Invasive Plant Science and Management 2011 4:000–000

Interactions between Two Biological Control Agents and an Herbicide for Canada Thistle (*Cirsium arvense*) Suppression

Joanna K. Sciegienka, Elai N. Keren, and Fabian D. Menalled*

We investigated the single and combined effects of two biological control agents, the stem-mining weevil *Hadroplontus litura* and the pathogen *Pseudomonas syringae* pv. *tagetis*, with a herbicide (reduced or full application of glyphosate: 0.63 kg ae ha^{-1}, or 3.78 kg ae ha^{-1}, respectively) on the growth of Canada thistle, *Cirsium arvense*. We hypothesized that first, although each control method would have a negative effect on Canada thistle shoot biomass, root biomass, and shoot number, the integration of more than one control method would have greater impact than individual control methods. Second, we hypothesized that the order in which control methods are applied affects the outcome of the management program, with a pathogen application following weevil infestation being more effective than one prior to it. Although control methods impacted Canada thistle growth ($P < 0.001$, expect for a nonsignificant impact of glyphosate on shoot number), the combined effect of the three control methods behaved, generally, in an additive manner. A marginal interaction between the pathogen and the herbicide ($P = 0.052$) indicated a slight antagonistic interaction between these control methods. An interaction between the two biological control agents tested ($P < 0.001$) indicated that application of a pathogen prior to the release of weevil larvae could be more deleterious to Canada thistle than a late application. The observed, mostly additive, relationship between biological control agents and herbicides implies that integrating control methods rather than using a single approach could lead to greater Canada thistle control.

Nomenclature: Glyphosate; *Pseudomonas syringae* pv. *tagetis* (Hellmers) Young et al.; stem-boring weevil, *Hadroplontus litura* (Fabricius); Canada thistle, *Cirsium arvense* (L.) Scop.

C1 **Nomenclature:** Integrated weed management; invasive species; biological control.

Increasing attention has been devoted in recent years to the development of integrative control protocols to reduce the spread and impacts of invasive plants (Ferrero-Serrano et al. 2008; Rayamajhi et al. 2010; Turner et al. 2010). It is of key importance to understand the biological and environmental factors conditioning the success of individual control practices as well the outcomes of their interactions (Hatcher 1995). These interactions can be direct, e.g., when insects consume fungal mycelia or spores, or when herbicides reduce the availability of pollen or nectar to support beneficial insect populations. Interactions among management practices can also be indirect, e.g., when alterations of the plant caused by herbicides affect behavioral responses by natural enemies. Identifying which suite of biological, cultural, mechanical, or chemical control practices will maximize control with minimal competition among them remains a challenge (Hunt-Joshi and Blossey 2005; Kaplan and Denno 2007).

Canada thistle [*Cirsium arvense* (L.) Scop.] is an aggressive, introduced creeping perennial that infests crops, pastures, rangelands, roadsides, and noncrop areas. This species originated in southeastern Eurasia and is one of the most problematic invasive weeds in the United States, Canada (Moore 1975), and northern Europe (Rydberg and Milberg 2000). In the Southern Hemisphere, Canada thistle has been introduced into South America, South Africa, Australia, and New Zealand (Tiley 2010).

DOI: 10.1614/IPSM-D-10-00061.1

* First and third authors: Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717-3120; second author: Department of Mathematical Sciences, Montana State University, Bozeman, MT 59717. Corresponding author's E-mail: menalled@montana.edu

Interpretative Summary

Canada thistle (*Cirsium arvense*) is one of the most problematic invasive plant species infesting crops, pastures, rangelands, roadsides, and noncrop areas in the United States, Canada, northern Europe, and New Zealand. Although many single methods have been developed to minimize Canada thistle spread and impact, this persistent invasive species continues causing problems in temperate regions of the world. Field data and mathematical models have shown that, if properly designed, integrated weed management can provide sustainable, economically viable, and successful weed control. In an integrated weed management program, control methods can interact synergistically, additively, or antagonistically, but few studies have specifically evaluated the existence and patterns of such relations. In repeated greenhouse experiments, we investigated the potential for suppressing Canada thistle using a combination of the stem-mining weevil *Hadroplontus litura,* the pathogen *Pseudomonas syringae* pv. *tagetis*, and reduced or full-rate applications of glyphosate (0.63 kg ae ha^{-1}, or 3.78 kg ae ha^{-1}, respectively). In general, individual control methods impacted Canada thistle growth and the combined effect of the three control methods behaved in an additive manner. A marginal interaction between the pathogen and the herbicide indicated an antagonistic interaction between these control methods. An interaction between the two biological control agents tested ($P < 0.001$) indicated that application of a pathogen prior the release of weevil larvae could be more deleterious to Canada thistle than a late application. The observed mostly additive relationship between biological control agents and herbicides implies that integrating management tactics rather than using a single approach could lead to improved Canada thistle control.

