Growth Inhibition of Dalmatian Toadflax, *Linaria dalmatica* (L.) Miller, in Response to Herbivory by the Biological Control Agent *Mecinus janthinus* Germar[1]

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Abstract Our study reports the results of field and garden experiments designed to quantitatively evaluate the impact of herbivory by a weed biological control agent, the stem-mining weevil *Mecinus janthinus* Germar, on the growth of its exotic host Dalmatian toadflax, *Linaria dalmatica* (L.) Miller. Herbivory by *M. janthinus* under both natural and manipulated environmental conditions inhibited *L. dalmatica* growth. Reductions in stem length, biomass, and growth were more pronounced for plants subjected to both exophagous (adult) and endophagous (larval) feeding injury than for plants exposed only to adult folivory. Decreases we observed in root biomass could additionally inhibit shoot production from lateral roots. This provides a plausible mechanism explaining anecdotal reports correlating the reduced spread of *L. dalmatica* with attack by *M. janthinus*. Our results indicate that *L. dalmatica* growth is compromised once a threshold density equivalent to 5 *M. janthinus* larvae per stem is exceeded. The consistency of growth responses observed in this study suggests that a mechanistic/quantitative approach, such as measuring the impact of *M. janthinus* herbivory on *L. dalmatica*, is a robust and relevant method for postrelease evaluations of weed biocontrol efficacy.

Key Words herbivory, plant-insect interactions, biological control, weed, Curculionidae

Herbivory affects plants at multiple demographic levels (Louda et al. 1990, Crawley 1989a, Karban and Baldwin 1997). At the level of the individual plant, insect herbivory has been linked to reductions in height, biomass, and growth rates (Louda et al. 1990); changes in root:shoot ratios (Vranjic and Gullan 1990); altered photosynthetic activity (Detling et al. 1979, Peterson 2001, Retuerto et al. 2004, Peterson et al. 2005); and reductions in reproductive rates (Notzold et al. 1998, Pratt et al. 2005).

Plasticity in plant response to herbivory necessitates the simultaneous evaluation of a number of plant performance variables under a range of injury levels (Foggo 1996). Garden experiments using plants grown from seed collected from a common source and cultivated under controlled conditions can be used to separate the effect of herbivore injury level from plant growth history and environmental conditions. Results obtained under controlled experimental conditions in a garden study may,

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however, have limited applicability due to the range of environmental variability encountered under field conditions (Cipollini and Bergelson 2002).

Dalmatian toadflax, *Linaria dalmatica* (L.) Miller (Plantaginaceae), is a self-incompatible, insect-pollinated, erect, short-lived perennial, invasive herb native to eastern Europe and the Mediterranean (Bruun 1937). *Linaria dalmatica* primarily invades natural areas, reducing the carrying capacity and value of U.S. and Canadian rangelands west of the 100th meridian where it forms dense stands (De Clerck-Floate and Harris 2002). Individual crowns produce 1 - 20 reproductive shoots averaging 40 - 100 cm tall, along with 3 - 40 decumbent to weakly ascending succulent vegetative stems (Alex 1962). Mature plants with 10 - 15 reproductive stems produce an estimated 400,000 - 500,000 seeds under ideal conditions (Robocker 1970, Lange and Wolfe 1954). Although the floral stems die at the end of each growing season, prostrate vegetative stems emerging from the crown and lateral roots in late summer and early fall persist over winter into the following spring (Robocker et al. 1972). Individual crowns live 1 - 3.5 years, but individual clones have the potential to persist indefinitely due to their ability to produce new crowns from lateral roots (Robocker 1970).

The toadflax stem mining weevil, *Mecinus janthinus* Germar (Coleoptera: Curculionidae), is native to south and central Europe to Kazakhstan, over an elevational range from sea level to subalpine (Jeanneret and Schroeder 1992). Adults emerge from late March to early April, and feed on host leaves and shoot meristems for a short period before mating. Oviposition begins by the end of May or beginning of June and continues into midJuly. Females chew a small cavity into which eggs are deposited; calluses eventually develop over oviposition sites (De Clerck-Floate and Miller 2002). Mining by *M. janthinus* larvae, especially when it occurs at high population densities, suppresses flowering and causes premature wilting of stems; this is exacerbated in drought-stressed plants (Jeanneret and Schroeder 1992). Suppression of flowering and stunting of shoot growth has been attributed to adult weevil feeding only (De Clerck-Floate and Miller 2002).

