THE IMPACTS OF A STEM BORING WEEVIL, *MECINUS JANTHINUS*, ON DALMATIAN TOADFLAX, *LINARIA DALMATICA*

by

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ABSTRACT

Classical biological control of weeds is generally considered an effective, safe, and cost effective tool for controlling widespread weeds in natural areas. However, only 60% of releases have become established and, of those, only 50% have led to control. Therefore, understanding the impacts of agents on target weeds across spatial scales, at different insect densities, and over time can give biological control practitioners the knowledge necessary to improve establishment and success rates. My studies characterized the impacts the biological control agent, *Mecinus janthinus*, on the rangeland weed Dalmatian toadflax (*Linaria dalmatica*) at individual plant and plant population scales. Individual plant studies were conducted in a garden and replicated on plants growing in the field, to measure the impact of agents on plant growth and primary physiology. The population study followed operational scale releases of *M. janthinus* for three to four years using intensive monitoring to characterize *L. dalmatica* cover, density, and population structure, and weevil establishment and population increase. I also evaluated which parameters were most important to measure to determine establishment and success. In the common garden experiment, *M. janthinus* injury was found to reduce relative plant growth, as well as root, stem, and reproductive biomass at medium and high adult densities. Trends of reduced photosynthetic, conductance, or transpiration rates with increasing *M. janthinus* density were observed. In the field experiment, *M. janthinus* injury led to reduced growth. Gas exchange rates decreased over the season and were lower in plants exposed to high larval and adult herbivore pressure. Field monitoring indicated successful *M. janthinus* establishment at releases across a range of elevations, slopes, and geographic locations, though high rates of overwintering mortality were observed at all sites. *Linaria dalmatica* cover was variable within watersheds and patches, and also differed between watersheds. Abundance of *L. dalmatica* decreased over time based on cover measurements, but did not change over time with respect to density. The proportion of mature *L. dalmatica* stems decreased over time. The majority of changes in *L. dalmatica* over time were the same in release and control transects and could not be attributed to *M. janthinus*.

CHAPTER 1

INTRODUCTION

Invasion Theory

For a time, ecological theory suggested that diverse communities were less prone to invasion than species deficient or monoculture communities (Elton 1958). This has been supported by numerous microcosm and controlled environment experiments aimed at isolating the local or neighborhood effects of diversity on invasions, as well as their underlying mechanisms (Levine 2000). Communities supporting diverse assemblages of endemic species have been considered most resistant to invasion pressure. However, a review of five papers that examined invasion potential on 24 nature reserves across a broad range of geographic locales where native diversity tended to be high, concluded that nature reserves across all locales (except Antarctica) had been colonized by invasive species (Usher 1988). Invasions have also been reported throughout arid lands, but tend to be most severe along perennial and intermittent waterways (Loope et al. 1988). These results support the arguments that all communities are potentially prone to invasion, but some are more likely to be invaded than others with the differences being due to propagule pressure, the availability of species able to survive in a given habitat (Williamson 1996), the identity of species present in a habitat (Crawley et al. 1999), the level of disturbance of a given habitat (Collins et al. 1995, Mackey and Currie 2001), and random chance.

High propagule pressure or large initial introductions can increase the likelihood that at least some propagules survive an initial introduction, or, that some individuals reproduce successfully (Williamson 1996). In a naked sedge (*Carex nudata* W. Boott) dominated plant community along the South Fork of the Eel River in northern California, individual tussocks of *C. nudata* form discrete islands supporting as many as 20 perennial herbs and bryophytes. In an experiment where individual tussocks were manipulated to control initial species richness and then over-seeded with *Agrostis stolonifera* L. and *Plantago major* L., diversity only explained 25% of the variation in success of the two "invaders" (Levine 2000). Levine (2000) concluded that propagule pressure was a more important factor than species richness in the success of the over-seeded species.

Communities in harsh environments appear more resilient to invasion, but this may be due to the limited number of species that can survive under harsh conditions (Williamson 1996). In a study on productive grassland plots at Silwood Park in England, Crawley et al. (1999) allowed seeded plots at four species richness levels (1, 2, 4, and 80 species) to become established for one year without invasion of non-seeded species. Plots had been fumigated with methyl bromide before seeding to kill vegetative propagules and seeds present in the soil. In the second year, unseeded species were allowed to invade. Plots were followed for eight years. Crawley et al (1999) found that for productive small scale grasslands there was no correlation between species richness and the number of species that invaded plots or the biomass of invasive species (Crawley et al. 1999). However, species identity was positively linked to number of invading species and their biomass.

Many species coming into a community as seed propagules may not be able to compete with established adult plants (Crawley et al. 1999), and rely on disturbance to establish in the new community. In both disturbed and undisturbed plots at low nutrient levels, belowground competition was the dominant interaction in a study where a perennial grass was sown with and without neighbors in disturbed or undisturbed plots that were either fertilized or not (Wilson and Tilman 1993). They found that light competition was reduced by disturbance, but was unaffected by nutrient level. In a complete randomized block experiment designed to determine the effects of disturbance, competition, and herbivory on tansy ragwort (*Senecio jacobaea* L.), McEvoy et al. (1993) found that when a large buried seedbank was combined with disturbance, ragwort outbreaks occurred, but that herbivory and competition either alone or together ameliorated some of the effects of disturbance.

The effects of the above factors (propagule pressure, availability of suitable species, identity of nearest neighbor, and disturbance) on invasion depend in part on the spatial and temporal scales at which invasions are studied and on the metric used to characterize the invasion. In an observational study of four long-term ecological research sites in three habitat types, Cleland et al. (2004) found that native species richness was positively correlated with invasive species richness, but negatively correlated with invasive species abundance. The relationships were stronger at larger spatial scales. They also found that the probability of invasion was reduced within years of high productivity and the year after, with the exception of their Chihuauan desert site, where they found the opposite trend. In a different study at 24 locations in Narragansset Bay, RI, Bruno et al. (2004) found that cover and richness of exotics varied and was not

strongly related to physical disturbance of the sites, neighboring habitat type, or substrate. They also found that native and exotic cover values were not negatively correlated. However, across sites Bruno et al. (2004) found that native and exotic species richness were positively correlated, although exotic cover was unrelated to native species richness.

Wildfires and prescribed fires constitute one type of disturbance that has received considerable attention with regard to effects on exotic species invasions. However, actual results have been inconsistent. Fires are unique in that they produce disturbance that is not uniform across the burn site, and along with removing litter and varying degrees of above ground organic matter, they can deposit biologically usable nutrients, thereby reducing competition for light while fertilizing the site. The degree to which the live vegetation is removed by fire is partially dictated by intrinsic fuel characters (such as stem and leaf moisture content) and extrinsic fuel characteristics such as the amount of fuel at the site, the horizontal continuity of the fuels, the vertical continuity of the fuels, how densely the fuels are packed (Brooks et al. 2004), and the topography of the site (Daubenmire 1968a).

Fires move on average two times faster on 10% slope than on flat ground, and move four times faster on a 20% slope than on flat ground (Daubenmire 1968a). Temperatures recorded in fires vary with soil layer measured, height above the ground temperature is measured at, direction of fire, and the type of vegetation the fire is moving through (White et al. 1973, Bailey and Anderson 1980, Jacobs and Sheley 2003a). The amount of damage a fire does to a given leaf depends on the fire temperature and if the plant material is exposed long enough for the leaf to reach the lethal threshold temperature (Daubenmire 1968a). The reported temperature that is lethal to most leaves

is 60 °C. Jacobs and Sheley (2003a) found that in a sage habitat in the foot hills of the Elkhorn Mountains in southwestern Montana, prescribed fires burned hottest at the top of the vegetation, effectively burning off the sage and pine species and opening the canopy. The location of the perennating buds on a plant can in part determine if a perennial plant will survive a given fire (Daubenmire 1968a). Perennating buds underground are less likely to be killed than buds at the soil surface.

Fire temperature can also affect the availability of nutrients released by a fire to colonizing plants and fire survivors. White et al. (1973) conducted a laboratory study where they collected samples of litter, mor layer, and A1 horizon from six ponderosa pine (*Pinus ponderosa* C. Lawson) forest locations in the Black Hills of South Dakota and subjected them to heating at different temperatures to determine the effects of heat on nutrient release. They recorded no loss in nitrogen at temperatures below 200 °C, and losses of 50 – 66% at 400 °C. Only trace amounts of N remained in samples heated to 500 °C. They recorded the greatest release of water soluble potassium at 200 °C. At temperatures between 200 and 500 °C, water soluble potassium content of the litter and mor decreased. The effect of heating the A1 horizon to temperatures above 200 °C varied with location, increasing potassium at some locations (by fracturing the samples), and decreasing it at other sites. These lab derived results have been corroborated with field studies (Sharrow and Wright 1977, Debano and Conrad 1978).

Hurlbert (1988) conducted an experiment to determine if fire effects on tallgrass prairie were due to removal of standing vegetation and competition for light or the addition of nutrients to the soil, litter removal, or soil heating. In the experiment, Hurlburt (1988) compared burned plots with plots treated to simulate known or suspected

effects of fire on the biomass and production of flowering stems in six dominant tall grass prairie species. Hurlbert (1988) concluded that the increased levels of solar radiation were the most important effects of burning – both in removing shading or competition from other species, and maintaining a higher soil surface temperature throughout the season than unburned plots.

The effects of burning on germination are mixed. A study on the effects of fire on germination of 45 tree, shrub, sub-shrub, and liana taxa found that the addition of powdered charred wood stimulated germination in a quarter of the species, and heat stimulated germination in another quarter of the species, while light was required for germination of approximately 1/3 of the species studied (Keeley 1987). In a laboratory based germination study on three post fire chaparral species (*Emmenanthe penduliflora* Benth., *Phacelia grandiflora* Benth., and *Salvia mellifera* Greene), Thanos and Rundel (1995) found that nitrate was the principal factor for induction of germination in *E. penduliflora* and *P. grandiflora*, but that light was the most important cue for germination in *S. mellifera*. Chaparral soils are generally very nitrogen poor, so the slight increase that might occur in low temperature fires would be enough of an increase to stimulate germination.

Non-native invasive species are often strong colonizers, and in nutrient poor habitats, fires that add nutrients while clearing the canopy could give exotic species an extra advantage. When a mix of native and non native seeds were sown in both annual and perennial grass sites, emergence and survival of native seedlings was enhanced in burned sites compared to unburned plots (Maret and Wilson 2000). When similar species were seeded over burned native bunch grass plots, they did not change the seedling

dynamics of the native species, but did enhance the dynamics of non-native species (Maret and Wilson 2000). The authors recommended burning with seeding to restore invaded sites but concluded burning pristine sites was a risky management tactic due to the potential for invasion by non-natives. Once established on burned sites, invasive species have the potential to alter the fire regime by altering intrinsic and extrinsic fuel properties. These effects can be short lived if the species is not adapted to the new fire regime, or can be long term if the change benefits the introduced species but not the prior vegetation (Brooks et al. 2004). The ability of exotic species to invade burned areas and their potential to change the fire regime of the areas they invade could have consequences for weed management and biological control.

Classical Biological Control

Classical biological control is one of three techniques of biological control that also includes augmentation and conservation biological control (McFadyen 1998). Classical biological control involves the utilization of non-native parasites, predators, and pathogens to regulate pest populations below a threshold level (Harris 1991). Harris (1991) also includes genetically modified organisms in his definition of classical biological control agents and implies that the agent must be alive and attack a pest to be considered a biological control agent. Pests controlled biologically can be plants or animals (including arthropods).

Weed biological control is considered an effective, safe, and cost effective management tool. However, only approximately 60% of releases have established (Julien et al. 1984, Julien 1989, McFadyen 1998) and of those established agents, only

about 50% have led to successful control (Julien 1989, McFadyen 1998, McEvoy and Coombs 2000). Julien et al. (1984) defined a release as "the liberation of a phytophagous species to control a target weed species. The same organism liberated in the same country against a different target species, or the same organism liberated in a different country against the same target species has been treated as a separate release." The number of programs leading to control may be under reported due to the general lack of long term monitoring in many systems (Blossey 1999) and the large time lags that can occur between agent release and visible impacts on the weed population (McEvoy et al. 1991).

Stages of a Classical Weed Biological Control Program

There are several stages involved in successfully implementing classical biological weed control programs. These stages span from the selection of a target weed as potentially controllable by an insect or pathogen (Peschken and McClay 1992) to the suppression of the target weed (Julien 1984, Syrrett et al. 2000) and restoration of the ecosystem. Once a plant is selected for biological control, control agents need to be selected (Goeden 1983), screened for host-specificity (, released, and established (Grevstad 1999a, 1999b). The established agent population then needs to increase, spread throughout the weed infestation (Crawley 1986), damage the weed, and control it by maintaining it at economically or ecologically acceptable levels (Crawley 1989b, Julien and Griffiths 1998, Syrett et al. 2000). Once control is achieved, restoration of the ecosystem becomes the main focus.

Choosing a Target Weed: Not all weeds are suitable for biological control. Biological weed control programs can take up to 20 years from control agent selection to successful weed control (Harris 1979). The cost associated with this has encouraged scientists to consider ways to select plants that are more likely to be successfully biologically controlled. Peschken and McClay (1992) have developed a scoring system to rank weeds for their potential to be successfully controlled with a classical biological control program. Their scoring system considers both weed biology and economic impacts. They give particular weight to plants that cause severe economic losses, plants whose current control practices cause high levels of environmental damage with low economic justification, plants with native ranges restricted to one continent, and plants that colonize stable habitats (Peschken and McClay 1992, McClay 1995).

Choosing Biological Control Agents: Control agents to be used in biological control programs need to be good invaders with high intrinsic rates of natural increase, *r*, high abundance in their native habitat, have good climate matching between the native range and area of introduction, and fill a vacant niche (Goeden 1983, Williamson 1996). Good control agents also need to have good host finding and dispersal abilities and high rates of survivorship from immature stages to reproductive adults (Harris 1973, Goeden 1983). Traditionally, host-specificity has been considered the most important quality in potential biocontrol agents. Relatively little emphasis has been placed on potential efficacy of agents that prove to be host-specific in tests (McFadyen 1998, McEvoy and Coombs 2000), despite early calls for choosing agents that attack a sensitive phenological stage of the target weed (Muller 1988). However, the recent increase in the number of

publications advocating incorporation of efficacy testing into agent selection protocols indicates a shift in approach (Briese 2005, 2006, Goolsby et al. 2006, Raghu et al. 2006, Schooler et al. 2006).

Biocontrol Agent Establishment: According to Williamson (1996), only about a third of species introduced for biological control become established. Julien (1989) reported that 65% of control agent releases (including multiple releases of the same organism in different locations) around the world have become established. The discrepancy in establishment rates could in part be due to the lack of well-defined criteria for establishment. One definition currently being used in Oregon is the recovery of a univoltine insect three years after release, or a population greater than 1000 individuals around the release point two years after release (Eric Coombs, Personal communication). The release strategy used can affect the establishment of control agents. Two strategies are often suggested: (1) large releases should be used to maximize mate finding and minimize Allee affects (an effect where smaller populations have reduced reproduction and survival rates than higher population levels), and (2) multiple releases should be made to reduce the risk that environmental conditions lead to local extinction (Beirne 1985, Grevstad 1999a, 1999b). However, in most cases there are a limited number of agents available for release and a trade-off needs to be made between size of release and number of releases made. Choosing insects with a high intrinsic rate of increase, *r*, can help reduce the extinction risk and increase the establishment rate for small populations of insects (Crawley 1986).

Increase and Spread of Biocontrol Agents: The ability of a biological control agent to affect a weed population depends on its ability to increase in density and spread throughout the weed infestation (Crawley 1986). There is a generally a positive linear relationship between the density of herbivorous insects and the damage they cause plants (Southwood and Norton 1972). A high rate of increase suggests a rapid increase in herbivore density. Andow et al. (1990) linked the rate of spread of biological control agents to their ability to suppress a weed population and move on to new host patches.

Suppression of Target Weed: The definition of successful biological control varies with the system, but usually refers to the reduction of the weed below levels existing prior to control (Crawley 1989b, Julien and Griffiths 1998, Syrett et al. 2000). In insect control there are often thresholds above which a pest will cause economic losses. An insect control program is then considered successful if the control agent maintains the pest below the economic threshold level. Many weed biological control programs are aimed at natural area infestations, where thresholds have yet to be established, so success of these programs is more difficult to measure. In some cases successful biological control agents reduce the competitive ability of a weed to a point where it can no longer dominate the ecosystem (Thompson et al. 1987).

Many models of biological control are based on the Nicholson-Bailey and Lotka-Volterra families of predator prey models. It has been argued that for biological control to work, the predator or parasite and prey need to maintain a stable relationship. Murdoch (1992) listed four mechanisms that he considered important for promoting stability in successful insect biological control programs : (1) parasitism is density

dependent through time, (2) parasitism is highly concentrated in a small fraction of pest individuals (aggregation), (3) parasitism is density dependent in space, and (4) considered less important, parasitoid sex ratio is density dependent through time. Weed biological control programs have some important differences from insect biological control programs. In insect biological control programs the prey can move to escape a predator or parasitoid, whereas in weed biological control programs the plants (prey) cannot escape parasitism (biological control agent) by moving in space.

For a program to be successful, the agents need to impact individual plants to the degree that wider population dynamics are affected. Ideally the change in target weed population will shift the plant community towards desired vegetation. Insects can affect plant populations directly by killing individual plants outright, suppressing reproduction and reducing future propagule pressure, or indirectly by slowing the growth of plants enough to allow other species in the community to compete more effectively (Crawley 1989a). The ability of a control agent to damage individual plants does not necessarily translate into successful suppression of the target weed population (Crawley 1983, McClay and Hughes 1995). Choosing a suite of control agents that attack several plant parts, increases the chance that the limiting life history stage is attacked (Muller 1988).

Insects may impact individual plants but not the plant population due to (1) low quality food preventing the biocontrol agent population from growing enough to affect the population, (2) control agents ceasing to feed before the target weeds stop growing and allowing plants to compensate for insect damage after the agent stops feeding, (3) soil seedbanks large enough that even dramatic reductions in seed output may not lead to reduced seedling recruitment, and (4) biocontrol agents remaining at low levels due to

predation or parasitism (Crawley 1989a). Harris (1997) suggests that it may not be worth monitoring weed population effects unless insects remove more than 20% of the resource they feed on, although that threshold may vary for agents with different modes of feeding.

There have been several studies on the impacts of biological control agents on individual plant performance, showing reduced growth, suppression of flowering, and reduced seed output, but very few studies have attempted to elucidate the physiological mechanisms behind the plant performance changes observed. There are also very few studies that catalog the effects of biological control agents at a population or community scale, (but see Huffaker and Kennett 1959, McEvoy 1985, McEvoy et al. 1993, McEvoy and Coombs 1999).

Stability of Control and Ecosystem Restoration: For a biological control program to be successful over the long term, it needs to maintain control in the face of ecosystem perturbations. Often, an area under biological control is simultaneously being managed for other purposes. If other management programs interfere with the biological control agents, control of the weed may not be maintained. Once the weed population is suppressed it is important to focus management onto restoring the ecosystem. In many cases, if no further management occurs, a new weed will replace the one controlled. What replaces a weed depends on what can colonize the site from the seed bank and from propagules coming in from outside the system.

Concerns About Biological Control

Major concerns surrounding biological control include success controlling the target weed, direct non-target effects (agents damaging non-targets), indirect non-target effects (competition between agents and native species), and community ecosystem effects (Simberloff and Stiling 1996). Through 1984, only 55 out of 151 biological control projects against 82 weeds were successful and 35 weeds species were controlled (39%) (Julien et al. 1984). One of the most expensive aspects of biological control is foreign exploration and host-specificity testing (Coombs et al. 2004). The introduction of many host-specific agents that do not impact the plant population drives up the costs associated with biological control and increases the chances of non-target effects (McEvoy and Coombs 2000). Direct non-target effects caused by control agents can be minimized by choosing host specific control agents and by selecting taxonomically isolated target species. The standards for importing weed biocontrol agents have become more stringent over time, and rigorous host-specificity testing is now required. Indirect non-target effects are more difficult to anticipate.

To improve weed biological control success, cost effectiveness, and safety, we need to improve our understanding of how agents impact plants and under what conditions the individual plant impacts translate into population impacts and shift control agent selection from being almost entirely host-specificity driven to a combination of host-specificity and potential efficacy. One way to improve our understanding of plant-insect interactions is to determine how the agents impact individual plant performance and the physiological mechanisms behind those changes. The next step is to determine a threshold agent population level required for impacts to occur and link the agents to changes in weed population in the field.

Impacts of Insects on Plants

Photosynthesis

Photosynthesis is the process of converting carbon from CO_2 to glyceraldehyde 3-phosphate (GAP), the basic building block for biosynthesis in plants. For this conversion to occur, plants need light to catalyze the electron transport chain that produces energy in the form of ATP and NAPDH for biosynthesis of primary and secondary metabolites.

When light strikes a pigment molecule, it excites electrons. Those electrons can lose excitation to their more stable ground states in one of four ways; energy is released a heat, fluorescence (energy is released as light with a slightly longer wavelength than originally absorbed), energy transfer (when energy is passed through a chain of molecules), and charge separation (Malkin and Niyogi 2000). Photosynthesis involves both energy transfer and charge separation. Under conditions when too much light is intercepted to be used in electron transport, plants exhibit higher rates of fluorescence (Larcher 1995).

Light hitting a leaf strikes light harvesting molecules embedded in the thylakoid membranes in chloroplasts, exciting electrons. The energy is passed through a series of chlorophyll molecules until charge separation occurs at the photosystem II reaction center. The electrons are then passed onto a pheophytin molecule and through two plastiquinones generating two hydrogen atoms. The hydrogen atoms help create an ion gradient that drives the synthesis of ATP. Two more hydrogen atoms are generated as the electrons continue to move through the electron transport chain into the cytochrome

b6f complex. After exiting the cyctochrome complex, the electrons move through photosystem I generating NADPH. The hydrogen atoms generated go through ATP synthase molecules, driving ATP synthesis. This electron transport chain is called the Z-scheme since the electrons being shuttled through the system move back and forth between the thylakoid lumen and the cell stroma.

The ATP and NADPH generated are the energetic currency used to convert CO2 to 3PGA. Once CO_2 enters the plant, in C3 plants, the enzyme rubisco combines it with ribulose-1,5-bisphosphate (RuBP) in a carboxylation reaction. This produces an unstable C6 molecule that is immediately broken down into two 3-phosphoglycerate (3PGA) which are reduced in two steps to two GAP molecules (Malkin and Niyogi 2000). For every three CO_2 molecules assimilated, six GAP molecules are produced. Five of those GAP molecules are used to regenerate RuBP in a ten step pathway, and one GAP is put in the triose phosphate pool and used for biosynthesis (Malkin and Niyogi 2000). Rubisco can also catalyze an oxygenation reaction with RuBP to produce one 3PGA and one 2-phosphoglycolate which needs to go through a photorespiration pathway to be converted into a usable form (Siedow and Day 2000). The oxygenation reaction reduces the photosynthetic efficiency of C3 plants. The concentration of CO_2 relative to O_2 in the intercellular airspaces and temperature can affect the selectivity of rubisco for CO_2.

It has been suggested that there are three limitations to photosynthesis: the availability or use of CO_2, ability to utilize light, or phosphorous (Sharkey 1985). These limitations can be distinguished by measuring gas exchange variables such as CO_2 assimilation, light use efficiency, stomatal conductance rates, and fluorescence, and assays to determine the activity and amount of enzymes and metabolites important in

reduction of CO_2 (Peterson 2001). Fluorescence can be used as a measure of the efficiency of the electron transport chain (Larcher 1995). Another way to determine if injury if impacting the electron transport chain is to examine light response curves (Peterson et al. 2004). The initial slope of the response curves gives the efficiency at which one photon of light can be used for CO_2 assimilation. The response curves also provide a CO_2 compensation point which is the point at which CO_2 assimilation is equal to respiration (the point where a plant is alive, but cannot grow). If these points do not change between injured and uninjured leaves, it indicates that the electron transport chain has not been inhibited by the injury.

The plant also needs to allow CO_2 to enter the plant. When stomata are open to allow CO_2 to enter a leaf, water also escapes from the leaf due to the lower relative humidity outside of leaves compared to that inside the leaf (which is considered to be 100%) (Sharkey 1985). The mass flow of water out of leaves can affect the partial pressure of CO_2 within intercellular air space which can in turn affect photosynthetic efficiency. Stomatal conductance (mol H_20 m^{-2} s^{-1}) is a measure of the movement of water out of the leaf. In general, as stomatal conductance increases, the transpiration rate will increase more than the CO_2 assimilation rate (Sharkey 1985). Assimilation curves over a range of CO_2 concentrations can be used as a measure of rubisco efficiency and RuBP regeneration (Peterson et al. 2004).

Interruption of metabolism transport can be measured by measuring the accumulation of carbohydrates in the leaves. At first light, photosynthesis leads to an accumulation of triose phosphates in the chloroplasts. These are transported into the cytosol and initiate sucrose production. As midday approaches, the amount of sucrose

being mobilized out of the cytosol cannot keep up with production of sucrose. The sucrose synthesis pathway is inhibited and the starch synthesis pathway is initiated in the cells (Dennis and Blakely 2000). Insects that inhibit the flow of sucrose in the phloem would lead to starch accumulation in the chloroplasts and high levels of sucrose in the cytosol.

Impact of Insects on Plant Primary Physiology

Although it has been widely accepted that arthropods can impact crop yield and plant fitness, there have been relatively few studies that pinpoint the mechanisms behind the yield and fitness losses. Photosynthetic rate, water vapor transfer, and respiration can affect plant yield and fitness (Peterson 2001). Previous studies have suggested that the type of physiological response elicited could depend specific herbivore feeding mechanisms. Suggested functional feeding groups include stem borers, leaf miners, leaf skeletonizers, fruit scarring and seed feeding (Peterson 2001). These can be further characterized by expected physiological responses, including population or stand reducers, leaf photosynthesis reducers, leaf mass reducers, those that alter leaf senescence, light reducers, architecture modifiers, phenology disrupters, assimilate removers, water-balance disruptors, and those that cause seed and fruit destruction (Peterson 2001).

The majority of studies evaluating impacts of insect herbivores on photosynthesis have focused on defoliators, due to the relative ease of both quantifying injury level and rearing study insects. Physiological responses reported for defoliators have been variable. Many studies have showed that removal of material from one leaf leads to

increased photosynthetic rates in other leaves on the same plant (Peterson and Higley 1993). Peterson and Higley (1993) also cite several papers linking increased photosynthetic rates to increased demand for primary metabolites after injury, reduced inter-leaf competition for mineral nutrients critical in cytokinin production, and delayed leaf senescence. Conversely, leaf feeding by a root weevil (*Artirus floridanus* Horn) on grapefruit led to a reduced photosynthetic rate proportional to the amount of leaf tissue removed (Syvertsen and McCoy 1985).

Feeding by the leaf defoliating beetle *Trirhabda* sp. reduced photosynthesis in goldenrod by decreasing leaf area, but had no impacts on per leaf photosynthetic rates on injured leaves or uninjured leaves (Meyer and Whitlow 1992). In a natural and simulated herbivory experiment on six species and cultivars of legumes, the authors found that defoliation of a single leaf had no effect on the gas exchange variables measured (Peterson et al. 2004). The type of leaf consumption may also play a role in photosynthetic response. Mexican bean beetle (*Epilachna varivestis* Mulsant), which skelentonizes leaves and often injures vascular tissue, caused different physiological responses in soybean (*Glycine max* (L.) Merr) than reported for other defoliators of the same species (Peterson et al. 1998). Peterson et al. (1998) observed that Mexican bean beetle defoliation reduced gas exchange variables measured and that leaflets did not return to pre-injury levels of photosynthesis after feeding stopped. They were also able to determine that reduced photosynthetic rates were not due to light use or stomatal conductance changes, but to the utilization of CO_2. Variability in plant photosynthetic response to defoliators could in part be due to specific feeding mechanisms and experimental limitations (Peterson 2001).

Comparatively few studies have been undertaken on injury by concealed feeders, including stem miners due to the inherent difficulty in controlling or simulating injury levels. Stem borer reduction in photosynthesis has been linked to interruption of vascular transport (Peterson and Higley 1993). However, Peterson and Higley (1993) caution that the results were based on studies without manipulated levels of herbivory and without replication. In a replicated but un-manipulated study on the impact of shoot mining by *Mecinus janthinus* Germar on photosynthetic rate of *Linaria. dalmatica* (L.) Miller, Peterson et al. (2005) reported that infested plants had significantly reduced photosynthetic rates 17 to 39% depending on field site and year compared to herbivore free plants. The authors were unable to suggest a mechanism for decreased photosynthesis apart from that it did not appear that the feeding resulted in stomatal limitations.

Recent studies in growth chamber and greenhouse studies on agricultural species have examined the impact of stem borer injury on plant primary physiology. In a growth chamber experiment, Macedo et al (2005) found that stem mining injury caused by larval wheat stem sawflies (*Cephus cinctus* Norton) led to significant reductions in the photosynthetic capacity of wheat as the plants senesced. In greenhouse studies on the impact of wheat stem sawfly on gas exchange rates in wheat at the grain filling stage, uninfested plants had photosynthetic rates 12% higher than infested plants (Macedo et al. 2006).

Resource Allocation

Metabolites produced by plants are moved to different parts of the plant for growth, flower and fruit production, and storage. Most studies of resource allocation patterns in plants use biomass as a method to measure allocation patterns because biomass seems to reflect allocation of other resources. However, it is important to consider that there may be some resource allocations that are not well correlated with biomass allocation (Weiner 2004). Traditionally, allocation patterns are considered as a proportional or partitioning process in which at any given time a plant has a finite amount of resources which are divided between different plant parts or processes. Under this view, different allocation patterns are then attributed to different priorities during the course of development (Harper and Ogden 1970, Weiner 2004). These allocation patterns are thought to show phenotypic plasticity; the ability of an organism to respond to environmental changes by changing their form, or rate of activity (West-Eberhard 2003).

The plastic responses of plants to biotic and abiotic environmental changes are often analyzed as ratios such as root:shoot ratios or whole plant:specific plant part ratios (Weiner 2004). Several studies have analyzed whether the variation in allocation ratios at different times are consistent with the optimal allocation theory, adapted from economic theory (Bloom et al. 1985), which states that plants will allocate resources to increase the uptake of limiting resources (Weiner 2004). Under this model, the true optimal allocation of resources is observed when all resources are equally limiting. Three studies of root:shoot ratios on the rapidly growing annual plants *Abutilon theophrasti* Medik, *Chenopodium album* L., *Polygonum pensylvanicum* L. (Gedroc et al. 1996,

McConnaughay and Coleman 1999) and 10 grass land species (Cahill 2003) concluded that the plasticity in biomass allocation and root:shoot ratios of test plants was only partially explained by the optimal allocation theory and that at least part of the variation observed was plasticity in ontogenic trajectories. Their conclusion suggests that allocation patterns change with plant size (Cahill 2003, Weiner 2004), and that some of the variation in biomass allocation ratios at different nutrient levels is actually due to plants in low resource environments being smaller than conspecifics grown in high resource environments. This plasticity could be defined as 'apparent plasticity' (Cahill 2003, Weiner 2004). Weiner (2004) argues that for biomass allocation to be considered truly plastic, the allometric trajectories have to be different, meaning that at a given size, plants in low resource environments would grow differently than similar sized plants in high resource environments.

In a number of studies, root-shoot ratios have been found to be plastic, but allometric trajectories have not. For example, a study that analyzed both biomass ratios and allometric trajectories for 27 herbaceous clonal species in 20 genera from 11 families found that most species had significantly different biomass allocations under different nutrient regimes, but that when the relationships were examined allometrically most of those differences turned out to be a consequence of plant size rather than changes in allometric growth trajectories (Muller et al. 2000). Further, Muller et al. (2000) discovered three types of effects of nutrients on allometric growth when the log of leaf biomass was compared to the log of root biomass. The first, found only in one of their test species (*Agrostis tenuis* Sibth.), was a difference in the slopes of the allometric trajectories, indicating that trajectory is size dependent. In three other species, *Eleocharis*

palustris (L.) Roem. and Schult., *Potentilla reptans* L., *and Prunella grandiflora* L., the intercepts differed indicating size independent effects. In seven species they found no differences in allocation trajectories by nutrient treatment. Another result reported was that in these clonal species the plant increased the number of ramets produced under higher nutrient levels, but the number of leaves per ramet remained constant. Due to the modularity and variation in ramets, growth rate is a more useful metric to analyze than plant size alone (Weiner 2004).

The idea of plasticity can be applied to allometric trajectories, but Weiner (2004) suggests that the level of plasticity be refined in a hierarchical structure in the following way:

1. Non-plastic traits that do not vary at all with the environment
2. Apparent plasticity traits are those where changes in allocation are size dependent (the suggested null hypothesis).
3. Modular plasticity include changes in the local traits such as the number of ramets or the number of shade vs. sun leaves.
4. Integrated plasticity which requires communication among plant parts or changes in one plant organ in response to variation in environment of a distant plant organ, such as induced defenses.

Biomass ratios can be assessed either between full plant biomass and biomass of a component part, or between two different plant parts, traditionally roots and shoots. Muller et al. (2000) cautions that using whole plant biomass and a component part may introduce some error because whole plant biomass is not independent of the component

being used. The classical formulation of an allometric equation is given in equation 1 (from Muller et al. 2000):

Equation 1: $Y = \alpha X^{\beta}$

Where β = the allometric exponent and α= the allometric coefficient. This equation is then log transformed to give equation 2.

Equation 2: $\log Y = \log \alpha + \beta \log (x)$

Where β becomes the slope and log α becomes the intercept. There is no consensus of the best way to estimate the regression model for allometric analysis, but two commonly used methods are least square (Muller et al. 2000) and reduced major axis regressions which assume the variance is the same magnitude in both response and covariate variables (Schmid and Bazzaz 1994). The method I will use to estimate the regression model will depend on the nature of the variance in the data. The slope and intercept of the allometric trajectories of plants grown under different environmental conditions can then be compared using regression to determine if they differ (Muller et al. 2000). In this case, the null hypothesis is that the allometric exponent and the allometric coefficients will not differ between plants subjected to different herbivory treatments. Biomass measurements need to be taken at least two times during the growing season to provide the two points required to make a line (Weiner and Fishman 1994).

The majority of the literature reviewed on the use of allometric analysis to describe changes in plant growth patterns centered on either competition or nutrient addition/deprivation. I believe this approach will be suitable for use in evaluating plant growth response to insect herbivory due to the nature of the study insect mode of action. We know insect feeding can reduce photosynthetic rate, and that in turn can reduce the

amount of resources available for plant growth, in a way analogous to the reduction of nutrient uptake or light availability caused by competition.

Study Organisms

Linaria dalmatica

Dalmatian toadflax is a successful invader from eastern Europe and the Mediterranean that has formed dense stands in rangelands in the United States and Canada west of the 100th meridian. *Linaria dalmatica* originally occurred from the Dalmatian coast of the former Yugoslavia to Northern Iran in a band from Transylvania and Moldavia in northern Romania, eastward around Black Sea to Bulgaria, Albania, Greece, Crete, Turkey, and Azerbaijan to northern Syria, northern Iraq and northern Iran (Alex 1962). It has been observed growing from 37° N to 47° N at elevations ranging from near sea level to 2800 m. In the native range Dalmatian toadflax is found primarily in open, sunny, rocky places and occupies uncultivated fields and vineyards, mountain meadows, sand hills, and limestone mountains (Alex 1962). *Linaria dalmatica* was brought to Europe for cultivation as an ornamental in the late 1500s. In Europe *L. dalmatica* evolved in plant communities subjected to moderate to intense grazing by sheep and goats, and to a lesser extent, cattle and cyclical wildfire (Lajeunesse 1999).

Taxonomy: The name *Linaria dalmatica* (L.) Miller is based on *Antirrhinum dalmaticum L.* It is a variable plant, and taxonomists studying a small selection of plant material would describe some specimens as species distinct from *L. dalmatica*. *Linaria grandiflora* Desfontaines is one of the earliest such determinations and is still widely

used. The classification is based on *L. dalmatica* having branched stems, narrower leaves, and flowers 1/3 smaller than *L. grandiflora* (Alex 1962). Alex (1962) considers *L. dalmatica*, *L. grandiflora*, *L. dalmatica* var *grandiflora*, and *L. dalmatica* ssp *grandiflora* to be synonyms. *Linaria dalmatica* was recently moved from the family Scrophulariaceae to Plantaginaceae and literature from the last four years refers to both families (May et al. 2004, Saez and Crespo 2005, Segarra-Moragues and Mateu-Andres 2007, Hamdi et al. 2008, Niketic and Tomovic 2008).

