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**Comparative Study of *Mecinus janthiniformis* (Coleoptera: Curculionidae) Attack and Simulated Mowing for Control of *Linaria dalmatica* spp. *dalmatica* (Plantaginaceae)**

Elizabeth J. Goulet<sup>1</sup>, Antonio Ditommaso<sup>2</sup>, and Elson J. Shields<sup>3\*</sup>

**Abstract**

Dalmatian toadflax, *Linaria dalmatica* spp. *dalmatica* (L.) Mill. (Plantaginaceae) has invaded over one million hectares in the western United States and Canada, in habitats similar to its native range. Two field studies were conducted to examine the impact of simulated mowing or insect herbivory on *L. dalmatica* growth and reproduction. Simulated mowing over the duration of the study decreased *L. dalmatica* total biomass per square meter, significantly reduced the total number of fruits and flowers per square meter, and resulted in significantly shorter flowering stems in the simulated mowing plots than in their controls. Plants in plots attacked by *Mecinus janthiniformis* Tošovskí and Caldara (Coleoptera: Curculionidae) had significantly less biomass per square meter, significantly fewer total numbers of fruits and flowers per square meter, and significantly fewer reproductive structures per stem than plants in paired control plots over the duration of the study. Specifically, both management tactics resulted in a negative impact on this invasive plant. With repeated tissue removal or damage, a reduction in numbers of fruits and flowers per stem on both the stems subjected to simulated mowing and *Mecinus*-attacked stems relative to their controls suggests that long term stress effects on the plants may be similar. The results of these studies suggest that mowing may warrant further evaluation as a possible method of control in areas where *M. janthiniformis* release is not effective.

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Dalmatian toadflax, *Linaria dalmatica* spp. *dalmatica* (L.) Mill. (Plantaginaceae), a native of the eastern Mediterranean and Black Sea regions of Europe and Asia, was introduced in the United States as an ornamental by 1894 and first planted in Canada in 1901 (Vujnović and Wein 1997). *L. dalmatica* has invaded over one million hectares in the western United States and Canada, in habitats similar to its native range (Sheley and Petroff 1999). 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). *L. 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 (Vujnović and Wein 1997), although sheep are reported to graze it without adverse effects (Sheley and Petroff 1999). Individual *L. dalmatica* plants are short-lived perennials and can survive up to

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four years (Robocker 1974), but stands can persist for long periods of time due to vegetative propagation and prolific seed production (Robocker 1970). Seeds can remain dormant in soil for up to 10 years under field conditions.

Mowing when root reserves are low has been identified as a control strategy for other perennial weeds (Peters and Lowance 1978, Bouhache et al. 1993), and work by Robocker et al. (1972) showed reduction in *L. dalmatica* carbohydrate root reserves after two seasons of mowing. However, mowing is not currently recommended as a control tactic for *L. dalmatica* (Sheley and Petroff 1999). Other perennial weeds, such as perennial sow thistle (*Sonchus arvensis* L.) have been successfully controlled with mowing as part of an integrated control strategy (Vanhala et al. 2006).

Biological control of *L. dalmatica* with *Mecinus* spp. (Coleoptera: Curculionidae) has shown promise in the US and Canada. Recent molecular data indicate that some individuals within the species *M. janthinus* Germar are actually members of the cryptic species *M. janthiniformis* Tosevski and Caldara, which prefers Dalamatian toadflax. *M. janthinus* prefers yellow toadflax (Tosevski et al. 2011). While the older literature reports that *M. janthinus* was originally released in the US, the preference of the released insect to attack Dalamatian toadflax suggests the insect released was actually *M. janthiniformis*. *M. janthiniformis* is a univoltine stem boring weevil specific to a small number of perennial Eurasian *Linaria* species with stem diameters greater than 0.9 cm. Like its *Linaria* spp. host plants, *M. janthiniformis* is native to southern and central Europe and southern Russia (McClay and De Clerck-Floate 2002). The beetle was found to be established in 100% of 22 release sites in British Columbia, Canada (1991-94), and all stems at some of the sites were attacked after only three years (De Clerck-Floate and Harris 2002, Van Hezewijk et al. 2010, Schat et al. 2011). Studies on post release feeding behavior showed no evidence of non-target herbivory on native plants growing at toadflax sites in the US where *M. janthiniformis* is established (Breiter and Seastedt 2007).

