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BIOLOGICAL-CONTROL HERBIVORES MAY INCREASE COMPETITIVE ABILITY OF THE NOXIOUS WEED *CENTAUREA MACULOSA*

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Abstract. Biocontrol organisms are generally applied in an attempt to reduce the vigor of target species and provide native species with an competitive advantage. We tested the effectiveness of a widely used biocontrol moth, *Agapeta zoegana* (knapweed root moth) for two years in the field and found that it had no significant direct effect on the biomass of *Centaurea maculosa* (spotted knapweed), one of the most destructive invasive plants in North America. Instead of releasing a native grass from competition, the reproductive output of *Festuca idahoensis* planted with *Centaurea* was significantly lower when neighboring *Centaurea* had been attacked by *Agapeta*. In a greenhouse experiment, we found that *Festuca* planted in pots with *Centaurea* that had been attacked by *Trichoplusia ni* (another nonnative herbivore) had significantly smaller root systems than when they were planted with *Centaurea* that were protected from herbivory. Root systems of *Centaurea* that had been attacked by *Trichoplusia* exuded higher levels of total sugars, but not total phenols. We hypothesize that moderate herbivory stimulated compensatory growth, induced the production of defense chemicals that also had allelopathic effects, or stimulated root exudates that altered the relationship between *Centaurea* and *Festuca* via soil microbes. Our data suggest that herbivory may increase the negative effects of *C. maculosa* on neighboring plants, and that some biocontrols may have indirect negative effects on native species that are not currently recognized.

Key words: *Agapeta zoegana* Lin; biocontrols; *Centaurea maculosa* Lam.; competition; consumers; exotics; *Festuca idahoensis* Elmer; herbivory; indirect and higher order interactions; invasive plants; knapweed; *Trichoplusia ni* Hübner.

INTRODUCTION

Exotic invasive species pose a serious threat to global biological diversity by causing rapid, local replacement and eradication of native species (Julien 1987, Drake et al. 1989). Many promising weapons in the fight against exotic invaders are biological: predators, herbivores, and parasites imported from the invasive species' place of origin. The use of biocontrols has been highly successful in some cases, reducing the spread of invasive plants (Huffaker and Kennett 1959, Cullen 1973, McEvoy et al. 1991) and providing "green" alternatives for pest management. However, biocontrols sometimes attack nontarget native species, compete with native species, and have unwanted community and ecosystem effects (Howarth 1991, Simberloff and Stiling 1996, Louda et al. 1997). Ecologists have also expressed alarm about the widespread use of biocontrols, because biocontrols may exert indirect effects that are not yet understood (Howarth 1991).

Centaurea maculosa Lam. (Asteraceae, spotted knapweed) is one of the most economically destructive exotic invaders in the northwestern United States and southwestern Canada, and it infests >4 Mha in North

America (Müller-Schärer and Schroeder 1993). *Centaurea* invasion is often characterized by dense stands and virtually complete competitive exclusion of native species. Although *Centaurea* species have been highly successful in North America, they are usually minor components of their native communities. Biological control agents were first introduced to control knapweed species in 1970, and now 11 species of insects have been introduced to North America for this purpose (Müller-Schärer 1991). One of the most promising is the moth *Agapeta zoegana* Lin (Tortricidae, knapweed root moth) the larvae of which are specialist herbivores on the taproots of a few related *Centaurea* species (Müller 1989). We have also observed other nonnative herbivores consuming *Centaurea*, including the common cabbage looper, *Trichoplusia ni* Hübner (Noctuidae).

The use of biocontrols to weaken or eliminate exotic plants is based on a general, but untested, assumption: invasive exotics are successful because they have escaped intense consumer pressure in their native habitat. Implicit in this assumption is that direct top-down effects in these systems are paramount and that indirect effects are weak. However, in some circumstances, indirect effects in natural communities can be stronger than direct effects (Strauss 1991, Wootton 1994). Despite the fundamental importance of understanding how

Manuscript received 3 July 1997; revised 11 May 1998; accepted 18 May 1998; final version received 29 June 1998.