In the literature on *L. dalmatica* in North America, De Clerck-Floate and Harris (2002) describe two varieties of *L. dalmatica* – a broadleaved variety which they consider a more important weed and a narrow-leaved form. Possibly due to this variation, synonyms have been published including *L. genistifolia* ssp *dalmatica* (L.) Marie and Petitmengin (Vujnovic and Wein 1997, Lajeunesse 1999). Nowierski (1992) reports that different agents seem to prefer broad or narrow leaved varieties with one agent *Rhimisa antirrhini* (Coleoptera: Curculionidae) only seen on *L. genisitifolia*, and *Calophasia lunula* (Lepidoptera: Noctuidae) as being unsuccessful at establishing on *L. dalmatica*. The name *L. gensistifolia* is still used today (Nowierski 1992, Lajeunesse 1999, Phillips and Crisp 2000). The issue may be further confused by the ability of *L. vulgaris* and *L. dalmatica* to hybridize under natural field conditions (Sing, personal communication). The hybrid low fertility was reported by Bruun (1937) may be an artifact of the generally low germination rates for *L. dalmatica* and *L. vulgaris*. Work has been undertaken using molecular techniques to determine if broad and narrow leaved toadflax should be considered one or two species (Sing, personal communication). Because most published

literature identifies Dalmatian toadflax as *L. dalmatica*, the term will be used in this document.

Introduction and Distribution in North America: Evidence suggests *L. dalmatica* was first introduced to North America for use as an ornamental in 1894 in a botanical garden in Massachusetts, however, the earliest authenticated voucher is a specimen dated 1920 from the San Gabriel Mountains in southern California (Alex 1962). There are also reports that *L. dalmatica* was introduced for production of yellow dye, for folk remedies, and use in xeriscaping (Lajeunesse 1999). The first report of *L. dalmatica* having escaped cultivation was from 1920 (Alex 1962). In North America, *L. dalmatica* is found from 33° N to 56° N, a broader latitudinal range than it is found in Europe (Alex 1962). As in Europe, *L. dalmatica* persists in coarse soils varying from sandy to loams to coarse gravel. By 1974 all states west of the 100th Meridian except New Mexico reported infestations (Robocker 1974), and *L. dalmatica* has become a major management issue in range and forest lands in southern interior British Columbia and southwest Alberta in Canada since 1980 (De Clerck-Floate and Harris 2002). In North America, the broadleaved form is more widely spread than the narrow leaved form, which is the opposite of what is reported for Europe (Lajeunesse 1999).

Linaria dalmatica reduces the carrying capacity and value of ranchlands (De Clerck-Floate and Harris 2002). *Linaria* leaves have been shown to contain iridoid glycosides (Ilieva et al. 1992), which are reported to be mildly poisonous to cattle. Some mild cases of bovine poisoning have been reported, but reports are rare due to the tendency of cattle to avoid grazing the plant (Mitich 1993, Lajeunesse 1999). In Canada,

thousands of hectares of grazing land are infested and Canadian ranches have listed *L. dalmatica* as their third priority for control after knapweeds (*Centauria* spp) and common houndstougue (*Cynoglossum officionale* L.) (De Clerck-Floate and Harris 2002).

Unlike yellow toadflax, which tends to be a problem in cultivated areas, *L. dalmatica* primarily invades natural areas and rangelands. The economic impact of *L. dalmatica* is not widely reported, but Lajeunesse (1999) did report that for one ranch in Montana where approximately 30% of the 431 hectare ranch was severely infested (25 – 100% of the vegetative cover) direct management costs in 1992 totalled $99 per hectare. The managers additionally reported reduced carrying capacity and a reduction of appraised value of the land.

Description: *Linaria. dalmatica* is a self incompatible, insect pollinated erect short lived perennial plant (Bruun 1937) with glaucous (covered with a whitish waxy coating), glabrous leaves that usually clasp the stem (Alex 1962). Leaves are linear lanceolate to broad ovate with broad to chordate bases. Annual reproductive stems are robust and sometimes woody at the base (Alex 1962). Individual crowns can produce one to twenty reproductive shoots that average 40 – 100 cm tall and 0.5 to 0.9 cm in diameter (Alex 1962). Crowns also produce three to forty decumbent to weakly ascending succulent vegetative stems (Alex 1962). Flowers are grouped into simple, often loose racemes 15 – 22 cm long on the main stems (Alex 1962). Flowers are light yellow to yellow and 3.3 to 4.5 cm long including the corolla (Alex 1962). *Linaria dalmatica* produces flowers in one flush and cannot compensate for damaged meristems by producing a second batch of flowers later in the season (Saner et al. 1994). The brown

seeds are three edged to somewhat compressed with a narrow wing (Alex 1962). Seeds are 0.7 to 1.3 mm long, 0.5 to 1 mm wide, and the wings are 0.2 mm (Alex 1962). *Linaria dalmatica* has deeply penetrating tap roots and horizontal roots 5 – 20 cm below the soil surface that can spread up to 3.6 m from the parent plant. (Alex 1962, Robocker 1974).

Life Cycle: Flowering stems are initiated from perennial crowns in early spring. Elongation of flowering stems requires temperatures greater than 10 °C and less than 20 °C. Flowering and seed production occur between May and August in lower warmer areas and June to September in more northern latitudes and higher elevations (Lange 1958, Vujnovic and Wein 1997). Variation seems to be linked to temperature (Vujnovic and Wein 1997). Seed capsules begin opening approximately six weeks after flower initiation) with 97% of seeds being released in the first 5 weeks, and although blooming continues, very little seed production has been recorded after August (Robocker 1970).

Main stem seed capsules contain an average of 250 seeds with branch capsules averaging 140 seeds (Robocker 1970). Under ideal conditions, Robocker (1970) estimated that a mature plant with 10 floral stems could produce 500,000 seeds, and Lange (1958) estimated that plants with 12 – 15 stems can produce up to 400,000 seeds. Seeds produced during peak seed production are about 7000 to the gram, but seeds produced later weigh one quarter of that amount (Robocker 1970). Soil moisture was listed as one variable that can affect seed weight. Seeds are primarily dispersed by wind, though cattle and deer can also spread ingested seeds (Robocker 1970).

Seeds can germinate the year they are produced, but appear to have a dormant period with maximum germination occurring 5 to 6 weeks after maturation. In dry storage in a lab, seeds can remain viable for over 13 years (Robocker 1970) and can remain viable for at least 10 years in the field (Robocker 1974). In germination experiments, seedlings were able to emerge from seeds buried within the top 2.5 cm of soil, though in the field emergence tends to be from depths of 5 mm or less (Robocker 1970). Laboratory studies indicate that the ideal conditions to maximize germination are an alternating light and dark regime with either temperature fluctuations (20°C in the light, and 10°C in the dark) or a steady temperature of 15°C (Robocker 1970).

Seedlings emerge late March to late April (in Spokane WA) coinciding with average maximum soil temperatures of 10°C at a depth of 2.5 cm (Robocker 1970). Vujnovic and Wein (1997), and Robocker (1970) reported some seedling emergence in late fall, but survival was low. Seedling recruitment is competition limited rather than seed or herbivore limited (Grieshop and Nowierski 2002). Robocker (1970) also found that soil disturbance and removal of perennial plants increased seedling survival. Gates and Robocker (1960) report that in disturbed soil once occupied by *Hypericum perforatum* L., *L. dalmatica* was able to invade rapidly. Seedlings produce one primary stem and up to three upright adventitious stems from the base. Plants grown from seed can produce flowering stems in their first year. Floral stems die at the end of each year, but prostrate vegetative stems emerge from the crown and lateral roots in late summer and early fall. These persist over winter into the following spring (Robocker et al. 1972). New crowns can form on lateral roots. Individual crowns live 1 to 3.5 years, but individual clones have the potential to persist indefinitely due to their ability to produce

new crowns from lateral roots. New crowns may stay attached to the parent plant for a year, but eventually separate off (Robocker 1974). Stand persistence varies between patches. Some patches disappear for a period of years before re-establishing. Other stands persist for long periods of time (13 years in one location) (Vujnovic and Wein 1997).

Factors Limiting Growth: Grieshop and Nowierski (2002) found that seedlings were competition limited, and Robocker (1974) determined that while lateral shoots on mature plants contributed to carbohydrate reserves, they were not necessary for development of flowering shoots. Clipping or removing flowering stems in the spring lead to a reduced size of regrowth and greatly reduced flowering (Robocker et al. 1972).

Control: Prior to 1968, levels of control obtained through herbicide application were variable and inconsistent (Robocker 1968). In their review of *L. dalmatica* control, Vujnovic and Wein (1997) reported successful management with a variety of herbicides. The most successful herbicides were picloram in the granular form applied in fall at 0.5 to 2.25 kg ha^{-1} (Robocker et al. 1972), and silvex applied either in early spring to prostrate stems at a rate of 2.2 to 3.4 kg ha^{-1} or during early bloom at a rate of 3.4 kg ha^{-1}(Robocker 1974). 2,3,6-trichlorobenzoic acid did not lead to control, and in fact, Robocker et al. (1961) found *L. dalmatica* establishment rates increased with increasing application rates. Triclopyr and fluroxpyr were ineffective when used alone (Vujnovic and Wein 1997) and 2,4-D ester gave inconsistent results (Lange 1958, Robocker et al. 1961). De Clerck-Floate and Harris (2002) remark that using herbicides over large infestations is uneconomical and damaging to the environment. They also note that

although picloram alone or in combination with fluroxypyr can be effective, it is less effective under dry conditions, leaches readily into coarse soils, and at high rates can damage non-target broadleaved plants. *Linaria dalmatica* tends to grow in dry regions with coarse soils where these limitations to picloram effectiveness are most likely to apply. Long term control requires reapplication every three to four years for up to 12 years (Vujnovic and Wein 1997).

Because of the ability of *L. dalmatica* to reproduce and spread vegetatively, mowing is not likely to be a long term control, and prescribed burns tend not to damage roots or buds below the soil surface (Vujnovic and Wein 1997). Robocker (1974) reported that clipping flowering stems near the end of June stimulated dormant buds to produce axillary stems. Gates and Robocker (1960) evaluated the establishment of *L. dalmatica* seedlings in cultivated and uncultivated plots seeded with adapted forage grasses and found that *L. dalmatica* seedlings were unable to establish in uncultivated plots, but were able to establish well in cultivated plots, suggesting that reducing soil disturbance and providing competition may deter *L. dalmatica* establishment from seed. Rose et al. (2001) compared the impact of seeding five cool season grasses into established stands of *L. dalmatica* and two other weeds. They found that in general *L. dalmatica* production declined with increasing grass production in spring seeded grasses, but was only significantly lower than controls for three of the five species. Toadflax was able to grow in all plots, however.

Fire and *Linaria dalmatica*: In a study designed to investigate the impact of prescribed fire on Flagstaff pennyroyal (*Hedeoma diffusa* Greene), Phillips and Crisp

(2000) report post-fire invasions of *L. dalmatica* in spring burn treatments. Their paper did not discuss the origin of the infestation, but suggests the *L. dalmatica* colonized from seed.

Jacobs and Sheley (2003a) investigated the impact of prescribed fire on *L. dalmatica* density, cover, biomass, and seed production at three different burn sites. They found that fire did not affect density or percent cover when burning took place in March and sampling occurred in September and early October, but total biomass, per plant biomass and seed production increased. The temperature of the fire was low and roots and buds were presumably not damaged, explaining the lack of decrease in density in the short term. The fire was hottest at the top of the vegetation, burning off large sagebrush and pine species, possibly reducing competition, along with potentially releasing nutrients bound up in living and dead plant material allowing for increased plant biomass and seed production. Fire does not decrease *L. dalmatica* in rangelands and may in fact increase dominance of the weed. Jacobs and Sheley (2003b) also report that when burning was combined with picloram or chlorosulfuron applications, *L. dalmatica* biomass, cover, and density were reduced to 90% of controls, though chlorosulfuron had less of a negative impact on species richness and diversity. *Linaria dalmatica* growing in burned sites in Montana can reach heights substantially greater than the one meter size range reported in the literature (Sing, personal communication).

Mecinus janthinus

Taxonomy: The toadflax stem boring weevil *M. janthinus* Coleoptera: Curculionidae) is in the subfamily Mecininae. Jeanneret and Schroeder (1992) report that

there are 30 Palearctic species in the genus *Mecinus*, 10 of which occur in Europe. The European species fall into three groups: four species that feed on the genus *Linaria*, two which feeds on *Antirrhinum*, and four which feed on *Plantago*.

European Distribution: Jeanneret and Schroeder (1992) report that the greatest diversity of *Mecinus* species are found in southwestern Europe. The distribution of three species extends into North Africa. *Mecinus janthinus* is found from south and central Europe to Kazakhstan. The weevil has been reported from sea level up to the subalpine zone in the Alps. For their studies, Jeanneret and Schroeder (1992) collected *M. janthinus* from Rome, the Upper Rhine Valley (between Basil and Strasbourg), the Jura mountains in Switzerland, and near Belgrade and Macedonia in the former Yugoslavia. The authors also hypothesize that the weevil can be found in southern Germany, Austria, Hungary, and the Balkans. They report *M. janthinus* from a broad range of ecological conditions from the maritime lowlands in western France and northern Germany, Mediterranean climates, sub continental regions with dry summers in eastern Europe. They collected *M. janthinus* from four species of *Linaria* (*Linaria vulgaris*, *L. dalmatica*, *L. genistifolia*, and *L. genistifolia linifolia*). They predicted that *M. janthinus* would be able to establish on *L. dalmatica* and *L. vulgaris* between 40° and 52° N in North America.

Life History: Adults emerge from late March to early April on *L. dalmatica*, but later on *L. vulgaris* (De Clerck-Floate and Miller 2002). Jeanneret and Schroeder (1992) report later emergence, during May in Yugoslavia. Adults feed on leaves and stems for a short period before mating. Oviposition begins by the end of May or beginning of June

and continues into mid-July in Yugoslavia. In the field, adults die shortly after completing oviposition. Females chew a small cavity into which eggs are laid. Plants eventually develop calluses over oviposition sites.

Under laboratory conditions, females lay on average 1.15 (S.D. = 0.73) eggs per day (Jeanneret and Schroeder 1992). Two to 100 eggs have been reported per growing stem, but only one egg per cavity (De Clerck-Floate and Miller 2002). Eggs (oval in shape, 0.66 mm wide and 0.65 mm long) hatch after 6 – 7 days and larval development takes 23 – 34 days in the lab. During the course of their development, larvae go through 3 larval instars and create mines between 1 and 3 cm long with the longest reported mine 7.5 cm long (Jeanneret and Schroeder 1992). A shoot diameter of 0.9 mm is required for successful larval development to occur, though females will oviposit in narrower stems (Jeanneret and Schroeder 1992). Stems with smaller diameters sometimes swell or split around developing larvae (personal observation).

Pupae form about 40 days after oviposition, are 3.0 to 4.5 mm long, and are initially white, turning black over time. The pupal stage lasts approximately three weeks with complete development from oviposition to adult taking 50 to 63 days (Jeanneret and Schroeder 1992). Adults overwinter in the pupal cells.

Mortality Factors: Jeanneret and Schroeder (1992) reported the greatest mortality factor for *M. janthinus* on potted plants under greenhouse conditions was the inability of larvae to develop in stems that are too small. They do not report mortality factors under field conditions, though they do state that approximately 10% of field collected larvae were parasitized. They were unable to rear adult parasitoids, but speculate that they were

in the families Eulophidae and/or Pteromalidae (Jeanneret and Schroeder 1992). De Clerck-Floate and Miller (2002) observed low rates of parasitism from Hymenopera (<10%) in Canada.

In a study designed to determine the factors affecting overwintering mortality in Canada, De Clerck-Floate and Miller (2002) found a significant negative relationship between absolute minimum temperature and overwintering mortality, although snow cover ameliorated the effects of low temperature for at least one of their study sites. The low temperature threshold was between -20° and -15 °C.

Mecinus janthinus as a Biocontrol Agent: An initial host-specificity test list was approved in 1987 with revisions accepted in 1988 (Jeanneret and Schroeder 1992). The test list contained 38 species, of which only eight were native to North America (Breiter and Seastedt 2007). Host-specificity testing showed adults were able to feed on a few non - *Linaria* species, but oviposition was restricted to four species, all in the genus *Linaria*. *Mecinus janthinus* did not oviposit on *Antirrhinum majus* L. when *L. vulgaris* was present and there have been no reports of *M. janthinus* attack on *A. majus* under field conditions in Europe. Larval development was restricted to *L. vulgaris* and *L. dalmatica*, species with stem diameters greater than 0.9 mm (Jeanneret and Schroeder 1992). Breiter and Seastedt (2007) evaluated *M. janthinus* host specificity post release along the Colorado Front Range using greenhouse choice tests, field cage choice tests, and release site monitoring for non-target effects. The greenhouse choice tests expanded the initial test list to include paintbrush (*Castilleja* spp.), yellow monkeyflower (*Mimulus guttatus* DC), purple monkeyflower (*Mimulus lewisii* Pursh), Texas toadflax, bearded sidebells

penstemon (*Penstemon secundiflorus* Benth.), beardless sidebells penstemon (*Penstemon virgatus* Gray), and low beardtongue (*Penstemon virens* Pennell ex. Rydb.). Field cage tests were conducted on *L. dalmatica*, *P. secundiflorus*, and *P. virens*. Their greenhouse and field experiments failed to demonstrate use of native plants by *M. janthinus* (Breiter and Seastedt 2007). They also recorded no evidence of herbivory on native species at field releases where *M. janthinus* was well established (Breiter and Seastedt 2007).

The initial releases of *M. janthinus* (29 – 65 individuals per release) in Canada were made in 1991 and 1992. Agents were recovered at 21 of 25 releases made between 1992 and 1994 (De Clerck-Floate and Harris 2002). De Clerck-Floate and Harris (2002) report attack rates of 100% were reached within three years at some sites. In a study in Alberta the average density of weevils per stem was 4.8 at non-outbreak sites, and 15 weevils per stem at outbreak sites (Carney 2003). At three field sites established in 2003, the attack rate on stems collected in 2004 ranged between 0 and 4 larvae per stem, with one quadrat with an average seven larvae per stem and one with 10 larvae per stem. Outbreak numbers of weevils have been reported 3 – 5 years post release with some large stems producing 100 adults (De Clerck-Floate and Harris 2002). Outbreak sites were considered sites where (1) weevils were released more than four years previously, (2) there was obvious widespread damage, and (3) more than 20 adult weevils are observed per minute during peak adult activity (Sing, personal communication).

Mining by larvae, especially under high population levels, suppresses flowering and causes premature wilting of stems and is exacerbated in areas where the plants are facing drought stress (Jeanneret and Schroeder 1992). De Clerck-Floate and Harris (2002) also report that the weevils suppress flowering and stunt shoot growth, but they

attribute this to adult weevils feeding on apical meristems, rather than to larval mining. Damage to main shoots by *M. janthinus* feeding may be compensated for by the development of secondary growth in places with summer rains or with water holding soils. Jeanneret and Schroeder (1992) found the highest attack rates on plants growing in dry coarse soils in "summer-warm" and "summer-dry" areas.

Those observations were corroborated in an experiment conducted by Saner et al. (1994). In their study, they caged one pair of adults on each plant for a month during the peak oviposition period and estimated the effects of the mining on shoot and root biomass. *Mecinus janthinus* decreased shoot biomass from 5.50 to 3.86 g ($F_{1,33} = 6.58$, $p = 0.015$) and decreased shoot/root ratio. They found no interaction between plant developmental stages (vegetative – six months old and flowering – 18 months old) and *M. janthinus* attack. *Mecinus janthinus* caused an increase in the proportion of dead prostrate stems in October (51% - 77%, $F_{1,15} = 3.50$, $P = 0.081$), as did drought stress (from 47% – 81%, $F_{1,15} = 3.96$, $P = 0.065$). However, *M. janthinus* mining did not affect root biomass. They hypothesized that the mining by larvae and the oviposition of adults lead to water stress in *L. dalmatica* plants. Attack can act as an energy sink by damaging prostrate stems which contribute carbohydrates to the roots (Robocker 1974). Although *M. janthinus* adults attacked both vegetative and flowering stems, larval survival in vegetative stems was less than in flowering stems. Saner et al. (1994) cite the inward collapse of dead prostrate stems when they desiccate, as opposed to the drying and woodiness of flowering stems as they desiccate, as the reason for poor larval survival in prostrate stems.

Other Dalmatian Toadflax Biocontrol Agents

Biocontrol of Dalmatian toadflax was initiated in the 1960s at the same time as biocontrol of yellow toadflax with release of the defoliating moth *Calophasia lunula* (Hufnagel) (Jeanneret and Schroeder 1992). *Calophasia lunula* was first released on *L. dalmatica* in 1980, but only became established at 40% of sites. Since 1991, releases of the root moth *Eteobalea intermediella* (Treitschke), root galling weevil *Rhinusa linariae* (Panzer), and a biotype of *Rhinusa antirrhini* (Paykull) seed weevil collected from *L. dalmatica* in Europe have been made in Canada (Jeanneret and Schroeder 1992). The European flower feeding beetle *Brachypterolus pulicarius* (L.) has been found adventively on *L. dalmatica* in both the United States and Canada (Jeanneret and Schroeder 1992).

Objectives

The purpose of this study was to determine the impacts of the biological control agent *M. janthinus* on individual *L. dalmatica* plants and link those individual plant responses to population changes occurring at an operational scale and to evaluate the establishment and increase of biocontrol agents under field conditions in Montana. Understanding the mechanism of control in this weed-agent system could aid in selection of biological control agents for future programs. Understanding the factors affecting release establishment and control agent increase will assist land managers in selecting release sites and determining timelines for expected results.

The impacts of *M. janthinus* weevils on plant primary physiology and growth were evaluated in common garden and field experiments. Different levels of herbivore

feeding injury from adult and adult plus immature insects were used to determine if there was a threshold level of injury required to cause changes in plant growth or gas exchange and to determine which stage caused those changes. *Linaria dalmatica* population changes were evaluated using data collected at biocontrol release sites over the course of four years. *Linaria dalmatica* population changes detected were compared between data collected at release and non-release areas to determine if changes could be linked to the influence of the biological control agents.

CHAPTER 2

IMPACT OF *MECINUS JANTHINUS* LARVAL AND ADULT INJURY ON GROWTH AND PRIMARY PHYSIOLOGY OF *LINARIA DALMATICA* UNDER CONTROLLED CONDITIONS

Abstract

Weed biological control has a relatively poor record with a 60% establishment rate and only 50% of established releases leading to control. For weed biological control programs to be successful, control agents must established, increase their populations, and significantly impact plant growth, primary physiology and/or reproductive success. Randomized complete block garden experiments were conducted to evaluate the impact of four levels of herbivore pressure on plant growth and primary physiology of *Linaria dalmatica*. Caging 0, 2, 8, or 16 pairs of adult *Mecinus janthinus* weevils on plants for 7 to 15 days led to different immature herbivore loads. The differences between adult density treatments were greatest for number of oviposition scars and smallest for the number of insects that completed development to the adult stage. Increasing herbivore loads led to decreased relative plant growth. Control plants had greater root, stem, and reproductive biomass at the end of the season than plants in the high adult density treatment. There were no differences in root-shoot ratios between adult density treatments. However, reproductive-root and reproductive-stem biomass ratios differed between adult density treatments. Allometric analyses indicated that high larval herbivore pressure did not change plant growth trajectories, but slowed them down. There were trends of decreasing gas exchange rates with increasing herbivore pressure.

The lack of a strong gas exchange response to herbivore injury was not similar to results of previous studies conducted under field conditions and could be due to differences in growth conditions between potted plants in a garden environment and plants growing under natural conditions.

Introduction

Herbivorous insects affect plant population dynamics through impacts on plant growth (Louda et al. 1990, Schat and Blossey 2005), root:shoot ratios (Vranjic and Gullan 1990, Blossey and Schat 1997), reduced reproductive rates (Harrison and Maron 1995, Notzold et al. 1998, Schat and Blossey 2005), and primary physiology (Peterson 2001). Although these potential effects are the basis for using insects in the biological control of weeds, few studies have been conducted on injury by concealed feeders including stem mining due to difficulty in controlling or simulating injury levels. Reduction in photosynthesis by stem boring insects has been linked to interruption of vascular transport (Peterson and Higley 1993). However, Peterson and Higley (1993) caution that reported results were often from studies lacking manipulated levels of herbivory or proper replication.

Recent growth chamber and greenhouse studies on agricultural species and field studies in wildland systems have examined the impact of stem borer injury on plant primary physiology. In a growth chamber experiment, Macedo et al. (2005) found that larval feeding injury by the wheat stem sawflies (*Cephus cinctus* Norton) resulted in significant reductions of photosynthetic capacity of wheat (*Triticum aestivum* L.) as the plants senesced. In greenhouse studies on the impact of wheat stem sawfly on gas

exchange rates in wheat at the grain filling stage, uninfested plants had photosynthetic rates 12% higher than infested plants (Macedo et al. 2006). In a replicated but un-manipulated study on the effect on photosynthetic rate of stem mining by *Mecinus janthinus* Germar larvae on Dalmatian toadflax (*Linaria dalmatica* (L.) Miller), Peterson et al. (2005) reported that injured plants had photosynthetic rates 17 to 39% lower (depending on field site and year) than uninjured plants. The authors were unable to suggest a mechanism for decreased photosynthesis apart from that it did not seem that the feeding resulted in stomatal limitations.

Weed biological control programs using insects have had low success rates. Approximately 60% of agents released have become established (Julien 1989, McFadyen 1998) and of the programs where successful establishment has occurred, 50% of program have led to control (Julien 1989, McFadyen 1998, McEvoy and Coombs 2000). For biological control to be successful, cost effective, and safe, the overall success rates need to be improved. Understanding how agents affect plants and under what conditions the impacts on individual plants translate into population impacts can help focus attention on agents likely to be effective rather than working with all agents that "pass" host specificity screening tests. Investigating impacts of insect feeding injury and minimal injury thresholds for effects can link plant primary physiology, plant growth, and plant fitness. There have been few studies of the impacts of concealed feeders on plant growth and physiology, and few studied that have considered both growth and primary physiological responses in the same study.

The objectives of this study were to 1) determine the impacts of different levels of *M. janthinus* feeding injury on *L. dalmatica* primary physiology and growth, 2) link

morphological responses of *L. dalmatica* to herbivory by *M. janthinus* to primary physiological mechanisms, and 3) determine if there is a threshold injury level in *L. dalmatica* to *M. janthinus* feeding injury that must be reached before changes in growth and gas exchange rates are realized. To do this, a controlled, randomized complete block experimental design was used to evaluate the effects of four levels of *M. janthinus* injury on *L. dalmatica* growth, photosynthesis, transpiration, and conductance.

Study Organisms

Linaria dalmatica, a short lived perennial plant, came to the United States from Eastern Europe and the Mediterranean in the 1890s for use as an ornamental (Alex 1962). Since being introduced, *L. dalmatica* has formed dense stands in the United States and Canada west of the 100th meridian (Robocker 1974). *Linaria* leaves have been shown to contain iridoid glycosides (Ilieva et al. 1992), which are reported to be mildly poisonous to cattle. Because cattle avoid *L. dalmatica*, infestations can reduce the carrying capacity and value of ranchlands (De Clerck-Floate and Harris 2002).

A biological control program to manage *L. dalmatica* was initiated in the 1960s but little control was realized until the stem mining weevil, *M. janthinus*, was released in North America in 1991 (De Clerck-Floate and Harris 2002). Adults of this stem mining weevil emerge in spring and feed on shoot tips. Eggs are laid individually in holes chewed in the stem, and development occurs entirely within the toadflax stems (Jeanneret and Schroeder 1992). Larvae mine the stems and the larval stage lasts approximately 40 days followed by a three week pupal stage. New adults remain in pupation chambers over the winter and emerge the following spring (Jeanneret and Schroeder 1992).

Materials and Methods

A randomized complete block experiment conducted in a common garden was used to determine the effects of *M. janthinus* larval feeding on *L. dalmatica* growth and primary physiology. The experiment was replicated once per year in 2005 and 2006 using new plants and insects each year (Table 1). Each year 144 plants were randomly assigned to one of four adult insect density treatments (0, 2, 8, or 16 pairs of adults per plant). Adults were caged on plants for a one to two week oviposition period before being removed. Stem length measurements were taken weekly throughout the summer until the plants senesced. Three times each summer gas exchange, and chlorophyll florescence measurements were taken on a randomly selected subset of the plants. The measured plants were then harvested to determine larval injury level (the density of insects at each stage of development), dried to a constant weight, and weighed.

The experiment was conducted at the MT USFS RMRS Forestry Sciences Laboratory in Bozeman, MT in a fenced garden area. Pots were arranged in blocks to account for potential variability in available light and temperature, as well as to allow for gas exchange measurements to be taken over a 2-day period if necessary. The blocks were tilled to loosen the soil in early spring 2005 for the 2005 experiment, and in the fall of 2005 for the 2006 experiment. The potted plants were then placed in the ground up to the pot rims (depth = 21 cm) with each block containing 36 plants arranged in six rows of six pots. In 2005, plants were spaced 1 m apart and in 2006 plants were arranged in three paired rows such that rows 1 and 2, 3 and 4, and 5 and 6 were each 10 cm apart, with a 50 cm gap between rows 2 and 3, and 4 and 5. Plants within rows were spaced 10 cm apart

in 2006. In 2005, the four blocks were arranged in a ”t” configuration and in 2006, plants were arranged in a single north-south line. In both years there was a 1-m space between blocks.

Irrigation

Plants were watered daily using an automatic drip irrigation system consisting of 16 mm polyethylene pipe connected to a garden hose and fitted with a 2.46 kg cm^{-2} pressure regulator (1.9 cm low flow; 2 to 20 lpm). Pressure compensating drippers were connected to 4-way, 76 cm multi-outlets at intervals so that each pot had a single dripper inserted at the base of the toadflax stem. The irrigation system was controlled by an electronic auto timer (Melnor Electronic Aqua Timer Model 3060, Melnor Inc, Winchester, VA) set to water for five minutes out of every 20 between 5 AM and 6 AM Mountain Standard Time daily.

Plants

All *L. dalmatica* plants used in the 2005 experiment originated from the Helena National Forest. They consisted of plants already potted and set in the ground, and plants growing directly in the soil. Potted plants were cultivated from roots collected from a population of mature plants located approximately 8 km into Magpie Gulch in the Helena National Forest, Townsend Ranger District. Plants were cultivated by APHIS PPQ and USFS RMRS personnel for experimental purposes. Plants growing directly in the soil were progeny of potted plants. Plants of similar above ground size were selected for use in the experiment. All plants used were transplanted into 4 L pots in a 50-50 mix by volume of Montana State University Plant Growth Center soil mix (MSU mix) and

Sunshine Mix #1 (Sun Gro Horticulture, Bellevue, WA). The MSU mix is a 1:1:1 ratio by volume of mineral soil, Canadian sphagnum peat moss, and washed concrete sand with 0.45 kg of Aqua-Gro 2000G wetting agent added per cubic meter of mixed soil. The soil was steam sterilized before use. After transplanting, the plants were randomly assigned to and arranged in the blocks in the garden, and dug into the ground up to their rims in May 2005. The plants used in 2006 were started from seeds from a common source and grown as described below.

For the 2006 experiment *L. dalmatica* plants were grown from seed in a greenhouse and transferred to the garden in late September 2005. The seeds were collected from mature plants near Wyola, MT. Seeds were stored in the dark at room temperature in re-sealable plastic bags after collection. Seeds were germinated by placing them in boxes lined with paper towel and moistened with a 4 ppm gibberellic acid solution. Germination boxes were maintained at room temperature in dark conditions for three days. Germinated seeds were then individually transferred to 24-well self-watering seed starting trays (Lee Valley Sel-Watering Seed Starter, Lee Valley Tools Ltd. Ogdensburg, NY) at a density of five germinated seeds per well. Seedlings were thinned to three seedlings per well at two weeks and transplanted individually into 10 cm square pots at four weeks, when the seedlings were approximately 10 cm tall. Seedlings were transplanted into 15 cm round pots at two months and into 4 L pots at four months. In all cases a 50-50 mix of MSU mix and Sunshine Mix #1 was used.

Treatments and Treatment Application

To determine if there is a threshold injury level at which *L. dalmatica* growth and primary physiology is affected by *M. janthinus* injury, four adult insect densities were used: a control (no insects), low (2 pairs of weevil adults/plant), medium (8 pairs of weevil adults/plant) and high (16 pairs of weevil adults/plant). Ovipositing adults were used due to difficulties associated with transferring stem boring larvae. Each year, weevils were collected in eastern Washington by USDA, APHIS, PPQ personnel and shipped to Bozeman via overnight air courier in paper containers containing tissue paper, *L. dalmatica* stems, and a small section of dampened sponge. Weevils were transferred to a refrigerator immediately after arrival. Adults were sorted by gender, using external rostral (Schat et al. 2007) and leg characters (Carney et al. 2004). Adult density treatments were applied within 48 hours of receipt of the adults from Washington. All *L. dalmatica* plants were caged with fine mesh bags constructed from mesh fabric (mesh size 70) secured around the rim of pots with elastic cording and held up with bamboo stakes place in pots (Figure 1A and B). After treatment application, the tops of the bags were folded over and tied securely. Irrigation remained in the pots while the cages were present. In 2005, possibly due to cool temperatures and rain, few signs of oviposition were observed on the high adult density treatments after one week so adults were retained on the plants for 15 days. In 2006, the weather was comparatively warmer and drier so adults were removed after seven days. Once weevils were removed, the cages were also removed.

Harvests

For each block, three randomly selected plants per adult density treatment were collected at each of three harvest dates during the summer. The first harvest occurred on the day the adult *M. janthinus* were removed from the plants (Table 1). Leaves were carefully stripped from stems of selected plants and stems were examined for oviposition scars. For each plant, the number of oviposition scars per plant was recorded and stems were dissected to determine the density of developing weevils. The number of eggs, living larvae, dead larvae, living pupae, dead pupae, and living adults were counted and total densities per plant were recorded in 2005, while total per densities stem and per plant were recorded in 2006.

Growth and Biomass Measurements

In 2005, stem length for the tallest, shortest, and representative stems were measured to the nearest 0.5 cm the day the weevils were removed (11 June 2005) and then once per week through 31 July 2005, and when the experiment was terminated on 24 August. In 2006 each individual stem was numbered and stems were measured weekly beginning when the adults were removed from the plants (9 June 2006) through 7 July 2006. On this date experimental plants were damaged due to unforeseen events. Due to the delicate state of the plants after the incident, stem length was only measured on two additional days: 21 July 2006, and when the plants were harvested on 31 August 2006.

Harvested, stems were clipped at soil level and stored in a refrigerator until they were dissected. Soil in the pots was carefully loosened and removed from the roots. Roots were then rinsed in cool water to remove any remaining soil, placed in paper bags,

and dried to constant weight. Dissected stems were separated into reproductive parts and non-reproductive parts and dried to constant weight. Dried plant components were weighed to the nearest 0.01 gram. In 2005, roots, stems, and reproductive parts (buds, flowers and seed pods) were weighed separately. The same components were measured in 2006, along with leaf biomass. To prevent underestimating seedpod biomass, in both years plants were examined daily and dried seedpods that were beginning to open were collected and stored in labeled coin envelopes until the plants were harvested.

Gas Exchange and Fluorescence Measurements

A handheld chlorophyll fluorometer (OS1-FL modulated chlorophyll fluorometer, Opti-Sciences, NH) was used to measure changes in photosynthetic fluorescence. Both light and dark chlorophyll fluorescence measurements were made. Leaves were allowed 10 minutes to adapt to dark conditions before dark adapted measurements were obtained. After dark adapted measurements were completed, the same leaves were measured again in a light adapted test and to asses gas exchange.

A LI-6400 portable photosynthesis system (Li-Cor Inc., Lincoln NE) with a 2-cm^2 leaf chamber was used to assess gas exchange of plants. The LI-6400 system allows users to set light intensity, color spectrum, flow rate, and reference CO_2 concentrations. Following Peterson et al (2005), leaves were illuminated with a light intensity of 1400 μmol photons m^{-1} s^{-1} from a light source inside the leaf chamber (10% blue light, 90% red light), a flow rate of 500 μmol air s^{-1}, and a reference CO_2 concentration of 400 μmol CO_2 mol^{-1} generated from an integral 12 g CO_2 cylinder. Net CO_2 exchange rate

(photosynthetic rate μmol CO_2 m^{-2} s^{-1}), stomatal conductance rate (mol H_2O m^{-2} s^{-1}), and transpiration rate (mmol H_2O m^{-2} s^{-1}) were measured.