Canada thistle is well known for its deep and creeping root system and colony-forming tendencies that make infestations hard to control and easy to spread. Although many single methods including herbicides (Almquist and Lym 2010; Enloe et al. 2007;), tillage (Donald 1990), competition (Wilson and Kachman 1999), grazing (Edwards et al. 2005), and biological control (Coombs et al. 2004) have been developed to minimize Canada thistle spread and impact, this persistent invasive species continues causing problems in temperate regions of the world (Tiley 2010, and references herein).

The realization that individual control methods do not provide long-lasting solutions has spurred research into the integration of mechanical, cultural, biological, and chemical practices in the management of Canada thistle. At the crux of an integrated weed management (IWM) program is the concept that although each single control method may be only marginally effective, when combined they may provide significant and long-lasting suppression (Liebman and Gallandt 1997). Field data and mathematical models have shown that, if properly designed, IWM can provide sustainable, economically viable, and successful weed control in croplands (Blackshaw et al. 2008; Westerman et al. 2005), rangelands (Ferrero-Serrano et al. 2008), prairies (Lym 2005), and pastures (Shea et al. 2006).

The stem-boring weevil, *Hadroplontus litura* (Fabricius), formerly *Ceutorhynchus litura,* (Coleoptera: Curculionidae) is a phytophagous insect that can be used as a biological control agent in a Canada thistle IWM program. This weevil originated in Europe (Zwölfer and Harris 1966) and releases were made in the United States and Canada in the early 1970s for biological control of Canada thistle (Rees 1990). In early spring, adult weevils emerge from the soil and feed on thistle rosettes. When the thistle plants bolt in late spring, female weevils lay groups of eggs in small feeding cavities on the underside of leaves. After stem elongations, larvae hatch and mine down the leaf midveins into the root crown, feeding on parenchymal tissue. Larval feeding results in significant damage to the inner stem cavity and could result in secondary infection by plant pathogens (Rees 1990). Mature third instars larvae burrow out of the plant and pupate in the soil before emerging as adults (Peschken and Wilkinson 1981). Although previous studies have shown mixed results on the ability of *H. litura* to suppress Canada thistle (Hein and Wilson 2004; Reed et al. 2006), it has been suggested that the stress imposed by the weevil's feeding might lead to improved control when combined with other control methods such as herbicides (Collier et al. 2007) or competition (Ferrero-Serrano et al. 2008).

Plant pathogenic microbes have also been tested as biological control agents to manage Canada thistle but showed low incidence of systemic infection (Frantzen 1994), high year- to-year impact variability (Bourdôt et al. 2006; Gronwald et al. 2002), and high unpredictability of pathogen survivorship (Tichich et al. 2006). Despite these limitations, the potential of combining phytophagous insects with plant pathogenic microbes in an IWM program has been explored because, many times, these biocontrol agents share the same host plants (Barbosa 1991). However, the integration of plant pathogens and phytophagous insects in an IWM program should be done cautiously because the interactions between biocontrol agents are idiosyncratic and strongly dependent on the identity of the species involved. For example, Kok et al. (1996) determined that the rust *Puccinia carduorum* (Jacky) did not interfere with the oviposition behavior of three biocontrol insects [*Cassida rubiginosa* (Müller), *Rhinocyllus conicus* (Froel.), and *Trichosirocalus horridus* (Panzer)] on musk thistle [*Carduus thoermeri* (Weinm.)] control. However, Kruess (2002) observed that the necrotrophic fungus *Phoma destructiva* (Plowr.) hindered the egg deposition and adult feeding of *C. rubiginosa* in Canada thistle.

