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Impact of *Mecinus janthinus* (Coleoptera: Curculionidae) on the growth and reproduction of *Linaria dalmatica* (Scrophulariaceae)

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Abstract

Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae), a native to the eastern Mediterranean and Black Sea regions of Europe and Asia, has invaded over one million hectares in the western United States and Canada, in habitats similar to its native range. Once established, the aggressive vegetative growth of the plant allows it to invade undisturbed habitats where it can out-compete most other vegetation, placing native plant communities at risk.

Biological control of *L. dalmatica* with *Mecinus janthinus* Thomson (Coleoptera: Curculionidae) has shown promise in the field. In both studies reported in this paper, the presence of insect attack reduced *L. dalmatica* plant growth and reduced plant reproductive potential. In a field sleeve cage study, insect-attacked stems were significantly shorter (18 cm) and had 50-70% fewer fruits and flowers than the control stems at the end of the study period. *M. janthinus* attacked stems showed little apical growth, fewer fruits and flowers, and lower stem biomass relative to control stems. Similar results were observed in the potted plant study where the influence of the extensive root system of the plant was eliminated. This negative impact by the insect is caused both by adult feeding in the apical portion of the plant and the physical destruction of the plant stem from larvae feeding. The decrease in the insect-attacked stem heights may also have an impact on seed dispersal from the mature reproductive structures.

A combination of decreased seed production through *M. janthinus* biological control and poor seedling competition in the moisture limited sites common to north-central Washington State and other similarly dry habitats may negatively influence *L. dalmatica* populations more than general models predict.

Dalmatian toadflax, *Linaria dalmatica* (L.) Mill. (Scrophulariaceae) was introduced in the United States as an ornamental by 1894 and first planted in Canada in 1901 (Vujnovic and Wein 1997). *L. dalmatica*, a native to the eastern Mediterranean and Black Sea regions of Europe and Asia, has invaded over one million hectares in the western United States and Canada. The invaded habitats are similar to its native range. Its closest relatives include yellow toadflax (*Linaria vulgaris* Mill.) and narrow leaved toadflax (*Linaria genistifolia* (L.) Mill.) also considered invasive species in North America (Sheley and Petroff 1999). Individual *L. dalmatica* plants are short-lived perennials and can survive up to four years (Robocker 1974), but stands can persist for long periods of time from

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vegetative propagation and prolific seed production (Robocker 1970). Seeds can remain dormant in soil for up to 10 years under field conditions.

Linaria dalmatica often invades well drained disturbed sites such as road cuts and overgrazed rangeland. Once established, the aggressive vegetative growth of the plant allows it to invade undisturbed habitats where it can out-compete most other vegetation, placing native plant communities at risk (Sheley and Petroff 1999). The plant is toxic to cattle (Vujnovic and Wein 1997) though sheep are reported to graze it without adverse effects (Sheley and Petroff 1999). Biological control of weeds is often unsuccessful because the control organism does not become established or fails to reduce the fitness of the target plant (McEvoy and Coombs 1999, Davis et al 2006). Biological control of *L. dalmatica* with *Mecinus janthinus* Thomson (Coleoptera: Curculionidae) has shown promise in the field. *M. janthinus* is a univoltine stem boring weevil specific to a small number of perennial *Linaria* species with stem diameters greater than 0.9 cm. Like its *Linaria* spp. host plants, *M. janthinus* is native to southern and central Europe and southern Russia and was first released in British Columbia, Canada in 1991 (McClay and De Clerck-Floate 2002). Monitoring of 22 release sites in British Columbia, Canada (1991-94) showed 100% establishment for the beetle and some of the sites achieved attack on 100 percent of stems after only three years (De Clerck-Floate and Harris 2002, Van Hezewijk et al. 2010). The damage to *L. dalmatica* stems by both adult and larval feeding has been described at both individual stem and stand levels in the field (De Clerck-Floate and Harris 2002).

While *M. janthinus* has successfully established, its role in controlling *L. dalmatica* has not been documented in the literature for the northwestern United States. In many field locations, attack may only occur on a small number of stems and the large root system of *L. dalmatica* may play a substantial role in compensatory growth in the plant after attack. Questions arise about root reserves and their influence on insect damaged *L. dalmatica* stems.