The effects of larval mining on shoot and root biomass were studied in an experiment where single pairs of adult weevils were caged on individual plants for a month during the weevils' peak oviposition period (Saner et al. 1994). Larval mining and adult feeding/oviposition activities were hypothesized to have led to water stress in *L. dalmatica*, in turn resulting in decreased shoot biomass and shoot:root ratio, increased proportion of dead prostrate vegetative (vs. erect reproductive) stems, but with no apparent impact on root biomass. An earlier study found that herbivory could, in effect, act as an energy sink in *L. dalmatica* through injury to the prostrate (rosette) shoots which produce the carbohydrates essential to crown persistence that are sequestered between growing seasons in the taproot (Robocker 1974). More recently, Hellström et al. (2006) found that removing the apex from shoots of a closely-related species, *Linaria vulgaris* Miller, had stronger negative effects on ramet performance than non-apex defoliation, possibly due to hormonal consequences and reproductive losses correlated with apical meristem removal. However, none of these earlier studies attempted to link manipulated variations in levels of herbivory to measured differences in host plant growth parameters.

Understanding plant responses to herbivory is key to accurately predicting trends and trajectories in population and community dynamics in both natural and agricultural ecosystems (Peterson 2001, Crawley 1989b). Despite the obvious utility of this approach, relatively few studies have been conducted on growth and physiological responses of invasive weeds to injury by insect herbivores, especially endophagous

species (Peterson et al. 2005, Carson et al. 2008). Regulatory demands for evidence supporting candidate agent safety undoubtedly influence the proportion of time and budget allocated to elective studies of agent efficacy (Raghu et al. 2006). Measuring fundamental host responses to specialized herbivory at the level of individual plants represents a strategic departure from the less mechanistic approaches now conventionally used in the postrelease evaluation of weed biological control agent efficacy. Such conventional approaches model population and community dynamics, fulfilling demands for holistic, system-wide postrelease impact assessments (Raghu et al. 2007, Kriticos et al. 2009, Morin et al. 2009). This may explain why few studies seek to explicitly quantify the influence of feeding injury by candidate or approved agents on specific target weed growth responses (Raghu et al. 2007).

Therefore, the objectives of this study were to: (1) determine the impacts of different levels of *M. janthinus* feeding injury on growth of *L. dalmatica*, and (2) determine if there is a threshold injury level to *M. janthinus* feeding injury that must be reached before changes in growth are realized. Experiments were conducted in both garden and field settings to evaluate the effects of different levels of *M. janthinus* injury on *L. dalmatica* growth.

Materials and Methods

The adult *M. janthinus* weevils used in all experiments were field-collected in late May each year in eastern Washington by U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine (USDA APHIS PPQ) personnel and express-shipped to Bozeman, MT, USA. Weevils were stored under refrigeration upon arrival then individually sorted by gender, using external rostral (Schat et al. 2007) and leg characters (Carney et al. 2004). Adult density treatments (0, 2, 8, or 16 pairs of adults per plant) were applied within 48 h of their arrival in Bozeman.

Garden experiments. Experiments were conducted within a fenced enclosure on the grounds of the Bozeman Forestry Sciences Laboratory (USDA Forest Service, Rocky Mountain Research Station). The randomized complete block experiment was replicated annually in 2005 and 2006 using new plants and insects each year. Each year, a total of 144 preflowering plants were randomly assigned to 1 of the 4 adult *M. janthinus* density treatments.

Plants used in the 2005 experiment were grown from rootstock originating from mature plants growing in a single drainage in south-central Montana. Because of variability in size of the plants in 2005, for the 2006 experiment, plants were cultivated in the greenhouse from seed collected at an extensive *L. dalmatica* infestation in southwestern Montana. Plants were cultivated for the duration of each study period in 4-L pots filled with 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, USA). The steam sterilized MSU mix was a 1:1:1 ratio by volume of mineral soil, Canadian sphagnum peat moss, and washed concrete sand amended with 0.45 kg of Aqua-Gro 2000G wetting agent/m^3 growing medium.

Plants were randomly assigned to blocks and within-block positions. Blocks were separated by at least 1 m; each block contained 36 plants arranged in 6 rows of 6 pots. Each pot was embedded in the ground with the soil level at the rim, resulting in a soil depth of $\cong$ 21 cm. Pots were watered daily via automatic drip irrigation. Each pot/plant was equipped with a nylon size 70-mesh sleeve cage that was secured with elastic

cording and bamboo stakes to prop up the plant. Irrigation continued while the cages were present. Adult *M. janthinus* were caged on individual plants for approx. a 1 wk oviposition period before cages and adults were removed. Adult weevils were carefully removed using forceps and counted to ensure that all had been recaptured and accounted for from each plant when the cages were removed.

For both years of the study, data were recorded on 3 dates (hereafter referred to as 'harvest dates') from 3 randomly selected plants per each of the 4 adult density treatments, from each of the 4 blocks (hereafter referred to as 'harvest plants'). On each harvest date all leaves were carefully removed from the stems of the harvest plants and examined for oviposition scars. After the number of oviposition scars per harvest plant was recorded, the 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. From these, total densities per stem and total density per harvest plant were recorded.

