa* was recorded exclusively on *L. vulgaris*. Under no-choice conditions, all 13 non-target *Linaria* species exposed to *R. pilosa* were accepted for oviposition and most were found to be suitable, to varying degrees, for gall and larval development. In Europe, historical field host records for *R. pilosa* reported in the literature need to be confirmed, since in most cases no distinction has been made between *R. pilosa* and *R. brondelii*. A recent population genetics study of weevils from the *R. pilosa–R. brondelii* complex, for example, highlighted what appears to be a significant host-associated, genetic divergence of *R. brondelii* collected from *L. genistifolia* and *L. purpurea* (Toševski, unpublished data). Even if only minor morphological differences can be found in the species of the *R. pilosa* group, it was concluded that all these taxa should be treated as very old and relict species, and may be very specific to the host plant with which they are associated. In two separate single-choice tests, *R. pilosa* produced significantly fewer oviposition scars and galls and no adults on *L. genistifolia* compared to *L. vulgaris*. This confirms that *L. genistifolia* is not a suitable host.

Linaria vulgaris from European and North American populations of *L. vulgaris* were similarly accepted by *R. pilosa* for oviposition. Both host populations also were able to support full gall and weevil development, despite adult feeding being lower on the North American *L. vulgaris* tested. Barnewall (2011) found that *R. pilosa* was able to gall and develop successfully on all Canadian populations of *L. vulgaris* tested, even though the plant growth variables measured were different among the populations at the set-up of the experiment, and there were population-dependent differences in plant growth responses post-galling.

The test plant list has taken into consideration, both ecologically and economically, the importance of the

now more speciose Plantaginaceae family, and also is more phylogenetically relevant for delineating the host range of candidate *Linaria* spp. biological control agents (Gaskin et al. 2011). Adult feeding and survival was minimal on native North American species in the weed tribe Antirrhineae. Moreover, oviposition was limited to only four out of 63 native North American species in no-choice tests. Of the native species attacked by *R. pilosa*, only three larvae developed to the adult stage on only one species, *S. virga*. Very little oviposition and gall development without larval development was recorded on *N. canadensis*, whose phylogenetic relationship with *Linaria* has been recently disputed (Fernández-Mazuecos et al. 2013). Oviposition on native North American species was further reduced in choice experiments.

Relative to impact on *R. pilosa*'s preferred host, *L. vulgaris*, gall development had apparently no negative impact on the growth of the one native plant species supporting gall and larval development, *S. virga*. In contrast, stem-galling had a highly significant impact on the vegetative growth of *L. vulgaris.* In a similar experiment, Barnewall (2011) found that galled *L. vulgaris* plants produced fewer flowering stems in comparison to controls. Although there was no significant difference between the galled and control plants in the total amount of dried, above-ground biomass, approximately 40 % of the total produced by galled plants constituted gall tissues. Thus, if gall biomass is subtracted from the total above ground biomass produced by the galled plants, it could be deduced that there was a significant loss in net plant productivity due to galling.

Under no-choice conditions, four plant species native to North America were found to be suitable for oviposition, and three supported gall development, i.e. *N. canadensis*, *S. nuttallianus* and *S. virga*. When galls form on these sub-optimal hosts, they are abnormal in shape and growth. Externally, they appear as elongated, slight swellings of the stem, which sometime cause stem contortions. Internally, the central gall cavity when present is often narrow and partially occluded by parenchymous tissue. Occasional abnormal stem tissue proliferation was observed to crush weevil eggs deposited within the pith of sub-optimal hosts (Barnewall 2011). This resulted in high egg mortality, and with very few exceptions, the plant's response did not allow normal larval development to the adult stage.

Host-range studies demonstrated that *L. vulgaris* is the most suitable host plant for *R. pilosa*. Choice tests with *L. vulgaris* and non-target species have confirmed that *R. pilosa* clearly prefers to oviposit on *L. vulgaris*, which was also the most suitable host for successful gall and larval development. *R. pilosa* is highly specific to *L. vulgaris* and the potential impact on non-target species is negligible under these conditions. *R. pilosa* has been associated in the field exclusively with *L. vulgaris*, and demonstrates the potential to exert significant impact on its host plant if introduced. Populations are expected to increase rapidly where they will be free from regulation by the gall intruder *R. eversmanni* and its other native range natural enemies. The Technical Advisory Group for the Biological Control of Weeds (TAG—–BCW) has recommended release of *R. pilosa* in September 2013.

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