Gas exchange and fluorescence measurements were made immediately before each harvest. Measurements were made on a single randomly selected leaf from the top third of each plant for all plants being harvested (3 plants per adult density treatment per block at each harvest). *Linaria dalmatica* leaves have no petioles and are small compared to the LI-6400 measuring head. This required the leaves being measured to be removed from the plant to obtain gas exchange measurements. Time constraints therefore necessitated a harvest of two blocks each day on two consecutive days.

Data Analysis

All data analyses were conducted using SAS 9.0 (SAS Institute Inc., Cary, NC) software. Each year's data were analyzed separately due to the differences in block orientation, spacing of plants within blocks, plant origin, and cultivation regimes (transplants vs seeded stock). The Proc-GLM procedure was used to determine varying *M. janthinus* adult densities produced differences in the numbers of oviposition scars, eggs, and developing weevils within the stems. Where analyses indicated significant effects ($\alpha = 0.05$), post-hoc Tukey's Studentized Range Tests were used to test for differences between groups. Tukey's test was chosen due to its conservative nature, its ability to handle uneven sample sizes, and because it controls experiment wide type I error (Ott 1993, Montgomery 1997, Underwood 1997). The number of oviposition scars was used as a measure of injury for all three harvests. Egg number was used as an additional measure of injury in the first harvest. Larvae and the sum of pupae and adults

(post larvae) were used as measures of injury in the second harvest. For the third harvest, oviposition scars and post egg stage insects (sum of larvae, pupae, and adults) were used as measures of injury.

Initial and final heights were used to calculate the change in plant height. This was converted to net relative change in height based on the initial size of the plant [(final height – initial height)/initial height]. Relative plant heights are typically calculated using natural log transformed data; however, several adult density treatments resulted in negative changes. To accommodate negative changes, relative changes in plant height were calculated in two ways: 1) using absolute values and 2) natural log transformed data where all stems that were shorter at the end of the season were coded as having zero growth. The same statistical procedures as described above were used to determine if there were growth differences between blocks and adult density treatments. Generalized linear models including a block variable, were used to examine the relationship between injury and change in height.

Biomass data were natural log transformed and compared between adult density treatments and harvests using Proc-GLM with a post-hoc Tukey test to determine the impact of herbivory on plant growth. *Mecinus janthinus* adult density treatment effects on *L. dalmatica* were also compared using root:shoot, reproductive:shoot, and reproductive:root biomass ratios using the same procedures. The numbers of insects dissected out of plants were log transformed and Proc-GLM models including block and adult density treatment were used to test the effects of insect density on biomass.

Biomass data were subjected to allometric analyses to determine if differences in biomass ratios were indicative of plastic responses in plant growth pattern or were due to

plants being at different points on a common allometric trajectory (Weiner and Fishman 1994, Muller et al. 2000, Weiner 2004). Component parts were first tested for significant correlations and then allometric coefficients and exponents were calculated based on equation 2, where Y is the variable of interest, X is a covariate, α is the allometric coefficient, and β is the allometric exponent (Muller et al. 2000).

Equation 2. $Y = \alpha X^{\beta} \leftrightarrow \log(Y) = \log(\alpha) + \beta \log(X)$

The specific equation used to calculate the allometric exponent and coefficient depended on if there were significant interactions between the covariate, time (harvest), and adult density treatment. Significant interactions indicate that the slope of the relationship between the variable of interest and covariate differed between adult density treatments and/or harvests (Muller et al. 2000). Proc-CORR was used to test for correlations between the biomass of plant parts and Proc-GLM to test for adult density treatment, harvest, and interaction effects. If there were no adult density treatment or time effects, data from all adult density treatments and harvests were pooled and allometric coefficients and exponents were calculated using Proc-REG. If adult density treatment effects were significant but there were no adult density treatment -covariate interactions, then parallel regression lines were fit for each adult density treatment and used to calculate the allometric equation. If adult density treatments and harvests and interactions were significant, separate regressions were fit for each adult density treatment and harvest to obtain the allometric variables.

Different measures of injury were used for each harvest so gas exchange and fluorescence data were analyzed separately for each harvest to test for adult density treatment and injury effects using Proc-GLM. In the first harvest measures of injury

were number of oviposition scars and number of eggs, while the second harvest injury measures were number of oviposition scars and number of larvae. For the third harvest, injury measures were number of ovipositon scars and number of post larval staged insects. Tukey comparisons were used to determine which adult density treatments differed from each other in models with significant results.

Results

Adult Density and Injury

The adult density treatments led to measurable differences in numbers of oviposition scars and developing insects in the stems (Figure 2). The adult density treatments differed more for the number of oviposition scars recorded than for the number of larvae or post larval insects across all three harvests. There were harvest and adult density treatment differences in the number of oviposition scars per plant in both 2005 ($F = 7.28$; d.f. = 2,134; $P = 0.001$ and $F = 80.61$; d.f. = 134; $P < 0.0001$, respectively) and 2006 ($F = 7.59$; d.f. = 2, 135; $P = 0.0007$ and $F = 233.04$; d.f. = 3,135; $P < 0.0001$, respectively).

Larvae were only observed in the second and third harvests. There were differences between adult density treatments for larvae per plant in 2005 ($F = 18.25$; d.f. = 3,87; $p < 0.0001$) and in 2006 ($F = 37.16$; d.f. = 3,135; $p < 0.0001$). In 2005 and 2006 the number of larvae dissected out of the low adult density treatments did not differ from the number of larvae found in control plants and there were no differences in the number of larvae per plant between medium and high adult density treatments in 2005. However, in 2006 there were more larvae in plants in the high adult density treatment than in the

medium adult density treatment. Post larval staged insects were observed only in the third harvest in 2005, and in the second and third harvests in 2006. In 2006 there were more post larvae in the third harvest than the second ($F = 52.15$; d.f. = 2, 135; $P < 0.0001$). There were no differences between medium and high adult density treatments. In 2005, the low adult density treatments did not differ in the number of pupae plus adults in the third harvest from the control plants or the medium and high adult density treatments. However, the control plants had fewer post larvae than medium and high adult density treatment plants.

Plant Growth and Biomass

Initial plant heights did not differ by adult density treatment or block and increasing the number of adults caged on plants led to decreased relative plant growth (Figure 3). There were highly significant block ($F = 9.05$; d.f. = 3, 37; $P = 0.0001$) and adult density treatment ($F = 5.76$; d.f. = 3,37; $P = 0.0024$) effects on net relative change in plant height. Growth data from 2006 were excluded due to an unforeseen loss of data due to plant damage. The relationships between net relative growth and injury in generalized linear models including block resulted in a negative relationship between growth and the number of oviposition scars ($F = 5.68$; d.f. = 1,39; $P = 0.022$). The model explained 42% of the variation in the net relative growth of the plants when block was included. When block was excluded, the R^2 value was reduced to 0.0375.

Biomass of component plant parts closely paralleled the plant height results. There were no differences between blocks in root, stem, or reproductive biomass ($P = 0.08$, $P = 0.2072$, and $P = 0.8787$, respectively). Although there were differences

between adult density treatments in root biomass ($F = 5.61$; d.f. = 3,135; $P = 0.0012$), there were no differences in root biomass across harvests ($F = 0.09$; d.f. = 2,135; $P = 0.9174$). There were significant adult density treatment ($F = 5.99$; d.f. = 3,135; $P = 0.0007$) and harvest ($F = 9.26$; d.f. = 2,135; $P = 0.0002$) differences in stem biomass. Plants in control groups had greater stem biomass at the second and third harvests than plants in the high adult density treatments (Figure 4). The differences were more pronounced with respect to reproductive biomass, with more reproductive biomass in control plants than medium or high adult density treatment plants ($F = 6.46$; d.f. = 3,135; $P = 0.0004$). However, there were no relationships between the numbers of oviposition scars, larvae, or post larvae and biomass of roots, stems, or reproductive biomass.

Biomass Ratios

Root-shoot ratios did not differ by block ($F = 1.56$; d.f. = 3,135; $P = 0.2030$) or adult density treatment ($F = 0.26$; d.f. = 3,135; $P = 0.8525$), although they did change with harvest ($F = 25.34$; d.f. = 2,135; $P < 0.0001$) (Figure 5). The reproductive biomass-root biomass ratios differed between both adult density treatments ($F = 8.02$; d.f. = 3,135; $P < 0.0001$) and harvest ($F = 25.39$ d.f. = 2,135; $P < 0.0001$), but not between blocks ($F = 0.09$; d.f. = 3,135; $P = 0.9629$) (Figure 6A). The ratios were lower for the first harvest than for the second or third harvest. The same pattern was observed for the reproductive biomass-shoot biomass ratios (Figure 6B). The reproductive biomass-shoot biomass ratios differed between both adult density treatments ($F = 9.31$; d.f. = 3,135; $P < 0.0001$) and harvest ($F = 27.07$, d.f. = 2,135; $P < 0.0001$), but not between blocks ($F = 0.04$; d.f. = 3,135; $P = 0.9877$).

Allometric Analyses

There were significant positive relationships between the variables of interest (stem, root, and reproductive biomass) and the covariates. The correlation between ln(stem biomass +1) and the covariate ln(root biomass +1) was 0.773 ($P < 0.0001$). The lowest correlation coefficient was for ln(reproductive biomass +1) and covariate ln(root biomass +1) at 0.498 ($p < 0.0001$). The correlation between the natural log of reproductive biomass and the covariate (the natural log of stem biomass) was 0.724 ($p < 0.0001$).

Results of the Proc-GLM procedures testing for significant adult density treatment, harvest, treatment*covariate, harvest*covariate, and treatment*harvest*covariate effects differed for each of the three biomass component pairs examined (Table 2). In the model with ln(stem biomass +1) as the dependent variable and ln(root biomass + 1) as the covariate, there were no significant adult density treatment effects, harvest effects, or interactions (Table 2) so the allometric coefficient and exponent were estimated from a regression on data pooled across adult density treatments and harvests. When reproductive biomass was the dependent variable and root biomass was the covariate, the adult density treatment effect and all three interaction terms were significant although there was no harvest effect (Table 2). For this case, allometric coefficients and exponents were calculated from separate regressions for each adult density treatment-harvest combination. In the third model, where the reproductive biomass was the dependent variable and the stem biomass was the covariate, there was a significant harvest effect and harvest*stem biomass interaction (Table 2), so regression

lines were fit for each harvest on pooled adult density treatment data to calculate the allometric parameters.

For the allometric relationship between natural log transformed shoot and root biomass, combining data from adult density treatments and harvests yielded a significant allometric coefficient (intercept) of 0.47325 ± 0.08946 (standard error) ($t = 5.29$; $P < 0.0001$) and an allometric exponent (slope) of 0.92997 ± 0.06393 ($t = 14.55$; $p < 0.0001$). There were very few plants with reproductive biomass in any adult density treatment in the first harvest and thus none of the regressions for the first harvest yielded non-zero slopes or intercepts for the ln(reproductive biomass)-ln(root biomass) relationships (Table 3). In the second harvest there were no non-zero intercepts and only the slopes for control and low adult density treatment plants (treatments where every plant had some reproductive biomass) yielded significant non zero slopes (Table 3). Those two models explained 57% and 53% of the variability in reproductive biomass for the control and low adult density treatments, respectively. For the third harvest, the control, low, and medium plants had intercepts of zero, and only the plants in the high adult density treatments had a non-zero intercept (allometric coefficient) (Table 3). All adult density treatments, except the control group, had significant non-zero slopes (allometric exponents) (Table 3). All three regression models constructed to determine the allometric relationships between the natural log of reproductive biomass (dependent variable) and the natural log of stem biomass (covariate) on data pooled across adult density treatments returned significant intercept and slope values (Table 4).

Gas Exchange and Chlorophyll Fluorescence

No adult density treatment differences in photosynthetic or conductance rates were detected in the first two harvests in 2005. There were significant adult density treatment effects in the third harvest where plants in the medium adult density treatment had photosynthetic rates and conductance rates just 13% of control plants (Table 5, Figure 7A and B). There were no adult density treatment effects for transpiration rates in the first harvest, but in both the second and the third harvests there were differences (Table 5, Figure 7C).

The patterns of photosynthesis, conductance, and transpiration in 2006 were similar to those in 2005 (Figure 8). Adult density treatment effects were recorded for photosynthesis and transpiration for plants in the first harvest, but no differences in the second or third harvests (Table 5, Figure 8A and C). There were no differences in conductance measures with respect to adult density treatment in any harvest (Table 5, Figure 8B). Plants in the high adult density treatment had significantly lower photosynthetic rates than plants in the low adult density treatments (Figure 8A). Although the general model suggested there were differences in transpiration rate with respect to adult density treatment in the first harvest ($F = 2.9$, d.f = 3,71; $P = 0.0409$), the post hoc Tukey comparisons did not detect any differences.

There were no relationships between photosynthetic rates or conductance rates and injury levels in 2005 except for a significant block term in harvest 2 for photosynthesis and harvests 1 and 2 for conductance (Table 6). There was a significant block effect in the first two harvests for transpiration and negative relationship between number of oviposition scars and transpiration rate for harvest 2 (Table 6). In 2006, there

were relationships between the number of eggs per plant and gas exchange rates for all three parameters for harvest 1, and block effects for conductance and transpiration rates (Table 6). In the second harvest there was a relationship between the number of larvae and photosynthetic rate, and block effects on all three gas exchange parameters (Table 6). There were no relationships between injury measures or block and gas exchange for the third harvest (Table 6). Although there were significant injury terms in a number of models, the models explained less than 35% of the variability in gas exchange parameters.

In 2005, none of the fluorescence values differed with adult density treatment or injury in any of the harvests. In 2006, there were no adult density treatment or injury effects on fluorescence values in the first and third harvests, although there were adult density treatment differences and a significant injury term in the Fo component (minimal fluorescence) of the dark adapted measurements in the second harvest (Table 7). Plants in the high adult density treatment had higher Fo values than plants in the control or low adult density treatments. Fo values increased as the number of oviposition scars increased.

Discussion

Herbivory by *M. janthinus* weevils decreased plant growth and primary physiology, although the effects on gas exchange were not as strong as expected. Observed decreases in plant growth and biomass were consistent with studies examining the impact of leaf defoliators on plant growth (Louda et al. 1990, Jeanneret and Schroeder 1992, Blossey and Schat 1997, De Clerck-Floate and Miller 2002, Schat and Blossey

2005). Schat and Blossey (2005) studied the effects of simulated defoliation and defoliation by the chrysomelid beetles, *Galerucella calmariensis* L. and *G. pusilla* Duft., with insect defoliation concentrated at active meristems and observed that while high rates of simulated herbivory did not decrease plant growth or biomass, low levels of herbivory decreased plant growth and biomass. In this study, *L. dalmatica* plants exposed to a high density of adult weevils had adult feeding injury concentrated at shoot tips. These plants had brown and wilted tops that crumbled off the plants over the growing season and reduced growth. Such injury to apical meristems could slow or prevent growth on injured stems and reduce production of reproductive structures. Plants in the high adult density treatment also had reduced root and reproductive biomass.

Results of this study were similar to observations from areas where *M. janthinus* has reached outbreak population levels and where *L. dalmatica* plants are generally smaller than plants at non-outbreak sites (Jeanneret and Schroeder 1992, De Clerck-Floate and Miller 2002). Jeanneret and Schroeder (1992) attributed the wilted shoot tips to larval feeding causing water stress in the plants while De Clerk-Floate and Miller (2002) linked repressed flowering and stunted growth to adult feeding induced meristem damage. Clipping studies have shown that *L. dalmatica* flowers in one flush and cannot compensate for damaged meristems by producing a second batch of flowers later in the season (Saner et al. 1994) and that spring clipping of stems leads to reduced regrowth and flowering (Robocker et al. 1972). The concentration of adult feeding around meristems and the resulting wilted stem tips support the hypothesis of De Clerk-Floate and Miller (2002). Observed decreases in stem biomass mirror results from a study in which single pairs of adult weevils were caged on plants for a month (Saner et al. 1994). However,

Saner et al. (1994) did not detect a change in root biomass, whereas I did. More work is necessary to determine if the differences in growth and biomass are due to adult feeding injury on active meristems, larval mining to stems, or a combination of the two. The separate effects of adult feeding injury and adult plus larval feeding injury were addressed in a field experiment (Chapter 3).

The pattern of biomass allocation to different parts of the plant changes as plants mature from seedlings to reproductive adults (Coleman and McConnaughay 1995). These phenotypic changes, also considered ontogenetic drift (Evans 1972), are frequently examined by comparing biomass ratios of component parts to other parts or to the whole across adult density treatments that are expected to affect plant growth (Muller et al. 2000). The differences in ratios between harvests, where the shoot biomass increased with respect to root biomass as the season progressed is expected as shoots grow over time. The differences in shoot-reproductive and root-reproductive biomass ratios between adult density treatments indicate that there was more shoot and root biomass relative to reproductive biomass in high adult density treatments than for control or low adult density treatment plants. This is consistent with the observation that plants in the high adult density treatments appeared to have meristem injury that could delay or reduce the ability of injured stems to produce flowers and seeds.

Differences in biomass ratios observed between harvests indicate the importance of including a time dimension when modeling the impact of adult density treatments on plant growth. Linking the differences in relative plant growth (stem length) to changes in biomass ratios suggest that differences in reproductive-shoot and reproductive-root ratios between adult density treatments may not be direct responses to larval mining injury, but

could be secondary responses due to injury induced reductions in plant growth (Samson and Werk 1986). Not surprisingly, given the lack of adult density treatment effect on root-shoot ratios, the allometric coefficient and exponent for the allometric relationship between root and shoot biomass were estimated from combined data. The ability to pool data across adult density treatments and harvests suggests that larval feeding injury did not alter the growth trajectory of stem and root biomass relationship. The allometric exponent of 0.93 indicates a nearly isotonic relationship in the growth of roots and shoots (Gould 1966, Poorter and Nagel 2000) suggesting "the maintenance of geometrical similarity with size increase" (Gould 1966).

The allometric parameters for the relationship between reproductive and root biomass were calculated separately for each adult density treatment-harvest combination due to significant treatment, harvest, and interaction terms. The relationships between the reproductive biomass and the covariate (root biomass) were all negative (allometric exponent was less than one) in the second and third harvests, indicating that increases in root mass were not matched by proportional increases in reproductive biomass (Gould 1966). The non-significant relationship between reproductive and root biomass in control plants at the third harvest might indicate that these plants had already completed reproduction where as the plants subjected to larval mining injury had not yet completed reproduction.

The allometric analysis of the relationship between reproductive biomass (dependent variable) and stem biomass (covariate) suggested that adult density treatments did not affect the growth trajectories of the plants with respect to these two variables, although time, or plant age and developmental stage did. As with the reproductive-root

relationships, the relationship between reproductive biomass and stem biomass was negative (the allometric exponent was less than one) at each of the three harvests. The discrepancy between significant adult density treatment effects on the reproductive-shoot ratios and the adult density treatment independent reproductive–shoot allometric relationships suggests that the treatment effects on that biomass ratio may be indicative of the treatments slowing development in high adult density treatment plants, and may thus be an effect of plant developmental stage or size rather than a change in growth trajectory.

The lack of strong differences in gas exchange rates measured in the carefully controlled garden experiment was not similar to the Peterson et al. (2005) field results where gas exchange was measured on *L. dalmatica* plants growing naturally in the field, either with or without oviposition scars.

The timing of significant differences, immediately after adults were removed from the plants in 2006 and at the end of the growing season in 2005, could be related to the type of feeding injury. A recent study also found that oviposition by a sawfly (*Diprion pini*) into Scots pine (*Pinus sylvestris*) resulted in decreased photosynthesis in the first three days after oviposition (Schroeder et al. 2005). The decrease in photosynthesis directly after adults were removed suggests that adult injury rather than early larval feeding was responsible for changes in primary physiology. *Linaria dalmatica* stems in medium and high adult density treatments appeared to have no internal tissue left in the stem by the end of the season. Larval mining in stems by a high density of immature insects could have damaged vascular tissue and impeded flow of resources from leaves to roots, causing reductions in photosynthesis in response to

negative feedback loops. (Dennis and Blakely 2000). In a greenhouse study on the effects of water availability and feeding injury by a stem mining sawfly (*Cephus cinctus*) Macedo et al. (2007b) found that *Triticum aestivum* plants grown under low water availability experienced reduced photosynthesis in response to larval feeding. They hypothesized that the sawfly larvae were causing injury to vascular tissues in drought stressed plants but not in plants grown with adequate water.

The discrepancy between growth and primary physiological responses could be due to type of injury and the ability of *L. dalmatica* to overcome the injury. Adult *M. janthinus* feeding on meristems early in the season could have lasting effects on growth patterns (Robocker et al. 1972, Saner et al. 1994). This is supported by the results of the allometric analysis. The weak primary physiological response observed, however, could reflect the conditions under which the garden experiment was conducted. Plants were watered daily and soil in the pots never became dry. The daily watering may have permitted *L. dalmatica* plants to compensate for *M. janthinus* feeding injury to a degree that plants in the field could not ordinarily do. The majority of the differences recorded in the garden in growth, biomass, and gas exchange were between minimally injured or uninjured control plants and plants in the high adult density treatments, suggesting a fairly high threshold level of injury is required for individual plant responses to be realized. Many plant species tolerate high levels of insect injury before measurable decreases in growth occur (Huffaker and Kennett 1959, McEvoy et al. 1991, Peterson 2001).

The results of this study suggest that *M. janthinus* can impact *L. dalmatica* populations by decreasing reproduction and root biomass. However, the spread of *L.*

dalmatica is unlikely to slow by reducing seed set, due to the low competitive ability of seedlings (Gates and Robocker 1960, Grieshop and Nowierski 2002) and the propensity of the plant to produce clones from lateral roots (Robocker 1974). Due to the ability to produce clones from lateral roots, the decrease in root biomass with increasing adult weevil density treatment severity is a more likely mechanism for reducing spread of *L. dalmatica*. Plant responses to insect herbivory can be influenced by biotic and abiotic conditions. To determine if the environmental conditions in the garden could have interacted with impacts of *M. janthinus* feeding injury on *L. dalmatica* growth and primary physiology, and to differentiate between adult and adult plus larval feeding injury, field experiments with similar adult density treatments was conducted in 2006 and 2007 (Chapter 3).

Table 1 Plant origin and experiment timelines, 2005-2006.

Year	Plant origin	Seedlings started	Placed in blocks	Weevils arrived	Treatments applied	Adults removed/ Harvest1	Harvest 2	Harvest 3
2005	Already in garden	N/A	5/2005	5/27/2005	5/28/2005	6/11/2005	7/18/2005	8/21/2005
2006	seed	4/5/2005	10/2005	6/1/2005	6/3/2006	6/10/2006	7/20/2006	8/30/2006

Table 2 Analysis of variance tables for allometric relationships. The biomass component listed first in each column is the dependent variable and the second the covariate. Models were run on natural log transformed biomass data. P-values in bold are significant at the 0.05 level.

		Shoot-root allometry			Reproductive-root allometry			Reproductive-shoot allometry		
Factor	d.f.	SS	F	P	SS	F	P	SS	F	P
Covariate	1	33.96	235.09	< **0.0001**	4.66	44.81	< **0.0001**	5.45	69.68	< **0.0001**
Treatment	3	1.14	2.64	0.0524	0.99	3.16	**0.0272**	0.50	2.14	0.0984
Harvest	2	0.79	2.73	0.0694	0.09	0.46	0.6349	1.01	6.45	**0.0021**
Covar*treatment	3	0.77	1.79	0.1534	1.06	3.40	**0.0198**	0.59	2.51	0.0619
Covar*harvest	2	0.65	2.27	0.1079	1.73	8.29	**0.0004**	2.33	14.89	<**0.0001**
Covar*treat*harvest	6	0.47	0.54	0.7739	2.04	3.26	**0.0051**	1.12	2.40	**0.0312**
Residual	126									

Table 3 Relationships between natural log transformed *Linaria dalmatica* reproductive and root biomass. Separate regressions were run for each harvest-*M. janthinus* adult density treatment combination. Adult densities were 0 insects, 2 pairs, 8 pairs, and 16 pairs of *M. janthinus* adults. Results of the regressions were used to obtain allometric coefficients (intercept) and exponents (slope) for the relationship between root and reproductive biomass. Reproductive biomass was the dependent variable and root biomass was the covariate. Cells where there is a '.' under t-value and p column heads indicate that it was not possible to calculate those values due to lack of reproductive biomass in the first harvest. P-values in bold type are significant at the 0.05 level.

Harvest/	Intercept (α)				Slope (β)			
Treatment	Value	SE	t-value	P > |t|	Value	SE	t-value	P > |t|
Harvest 1								
Control	0.04154	0.03730	1.11	0.2915	-0.00324	0.02283	-0.14	0.8899
Low	0	0	.	.	0	0	.	.
Medium	-0.06684	0.05778	-1.16	0.2743	0.06718	0.04051	1.66	0.1282
High	0	0	.	.	0	0	.	.
Harvest 2								
Control	-0.00760	0.25217	-0.03	0.9765	0.55833	0.14144	3.95	**0.0027**
Low	-0.40538	0.26481	-1.53	0.1568	0.77478	0.20984	3.69	**0.0042**
Medium	-0.20317	0.28810	-0.17	0.4968	0.38559	0.20400	1.89	0.0880
High	-0.02147	0.12365	-0.17	0.8656	0.20410	0.10788	1.89	0.0878
Harvest 3								
Control	1.04452	0.43226	2.42	0.0630	-0.22639	0.25720	-0.88	0.3994
Low	-0.30060	0.29667	-1.01	0.3348	0.84222	0.19577	4.30	**0.0016**
Medium	-0.23684	0.21107	-1.12	0.2880	0.55571	0.16808	3.31	**0.0079**
High	-0.32170	0.09230	-3.49	**0.0059**	0.55418	0.08670	6.39	**<0.0001**

Table 4 Results of regression analyses on the relationship between natural log transformed *Linaria dalmatica* reproductive and stem biomass. Separate regressions were run for each harvest on data pooled across adult density treatments. Results of the regressions were used to obtain allometric coefficients (intercept) and exponents (slope) for the relationship between shoot and reproductive biomass. Reproductive biomass was the dependent variable and shoot biomass was the covariate.

	Intercept (α)				Slope (β)			
Harvest	Value	SE	t-value	P > |t|	Value	SE	t-value	P > |t|
Harvest 1	-0.03658	0.01451	-2.52	0.0152	0.04032	0.01041	3.87	0.0152
Harvest 2	-0.48940	0.13141	-3.72	0.0005	0.54555	0.06934	7.87	< 0.0001
Harvest 3	-0.58566	0.15337	-3.82	0.0004	0.57956	0.07485	7.74	< 0.0001

Table 5 Impact of *Mecinus janthinus* adult density on *Linaria dalmatica* gas exchange parameters, 2005, 2006. Adult density treatments were 0, 2 pairs, 8 pairs, or 16 pairs of *M. janthinus* per plant.

Harvest/	2005			2006		
Model	d.f.	F	P	d.f.	F	P
Harvest 1						
Photosynthesis						
Block	3, 56	2.06	0.1162	3,71	2.74	**0.0499**
Treatment	3, 56	0.32	0.8134	3,71	4.00	**0.0109**
Conductance						
Block	3, 56	5.46	**0.0023**	3,71	5.25	**0.0025**
Treatment	3, 56	0.46	0.7106	3,71	2.52	0.0649
Transpiration						
Block	3, 56	4.60	**0.0060**	3,71	2.83	**0.0430**
Treatment	3, 56	0.31	0.8164	3,71	2.90	**0.0409**
Harvest 2						
Photosynthesis						
Block	3, 56	3.83	**0.0145**	3,57	3.14	**0.0321**
Treatment	3, 56	1.85	0.1489	3,57	1.96	0.1299
Conductance						
Block	3, 56	5.00	**0.0038**	3,57	6.68	**0.0006**
Treatment	3, 56	1.59	0.2100	3,57	1.37	0.2611
Transpiration						
Block	3, 56	5.14	**0.0033**	3,57	6.49	**0.0007**
Treatment	3, 56	3.25	**0.0284**	3,57	1.02	0.3890
Harvest 3						
Photosynthesis						
Block	3, 40	1.94	0.1383	3,31	0.31	0.8165
Treatment	3, 40	4.10	**0.0126**	3,31	1.06	0.3791
Conductance						
Block	3, 40	0.66	0.5810	3,31	0.69	0.5675
Treatment	3, 40	3.56	**0.0225**	3,31	1.13	0.3507
Transpiration						
Block	3, 40	0.54	0.6588	3,31	0.34	0.7943
Treatment	3, 40	3.29	**0.0302**	3,31	1.00	0.4055

Table 6 Impact of *Mecinus janthinus* oviposition scars, eggs, larvae, and post larval stage insects on *Linaria dalmatica* gas exchange parameters, 2005-2006. P-values in bold indicate significant terms at the 0.05 level.

Harvest	2005			2006			
	Model	d.f.	F	P	Model	d.f.	F	P
Harvest 1								
Photosynthesis								
					Block	3,72	2.43	0.0725
	Oviposition scars	1,45	1.21	0.2766	Oviposition scars	1,72	1.88	0.1751
	Eggs	1,45	1.68	0.2016	Eggs	1,72	4.68	**0.0338**
Conductance								
	Block	3,42	6.20	**0.0014**	Block	3,72	4.88	**0.0038**
	Oviposition scars	1,42	1.07	0.3066	Oviposition scars	1,72	0.44	0.5072
	Eggs	1,42	0.88	0.3538	Eggs	1,72	5.80	**0.0186**
Transpiration								
	Block	3,42	5.28	**0.0035**	Block	3,72	3.04	**0.0345**
	Oviposition scars	1,42	1.90	0.1758	Oviposition scars	1,72	0.51	0.4768
	Eggs	1,42	1.68	0.2014	Eggs	1,72	7.71	**0.0070**
Harvest 2								
Photosynthesis								
	Block	3,42	4.60	**0.0072**	Block	3,58	3.72	**0.0163**
	Oviposition scars	1,42	3.92	0.0543	Oviposition scars	1,58	0.74	0.3919
	Larvae	1,42	3.35	0.0743	Larvae	1,58	4.75	**0.0334**
Conductance								
	Block	3,42	5.24	**0.0037**	Block	3,58	7.49	**0.0003**
	Oviposition scars	1,42	4.06	0.0505	Oviposition scars	1,58	0.57	0.4522
	Larvae	1,42	2.87	0.0977	Larvae	1,58	3.46	0.0678
Transpiration								
	Block	3,42	5.26	**0.0036**	Block	3,58	7.28	**0.0003**
	Oviposition scars	1,42	4.49	**0.0401**	Oviposition scars	1,58	0.80	0.3745
	Larvae	1,42	2.86	0.0981	Larvae	1,58	3.44	0.0688

Table 6 Continued.

Harvest	2005			2006			
Model	d.f.	F	P	Model	d.f.	F	P
Harvest 3							
Photosynthesis							
Oviposition scars	1,43	0.00	0.9623	Block	3,32	0.31	0.8185
Larvae	1,43	0.02	0.8821	Oviposition scars	1,32	0.30	0.5862
Post larvae	1,43	2.33	0.1346	Post egg	1,32	0.02	0.9019
Conductance							
Oviposition scars	1,43	0.06	0.8058	Block	3,32	0.78	0.5135
Larvae	1,43	0.05	0.8158	Oviposition scars	1,32	0.90	0.3492
Post larvae	1,43	2.57	0.1160	Post egg	1,32	0.51	0.4798
Transpiration							
Oviposition scars	1,43	0.12	0.7318	Block	3,32	0.28	0.8368
Larvae	1,43	0.03	0.8611	Oviposition scars r	1,32	1.32	0.2583
Post larvae	1,43	3.22	0.0796	Post egg	1,32	0.94	0.3394

Table 7 Impact of *Mecinus janthinus* adult density, oviposition scars, and larvae on *Linaria dalmatica* minimal fluorescence in the dark-adapted fluorescence tests. Only tables for significant models are presented. Treatments are 0, 2 pairs, 8 pairs, and 16 pairs of adult *M. janthinus* caged per plant.

	2006		
Model	d.f.	F	P
Fo			
Block	3,55	3.88	0.0137
Treatment	3,55	4.93	0.0042
Fo			
Block	3,56	2.93	0.0413
Oviposition scars	1,56	4.19	0.0453
Larvae	1,56	0.02	0.8900

Figure 1 Mesh bag cages used to enclose *Mecinus janthinus* weevils on plants. **(**A**)**. Applying treatments, (B). Closed cages.

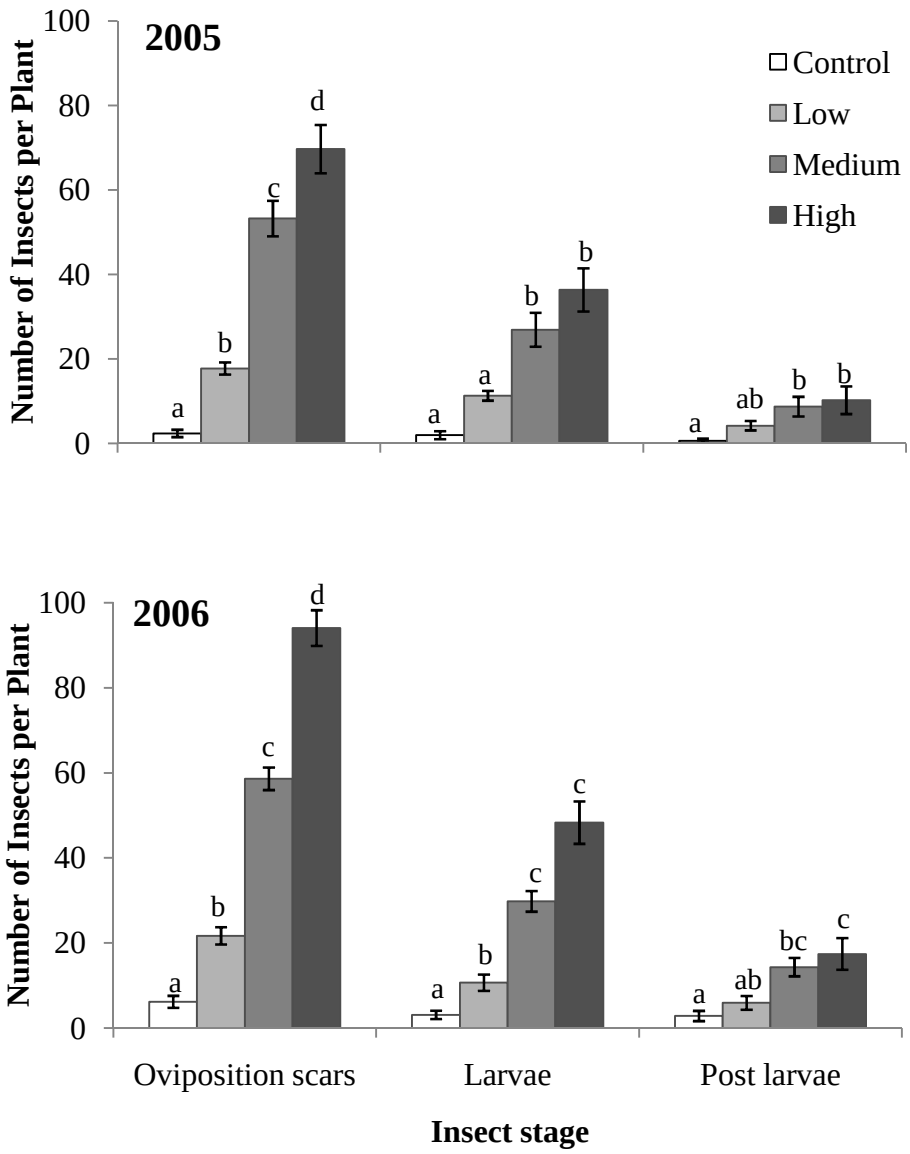

Figure 2 The number of oviposition scars and immature *Mecinus janthinus* dissected out of *Linaria dalmatica* plants from each treatment, 2005-2006. Control plants had no adults caged on them, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively. Letters indicate differences between treatments (Tukey $\alpha = 0.05$) within injury types (oviposition scars, larvae, and post larval staged insects).Data were pooled across harvests.