Garden study measurements. In 2005, the length (measured to the nearest 0.5 cm) of the tallest, shortest, and representative (i.e., estimated average) stem in each pot was recorded on the day the adult weevils were removed (11 June), and weekly thereafter through 31 July, and for the last time when the experiment was terminated (24 August). In 2006, all stems were numbered and measured approximately weekly, beginning when the adults were removed from the plants (9 June) through 31 August.

In addition, on each of the 3 harvest dates, all stems from the randomly selected harvest plants were clipped at soil level and refrigerated until they could be dissected. Soil was then carefully loosened and removed from the harvest plant roots. The roots were rinsed in cool water to remove the remaining soil, placed in paper bags, and dried to constant weight. Dissected stems were separated into reproductive (buds, flowers, and seed pods) and nonreproductive biomass before drying to a constant weight. Dried plant components were weighed to the nearest 0.01 g. Roots, stems, leaves, buds, flowers, and seed pods were weighed separately. To prevent underestimating seed pod biomass, plants were examined daily, and dried seed pods that were beginning to open were collected and stored in labeled coin envelopes until the plants were harvested.

Field experiments. Field experiments were established and replicated in space and time at 2 field sites in 2006 and 2007, both to corroborate garden study results and to incorporate the influence of less controlled environmental conditions typically encountered during weed operational management. The experimental design was similar in both years, although in 2007 the number of replicates per adult density treatment was increased and 2 additional adult density treatments were included. One of the 2 sites used in 2006 was used again in 2007 (Pond Flat), with a new site selected for the second 2007 experimental location.

All 3 sites used over the 2-yr field study were located near Gardiner, MT, USA. In 2006, the study was conducted at Pond Flat (N45°02.61', W110°40.09', 1897m) and Eagle Creek (N45°02.96', W110°40.57', 1922m). The 2007 study plots were located at the Pond Flat site and Slope Creek (N45°03.49', W110°38.84', 1935m). The second study plot was set up in Slope Creek in 2007 because the Eagle Creek site lacked the number of host plants needed to accommodate the additional treatments and replicates per treatment added in the second year of the study. Study sites used in both years were located in close in proximity to each other and similar in elevation.

Treatments were restricted to plants with a single reproductive stem that was randomly selected from among the plant stand at each study location. Each of the selected study plants was randomly assigned 1 of 3 adult density treatments in 2006, or 1 of 5 adult density treatments in 2007. Study plants were individually caged using the same materials as described for the garden experiments and exposed to feeding and oviposition by *M. janthinus* adults for approx. 1 wk before insects and cages were removed. Stem length was measured every second week throughout the summer until the plants senesced. Treatment plants were harvested after the last measurement event and dissected to determine the number of larvae, pupae, and adults in each.

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 (2 males and 2 females), and a high adult and larval injury treatment (16 males and 16 females). A low adult-only injury (4 males) and a high adult-only injury (32 males) treatment was added in 2007.

Field study measurements. Stem lengths were measured to the nearest 0.5 cm, beginning the week the adult weevils were applied. Measurements were repeated when the adult weevils were removed and every 2 wks 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. 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 and hatched progeny (dead and live larvae, pupae, and F1 adults) were counted.

Data analysis. All data analyses were conducted using SAS 9.0 (SAS Institute Inc., Cary, NC, USA) software. The PROC-GLM procedure was used to determine if varying the treatment densities of *M. janthinus* adults produced differences in the number of oviposition scars, eggs, and weevil progeny developing within garden or field study stems. Where analyses indicated significant effects ($\alpha = 0.05$), posthoc Tukey's Studentized Range Tests were used to test for differences between groups. Tukey's test was chosen because of its conservative nature, its ability to handle uneven sample sizes, and its control of experiment-wide type I error (Montgomery 1997).

The number of oviposition scars was used as a partial estimate of injury at all 3 garden study harvest dates and for the single field study harvest date. Egg number was used as an additional estimate of injury on the first garden harvest date. Larvae and 'post larvae' (a variable defined as the sum of pupae and F1 adults) were used as additional estimates of injury at the second garden harvest date. For the garden and field harvests at the end of the season, the total number of 'post eggs' (a variable defined as the sum of larvae, pupae, and F1 adults) was used as the measure of feeding injury.

