Integrated Management of Canada Thistle (*Cirsium arvense*) with Insect Biological Control and Plant Competition under Variable Soil Nutrients

Erin E. Burns, Deirdre A. Prischmann-Voldseth, and Greta G. Gramig*

Because of economic and environmental constraints, alternatives to chemical management of Canada thistle (*Cirsium arvense*) are frequently sought, but adequate nonchemical suppression of this invasive species remains elusive. Combining biological control with other tactics may be an effective approach to suppress Canada thistle, but more information is needed about how environmental conditions affect interspecific interactions. We investigated effects of a biocontrol agent (*Hadroplontus litura*, a stem-mining weevil) and a potential plant competitor (common sunflower, *Helianthus annuus*, native annual) on Canada thistle under two soil nutrient regimes in outdoor microcosms. Larval mining damage was relatively light, and weevils negatively impacted only main shoot height and flower number. All measures of Canada thistle performance were reduced when plants were grown with common sunflower or in reduced nutrients, although effects of the latter on root biomass were not significant. Effects of common sunflower and soil nutrients on Canada thistle were generally additive, though a marginally insignificant interaction indicated a trend for greatest flower number with high nutrients and absence of common sunflower. Effects of weevils and common sunflower on Canada thistle were also additive rather than interactive. Although larval damage ratings were significantly greater on plants grown in high-nutrient soil, under our experimental conditions weevils and soil nutrients did not have a significant interactive effect on Canada thistle plants. Our results indicate that *H. litura* is a relatively weak biological control agent, but when combined with competitive desirable vegetation, some level of Canada thistle suppression may be possible, especially if soil nutrient levels are not highly enriched from agricultural runoff. Assessing the true ecological impacts of Canada thistle infestations may be an important direction for future research.

Nomenclature: Stem-mining weevil, *Hadroplontus litura* Fabricius; Canada thistle, *Cirsium arvense* (L.) Scop.; common sunflower, *Helianthus annuus* L.

Key words: Integrated pest management, biological control, cover crop.

Canada thistle [*Cirsium arvense* (L.) Scop.] is a problematic invasive weed in many croplands, rangelands, and recreational areas in cooler temperate regions of the northern hemisphere, including the United States and Canada. This invasive weed thrives in disturbed or moist environments and can form large patches, potentially outcompeting more desirable vegetation (McClay 2002; McLennan et al. 1991;

O'Sullivan et al. 1982, 1985). Canada thistle is a clone-forming perennial with a deep root system that can spread extensively (Donald 1994; McClay 2002) and give rise to adventitious shoots from root buds throughout the growing season (Tiley 2010). The widespread invasiveness of this weed is often attributed to these characteristics. Canada thistle's persistence and vegetative spread has also been associated with the plant's root carbohydrate reserves (Tworkoski 1992). In general, root carbohydrate levels are lowest in spring and early summer as a result of active shoot growth and begin to increase in late summer and fall in preparation for overwintering (Hein and Wilson 2004).

Despite extensive research, managing Canada thistle remains challenging (Cripps et al. 2011; Tiley 2010).

Management Implications

Canada thistle is a problematic invasive plant in many temperate regions of the northern hemisphere, and management continues to be a challenge. Research suggests that integrating multiple control tactics enhances suppression of Canada thistle, but that effects often depend on environmental context. In our microcosm study, weevil herbivory, common sunflower competition, and reduced levels of soil nutrients had substantial negative effects on many measures of Canada thistle growth and reproductive capacity. Overall, the latter two factors exerted the strongest negative effects; however, weevil damage was generally light. Canada thistle grown in high-nutrient soils had greater main-stem biomass, numbers of side shoots, and numbers of flowers, even though larval stem-mining damage was greater for these plants. Common sunflower proved to be a good competitor against Canada thistle, and sowing this type of fast-growing annual forb, which occupies a similar soil niche to Canada thistle, alongside perennial native plants (e.g., competitive grasses) may enhance restoration outcomes.

Numerous control tactics for suppression of Canada thistle have been investigated, including herbicides (reviewed by Donald 1990) and mechanical tactics such as frequent mowing (Lukashyk et al. 2008), hoeing (Graglia et al. 2006), and tillage (Pekrun and Wilhelm 2004). Although these practices can be effective, they are management intensive and often costly (Graglia et al. 2006). Although individual management tactics such as herbicides and mechanical methods have been moderately successful (Liu et al. 2000), these tactics often do not provide long-term results (Evans 1984; Travnicek et al. 2005), and therefore can be prohibitively expensive (Sciegienka et al. 2011; Tichich and Doll 2006). Overall, results from previous research suggest that integrated pest management (IPM) may provide longer-lasting Canada thistle suppression when compared to relying on a single chemical or mechanical control tactic (Ferrero-Serrano et al. 2008; Sciegienka et al. 2011).