The purpose of the studies reported herein was to assess the impact of *M. janthinus* feeding on *L. dalmatica* growth and reproduction. In the field, single stems from a ramet were selected as the experimental unit to reduce plant-to-plant variation. A related study, utilizing a single stem from a potted rhizome, was initiated to suggest if resource sharing was evident between stems when only a single stem was attacked by the insect.

Materials and Methods

Sleeve cage study. The sleeve cage study was conducted at a municipal water supply well site, 341 m in elevation near Leavenworth, Washington, USA (47º 34.33'N, 120º 40.11'W) in an area approximately one hectare in size. The study location was composed of sand, gravel and cobbles and was a flat, highly mechanically disturbed area. Native plants were sparse and limited to some hardy shrubs. Dalmatian toadflax (*L. dalmatica*) was present throughout the site and *M. janthinus* was absent from the site.

Thirty-three pairs of stems were selected for the cage study on 1 and 3 June 2005. Each pair of stems was selected from the same ramet of stems and was similar in height (± 3 cm), composed of approximately equal stem diameters and numbers of flowers. Stem breakage resulted in eight of the replicates being removed from the study, leaving 25 remaining pairs. The likelihood that the paired stems were from the same genotype was very high since the stems were in close proximity to each other and *L. dalmatica* is a rhizomatous species. Before the stems were caged, stem height, number of flowers per stem, and number of fruits per stem were recorded. Sleeve cages, approximately 90 cm long and 30 cm wide, were placed over each stem and held around the stem with paper-covered wire twist ties.

All of the insects used in this study were collected at the USDA APHIS PPQ insectary near Danville, Washington (48º 59.31'N, 118º 30.29'W), two days before being placed in the cages in Leavenworth, a distance of 140 miles by direct route. Insects were stored in cardboard containers with *L. dalmatica* stems and moist sponge cubes in a cooler with ice until they were placed on the study plants. For each pair of stems, one stem was randomly selected to receive 12 adult *M. janthinus* in the sleeve cage and the second stem served as the control. All stems were caged for nine days. Cages and insects were then removed from the stems, with both stems in each set receiving the same amount of handling.

An additional 14 stems of similar size and height, selected from existing study ramets, served as uncaged controls to test for cage effects. These control stems were handled similarly to their caged counterparts. When the study was initiated, stem height, number of flowers per stem, and number of fruits per stem were recorded for the control stems.

Once cages and insects were removed, stem height, number of flowers per stem, and number of fruits per stem were recorded for all of the stems in the study, including the uncaged controls. All stems were allowed to grow without cages for 46-48 d. On July 28-29, 2005, all stems were cut, measured (stem height, number of flowers, number of fruits, number of branches, and the stem diameter at the base), and dissected to determine the number of *M. janthinus* larvae. Once *M. janthinus* larvae were counted and removed, stems were oven-dried for 48 h and the weight of each stem including fruits was measured to the closest 0.01 g.

Potted plant study. *Linaria dalmatica* rhizomes were selected from four locations in Chelan and Douglas County, Washington, USA in mid-March 2006. At each location, eight to 10 overwintering rosettes were collected leaving 30-60 cm lateral roots. Rosettes were packed in soil, wrapped in plastic, and kept in a cold dry storage area until the initiation of the study. Sections of rhizomes with roots attached from each location were cut to 0.5 cm lengths with one visible stem bud. The rhizome sections were placed in large aluminum roasting pans, covered with sterilized potting soil and watered daily until emerging shoots had grown to at least 5 cm height. Sprouted rhizomes were individually transferred to 20 cm by 14 cm plastic pots containing local soil from Chelan County. Pots were watered daily, placed in two rows outside the west facing side of a building where plants received midday to mid-afternoon sun, and any new stems were clipped, restricting each pot to only one stem. The locations of the pots were rotated every 2 days to minimize the effect of pot location on results. Plants were grown in these pots for two years before the initiation of the study.