For both the garden and field experiments, initial and final lengths were used to calculate the changes in stem length for the entire season. This value was then converted to a net relative change in stem length based on initial stem length [(final length – initial length)/initial length]. Relative lengths are typically calculated using natural log transformed data; however, several of our adult density treatments actually resulted in negative changes. To accommodate these negative changes, relative changes in stem length were calculated in 2 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 among blocks and adult density treatments in the garden study. Growth data from the garden experiment in 2006 were excluded due to unplanned plant damage. Generalized linear models also were used to examine the relationship between injury level and change in stem length. For the field study, the PROC-GLM procedure was additionally used to assess differences between study sites and adult density treatments (coded as a categorical variable), which were determined using posthoc Tukey comparisons. Sites were tested separately where significant interactions for site-by-adult density treatments occurred.

Weight data were natural log transformed and compared between adult density treatments and harvest dates using PROC-GLM with a posthoc Tukey test to determine the impact of herbivory on plant biomass accumulation. 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 number of *M. janthinus* dissected out of the plants was log transformed and PROC-GLM models including block and adult density treatment were used to test the effects of insect density on plant biomass.

Results

Insect responses. In the garden experiment, varying the adult *M. janthinus* treatment density led to quantitative differences in specific injury measures, such as the number of oviposition scars, larvae, and post larvae dissected from the treated *L. dalmatica* plants (Table 1). Treatment density affected the number of oviposition scars more than the density of post egg progeny, for both years of the study. Differences in the number of oviposition scars recorded per plant were correlated with harvest date and density treatment in both 2005 ($F = 7.28$; $df = 2,134$; $P = 0.001$ and $F = 80.61$; $df = 134$; $P < 0.0001$, respectively) and 2006 ($F = 7.59$; $df = 2, 135$; $P = 0.0007$ and $F = 233.04$; $df = 3,135$; $P < 0.0001$, respectively). In both years of the field study, varying the number of adult *M. janthinus* caged on stems led to differences in oviposition scar and post egg progeny density ($P < 0.0001$ for each model tested) (Table 2).

Plant growth and biomass responses – garden study. Although trends were consistent across years, the 2005 experiment data are more complete than the 2006 data because in the second year the tips of several stems were broken off the plants late in the growing season {Schat 2008}. Consequently, results are only presented here for the 2005 garden experiment. No density treatment or block differences were detected in initial *L. dalmatica* stem lengths measured on the day the adult weevils were applied. Increasing the number of adult weevils caged on the plants led to decreased relative plant growth (Fig. 1). Net relative change in stem length was significantly affected by treatment density ($F = 5.76$; $df = 3,37$; $P = 0.0024$) and block ($F = 9.05$; $df = 3, 37$; $P = 0.0001$). A negative relationship was additionally detected between net relative growth and injury ($F = 5.68$; $df = 1,39$; $P = 0.022$).

Differences in root biomass (Fig. 2) were influenced by density treatment ($F = 5.61$; $df = 3,135$; $P = 0.0012$) but not harvest date (date when data reported) ($F = 0.09$; $df = 2,135$; $P = 0.9174$). Conversely, stem biomass (Fig. 2) was affected by both density

Table 1. Garden study (2005 - 2006): Effect of *Mecinus janthinus* adult density treatments on the number of oviposition scars, larvae, and post larvae dissected from *Linaria dalmatica* stems. Pairs of adult male-female weevils were caged on individual plants according to a randomly assigned density treatment: control (0 pr), low (2 pr), medium (8 pr), or high (16 pr).

		Number Per Plant Mean ± SE		
Treatment	Year	Oviposition Scars	Larvae	Post Larvae
Control (0 pr)	2005	2.3 ± 0.87 a*	1.9 ± 0.96 a	0.6 ± 0.5 a
Low (2 pr)		17.7 ± 1.44 b	11.3 ± 1.16 a	4.2 ± 1.12 ab
Medium (8 pr)		53.2 ± 4.18 c	26.9 ± 4.04 b	8.7 ± 2.33 b
High (16 pr)		69.6 ± 5.73 d	36.3 ± 5.12 b	10.2 ± 3.26 b
Control (0 pr)	2006	6.2 ± 1.42 a	3.1 ± 0.97 a	2.8 ± 1.2 a
Low (2 pr)		21.7 ± 2.02 b	10.7 ± 1.91 b	8.9 ± 1.64 ab
Medium (8 pr)		58.6 ± 2.66 c	29.8 ± 2.43 c	14.3 ± 2.17 bc
High (16 pr)		94.1 ± 4.18 d	48.3 ± 4.98 c	17.4 ± 3.73 c

* Letters indicate significant differences detected among density treatments within study year and each injury/stage type ($\alpha = 0.05$).

treatment ($F = 5.99$; $df = 3,135$; $P = 0.0007$) and harvest date ($F = 9.26$; $df = 2,135$; $P = 0.0002$). The stem biomass of control plants was greater at the third harvest date than for plants subjected to the high density treatment (Fig. 2). No other relationships were evident between the various measures of injury and the biomass of *L. dalmatica* roots, stems, or reproductive parts.