Biological control is often an important component of IPM programs for invasive weed management. Biological control is an attractive option for many land managers because, if released agents establish, persist, and reduce invader abundances, the expense and potential environmental impacts associated with repeated herbicide applications can be avoided (Liu et al. 2000). Although several insects are approved for biological control of Canada thistle (Winston et al. 2008), *Hadroplontus* (formerly *Ceutorhynchus*) *litura* Fabricius, a phytophagous stem-mining weevil (McClay 2002), is typically considered one of the most effective agents in North America (Coombs et al. 2004). Adult *H. litura* overwinter in the soil and emerge in early spring in synchrony with Canada thistle emergence (Zwolfer and Harris 1966). Females lay groups of one to five eggs in round feeding cavities on the leaves of Canada

thistle rosettes (Zwolfer and Harris 1966; E. Burns, unpublished data). Larvae emerge after 5 to 9 d and mine the midvein of the leaf, eventually tunneling into the stem (Zwolfer and Harris 1966). Each infested stem is mined by an average of three to six larvae and becomes discolored because of larval feeding and frass (Burns 2012; Rees 1990; Zwolfer and Harris 1966). Mature third instars exit the plant and pupate in the soil (Zwolfer and Harris 1966), which is generally in late June to mid-July in eastern North Dakota when plants are at the prebud to bud stage (Burns 2012).

Stem mining by *H. litura* larvae during the midsummer is thought to cause more damage to Canada thistle than the foliar chewing damage done by adult weevils in the spring and fall (Liu et al. 2000; Zwolfer and Harris 1966). Though larval mining stresses the plant, it is able to continue growth during and after attack because vascular bundles are not damaged by weevil feeding (Peschken and Wilkinson 1981). Larval feeding may lead to reduced overwinter survival (Rees 1990), reduction in early season root sugar (Peschken and Derby 1992) and starch content (Hein and Wilson 2004), and increased susceptibility to pathogens and/or adverse environmental conditions (Rees 1990). Overall, previous research results concerning *H. litura* efficacy are mixed, and suggest that *H. litura* alone does not adequately control Canada thistle (Peschken and Derby 1992; Reed at al. 2006), but that combining additional management tactics might improve Canada thistle suppression (Bacher and Schwab 2000; Ferrero-Serrano et al. 2008; Friedli and Bacher 2001).

One potential option is seeding highly competitive native vegetation along with releasing biocontrol agents. Ferrero-Serrano et al. (2008) found that combining *H. litura* and a native cool season grass greatly reduced Canada thistle root biomass and hypothesized that *H. litura* had a positive indirect effect on the grass by decreasing the competitive ability of Canada thistle. Other plants, such as common sunflower (*Helianthus annuus* L.) may have even greater competitive abilities. Common sunflower is an annual dicot native to North America and found throughout the United States, Canada, and Mexico (Burke et al. 2002). It is similar to Canada thistle in several ways (i.e., it is fast growing and often thrives in disturbed areas) (Burke et al. 2002; Perry et al. 2009), which likely enhances its ability to compete against Canada thistle for sunlight, nutrients, and water. As one example, Perry et al. (1990) reported that competition from common sunflower reduced Canada thistle aboveground biomass in greenhouse experiments.

Assuming that biological control and plant competitor impacts will be similar at all locations and under all conditions is naïve (Shea et al. 2005). For instance, in North Dakota, anecdotal evidence suggests that *H. litura* releases have resulted in Canada thistle suppression in some

geographical areas, but not in others (Gramig, personal observation). Environmental variability and differences among various Canada thistle biotypes in their response to *H. litura* are explanations for these observations. Environmental variables, such as water or soil nutrient availability, can mediate plant–plant and insect–plant interactions and thus may impact efficacy of biological control (Shea 2005). For example, a study that investigated interactive effects of soil nitrogen, plant competition, and various insect biological control agents found that a flower head weevil, *Larinus minutus* Gyllenhal, reduced spotted knapweed [*Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek] seed production most severely in low-nitrogen soils and that reduced plant competition was associated with increased *L. minutus* numbers per flower (Knochel and Seastedt 2010). Conversely, soil nitrogen and plant competition did not significantly affect impacts of a root-feeding weevil, *Cyphocleonus achates* Fahr (Knochel and Seastedt 2010). These results demonstrate that generalizing across species about the effects of soil resources on responses to insect herbivory or plant competition is problematic.