Adult insects feed on new *L. dalmatica* stems in the spring, most often feeding on leaf tissue near the tops of stems. For several months after mating, females oviposit singly in cavities chewed in the stem. Larvae feed on the internal stem tissue of the host and pupate in the stem after about 40 days. The insect overwinters as a non-feeding adult and emerges from dead toadflax stalks in spring (Jeanneret and Schroeder 1992).

We examined the effects of either simulated mowing or herbivory on *L. dalmatica* in order to gain insight into the impact of each treatment on *L. dalmatica* and its potential for area wide management in Washington State and surrounding states with similar habitats.

## Materials and Methods

**Mowing Study.** This study was established in 2004 utilizing three study sites, each comprised of five sets of plots to compare the potential control of *L. dalmatica* using mechanical control (simulated mowing).

**Sites.** Ski Hill Top (47°56.19'N, 120°03.35'W) and Ski Hill Bottom (47°56.20'N, 120°03.34'W) were established at the Echo Valley Ski Hill, approximately 10 km from the town of Chelan. The Cooper site (47°56.42'N, 120°03.39'W) was within 1 km of the ski hill sites on a south-facing slope. All mowing sites are in Chelan County, Washington, USA. All sites were in open habitats with little to no woody vegetation.

Ski Hill Top site (0.30 ha) was primarily flat and had been cleared of all vegetation with a bulldozer in 2002, leaving compacted soil with *Centaurea diffusa* Lam. (diffuse knapweed) and *L. dalmatica* as the main vegetative cover. *L. dalmatica* had dense but patchy distribution throughout the site.

Ski Hill Bottom site (0.40 ha) was primarily flat and was bulldozed approximately 10 years prior the start of this study. This site had greater plant species diversity and a more even distribution of *L. dalmatica* than the Ski Hill Top site. Both *Purshia tridentata* (Pursh) (bitterbrush) and *Artemisia tridentata* Nutt. (sagebrush) were present, as were *Bromus tectorum* L. (cheatgrass), *Stipa* spp., *Achillea millefolium* L. (common yarrow), *Brassica* spp., and *Secale cereale* L. (common rye grass).

Cooper Road site (0.60 ha) was a south facing slope with a gentle grade and had the highest native plant component of the three mowing sites. *L. dalmatica* was evenly distributed throughout the site at densities similar to those at the Ski Hill Bottom site. Sagebrush was the most abundant shrub with bitterbrush and *Ribes* spp. (currant) also present. The shrub cover and density were both low. The main grass cover was cheatgrass, a non-native invasive species. Other species included the native grass *Agropyron spicatum* (Pursh) and several *Stipa* spp. Also present were native forbs, including *Lupinus* spp., *Brodiaea douglasii* S. Wats., *Balsamorhiza sagittata* (Pursh) Nutt., *Aster* spp., *Calochortus macrocarpus* Dougl., and the non-native but naturalized *A. millefolium*.

Five circular 1 m<sup>2</sup> permanent plots were randomly established in each of the three mowing study sites. At each of the mowing sites, a replicate set of control plots was established in a random direction from each of the five permanent plots at a distance between 0.8 m and 2 m from the permanent plots.

All *L. dalmatica* stems and all other vegetation in each of the permanent mowing plots were cut annually with scissors to simulate mowing. Cutting in year one (2004) was timed to match mowing by local landowners, which occurred in mid- to late July. From 2005 to 2007, cutting was timed to closely match the maturation of the flowering stems in year one. The time of cutting was determined by calculating the ratio of fruit to flowers in a random selection of 20 stems in the cutting areas weekly, starting in the first week of July. Stems were cut at the sites when the ratio of flowers to fruits was within 10% of the average ratio calculated for the site for the first year's cut. By matching the time of mowing and post-treatment data collection closely to the maturity of the flowering stems from year one, a more accurate representation of actual effects on the plants across years can be made.

**Data Collection and Analysis.** The height of each stem within all plots was measured in cm, its reproductive phenology (flowering or non-flowering) recorded, and the number of flowers and fruits for each flowering stem counted. Stems in all of the plots were clipped at ground level, separated by phenological grouping, oven-dried at 60° C for 48 hours, and weighed to determine biomass. Total vegetative (i.e., non-flowering) stem biomass and total flowering stem biomass for each plot were recorded separately. All simulated mowing was conducted between 15 July and 4 August for all field seasons.