Linaria dalmatica root:shoot ratios (Fig. 3) did not differ by block ($F = 1.56$; $df = 3,135$; $P = 0.2030$) or weevil density treatment ($F = 0.26$; $df = 3,135$; $P = 0.8525$), although they did change with harvest date ($F = 25.34$; $df = 2,135$; $P < 0.0001$). Reproductive biomass:root biomass ratios differed between density treatments ($F = 8.02$; $df = 3,135$; $P < 0.0001$) and harvest date ($F = 25.39$ $df = 2,135$; $P < 0.0001$), but not between blocks ($F = 0.09$; $df = 3,135$; $P = 0.9629$) (Fig. 3). The same pattern was observed for the reproductive biomass:shoot biomass ratios (Fig. 3). Reproductive biomass:shoot biomass ratios differed between density treatments ($F = 9.31$; $df = 3,135$; $P < 0.0001$) and harvest dates ($F = 27.07$, $df = 2,135$; $P < 0.0001$), but not between blocks ($F = 0.04$; $df = 3,135$; $P = 0.9877$).

Plant growth and biomass responses – field study. Initial stem length was greater at the Pond Flat site than either of the other field study sites (Eagle Campground - 2006: $F = 4.51$; $df = 1,26$; $P = 0.0433$; Slope Creek - 2007: $F = 11.08$; $df = 1,104$; $P = 0.0012$). No significant within-site differences in initial stem length were detected before application of the various weevil density treatments (2006: $F = 0.96$; $df = 2,26$; $P = 0.3954$; 2007: $F = 0.62$; $df = 4,104$; $P = 0.6507$).

Table 2. Field study (2006-2007): Effect of *Mecinus janthinus* adult density treatments on number of oviposition scars (A) and postegg progeny (B) dissected from *Linaria dalmatica* stems. In 2006, pairs of adult male-female weevils were caged on individual stems according to a randomly assigned density treatment: control (0 pr), low (2 pr), or high (16 pr). In 2007, adult weevils were caged on individual stems according to a randomly assigned density category, number and gender treatment: control (0 pr), low mix (2 pr), low male (4 males), high mix (16 pr), and high male (32 males).

		Number Per Plant Mean ± SE	
Treatment	Year/Location	Oviposition Scars	Post Eggs
Control (0 pr)	2006	0 ± 0 a*	0 ± 0 a
Low (2 pr)		14.1 ± 1.67 b	9.8 ± 1.54 a
High (16 pr)		77.1 ± 6.65 c	36.9 ± 5.02 b
Control (0 pr)	2007/Pond Flat	0 ± 0 a	0 ± 0 a
Low Mix (2 pr)		24.5 ± 2.22 b	16.2 ± 1.64 b
Low Male (4 males)		9.1 ± 1.5 ab	1.4 ± 1.3 a
High Mix (16 pr)		146.6 ± 9.85 c	69.6 ± 7.17 c
High Male (32 males)		45.6 ± 3.41 d	4.7 ± 2.32 a
Control (0 pr)	2007/Slope Creek	0 ± 0 a	0 ± 0 a
Low Mix (2 pr)		23.5 ± 1.09 b	18.4 ± 0.8 b
Low Male (4 males)		6.6 ± 0.94 a	0 ± 0 a
High Mix (16 pr)		127.4 ± 6.55 c	68.9 ± 6.4 c
High Male (32 males)		32.8 ± 4.81 b	1.5 ± 0.89 a

* Letters indicate significant differences detected among density treatments within study year and each injury/stage type ($\alpha = 0.05$).

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 in the analysis (Table 3). In 2006, high density treatment stems decreased in length over the measurement period (Fig. 4; Table 3). Differences in net relative change in stem length were detected in 2007 between sites ($F = 8.22$; $df = 1,103$; $P = 0.0005$) and density treatments (Fig. 4, Table 3). Net relative change in the length of control stems increased significantly more than for stems exposed to any *M. janthinus* treatment density on either study site in 2006 or 2007 (Fig. 4, Table 3). Stems subjected to either of the high adult density treatments (16 male-female pairs or 32 males)