In late June 2008, 18 stems of similar vigor with heights ranging from 27 - 142 cm were selected for the study. Pots were divided into three groups of six plants each with similar stem heights. An additional 10 stems of similar height and vigor were selected as untreated controls. Data collected from each stem included height, number of branches and number of leaves in the top 10 cm of the stem. All stems except controls were covered with net sleeve cages, held away from the plant with wire. *M. janthinus* adults used for the study were collected in late June 2008 at the USDA APHIS PPQ insectary near Danville, Washington. Stems in each of the three groups randomly received between two to eight pairs of insects with each stem within a group receiving a different number of insect pairs. Each stem was labeled and the number of insect pairs introduced into the cage was recorded. The study design provided six different numbers of insect pairs on stems, replicated three times. After six days, cages and insects were removed from the stems. Stems were then allowed to grow cage free for the rest of the growing season and watered daily. Although no cages were placed over the control stems, the stems were handled in a similar manner as the caged plants to equalize the negative impact of handling across all treatments (Niesenbaum et al. 2006).

In mid-November 2008 all potted plants were clipped at the soil surface level and stems dissected to determine the number of live and dead *M. janthinus* adults found in each stem. The remainder of the underground stem was clipped from the root and added to the aboveground stem for biomass measurements. Data recorded included stem height, number of branches, number of fruits and flowers, and the number of leaves in the top 10 cm of the main stem. Stems and roots were then rinsed with water, bagged individually and oven-dried to determine biomass.

Data analysis

Sleeve cage study. Comparisons among *L. dalmatica* stem heights and the numbers of fruits and flowers were calculated across treatments using pre-treatment and post-treatment data. The general model, *Dependent variable = treatment type + block size + significant interactions,* was used for the analysis.

Stems from each ramet served as a block with either two stems (insect attacked and cage only) or three stems (an additional uncaged control). The analysis of numbers of fruits and flowers included blocks in which stems from all three treatments had at least one flower or fruit post treatment. Mixed model analyses were used on both a comparison of dry stem biomass (g) and mean dry fruit weight.

The relationship between number of larvae in stems and the growth of *M. janthinus*-attacked stems was evaluated using regression analysis. The relationship between number of larvae in stems and the change in number of fruits and flowers in insect-attacked stems was evaluated using the same regression model as for stem height. Natural log (ln) transformation was used for stem height, number of fruits and flowers, and biomass values to normalize data. Fruit weight and numbers of insects were normally distributed and were not transformed for analyses. A Bonferroni adjustment was made to control for error rate in the linear mixed model analysis. All statistical analyses were conducted using SAS version 9.1 (SAS Institute 2006) and *P* values greater than 0.05 were considered not significant.

Potted plant study. A comparison between attacked and control *L. dalmatica* stem heights was calculated across treatments and across time with a linear mixed model with treatment and time as fixed effects in the model. A Bonferroni adjustment was used to control for error rate. T-tests were used to examine differences in the number of fruits and flowers, root biomass, and stem biomass data.

The relationship between number of larvae and root biomass as well as stem height was assessed with regression analyses. Stem heights and above ground biomass were converted to natural log values to normalize data for analysis and square root transformation was used to normalize values in the fruits and flowers analysis. All statistical analyses were conducted using SAS version 9.1 (SAS Institute 2006) and *P* values greater than 0.05 were considered not significant.

Results

Sleeve cage study. Comparison between the caged controls and uncaged controls showed no cage effect on stem height in the experiment (n=13). Unlike control stems, the *M. janthinus* attacked stems did not grow over the duration of the study and were 11% shorter than both the caged control (t = -6.37, df = 24, P = <0.001) and uncaged control stems (t = -4.45, df = 24, P = <0.001) after treatment (Table 1). There was no height difference in the insect-attacked stems before and after treatment (t = 0.30, df = 24, P = 1.00). In addition, insect-attacked stems weighed 17% less than control stems (t = 5.71, df = 21, P = 0.0079) (Table 2). While initially there were no differences in flower and

fruit numbers across the treatments, significant differences were recorded at the end of the study. Post treatment comparisons indicated that insect-attacked stems had fewer flowers and fruits than both the caged stems ($t = $ -3.35, df = 14, $P = $ 0.019) and control stems ($t = $ -3.31, df = 14, $P = $ 0.021) (Fig 1, Table 3). However, there were no differences in individual fruit weight between any of the treatments. Comparison between the caged controls and uncaged controls showed no cage effect on flower and fruit numbers in the experiment.

The number of larvae ranged from four to 37 per stem. No relationship was found between change in stem height and the number of larvae in a stem ($F = 1.01$, $r^2 = 0.096$, $P = 0.382$,). In addition, the number of larvae per stem did not predict the change in numbers of fruits and flowers per stem ($F = 1.61$, $r^2 = 0.244$, $P = 0.247$).