The goal of this study was to investigate the combined impact of *H. litura* and common sunflower on Canada thistle under different nitrogen, phosphorus, and potassium (hereafter N, P, and K) concentrations in outdoor microcosms. We focused on assessing plant responses associated with growth and reproductive potential, including root biomass. We hypothesized that weevil herbivory and plant competition would reduce Canada thistle root biomass, shoot height, shoot biomass, flower number, and number of side shoots under low nutrient concentrations but not under high nutrient concentrations.

Materials and Methods

Experimental Design. Effects of *H. litura*, common sunflower, and soil nutrients on Canada thistle growth and reproductive output were determined with the use of outdoor microcosm experiments. During 2010 and 2011, experiments were conducted in a completely randomized design with four replications and three factorially combined treatments: (1) common sunflower present vs. absent, (2) *H. litura* weevil present vs. absent, and (3) high vs. low soil nutrient (N–P–K) concentrations.

Experiments were conducted from June 2 to September 8, 2010 and June 4 to August 8, 2011 outdoors on the campus of North Dakota State University in Fargo, ND. Microcosms were established outdoors to take advantage of natural light and temperature fluctuations. Microcosms consisted of 18 kg of lightly compacted, finely sieved (3 mm [0.1 in.] sieve) Ulen fine sandy loam (sandy, mixed, frigid Aeric Calciaquolls) soil (hereafter field soil) placed in 19-L (5 gal) white plastic buckets with two drainage holes in the bottom spaced 60 cm apart from adjacent microcosms.

Collection and Propagation of Plant Materials. In 2010, Canada thistle plants were propagated from vegetative root cuttings excavated from a small infestation on the campus of North Dakota State University (Fargo, ND). Vegetative root cuttings were approximately 8 to 10 cm in length and treated with 0.1% indole-3-butyric-acid powder (Bonide Products Inc., Oriskany, NY 13424) to promote rooting prior to planting. Root cuttings were grown in field soil in plastic pots measuring 8 cm in diameter and 9 cm in depth. Plants were grown in the greenhouse (24 to 26 C [75 to 79 F], 16 : 8-hr light : dark photoperiod) for 3 wk then transplanted on June 1, 2010 and June 2, 2011 into outdoor microcosms as small rosettes. Because of insufficient propagation in 2011, some Canada thistle plants were propagated as previously described, whereas others were extracted as small rosettes directly from the field.

Common sunflower plants were grown from seed collected in the fall of 2009 and 2010 from plants growing on the campus of North Dakota State University in Fargo, ND. Approximately five seeds were planted 3 mm deep into 4.5-cm-diam, 4-cm-high plastic pots containing Sunshine Mix No. 1 (SunGo Horticulture Canada Ltd., Bellevue, WA 98008) on May 5, 2010 and May 11, 2011. After emergence (approximately 5 d after planting), sunflowers were transplanted into 8-cm-diam, 9-cm-high plastic pots containing field soil. Plants were grown in a greenhouse (24 to 26 C, 16 : 8-h light : dark photoperiod) until approximately the four-leaf stage, when they were transplanted into microcosms on June 2, 2011 and June 1, 2012. When grown alone each Canada thistle plant was located in the center of the microcosm, and if grown with common sunflower each plant was 10 cm from the microcosm center.

***Hadroplontus litura* Treatment.** Canada thistle and sunflower plants were allowed to establish for 1 d, after which they were subjected to *H. litura* weevils at a rate of 10 adults per microcosm. The average height of Canada thistle plants at this time was 7 cm, which is approximately the same height of plant that adult weevils have been observed to prefer in the field (Gramig, personal observation). Adult weevils collected from Canada thistle infestations were purchased from a commercial source (Copeland Biological Inc., Bozeman, MT 59715) and shipped in containers along with Canada thistle foliage. Before being placed in microcosms, weevils were stored in a refrigerator (4 C) with Canada thistle foliage for 23 d in 2010 and 12 d in 2011. Weevil gender was not assessed, but assuming equal sex ratios, the probability that all 10 insects would be the same sex is approximately 0.001 (Ferrero-Serrano et al. 2008). To prevent beetle migration, microcosms (including microcosms without weevils) were caged while adult weevils were present. Cages were constructed of 75-cm-diam by 150-cm-high nylon mesh

sleeves draped over two overlapping 1.5-m (5 ft) metal wires embedded into the soil of each microcosm. Weevil adults and cages were removed 7 to 9 d after weevil release.