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biocontrols indirectly affect native plants, examination of the effects of biocontrols are almost always limited to the direct effects of the biocontrol on the target weed and the potential direct effects of the biocontrol on natives related to the target weed. Here, we studied the direct effects of *Agapeta* and *Trichoplusia* on the target species *C. maculosa* and its indirect effects on a non-target native bunchgrass in common-garden plots and in greenhouse conditions.

METHODS

Common garden experiment

A field experiment was designed to test the direct effect of *Centaurea* on *Festuca idahoensis* Elmer., the direct effects of *Agapeta* on *Centaurea*, and the indirect effect of *Agapeta* on *Festuca*. This experiment was carried out at the Diettart Experimental Gardens in Missoula, Montana, USA. Soils at the site are in the Bigarm Gravelly Loam Series (Loamy-skeletal, mixed, frigid Typic Haploxerols). Twelve *Centaurea* and 12 *Festuca idahoensis* individuals were planted in each of 20 0.25-m² plots, with individual plants alternating by species in rows and columns of a grid. The locations of these plots within the sample area were chosen randomly, and each plot was 50 cm from neighboring plots on all sides. Mesh cages were placed over all 20 plots, and four *Agapeta zoegana* moths were introduced into each of a randomly chosen subset of 10 plots in June 1994 (see Müller-Schärer 1991). *Agapeta zoegana* is a native of Eurasia, as is *Centaurea*, and is now being used extensively in efforts to control the spread of *Centaurea*. We acquired *Agapeta* from the Montana State University Experiment Station in Corvallis, Montana. *Agapeta* moths lay their eggs on the stems and at the root-shoot interface of *Centaurea* plants, and the larvae hatch from the eggs in 7–10 d and immediately burrow into the taproot to feed (Weeden et al. 1997). In western Europe, the moth is univoltine, and the main period of feeding occurs during the fall and spring (Müller et al. 1988). Adult moths emerge from knapweed roots in early July–early September. In another 10 plots, *Festucas* were planted with conspecific competitors, rather than *Centaurea*. These plots were also treated with cages, although no moths were introduced. Herbivory on *Festuca* by native insects was not controlled, but vertebrate herbivores were excluded from the experimental garden. In October 1994 we measured the basal areas of the four central *Festuca* individuals in each plot. In September 1995, two growing seasons after introducing *Agapeta*, total aboveground biomass of *Centaurea* and *Festuca* was harvested, and the total number of *Festuca* florets in each plot were counted. Bunchgrass meristems accrue laterally; thus, basal diameter is an important measure of vegetative growth. We did not reapply *Agapeta* in the second year of the experiment. In the plots with only *Festuca* plants, total floret number was halved. This was done in order to

compare reproductive production between single-species plots and plots containing both *Centaurea* and *Festuca*.

To provide the abiotic background for this experiment, and to investigate the potential confounding effects of soil water availability on treatments, soil moisture was measured using Frequency Domain Reflectometry (Troxler [Triangle Park, North Carolina], Sentry 200-AP). To investigate the potential confounding effects of differences of soil nutrient availability on treatment effects, ion exchange resin bags were used to accumulate available nitrogen and phosphorus from 15 April–15 October 1994 (Binckley and Vitousek 1991). Resin bags were buried 15 cm deep near the center of each of the 30 garden plots. Nitrogen was measured as 1 mol/L KCl extractable ammonium and nitrate, and phosphorus was measured as 1 mol/L KCl extractable phosphorus.

Signs of *Agapeta* herbivory were observed in the taproots of numerous plants in each of the treatment plots at the time of the harvest, but root damage was not quantified. We observed mature *Agapeta* moths in *Centaurea* foliage 2 yr after establishing the treatments, suggesting that *Agapeta* may have dispersed to treatment plots that were intended to be free from herbivory. However, we observed no sign of larval damage in any of the *Centaurea* taproots in the no-herbivory treatments. All ANOVA comparisons were conducted with fixed-effect models.