Pseudomonas syringae pv. *tagetis* (PST), a bacterial pathogen that causes leaf spot and apical chlorosis in members of the Asteraceae family (Gronwald et al. 2002; Rhodehamel and Durbin 1985) can be used in a Canada thistle IWM program. This pathogen produces a tageti-

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toxin that directly inhibits chloroplast RNA synthesis (Mathews and Durbin 1994) and has been shown to reduce Canada thistle growth and plant height (Gronwald et al. 2004; Hoeft et al. 2001). Although PST does not always lead to high levels of Canada thistle control (Tichich and Doll 2006) and its abundance is affected by environmental conditions (Tichich et al. 2006), it has the potential to be more successful when paired with other tactics. For example, the joint application of PST and glyphosate increases disease levels in the plants, reducing fresh and dry biomass (Bailey et al. 2000).

The outcome of an IWM program depends on the degree to which control methods influence each other as their interactions can be synergistic, additive, or antagonistic (Hatcher 1995). From an IWM perspective, synergistic interactions would be the best scenario but few studies have specifically evaluated the existence and patterns of such interactions and results are far from conclusive (Ainsworth, 2003). For example, although Kluth et al. (2003) observed some synergism between repeated Canada thistle cutting and the inoculation of the rust *Puccinia punctiformis* [(F. Strauss) Röhl.] , Collier et al. (2007) established that combining 2,4-D, glyphosate, or clopyralid with *H. litura* led to additive Canada thistle control. Also, Hoeft et al. (2001) failed to detect synergistic interactions between PST application and the use of a highly competitive soybean [Glycine max (L.) Merr.] variety for the management of Canada thistle in conservation tillage systems. Finally, Cripps et al. (2010) observed that combining the leaf-feeding beetle *C. rubiginosa* with interspecific plant competition to control Canada thistle produced weak additive or antagonistic responses, depending on the measured variable.

To our knowledge, no study has systematically evaluated the existence and patterns of interactions when more than two control methods are applied on Canada thistle. The goal of our experiments was to assess the existence and patterns of interactions between two biological control agents and a herbicide when combined in an IWM strategy for Canada thistle control. Specifically, we assessed the individual and joint impact of *H. litura,* timing of PST application, and two rates of glyphosate on Canada thistle emergence and growth. We hypothesized that first, although each treatment would have a negative effect on Canada thistle growth, the integrated strategies would have higher impact than individual control methods. Second, it is possible that the exit holes left in the root crown by *H. litura* larvae make Canada thistle more susceptible to PST. Therefore, a PST application following weevil infestation would be more effective than one prior to it.

Materials and Methods

This study was conducted in a light- and temperature-controlled greenhouse at Montana State University, Bozeman, MT. Two different experiments were run between April 2006 and October 2008, each with the same treatments. Each experiment followed a completely randomized design with five replicates per treatment. Greenhouse lights[1] supplemented ambient light to provide a 16-h/8-h light/dark photoperiod to facilitate plant emergence and growth. Temperature in the greenhouse ranged from a nightly low of 13 to 15 C (55.4 to 59 F) to a daily high of 30 to 40 C.

Canada thistle root fragments to be used in the 2006 experiment were dug in September 2005 from the top 30 cm of soil in an upland grassland site near Cheyenne, WY (41°N, 105°W, 1,843 m [6,047 ft] elevation). Root fragments were planted into 10.6-L pots containing topsoil collected in Laramie, WY. The resultant Canada thistle plants were maintained in a temperature-controlled greenhouse at the University of Wyoming greenhouse facility until shipped overnight to Bozeman, MT, and transplanted for the experiments. Root fragments used in the 2008 experiment were dug the previous fall at the Montana State University Arthur H. Post Agronomy Farm in Bozeman, MT (45°N, 11°W, 1,423 m elevation). These root fragments were stored in Canadian sphagnum peat moss at 4 C until transplanted for the experiments.