A repeated measures analysis was used with 2004–2006 data to test for effects between treatments over three years for the mowing sites. Least squares means with Bonferroni adjustment were used to determine differences in means for data group-by-year combinations. Differences in means between years as a simple factor were determined using a least squares means comparison for years only. All analyses used SAS version 9.1 mixed model procedures (SAS Institute 2006).

When insufficient data were available to run either of these models, a block t-test or simple t-test was used for analysis. A t-test comparing 2004 and 2005 stem density on mowing sites also was performed. Vegetative stem heights and numbers of fruits and flowers per stem were natural log transformed for all within year analyses. A square root conversion was used on the total numbers of fruits and flowers per square meter and the stem density data for among year analysis in mowing sites. Stem heights were square root transformed for

across year analysis of mowing sites. A square root transformation was used for total biomass data in t-tests with 2006 and 2007 comparisons on mowed plots. Flowering stem data were distributed normally.

**Biological Control Study.** This study was established in 2004 utilizing one site, comprised of five sets of plots to evaluate the potential control of *L. dalmatica* using *M. janthiniformis* as an insect biological control. In 2006, two additional *M. janthiniformis* biological control sites were added to the study with five additional sets of plots at each site.

**Sites.** The Gracey site was located near Danville, in Ferry County, Washington (48°59.73'N, 118°30.79'W). The Railroad site was located 1.5 km northeast of the Gracey site near a railroad track approximately 150 m south of the Canadian border (48°59.94'N, 118°29.62'W). The Gebbers site was located approximately 5.6 km from State Highway 150 in Chelan County, Washington (47°55.82'N, 120°03.25'W). All sites were in open habitats with little to no woody vegetation.

Gracey site was a fallow field which had last been planted in 1999 with alfalfa. The *Mecinus*-attacked plots were located in the north side of the field in a *L. dalmatica* stand of approximately 1.2 ha, which experienced severe *M. janthiniformis* attack. The *Mecinus*-free controls were located in a 0.60 ha stand of *L. dalmatica*, approximately 300 m south from the *Mecinus*-attacked plots and had little to no *M. janthiniformis* activity and damage in 2004. *L. dalmatica* was present at high densities and fairly evenly distributed throughout the Gracey field. The primary species present in locations where *M. janthiniformis* was successfully limiting *L. dalmatica* was *Lepidium draba* L. (hoary cress) with both cheatgrass and *Amsinckia* spp. (fiddleneck) also present. Gracey-north *Mecinus*-attacked plots were flat or had a gentle southwesterly grade, and Gracey-south control plots were flat.

Railroad site (1.0 ha) was approximately 150 m south of the Canadian border near railroad tracks in very rocky soil. The *Mecinus*-attacked sites were on the east side of the tracks up a slight embankment from the railroad cut, and the controls were located along the west side of the tracks approximately 200 m from the attacked plots. Both fiddleneck and cheatgrass were common at the site, and *L. vulgaris* (yellow toadflax) also was present in the *Mecinus*-attacked section of this site.

Gebbers site (0.80 ha) was located on a relatively undisturbed slope above a road cut edge. *Mecinus*-attacked plots were located approximately 400 m from the control plots along the same road. The vegetation at this site was very similar to the Cooper mowing site, which was located approximately 6 km away.

At each of the three *M. janthiniformis* biological control sites, five circular 1 m<sup>2</sup> permanent plots were randomly established. The non-*Mecinus*-attacked control plots were established in *L. dalmatica* stands closest to the permanent attacked plots, which had not yet been heavily colonized by *M. janthiniformis*. Each year, a new set of control plots were selected at each site in a different direction and distance than control plots from all previous years to eliminate any potential effect of vegetation removal from the previous year's control plot on the current year data.