Greenhouse experiment

A greenhouse experiment was designed to more carefully control the intensity of herbivory and to test the effects of a leaf herbivore. Twenty-six *Centaurea* at the rosette stage were collected in the field, and each was transplanted into an 8-L pot containing field soil of the same type used in the common garden experiment and grown in a naturally lighted greenhouse. On sunny days, irradiation ranged from 450–1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and temperatures ranged from 24–32°C. *Festuca* were grown from seed in silica sand for 8 wk, and then one individual was transplanted into each pot with a *Centaurea*. Five days after transplanting the *Festucas*, a single cabbage looper caterpillar (*Trichoplusia ni*) was placed inside a cage (made from transparent acetate and fine mesh) around the *Centaurea*–*Festuca* pairs in 13 pots. These were left until 10–25% of the *Centaurea* leaf area was either eaten or was discolored, due to caterpillar herbivory. We acquired *Trichoplusia* from the Cornell University Experiment Station, Geneva, New York, USA. *Trichoplusia* is not currently used as a biocontrol for *C. maculosa*, but we have observed *Trichoplusia* eating *Centaurea* in the field, and, in earlier experiments, we found it to consume the weed rapidly. Our primary reasons for using *Trichoplusia* were the ease of application and estimating rates of herbivory, which was not possible with *Agapeta*. Another 13 *Centaurea*–*Festuca* pairs were protected from

caterpillar herbivory with the same cages used for the herbivory treatment. Plants were harvested 8 wk after *Trichoplusia* were removed. They were then dried at 60°C and weighed. During the 8 wk of the experiment, we did not observe other insects on our test plants; however, in the eighth week aphids were seen on several *Centaurea* plants, and the experiment was abruptly terminated.

A single 2 cm diameter polyester capsule (Unibest, Bozeman, Montana, USA) containing 1100 m² surface area of nonionic carbonaceous resin (Ambersorb 563, Rohm and Haas, Philadelphia, Pennsylvania, USA) was buried within the root mass of each *Centaurea* at the time of transplanting. When the plants were harvested, capsules were removed and frozen until analysis. Capsules were thawed to room temperature, and exudates were eluted from the resins using a sequential extraction of five pore volumes (25 ml) of distilled deionized water, followed by five pore volumes of 50% redistilled methanol. The extracts were then analyzed for total hydrolyzable sugars, total phenols, and ultraviolet (UV) absorption spectra. Total sugars were determined by reacting 5 ml of extract with 10 ml of anthrone reagent, followed by analysis against glucose standards at 625 nm (Brink et al. 1960). Total phenolic compounds were determined in methanol extracts, as described by Price and Butler (1977). Ultraviolet absorption spectra were recorded for all extracts, by performing a scan of light absorption in the range 350–190 nm.

RESULTS

Plots in all treatments were very similar in soil water status, ammonium, and nitrate. Soil moisture content at 15- and 30-cm depths did not vary significantly between treatments in either year (repeated-measures ANOVA, $F_{\text{treatment} \times \text{time at 15 cm}} = 0.93$; $df = 5, 59$; $P = 0.606$; $F_{\text{treatment} \times \text{time at 30 cm}} = 1.04$; $df = 5, 59$; $P = 0.412$). Likewise, soil nutrients did not vary among treatments. Available soil nitrate for the *Festuca* alone, *Festuca*–*Centaurea*, and *Festuca*–*Centaurea*–*Agapeta* treatments, combined, were the following: 5.25 ± 1.31 µg/g resin (mean \pm 1 SE) for nitrate, 4.17 ± 0.93 µg/g for ammonium, and 0.49 ± 0.13 µg/g for phosphorus. These values did not vary significantly between treatments during the first year of the study (ANOVA, NO_3^- : $F_{\text{treatment}} = 0.149$; $df = 2, 29$; $P = 0.862$; NH_4^+ : $F_{\text{treatment}} = 0.319$; $df = 2, 29$; $P = 0.729$; Phosphorus: $F_{\text{treatment}} = 1.74$; $df = 2, 29$; $P = 0.194$).