Root fragments of approximately uniform diameter were divided into 10-cm (4-in) sections and planted 4 to 5 cm deep singly into 43-cm-tall pots constructed with 15-cm-diam thin-walled polyvinyl chloride irrigation pipes. Prior to planting, each pot was lined with a 0.1-mm-thick plastic liner to create a bottom for the pot. Approximately 7.6 L (2 gal) of a soil blend of a 1 : 1 : 1 ratio of mineral soil collected near Bozeman, MT, Canadian sphagnum peat moss, and washed concrete sand was put into each pot. A wetting agent[2] was added at a rate of 5.9 g L^{-1}. Treatments were insects (absent or present), timing of PST application (none, early [before insect release], or late [after insect release]), and glyphosate[3] rate (none; one-sixth of the labeled rate, 0.63 kg ae ha^{-1} [0.56lb ae ac^{-1}], or a full labeled rate −3.78 kg ae ha^{-1}),

Adult *H. litura* collected from late April to early May near Bozeman, MT, were purchased from a commercial source[4] and maintained on Canada thistle foliage for 2 to 9 d. When rosettes were 5 to 10 cm in diameter, five adult weevils were placed on each experimental thistle plant receiving an insect treatment. At the time of the weevil release, all pots contained one single Canada thistle plant and were individually caged with 40-cm-wide, 40-cm-long mesh sleeves made from mosquito netting.[5] Weevils were allowed to feed and oviposit on thistle plants for 1 wk, after which all weevils and cages were removed. Newly emerged second-generation weevils were removed when appropriate.

The PST was grown in the laboratory on tryptic soy agar and suspended in a cryo-solution of 1% tryptic soy broth and 10% glycerol before storage in a freezer at −20 C. It

Table 1. *F*-values and degrees of freedom from ANOVA assessing the effect of glyphosate, *Pseudomonas syringae* pv. *tagetis*, and *Hadroplontus litura* weevils on Canada thistle shoot weight and root weight growing in greenhouse conditions The impact of control methods on Canada thistle shoot number was evaluated using a Poisson regression.[a,b] ?1

Factor	Root biomass	Shoot biomass	Shoot number	df[c]
Glyphosate	26.93***	15.54***	2.07$^{n.s.}$	2
H. litura	34.99***	35.97***	19.88***	1
PST	26.56***	24.35***	13.88***	2
Glyphosate × *H. litura*	1.06$^{n.s}$	0.20$^{n.s.}$	0.23$^{n.s.}$	2
Glyphosate × PST	0.73$^{n.s.}$	2.40*	0.21$^{n.s.}$	4
H. litura × PST	1.88$^{n.s.}$	0.55$^{n.s.}$	4.22***	2
Glyphosate × *H. litura* × PST	1.91$^{n.s.}$	1.43$^{n.s.}$	1.25$^{n.s.}$	4
Year	0.90$^{n.s.}$	0.93$^{n.s.}$	4.88**	1

[a] Abbreviations: df, degrees of freedom; PST, *Pseudomonas syringae* pv. *tagetis*.

[b] Statistical significance is indicated as follows: n.s., not significant ($P > 0.05$); *$P = 0.05$; **$P < 0.05$; ***$P < 0.001$.

[c] Denominator degrees of freedom = 161.

was first thawed from this frozen state, then 100 ml of this solution was spread onto a petri dish containing tryptic soy agar at 50% strength and incubated at 25 C for at least 48 to 54 h. For each greenhouse trial, one plate of PST containing approximately 10^{10} bacteria cells ml^{-1} was mixed with 500 ml (17 oz) of water to create a solution that contained approximately 2×10^7 bacteria ml$^-$1. A organosilicone surfactant[6] was added to the suspension at a rate of 0.4% final volume to facilitate spreading, wetting, and absorption (Bailey et al. 2000; Zidack et al. 1992). A solution of the organosilicone surfactant alone was sprayed on all control plants. The PST was applied at two different times: either 1 d before the insects were released on Canada thistle (early application) or 1 wk after the insects were removed (late application).

Herbicide treatments were applied prior to the flowering stage of Canada thistle, 77 d after planting (DAP) in 2006 and 84 DAP in 2008. In 2006, the glyphosate was applied outside the greenhouse using an 8001 flat-fan nozzle[7] at a pressure of 276 kPa and a spray volume of 93.5 L ha^{-1}. In 2008, the glyphosate was applied in a spray chamber with a single 8002 even flat-fat nozzle[7] at a pressure of 276 kPa and a total spray volume of 131 L ha^{-1}.

Approximately 90 d after herbicide application, we recorded the number of shoots subsequently produced by each individual Canada thistle plant. At that time, all Canada thistle shoot and roots were harvested. The shoots were clipped at the soil surface, dried in a 65 C oven for at least 72 h, and weighed. The roots were gently washed before drying in a 65 C oven for at least 72 h and weighing.