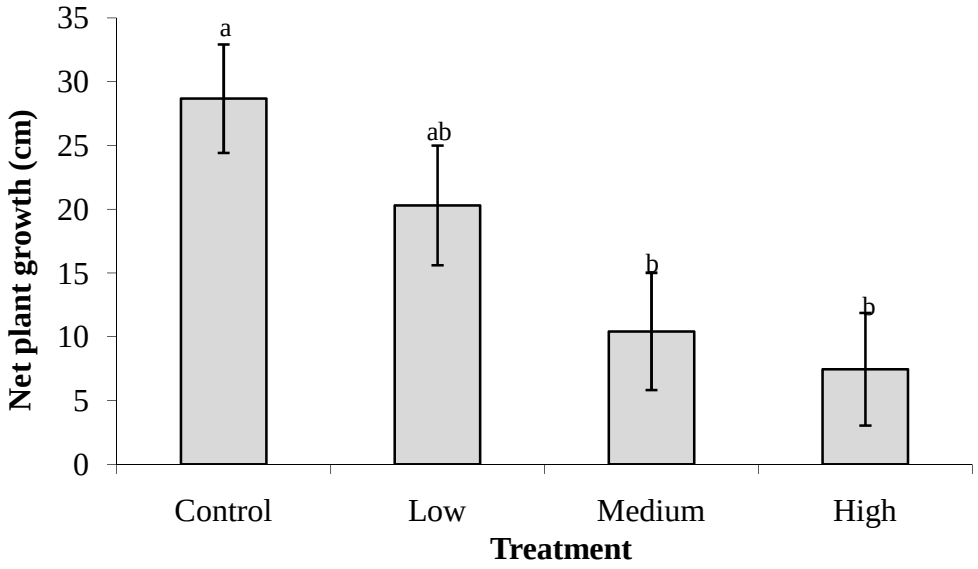

Figure 3 Impacts of *Mecinus janthinus* density on *Linaria dalmatica* growth, 2005. Letters indicate significant differences between treatments (Tukey $\alpha = 0.05$). Control plants had no adults caged on them, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively.

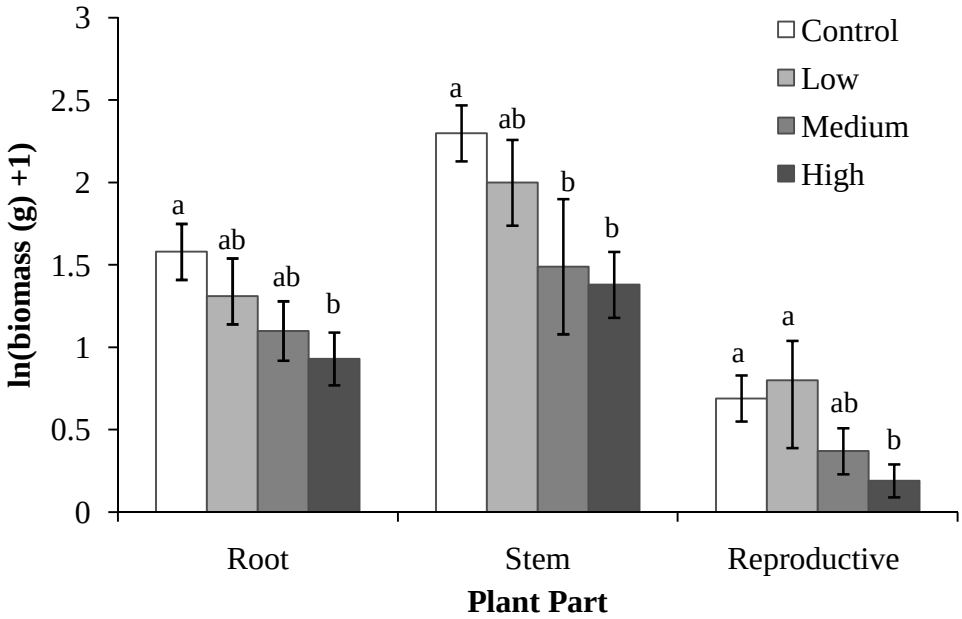

Figure 4 Biomass of component of *Linaria dalmatica* plant parts from the third harvest, 2005. Letters indicate significant differences between treatments within type of biomass. Control plants had no adults, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively.

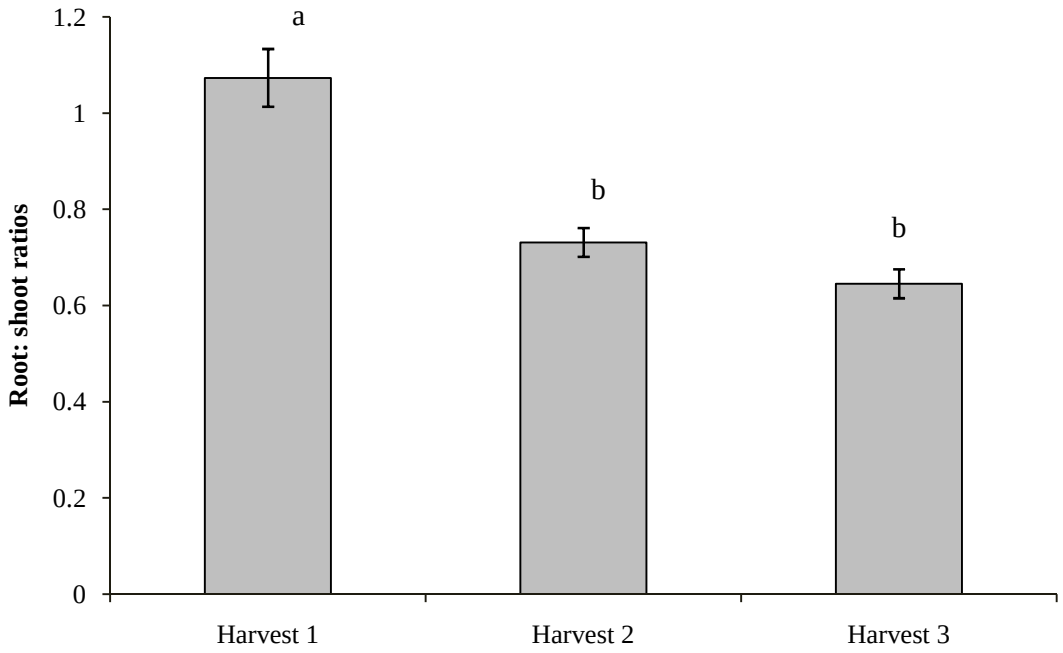

Figure 5 Impact of *Mecinus janthinus* density on *Linaria dalmatica* root-shoot biomass ratios over time (harvests), 2005. Letters indicate differences between harvests.

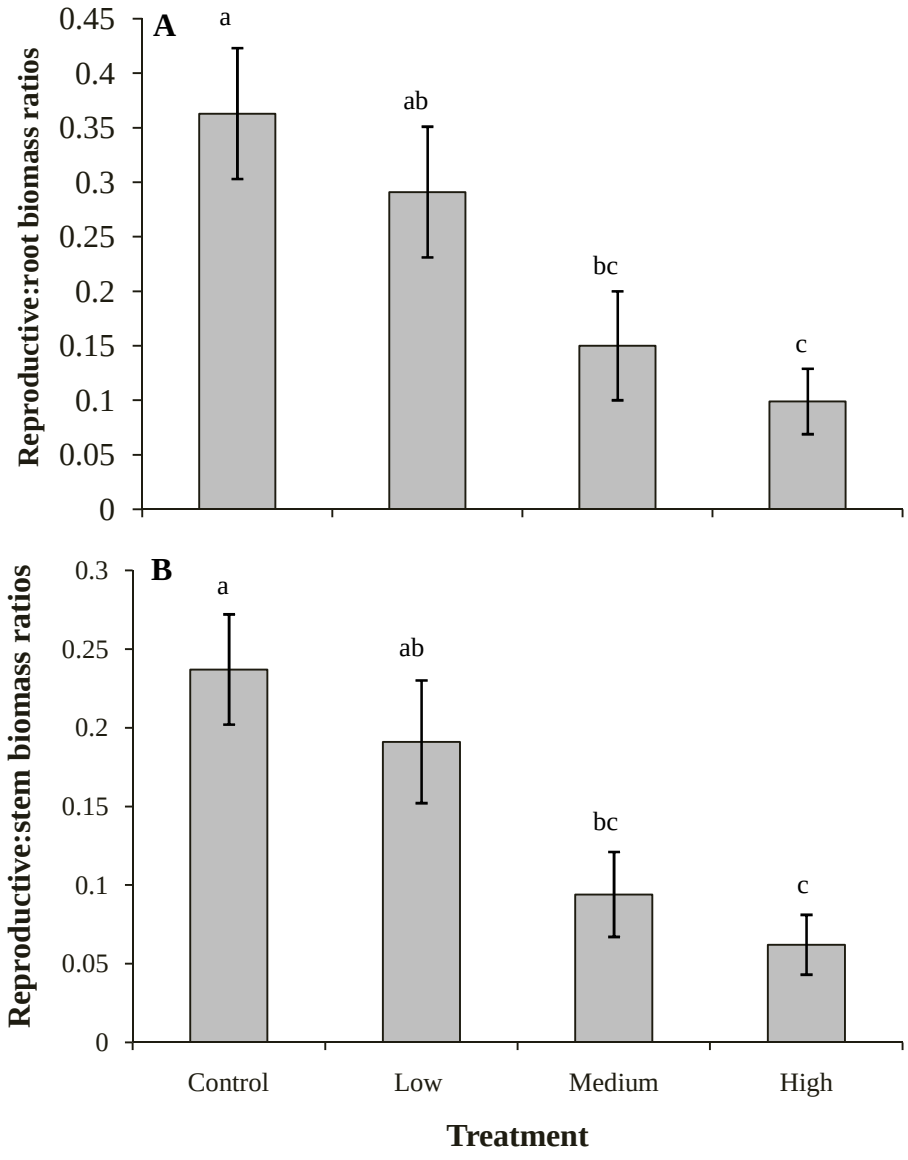

Figure 6 Impact of *Mecinus janthinus* density on *Linaria dalmatica* reproductive biomass to root biomass ratios (A) and reproductive biomass to stem biomass ratios (B) separated by treatment, 2005. Ratios were calculated from natural log transformed data. Letters indicate differences between treatments. Control plants had no adults, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively.

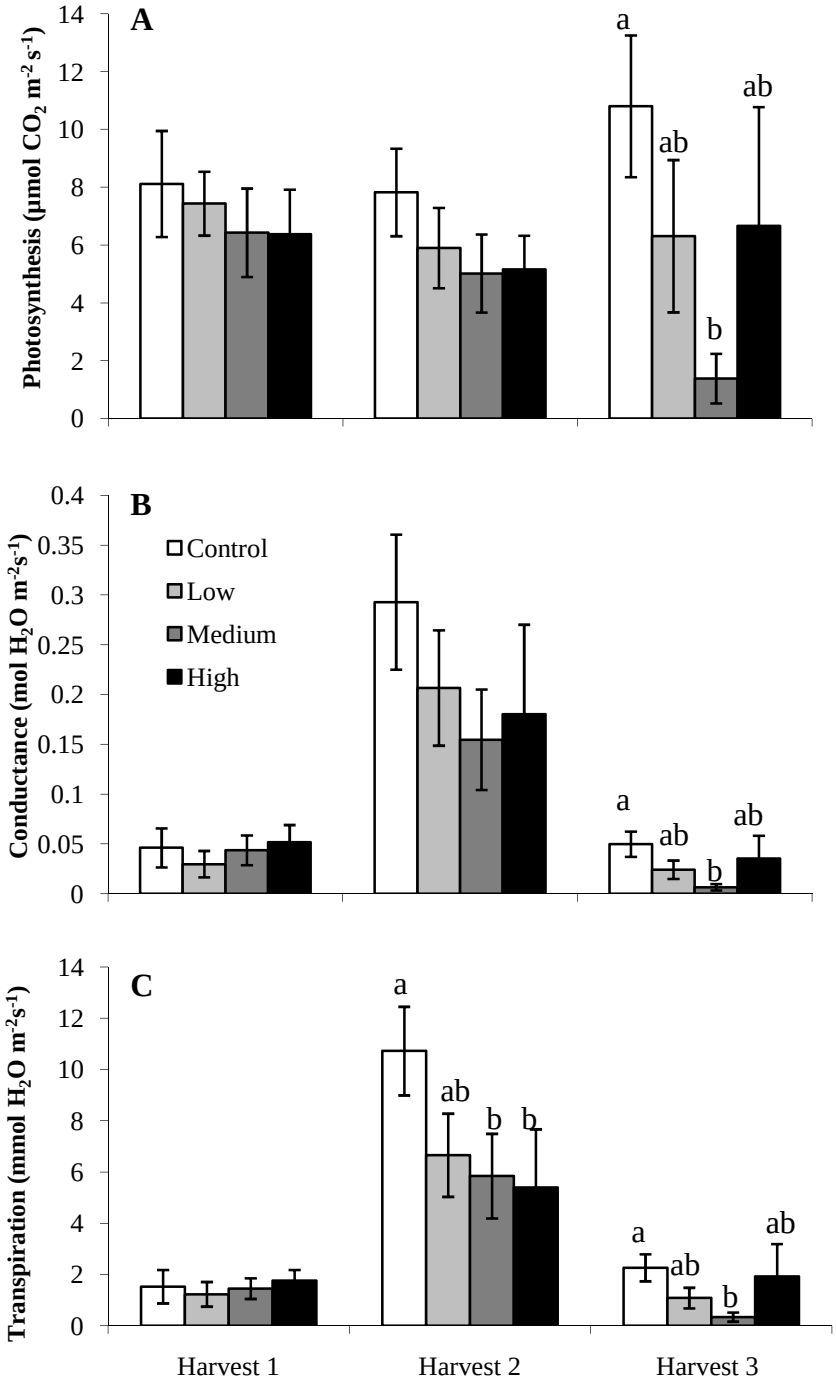

Figure 7 Impact of *Mecinus janthinus* density on *Linaria dalmatica g*as exchange parameters collected at three times over the summer, 2005. Separate models were used for each harvest. Letters indicating differences apply only to treatments within each harvest. Control plants had no adults, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively.

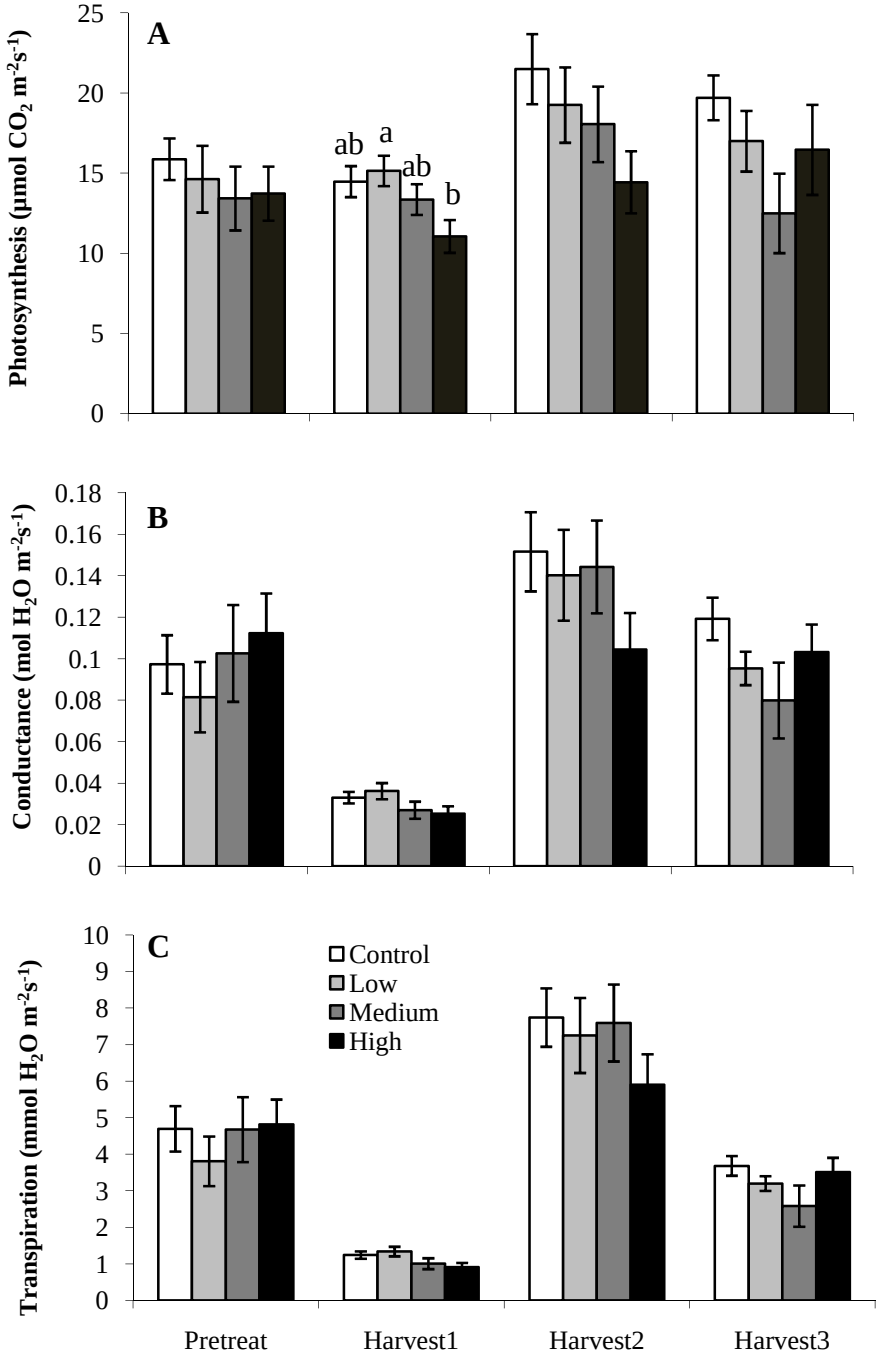

Figure 8 Impact of *Mecinus janthinus* density on *Linaria dalmatica g*as exchange rates measured before treatments were applied, and at three times over the summer, 2006. Separate models were used for each harvest. Letters indicating differences apply only to treatments within each harvest. Control plants had no adults, low, medium, and high treatments had 2, 8, and 16 pairs of adults caged on them, respectively.

CHAPTER 3

IMPACT OF *MECINUS JANTHINUS* INJURY ON GROWTH AND PRIMARY PHYSIOLOGY OF *LINARIA DALMATICA* IN THE FIELD

Abstract

Herbivorous insects can affect plant growth, biomass, gas exchange rates, and reproductive rates. These potential impacts are the rationale for using insect agents in the biological control of weeds. Because environmental conditions can affect how plants respond to herbivores, studies often use controlled garden or greenhouse experiments to isolate herbivore effects from potentially confounding environmental influences. However, the applicability of results obtained through experiments with controlled conditions to weed control in natural settings might be limited. This study reports the results of a field study designed to determine the impact of variable insect densities and the type of insect injury (adult feeding and larval feeding) on plant growth and primary physiology. Dalmatian toadflax (*Linaria dalmatica*) plants growing near Gardiner, MT were used as the study plant, and a stem boring weevil (*Mecinus janthinus*) released as a biological control agent was the herbivore. A range of larval densities was obtained by caging four to 32 adult weevils on individual stems for one week. Stem length and gas exchange rates were measured every two weeks after adults were removed. The number of oviposition scars and immature insects in stems was positively correlated with the density of adult weevils initially caged on the stems. In 2006, plants in control and low adult density treatments had greater increases in stem length than plants in the high

density treatments. In 2007, control plants had the greatest increase in stem length and high adult density plants experienced net negative changes in stem length over the summer. No significant differences in gas exchange rates between adult density treatments or resulting numbers of oviposition scars and immature insects were found in 2006. At one of the two sites in 2007, plants receiving the highest density of adult weevils had lower photosynthetic, conductance, and transpiration rates than all other adult density treatments.

Introduction

Herbivory can have important consequences for the performance of individual plants, plant populations, and plant communities (Crawley 1989b, Louda et al. 1990, Karban and Baldwin 1997, Kessler et al. 2004). Common consequences of insect herbivory include reductions in height, biomass, and growth rates (Louda et al. 1990, Schat and Blossey 2005); changes in root:shoot ratios (Vranjic and Gullan 1990, Blossey and Schat 1997); reduced or increased photosynthetic activity (Detling et al. 1979, Peterson 2001, Retuerto et al. 2004, Peterson et al. 2005); and reduced reproductive rates (Notzold et al. 1998, Schat and Blossey 2005). Understanding plant responses to herbivory is important for improving our ability to predict trends and trajectories in community dynamics in both natural and agricultural ecosystems and for selecting more effective biological control agents.

The plasticity of plant response requires ecological studies to simultaneously investigate a number of variables for plant performance (Foggo 1996) under a range of

injury levels. However, combinations of biotic and abiotic conditions, such as history of herbivory (Brown and Weiss 1995, Harrison and Maron 1995, Root 1996a), drought stress, and nutrient status (Louda et al. 1990, Levine and Paige 2004) can influence how individual plants respond to herbivore injury. To separate the effect of herbivore injury level from plant growth history and environmental conditions, garden experiments using plants grown from seed collected from a common source and cultivated under identical conditions are often used. However, experimental conditions that deviate substantially from field conditions can lead to disparate results.

In a controlled garden experiment (Chapter 2) designed to reduce confounding factors such as plant age, drought stress, and injury history on plant response to herbivory, equivocal results were found in primary physiological responses of Dalmatian toadflax (*Linaria dalmatica* (L.) Miller), even to high levels of stem boring weevil *(Mecinus janthinus* Germar) larval injury, despite reductions in plant growth. Trends of decreasing gas exchange rates with increasing adult *M. janthinus* density were found. In an observational field study of primary physiological responses of *L. dalmatica* to apparent injury by *M. janthinus*, Peterson et al. (2005) showed that plants with calluses and swollen stems indicative of oviposition activity by the weevil had lower gas exchange rates than plants with no signs of oviposition. The discrepancy between the experimental results obtained in a controlled garden experiment and the field study suggests that environmental conditions in the garden, such as moisture availability, may have allowed the plants to compensate physiologically for injury.

This study was designed to replicate the garden experiment described in chapter 2 on plants growing under typical field conditions. Using plants growing in the field resulted in a loss of control over plant age and growth history, but replicated conditions likely to be found in naturally occurring *L. dalmatica* populations where *M. janthinus* field releases would be made. Because the environmental conditions experienced by perennial plants in previous years can continue to influence plant responses to herbivory, this field experiment should provide a means to more accurately describe the impact of *M. janthinus* on *L. dalmatica* growth and primary physiology than the garden experiment. This study will also serve as a link between plant responses observed at field releases of *M. janthinus* and potential mechanisms of biological control, to determine if there is a minimum level of injury required for changes in plant growth and primary physiology to occur. Finally, this study was designed to differentiate plant responses to adult feeding injury from responses to adult plus larval feeding injury. This study is unique in that it evaluates both growth and gas exchange responses to a concealed feeder in a field experiment.

I hypothesized that adult *M. janthinus* feeding injury and adult plus larval *M. janthinus* feeding injury would reduce stem growth and photosynthetic rates of *L. dalmatica*. I also expected there to be a minimum density of adults per stem required to obtain measurable decreases in plant growth and gas exchange rates.

Study Organisms

Linaria dalmatica, a short lived perennial plant, was introduced to the United States from Eastern Europe and the Mediterranean in the 1890s for testing as an ornamental (Alex 1962). *Linaria dalmatica* has since formed dense stands in the United States and Canada west of the 100th meridian, reducing the carrying capacity and value of rangeland (De Clerck-Floate and Harris 2002). *Linaria* spp. leaves typically contain iridoid glycosides (Ilieva et al. 1992), which are reported to be mildly poisonous to cattle.

A biological control program to manage *L. dalmatica* was initiated in the 1960s, but little control was realized until *M. janthinus* was released in North America in 1991 (De Clerck-Floate and Harris 2002). Adult *M. janthinus* emerge from late March to early April on *L. dalmatica* (De Clerck-Floate and Harris 2002) and feed on host leaves and stems for a short period before mating. Oviposition occurs from late May through mid July. Eggs are laid individually in cavities chewed into the stems. Eventually the plant forms distinct calluses over oviposition sites that are visible throughout the growing season. Larvae mine the stem for approximately 40 days until the onset of pupation. The pupal stage lasts three weeks with adults overwintering in the natal stem (Jeanneret and Schroeder 1992).

Materials and Methods

A randomized experiment was established in a field setting to study the impact of variable *M. janthinus* adult only or combined adult and larval feeding injury on *L. dalmatica* growth and primary physiology. The experiment was replicated in space and

time at two field sites for two years, 2006 and 2007 (Table 8). The experimental design was similar in both years, although in the second year (2007) the number of replicates per adult density treatment was increased and two additional adult density treatments were added. One of the two sites used in 2006 was used again in 2007, with a new site selected for the second 2007 experimental location.

All three sites used over the two year study were located near Gardiner, MT. Each site had similar plant community composition and a naturally occurring population of *L. dalmatica*. Stems used were randomly selected such that all stems occurred at a similar elevation. Each stem was then randomly assigned to one of three adult density treatments in 2006, or one of five adult density treatments in 2007 (Table 9). Stems were caged and exposed to feeding and oviposition by adult weevils for approximately one week before the adult insects and cages were removed. Stem length and primary physiological parameters (photosynthetic rate, stomatal conductance, and respiration rate) were measured every second week throughout the summer until stems senesced. Stems were harvested after the last measurement event and then dissected to determine the number of larvae, pupae, and adults in individual stems.

Experimental Sites and Transect Layout

The three study sites were located immediately north of Gardiner, MT near Jardine Rd (National Forest Road 493). In 2006, the sites used were Pond Flat (N45º02.61', W110º40.09', 1897m) and Eagle Creek (N45º02.96', W110º40.57', 1922m, SSE aspect). Neither site had received biocontrol agent releases before the 2006 experiment, although there was a release made in 2004 within 2 km of the Eagle Creek

site. The Pond Flat site was used again in 2007, after it was determined that a measurable population of *M. janthinus* had not established as a result of the 2006 experimental releases. The second 2007 experimental replicate was set up at Slope Creek (N45º03.49', W110º38.84', 1935m, SE aspect), rather than at Eagle Creek, due to that sites lack of host stem density required to accommodate the additional treatments and replicates per treatment. The Pond Flat experimental site was situated at the flat base of a gentle slope parallel to and approximately 10 meters from an irrigation ditch. Stems selected were all within two meters of a transect that ran the length of the *L. dalmatica* stand, and through the densest part of the stand. The Eagle Creek experimental site was situated on the bank of a dry gully with the transect following the bank of the gully and all stems falling within two meters of either side of this transect. There was no standing or running water in the vicinity of the Eagle Creek plants. Slope Creek was set up across the fall line near the bottom of a hill approximately three meters from the same irrigation ditch that runs past the Pond Flat site. As with the Pond Flat site, the transect ran through the densest part of the stand and stems selected were within two meters of the transect.

Experimental stems were selected by stretching a tape measure through the densest section of a *L. dalmatica* stand perpendicular to the fall line of the slope. A random number table was then used to select experimental stems by location. The stem closest to the location indicated by the random number table was selected; in some cases the closest corresponding stem occurred up to two meters above or below the transect. If the end of the transect was reached before enough stems could be identified then additional plants were added from the beginning of the transect using the same approach.

To reduce the chance of selecting two stems from the same plant, the closest stem above or below the transect line that was also at least 0.5 meters from an already marked stem was selected. Selected stems were then randomly assigned to one of three or five adult density treatments, depending on the year (Tables 8 and 9).

Treatments and Treatment Application

Adult density treatments were applied to obtain a range of adult plus larval feeding injury levels and a range of adult only feeding injury levels. In 2006, three adult density treatments were applied: a control (no *M. janthinus* feeding injury), a low adult and larval injury treatment (two male and two female weevils), and a high adult and larval injury treatment (16 male and 16 female weevils). A low adult only injury (4 male weevils) and a high adult only injury (32 male weevils) treatment were added in 2007 (Table 9).

Adult insects were collected in eastern Washington by USDA-APHIS-PPQ personnel and shipped via overnight delivery to Bozeman. External rostral (Schat et al. 2007) and leg characters (Carney et al. 2004) were used to rapidly separate the weevils by gender. Weevils were caged on stems within 48 hours of being sorted. In 2006, the weevils used in the treatments described above had been previously used in a garden experiment (Chapter 2) before being re-sorted and placed on the plants in the field. In 2007, the weevils were used only for this field experiment.

Due to the inherent difficulty in distinguishing between individual plants growing within a stand of *L. dalmatica* without destructively digging up plant root masses, adult weevils were applied to individually caged stems. To reduce the probability of selecting

multiple ramets from a single root system, experimental stems were selected from discrete clumps of stems separated by at least 0.5 meters from other stems. Experimental stems were not excavated in either year to verify if selected stems had oridinated from discrete plants.

Cages fabricated from size 70 nylon mesh (approximately 0.211 mm holes) 30 cm diameter and 1 m tall were placed over stems and secured around their base with duct tape. Insects were then released onto the stems and the bags were tied closed at the top. To avoid damaging the plants, the tops of the bags were attached to bamboo stakes on either side of the caged stems to anchor the bags vertically. Cotton balls were placed as a physical buffer between the base of the bag and the stem to avoid crushing the stems when bags were secured at the base with the tape. Weevils remained caged on the stems for seven days in 2006 and 10 days in 2007 (Table 8). A hand-held aspirator was used to remove the insects from the stems; 90% of the weevils were recovered. On subsequent visits, all experimental stems were examined and any adult weevils observed were removed. Each experimental stem was collected at the end of the experiment and dissected to quantify larval feeding injury as a function of the density of insects by developmental stage (e.g. oviposition scars, larvae, pupae, and adults).

Measurements

Linaria dalmatica stem lengths were measured to the nearest 0.5 cm, beginning the week treatments were applied. Measurements were repeated when adult insects were removed and every two weeks thereafter until plants senesced and stems were harvested. Stem lengths were measured from the point where they emerged from the ground to the

tip of the active meristem of the main stem; length of secondary shoots was not considered. Adult feeding damage was scored when adult insects were removed from plants, and each stem was photographed on each measurement date to document visual changes in the stems over time. Harvested stems were dissected and the number of oviposition scars, post egg stage immature insects (larvae and pupae), and new adults were counted.

Photosynthetic rates, stomatal conductance, and transpiration rates were measured with a LI-6400 portable photosynthesis system (LI-Cor Inc., Lincoln NE) on a single leaf randomly selected from the top third of each experimental stem every two weeks. In 2006, the first gas exchange readings were taken on 22 June, the day the adult insects were removed from the plants, and every other week thereafter until the stems were harvested on 24 August. In 2007, gas exchange rates were measured the day adults were placed on the plants, the day after adult insects were removed from the stems, and then every second week after that until the plants senesced. The only exception to this measurement schedule occurred when I was unable to take the mid August measurements at the Slope Creek site in 2007. Gas exchange rates were measured every second week to minimize the number of leaves removed from the stems. Due to the generally small leaf size and lack of petioles, *L. dalmatica* leaves had to be removed from the stems before they could be placed in the LI-6400 leaf chamber. The LI-6400 settings used in this series of measurements matched those used by Peterson et al (2005), and those used in the garden experiment (Chapter 2). Leaves inside the chamber were illuminated with a light intensity of 1400 μmol photons m^{-1} s^{-1} from a light source inside the leaf chamber

(10% blue light, 90% red light), and exposed to a flow rate of 500 μmol air s^{-1}, and a reference CO_2 concentration of 400 μmol CO_2 mol^{-1} (generated from a 12 g CO_2 cylinder connected to the LI-6400).

Data Analysis

All data analyses were carried out using SAS 9.0 (SAS Institute Inc., NC, USA) software. Each year's data were analyzed separately due to changes in experimental design between years. Where significant site by adult density treatment interactions occurred, sites were also tested separately. The Proc-GLM procedure was used to conduct analyses of variance to determine if different treatments (as a categorical representation of the combined density and sex of adults that had been caged on the plants) resulted in different numbers of oviposition scars and post egg stage insects. An α value of 0.05 was used to determine significance. Differences between sites and adult density treatments (coded as a categorical variable) were determined using conservative post-hoc Tukey comparisons (α 0.05). The number of oviposition scars and surviving post egg stage individuals (sum of larvae, pupae, and adults) were used in regression analyses to diagnose potential relationships between injury and growth or gas exchange parameters.

Initial and final heights were used to calculate the change in stem length and the relative change in stem height based on the initial length of the stem. Relative plant heights are typically calculated using natural log transformed data; however, some of the adult density treatments resulted in negative changes in stem height. To accommodate such negative changes, relative changes in stem length were calculated in two ways,

using: 1) absolute values, and 2) natural log transformed data where all stems that were shorter at the end of the season were coded as having zero growth. Analyses of variance and Tukey comparisons were used to determine growth differences between sites or adult density treatments. Regression analyses were used to examine the relationship between injury (number of oviposition scars or number of post egg stage individuals) and change in stem length.

Gas exchange data were analyzed separately for each date, both as a function of adult gender and density (0, 4, or 32 adult *Mecinus janthinus*), as well as a function of injury using ANOVAs and regression analyses, respectively. Tukey comparisons were used to determine the adult density treatments that differed from each other in models with significant results ($P < 0.05$). Differences in gas exchange parameters over time were examined using the Proc-Mixed procedure with a repeated statement, with Julian date as the measure of time.

Results

Plant Size and Growth

Varying the number of adult *M. janthinus* caged on stems led to differences in oviposition scar and post egg stage insect density in both years ($P < 0.0001$ for each model tested) (Figure 9, 10). Initial stem length was greater at the Pond Flat site than either of the other sites ($F = 4.51$; d.f. = 1,26; $P = 0.0433$ in 2006, and $F = 11.08$; d.f. = 1,104; $P = 0.0012$ in 2007). We confirmed that within sites and before treatment application, there were no differences in stem lengths for stems randomly assigned to the

different adult density treatments ($F = 0.96$; d.f. = 2,26; $P = 0.3954$ in 2006, and $F = 0.62$; d.f. = 4,104; $P = 0.6507$ in 2007).

In 2006, relative change in stem length did not differ between sites when absolute values were used (allowing for negative changes), or when negative changes were coded as no growth and natural log transformed data were used (Table 10). Relative increases in stem length were the same for control stems and low adult density stems, and greater than in the high adult density stems which became shorter over the measurement period in 2006 (Figure 11A, Table 10). In 2007, there were differences in net relative change in stem height between sites ($F = 8.22$; d.f. = 1,103; $P = 0.0005$) and adult density treatments (Figure 11B and C, Table 10). At both sites, control stems grew significantly more than any of the stems exposed to adult weevils. Stems in both of the high adult density treatments had severe tip damage that resulted in a net negative change in stem length over the course of the summer.

In 2006, no differences were observed between sites with regard to growth response to injury so the data set was combined across sites. Regression analysis showed net stem growth decreased with increasing numbers of oviposition scars ($y = -0.1881x + 8.5739$, $r^2 = 0.47$, $P < 0.0001$). A decrease in net stem growth was accompanied by an increase in the number of post egg stage insects ($y = -.03364x + 8.1032$, $r^2 = 0.38$, $P = 0.0005$).

In 2007, data from the two study sites were analyzed separately due to differences in stem growth between sites. Decreases in net change in stem length occurred as the number of oviposition scars (Figure 12A and C) and density of post-egg stage insects

(Figure 12B and D) increased, although these injury variables accounted for much less of the variability in change in stem length than in 2006.

Gas Exchange

When gas exchange measurements from each sample event were analyzed for 2006, site differences occurred on most sample dates. There were also trends in gas exchange parameters for the sample event occurring three weeks after the adults weevils were removed. In 2007, gas exchange rates differed between site every time measurements were taken. Furthermore, gas exchange rates differed within sites for several measured dates. Photosynthetic and conductance rates were higher across all dates and adult density treatments at Pond Flat, compared to those recorded at Slope Creek. At Slope Creek, the only gas exchange differences observed with respect to adult density treatments were in conductance and transpiration rates six weeks after the adults were removed (Table 11, Figure 13); however, the post hoc Tukey comparisons did not indicate adult density treatment differences for either parameter on that sample date. At Pond Flat, differences in photosynthetic, conductance, and transpiration rates occurred the day after adults were removed from plants, and six and eight weeks after adults were removed (Table 11, Figure 14).