Potted plant study. The untreated control stems grew on average an additional 18.7 cm through the season; a significant height increase compared with the pre-treatment measurements ($t = 4.78$, df = 5, $P = 0.0008$). In contrast, the height of the *M. janthinus* attacked stems remained unchanged throughout the growing season ($t = 0.74$, df = 15, $P = 1.00$) (Table 4).

When the study was initiated, no stems had flowers. At the end of the study, most stems (treated or untreated) did not produce flowers or fruits. They were, however, produced on five *M. janthinus*-attacked stems (9.8 ± 2.3 per stem) and seven untreated control stems (20.4 ± 4.2 per stem) and the two groups were significantly different from each other ($t = $ -2.17, df = 4, $P = 0.0551$).

At the end of the study, the biomass of the untreated control stems (2.03 ± 0.293 g) was significantly higher than the insect attacked stems (1.39 ± 0.210 g) ($t = 2.08$, df = 9, $P = 0.0571$). There was no difference in root biomass between the two groups.

A correlation between the number of insect pairs placed on each treated plant and the change in stem height through time was noted. The model, using the difference in stem height as the dependent variable and the starting height of the stem as a covariant, indicated differences were significant ($F = 4.78$, $R^2 = 0.42$, $P = 0.0279$). Parameter estimates showed that numbers of pairs of insects was significant ($t = $ -2.90, df = 5, $P = 0.0123$) and the covariate was not significant ($t = 0.43$, $P = 0.675$). There was no relationship, however, between the number of larvae in the stems and number of fruits and flowers, difference in stem height, or stem biomass.

Discussion

In both studies, the presence of insect attack reduced *L. dalmatica* plant growth and reduced plant reproductive potential. In the field sleeve cage study, insect-attacked stems were significantly shorter (18 cm) and had 50-70 percent fewer fruits and flowers than the control stems at the end of the study period. *M. janthinus* attacked stems showed little apical growth, fewer fruits and flowers, and lower stem biomass relative to control stems. Similar results were observed in the pot study where the influence of the extensive root system of the plant was eliminated. This negative impact by the insect is caused both by adult feeding in the apical portion of the plant and the physical destruction of the plant stem from larvae feeding.

It is interesting to note that there appears to be no relationship between the number of *M. janthinus* larvae in the stem and their impact on stem height, though work by Van Hezewijk et al. (2010) found a strong negative correlation between *M. janthinus* density and stalk length across years at study sites in British Columbia. No correlation between insect density and stalk density was determined in their work. In addition, our data suggest that the number of *M. janthinus* larvae in stems appears to be a poor predictor of the degree of

Table 1. Mean height (± SE) of uncaged control, caged control, and *M. janthinus*-attacked *L. dalmatica* stems pre and post treatment. Different letters indicate significant difference at the α = 0.05 level.

Treatment	Time	Number of stems	Stem height (cm) ± se	
Uncaged control	Pre treatment	13	72.5 ± 9.1	a
	Post treatment	13	80.9 ± 11.7	b
Caged control	Pre treatment	25	71.3 ± 5.5	a
	Post treatment	25	79.9 ± 6.9	b
Insect-attacked	Pre treatment	25	71.1 ± 5.6	a
	Post treatment	25	71.0 ± 5.6	a

Table 2. Mean biomass (g) (± SE) of *M. janthinus*-attacked, caged control, and uncaged control *L. dalmatica* stems post treatment. Different letters indicate significant difference at α = 0.05 level.

Treatment	Number of stems	Stem biomass (g) ± se	
Insect-attacked	22	3.26 ± 0.52	a
Caged control	22	3.93 ± 0.63	b
Uncaged control	10	3.49 ± 0.98	ab