Soil Nutrient Treatment. The low soil nutrient treatment consisted of unamended field soil with 60 kg ha^{-1} (53 lb ac^{-1} N, 15 kg ha^{-1} P, and 132 kg ha^{-1} potassium. The high soil nutrient treatment consisted of 142 kg ha^{-1} N, 55 kg ha^{-1} P, and 179 kg ha^{-1} K added to the field soil as ammonium nitrate (NH_4NO_3), potassium chloride (KCl), and triple superphosphate (Ca[H_2PO_4]$_2$ · H_2O). Microcosms were watered periodically to field capacity, a water regime reflective of typical soil water conditions in many areas where Canada thistle is a problem (Gramig, personal observation).

Data Collection. Adult weevil damage to Canada thistle foliage was qualitatively assessed by visually inspecting plants after insect attack and determining the relative level of adult feeding damage on upper and lower leaf surfaces. At the end of the growing season, Canada thistle main stem (maximum) shoot height, number of flowers (including developing buds), and number of side shoots were quantified. Microcosms were moved to a greenhouse on September 8, 2010 and August 3, 2011 (24 to 26 C, 16 : 8-h light : dark photoperiod) so plants could dry until visibly wilted (approximately 10 to 14 d) to aid root dyeing (Murakami et al. 2006). At the time of harvest, 13 and 10 wk after weevil removal in 2010 and 2011, respectively, all Canada thistle shoots had begun to senesce. At this time, Canada thistle main stem shoots were cut 5 cm above the soil surface, split open with the use of a scalpel, and assessed for weevil larval damage on a qualitative scale: 1 = undamaged (stem pith fleshy and 100% white/green), 2 = lightly damaged (stem pith mostly white with <25% stem damaged with thin, light brown mines), 3 = moderate damage (25 to 50% stem pith mined and discolored brown), and 4 = severe damage (>50% stem pith severely mined and blackened). Side shoots, defined as small shoots emerging adjacent to the main stem during the last third of the experimental time period, were harvested at the same time. Shoot material was placed in paper bags and dried at 70 C to a constant mass. After harvesting aboveground plant parts, we followed a root dyeing procedure developed by Murakami et al. (2006) to aid in separating roots by species. After separating by species, roots were dried to a constant mass at 70 C.

Statistical Analysis. For all plant response variable data, Levene's test was performed to assess homogeneity of residual variance and normality was assessed via the Shapiro-Wilk test (Proc Univariate in Statistical Analysis Systems (SAS) Version 9.2 (SAS 2008). Data for all response variables met the assumptions of ANOVA and were not transformed but analyzed separately using Proc Mixed in SAS Version 9.2 (SAS 2008). Initial Canada thistle height was included as a covariate in all analyses. Weevil attack, common sunflower presence, and soil nutrient level were modeled as fixed effects and year as a random effect. Significant differences among treatment means were determined with the use of Tukey's honestly significant difference post hoc tests (95% confidence level).

Effects of common sunflower presence and soil nutrients on the frequency of larval weevil damage to Canada thistle plants in weevil-addition treatments were analyzed with the use of frequency tables and Pearson's chi-square statistic (SYSTAT Software, 2007). Differences in the intensity of larval damage among treatments receiving weevils were assessed with the use of factorial ANOVA, with year, common sunflower presence, and soil nutrient level as the independent variables and larval damage rating as the dependent variable.

Results and Discussion

Weevil Pressure. Canada thistle foliage in weevil addition treatments sustained similar amounts of heavy feeding damage by adult weevils. The majority (72%) of Canada thistle stems subjected to adult weevils sustained larval damage and the frequency of damage was similar among treatments (P > 0.05 for all interactions and main effects). Larval damage ratings were consistently greater under high (mean ± SE = 2.44 ± 0.24) versus low nutrient levels (1.75 ± 0.21; soil nutrients P = 0.045), although larval damage was similar whether common sunflower was present (2.00 ± 0.24) or not (2.19 ± 0.25; P > 0.05 for all relevant interactions and main effects). The development and performance of herbivorous insects is often linked to plant nitrogen, and endophagous insects (e.g., stem-miners, gall makers) often perform better on vigorous plants growing in resource rich environments (De Bruyn et al. 2002; Price 1991, 1997).