Gas Exchange Regression Analyses

In 2006, there were no relationships between any of the three gas exchange parameters and injury level (number of oviposition scars, or number of post egg stage insects). Due to site differences in injury level in 2007, the relationships between injury

and gas exchange parameters were analyzed separately by site. At Pond Flat, there were negative relationships between photosynthetic and conductance rates and the number of oviposition scars for the day after the adults were removed from plants as well as five, seven, and nine weeks after adult removal (Table 12). Transpiration rates were negatively related to the number of oviposition scars the day after insects were removed and on the five and seven week measurement dates (Table 12). There were negative relationships between all three gas exchange parameters and the number of post egg stage insects at Pond Flat the day after the adults were removed as well as five, seven, and nine weeks after the adult weevils were removed (Table 12). At Slope Creek, there were negative relationships between conductance and transpiration rates the number of oviposition scars and number of post egg insects the seventh week after adult insects were removed from plants (Table 12). However, the adjusted R^2 values for significant models from Pond Flat ranged from 0.073 to 0.2381 for photosynthesis, 0.0732 to 0.2373 for conductance, and 0.088 to 0.2495 for transpiration. The four significant models from Slope Creek explained 12.2 to 15.3 percent of the variability in conductance and transpiration values.

Gas Exchange Over Time

Gas exchange rates changed over time in both 2006 and 2007 with the highest rates for all three parameters observed early in the season. In models testing for differences in gas exchange parameters with respect to site, adult density treatment, and Julian date for the 2006 data, there were differences in photosynthetic rate, conductance, and transpiration between sites and dates, but not adult density treatments (Table 13).

Models with injury values detected differences in photosynthetic rate with respect to site, Julian date, and number of oviposition scars as well as for a model of photosynthetic rate with respect to site, Julian date and number of post egg stage insects (Table 13). There were site and time differences in conductance and transpiration rates in models testing injury, but no injury effect was detected. There were significant or marginally significant site by date interactions in analyses of differences in gas exchange with respect to adult density treatment, number of oviposition scars, and number of post egg insects.

In 2007, there were significant site, adult density treatment, and time effects on all three gas exchange parameters (Table 14). Gas exchange rates decreased over time (Figure 8) for all gas exchange variables measured. When gas exchange rates were compared to injury in the form of number of oviposition scars or number of post egg stage insects, as well as site and Julian date, significant relationships between gas exchange and the three explanatory variables in each model were found (Table 14). There were significant site by time interactions in conductance and transpiration rates in models using categorical treatment, number of oviposition scars, and number of post egg stage insects.

Weevil Injury

Pond Flat had consistently higher injury levels for all adult density treatments than Slope Creek. At Pond Flat, significantly more oviposition scars were recorded in the high density mixed sex treatment (16 males and 16 females) than any of the other treatments. The high density male only treatment (32 males) had the second highest number of “oviposition scars”. The two low adult density treatments did not differ from

each other (Figure 10A, Table 15). Sexing was not 100% accurate nor were all adult weevils recovered when cages were removed, and some pupae and adults were dissected out of stems in the all-male treatments. When larval, pupal, and adult counts were added together to obtain a total insect load per stem, the high density mixed sex treatment had a significantly higher insect load than any of the other treatments (Figure 10B). The low density mixed sex treatment had the second highest mean number of post egg stage insects per stem but did not differ statistically in insect load from the high density male only treatment. Insect loads for both the high and low density male only treatments did not differ from the control stems. The results for Slope Creek followed similar trends to those observed at Pond Flat, although the adult density/sex treatments that differed statistically from each other were not the same for both sites (Figure 10A and B, Table 15). There were no post egg stage insects in the control or low density male only treatments, and the low number of insects found in the high density male only treatment did not differ significantly from zero. Control stems and stems with four adults caged on them appeared similar with little obvious injury to foliage. Stems in the treatments that received 32 adults, regardless of sex mix, all had very distinct leaf injury with severe feeding damage and dry wilted tips.

Discussion

Mecinus janthinus adult and adult plus larval feeding injury significantly affected *L. dalmatica* stem growth, gas exchange rates, and appearance. However, the results differed between growth and primary physiological responses, sites, and type of feeding

injury. In both years and at all three study sites, the high density adult treatments led to decreased growth. This effect was more pronounced for plants that experienced both adult and larval feeding injury than for plants that received only adult (e.g. all male) feeding injury. The visual damage observed in the study of reduced growth and dry, wilted shoot tips is consistent with decreased growth observed on *M. janthinus* release sites in Canada (Jeanneret and Schroeder 1992, De Clerck-Floate and Miller 2002). Dry wilted shoot tips in *L. dalmatica* populations with high *M. janthinus* populations have been attributed to both larval mining (Jeanneret and Schroeder 1992) and to adult feeding (De Clerck-Floate and Miller 2002). Jeanneret and Scroeder (1992) reported that the wilting was exacerbated in areas where plants were experiencing drought stress.

Other studies of foliar feeding insects have shown that many of these species prefer young foliage at shoot tips, which can lead to meristem damage and reduced growth (Louda et al. 1990). In a common garden experiment comparing the impact of simulated and actual leaf feeder injury on a perennial plant, Schat and Blossey (2005) found that feeding injury caused by the chrysomelid beetles *Galerucella calmariensis* L. and *G. pusilla* Duft. was concentrated around meristems and resulted in reduced primary stem growth, increased branching, and led to shorter, bushier plants. Plants with simulated herbivory that left meristems uninjured did not exhibit reduced growth or increased branching. In this study, growth of stems exposed to either of the high adult density treatments was significantly decreased. There was no corresponding statistical difference between the magnitude of decreased growth between stems exposed to adult plus larval feeding injury and adult only injury. The trends in growth reported from this

study on *L. dalmatica* are consistent with adult defoliation injuring meristems and reducing growth, rather than effects typical of internally feeding larvae.

The decreased gas exchange rates observed only in stems with high levels of both adult and larval feeding injury did not match changes in growth, but is consistent with other studies on the impact of insect feeding injury on photosynthesis. Results of defoliation by insects on plant primary physiology have been mixed. A number of studies have shown that gas exchange rates in uninjured leaves located near defoliated leaves had increased photosynthetic rates (Peterson and Higley 1993). Other studies have found that defoliation has, conversely, led to decreased photosynthesis (Meyer and Whitlow 1992, Peterson et al. 1998) or had no apparent effect on gas exchange (Peterson et al. 2004). Fewer studies have been conducted on concealed feeders, but stem borer-induced reductions in photosynthesis have been linked to interruptions of vascular transport (Peterson and Higley 1993). Although they did not determine a mechanism, Peterson et al. (2005) observed that *L. dalmatica* plants with *M. janthinus* oviposition scars and swollen stems had lower gas exchange rates than uninjured plants. Despite highly significant reductions in relative stem growth in both of the high adult density treatments, decreases in gas exchange parameters were only observed in the high density mixed sex treatments. These results suggest that the observed reduced photosynthetic rates are most likely linked primarily to larval feeding injury.

The effects of *M. janthinus* feeding on *L. dalmatica* were more pronounced at Pond Flat than at Slope Creek. The difference in results between the two sites and differences in results between the garden experiment (Chapter 2) and this experiment

underscore the important role that site characteristics play in the impact of insect herbivory on plants. Differences in environmental characteristics such as water availability and nutrient levels (Louda et al. 1990, Levine and Paige 2004) can influence the degree to which insect herbivores affect plant growth and physiology, as can the history of prior herbivory on perennial species (Brown and Weiss 1995, Harrison and Maron 1995, Root 1996b). The sites in the field experiment differed in several ways. The plants at Pond Flat were growing on a flat area and close to an irrigation ditch and the plants remained green later into the season than at Slope Creek. There were also fewer trees and animal dung was more abundant at the Pond Flat site than at Slope Creek. In a greenhouse study where wheat was grown under a range of soil moisture levels and with or without feeding by a stem boring sawfly, Macedo et al. (2007a) found that drought stressed plants subjected to insect feeding had lower photosynthetic rates at the grain filling stage than similarly injured plants that were not drought stressed. The differences we observed between our garden and field experiments are consistent with the results reported by Macedo et al. (2007a). Plants in the garden experiment were watered daily and the soil in the pots was moist throughout the growing season. Plants in the field experiment received no supplemental moisture. It is therefore possible that the observed differences could be attributed to the plants receiving water being better able to compensate for insect injury than water stressed plants.

These herbivore density treatments were successful in creating a range of injury levels and the differences between treatments were most distinct when comparing numbers of oviposition scars across treatments. Fewer insects were dissected out of

stems than there were corresponding oviposition scars in all adult density treatments. This suggests that insects do not oviposit in all holes chewed in the stems, and/or there is a high degree of mortality as the immature insects develop inside the stem. There is evidence supporting both factors in this study. In the male only treatments, so called "oviposition scars" were observed on stems that had no signs of mining, suggesting males as well as females feed on stems even when apparently undamaged and healthy leaves are available to be fed upon. Dead black larvae and larvae with ecto-parasitoids were also observed during stem dissections. There were also several empty mines with adult parasitoids in them, or characteristic exit holes produced by adult parasitoids. The adult parasitoids recovered from study stems were members of the family Pteromalidae, and have been submitted for expert identification at a finer taxonomic level. The similarity in the number of post egg stage insects produced by all adult density treatments despite differences in numbers of oviposition scars suggests that higher mortality was occurring in the higher density treatments, even though parasitoids were recovered from stems with low immature insect densities as well.

Jeanneret and Schoeder (1992) reported that 10% of *M. janthinus* larvae collected in the native range were parasitized, and De Clerck-Floate and Miller (2002) reported parasitism rates in established North American populations of less than 10%. De Clerk-Floate and Miller (2002) did not report the identity of parasitoids, and Jeanneret and Schoeder (1992) were unable to rear parasitoids to adults, but speculated that they were either eulophids or pteromalids. In our high adult density treatments there were 50% fewer post egg-stage insects than oviposition scars. If a 10% parasitism rate is universal,

that alone does not account for the discrepancy between the umber of oviposition scars and density of post egg stage insects.

The consistency of the growth response across the garden experiment and all three sites in the field experiment, as well as in studies conducted in Canada, and the similarity of symptoms of high levels of injury (brown wilted shoot tips) suggest that growth responses are a better predictor of the impacts of *M. janthinus* on *L. dalmatica*. However, the discrepancy between the number of oviposition scars and the density of post egg stage insects, and the confirmed occurrence of parasitism suggest that there is a threshold density of insects required before growth reduction and primary physiological impairment occur. The adult density treatment with the lowest injury level that resulted in a negative change in stem length had 45.6 (SE = 3.4) oviposition scars per stem and 4.73 (SE = 2.3) post egg stage insects per stem. The density of oviposition scars and post egg stage insect required for a decrease in gas exchange rates was higher at 146 (SE = 9.9) oviposition scars and 69.6 (SE = 7.2) post egg stage insects per stem. Those injury levels required 32 adults per stem. Therefore, given a high enough density of adult insects at a site to cause injury equivalent to 5 post egg stage immature insects per stem, *M. janthinus* has the potential to impact plant growth and physiology in the field.

Table 8 Experimental design and time line for the three study sites, 2006-2007.

Year	Site	Number of treatments	Replicates per treatment	Date treatments applied	Date adults removed	Date plants harvested
2006	Pond Flat	3	5	6/15/2006	6/22/2006	8/24/2006
	Eagle Creek	3	5	6/15/2006	6/22/2006	8/24/2006
2007	Pond Flat	5	11	6/8/2007	6/18/2007	8/17/2007
	Slope Creek	5	11	6/8/2007	6/18/2007	8/16/2007

Table 9 *Mecinus janthinus* adult density treatments. In 2006 only treatments A, B, and D were used; A-E were used in 2007

Treatment	Number of males	Number of females	Total number of adults
A	0	0	0
B	2	2	4
C	4	0	4
D	16	16	32
E	32	0	32

Table 10 Impact of *Mecinus janthinus* adult density treatments on net relative change in *Linaria dalmatica* stem length, 2006-2007. Analysis was performed on pooled data in 2006 as no between site differences were detected. In 2007 there were growth differences between sites so data were analyzed separately. Treatments were no adults, 2 pairs, 4 males, 16 pairs, or 32 males.

Source	Sum of squares	d.f.	F-ratio	P
2006				
Site	0.0067	1	0.47	0.4990
Treatment	0.4827	2	16.87	<0.0001
2007				
Pond flat				
Treatment	1.6702	4	15.19	<0.0001
Slope Creek				
Treatment	0.9898	4	11.37	<0.0001

Table 11 Impact of *Mecinus janthinus* adult density treatments on *Linaria dalmatica* gas exchange parameters, 2007. Data from the two sites were analyzed separately and only data from the dates where significant differences were observed are presented. Treatments were no adults, 2 pairs, 4 males, 16 pairs, or 32 males.

Model	Sum of squares	d.f.	F-ratio	P
Pond Flat				
6/19/2007				
Photosynthesis	1219.21	4, 50	5.52	0.0009
Conductance	0.037	4, 50	4.82	0.0023
Transpiration	82.24	4, 50	5.34	0.0012
7/31/2007				
Photosynthesis	517.99	4, 50	5.53	0.0009
Conductance	0.0051	4, 50	4.00	0.0068
Transpiration	20.87	4, 50	4.05	0.0064
8/17/2007				
Photosynthesis	748.98	4, 50	5.35	0.0012
Conductance	0.015	4, 50	3.35	0.0167
Transpiration	38.00	4, 50	4.03	0.0066
Slope Creek				
7/30/2007				
Photosynthesis	187.79	4, 50	1.03	0.3992
Conductance	0.0013	4, 50	2.95	0.0289
Transpiration	8.15	4, 50	3.55	0.0126

Table 12 Relationship between number of *Mecinus janthinus* oviposition scars and post egg stage insects, and *Linaria dalmatica* gas exchange parameters for Pond Flat and Slope Creek, 2007. Data from each date were analyzed separately. *There are no data for week 9 from Slope Creek due to high smoke levels from a nearby wildfire. There were no significant relationships in week 3 for either site so those data are not presented.

Site, parameters		**Week 1**				**Week 5**				**Week 7**			
	d.f.	**Slope**	**t**	**P**	**r^2**	**Slope**	**t**	**P**	**r^2**	**Slope**	**t**	**P**	**r^2**
Pond Flat													
Photosynthesis													
Oviposition scars	1	-0.0730	-3.39	**0.0003**	0.22	-0.0420	-2.34	**0.0232**	0.09	-0.0490	-4.09	**0.0001**	0.24
Post egg insects	1	-0.1260	-3.39	**0.0013**	0.17	-0.0820	-2.34	**0.0233**	0.09	-0.0980	-4.23	**<0.0001**	0.25
Conductance													
Oviposition scars	1	-0.0004	-4.22	**<0.0001**	0.25	-0.0001	-2.38	**0.0208**	0.10	-1E-04	-3.65	**0.0006**	0.20
Post egg insects	1	-0.0008	-3.85	**0.0003**	0.22	-0.0002	-2.39	**0.0204**	0.10	-3E-04	-3.65	**0.0006**	0.20
Transpiration													
Oviposition scars	1	-0.0200	-4.35	**<0.0001**	0.26	-0.0080	-2.51	**0.0150**	0.11	-0.0100	-3.55	**0.0008**	0.19
Post egg insects	1	-0.0370	-3.90	**0.0003**	0.22	-0.0160	-2.53	**0.0143**	0.11	-0.0200	-3.69	**0.0005**	0.20
Slope Creek													
Photosynthesis													
Oviposition scars	1	-0.0750	-1.98	0.0538	0.08	-0.0040	-0.14	0.8920	0.01	-0.0340	-1.76	0.0837	0.06
Post egg insects	1	-0.0710	-1.10	0.2785	0.02	0.0360	0.74	0.4644	0.01	-0.0370	-1.10	0.2761	0.02
Conductance													
Oviposition scars	1	-0.0003	-1.97	0.0544	0.08	-0.0001	-1.46	0.1498	0.04	-9E-05	-2.89	**0.0055**	0.14
Post egg insects	1	-0.0003	-1.31	0.1979	0.03	-0.00003	-0.90	0.3732	0.02	-1E-04	-2.94	**0.0048**	0.14
Transpiration													
Oviposition scars	1	-0.0200	-2.35	0.0229	0.11	-0.0040	-1.59	0.1178	0.05	-0.0070	-3.10	**0.0031**	0.16
Post egg insects	1	-0.0260	-1.74	0.0879	0.06	-0.0050	-1.13	0.2653	0.02	-0.0120	-3.25	**0.0020**	0.17

Table 12 Continued.

Site, parameters		Week 9			
	d.f.	Slope	t	P	r^2
Pond Flat					
Photosynthesis					
Oviposition scars	1	-0.0440	-2.82	**7E-04**	0.13
Post egg insects	1	-0.1000	-3.40	**0.0010**	0.18
Conductance					
Oviposition scars	1	0.0002	-2.29	**0.0260**	0.09
Post egg insects	1	-0.0004	-2.72	**0.0090**	0.12
Transpiration					
Oviposition scars	1	-0.0099	-2.49	**0.0160**	0.10
Post egg insects	1	-0.0220	-2.98	**0.0040**	0.14
Slope Creek					
Photosynthesis					
Oviposition scars	1	*	*	*	*
Post egg insects	1	*	*	*	*
Conductance					
Oviposition scars	1	*	*	*	*
Post egg insects	1	*	*	*	*
Transpiration					
Oviposition scars	1	*	*	*	*
Post egg insects	1	*	*	*	*

Table 13 Impact of *Mecinus janthinus* adult density treatments on *Linaria dalmatica* gas exchange parameters over time, 2006. Julian date was used to code for the dates measurements were taken. Models tested were each gas exchange parameter as a function of site, adult density and gender treatment (0, 2 pairs, 4 males, 16 pairs, or 32 males) or injury (number of oviposition scars or number of post egg insects), and Julian date with measure repeated on individual plants.

Parameter	d.f.	F-ratio	P
Photosynthesis			
Site	1,26	6.05	**0.0209**
Treatment	2,26	3.31	0.0525
Julian date	1,111	46.61	**<0.0001**
Conductance			
Site	1,26	12.76	**0.0014**
Treatment	2,26	1.00	0.3814
Julian date	1,111	13.11	**0.0004**
Transpiration			
Site	1,26	15.83	**0.0005**
Treatment	2,26	2.03	0.1511
Julian date	1,111	26.74	**<0.0001**
Injury			
Photosynthesis			
Site	1,25	7.15	**0.0130**
Oviposition scars	1,25	4.96	**0.0352**
Julian date	1,109	48.89	**<0.0001**
Conductance			
Site	1,25	12.85	**0.0014**
Oviposition scars	1,25	1.80	0.1923
Julian date	1,106	11.75	**0.0009**
Transpiration			
Site	1,25	15.30	**0.0006**
Oviposition scars	1,25	3.82	**0.0619**
Julian date	1,106	23.10	**<0.0001**
Photosynthesis			
Site	1,25	6.93	**0.0143**
Post egg insects	1,25	5.09	**0.0331**
Julian date	1,109	48.69	**<0.0001**
Conductance			
Site	1,25	12.42	**0.0017**
Post egg insects	1,25	2.14	0.1561
Julian date	1,106	11.85	**0.0008**
Transpiration			
Site	1,25	14.64	**0.0008**
Post egg insects	1,25	4.92	**0.0359**
Julian date	1,106	23.44	**<0.0001**

Table 14 Impact of *Mecinus janthinus* adult density treatments on *Linaria dalmatica* gas exchange parameters over time, 2007. Julian date was used to code for the dates measurements were taken. Models tested were each gas exchange parameter as a function of site, adult density and gender treatment (0, 2 pairs, 4 males, 16 pairs, or 32 males) or injury (number of oviposition scars or number of post egg insects), and Julian date with measure repeated on individual plants.

Parameter	d.f.	F-ratio	P
Photosynthesis			
Site	1,104	108.21	**<0.0001**
Treatment	4,104	10.28	**<0.0001**
Julian date	1,378	279.38	**<0.0001**
Conductance			
Site	1,104	69.73	**<0.0001**
Treatment	4,104	8.23	**<0.0001**
Julian date	1,378	299.95	**<0.0001**
Transpiration			
Site	1,104	107.30	**<0.0001**
Treatment	4,104	7.13	**<0.0001**
Julian date	1,378	209.26	**<0.0001**
Injury			
Photosynthesis			
Site	1,106	111.44	**<0.0001**
Oviposition scars	1,106	31.36	**<0.0001**
Julian date	1,376	277.45	**<0.0001**
Conductance			
Site	1,106	11.65	**<0.0001**
Oviposition scars	1,106	24.51	**<0.0001**
Julian date	1,376	209.31	**<0.0001**
Transpiration			
Site	1,106	72.97	**<0.0001**
Oviposition scars	1,106	26.90	**<0.0001**
Julian date	1,376	299.84	**<0.0001**
Photosynthesis			
Site	1,106	103.07	**<0.0001**
Post egg insects	1,106	21.57	**<0.0001**
Julian date	1,376	272.37	**<0.0001**
Conductance			
Site	1,106	105.96	**<0.0001**
Post egg insects	1,106	22.33	**<0.0001**
Julian date	1,376	208.58	**<0.0001**
Transpiration			
Site	1,106	68.29	**<0.0001**
Post egg insects	1,106	25.57	**<0.0001**
Julian date	1,376	299.28	**<0.0001**

Table 15 Impact of adult *Mecinus janthinus* caged on *Linaria dalmatica* plants on number of oviposition scars and density of immature insects, 2007. Data from each site were analyzed separately.

Model	sum of squares	d.f.	F-ratio	P
Pond Flat				
Oviposition scars	154531.93	4	151.47	<0.0001
Larvae	73.56	4	2.04	0.1033
Pupae	1054.11	4	55.07	<0.0001
Adults	8304.11	4	17.95	<0.0001
Post egg stage	37764.98	4	69.83	<0.0001
Slope Creek				
Oviposition scars	109113.73	4	201.14	<0.0001
Larvae	1175.96	4	31.91	<0.0001
Pupae	2205.48	4	18.41	<0.0001
Adults	1306.05	4	4.72	<0.0001
Post egg stage	35970.59	4	113.1	<0.0001

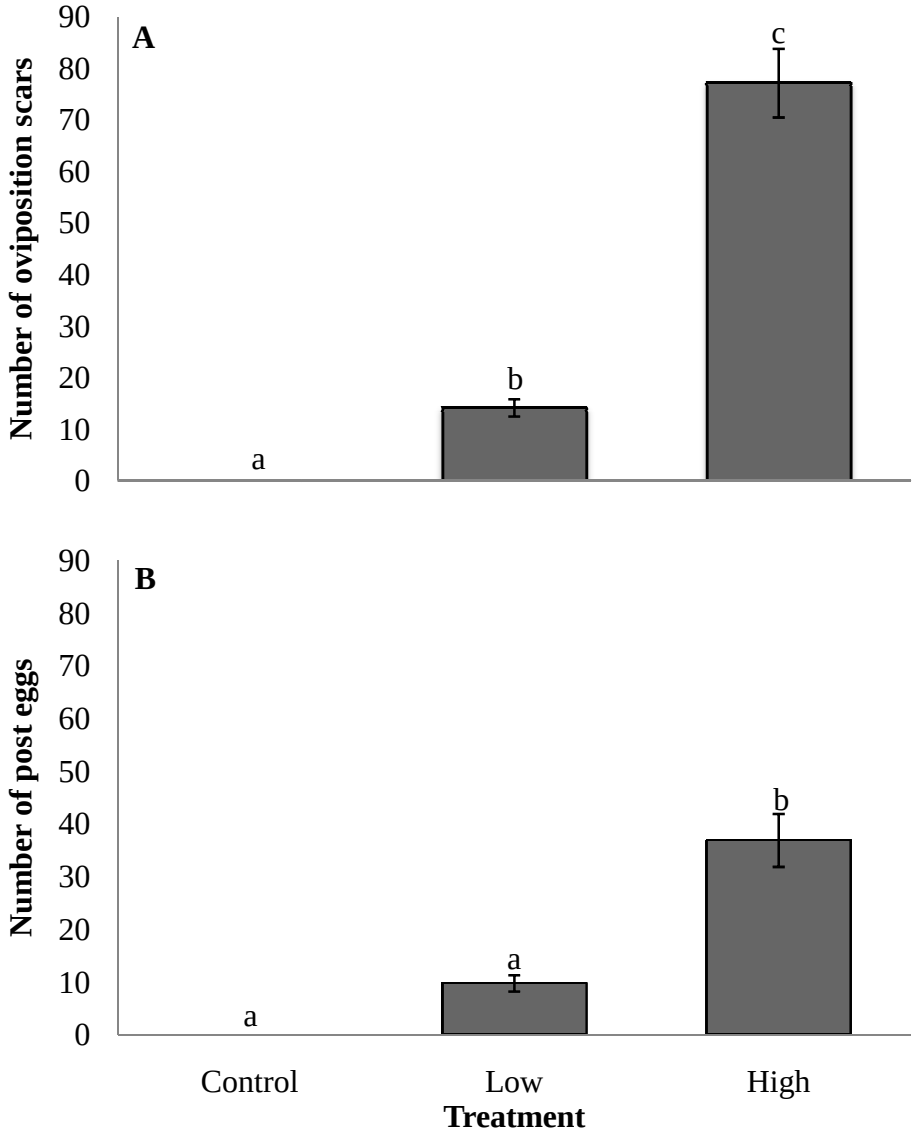

Figure 9 The number of *Mecinus janthinus* oviposition scars (A) and post-egg stage insects (B) dissected from *Linaria dalmatica* stems, 2006. Letters above bars indicate results of post hoc Tukey comparisons of treatments. Treatments were no insects (control), 2 pairs of adult insects (low), and 16 pairs of adult insects (high) caged on plants at the beginning of the experiment.

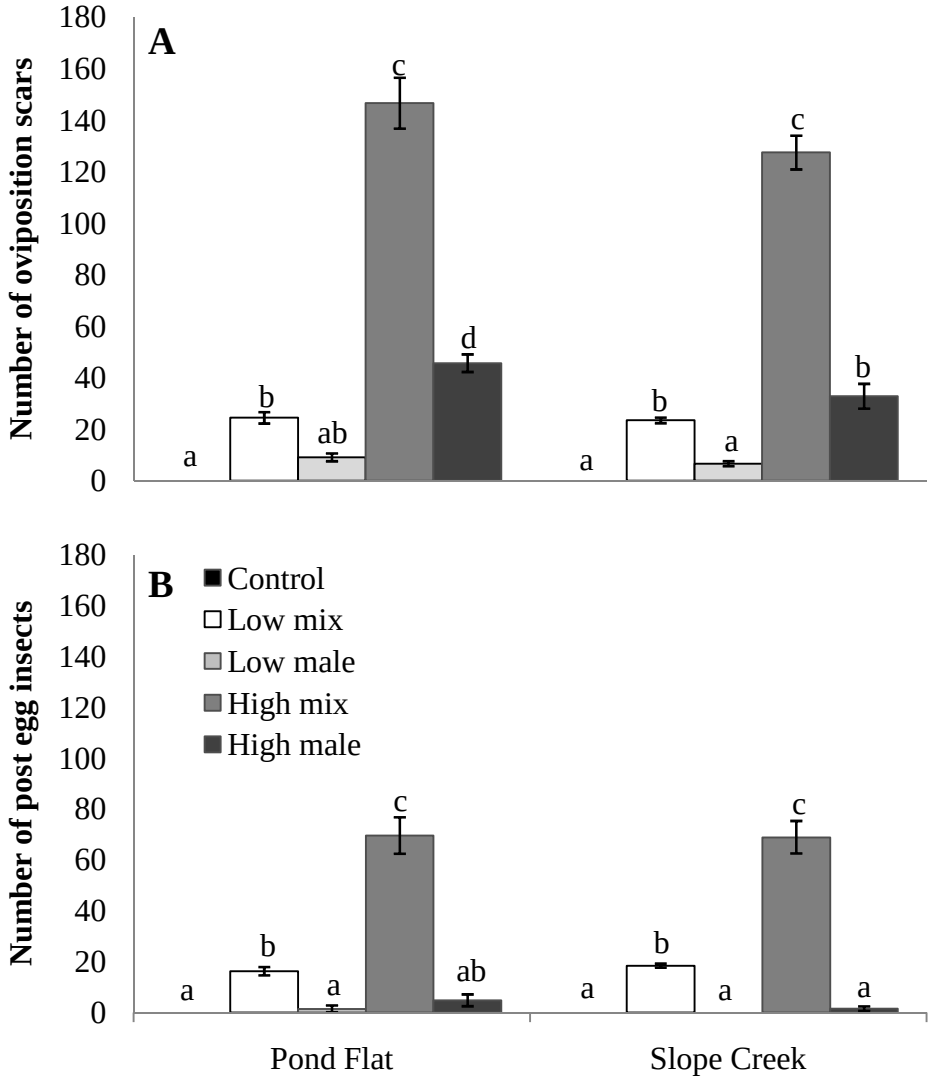

Figure 10 The number of *Mecinus janthinus* oviposition scars (A) and post-egg stage insects (B) dissected from *Linaria dalmatica* stems at Pond Flat and Slope Creek, 2007. Letters above bars indicate results of post hoc Tukey comparisons of treatments within sites. Treatments were no insects (control), 2 pairs (low mix), 4 males (low male), 16 pairs (high mix), and 32 males (high male) caged on stems at the beginning of the experiment.

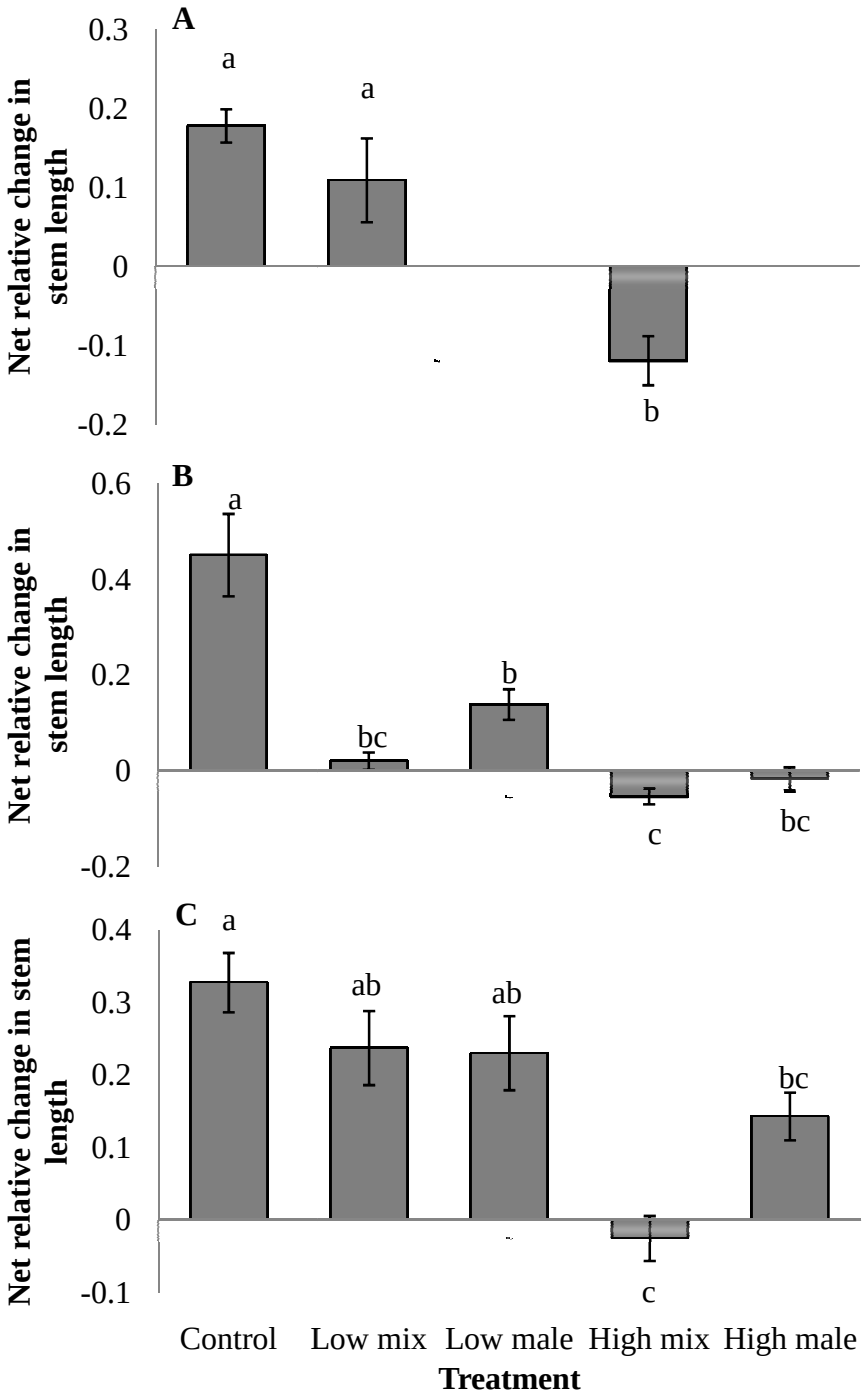

Figure 11 Impact of *Mecinus janthinus* adult density on net relative change in *Linaria dalmatica* stem length over the course of the growing season, 2006-2007. (A) n=both sites combined in 2006 and in 2007 at Pond Flat (B) and Slope Creek (C). Letters indicate results of post hoc Tukey comparisons between treatments. Treatments were no insects (control), 2 pairs (low mix), 4 males (low male), 16 pairs (high mix), and 32 males (high male) caged on stems at the beginning of the experiment.

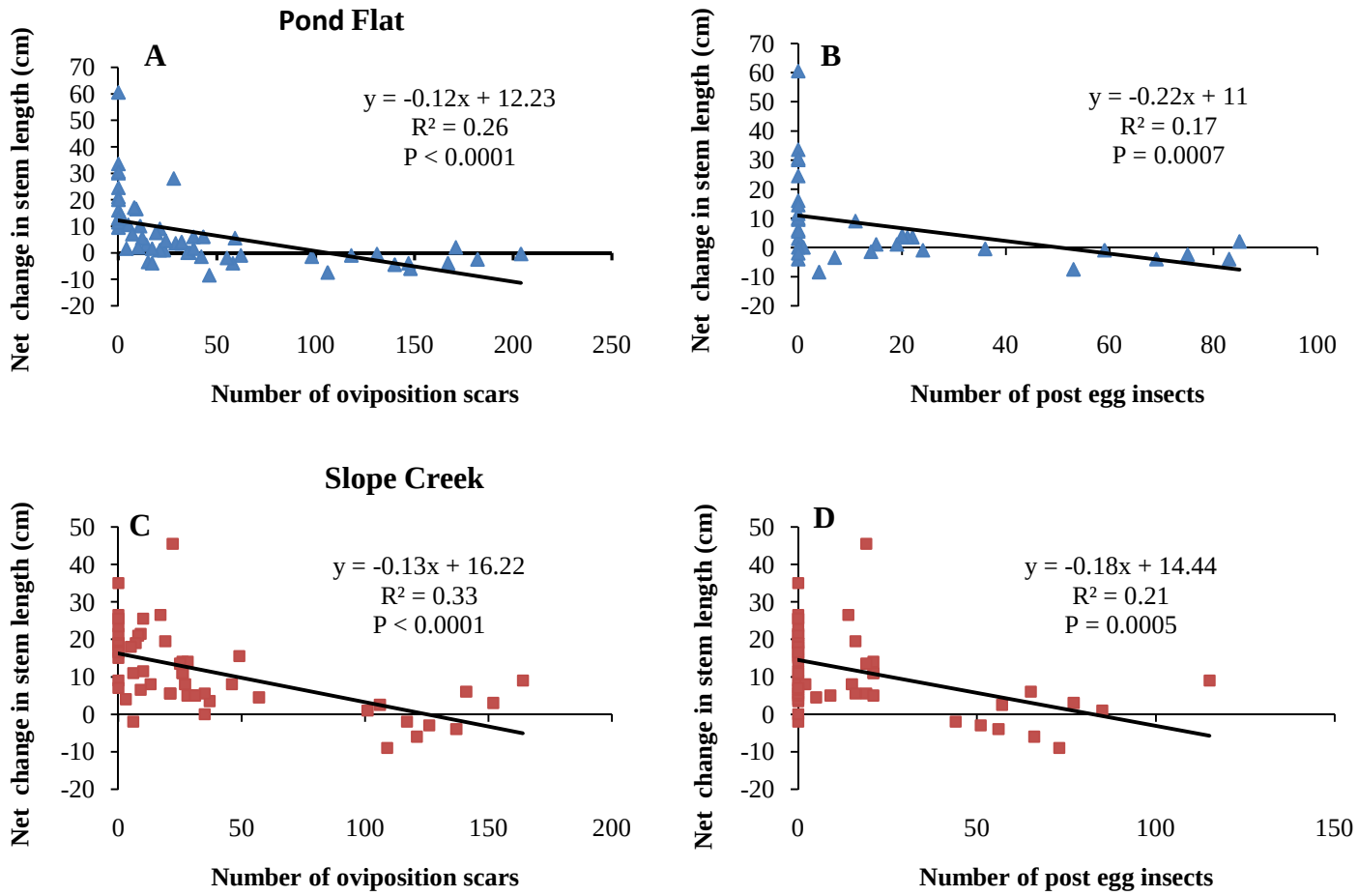

Figure 12 Relationship between *Mecinus janthinus* oviposition scars and post-egg stage insects in *Linaria dalmatica* stems at Pond Flat (A and B) and Slope Creek (C and D) and the net relative change in stem length, 2007.