In the field, *Centaurea* severely suppressed *Festuca* reproduction (Fig. 1) and aboveground biomass (*Festuca* alone, 0.85 ± 0.17 g/plant, *Festuca* with *Centaurea*, 0.33 ± 0.11 g/plant; ANOVA, $F = 7.62$; $df = 1, 19$; $P = 0.012$), but *Centaurea* biomass was not significantly affected by exposure to *Agapeta* herbivory for two growing seasons (ANOVA, $F = 1.68$; $df = 1, 19$; $P = 0.21$). We conducted a power test (Cohen 1988) on data for *Centaurea*'s response to herbivory and

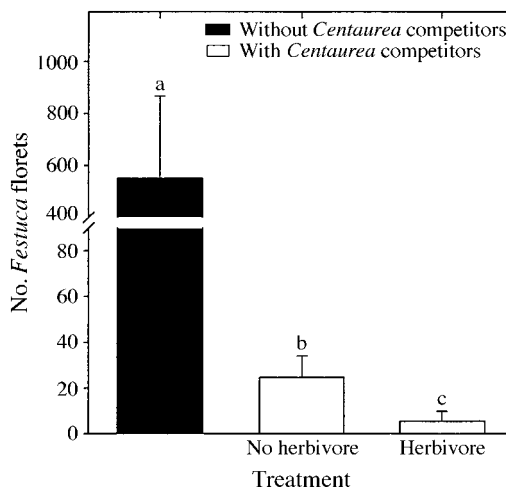


FIG. 1. Total number of florets produced in 0.25 m common-garden plots by *Festuca idahoensis* without *Centaurea maculosa* neighbors (corrected for the number of *Festuca*), or with *C. maculosa* neighbors and either with or without *Agapeta zoegana* biocontrols. Error bars show +1 SE. Significant differences among treatments are denoted by different letters (a, b, and c) above the error bars (ANOVA, post-ANOVA Tukey).

found that it would have taken a sample size of 50 to have an 80% chance of detecting a biologically real difference of the observed size. We found no significant effects of water or soil nutrients as covariates. *Festuca* did not benefit from herbivory on *Centaurea*. In fact, when *Agapeta* were allowed to attack *Centaurea*, the number of *Festuca* florets produced per plot decreased (Fig. 1; ANOVA, including soil moisture in plots as a covariate, $F_{\text{treatment}} = 4.43$; $df = 1, 19$; $P = 0.050$). When neighbor *Centaureas* were exposed to herbivory, individual *Festuca* basal area was 4.49 ± 1.45 cm², compared to 7.25 ± 1.25 cm² when there was no herbivory on *Centaurea*, but the difference was not significant (treatment by plot ANOVA, $F_{\text{treatment}} = 2.14$; $P = 0.149$), and there were no significant effects of water or nutrients as covariates. *Festuca* biomass was 0.49 ± 0.16 g when grown with *Centaurea* without *Agapeta*, and 0.33 ± 0.05 g when *Agapeta* was applied to *Centaurea* (ANOVA, $F = 1.380$; $df = 1, 19$; $P = 0.268$, no significant effects of covariates).

We could not accurately measure root biomass in the field, although it was measured in the greenhouse. In the greenhouse experiment, *Festuca* root biomass was 0.33 ± 0.04 g when neighboring *Centaurea* had been attacked by cabbage loopers, but was 0.41 ± 0.04 g when *Centaurea* was protected from herbivory (Fig. 2; ANOVA, initial *Festuca* size as covariate, $F_{\text{treatment}} = 6.75$; $df = 1, 25$; $P = 0.016$). Herbivory on *Centaurea* did not significantly reduce the shoot or total biomass of the invasive weed. *Centaurea* did not flower during the experiment; therefore, we do not have measurements of reproduction to compare to the field experiments. Total sugars sorbed to nonionic resins and ex-