Stems in the permanent *Mecinus*-attacked plots at the *M. janthiniformis* sites were not cut, since clipping effects on stems would confound effects from insect attack. Instead, a 'matched' plot was selected in a random direction and distance within two meters of the permanent *Mecinus*-attacked plot, and data from these stems were used to estimate annually the biomass and insect numbers for *Mecinus*-attacked plots. All other data for *M. janthiniformis* attacked stems were collected directly from the stems within the permanent plots. The *Mecinus*-attacked 'matched' plots and their controls were cut at the same maturation stage as stems from the clipped sites as described previously.

**Data Collection and Analysis.** The height of each stem within all plots was measured in cm, its phenology (flowering or non-flowering) recorded, and the number of flowers and fruits for each flowering stem was counted. Stems in all of the plots were clipped at ground level, separated by phenological grouping, oven-dried at 60° C for 48 hours, and weighed to determine biomass. Total vegetative (i.e., non-flowering) stem biomass and total flowering stem biomass for each plot were recorded separately.

An estimate of *L. dalmatica* biomass in the *Mecinus*-attacked permanent plots each year was made by using the vegetative and flowering biomass values calculated from the 'matched' plots associated with each permanent plot for that year. A biomass per centimeter stem length was calculated by dividing total biomass by the total stem length. This biomass per centimeter value was then multiplied by the stem lengths in the permanent *Mecinus*-attacked plots to estimate the biomass in the non-clipped permanent *Mecinus* plots. Likewise, an estimate of the average number of insects per stem length in *M. janthiniformis* attacked stems was calculated using data from the 'matched' plot stems. All stem collection was conducted between 15 July and 4 August for all field seasons.

Split-plot analysis was used to directly compare the *Mecinus*-attacked treatments within a given year (2006 and 2007). Significant differences between the treated and control stems were determined by least squares means comparisons. The covariance structure was limited to within group stems for all split-plot analysis.

Differences in all stem variables from the *Mecinus*-attacked sites across two years were analyzed using a split-plot model with two years (2006 and 2007) and two treatments (*Mecinus*-attacked or control). Significant differences between treatments within and between years were determined by least squares means comparisons. A Tukey's adjustment controlled for error rate. All split-plot, repeated measures, and covariate analyses used SAS version 9.1 mixed model procedures (SAS Institute 2006).

When insufficient data were available to run either of these models, a block t-test or simple t-test was used for analysis. A regression of flowering biomass per plot on total number of flowers and fruits within a given plot using 2006 data also was calculated using a standard regression procedure in SAS version 9.1 (SAS Institute 2006).

*Linaria dalmatica* stem densities and stem heights for split-plot analyses were natural log transformed to normalize the data. Vegetative stem heights and numbers of fruits and flowers per stem were natural log transformed for all analyses. Natural log transformations were used for stem density, stem height, and numbers of fruits and flowers per square meter in *M. janthiniformis* 2006–2007 comparisons. Flowering stem data were distributed normally.

## Results

Impact of mowing on *L. dalmatica*. Whenever *M. janthiniformis* was found in toadflax stems from a mowing plot, the plot was dropped from the analysis. The presence of *M. janthiniformis* confounded the results by either affecting control measurements or by interaction with the mowing treatment effect. This situation occurred in two plots in 2006 and four additional plots in 2007. One of the mowing plots had no stems in the final year of the study and was excluded from the final year analysis.

An analysis of 2004–2006 data showed that total *L. dalmatica* biomass per square meter decreased in the mowed plots in 2006 when compared to 2004 and 2005 data ( $t = -4.86$ ;  $P < 0.001$ ), while there was no change in total biomass production in the control plots. However, data were not robust enough for analysis of biomass production over time for the 2005–2007 growing seasons. This is primarily due to the required removal from data analysis of *M. janthiniformis* attacked stems in 2007 (Fig. 1).