Root dry biomass was natural-log-transformed to meet normality assumptions for ANOVA. We a used the Mixed procedure in SAS[8] to perform a multifactor ANOVA with glyphosate, pathogen, and insects as fixed factors and year and rep nested in year as random effects. Significant main effects were further analyzed for differences among levels using a Tukey multiple comparisons test. Poisson regression was used to analyze shoot number count data with Genmod in SAS.[8] Significant interactions between main effects ($P < 0.05$) suggest synergism or antagonism whereas lack of interactions suggests that the control methods were additive (Collier et al. 2007). Interactions were further sliced and analyzed with a Bonferroni multiple comparisons procedure to determine differences among levels (Littell et al. 1996).

Results and Discussion

We failed to detect a significant impact of year on shoot weight ($P = 0.37$), root weight ($P = 0.35$), or repetitions ($P < 0.98$). Furthermore, there were no significant interactions among random effects (year and repetition nested in year) and the fixed effects (glyphosate, PST, or insects) for either shoot or root weight. The shoot weight and root weight data were pooled across repetitions and years for all further analysis.

Individual control methods impacted Canada thistle growth (Table 1). In the absence of other control methods, *H. litura* decreased root biomass by an average of 18% and PST decreased it by an average of 33% (late application) and 69% (early application) (Figure 1). When compared to the control plants, the application of reduced and full rates of glyphosate decreased root biomass by an average of 45 and 86%, respectively (Figure 1). The existence of nonsignificant second- or third-order interactions between *H. litura*, PST, and glyphosate on Canada thistle root biomass (Table 1) indicates that, in accordance with previous studies (Collier et al. 2007; Cripps et al. 2010; Hoeft et al. 2001), the integrated effects of control methods were additive rather than antagonistic or synergistic.

Canada thistle shoot biomass was impacted by the individual application of *H. litura,* PST, or glyphosate (P

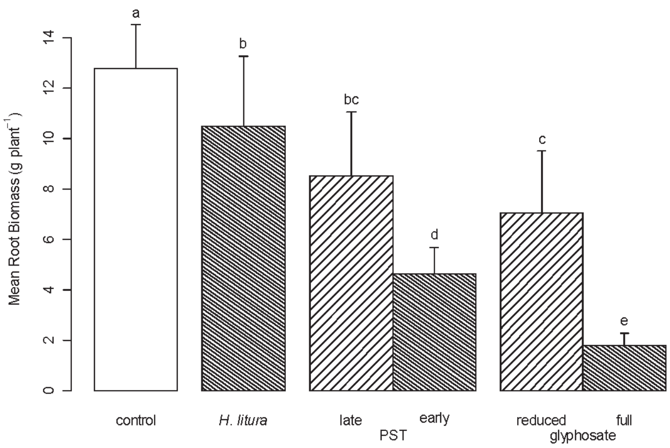

Figure 1. Impact of *Hadroplontus litura*, *Pseudomonas syringae* pv. *tagetis* (PST), and glyphosate on Canada thistle dry root biomass in greenhouse conditions. Bars indicate mean values plus standard error of the mean. Bars labeled with different letters differ ($P < 0.05$).

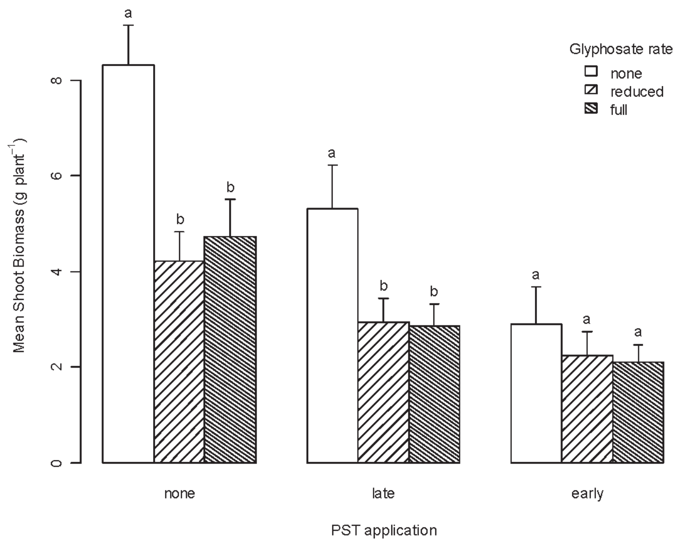

Figure 2. Impact of glyphosate and *Pseudomonas syringae* pv. *tagetis* (PST) on Canada thistle dry shoot biomass in greenhouse conditions. Bars indicate mean values plus standard error of the mean. For each PST level, bars labeled with different letters differ ($P < 0.05$).