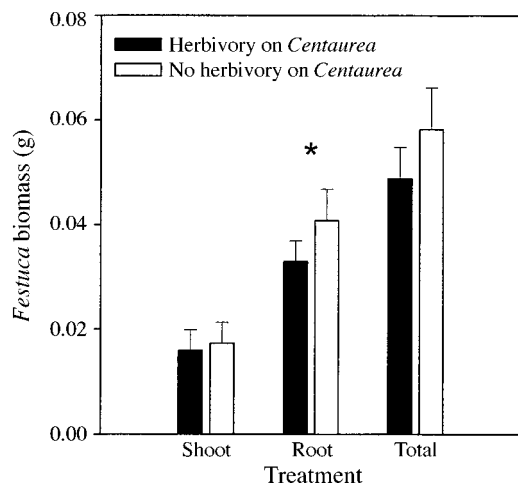


FIG. 2. Biomass of *Festuca idahoensis* with *Centaurea maculosa* neighbors that had either been grazed by *Trichoplusia ni* or protected from grazing. Error bars show +1 SE. The asterisk denotes a significant difference between treatments; ANOVA, $P < 0.05$, initial size of *Festuca* was used as a covariate.

tracted by water or methanol were significantly greater in resin capsules under *Centaurea* that had been attacked by *Trichoplusia* (Fig. 3; ANOVA, $F = 5.602$; $df = 1, 24$; $P = 0.027$). Total methanol-extractable phenols captured on the resin capsules did not differ among the treatments, but they tended to be greater under *Centaurea* that had been exposed to herbivory. (ANOVA with cosine-transformed data, $F = 0.697$; $df = 1, 25$; $P = 0.413$).

DISCUSSION

Contrary to expectations for biocontrols in general, in both experiments, *Centaurea* that had been exposed to herbivory had greater negative effects on *Festuca* than *Centaurea* that were kept free from herbivory; and, in the field experiment, *Agapeta* did not significantly decrease *Centaurea* biomass. Müller-Schärer (1991) and Steinger and Müller-Schärer (1992) also found that intermediate levels of *Agapeta* herbivory did not reduce shoot biomass, shoot number, or seed output of *C. maculosa*; but such levels of herbivory did reduce plant height and reproductive mass.

The mechanisms for the indirect effect of these herbivores on *Festuca* are unclear; however, there are three salient hypotheses that are applicable to both experiments. First, *Centaurea* plants may have grown faster after they experienced herbivory and, thereby, reduced resources available for other species. This is supported by the absence of a difference in total biomass between *Centaurea* exposed to herbivory and *Centaurea* protected from herbivory in the greenhouse and field experiments. Such herbivory-induced growth is termed "compensatory growth," and it has been widely reported and debated (Maschinski and Whitham 1989,

Alward and Joern 1993, Belsky et al. 1993). However, we know of no instance in which a compensatory response has increased the competitive ability of a plant. The intensity of compensatory growth is highly dependent on particular abiotic and biotic conditions (Maschinski and Whitham 1989, Alward and Joern 1993), and, if compensatory effects are restricted to certain soil conditions (high-nitrate low-phosphorus conditions, in our study), it may not be important in other environments. In Switzerland, Steinger and Müller-Schärer (1992) found that *Centaurea maculosa* ssp. *rhenana* exhibited root compensatory growth when consumed by *Agapeta* and *Cyphocleonus achates* (which is another root herbivore used as a biocontrol) and that the compensatory response was affected by soil nitrogen content and competition from *Festuca pratensis*.

A second hypothesis is that biocontrols induced the production of defensive secondary metabolites that also functioned as antiplant allelopaths. Others have found dual antiherbivore-allelopathic roles in inducible plant metabolites (Lovett and Holt 1995), and increased allelopathic productivity under stress (Tang et al. 1995). Cnicin, a defensive chemical in *Centaurea* species that has been found to deter herbivory (Landau et al. 1994), is also suspected to have allelopathic properties (Fletcher and Renney 1963, Muir and Majak 1983, Kelsey and Locken 1987). However, cnicin is primarily restricted to the trichomes on *Centaurea* leaves, and herbivory is unlikely to increase its production (R. Kelsey, *personal communication*). *Centaurea maculosa* roots have been found to reduce the growth rates of *F. idahoensis* roots near them, and this effect was ameliorated by activated carbon (Belliveau and Callaway, *unpublished manuscript*). Activated carbon is a potent