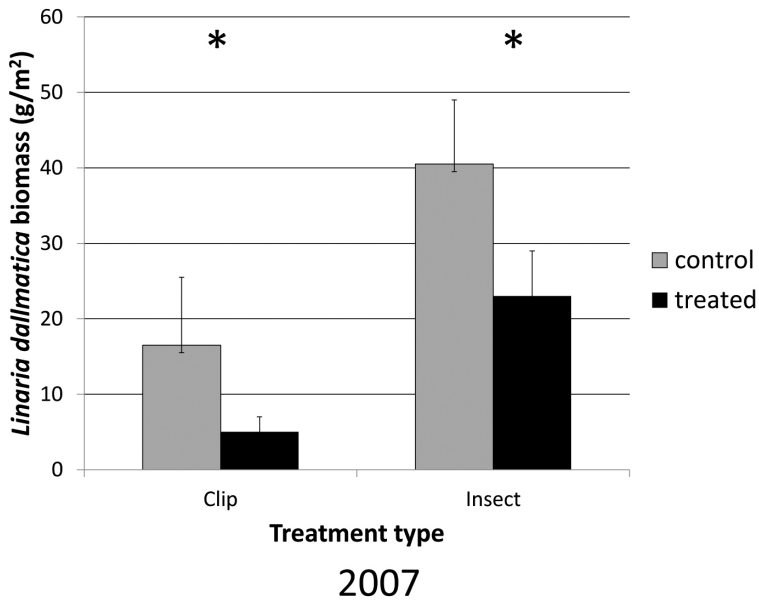
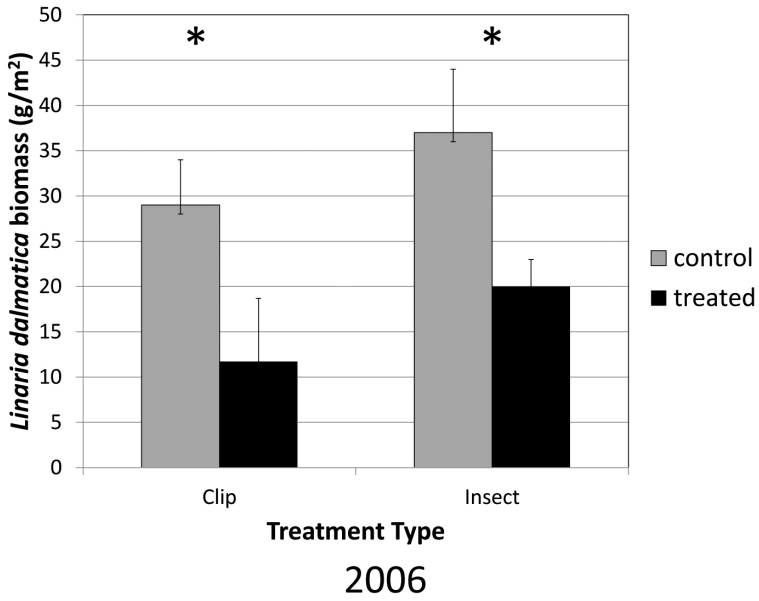


Figure 1. Mean *L. dalmatica* biomass (g/m<sup>2</sup>) ( $\pm$  se) in mowed, *M. janthiniformis*-attacked, and control plots in July 2006 and July 2007. Asterisk (\*) indicates significant difference between treated and control stems within treatment type group at  $\alpha = 0.05$  level.

To test if trends from earlier analyses were supported, t-tests comparing 2006 and 2007 data were used. A decreasing trend in total biomass in the 2007 mowed plots compared to 2006 mowed plots was observed (3.4 g/m<sup>2</sup> vs. 5.6 g/m<sup>2</sup>), but the decrease was not significant due to high variance. Stem densities decreased in the mowing plots compared to their controls, when data were analyzed from 2004 to 2006 ( $F = 16.07$ ;  $P \leq 0.0001$ ); treatment by year interactions were significant ( $F = 16.10$ ;  $P = 0.0001$ ). When 2006 data were compared with 2007 data, the decrease in stem density relative to controls continued ( $t = -3.76$ ;  $P = 0.0008$ , 2006;  $t = -2.06$ ;  $P = 0.0499$ , 2007). Control plots showed no significant differences in stem density between years. There were no significant differences in stem height between plants in the control plots and mowed plots either within or between years.

The total number of fruits and flowers per square meter was significantly less in the mowed plots compared with their controls over the duration of the study (2004–2007) ( $t = -3.80$ ;  $P = 0.009$ ). Most notable was a decrease in total fruits and flowers in the treated plots in 2006 compared with 2005 ( $t = -4.09$ ;  $P = 0.0018$ ) and in 2007 compared with 2005 ( $t = -4.34$ ;  $P = 0.0006$ ). There was no significant difference in the number of total fruits and flowers per square meter in the control plots between years (Fig. 2).