< 0.001, Table 1) with a marginally significant interaction between PST and glyphosate ($P = 0.052$). The slicing of the data across pathogen treatments showed that in the absence of PST or when PST was applied after *H. litura* (late), the reduced and full rate of glyphosate impacted shoot biomass. However, for the early PST application both rates of glyphosate were equivalent to the control indicating no additional benefit from the combination of these two control methods (Figure 2). It is possible to speculate on two mechanisms responsible for the antagonistic interaction between glyphosate and the early PST application. First, although glyphosate could have reduced PST's efficacy by killing shoot tissue, PST could have also interfered with glyphosate's activity by metabolizing or degrading the herbicide's active ingredient. Boyette et al. (2008) suggests a similar mechanism to explain antagonistic effects between glyphosate and the fungus *Colletotrichum truncatum* [(Schwein.) Andrus & W.D. Moore]. Similar to the tagetitoxin that PST produces, a secondary effect of glyphosate is to inhibit the production of chlorophyll in meristematic tissue (Hunter 1996; Mathews and Durbin 1990). Second, it is possible that the combined impact of glyphosate and PST was masked by the already heavy impact that each control method had on the Canada thistle shoots.

The application of glyphosate did not impact the number of shoots produced by Canada thistle plants whereas *H. litura* and PST had a significant effect (Table 1). Furthermore, we observed significant differences across years ($P < 0.01$) and an interaction between *H. litura* and PST ($P = 0.01$), between year and *H. litura* ($P = 0.01$), and between year and PST ($P = 0.01$) on the number of Canada thistle shoots produced. An inspection of these interactions indicated that in 2006 there were no differences in the number of Canada thistle shoots produced between the early and late application of PST when *H. litura* was absent. However, fewer Canada thistle shoots were produced in the early PST treatment than in the late PST treatment when insects were included in the study (Figure 3). In 2008, no differences were observed across insect and pathogen levels. It is possible that the different patterns observed across years in the interaction between biological control agents resulted from the genetic characteristics of the Canada thistle population used in this study. The two experimental runs were conducted using Canada thistle collected in different regions (Wyoming and Montana). Previous research (Solé et al. 2004) determined high levels of genotypic and genetic diversity across Canada thistle populations which in turn

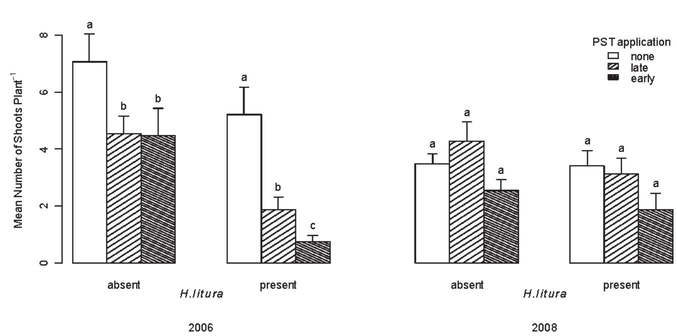

Figure 3. Impact of *Pseudomonas syringae* pv. *tagetis* (PST) and *Hadroplontus litura* on Canada thistle shoot number in greenhouse conditions. Bars indicate mean values plus standard error of the mean. For each *H. litura* and year level, bars labeled with different letters differ ($P < 0.05$).

could affect the incidence of biocontrol agents (Smith et al. 1999). Previous studies (Menalled et al. 2004) have also highlighted the impact of environmental conditions on the performance of biological control agents.