Canada Thistle Size. *Canada Thistle Height.* Canada thistle main stem height was negatively affected by weevil herbivory, common sunflower, and reduced soil nutrients, but interactions among treatments were not detected (Table 1). Canada thistle plants exposed to weevils produced main stems that were 15% shorter (Figure 1), although this was not accompanied by a significant decline in shoot biomass (Figure 2). Many authors have reported a positive association between *H. litura* and Canada thistle height under field conditions, although results can be variable (Peschken and Beecher 1973; Reed et al. 2006) and differences may be due to timing of plant emergence rather than insect damage stimulating elongation responses (Liu et al. 2000, Peschken and Wilkinson 1981).

Common sunflower reduced Canada thistle main stem heights by 28% (Figure 1). Cripps et al. (2010) showed

Table 1. P values for Canada thistle main stem shoot height, main stem biomass, flower number, side shoot number, and root biomass as affected by *Hadroplontus litura* weevils, common sunflower, soil nutrients, and their interactions.

	Canada thistle responses				
Treatment	Main-stem shoot height	Main-stem shoot biomass	Flower number	Side-shoot number	Root biomass
W[a]	0.028	NS	0.039	NS	NS
CS[b]	<0.001	<0.001	<0.001	<0.001	<0.001
SN[c]	<0.001	<0.001	<0.001	<0.001	NS
W × CS	NS[d]	NS	NS	NS	NS
W × SN	NS	NS	NS	NS	NS
CS × SN	NS	NS	0.053	NS	NS
W × CS × SN	NS	NS	NS	NS	NS

[a] W = weevil (*H. litura*) (presence, absence).

[b] CS = common sunflower (*Helianthus annuus*) (presence, absence).

[c] SN = soil nutrient level (low, high).

[d] NS indicates not significant at 95% confidence level.

that competition from perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) did not affect Canada thistle height. In our study, common sunflower plants grew large enough to shade Canada thistle plants partially. However, plant height often increases in response to shade (Aarssen 1995; Jaremo et al. 1996) and furthermore, Canada thistle shoot growth is often not reduced much by shade (Zimdahl et al. 1991). The reduction of Canada thistle height by common sunflower was therefore probably due to competition for soil resources rather than shading. Differences in experimental context (e.g., size of pot, plant density) and competitor functional group (e.g., grass vs. forb, N fixing status) impact intensity of competition for belowground resources between plant neighbors. The above-referenced study by Cripps et al. was conducted in 15-L pots with a perennial

grass and an N-fixing forb as potential competitors planted at a density representative of a typical pasture around one Canada thistle plant. Because the pot sizes were similar (15 vs. 19 L) between our study and the Cripps et al. study, differences in the effect of plant competition on Canada thistle height could have been due to differences in plant density or differences in the functional attributes of the competitors. Competition for soil nutrients would likely be more intense between two plants with similar root-system architectures that both lack the ability to fix N, as in our study.

Canada thistle plants grown in reduced-nutrient soil produced main stem shoots that were 30% shorter (Figure 1). Most previous results regarding Canada thistle growth responses to nutrients are from studies that focused
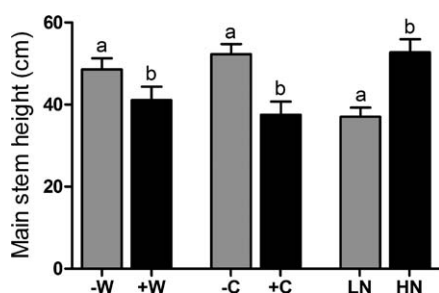

Figure 1. Canada thistle main stem height with (+W) and without (−W) *Hadroplontus litura* weevil additions, with (+C) and without (−C) common sunflower additions, and under low (LN) and high (HN) soil nutrient levels. Bars indicate mean values and whiskers indicate two standard errors. In comparing weevil present to absent, common sunflower present to absent, and nutrient addition to no nutrient addition, bars labeled with different letters differed significantly (P < 0.05).
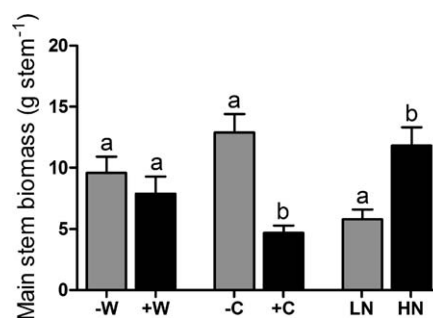