Flowering stems from mowed plots were significantly shorter than their controls ( $t = -2.88$ ;  $P = 0.0231$ ). Repeated measures analysis of *L. dalmatica* flowering stem height for 2004–2006 data showed significant differences between years ( $F = 36.81$ ;  $P < 0.0001$ ) and between treatments among years ( $F = 3.55$ ;  $P = 0.0292$ ). The 2007 flowering stem data were not robust enough for an analysis among all four years.

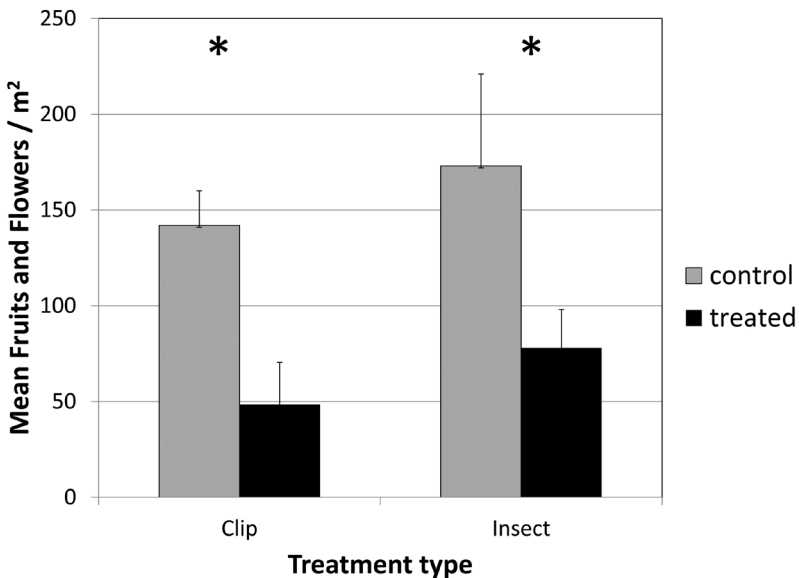


Figure 2. Total number of *L. dalmatica* fruits and flowers ( $\pm$  se) per square meter in mowed, *M. janthiniformis*-attacked, and control plots in July 2006.  $n = 13$  plots for each treatment. Asterisk (\*) indicates significant difference between treated and control stems within treatment type group.



A set of t-tests comparing 2007 and 2006 control and mowed flowering stems indicated that the height of mowed flowering stems in 2007 decrease relative to the 2006 flowering mowed stems ( $t = -1.84$ ;  $P = 0.0736$ ). Also notable was the decrease in height of clipped flowering stems in 2007 relative to their controls ( $t = 2.72$ ;  $P = 0.0104$ ). In contrast, there was no difference in height of control flowering stems between the two years.

There were no significant differences in the number of reproductive structures per stem between the control plots and the mowed plots over time (2004–2007). While the data showed trends toward fewer flowers or fruits per stem in the mowed plots, these were not significant.

Impact of *Mecinus janthiniformis* on *Linaria dalmatica*. Over the duration of the study, the control plots remained free of *M. janthiniformis* until 2007, when four control plots were attacked by the insect. In addition, one of the *M. janthiniformis* plots had no stems in the final year of data collection. Data from these plots were not included in the analysis.

Analysis of 2004–2006 data showed that plants had significantly less biomass per square meter in *M. janthiniformis* attacked-plots compared with their control plots in 2006 ( $t = 2.28$ ;  $P = 0.0286$ ). Analysis of biomass data from the nine useable pairs of *M. janthiniformis* attacked plots from all sites indicated significantly less biomass was present in the insect attacked plots relative to their controls ( $t = -2.16$ ;  $P = 0.0442$ ) (Fig. 1).