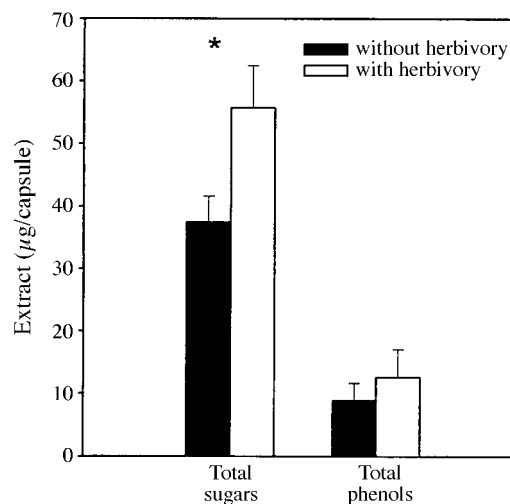


FIG. 3. Total sugars and phenols extracted from resin capsules that had been inserted in *Centaurea* roots. Error bars show +1 SE. The asterisk denotes a significant difference between treatments; ANOVA, $P < 0.05$.

adsorbent of charged organic molecules (Cheremisinoff and Ellerbusch 1978). Biologically active volatile polyacetelylenes have also been isolated from the roots of other *Centaurea* species (Bohlmann et al. 1966) and the roots of *C. maculosa* (R. Kelsey, *personal communication*). We found no significant difference in phenol concentrations in the extracts of resin capsules under plants, with and without herbivory, in the greenhouse; but the trend was similar to that of total sugar concentrations. We do not know if root herbivory in field conditions also altered root exudation.

Sugars, or compounds exuded from the roots attached to sugars, may have altered microbe-plant and plant-plant interactions. Sugar release to the rhizosphere of plants, which increased for *Centaurea* when grazed by *Trichoplusia*, is an important and readily available source of energy for microorganisms (Ocampo and Azcon 1985). Sugars in the rhizosphere help drive a number of important processes, including free-living N fixation and mineral immobilization and solubilization (Tate 1995). Relationships between root exudation of sugars and mycorrhizal infection has been reported (Ratnayake et al. 1978, Graham et al. 1981, Same et al. 1983). Differences in root exudate quantity or composition may also affect mycorrhizal mediation of the interaction between *Centaurea* and *Festuca*. UV-absorption spectrographs of fescue, knapweed, and bulk soil resin extracts (T. H. DeLuca, *unpublished data*) indicated that a significant portion of carbon detected in the methanol had UV absorption spectra similar to that of flavonoid compounds. Recently, root flavonoids have been identified as important signals of microbial response in the rhizosphere at micromolar and even nanomolar concentrations (Becard et al. 1992). Higher levels of sugars in the exudates of attacked *Centaurea* may indicate higher levels of exuded flavonoids, because flavonoids that are released as root exudates are often glycosylated to increase their solubility (Markham 1981, Varin 1992) and transport out of the root. The anthrone reagent used in this study hydrolyzes complex polysaccharides and glycosides and reacts with the released monosaccharides; thus, detection of soluble sugars in these studies may, in part, have been related to the presence of glycosylated flavonoids. Exudate effects on mycorrhizae have the potential to be important, as our previous research has shown that *Centaurea* develops substantial arbuscular mycorrhizae (AM) infections in the field in intermountain grasslands, and they have large effect on interactions between *Centaurea* and *Festuca idahoensis* (Marler et al. 1999).

The results of our experiments corroborate each other, but the greenhouse experiment does not provide an explicit test of the field experiment. We used a generalist herbivore in the greenhouse to facilitate precise levels of herbivory; however, *Trichoplusia* ate leaves, and herbivory occurred in a short intense bout. In contrast, *Agapeta* ate roots, and over a long period of time.