Figure 2. Canada thistle main stem biomass with (+W) and without (−W) *Hadroplontus litura* weevil additions, with (+C) and without (−C) common sunflower additions, and under low (LN) and high (HN) soil nutrient levels. Bars indicate mean values and whiskers indicate two standard errors. In comparing weevil present to absent, common sunflower present to absent, and nutrient addition to no nutrient addition, bars labeled with different letters differed significantly (P < 0.05).

on only the effect of nitrogen. One previous study (McIntyre and Hunter 1975) found that Canada thistle shoot length was reduced by 53% when plants were grown with 31.5 kg ha^{-1} N compared to 315 kg ha^{-1} N (for comparison the N levels in this study were 60 and142 kg ha^{-1}). Because we varied N, P, and K levels in our study, we cannot determine if N was the primary limiting resource.

Canada Thistle Shoot Biomass (Main Stem). We did not detect a significant impact of *H. litura* on Canada thistle main stem biomass (Table 1, Figure 2). These findings are similar to those reported by Ferrero-Serrano et al. (2008), who found no effect of weevils on Canada thistle shoot biomass in greenhouse experiments. Conversely, Sciegienka et al. (2011) reported that *H. litura* herbivory negatively impacted Canada thistle shoot biomass, and Collier et al. (2007) found inconsistent negative effects.

Canada thistle plants grown with common sunflower neighbors or in reduced-nutrient soil produced 64 and 74% less main stem shoot biomass, respectively (Table 1; Figure 3). This result is similar to results from a greenhouse study with common sunflower (Perry et al. 2009) and a field study (Friedli and Bacher 2001) investigating perennial ryegrass, Italian ryegrass [L. perenne L. ssp. multiflorum (Lam.) Husnot], and orchardgrass (*Dactylis glomerata* L.) as potential competitors against Canada thistle. In contrast, Canada thistle shoot biomass was not affected when it was grown with alkali sacaton [*Sporobolus airoides* (Torr.) Torr.] and needle and thread grass [*Hesperostipa comata* (Trin. & Rupr.) Barkworth] (Ferrero-Serrano et al. 2008).

Canada Thistle Reproductive Potential. *Canada Thistle Flower Number.* Flowers were produced only by Canada thistle main shoots and not by side shoots. Weevil presence, common sunflower presence, and soil nutrients each influenced Canada thistle flower number, and there was a weak but marginally insignificant interaction between the latter two factors (Table 1). Canada thistle plants exposed to *H. litura* produced 40% fewer flowers (Figure 3) Stems with weevils tended to produce fewer flowers per gram biomass (1.7 vs. 2.4), suggesting that the reduction in flower number did not scale strictly with size, though this effect was marginally insignificant (P = 0.115). With more statistical power we may have been able to demonstrate a difference. Our study is the first (to our knowledge) to document a negative impact of *H. litura* on Canada thistle flower number, but we did not quantify seeds per flower. A previous study found that exposure to *H. litura* and seed-head parasites did not reduce the number of seeds per seed head (Larson et al. 2005). Also, Canada thistle is a dioecious plant and male Canada thistle shoots have been shown to produce fewer flowers than female shoots (Becker et al. 2008). We did not identify the gender of individual
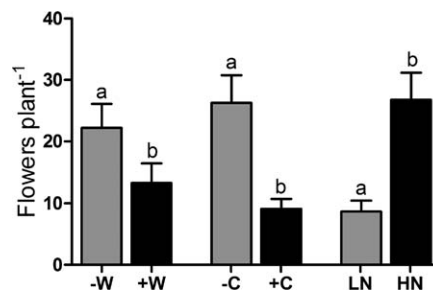

Figure 3. Canada thistle flowers per plant with (+W) and without (−W) *Hadroplontus litura* weevil additions, with (+C) and without (−C) common sunflower additions, and under low (LN) and high (HN) soil nutrient levels. Bars indicate mean values and whiskers indicate two standard errors. In comparing weevil present to absent, common sunflower present to absent, and nutrient addition to no nutrient addition, bars labeled with different letters differed significantly (P < 0.05).

Canada thistle plants, so this could be a source of error in our study.