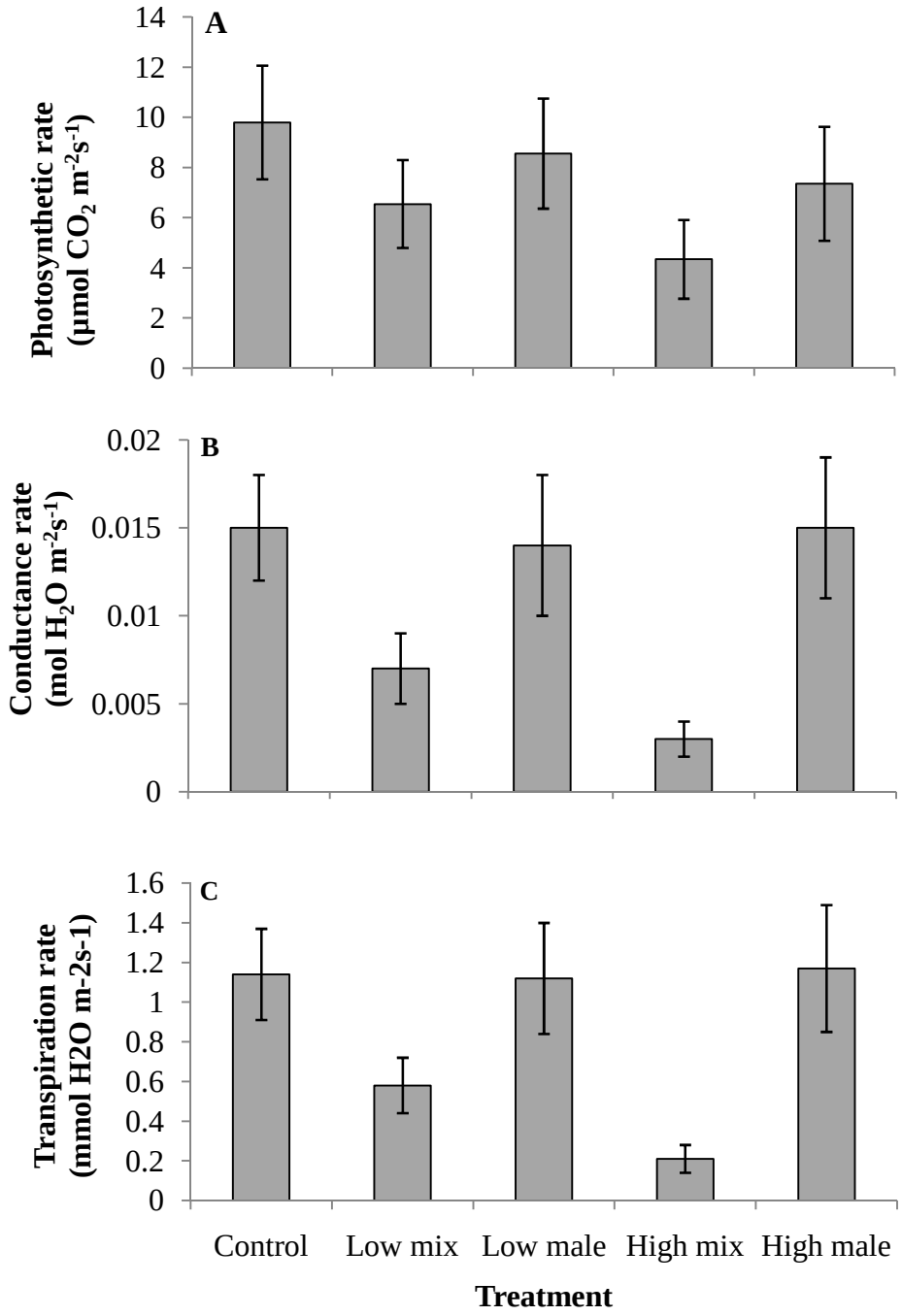

Figure 13 Impact of *Mecinus janthinus* adult density on *Linaria dalmatica* gas exchange rates by treatment for Slope Creek on July 30, 2007. Tukey tests did not return any significant differences between adult density treatments. Treatments were no insects (control), 2 pairs (low mix), 4 males (low male), 16 pairs (high mix), and 32 males (high male) caged on stems at the beginning of the experiment.

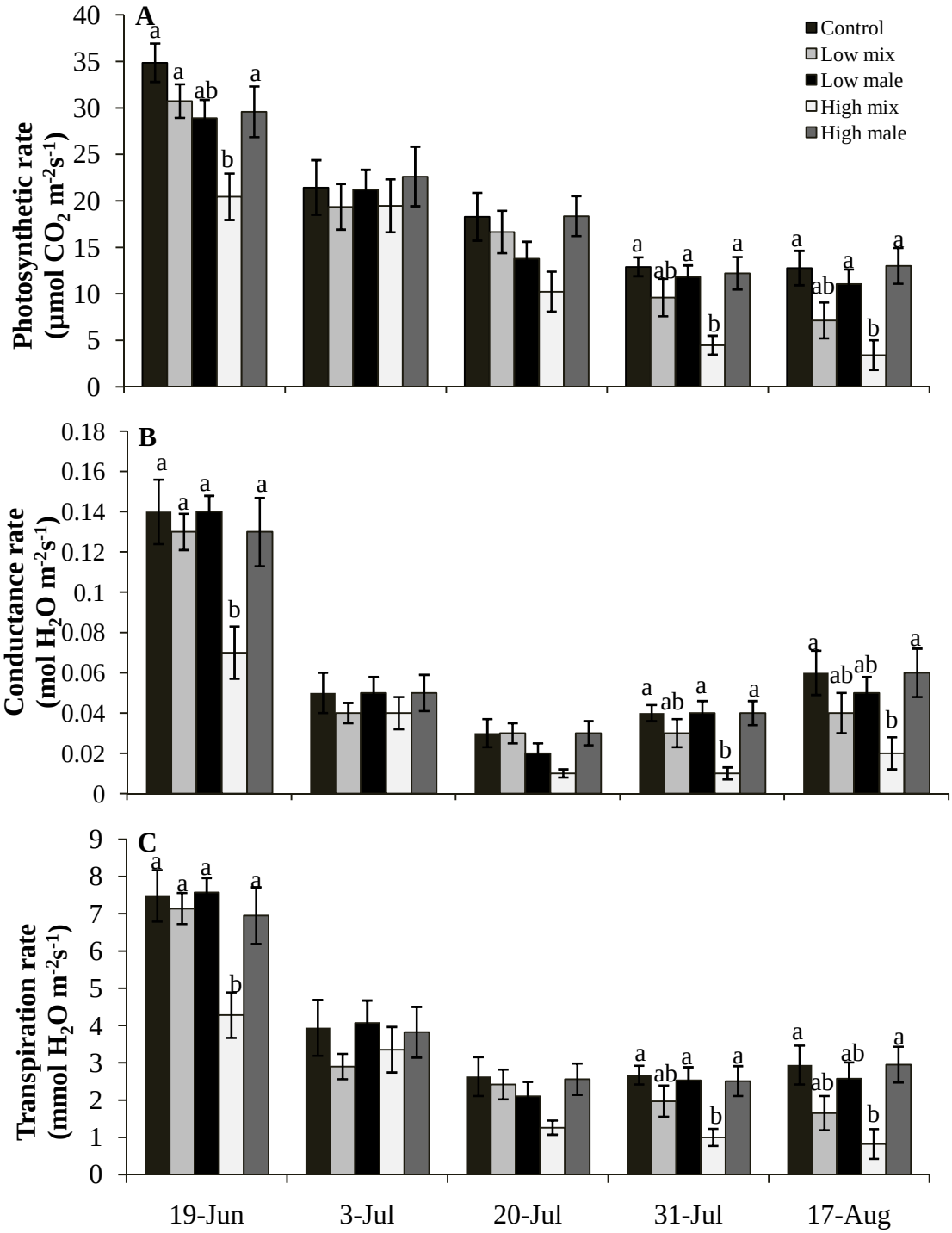

Figure 14 Impact of *Mecinus janthinus* adult density on *Linaria dalmatica g*as exchange rates by treatment for the Pond Flat site over time in 2007. Letters indicate significant differences between treatments within dates. Treatments were no insects (control), 2 pairs (low mix), 4 males (low male), 16 pairs (high mix), and 32 males (high male) caged on stems at the beginning of the experiment.

CHAPTER 4

MONITORING POST-RELEASE ESTABLISHMENT AND IMPACTS OF *MECINUS JANTHINUS* ON *LINARIA DALMATICA* IN MONTANA

Abstract

Biological control is a multi-stage process and for a program to be considered successful, the biocontrol agents, once identified, tested, and released need to become established at sufficient densities to significantly affect target pest populations. It is also important that any successful decrease in the target pest leads to shifts towards desirable species in the community. The goals of this study were to 1) determine if *Mecinus janthinus* weevils were able to establish in Montana and could increase their populations enough to impact *Linaria dalmatica* populations, and 2) characterize changes in *L. dalmatica* populations. Four watershed scale sites (Skytop, Coxcy Gulch, Melstone, and Phelps Creek) were identified and multiple releases were made within each watershed. Paired permanent monitoring transects were established and monitored on a yearly basis for three to four years. Data collected included *L. dalmatica* density and cover. Adult *M. janthinus* feeding injury was classified and stems were dissected for evidence of development and successful emergence of adult weevil progeny. The majority of the *M. janthinus* releases led to establishment, but the rate of population increase as estimated from stem dissections was lower than expected from previous reports. *Mecinus janthinus* overwintering mortality could be a major factor influencing the observed slow population growth. *Linaria dalmatica* populations were variable over time based on cover measurements, but not in terms of stem density measurements. There were no

differences between release and control transect pairs within years with respect to *L. dalmatica* cover. Although total stem counts did not change over time, the proportion of mature stems decreased, and decreased more in release transect pairs at the Skytop site than in control transect pairs. These results suggest that the shift in proportion of mature stems at the Skytop site could have been in part due to the presence of *M. janthinus*. Based on these results we have confirmed that the weevils were able to become established at all sites, but did not significantly affect *L. dalmatica* density or cover.

Introduction

Weed biological control has often been described as an effective, safe, and cost-effective control measure (Andres and Goeden 1971). However, only 30 to 60% of biological control releases have established (Julien 1989, Williamson 1996, McFadyen 1998) and of those only about 50% have led to successful control (Julien 1989, McFadyen 1998, McEvoy and Coombs 2000). However, the number of programs leading to control may be under-reported due to the general lack of long term monitoring (Blossey 1999) and the large time lags that can occur between agent release and visible impacts on the weed population (McEvoy et al. 1991). Furthermore, concerns have been raised about the safety of releasing apparently ineffective control agents (Pearson et al. 2000, Pearson and Callaway 2003). Long term monitoring programs at a multiple release sites with diverse biotic and abiotic characteristics can improve our understanding of those that might increase successful control.

Linaria dalmatica (L.) Miller, a short lived perennial plant introduced to North America from eastern Europe in 1894, has been the target of biological control programs

since the 1980s (Jeanneret and Schroeder 1992). Despite an ongoing long term control program including the release and redistribution of five classical biological control insects (Jeanneret and Schroeder 1992), *L. dalmatica* is still considered a significant management problem (De Clerck-Floate and Harris 2002). The economic impact of *L. dalmatica* has not been widely reported, although Lajeunesse (1999) documented that for one ranch in Montana where approximately 30% of the 431 hectare ranch was severely infested (25 – 100% of the vegetative cover), direct management costs in 1992 totaled $99 per hectare. The ranch's managers also reported reductions in both carrying capacity and appraised value of the land as additional costs accrued as a result of the *L. dalmatica* infestation.

According to the Montana State Noxious Weed List (http://agr.mt.gov/weedpest/pdf/weedlist3-08.pdf; accessed Oct 2008) and the USDA-NRCS Plants Database (http://plants.usda.gov/java/profile?symbol=LIDA accessed Oct 2008), *L. dalmatica* is a Category 1 weed, indicating that it is currently widely established in the state (reported in 43/54 counties). Management criteria for Category 1 weeds in Montana include awareness and education campaigns, treatment of current populations, and efforts to limit the spread and establishment of new populations. Fire prone areas are an even greater concern for land managers because fire does not decrease *L. dalmatica* in rangelands and may increase dominance of the weed (Phillips and Crisp 2000, Jacobs and Sheley 2003a). Jacobs and Sheley (2003a) found that toadflax biomass and cover increased two-fold within one season in experimental plots where prescribed burned treatments were applied.

Currently, the most effective biocontrol agent against *L. dalmatica* is the stem boring weevil, *Mecinus janthinus* Germar, which was first released in North America in 1991 (De Clerck-Floate and Harris 2002). *Mecinus janthinus* has established and proliferated on many sites throughout western Canada and the United States (De Clerck-Floate and Miller 2002). Harris et al. (2002) report that *M. janthinus* attacked up to 100% of available *L. dalmatica* stems within three years on initial release sites in Canada.

To maximize control of *L. dalmatica* and minimize management costs, it is important to understand factors that influence the establishment and impact of biological control agents. To evaluate establishment and impact of *M. janthinus* on *L. dalmatica*, a watershed-scale monitoring study was initiated at four sites in western and central Montana. Data collected were used to evaluate the control of *L. dalmatica* by *M. janthinus*.

Objectives and Hypotheses

The first objective of this study was to determine if *M. janthinus* could establish and exhibit detectable population increase under typical Montana field conditions. *Mecinus janthinus* populations were expected to become established at all studied sites and experience population increases over the course of our three year study period. The second objective of this study was to determine if *L. dalmatica* populations decreased over time and if changes could be attributed to *M. janthinus*. I expected to see measurable decreases in *L. dalmatica* populations on our study sites due to *M. janthinus* herbivory within three years of initial releases.

Study Sites

Four study sites (Skytop, Coxcy Gulch, Melstone, and Phelps Creek) were selected to reflect the range of conditions under which *L. dalmatica* grows in Montana. Sites received no chemical control applications during the course of the study. The selected sites had a variety of burn histories, and included public and private lands, relatively undisturbed to heavily disturbed areas, as well as a range in elevations (Table 16). Three of the four sites were established in 2003, and the fourth in 2004. *Mecinus janthinus* releases were made throughout each watershed (see Appendix A for full details of releases and transect pairs).

The Skytop site was located 7.5 km north of Boulder, MT on a private ranch bordered by Bureau of Land Management (BLM) land. Releases were made in early August 2003 and transect pairs were established, vegetation attributes recorded, and all dead toadflax stems from previous years were removed between 19 – 22 August 2003. Subsequent vegetation sampling and stem collection occurred 19 – 21 July 2004, 28 – 29 June 2005, and 19 – 21 June 2006.

The Coxcy Gulch site was located in the Helena National Forest, Townsend Ranger District 31.5 km southeast of Helena, MT. Wildfires swept through the area in 1996 and 2000. Insect releases were made on 30 May 2003 and transect pairs were set up and vegetation attributes were recorded between 6 – 12 August 2003. Vegetation and control agent attributes were measured again between 28 – 29 July 2004, 28 – 29 June 2005, and 19 – 21 June 2006.

The Melstone site was located 8.6 km southeast of Musselshell, MT and 17.2 km southwest of Melstone, MT, on private property surrounded by BLM land. Insect releases were made in 2003 and seven of the transect pairs were established and vegetation attributes were obtained between 7 – 8 August 2003. The eighth transect pair was set up and vegetation attributes recorded on 18 August 2003. Vegetation and control agent attributes were recorded again on 5 – 6 August in 2004, 30 June (transect pairs 1 – 7) and 1 August (transect pair 8) in 2005, and 3 – 4 July 2006. Other releases of *M. janthinus* had been made on this ranch before the initiation of this study.

Phelps Creek, an unburned site located 2.9 km north of Gardiner, MT was established on the Gallatin National Forest, Gardiner Ranger District in 2004. Phelps Creek was the study site least disturbed by human intervention. Releases were made on 28 May 2004, and the transect pairs were established and vegetation attributes were recorded on 8 – 15 June 2004. Vegetation attributes and weevil parameters were measured again on 12 – 13 July 2005, and 10 – 12 July 2006.

Methods

Mecinus janthinus Releases

Within each watershed, release localities were selected in areas not heavily or likely to be disturbed by ATVs or cattle, and not prone to flooding. Each release point had a unique slope, aspect, or apparent community composition. Geo-referencing coordinates, a written site description, and photo reference points were taken at each release point. Releases were also marked with orange plastic stakes to aid in re-locating releases.

The weevils released were collected in Washington state, and shipped to Montana via overnight delivery. When the weevil shipments arrived, weevils were kept cool and released as soon as possible after being received. To avoid immediate dispersal flights, weevils were released early in the morning or late in the day. At the release sites, weevils were sprinkled at the base of toadflax plants over a small area around the release stake.

Transect Pair Establishment

The same protocol was used at all field sites to determine where to make releases and for setting up and reading transect pairs. Two types of monitoring transect pairs were established: release transect pairs and control transect pairs. Once release points were identified, one or more release transect pairs were set up within 10 m of each release. Additional control transect pairs remote from any release (more than 100 m) were set up at three of the four sites to monitor short term *L. dalmatica* population changes in the absence of *M. janthinus*. Transect pairs were set up and monitored at the time releases were made or shortly thereafter.

Release transect pairs were established in one of two ways: (1) with the release point between the two transects approximately half way up the length of the transect pair, or (2) with the release point several meters down-slope of the transect pair. All transects were 20 m in length and were oriented up the fall line of the hill. Most transect pairs were set up with the transects parallel to each other with 5 m between transects. A few pairs had non parallel transects or transects more than 5 m apart. The beginning and end of each transect (e.g. 0 m and 20 m points) and each meter point along each transect was

permanently marked. UTM coordinates were recorded at the 0 m and 20 m marker for each transect.

Data Collection

Twenty 0.5 m x 0.20 m modified Daubenmire cover quadrats (Daubenmire 1968b) were sampled per transect, one at each meter marker. In 2003 and 2004 crews measured 25 variables including *L. dalmatica* cover and density measures, and *M. janthinus* adult feeding injury classes (Table 17). All dead toadflax stems in each quadrat were collected yearly. Stems were brought back to the lab and six *M. janthinus* population variables were measured: (1) number of empty pupation chambers with emergence holes (successful emergence), (2) number of empty pupation chambers without emergence holes (predation of pre-emerged adults or pupae), (3) number of dead adults in chambers with emergence holes (unsuccessful adult emergence), (4) number of dead adults in pupation chambers without exit holes (overwintering mortality), (5) number of dead pupae (developmental mortality), and (6) number of dead larvae (developmental mortality). Exit holes are easily distinguished and remain obvious throughout the season of emergence, allowing estimates of weevil emergence in each plot per year.

Field sites were visited between mid-June and mid-August annually. Each year, the exact timing of site visits depended on growing conditions with the goal of sampling sites when *L. dalmatica* had reached its maximum height. Due to the environmental conditions and elevation at each site, Coxcy Gulch was sampled first, then Melstone, followed by Skytop, and Phelps Creek last.

Data Analyses

Data analyses were divided into several parts. Each of the objectives was addressed in turn, analyzing the *M. janthinus* data, then the *L. dalmatica* data. All data analyses were conducted using SAS 9.0 (SAS Institute Inc., NC, USA).

Mecinus janthinus Population Data

To determine if *M. janthinus* established, evidence of their presence (adult feeding injury, visual sightings of adults in the field, and evidence of oviposition the previous year from stem dissections) was evaluated on a transect pair scale. *Mecinus janthinus* were considered established if evidence of the insect was observed three years after release (Coombs Personal communication). To determine if the populations of *M. janthinus* were increasing through time, the change in amount and severity of adult feeding injury was scored (Table 17), the number of exit holes indicating successful emergence were counted, and the insect load per dead stem was calculated. Mortality was also evaluated using number of dead immature insects from stem dissections (unsuccessful development) and dead adults (unsuccessful emergence).

The proportion of quadrats per transect pair containing *L. dalmatica* where adult feeding injury was recorded was compared over time using a Proc-Mixed procedure with a repeated statement, with transect pairs as the sample units being measured over time. Each site was analyzed separately because we wanted to ensure that inherent differences between watersheds did not obscure differences between transect pairs within watersheds. The relationships between the proportion of quadrats with feeding damage per transect pair and site, year, type of transect pair (control or release), and interactions

were examined using Proc-Mixed. Regression analyses were conducted separately for each study site and year to determine if there was a relationship between the prevalence of adult feeding injury and average *L. dalmatica* density when *L. dalmatica* was present. Average injury scores on a transect pair basis were also compared over time using a Proc-Mixed procedure to determine if adult feeding injury increased in severity over time.

All dissection data were pooled across the 40 quadrats per transect pair due to the low number of quadrats per transect pair where dead stems were recovered, and the generally low level of insect load per stem. Total insect load (successful emergence, dead adults, dead pupae, and dead larvae) per transect pair was compared over time using Proc-Mixed with a repeated statement on transect pair, to compare potential weevil population over time. The routine was run on each study site with total insect load as the dependent variable and year and type of transect pair (control or release) as explanatory variables.

Similar Proc-Mixed-repeated routines were used to compare proportion of successfully emerged adults with the number of insects that successfully completed development to the adult stage over time, and between the first year after release and last year sampled. The number of dead insects dissected out of stems from each transect were examined to give an indication if the number of eggs laid per year had increased. The proportion of the total insect load that fell into each category (successful emergence, overwintering mortality, developmental mortality) was examined to obtain a clearer picture of the weevil population dynamics at each site. Proportions of dead immature insects, dead adults, and empty pupation chambers with exit holes were examined within sites over time. The probability of under-representing the weevil population was

evaluated by comparing the number of growing stems counted in one year that were then collected as dead stems the following year using a Proc-Mixed routine.

Linaria dalmatica Population Data

To accommodate the typically patchy nature of *L. dalmatica* growth habits (Robocker 1970, 1974, Vujnovic and Wein 1997), *L. dalmatica* density data were pooled across the 40 quadrats for each transect pair and used as the sample unit. Cover data were averaged across all 40 quadrats and across only those quadrats that contained *L. dalmatica*. Proc-Mixed routines with repeated statements were used to compare *L. dalmatica* density, frequency, and cover data across sites, years, and transect pair type (release or control) for each site.

Results

Establishment and Increase of *Mecinus janthinus*

Because of the need to monitor study sites at peak *L. dalmatica* growth, monitoring usually occurred after peak adult *M. janthinus* activity, and it was not possible to assess the population of weevils near releases by counting apparent adults. Using adult feeding damage and stem dissection data as proxy measures of *M. janthinus* presence, evidence of weevils were recorded each of the three years after releases were made at Skytop, Coxcy Gulch, and Melstone, and for two years after releases were made at Phelps Creek. Establishment could not be confirmed at all releases within the study period using these criteria (Table 18).

No change was recorded in the proportion of quadrats per transect pair containing *L. dalmatica* with adult feeding injury (d.f. = 1,38; P = 0.0596), and there was no difference in the prevalence of injury between control and monitoring transect pairs (d.f. = 1,11; P = 0.1462) (Figure 15) at Skytop. At Coxcy Gulch the prevalence of adult feeding injury increased over time (d.f. = 1,29; P = 0.0003) and the marked difference in adult feeding injury between release (present) and control (absent) transect pairs remained constant over the duration of the study (d.f. = 1,8; P = 0.0091) (Figure 16). Melstone, a site with only release transect pairs, was the only site where the frequency of quadrats exhibiting adult feeding injury increased significantly over time (d.f. = 1,23; P < 0.0001) (Figure 17). At Phelps Creek the proportion of quadrats with adult feeding injury increased between 2004 and 2006 (d.f. = 1,33; P = 0.0003), but no differences in feeding injury were detected between control and monitoring transect pairs (d.f. = 1,15; P = 0.2157) (Figure 18).

At Coxcy Gulch there was a trend toward an increase in the proportion of quadrats with adult feeding injury with increasing *L. dalmatica* density ($y = 0.0044x – 0.1078$; $R^2 = 0.521$; P = 0.067) in 2003, the year the insects were released. At Melstone there were positive relationships between the prevalence of adult feeding injury and *L. dalmatica* density 2003 ($y = 0.0039x – 0.1446$; $R^2 = 0.541$; P = 0.03747) and 2006 ($y = 0.0132x + 0.0357$; $R^2 = 0.745$, P = 0.005752).

Adult feeding injury class (Table 17) did not differ over time or between transect types. With the exception of seven quadrats in 2006, all feeding injury recorded fell into the ‘hard to detect’ category where fewer than 5% of the stems had any detectable feeding injury. The seven quadrats that were categorized as “minimal damage” in 2006

had feeding injury on 5.5 to 20% of stems. There were no quadrats with "obvious damage" (more than 20% of the stems with feeding injury).

The proportion of dead stems collected to live stems recorded in the same quadrats the previous year was variable and differed by site (d.f. = 3,43; P = 0.0174) but not with year (d.f. = 1,78; P = 0.3464), or between release and control transect pairs (d.f. = 1,43; P = 0.207). When stem data were pooled across the 40 quadrats per transect pair, 1 – 55% of the stems counted in 2003 were recovered in 2004 across all sites. In 2005, 5 -83% of the stems growing in 2004 were recovered. In 2006, 0 – 71% of the growing stems counted in 2005 were recovered.

Despite our relatively low stem recovery rate, evidence of weevil oviposition was detected at all sites. Dissections of stems collected the year the releases were made indicated *M. janthinus* were present before the releases were made for this study at Coxcy Gulch transect pair 7, Skytop transect pairs 11 and 13, and Melstone transect pairs 4 – 8. There was no evidence of weevil establishment at Phelps Creek before the initiation of this study.

The total insect load recorded from stem dissections increased over time at Coxcy Gulch (d.f. = 1,29; P=0.0217) and there was a trend towards increasing insect load at Melstone (d.f. = 1,23; P=0.0749), but there were no relationships between insect load and year at Skytop or Phelps Creek. Total insect load per stem was low across all sites. Three years after the release made for this study, at the transect pair with the highest pooled total insect load (Coxcy Gulch transect pair 7), there were 159 insects and empty chambers. However, only 75% of the dead stems collected in that transect pair showed signs of *M. janthinus* infestation giving an average of 0.7 insects per stem. This transect

pair also had signs of insects present before the initiation of this study and previous releases near this site were made five years before the last sampling date.

The proportion of insect load that resulted in successful emergence decreased over time at Melstone (d.f. = 1,14; P = 0.0031) and Coxcy Gulch (d.f. = 1,13; P = 0.0005). There was a marginally significant difference across years at Skytop (d.f. = 1,7; P = 0.0697), and no differences at Phelps Creek. No differences in overwintering mortality were found between control and release transect pairs at any of the three sites with both transect pair types. Increases in overwintering mortality over time were recorded from Coxcy Gulch (d.f. = 1,13; P = 0.0104), and Melstone (d.f. = 1,14; P = 0.0112). Overwintering mortality decreased over time at Skytop (d.f. = 1,7; P = 0.0486). The proportion of dead immature insects dissected out of stems (dead pupae and dead larvae) differed across years at Skytop (d.f. = 1,7; P = 0.0036) while there was a marginally significant year effect at Coxcy Gulch (d.f. = 1,13; 0.0584) and no difference at Phelps Creek. Overall, the majority of the insects dissected out of stems were dead adults. The mean (±SE) proportion of dead adults (overwintering mortality) across all years was 0.45 ± 0.05 at Coxcy Gulch, 0.59 ± 0.06 at Melstone, 0.46 ± 0.09 at Skytop, and 0.55 ± 0.16 at Phelps Creek.

Changes in *Linaria dalmatica* Populations

Changes in *L. dalmatica* populations were evaluated both by basal cover and density of stems. There were differences in the number of quadrats per transect pair that contained *L. dalmatica* between sites (F = 0.22; d.f. = 3, 43; P < 0.0001) but not year (F = 0.38; D.F. = 3,124; P = 0.7657) or type of transect (F = 0.22; d.f = 1,43; P = 0.6408). On

average (± SE) over all years, 51% ± 4.7 of quadrats contained *L. dalmatica* at Skytop, 67% ± 6 at Coxcy Gulch, and 71% ± 5 at Melstone. Phelps Creek had the lowest frequency of *L. dalmatica* with just 21% ± 4 of quadrats containing *L. dalmatica* (Figure 19). Due to the relatively low frequency of *L. dalmatica* occurrence in sample quadrats, average cover was evaluated both in terms of average for all 40 quadrats per transect pair and average cover for only those quadrats where *L. dalmatica* was present.

Although frequency of *L. dalmatica* did not change over time, cover decreased over time ($F = 119.32$; d.f. = 3,124; $P < 0.0001$) and differed between sites ($F = 18.24$; d.f. = 3,43; $P < 0.0001$) but did not differ between release and control transect pairs ($F = 0.24$; d.f. = 1,43; $P = 0.6302$) (Figure 20). Over all years studied, Coxcy Gulch had the highest mean cover (± SE) (3.4% ± 0.43) followed by Melstone (3.26% ± 0.45), Skytop (2.64% ± 0.32), and Phelps Creek with the lowest average cover (0.61 ± 0.11). When averages were calculated only for quadrats that contained *L. dalmatica* to account for the low frequency of *L. dalmatica* in quadrats, the results differed. Average cover and average cover when *L. dalmatica* was present decreased significantly with year at all four sites (Table 19). Average cover when *L. dalmatica* was present differed between transect types only at Skytop (Table 19, Figure 21).

Study-wide, the maximum cover recorded for *L. dalmatica* within each transect pair differed between sites ($F = 4.87$; d.f. = 3,43; $P=0.0053$) and decreased over time ($F = 49.98$; d.f. = 3,124; $p < 0.0001$), but there were no differences between transect type ($F = 1.23$; d.f. = 1,43; $P = 0.2740$) (Figure 22). Skytop, Coxcy Gulch, and Melstone all had similar maximum cover readings when averaged across transect pairs and years (13.79 ± 1.98, 12.27 ± 1.55, and 11.09 ± 1.61, mean ± SE for each site respectively) and Phelps

creek had the lowest maximum cover readings (4.31 ± 0.64). The proportion of the total vegetative cover per plot made up by *L. dalmatica* when *L. dalmatica* was present differed between sites ($F = 11.07$; d.f. = 3,43; $P < 0.0001$), and the proportion decreased over time ($F = 54.34$; d.f. = 3,124; $P < 0.0001$). There were no differences between type of transect ($F = 2.11$; d.f. = 1,43; $P=0.1532$) (Figure 23).

Mean stem density in transect pairs as well as the mean proportion of total stems that were mature were also examined. Study wide, when pooled across transect pairs, the density of *L. dalmatica* differed between sites ($F = 26.18$; d.f. = 3,43; $P < 0.0001$), but did not change with time ($F = 0.69$; d.f. = 3,124; $P = 0.5611$) or differ between control and release transect pairs ($F = 0.32$; d.f. = 1,43; $P = 0.5773$) (Figure 24). When each site was examined separately only Skytop had a significant change, a decrease, in stem density ($F = 3.18$; d.f. = 3,36; $P = 0.0353$).

Even though the total stem density did not change over time on a study wide basis, the proportion of the stems that were mature did change over time ($F = 8.34$; d.f. = 3,124; $P < 0.0001$) and differed between sites ($F = 4.38$; d.f. = 3,43 $P = 0.0089$), but not between control and release transects ($F = 1.21$; d.f. = 1,43; $P = 0.277$) (Figure 25). When each site was considered separately, all four sites showed significant changes over time (Table 20). There were significant differences in the proportion of mature stems between control and release transects at Coxcy Gulch and an almost significant difference between type of transect pair at Skytop (Table 20). At Coxcy Gulch there was a greater proportion of mature stems in control transect pairs than release transect pairs and there was a trend toward a greater proportion of mature stems in control transect pairs at Skytop (Figure 26).

Discussion

Establishment and Increase of *Mecinus janthinus*

There are no well defined criteria for what constitutes successful establishment of a biological control organism. A rule of thumb being used by the Oregon Department of Agriculture for considering establishment success in their weed biocontrol programs is recovery of univoltine species three years after release, or an observed population of more than 1000 individuals around the release point two years after release (Coombs, Personal communication). Based on that rule of thumb, successful establishment occurred at the three study sites where releases were made in 2003. On a study wide basis, the number of releases that became established (37/41) is consistent with the establishment rate reported by De Clerck-Floate and Miller (2002) in Canada where 21 of 25 initial Canadian releases became established. Although weevils established at each of the Montana sites from this study, outbreak populations were not recorded at any of the releases made for this study based on adult feeding injury evaluations. De Clerck-Floate and Harris (2002) reported that some of their sites recorded 100% of stems attacked within three years of release, and Carney (2003) reported outbreak levels of insects (approximately 15 immature weevils per stem, and some large stems with as many as 100 insects per stem) within three to five years of release. Although increasing prevalence of adult feeding damage over time was not recorded at Coxcy Gulch, Melstone, or Phelps Creek, trends of increasing *M. janthinus* population density are suggested by increasing insect loads over time only at Coxcy Gulch and Melstone. The discrepancy between insect load from stem dissections and prevalence and abundance of adult feeding and the

low infestation levels could partially be explained by the poor recovery rate of the previous season's stems, and thus subsequent under reporting of actual insect population levels.

The proportion of the insects recorded in dead stems that successfully emerged decreased by a factor of four over the course of the study at Coxcy Gulch and Melstone, despite the actual number of insects emerging (number of empty pupation chambers with exit holes) not changing over time. The decreases in successful emergence at Coxcy Gulch and Melstone were accompanied by a significant increase in the proportion of dead adults dissected out of stems, indicating successful development to adulthood but not survival through the winter. Because the majority of insects counted were dead adults, I believe that those insects that did survive the winter were successful in finding mates and laying eggs, and that conditions were suitable for development, but that the weevils were ultimately unable to survive the winters. De Clerck-Floate and Miller (2002) found a strong relationship of increasing overwintering mortality with decreasing absolute minimum temperature with a threshold between -15 – -20 ºC. Their results additionally suggested that the insulating properties of snow cover could ameliorate those lethal temperature effects. In that study, minimum temperature explained 70% of the variability in mortality. Temperatures at all of my sites have normal minimum temperatures above those thresholds. The lowest mean monthly temperatures at our study sites were near the thermal thresholds (NOAA 2002), and absolute low temperatures at all sites were below those thresholds, suggesting ambient temperatures in Montana could be contributing to overwintering mortality. This study occurred during years where snow pack in Montana

was below average (NOAA 2002), and this could also have contributed to higher mortality rates than expected.

Changes in *Linaria dalmatica* Populations

Linaria dalmatica average cover, maximum cover, and proportion of total cover were similar with differences between site and decreasing over time. However, the total stem density and the proportion of quadrats containing *L. dalmatica* also differed between sites but did not change over time. Overall, *L. dalmatica* was found in fewer than half the quadrats despite transect pairs being established through the densest portion of stands. The relatively low cover of toadflax when present and the large variability in density per transect pair matches reports in the literature of *L. dalmatica* having a patchy growth habit that may be in part due to the short life span of individual root crowns, and the clonal spread of the plant through lateral roots (Robocker 1974, Vujnovic and Wein 1997).