It has been suggested that insect injury to Canada thistle can provide vectors for pathogen infection, enhancing the results of integrated control methods (Friedli and Bacher 2001; Kluth et al. 2001). Our results did not support these observations as more root biomass was observed in the late PST application than the early one (Figure 1). A similar trend was observed for shoot biomass produced in the absence of glyphosate (Figure 2) and for the number of shoots produced when *H. litura* was combined with a late PST application (Figure 3). In accordance, Kruess (2002) found that the joint use of the ovipositing leaf beetle *C. rubiginosa* and necrotrophic fungus *P. destructiva* could be counterproductive when used to manage Canada thistle. Two mechanisms could explain the impact of the relative time of pathogen application on the number of Canada thistle plants. First, the response could be due to the fact that PST is only active on newly forming tissue in the plant (Mathews and Durbin 1990). Since the early application was sprayed on developing shoots, the effects of the infection could have been more pronounced than when applied to more mature plants. Second, it is possible that the presence of *H. litura* resulted in compensatory growth of Canada thistle plants. Compensatory growth due to *H. litura* has been observed in Canada thistle (Hein and Wilson 2004) as higher concentration of glucose and fructose was observed in the roots of plants that have experienced herbivory.

In recent years, there has been a growing interest in reduced herbicide rates for weed control (Blackshaw et al. 2006). In this study, when applied alone, a reduced rate of glyphosate, although reducing the thistle's growth, did not provide effective control limiting the applicability of this management approach. However, and in accordance with previous studies (Williams et al. 2004; Zhang et al. 2000), our results showed that when combined with other control methods, a reduced herbicide rate of glyphosate significantly impacted Canada growth.

Although we were unable to demonstrate synergism between control methods, several conclusions can be drawn from this work. First, this study highlights the importance of testing the existence and patterns of multiple interactions between control methods in the development of an IWM program. Although previous studies have evaluated the combined used of two single control methods for Canada thistle control (Ferrero-Serrano et al. 2008; Kluth et al. 2003; Travnicek et al. 2005), the literature is lacking in detailing interactions between more than two control methods. Specifically, the existence of interactions among insects, herbicides, and pathogens is still largely unexplored. While this study evaluated the short-term impact of multiple control methods on Canada thistle growth, future studies should evaluate the mid- and long-term impact on this species' temporal and spatial dynamics. Also, future research should assess the existence and patterns of interactions among the evaluated control practices across a wide range of environmental conditions.

Second, this work adds to the growing body of research that emphasizes the use of IWM over individual tactics (Chikowo et al. 2009). Our results show that, in general, integrated methods work equally well as or more effectively than the best individual control method. Although beyond to the scope of this study, a cost–benefit analysis performed in a typical management setting would be a useful accompaniment in the development of IWM programs (Pardo et al. 2010). This analysis should address the logistic challenges of integrating several control practices into holistic weed management programs. Major barriers to implementation of IWM include social, economic, and regulatory factors and time and labor constraints, as well as the development of an educational infrastructure that will support farmers and land managers (Jordan et al. 2006).

Finally, although these results did not display synergism, there is a possibility that synergistic interactions could still develop in field conditions due to the added abiotic and biotic stressors that plants face (Bostock et al. 2001) or if a different herbicide is used. Conversely, it is also important to determine whether interactions are antagonistic in field conditions to prevent unsuccessful management. Overall, the observed additive relationship between biological control agents and herbicides implies that integrating management tactics rather using than a single approach leads to greater Canada thistle control.

Sources of Materials

[1] GE Multi-Vapor MVR1000/C/U metal halide lamps, General Electric Co., 3135 Easton Turnpike, Fairfield, CT 06828.

[2] Aqua-Gro 2000G, Aquatrols Co., 1273 Imperial Way, Paulsboro, NJ.

[3] Roundup Original Max®; Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167.

[4] Biological Control of Weeds Inc., 1418 Maple Dr., Bozeman, MT 59715.

[5] Rockywoods Outdoor Fabrics, 418 8th St. S.E., Loveland, CO 80537.

[6] Silwet L-77®, polyaklyleneoxide modified heptamethyltrisiloxane, GE Silicones, 3500 South State Route 2. Friendly, WV 26146.

[7] TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189-7900.

[8] SAS Version 9.1, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

Acknowledgments

This project was funded by USDA/Western IPM, the Montana Noxious Weed Trust Fund, and the Montana Alfalfa Seed Committee. The authors wish to thank Dr. Timothy Collier and Dr. Stephen Enloe for their technical support; Dr. Nina Zidack for providing the PST; Libby Flanagan, Katrina Hartman, Sue Jerrett, Kristin Harbuck, Fred Pollnac, Evette Allison, Justin O'Dea, Jessie Wiese, Phil Davis, Matt Hanson, and Ed Davis for their assistance with greenhouse and field work; and two anonymous reviewers.

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Received August 11, 2010, and approved November 30, 2010.

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