Canada thistle plants grown with common sunflower neighbors produced 65% fewer flowers (Figure 3). This parallels results from Friedli and Bacher (2001), who saw an 81% reduction in Canada thistle flower production when grown with a mixture of three grass species (perennial ryegrass, Italian ryegrass, and orchardgrass). In our study, flower number : main stem shoot biomass ratios did not differ between common sunflower treatments (P = 0.275, data not shown), indicating that the decrease in flower number scaled with biomass reduction. Canada thistle plants grown in reduced nutrients produced 68% fewer flowers. The ratio of flowers:main stem biomass was 45% greater for Canada thistle plants grown in high-nutrient soil (P = 0.006, data not shown). This demonstrates that the increase in flower number in high-nutrient soil was not a simple function of increasing plant biomass. These results support a theoretical model describing energy partitioning in clonal plants that predicted increased soil nutrients would be associated with a decrease in the cost of producing sexual reproductive structures, leading to increased seed production per unit biomass (Loehle 1987).

Canada Thistle Side Shoot Number. Side shoots contribute to the expansion of Canada thistle patches (Tiley 2010), increase the amount of photosynthetically active tissue, and eventually produce seed. We failed to detect any impact of *H. litura* on Canada thistle side shoot number (Table 1, Figure 4), which contrasts with results from a greenhouse microcosm study where Canada thistle plants exposed to *H. litura* produced an average of 29% fewer side shoots (Sciegienka et al. 2011).

Canada thistle side shoot number was affected by both plant competition and soil nutrient level (Table 1). Canada thistle plants grown with common sunflower plants
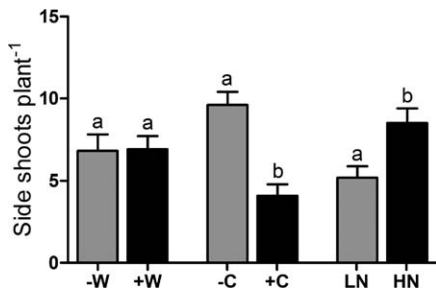
Figure 4. Canada thistle side shoots per plant with (+W) and without (−W) *Hadroplontus litura* weevil additions, with (+C) and without (−C) common sunflower additions, and under low (LN) and high (HN) soil nutrient levels. Bars indicate mean values and whiskers indicate two standard errors. In comparing weevil present to absent, common sunflower present to absent, and nutrient addition to no nutrient addition, bars labeled with different letters differed significantly (P < 0.05).

Figure 5. Canada thistle root biomass per plant with (+W) and without (−W) *Hadroplontus litura* weevil additions, with (+C) and without (−C) common sunflower additions, and under low (LN) and high (HN) soil nutrient levels. Bars indicate mean values and whiskers indicate two standard errors. In comparing weevil present to absent, common sunflower present to absent, and nutrient addition to no nutrient addition, bars labeled with different letters differed significantly (P < 0.05).

produced 57% fewer side shoots (Figure 4). These results are not consistent with another microcosm experiment, where there was no impact of competitors (i.e., alkali sacaton and needle and thread grass) on the number of side shoots produced by Canada thistle (Ferrero-Serrano et al. 2008).

Canada thistle plants grown in reduced nutrient soil produced 39% fewer side shoots (Figure 4). The negative impact of low soil nutrients on Canada thistle side shoot production is similar to findings by McIntyre and Hunter (1975), who reported a 68% decrease in side shoot production in soil treated with 21 versus 210 ppm N.

Canada Thistle Root Biomass. Canada thistle root biomass tended to be lower where *H. litura* was added, although effects were not significant (Table 1, Figure 5). The standard errors associated with the means were high, especially for mean root biomass for plants not exposed to weevils. Therefore, statistical power may have been insufficient to detect differences in root biomass between these two treatments. Several greenhouse experiments have shown negative effects of *H. litura* on root biomass (Ferrero-Serrano et al. 2008; Sciegienka et al. 2011), although results were not always consistent (Collier et al. 2007). An interesting question is, why would root biomass be more variable for plants without weevils?