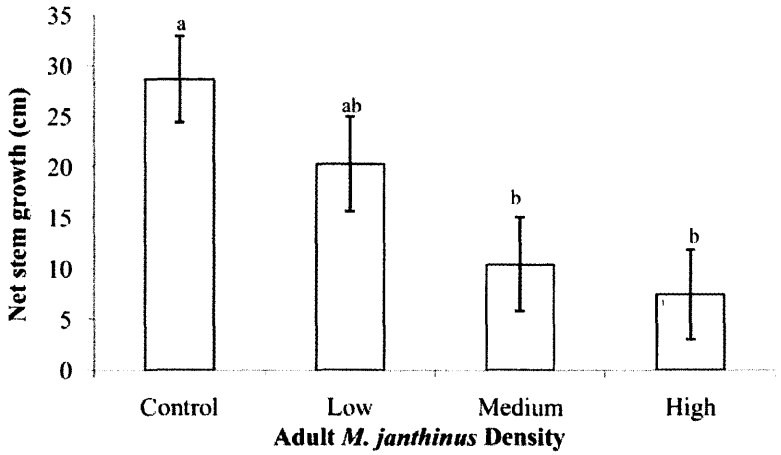

Fig. 1. Garden study (2005): Effect of *Mecinus janthinus* density on *Linaria dalmatica* net stem growth. Male-female pairs of adult weevils were caged on individual study plants treated at a randomly selected density: control (0 pr), low (2 pr), medium (8 pr), or high (16 pr). Letters indicate significant differences detected among densities (α = 0.05).

sustained severe shoot tip injury (Fig. 4). However, the high density mixed-sex treatment exposing stems to both folivory (adult weevils) and stem mining (larval weevils) resulted in a net negative change in stem length over the course of the growing season (Fig. 4).

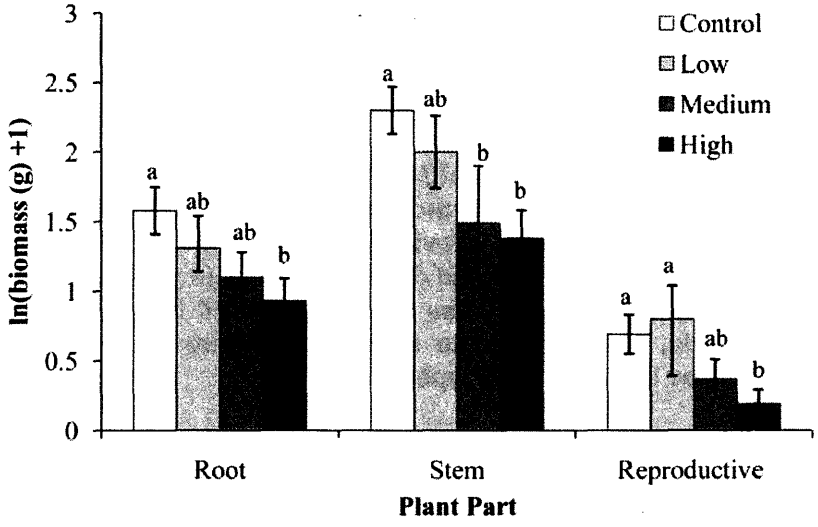

Fig. 2. Garden study (2005): Effect of *Mecinus janthinus* density on *Linaria dalmatica* root, stem or reproductive biomass at third harvest. Male-female pairs of adult weevils were caged on individual study plants treated at a randomly selected density: control (0 pr), low (2 pr), medium (8 pr), or high (16 pr). Letters indicate significant differences detected among densities (α = 0.05).