Second, we could not measure root biomass in the field nor reproductive output in the greenhouse (plants did not flower). Therefore, direct comparisons of the experiments are unclear. However, the fact that herbivory on *Centaurea* showed significant negative indirect effects on *Festuca*, under two very different sets of experimental conditions, suggests that these general indirect effects are robust.

Many invasive plants, such as a number of *Centaurea* species, are rapidly replacing native communities, and some risk in the application of biocontrols may be warranted. Nevertheless, our data suggest that herbivory by biocontrols can make an already superior competitor even better, especially if biocontrols do not badly damage or kill the target plant. Thus, evaluation of biocontrols prior to their use should include some screening for their indirect effects as has been suggested by others (Simberloff and Stiling 1996). A fundamental rationale for using biocontrols to suppress invasive plants is that, by weakening the invader, natives may gain a competitive advantage. Our data suggest that this may not always be the case.

ACKNOWLEDGMENTS

We thank Erick Greene for first observing unusual patterns in our data, and Tom Martin and Cathy Zabinski for helpful suggestions throughout. We thank Erik Ashehoug, Jennifer Costich, Shannon Garner, Marilyn Marler, Paul Ridenour, Raven Stevens, Michael Wojdylak, and Todd Wojtowicz for fruitful discussion or field assistance. We are indebted to Jim Story at the Montana State Experiment Station for supplying us with *Agapeta*, and providing us with valuable information, and to the Cornell Experiment Station at Geneva for supplying us with *Trichoplusia*. This work was supported in part by NSF EPSCoR grant OSR-955450 to Dr. Jack Stanford at the University of Montana and NSF DEB 9726829 to R. M. Callaway and C. Zabinski.

LITERATURE CITED

- Alward, R. D., and A. Joern. 1993. Plasticity and overcompensation in grass responses to herbivory. *Oecologia* **95**: 358–364.
- Becard, G., D. D. Douds, Jr., and P. E. Pfeffer. 1992. Extensive hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO₂ and flavonols. *Applied and Environmental Microbiology* **58**:821–825.
- Belsky, A. J., W. P. Carson, C. L. Jensen, and G. A. Fox. 1993. Overcompensation by plants: herbivore optimization or red herring? *Evolutionary Ecology* **7**:109–121.
- Binkley, D., and P. Vitousek. 1991. Soil nutrient availability. Pages 75–96 in R. W. Pearcy, J. R. Ehleringer, H. A. Mooney, and P. W. Rundel, editors. *Plant physiological ecology*. Chapman and Hall, London, UK.
- Bohlmann, F., K.-M. Rode, and C. Zdero. 1966. Neue polyine der Gattung *Centaurea* L. *Chemische Berichte* **99**:3544–3551.
- Brink, R. H., P. Dubach, and D. L. Lynch. 1960. Measurement of carbohydrate in soil hydrolysates with anthrone. *Soil Science* **89**:157–166.
- Cheremisinoff, P. N., and F. Ellerbusch. 1978. *Carbon adsorption handbook*. Ann Arbor Science Publications, Ann Arbor, Michigan, USA.
- Cohen, J. 1988. *Statistical power analyses for the behavioral sciences*. Second edition. Lawrence-Erlbaum, Hillsdale, New Jersey, USA.