No significant differences were recorded in stem densities between the insect-attacked plots and their controls in 2006 or 2007. However, differences were found between the *Mecinus*-attacked and insect control stem heights ( $t = -4.93$ ,  $P < 0.0001$ ) with *Mecinus*-attacked stems shorter than their controls ( $t = -5.01$ ;  $P < 0.0001$ ). The total number of fruits and flowers per square meter was significantly less for *M. janthiniformis* attacked plots than controls when compared between years (2006–2007) ( $t = -2.25$ ;  $P = 0.0341$ ) (Fig. 2). In addition, control stems had significantly more reproductive structures than *Mecinus*-attacked stems ( $F = 17.95$ ;  $P < 0.0001$ ) and produced more than twice as many fruits and flowers as attacked stems, averaging  $59.1 \pm 8.76$  per stem compared with  $24.4 \pm 4.74$  for the *Mecinus*-attacked stems. *Mecinus*-attacked flowering stems also were significantly shorter in 2006 than their controls ( $t = -5.89$ ;  $P \leq 0.0001$ ). Flowering stem height data from 2007 were insufficient for analysis.

Using data from dissected stems in 2006 *Mecinus*-attacked plots that had at least one *M. janthiniformis* present ( $n = 63$ ), a positive correlation was found between the number of *M. janthiniformis* larvae per stem and the number of flowers ( $r = 0.47$ ;  $P < 0.0001$ ). A significant but weaker correlation also was found between the number of larvae per stem and height ( $r = 0.27$ ;  $P = 0.0029$ ). Using a stepwise regression with the number of *M. janthiniformis* per stem as the dependent variable and 1) stem height, and 2) the number of fruits and flowers per stem as independent variables, stem height had no effect on the abundance of insects per stem.

## Discussion

Both management tactics had a negative impact on this plant and resulted in lower plant biomass in treated plots compared with matched control plots. However, stem density increased in simulated mowing plots in the year following first cutting. This indicates that *L. dalmatica* stems may respond differently to major stress factors, such as clipping or mowing, than to sustained but less severe damage, such as insect feeding. For example, perennial plants often will respond to initial tissue removal with a flush of new or compensatory growth,

when subjected to grazing (McNaughton 1983, Gold and Caldwell 1990). The extensive root systems of *L. dalmatica* plants likely provides the nutrients and carbohydrates necessary for plants to resume growth quickly after a single disturbance event such as clipping or mowing, but these resources are likely exhausted in subsequent years if tissue removal continues to occur. Vujnovic and Wein (1997) suggest that prolific growth from *L. dalmatica*, after removing aboveground plant parts by grazing, mowing, or burning, limits the effectiveness of these cultural control methods. Our data indicates that this biomass reduction in simulated mowing sites may initially be influenced by a reduction in stem numbers and a decrease in flowering stem height after continued stress. Research on the invasive perennial legume, Chinese lespedeza, *Lespedeza cuneata* (Dum. Cours.), suggests that clipping can decrease plant density while mowing can increase it (Brandon et al. 2004). Thus, assessing the response of *L. dalmatica* response to full field mowing could help clarify its response to large-scale stem removal.

Our study design did not allow determination of whether weevil attack was the causal factor for the decreased biomass observed on toadflax plants in *M. janthiniformis* sites. In a follow up study utilizing individually caged stems and potted plants, this relationship was established (Goulet et. al 2013).

The negative effect of biological control agent herbivory on biomass of other invasive plant species is well documented (Häfliger et al. 2006). Since the initial stem densities of *Mecomis*-attacked *L. dalmatica* stands was not known, presumptions about biological control impact on stem density over time are limited. The fact that toadflax stem density did not vary between the attacked and control plots suggests that stem height and fruit and flower production were most negatively impacted in these plots. However, Van Hezewijk et al. (2010) showed decreased density of *L. dalmatica* stems in patches colonized by *M. janthiniformis* after seven years. Reductions in stem height from biological control agent herbivory have been demonstrated in other studies (Franks et al. 2006, Häfliger et al. 2006).

Reduced fruit and flower production by stems subjected to *M. janthiniformis* attack or simulated mowing relative to their paired controls indicates that reproductive capacity also was negatively affected by both treatments. Initially, reductions in fruits and flowers may have been a result of decreased stem density in simulated mowing sites rather than a reduction in fruits and flowers produced on each stem. However, with repeated tissue removal or damage, a reduction of fruits and flowers per stem in both the mowed and *Mecinus*-attacked stems suggests that long-term effects on the plants may be similar.

The results from our two studies suggest that mowing may warrant further evaluation as a possible method of control in areas where *M. janthiniformis* release is not appropriate or feasible. Some of these locations might include land adjacent to orchards or other areas where insecticides may negatively impact the efficacy and longevity of the beetle.

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