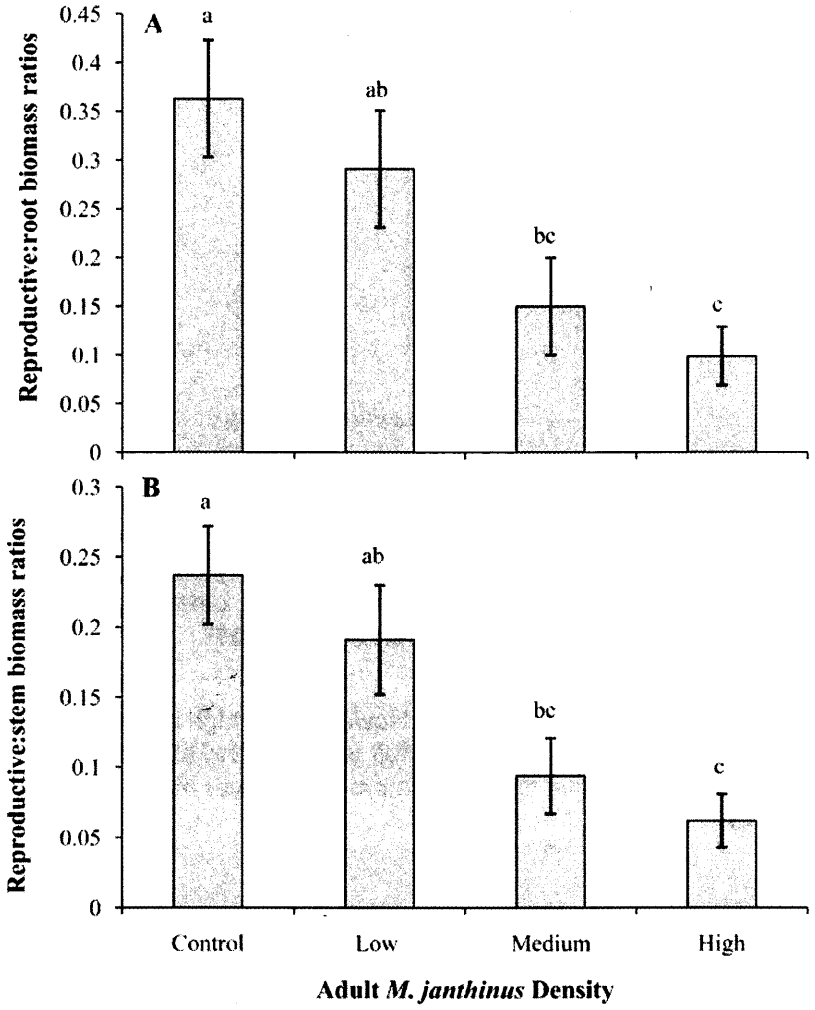

Fig. 3. Garden study (2005): Effect of *Mecinus janthinus* density on *Linaria dalmatica* biomass ratios, (A) reproductive:root and (B) reproductive:stem. Male-female pairs of adult weevils were caged on individual study plants treated at a randomly selected density: control (0 pr), low (2 pr), medium (8 pr), or high (16 pr). Biomass ratios calculated from natural log transformed data. Letters indicate significant differences detected among densities, by biomass ratio type ($\alpha = 0.05$).

Discussion

Host plant growth and biomass decreased as *M. janthinus* herbivory intensified. Reductions in plant growth and biomass observed in our study are consistent with the results of other studies examining the impact of folivores on plant growth (Jeanneret and Schroeder 1992, Louda et al. 1990, De Clerck-Floate and Miller 2002, Blossey and Schat 1997, Schat and Blossey 2005). We found that *L. dalmatica* plants exposed to a high density of adult weevils tended to have foliar feeding injury concentrated at the shoot tips; the brown and wilted tops generally withered away from the plants over

Table 3. Field study (2006 - 2007): Effect of *Mecinus janthinus* adult density treatments on net relative change in the length of *Linaria dalmatica* stems. Analysis was performed on pooled data in 2006 because there were no between site differences. Differences detected between sites in 2007 necessitated analyzing data from each site separately. 2006 treatments (male-female pairs only): control (0 pr), low (2 pr), or high (16 pr). 2007 density category and gender treatments (mix=male-female pair; male=males only): control (0 pr), low mix (2 pr), low male (4 males), high mix (16 pr), and high male (32 males).

Source	Sum of squares	*df*	*F*	*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

the growing season and reduced overall gains in stem length. The observed, consistent injury to apical meristems slows or prevents growth in injured stems and reduces normal differentiation and productivity of reproductive tissue.

Our study results reflect plant demographic trends frequently observed when well-established *M. janthinus* populations attain outbreak densities (Van Hezewijk et al. 2010). Intensified herbivory, typified by the focused feeding by adult weevils on apical meristems, may account for localized reductions in *L. dalmatica* density, stature, and reproductive output at outbreak sites (Jeanneret and Schroeder 1992, De Clerck-Floate and Miller 2002). Clipping studies indicated that *L. dalmatica* flowers in one flush and, therefore, cannot adequately compensate for damaged meristems by producing a second flush of flowers later in the season (Saner et al. 1994). Regrowth and flowering are known to be reduced in *L. dalmatica* stems that have been clipped in the spring (Robocker et al. 1972).

The decreases in stem biomass we report here are similar to results from an earlier study in which single pairs of adult weevils were caged on plants for a month (Saner et al. 1994). However, unlike our study, Saner et al. (1994) did not detect a change in root biomass. Root and reproductive biomass were both reduced in our high density treatment plants.

Patterns in *L. dalmatica* biomass allocation change with maturation from seedlings to reproductively mature plants (Coleman and McConnaughey 1995). Changes in resource allocations are frequently examined by comparing biomass ratios of component parts to other parts or to the whole across adult density treatments (Muller et al.