Adult and larval mining reduced flowering in *L. dalmatica* (Jeanneret and Schroeder 1992, Saner et al. 1994, De Clerck-Floate and Harris 2002) and clipping or removing flowering stems in spring, a time when adults are actively feeding on developing primary stems, can lead to reduced growth and subsequent flowering (Robocker et al. 1972, Robocker 1974). I therefore anticipated that the proportion of mature stems would decrease over time and the proportion of mature stems would be higher in control transect pairs than release transect pairs. A decrease in the proportion of mature stems was observed at Skytop and Coxcy Gulch, but not at Melstone, where the proportion of mature stems increased from 2004 to 2006. The high proportion of mature

stems at Melstone in 2003 could be due to sampling in August as opposed to late June and July, indicative of first year plants that could have had a chance to mature. The expected difference between transect types was observed at Coxcy Gulch, and there was a trend towards a greater proportion of mature stems at control vs. release transects at Skytop. The decrease in proportion of mature stems can therefore be correlated to the presence of *M. janthinus* at those sites. However, the changes in cover measures over time cannot be linked to *M. janthinus* because those changes did not differ between control and release transects.

Conclusions

Although there was a high rate of establishment of *M. janthinus* at release sites, their population growth at the sites monitored in this study was lower than reported at Canadian release sites. The high rate of overwintering mortality encountered is likely to be one factor in the slower-than-expected weevil population growth and the lack of evidence that the weevils had an impact on *L. dalmatica* abundance. However, De Clerck-Floate and Miller (2002) found that the weevils persisted at the majority of their sites and that *M. janthinus* populations increased despite the high mortality rates reported. The differences in *M. janthinus* population growth rates between the Canadian releases and the releases monitored for this study could also be due to differences between sites and years of release. Although previous studies assessing *M. janthinus* establishment and impact do not report *L. dalmatica* densities (De Clerck-Floate and Miller 2002, De Clerch-Floate and Harris 2002, Carney 2003), they do indicate that all releases were made in dense stands of *L. dalmatica*. The *L. dalmatica* densities recorded at my study

sites at the time the weevils were released ranged from fewer than 10 stems/m^2 at Phelps Creek to 40 stems/m^2 at Coxcy gulch. The density of mature stems at release sites could also impact *M. janthinus* population growth rates. Carney (2003) reported higher *M. janthinus* oviposition and a 10% greater survival rate to the adult stage on mature stems than vegetative stems. Differences in climate variables between years and locations and *L. dalmatica* population characteristics could have led to differences in growth rate.

There were releases made near the study sites at Melstone, Skytop (Sing, Personal communication), and Coxcy Gulch (Anthony 2005) before this study was initiated. The transect pair at Coxcy Gulch closest to releases made in 2001 (Anthony 2005) had the highest proportion of toadflax containing quadrats with adult feeding injury, and four times the total insect load dissected out of dead stems than the other transects at Coxcy Gulch in 2006. Based on the evidence from Coxcy Gulch and the studies in Canada (De Clerck-Floate and Miller 2002), *M. janthinus* populations should continue to increase at the study release sites over time.

The only potential weevil impact found was in the magnitude of decrease in the proportion of mature stems over time at Skytop, where the proportion of mature stems decreased more in release transect pairs than in control transect pairs. Those results are consistent with reported impacts of *M. janthinus* feeding on plants (Saner er al 2004). Results from the garden and field experiments (Chapters 2 and 3, respectively) indicate that a corresponding threshold density of oviposition scars and developing weevils is required where measureable reductions in plant growth and primary physiology are observed. The *M. janthinus* populations at the field releases in this study were low with an average total load per stem of less than one weevil.

The amount of time it takes weed biological control agents to reach population levels where impacts on the target weed are readily observed varies according to weed-agent combinations, and between sites within weed-agent combinations. The time lag between *M. janthinus* releases and measurable impacts on *L. dalmatica* populations in Canada fall within the range for other biological control systems. De Clerck-Floate and Miller (2002) and Carney (2003) recorded populations of *M. janthinus* high enough to cause measurable impacts on *L. dalmatica* in three to five years in Canada. At a release site near Wyola, MT, *M. janthinus* reached outbreak levels five years after release (Sing, Personal communication). Huffaker and Kennett (1959) recorded decreases in *Hypericum perforatum* L. six years after the release of the chysomelid *Chrysolina quadrigemina* Suffrian. In Oregon, McEvoy et al. (1991) recorded measurable decreases in *Senecio jacobaea* L. in response to feeding injury by the flea beetle *Longitarsus jacobaeae* Waterhouse three years after release and control four years after release. In South Africa, Moran and Zimmerman (1991) recorded repeated cycles of increasing populations of the cochineal insect *Dactylopius austrinus* De Lotto and decreasing populations of *Opuntia aurantiaca* Lindley, followed by crashes in *D. austrinus* populations and increases in *O. aurantiaca* populations. The length of time between *D. austrinus* population boom and bust cycles varied with site and year. However, they reported that after a population crash, *D. austrinus* took three to four years to attain population levels that caused decreases in *O. aurantiaca* populations. In Oregon, Schat (2002) found that it took *Galerucella calmariensis* and *G. pusilla* beetles four years to reach population levels high enough to decrease *Lythrum salicaria* populations at one field site but it took seven years at another field site 20 km away. The field sites used in

this study fall within the time ranges described above. Therefore as weevil populations increase, measurable impacts of *M. janthinus* on *L. dalmatica* should become more apparent.

Table 16 Details of the field sites used in a study of the impact of *Mecinus janthinus* on *Linaria dalmatica*.

	Site			
	Skytop	Coxcy Gulch	Melstone	Phelps Creek
UTM Easting	12T 414937.77	12T 450998.21	13T 781496.00	12T 523229.81
UTM Northing	5130601	5170785.71	2900000	4988203.88
Nearest Landmark	Boulder, MT	Canyon Ferry Lake, MT	Musselshell, MT	Gardiner, MT
Release year	2003	2003	2003	2004
Number of releases	12	7	8	11
Number of release transect pairs	10	7	8	12
Number of control transect pairs	3	3	0	4
Elevation	1630 - 1745 m	1508 -1764 m	1035 -1055 m	1955 - 2210 m
Slope	0 - 10 %	0 - 40%	0 - 5%	5 - 55%
Initial *L. dalmatica* cover	0 - 35%	0 - 30%	0 - 35%	0-10%

Table 17 Variables measured in each quadrat, 2003-2004.

Variable		Description
Abiotic variables		
1	Aspect	
2	Slope	
Toadflax density		
3	# mature stems	Count of live stems only (flowering stems)
4	# of immature stems	Count of live stems only (non-flowering stems)
5	# mature dead stems	
Toadflax size		
6	Height of tallest mature stem	Live stems only
7	Height of shortest mature stem	Live stems only
8	Height of average mature stem	Estimated and measured in the field
9	Height of tallest immature stem	Live stems only
10	Height of shortest immature stem	Live stems only
11	Height of average immature stem	Estimated and measured in the field
Cover by life form		For plants this is the estimate of basal cover, not canopy, and only of plants rooted inside the quadrat. Trace amounts were recorded as 5%
12	% cover of grasses	
13	% cover of forbs	
14	% cover of woody species	
15	% cover of bare ground	
Cover if noxious weeds		The sum of cover for all noxious forbs should be less than or equal to the total cover of forbs. If there are trace amounts of two noxious forbs, total forbs was recorded as 10% and cover of each weed was recorded as 5%
16	% cover of *L. dalmatica*	
17	% cover downy Brome	
18	% cover knapweed	
19	% cover leafy spurge	
20	% cover Canada thistle	
21	% cover musk thistle	
22	% cover sulfur cinquefoil	
23	% cover hounds tongue	
Insect variables		Average damage to all stems in the quadrat. Damage was evaluated in classes with 0 = no damage, 1 = 1 – 5% of the total toadflax foliage affected by any detectable feeding damage (hard to detect) 2 = 5.5 – 20% toadflax foliage affected by feeding damage (minimal damage) 3 = more than 20% foliage injured (obvious damage)
24	Damage class for mature stems	
25	Damage class for immature stems	

Table 18 Proportion of release transect pairs at each site with evidence of *Mecinus janthinus* establishment and emergence three years after release (2006).

		Proportion of transect pairs with evidence of	
Site	N	Establishment	Emergence
Skytop	10	0.60	0.20
Coxcy Gulch	7	1.00	0.86
Melstone	8	1.00	0.62
Phelps Creek[1]	13	0.54	0.08

[1]Releases at Phelps Creek were made in 2004, so these results are for two years after release

Table 19 Differences in *Linaria dalmatica* cover at four sites in Montana with respect to year and transect type. The two types of cover tested were cover averaged over all 40 quadrats per transect pair, and cover averaged only over the quadrats that contained *L. dalmatica*. Transect types were release and control transects. Separate analyses were run for each site and for each cover type.

Variable				
Site	Factor	d.f.	F	P
Average Cover				
	Skytop			
	Year	3,36	70.34	<0.0001
	TP Type	1,11	0.05	0.8268
	Coxcy Gulch			
	Year	3,27	23.65	<0.0001
	TP Type	1,8	1.69	0.2295
	Melstone			
	Year	3,21	26.64	<0.0001
	Phelps Creek			
	Year	2,32	15.46	<0.0001
	TP Type	1,15	1.66	0.2167
Average cover where *L. dalmatica* was present				
	Skytop			
	Year	3,36	103.10	<0.0001
	TP Type	1,11	7.09	0.0221
	Coxcy Gulch			
	Year	3,27	74.39	<0.0001
	TP Type	1,8	0.22	0.6537
	Melstone			
	Year	3,21	78.10	<0.0001
	Phelps Creek			
	Year	2,32	230.03	<0.0001
	TP Type	1,15	0.01	0.9096

Table 20 Proportion of mature *Linaria dalmatica* stems at four sites in Montana with respect to year and type of transect pair. Transect pairs were either release transects or control transects. Separate analyses were run for each site.

Site/Factor	d.f.	F-Stat	P-value
Skytop			
Year	3,36	21.26	<0.0001
TP Type	1,11	4.70	0.0529
Coxcy Gulch			
Year	3,27	22.39	<0.0001
TP Type	1,8	7.48	0.0257
Melstone			
Year	3,21	13.96	<0.0001
Phelps Creek			
Year	2,32	34.90	<0.0001
TP Type	1,15	0.63	0.4410

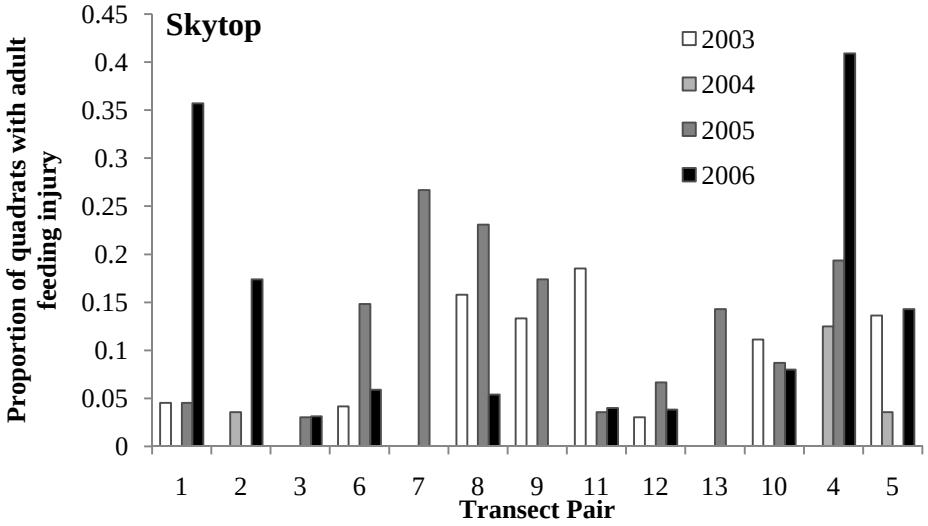

Figure 15 Skytop: Proportion of quadrats per transect pair containing *Linaria dalmatica* with *Mecinus janthinus* adult feeding injury, 2003-2006. The three transect pairs on the far right (10, 4, and 5) were control transects.

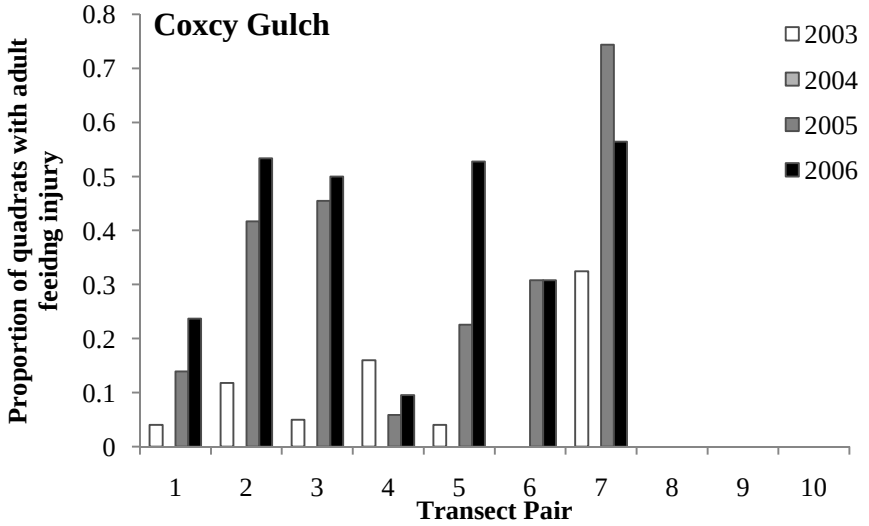

Figure 16 Coxcy Gulch: Proportion of quadrats per transect pair containing *Linaria dalmatica* with *Mecinus janthinus* adult feeding injury, 2003-2006. The three transect pairs on the far right (8, 9, and 10) were control transects.

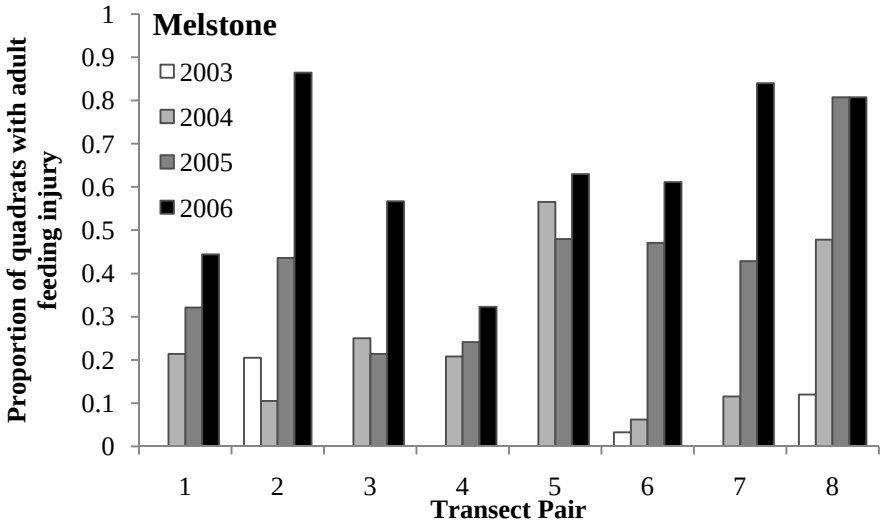

Figure 17 Melstone: Proportion of quadrats per transect pair containing *Linaria dalmatica* with *Mecinus janthinus* adult feeding injury, 2003-2006. All eight transect pairs were associated with releases.

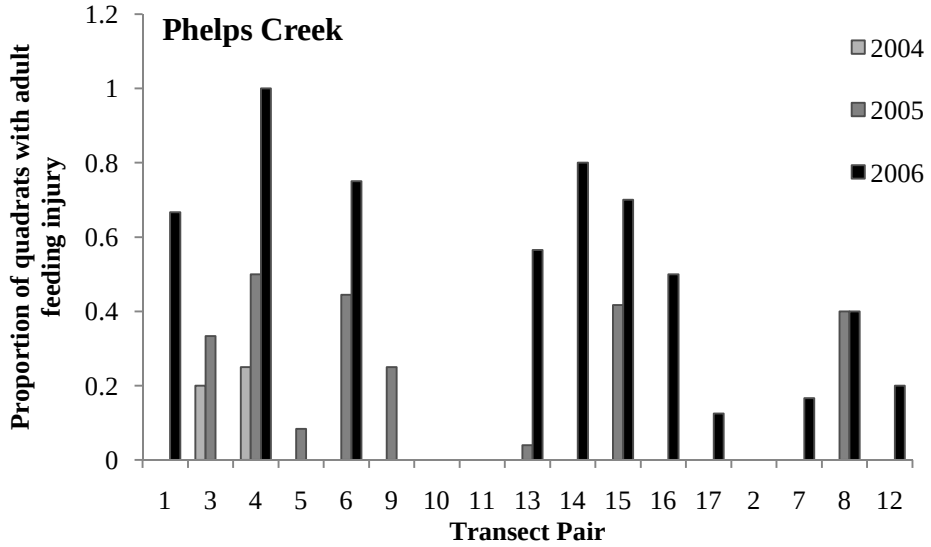

Figure 18 Phelps Creek: Proportion of quadrats per transect pair containing *Linaria dalmatica* with *Mecinus janthinus* adult feeding injury, 2004-2006. The four transect pairs on the far right (2, 7, 8, and 12) were control transects.

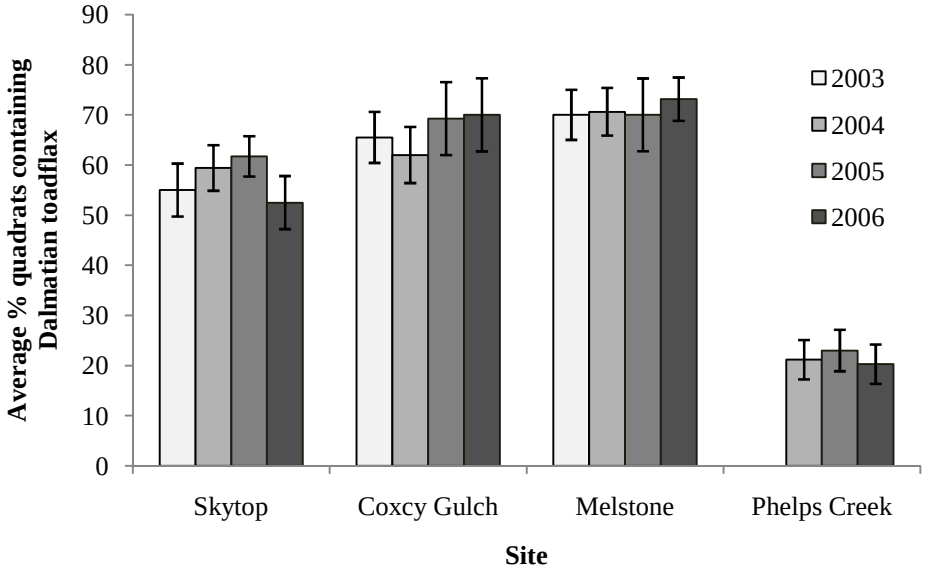

Figure 19 Average proportion of quadrats per transect pair containing *Linaria dalmatica*, 2003 – 2006.

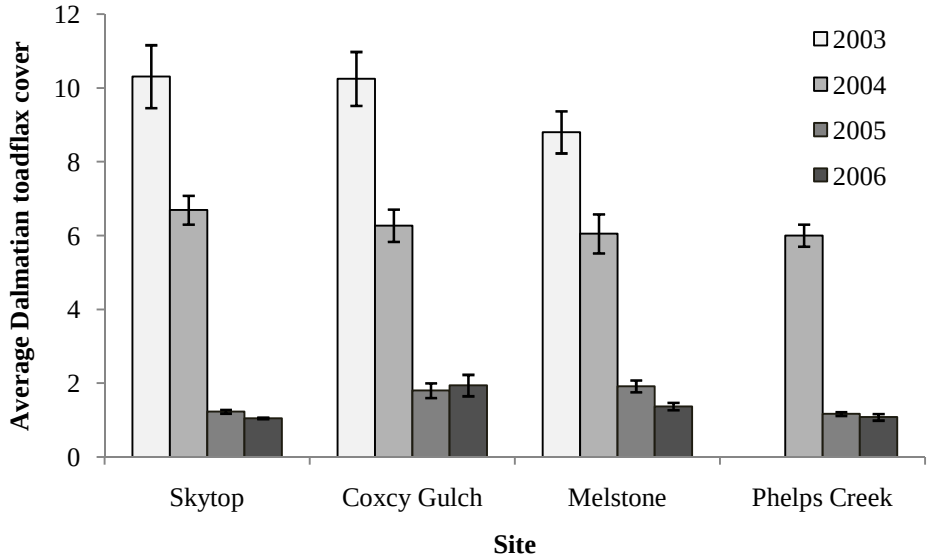

Figure 20 Average *Linaria dalmatica* cover where *Linaria dalmatica* was present, 2003 – 2006.

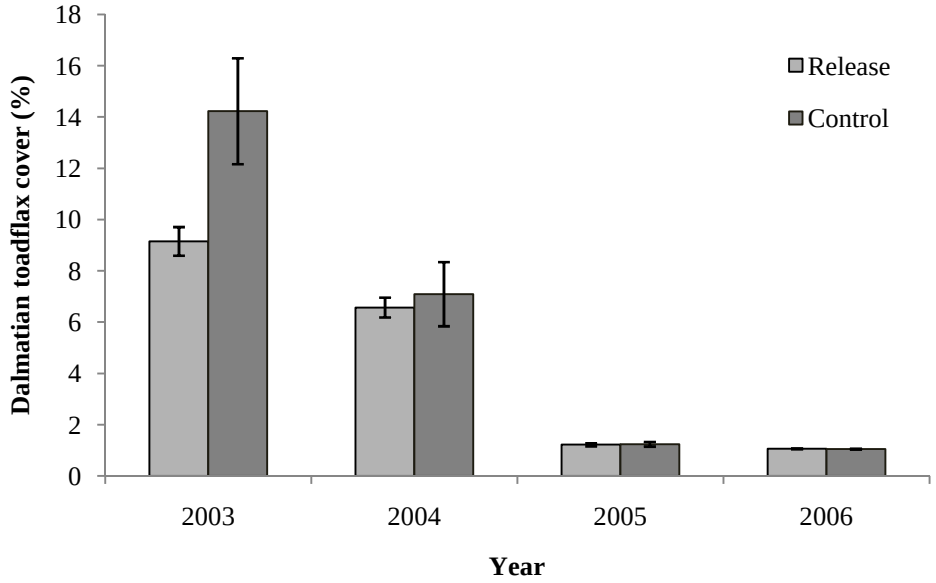

Figure 21 Skytop: Average *Linaria dalmatica* cover where *Linaria dalmatica* was present for control and release transects, 2003 – 2006.

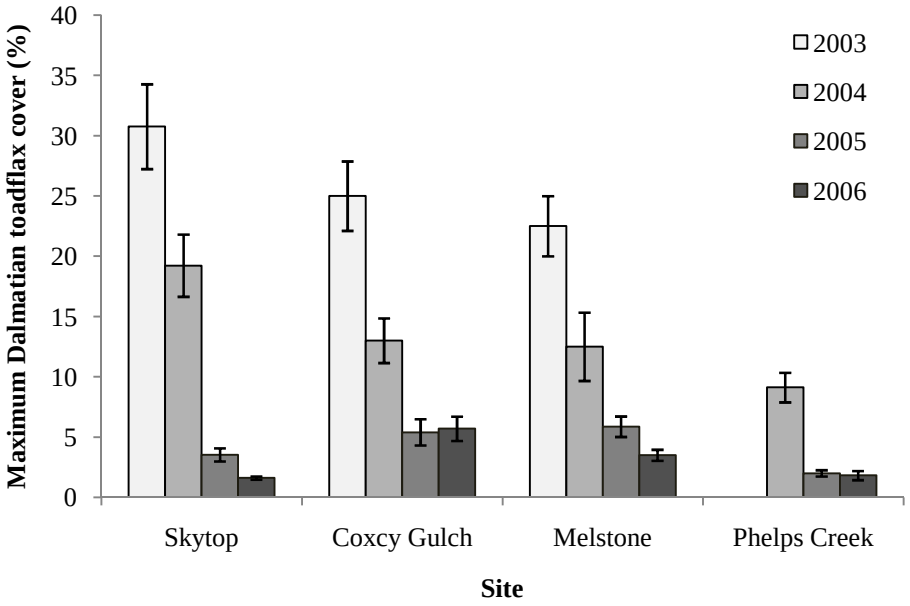

Figure 22 Average maximum *Linaria dalmatica* cover recorded in transect pairs, 2003 – 2006.

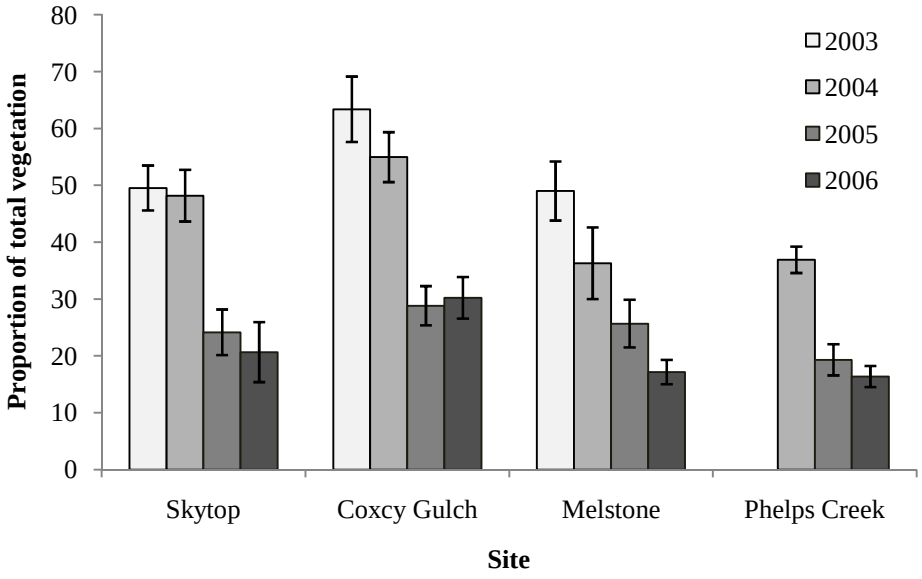

Figure 23 Average proportion of total vegetation cover made up of *Linaria dalmatica* when *Linaria dalmatica* was present, 2003 – 2006.

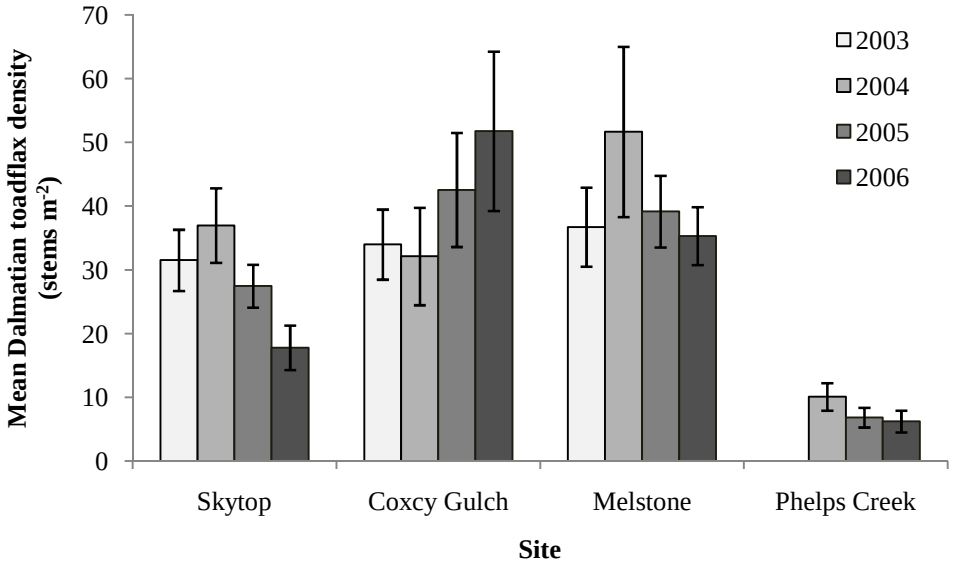

Figure 24 Mean *Linaria dalmatica* stem density, 2003 – 2006.

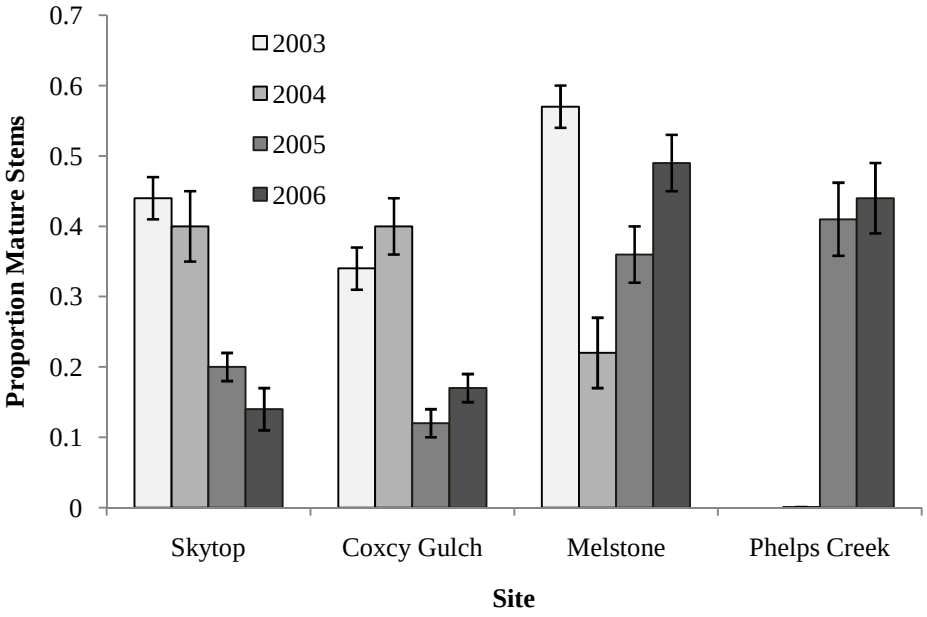

Figure 25 Mean proportion of mature *Linaria dalmatica* stems, 2003 – 2006.

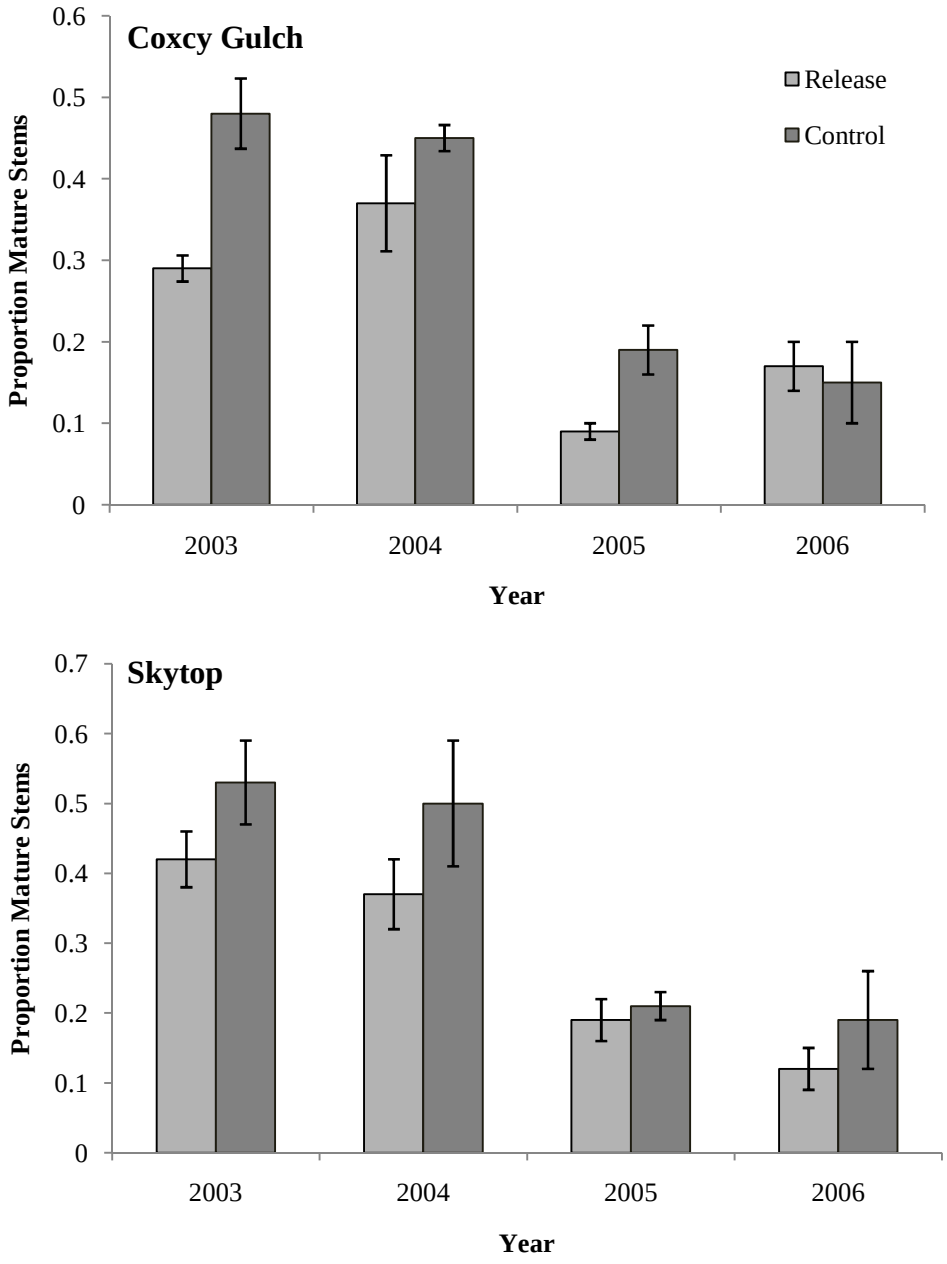

Figure 26 Mean proportion of total *Linaria dalmatica* stems that were mature stems for control and release transect pairs at two sites, 2003 – 2006.

CHAPTER 5

CONCLUSION

The results from these garden and field experiments (Chapters 2 and 3, respectively) indicate that a high level of *M. janthinus* herbivory is necessary to reduce *L. dalmatica* growth and affect gas exchange parameters. The results of the field monitoring study (Chapter 4) indicate that *M. janthinus* was able to establish at four diverse watershed scale field sites. However, *M. janthinus* populations at releases made in 2003 were not sufficient to reach the injury threshold required to cause decreases in plant growth and gas exchange rates by three years after the releases were made.

The differences in gas exchange results between the garden and field site underscore the importance of environmental factors on photosynthetic, stomatal conductance, and transpiration rates in response to herbivory. The pattern of decreasing gas exchange with increasing herbivore density was similar across all three locations, but the magnitude and significance of these responses differed. Further studies adding moisture gradients to the present study design would be needed to determine if daily watering in the garden experiment allowed plants to compensate for *M. janthinus* injury with respect to gas exchange. The decreases in gas exchange rates observed in the field were likely due to oviposition and larval feeding injury. However, I did not determine if feeding behaviors by adult males and adult females are similar, so there is a possibility the decreases observed in the plants in the high mixed treatments could be influenced by differences between male and female adult feeding. Results from previous greenhouse studies on a different plant-shoot mining insect system indicate that oviposition itself can

affect gas exchange, and that drought stress amplified those effects. The sampling dates on which I observed gas exchange rate differences (immediately after adults were removed from plants, and at the end of the growing season) indicate that oviposition behavior might be an important factor in the decreases I observed in the field.

The impact of *M. janthinus* injury on plant growth was more consistent across variable environmental conditions. Injured plants grown from both established roots (2005) and seed (2006) in the garden and three field sites had lower relative growth than plants without injury. These results are consistent with reports of reduced growth and a reduction and delay in flowering in plants in clipping and herbivore studies. Two hypotheses in the literature explain the observations: 1) that larval mining causes a drought-like response and subsequent reduced growth in *L. dalmatica,* and 2) that adult feeding reduces growth by injuring meristems. Results from the field experiment indicated plants with high levels of adult only feeding injury and plants with both adult and larval feeding injury experienced similar reductions in growth, supporting the adult injury hypothesis. Differences in plant growth and gas exchange occurred only between uninjured control and high herbivore pressure treatments, where approximately 100 oviposition scars were recorded per plant and approximately 20 adults completed development per plant.