Common sunflower caused an 83% reduction in Canada thistle root biomass (Table 1, Figure 5). Previous studies (Ferrero-Serrano 2008; Friedli and Bacher 2001) have reported reductions in Canada thistle root biomass resulting from competition with grass species. The exact mechanism by which competition reduced root biomass is unclear. Presumably, the primary mechanism would be belowground competition for soil nutrients. If this were the case then reduced soil nutrients should have also decreased root biomass, which was not observed. Canada thistle root biomass tended to be lower in pots with reduced soil
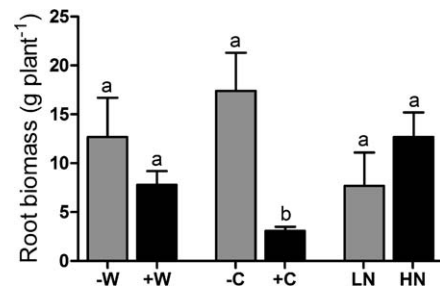
nutrients, but effects were not significant (Table 1, Figure 5), so the effect was not as strong as the effect from competition. In contrast, a previous study showed that Canada thistle root biomass from the 0 to 20–cm soil depth increased ~300% with the addition of 100 kg ha^{-1} nitrogen fertilizer (Nadeau and Vanden Born 1990).

Another possible mechanism for decreased Canada thistle root mass associated with common sunflower could be changes in root : shoot ratio caused by plant responses to shading or decreased red : far-red light ratios. Exposure to both shading and/or decreased red : far-red light ratios has been shown to cause plants to allocate more biomass to shoots vs. roots, thus enhancing the ability to compete for light (Smith 1982). In our study, Canada thistle plants grown with common sunflowers had 19% lower root : shoot ratios (P = 0.001, data not shown), lending some support to this explanation.

Summary

Though interactive effects among *H. litura* herbivory, common sunflower, and soil nutrients were generally not detected, these treatments did have substantial individual and additive effects on many measures of Canada thistle growth and reproductive output. Our research demonstrated the utility of using common sunflower as a plant competitor against Canada thistle, because it negatively impacted multiple growth and reproductive responses of Canada thistle, most notably a reduction in root biomass. Difficulties controlling Canada thistle are often attributed to its deep, extensive, and regenerative root system, which facilitates invasiveness, persistence, and tolerance to control measures (Lukashyk et al. 2008; Tiley 2010). Similar previous studies investigating the impacts of plant competition on Canada thistle did not quantify root biomass impacts (Edwards et al. 2000; Perry et al. 2009).

Weevils had a significant negative impact on Canada thistle height and flower number, but negative effects on shoot and root biomass were not statistically significant. In general we demonstrated fewer negative impacts of weevils on Canada thistle plants than have been reported from other similar experiments (e.g., microcosms; Collier et al. 2007; Ferrero-Serrano et al. 2008; Sciegienka et al. 2011). There are two possible explanations for this. First, in our study, not every Canada thistle plant that received adult *H. litura* treatment sustained visible larval stem-mining damage, and damaged plants generally had less than 25% of the stem pith mined. Previous studies did not report the extent of *H. litura* larval mining, but if the mining extent was greater, this could account for the discrepancy. Second, these previous studies were conducted in ~7.6-L plastic containers, whereas we used larger microcosms (19 L). Experiments have demonstrated that small pot sizes can negatively affect plant growth and lead to plants becoming root bound (Townend and Dickinson 1995), which may have contributed to general plant stress and enhanced negative treatment effects in previous experiments using smaller pots. Furthermore, results from microcosm studies should be interpreted cautiously, especially when studying clonal plants that can shift resources among interconnected ramets in response to herbivory (Gómez et al. 2007).

Our results suggest that *H. litura* weevils acting alone (at least at the level of weevil pressure present in our study) will have minor impacts on the growth and spread of Canada thistle, especially because weevils did not appear to reduce root biomass consistently. Practitioners often acknowledge that a single biological control agent is rarely sufficient to suppress an invasive weed species (DiTomaso 2000). Most known Canada thistle herbivores feed and develop in stems and leaves, where their impact is minimal (Friedli and Bacher 2001). Also, Canada thistle is a problematic weed in its home range, where it thrives among its natural enemies (Larson et al. 2005). For these reasons, successful direct biological control of Canada thistle solely via herbivorous insects seems unlikely. Some evidence suggests that the most important negative impact of Canada thistle insect biocontrol agents may be providing entry to disease pathogens and other detrimental organisms such as nematodes (Friedli and Bacher 2001; Liu et al. 2000). Combining insect agents with various pathogens may therefore hold some promise. Also, establishing and maintaining competitive beneficial plant species will help to suppress Canada thistle. Where feasible and economical, treatment with herbicides remains a viable option for reducing the impact of Canada thistle, especially in small accessible areas where dense monocultures have formed. Finally, given the widespread introduced range of this weed, perhaps assessing its real rather than perceived negative ecological impacts could improve development of future management policies and approaches.

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