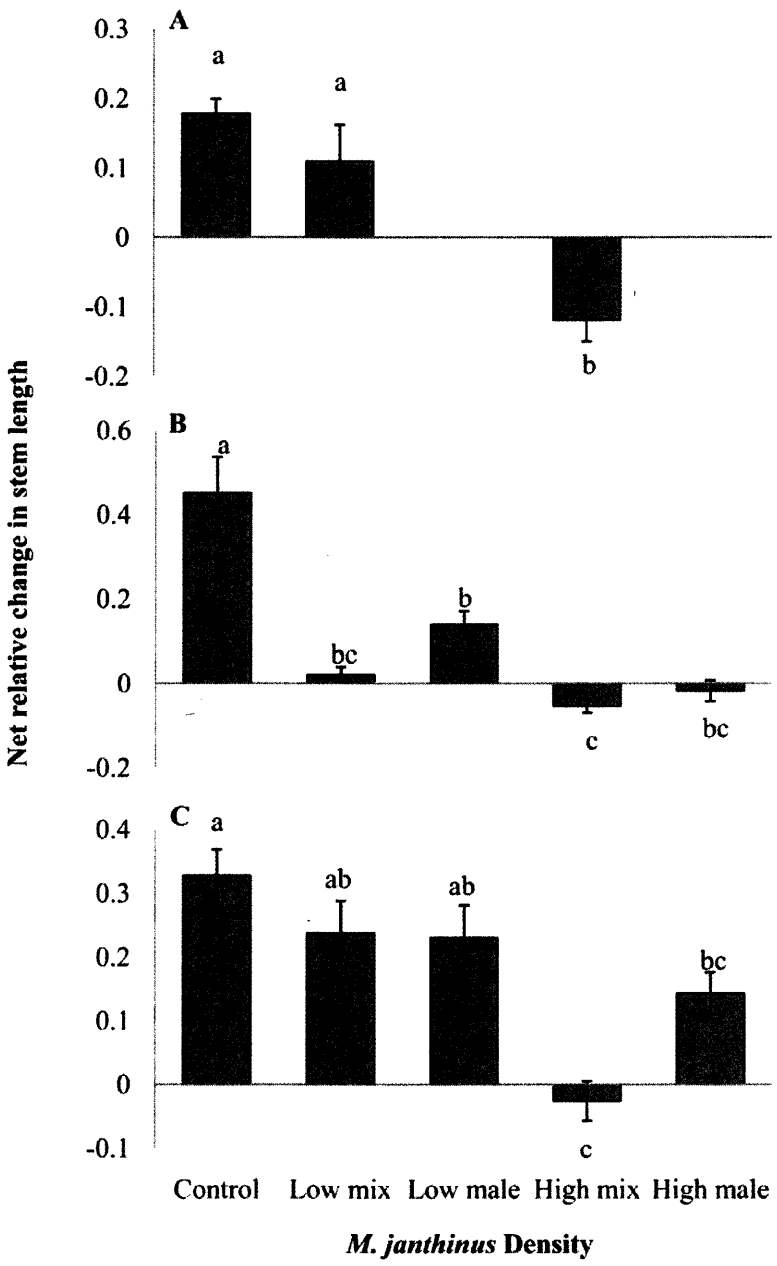

Fig. 4. Field study (2006 - 2007): Effect of *Mecinus janthinus* density on net relative change in *Linaria dalmatica* stem length. 2006 data pooled for both sites (A); 2007 Pond Flat (B) and Slope Creek (C). 2006 treatments (male-female pairs only): control (0 pr), low (2 pr), or high (16 pr). 2007 density category and gender treatments (mix=male-female pair; male=males only): control (0 pr), low mix (2 pr), low male (4 males), high mix (16 pr), and high male (32 males). Letters indicate significant differences detected among densities, by year or year and site (α = 0.05).

2000). The differences we observed in biomass ratios between harvests, where the shoot biomass increased with respect to root biomass as the growing season progressed, were anticipated. Differences detected in shoot:reproductive and root:reproductive biomass ratios between density treatments indicate that there was more shoot and root biomass relative to reproductive biomass in high density treatment plants than in control or low density treatment plants. This finding is consistent with the observation that plants in the high density treatments had meristem injury that could delay or reduce the ability of injured stems to produce flowers and seeds.

The results of our garden 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 be slowed by reducing seed set because of the low competitive ability of seedlings (Gates and Robocker 1960, Grieshop and Nowierski 2002) and the propensity of this species to produce clones from lateral roots (Robocker 1974). Consequently, the decrease in root biomass with increasing injury is a more likely mechanism for reducing spread of *L. dalmatica*.

Growth responses were consistent across years, locations, and modest differences in treatment designs. This consistency and the results from other studies conducted in Canada (De Clerck-Floate and Miller 2002) suggest that growth responses are a good predictor of the impacts of *M. janthinus* on *L. dalmatica*. 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) postegg stage insects per stem. Therefore, given that a sufficient density of adult insects at a site will cause injury equivalent to 5 postegg stage immature e insects per stem, *M. janthinus* has the potential to impact *L. dalmatica* growth in the field.

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