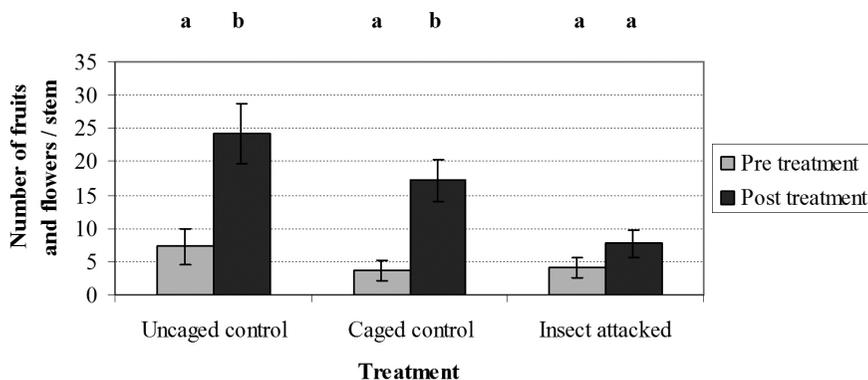Figure 1. Mean number of fruits and flowers (± SE) on *M. janthinus*-attacked, unattacked and control (uncaged) *L. dalmatica* stems. *N* = 15 stems for insect attacked and caged control. *N* =6 stems for uncaged control. Different letters indicate significant difference at α = 0.05 level.

Table 3. Mean number of fruits and flowers (± SE) on *M. janthinus*-attacked, caged control, and uncaged control *L. dalmatica* stems pre and post treatment. Different letters indicate significant differences at α = 0.05 level.

Treatment	Time	Number of stems	Number of fruits and flowers ± se	
Insect-attacked	Pre treatment	15	4.1 ± 1.54	a
	Post treatment	15	7.7 ± 2.14	a
Caged control	Pre treatment	15	3.7 ± 1.49	a
	Post treatment	15	17.2 ± 3.15	b
Uncaged control	Pre treatment	6	7.3 ± 2.65	a
	Post treatment	6	24.2 ± 4.54	b

Table 4. Mean height (cm) (± SE) of *L. dalmatica* potted plant stems before and after *M. janthinus* attack. Different letters indicate significant difference at α = 0.05 level.

Group	Time	Number of stems	Height (cm) ± SE	
Control	Pre treatment	6	28.0 ± 1.53	a
	Post treatment	6	46.7 ± 5.02	b
Insect-attacked	Pre treatment	16	31.6 ± 1.84	a
	Post treatment	16	33.3 ± 1.97	a

decrease in fruit and flower number in stems that are attacked. These data indicate that the impact of the insect is more closely related to their presence/absence than the actual number of insects present on plants. No relationship could be discerned between the numbers of larvae in the stem and changes in plant growth or reproductive capacity. However, Jeanneret and Schroeder (1991, 1992) reported that larval mining in high density or outbreak populations of *M. janthinus* caused premature stem wilting and suppression of flower formation, and consequently, reduction in seed production.

Results from the pot study, however, suggest that the number of *M. janthinus* adult insects on the stem does correlate with the negative impact of the beetle on change in stem height. This relationship can be partially explained by the impact of adult feeding in the apical portion of the plant. Greater damage to the apical meristem from a larger number of adult insects feeding would be expected to have a more profound effect on the ability of the host plant to grow after damage.

Beetles reduced flowering both when root reserves were available and when they were not. In both the potted plants and field stem study, attacked plants had comparable numbers of flowers (9.8 average in potted plants and 7.7 in field stems). In the pot study, no differences were noted in the root biomass by treatment comparisons. Since the pot study was a single year study, these results may not reflect the cumulative impact of the insect on root biomass in

situations where the insect has attacked the plant for several seasons, resulting in reduced growth for several seasons.

The lack of difference in individual reproductive flower/fruit/seed weights between the insect-attacked stems and the controls suggests that the negative effect of *M. janthinus* feeding on the reproductive capacity of the plant may be due to decreased flower and fruit production but not due to an actual decrease in quality of the fruit and seeds. A study comparing seed numbers from fruits collected from both attacked and unattacked stems could further clarify whether there is a decrease in quality, or only quantity, of reproductive structures as a result of *M. janthinus* damage. The decrease in the insect-attacked stem heights may also have an impact on seed dispersal from the mature reproductive structures.

Some biological control models suggest that as much as 95% or more of seeds need to be destroyed in some invasive plants if plant density is to be reduced (Myers and Bazely 2003). In addition, *L. dalmatica* seedlings are known to be poor competitors for moisture (Sheley and Petroff 1999). A combination of decreased seed production through *M. janthinus* biological control coupled with poor competition of seedlings in the moisture limited sites common to north-central Washington State and other similarly dry habitats may negatively influence *L. dalmatica* populations more than general models predict.

Acknowledgments

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