Although weevils were able to establish across a range of environmental conditions in this study, the population seems to be increasing more slowly than expected based on reports in the literature from other North American locations. Even at the release that had the highest insect load for stems dissected in 2006, emergence of less than one adult per dead stem was recorded. The low number of developing insects to

adults per stem and the low levels of adults feeding (fewer than 5% of stems had adult feeding injury) were below the threshold levels determined in my experiments that led to measurable growth and primary physiological responses in the garden and field experiments. At sites in Canada, where *M. janthinus* herbivory has led to control of *L. dalmatica*, extensive adult feeding was reported along with high weevil loads per stem. Although the low *L. dalmatica* density at my sites and the low recovery rate of the previous year's stems for dissection could have led to under-estimates of weevil populations, the populations were low enough three years after releases that no impacts on *L. dalmatica* cover or density were detected.

Reports from Canada indicate that although there were high rates of overwintering mortality, weevil populations persisted and over time increased (De Clerck-Floate and Miller 2002). The overwintering mortality observed in this study is comparable to that reported in the literature, and I also found that weevils were able to persist at the majority of the releases I studied. Although *M. janthinus* has not affected *L. dalmatica* density at the studied sites, given sufficient time for weevil populations to increase to the point of being able to inflict injury levels above the individual plant impact threshold, *M. janthinus* has the potential to reduce *L. dalmatica* levels in Montana.

APPENDICES

APPENDIX A

DETAILS OF FIELD RELEASES AND TRANSECT PAIRS USED FOR MONITORING POST RELEASE ESTABLISHMENT AND IMPACTS OF *MECINUS JANTHINUS* ON *LINARIA DALMATICA*

Table 21 Transect pair and release information for the Skytop field site.

Transects	Elevation (m)	Monitoring or release?	Nearest release(s)	# agents released	Distance* (km)
1AB	1649	Release	R1	250	0.03
3A	1639	Release	R2	250	0.1
			R3	500	0.08
3B	1636	Release	R2	250	0.03
			R3	500	0.01
3C	1635	Release	R2	250	0.02
			R3	500	0.02
3D	1635	Release	R2	250	0.03
			R3	500	0.03
3EF	1637	Monitoring	-	-	0.11
3GH	1637	Monitoring	-	-	0.12
5AB	1632	Release	R5	250	0.02
9AB	1640	Release	R9	250	0.02
10AB	1744	Release	R10	250	0.02
11AB	1724	Release	R11	250	0.07
12AB	1720	Monitoring	-	-	0.11
13AB	1692	Release	R12	250	0.04
14AB	1686	Release	R12	250	0.05
15AB	1669	Release	R11	250	0.2

* Distance between TP and nearest release(s)

Table 22 Transect pair and release information for the Coxcy Gulch field site.

Transects	Elevation (m)	Monitoring or release?	Nearest release(s)	# agents released	Distance* (km)
1AB	1508	Release	R1	250	0.01
2AB	1713	Release	R2	250	0.01
3AB	1736	Release	R3	500	0.01
4AB	1745	Release	R4	500	0.01
5AB	1674	Release	R5	750	0.01
6AB	1537	Release	R6	500	0.03
7AB	1596	Release	R6	500	0.03
8AB	1731	Monitoring	-	-	0.23
9AB	1721	Monitoring	-	-	0.29
10AB	1764	Monitoring	-	-	0.48

* Distance between TP and nearest release(s)

Table 23 Transect pair and release information for the Melstone field site.

Transects	Elevation (m)	Monitoring or release?	Nearest release(s)	# agents released	Distance* (km)
1AB	1049	Release	R1	250	
2AB	1050	Release	R2	250	
3AB	1052	Release	R3	250	
4AB	1052	Release	R4	250	
5AB	1051	Release	R5	250	
6AB	1050	Release	R6	250	
7AB	1048	Release	R7	250	
8AB	1036	Release	R8	250	

* Distance between TP and nearest release(s)

Table 24 Transect pair and release information for the Phelps Creek field site.

Transects	Elevation (m)	Monitoring or release?	Nearest release(s)	# agents released	Distance* (km)
1AB	2073	Release	R1	200	0.01
2AB	2115	Monitoring	-	-	0.1
3AB	2096	Release	R2	200	0.01
4AB	2176	Release	R2	200	0.04
5AB	2181	Release	R3	200	0.01
6AB	2196	Release	R4	200	0.01
7AB	2206	Monitoring	-	-	0.12
8AB	2185	Monitoring	-	-	0.1
9AB	2205	Release	R5	200	0.01
9CD	2207	Release	R5	200	0.04
10AB	2191	Release	R6	200	0.01
11AB	2122	Monitoring	-	-	0.12
12AB	2090	Release	R7	200	0.01
			R8	200	0.03
13AB	2089	Release	R7	200	0.03
			R8	200	0.05
14AB	2048	Release	R9	200	0.01
15AB	2006	Release	R10	200	0.01
16AB	1954	Release	R11	200	0.01

* Distance between TP and nearest release(s)

APPENDIX B

SAMPLE SAS PROGRAMS USED IN EACH CHAPTER

Chapter 2 Codes

Variables of Interest as a Function of Adult Density Treatments

Variables examined included injury variables (number of oviposition scars, number of eggs, number of larvae, number of post egg insects), growth variables (net relative growth using ln transformed data, and original data, ln biomass of roots, shoots, and reproductive parts, biomass ratios, photosynthesis, conductance, transpiration, and fluorescence variables.

```
Proc glm data=XXX;
  Title “Garden experiment treatment vs. variables of interest”;
  Class treatment block harvest;
  Model variable of interest=treatment block harvest;
  Means treatment/tukey;
  Means block/tukey;
  Means harvest/tukey;
Run;
```

Relationships Between Variables of Interest and Injury

Variables of interest were the same as above. Injury variables depended on the harvest being examined and included number of oviposition scars, number of eggs, number of larvae, and the number of post egg insects.

```
Proc glm data=XXX;
  Title “Garden experiment relationship between variables of interest and injury”;
  Class block
  Model variable of interest=block injury;
  Means block/Tukey;
Run;
```

Allometry Analyses

The allometric analyses included several parts: test to determine if there was correlations between variables of interest, Analyses of variance to test for treatment, time (harvest), and interaction effects, regressions for pooled data where there were no treatment or time effects or for each group separately.

```
Proc corr  Data=marjolib.Garden05_biomass2 Nosimple;
  Title "analysis of covariation for log transformed";
  Var lnastem lnroot;
Run;

Proc anova data=marjolib.Garden05_biomass2;
  Title "anovas testing for time and treatment effects";
  Class treat harvest;
  Model lnroot = treat harvest;
Run;

Proc GLM Data=marjolib.Garden05_biomass2;
  Title "tests for differences in slopes ";
  Class treat harvest;
  Model lnastem= lnroot treat harvest lnroot*treat lnroot*harvest lnroot*treat*harvest
/ss3;
Run;

Proc Reg data=Marjolib.Garden05_allometry;
  Title "regression to determine allometric coefficients";
  Model lnastem=lnroot;
Run;
```

Chapter 3 Codes

In 2006 there were no site by treatment interactions and data were combined. In 2007 sites were analyzed separately.

```
Proc glm data=XXX;
  Title “generalized linear model to test for differences in variables of interest between
adult density treatments”;
  Class treatment
```

```
  Model variable = treatment;
  Means treatment/Tukey;
Run;

Proc glm data=XXX;
  Title “generalize linear model to test for relationships between variables of interest and
injury”
  Model variable of interst = measure of injury;
Run;

Proc mixed data=XXX;
  Title “proc mixed repeated measure variable of interest as a function of treatment or
injury type”;
  Class plant treatment
  Model variable = treatment Julian_date;
  Repeated/subject = plant;
Run;
```

Chapter 4 Codes

Proc-Mixed analyses with repeated statements were conducted separately for each site.

```
Proc mixed Data = XXX;
  Title “Proc mixed repeated measures proportion of quadrats per transect…”;
  Class transect_pair transect_type;
  Model Variable = year transect_type;
  Repeated/subject=transect_pair;
Run;
```

APPENDIX C

MODELING PRESENCE AND ABUNDANCE OF THREE SPECIES USING GENERALIZED LINEAR MODELS, GENERALIZED ADDITIVE MODELS, AND TREES.

Introduction

Ecological theory has traditionally held that diverse habitats were less prone to invasion than species poor or monoculture habitats (Elton 1958). This has been supported with numerous microcosm and controlled environment experiments aimed at isolating the local or neighborhood effects of diversity on invasions as well as the underlying mechanisms (Levine 2000). Communities supporting diverse assemblages of endemic species have generally been considered most robust in the face of invasion pressure. However, in a review of five papers aimed at examining the invasion potential on 24 nature reserves across a broad range of geographic locales where native diversity tends to be high, Usher (1988) concluded that all nature reserves across all locales (except Antarctica) contained invasive species. These results support the arguments that all communities have the potential to be invaded, but that some are more likely to be invaded than others with the differences being due to propagule pressure, the availability of species able to survive in a given habitat (Williamson 1996), the identity of species present in a habitat (Crawley et al. 1999), and the level of disturbance of a given habitat (Collins et al. 1995, Mackey and Currie 2001).

Biotic and abiotic factors could affect the spread of weeds and the abilities of the biological control agents to impact the weeds. Modeling the presence/absence or abundance of a weed and determining if biocontrol agent variables impact those variables given the other conditions at the site could help choose locations where biological control is more likely to succeed. Model of this sort can be created using monitoring data from previous releases.

Monitoring data can be complex, including detailed measurements of species of interest, abiotic variables, community variables, and analysis of those data sets can be difficult. There are a variety of statistical tools available to test for differences over time or between treatments; and predict presence-absence or abundance of specific species, by incorporating community data into analyses. A commonly used approach to modeling species distributions is multiple linear regression using least squares where the dependent variable is typically species presence-absence or cover. Least squares regression assumes that errors are normally distributed with a mean of zero and a constant variance, and that the dependent variable is unbounded (Underwood 1997). However, the unbounded dependent variable assumption is not met with presence-absence data (Austin 1990, Huisman et al. 1993). Environmental and community data from collected from seven watershed scale sites in Montana where a stem mining weevil, *Mecinus janthinus* Germar, has been released to control Dalmatian toadflax, *Linaria dalmatica* (L.) Miller were used to compare three alternative inferential statistics models that do not require unbounded data or Gaussian responses: Generalized Linear Models (GLMs), Generalized Additive Models (GAMs), as well as both classification and regression trees.

Methods

This study used the same sites and data described in detail in Chapter 4 including the four main sites described in Chapter 4 (Skytop, Coxcy Gulch, Melstone, and Phelps Creek) as well as four additional "mini sites" located near the Phelps Creek site (North Fork of Bear Creek, Athas, Deckerd Flat, and Eagle Creek Campground). The mini sites each had two transect pairs with the exception of Eagle Creek Campground which only

had one transect pair. Environmental data used in the analyses included the percent cover for grasses, forbs, downed wood, rock, shrubs, bare ground, slope, elevation, latitude and longitude in decimal degrees, aspect in degrees, a categorical variable coding for site, burn history of the site (unburned, moderate, and severe), land use (BLM, USFS, Ranch), distance to the nearest biocontrol agent release, years since agents were released, and the number of agents released at the closest release. Only data from 2005 were used since there were no differences in species richness or diversity between 2005 and 2006, and community data were not collected in 2003 and 2004.

A total of 2200 quadrats were sampled in 2005, but only 1940 were included in the analysis. The 260 quadrats excluded from the analysis were for transects where individual species cover was not estimated due to time constraints. All analyses were conducted using R2.2.1 (R Foundation for Statistical Computing, ©2005). Values for aspect were entered as NA where slope was zero. Before being analyzed, aspect data, which were recorded as verbal descriptions (i.e., north, north east, east, etc.) were converted to degrees where north was recorded as 0, east as 90, south as 180, and west as 270. Aspects that had been recorded as NE, SE, SW, or NW were considered to fall equidistant between the two cardinal directions indicated in their codes. This was then converted from the circular scale of degrees to a more linear scale (aspect value) that is suitable for statistical analysis using equation 1 (Moisen and Frescino 2002).

Equation 3:
$$Aspectvalue = \frac{\cos(\frac{Aspect - 30}{180 * pi}) + 1}{2}$$

The cosine transformation converts aspect into solar radiation equivalents. The 30° correction reflects relative heat in the atmosphere at the time of peak radiation. The maximum value that can be obtained is 1.0 and is equivalent to an aspect of 30º, typically the coolest and wettest orientation (Moisen and Frescino 2002) and the minimum possible value is equivalent to an aspect of 210º (Roberts 2008).

The species selected for predictive models were the two species that were most widespread in the sample areas, though had low percent cover when found, as well as one species that was present in only 10% of the plots sampled, but had fairly high mean cover when present (Figure 27). The two frequently encountered species modeled were Dalmatian toadflax, the target weed of a biological control program, and downy brome, *Bromus tectorum* L., another weed that is adapted to frequent fires and can dominate plant communities after fires move through (Young et al. 1987, D'Antonio and Vitousek 1992, Mack et al. 2000). In the intermountain west, in the early 1990's, at least 40 million hectares of sage brush perennial bunchgrass habitat had become dominated by monospecific stands of downy brome (D'Antonio and Vitousek 1992). Due to the invasive potential of downy brome and the high cost associated with controlling downy brome fueled fires, estimated to be $20 million/yr (Knapp 1996), this is a species of concern. The third species considered was big sagebrush (*Artemesia tridentata* Nutt), chosen as a native species as well as to test the models on a species on the other end of the frequency and abundance spectrum from the other two species modeled.

Community data were recorded as percent cover of each species per quadrat. Species that were present only in trace amounts were assigned a cover value of 1%. Cover for functional classes of vegetation (shrubs, forbs, and grasses) were calculated by

adding covers for each species encountered that fell into those groups, as well as "checked" by being estimated outright in the field. To model presence-absence of the species of interest, the cover data were converted to a true false matrix where all cover values of 1% or greater were assigned the value of true (1) and all zeros were assigned values of false (0).

Models

Analyses were divided into several parts. Empirical cumulative density functions were used to characterize whole study patterns and box-and-whisker pots to graphically visualize elevation, aspect value, and slope at each site and the within site variability for those variables. Even though latitude and longitude were recorded for each transect pair, those values were very closely linked to site, and site name was used to represent geographic location of the sites within the state.

Generalized linear models (GLMs) and generalized additive models (GAMs) were tested for their ability to predict both presence-absence as well as abundance. For all presence absence models, the variance family was assigned to binomial. Original cover data were used to model abundance. Although cover data are theoretically bounded by 0 and 100, individual species cover per plot rarely reached 100%, so data were modeled as if there was no upper bound, and a Poisson distribution was used to describe deviance in GLMs and GAMs.

Based on the results of data exploration, GLMs were constructed based on three different sets of parameters. The first set of variables modeled was elevation and slope, the second set was total vegetative cover per plot and plot species richness, and the final

model was site name (Table 25). Models were initially compared on the basis of Akaike's Information Criterion (AIC). If there were two models with low AIC's from different sets of variables, the two sets were combined to create another model. How much better the model did at predicting presence or abundance of each species than the null model was calculated by dividing the residual deviance of a model by the null deviance and subtracting that value from one. Analysis of variance using a Chi-squared test was used to determine if the addition of a quadratic term or a new variable significantly reduced deviance. The same models were created for both the presence-absence data and the cover data. The variables that were incorporated in the best GLMs were then used in constructing GAMs.

Classification trees and regression trees used all the environmental variables collected. A 10-fold cross validation was used to evaluate over fitting and the trees were pruned where the cross validation showed the deviance was lowest. Cross validation and pruning led to the exclusion of some variables from final trees. The trees do not incorporate plots where environmental variables have missing values. There were 300 plots where the slope was zero and there was no associated aspect, so the trees were based on data from 1640 plots whereas the GLMs and GAMs were based on data from 1940 plots.

The three 'best' presence-absence models for each species were compared by examining the contingency tables and calculating percent correct classification. The three abundance models for each species were compared using root square errors. Root square errors (RSE) were calculated using equation 2 for regression trees and equation 3 for GLMs and GAMs.

Equation 4: $$RSE = \sqrt{\frac{\sum (observed - pred(tree))^2}{\#\ plots}}$$

Equation 5: $$RSE = \sqrt{\frac{\sum observed - fitted(GLMorGAM))^2}{\#\ plots}}$$

Results

Presence-Absence GLMs

For all three species, models 3 and 10 (Table 25) had the lowest AIC values (Table 26). When models 3 and 10 were combined into one model (model 11), the resulting AIC's were reduced further (Table 26). When the full model (model 11) was compared to the nested model 10 using analysis of variance with a Chi-squared test, the reduction of deviance from the nested model to the full model was highly significant for all three species (P(>|Chi|) = 2.658e-07, 1.244e-40, and 0.004171, respectively, for *L. dalmatica*, *B. tectorum*, and *A. tridentata*). For *L. dalmatica and B. tectorum* all five variables (site, elevation, elevation^2, slope, and slope^2) contributed significantly to a reduction in deviance in the model. For *A. tridentata* 3 of the five variables (Site, elevation, and slope^2) in model 11 contributed to a decrease in model deviance.

For *L. dalmatica*, the model predicts a decrease in probability of occurrence with increasing elevation, with the variability in probability of occurrence at any given

elevation influenced by slope and site (Figure 28). The model fit was low (0.14) and correctly predicted the presence or absence of *L. dalmatica* in 70% of the plots. The largest error was the absence of *L. dalmatica* in plots where it was predicted to be present.

For *B. tectorum*, the model predicts the highest probability of occurrence at the lowest elevation where downy brome was recorded (1000m), but a variable probability of occurrence between elevations of 1500 and 2200 m (Figure 29). The overall fit of the model was low 0.20, and much better than the fits of models 3 (0.07) and 10 (0.12). The model correctly predicted the presence of *B. tectorum* in 79% of the plots with the largest error being the presence of *B. tectorum* in plots where it was predicted to be absent.

For *A. tridentata*, the model predicts the highest probability of occurrence at the highest elevation plots. However, the highest probability recorded was only 0.3 (Figure 30). The overall fit of the model was low (0.20), and was only slightly better than the fit of models three and 10 (both 0.19). Despite the low fit, the model correctly predicted the absence of sage in 93% of the plots. The model did not predict presence of sage in any plots.

Abundance GLMs

The models with the lowest AIC values were different for each species in the abundance models (Table 27). For *L. dalmatica*, models 3 and 10 were combined to form model 11 (Site, elevation, elevation^2, slope, and slope^2). Model 11 had an AIC value of 4330.2, a value only slightly lower than the AIC for either model three or ten, but the deviance was significantly lower than models 3 and 10 (P(>|Chi|) = 2.205e-12 and

2.098e012 respectively). Four of the five variables in the GLM (Site, elevation, slope, and slope^2) contributed significantly to a reduction in deviance in the model (P(>|Chi|) = 50.36e-110, 1.599e-04, 2.713e-09, and 1.428e-03, respectively). The model predicts a decrease in abundance of toadflax with increasing elevation. The overall fit of the model was 0.20.

For *B. tectorum*, models 9 and 10 had the lowest AIC values and were combined into a model with site, total vegetative cover per plot, (total vegetative cover per plot)^2, species richness per plot, and (species richness per plot)^2. When the full model was compared to models 9 and 10 with analyses of variance, the reduction in deviance from the nested model to the full model was highly significant (P(>|Chi|) = 9.104e-289 and 1.372e-260 respectively). All five of the variables in the GLM contributed significantly to a reduction in deviance in the model (P(>|Chi|) = 1.744e-285, 9.129 e-171, 1.466e-71, 2.044e-22, and 1.501e-05, respectively). The model predicts an increase in the abundance of *B. tectorum* with an increase in total vegetative cover per plot. The overall fit of the model was 0.28.

The model with the lowest AIC values for *A. tridentata* was nested model model 9 (total vegetative cover per plot, (total vegetative cover per plot)^2, species richness per plot, and (species richness per plot)^2), so no new model was created. The reduction in deviance from the two nested models (models 6 and 8) to the full model was highly significant (P(>|Chi|) = 1.559e-83 and 0.0 respectively). All four of the variables in the GLM contributed significantly to a reduction in deviance in the model (P(>|Chi|) = 0.0, 4.776e-98, 1.052e-79, and 9.783e-07, respectively). The model predicts an increase in the abundance of *A. tridentata* with an increase in total vegetative cover per plot up to a

total vegetation cover of approximately 75% and then decreasing *A. tridentata* cover. The overall fit of the model was 0.44.

Presence-Absence GAMs

When site, slope, and elevation were entered into a GAM modeling the presence of *L. dalmatica*, the resulting model showed a decrease of log odds at middle elevations and then a slight increase again at higher elevations. The probability of occurrence also decreased with slope. The error was larger at low elevations than at mid or high elevations. The model explained 19% of the deviance and the model fit (adjusted R-square) was 0.23. The model correctly predicted presence or absence of toadflax in 70% of the plots. The largest error was predicting the presence of toadflax where sampling indicated *L. dalmatica* was absent.

When site, slope, and elevation were entered into a GAM modeling the presence of *B. tectorum*, the resulting model showed a decrease in log odds of *B. tectorum* with increasing elevations from 1000 to 1400m, and then no change to 1800 meters before decreasing again. The probability of occurrence decreased with increasing slope. The error was greatest at low elevations and decreased as elevation increased. The model explained 27% of the deviance and the model fit was 0.30. The model correctly predicted the presence or absence of *B. tectorum* in 77% of the models, with the largest error being predicting the absence of *B. tectorum* where it was present.

When site, slope, and elevation were entered into a GAM modeling the presence of *A. tridentata*, the resulting model showed a fairly linear increase in the log odds of *A. tridentata* occurrence with increasing elevations. The error was greatest at the lower

elevations and decreased almost zero at 1800 m and then increased again between 1800 and 2200 m. The model explained 22% of the deviance and the model fit was 0.12. The model correctly predicted the absence of *A. tridentata* in 93% of the plots, but it never predicted that *A. tridentata* would be present, even though *A. tridentata* was recorded in 6% of the plots.

Abundance GAMs

When site, slope, and elevation were entered into a GAM modeling the abundance of *L. dalmatica*, the resulting model showed a fairly linear decrease in abundance with increasing elevation. The error was larger at low elevations and decreased to almost zero at 1800 m and increasing again between 1800 and 2200m. The model explained 25% of the deviance and the model fit (adjusted R-square) was 0.20.

When site, total vegetative cover per plot, and species richness per plot were entered into a GAM modeling the abundance of *B. tectorum*, the resulting model predicted an increase in abundance with total vegetative cover, and a decrease in abundance as species richness increased. The error was highest at the very low and the very high ends of species richness gradient. The model explained 35.6% of the deviance and the model fit was 0.46.

When total vegetative cover per plot, and species richness per plot were entered into a GAM modeling the abundance of *A. tridentata*, the resulting model showed a flat line around zero for total vegetative cover. The model predicted abundance of *A. tridentata* increased with total vegetative cover, and decreased with number of species.

The error was highest at the very low and very high ends of the species richness spectrum. The model explained 50.4% of the deviance and the model fit was 0.60.

Classification Trees

The initial classification tree produced for *L. dalmatica* presence had five terminal nodes and cross validation suggested no pruning was necessary. The variables that were included in the tree were percent cover of forbs, study site as a categorical variable, and longitude. The tree predicted that there would be *L. dalmatica* in plots with more than 0.5% forb cover and the plot was at Coxcy, North Fork of Bear Creek, Deckerd Flat, Melstone, or Skytop. The final terminal decision points were based on longitude, and both choices led to the same prediction, and this tree could have been pruned to three branches. The tree correctly predicted the presence of *L. dalmatica* in 76% of the cases and had a residual deviance of 0.97.

The initial classification tree produced for *B. tectorum* presence had nine terminal nodes and cross validation indicated that model was overfit and the lowest deviance was at seven nodes. The variables that were included in the pruned tree were percent cover of grass, latitude, longitude, slope, and aspect. The tree predicted the presence of *B. tectorum* in plots with greater than 0.5% grass cover and a longitude greater than -110.633 or in plots with more than 0.5% grass cover, a longitude less than -110.633, latitude less than 45.0586, and a slope greater than 16%. The tree correctly predicted the presence of *B. tectorum* in 81% of the cases and had a residual deviance of 0.76.

The initial classification tree produced for *A. tridentata* presence had five terminal nodes and cross validation suggested pruning the tree at node 4. The variables that were

included in the pruned tree were percent cover of shrubs, Site as a categorical variable, and elevation. The model predicted the presence of *A. tridentata* in plots with more than 0.5% cover of shrubs, that were at North Fork of Bear Creek, Phelps Creek, or Skytop. At the sites where *A. tridentata* is predicted to be present, the final decision is based on elevation, but both choices led to the prediction of *A. tridentata* being present. This node could be pruned out from an ecological sense, but it did lead to a small decrease in deviance. The tree correctly predicted the presence of *A. tridentata* in 74% of the cases and had a residual deviance of 0.11.

Regression Trees

The original regression tree for *L. dalmatica* had 11 terminal nodes. The cross validation of that tree indicated the lowest deviance was obtained at five nodes. The variables important in estimating cover for *L. dalmatica* in the pruned tree were latitude, site as a categorical variable, percent cover of forbs, and aspect. The model predicted the highest *L. dalmatica* cover at higher latitude plots with over 3.5% forb cover and an aspect more southerly than east-south east. The lowest toadflax cover was predicted for sites at lower latitudes, and for Phelps Creek in particular. The model residual mean deviance is 0.8307 with slightly skewed residuals.

The full regression tree for *B. tectorum* had 10 nodes, but the plot of the cross validation suggested that to avoid over fitting, the tree should be pruned back to seven nodes. The variables included in the pruned tree were longitude, latitude, total percent cover of grasses per plot, and slope. The tree predicted the highest *B. tectorum* cover at plots that have a longitude lower than -110.633° and a latitude less than 45.0586° that

also have over 18% grass cover, or in plots with higher longitudes that have more than 13.5% total grass cover and slopes steeper than 22.5%. The residual mean deviance for this model was 8.4.

The full regression tree for *A. tridentata* had seven terminal nodes, and the most parsimonious tree suggested by the cross validation had six nodes. The three variables included in the model were total percent cover of shrubs per plot, latitude, and total percent cover of grass per plot. The model predicted the highest *A. tridentata* cover in plots that have more than 27.5% total shrub cover, with less than 0.5% grass cover. The second highest abundance was predicted for plots with high shrub cover and grass cover over 0.5%. The lowest *A. tridentata* cover (0%) was predicted for plots with between 9.5 and 27.5% shrub cover and a latitude over 45.0686°. The residual mean deviance for the model was 3.5%.

Discussion

For *L. dalmatica*, the GAM containing smoothed elevation and slope, and the categorical variable site had a significantly smaller residual deviance than the quadratic GLM with the same variables (P(>|Chi|) 1.231e-17). Although the residual deviance was lower in the GAM model, both the GLM and the GAM predicted the presence or absence of *L. dalmatica* correctly 70% of the time. The classification tree had a lower residual deviance (0.96) than either the GLM (1.18) or the GAM (1.12) and had a higher correct prediction rate at 75% than the GLM or the GAM, indicating it was the best model for predicting where *L. dalmatica* would occur. The only variable that was in the classification tree as well as the GLM and GAM was site.

For *B. tectorum*, when residual deviance for each model type was divided by the total number of plots that were considered for the model, the classification tree had a lower residual deviance (0.78) than either the GLM (0.98) or the GAM (0.90). The classification tree also had the highest percent correct prediction at 81% indicating it was the best model for predicting the presence or absence of *B. tectorum*. The only variable that was represented in both the Classification trees and the GLM/GAM was slope.

Based on residual deviance and percent correct classification, the classification tree was the best model for predicting the presence or absence of *A. tridentata*. Neither the GLM nor the GAM predicted the presence of *A. tridentata* in any plots even though we recorded *A. tridentata* in 128 plots (6% of the plots included in the analysis). The classification tree for *A. tridentata* shared two of the three variables that were in the GLM and GAM models. The results from the *A. tridentata* GLM and GAM models underscore the challenges associated with constructing predictive models for species encountered infrequently but in high abundances when they are encountered.

It is not surprising that the classification trees were better predictors of presence or absence because the tree for each species included a variable that incorporated the abundance of that species; total percent forb cover, total percent grass cover, and total percent shrub cover. For each tree the first decision was whether there was more or less than 0.5% cover for the functional group the species being modeled belonged to. The next steps for each weed would be to construct GLM and GAM models using the variables that were used in the classification trees and to create classification trees that exclude the functional group cover variables that the species being modeled belongs to.

For *L. dalmatica* and *A. tridentata*, the regression trees had the lowest root square error of the three model types, however the differences between models were minimal for *L. dalmatica* (Table 28). For *A. tridentata* the differences were striking. The GAM had the lowest root square error of the three models for *B. tectorum* (Table 28). The variables used in the regression trees differed from those used in the GLMs and GAMs for all three species. The regression trees for each species also all included the total percent cover for the functional class to which the species being modeled belonged.

Incorporating the functional class variables in classification and regression can be circular for use in predicting abundance of a target species. However, from a sampling logistics point of view, it takes less time in the field to estimate functional class percent cover than percent cover for each individual species. If the model fit is good, a model could be used to predict or estimate presence or abundance of species of interest with data collected at the functional group level. However, in this study the classification and regression trees explained only a small portion of the deviance in the data despite having the best fit for presence-absence data for all three species and for abundance data for *L. dalmatica* and *B. tectorum*.

From a biological control standpoint, the most interesting result was that the biocontrol related environmental variables (number of agents released and distance from release) were not important for predicting presence-absence or abundance of *L. dalmatica*. Those variables were included in model statements for both classification and regression trees but the resulting decision trees did not use those variables. This may indicate that the agent population has not reached a level where it is impacting *L. dalmatica* abundance. In British Columbia it took four years for agents to reach outbreak

population levels and strongly impact toadflax abundance (De Clerck-Floate and Miller 2002, Carney 2003). The oldest releases in this study were only two years old when the data used in these models were collected. Detailed analyses of insect populations presented in Chapter 4 indicate that biological control agent populations were still low and could not be linked to *L. dalmatica* abundance changes even with an additional year of data.

Table 25 Explanatory variables tested with GLM models for prediction of presence/absence and abundance of *Linaria dalmatica*, *Bromus tectorum*, and *Artemesia tridentata*.

Model	
1	Elevation
2	Elevation + Slope
3	Elevation + Elevation^2 + Slope + Slope^2
4	Elevation + Elevation^2
5	Total vegetation cover
6	Total vegetation cover + (Total vegetation cover)^2
7	Species Richness
8	Species Richness + (Species Richness)^2
9	Model 6 + Model 8
10	Site

Table 26 Akaike's information criterion (AIC) for generalized linear models created for the presence-absence data for *Linaria dalmatica*, *Bromus tectorum*, and *Artemesia tridentata*.

	AIC values		
Model	*L. dalmatica*	*B. tectorum*	*A. tridentata*
1	2243.5	2371.1	780.4
2	2440.1	2347.8	780.8
3	2375.2	2230.9	776.4
4	2396.8	2263.0	777.2
5	2674.8	2403.9	889.0
6	2673.3	2391.2	877.8
7	2555.5	2390.5	944.4
8	2555.2	2362.4	939.3
9	2411.0	2360.6	873.7
10	2340.8	2126.9	779.7
11	2312.6	1942.0	772.4

Table 27 Akaike's information criterion (AIC) for general linear models created with abundance (as percent cover) for *Linaria dalmatica*, *Bromus tectorum*, and *Artemesia tridentata*.

	AIC values		
Model	*L. dalmatica*	*B. tectorum*	*A. tridentata*
1	4572.5	10682.5	6795.0
2	4524.6	10618.4	6785.6
3	4384.7	10180.0	6692.5
4	4487.0	10345.4	6768.7
5	4888.6	10058.5	5978.6
6	4890.5	9910.1	5539.0
7	4864.0	10792.7	8803.8
8	4855.4	10747.0	8792.0
9	4820.4	9623.9	5161.7
10	4382.9	9486.3	6911.0

Table 28 Root square error for three model types for *Linaria dalmatica*, *Bromus tectorum*, and *Artemesia tridentata*. The models considered were general linear models (GLM), general additive models (GAM), and regression trees (Tree).

	Model type		
Species	GLM	GAM	Tree
L. dalmatica	0.024	0.023	0.022
B. tectorum	0.073	0.063	0.071
A. tridentate	0.078	0.062	0.046

s

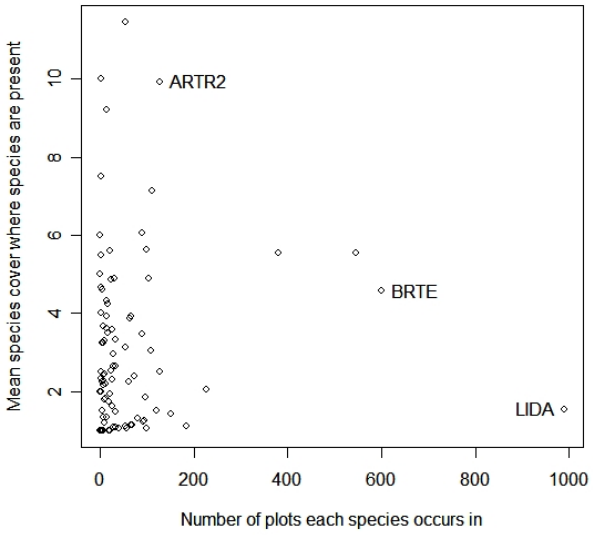

Figure 27 Frequency and mean cover, when present, of the three focal species in this study. The focal species were big *A. tridentata* (ARTR2), *B. tectorum* (BRTE) and *L. dalmatica* (LIDA).

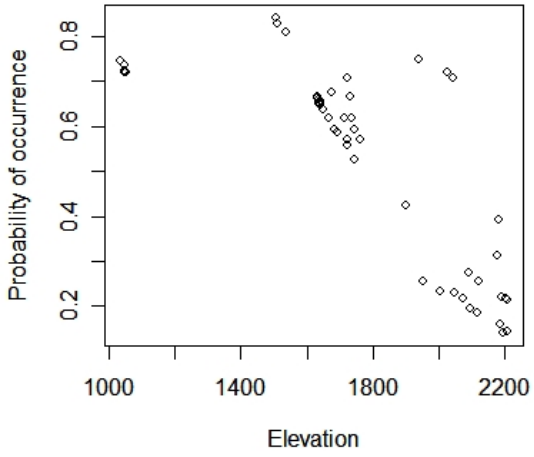

Figure 28 Fitted model of probability of occurrence of *Linaria dalmatica* plotted against elevation. The variability of points at a given elevation is due to slope of the plots and the site the plot is located.

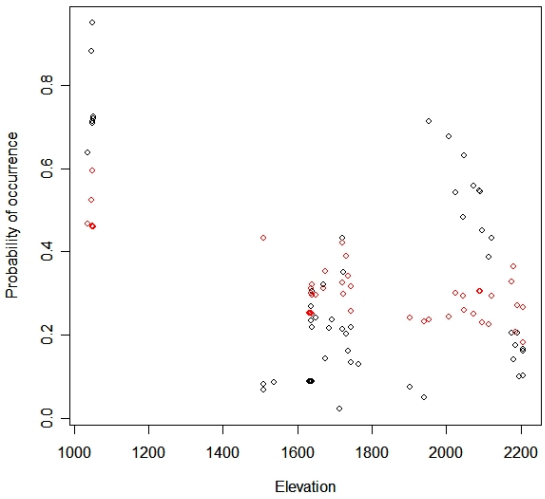

Figure 29 Fitted model of probability of occurrence of *Bromus tectorum* plotted against elevation. The variability of points at a given elevation is due to slope of the plots and the site the plot is located.

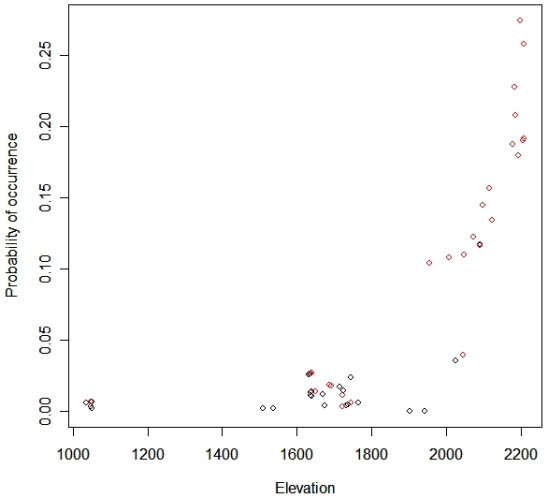

Figure 30 Fitted model of probability of occurrence of *Artemesia tridentata* brush plotted against elevation. The variability of points at a given elevation are due to slope of the plots and the site the plot is located.

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