- Cullen, J. M., P. F. Kable, and M. Catt. 1973. *Nature* **244**: 462–464.
- Drake, J. A., H. A. Mooney, F. Di Castri, R. H. Groves, F. J. Krueger, M. Rejmanek, and M. Williamson. 1989. *Biological invasions: a global perspective*. John Wiley and Sons, Chichester, UK.
- Fletcher, R. A., and A. J. Renney. 1963. A growth inhibitor found in *Centaurea* spp. *Canadian Journal Plant Science* **43**:475–481.
- Graham, J. H., R. T. Leonard, and J. A. Menge. 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. *Plant Physiology* **68**:548–552.
- Howarth, F. G. 1991. Environmental impact of classical biological control. *Annual Review of Entomology* **36**:485–509.
- Huffaker, C. B., and C. E. Kennett. 1959. *Journal of Range Management* **12**:69–82.
- Julien, M. H. 1987. *Biological control of weeds—a world catalogue of agents and their target weeds*. Second edition. C. A. B. International, Wallingford, UK.
- Kelsey, R. G., and L. J. Locken. 1987. Phytotoxic properties of cnicin, a sesquiterpene lactone from *Centaurea maculosa* (spotted knapweed). *Journal of Chemical Ecology* **13**:19–33.
- Landau, I., H. Müller-Schärer, and P. I. Ward. 1994. Influence of cnicin, a sesquiterpene lactone of *Centaurea maculosa* (Asteraceae), on specialist and generalist insect herbivores. *Journal of Chemical Ecology* **20**:929–942.
- Louda, S. M., D. Kendall, J. Connor, and D. Simberloff. 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* **277**:1088–1090.
- Lovett, J. V., and A. C. H. Holt. 1995. Allelopathy and self-defense in barley. Pages 170–183 in Inderjit, K. M. M., Dakshini, and F. A. Einhellig, editors. *Allelopathy*. American Chemical Society, Washington, D.C., USA.
- Markham, K. R. 1981. *Techniques of flavonoid identification*. Academic Press, New York, New York, USA.
- Marler, M. J., C. A. Zabinski, and R. M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology*, *in press*.
- Maschinski, J., and T. G. Whitham. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability and timing. *American Naturalist* **134**:1–19.
- McEvoy, P., C. Cox, and E. Coombs. 1991. Successful biological control of ragwort. *Ecological Applications* **1**: 430–442.
- Muir, A. D., and W. Majak. 1983. Allelopathic potential of diffuse knapweed (*Centaurea diffusa*) extracts. *Canadian Journal of Plant Sciences* **63**:989–996.
- Müller, H. 1989. Structural analysis of the phytophagous insect guilds associated with the roots of *Centaurea maculosa* Lam., *C. diffusa* Lam., and *C. vallesiaca* Jordan in Europe. I. Field observations. *Oecologia* **78**:41–52.
- Müller, H., D. Schroeder, and A. Gassmann. 1988. *Agapeta zoegana* (L.) (Lep.: Cochylidae), a suitable prospect for the biological control of spotted and diffuse knapweed, *Centaurea maculosa* Lam. and *C. diffusa* Lam. (Compositae) in North America. *Canadian Entomologist* **120**:109–124.
- Müller-Schärer, H. J. 1991. The impact of root herbivory as a function of plant density and competition: survival, growth, and fecundity of *Centaurea maculosa* in field plots. *Journal of Applied Ecology* **28**:759–776.
- Müller-Schärer, H., and D. Schroeder. 1993. The biological control *Centaurea* spp. in North America: do insects solve the problem? *Pesticide Science* **37**:343–353.
- Ocampo, J. A., and R. Azcon. 1985. Relationship between the concentration of sugars in the roots and VA mycorrhizal infection. *Plant and Soil* **86**:95–100.
- Price, M. L., and L. G. Butler. 1977. Rapid visual estimation and spectroscopic determination of tannin content of sorghum grain. *Journal of Agriculture and Food Chemistry* **25**:1268–1273.
- Ratnayake, M., R. T. Leonard, and J. A. Menge. 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. *New Phytologist* **81**:543–552.
- Same, B. I., A. D. Robson, and L. K. Abbott. 1983. Phosphorus, soluble carbohydrates, and endomycorrhizal infection. *Soil Biology and Biochemistry* **15**:593–597.
- Simberloff, D., and P. Stiling. 1996. How risky is biocontrol? *Ecology* **77**:1965–1974.
- Steinger, T., and H. Müller-Schärer. 1992. Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia* **91**: 141–149.
- Strauss, S. Y. 1991. Indirect effects in community ecology: their definition, study, and importance. *Trends in Ecology and Evolution* **6**:206–210.
- Tang, C., W. Cai, K. Kohl, and R. K. Nishimoto. 1995. Plant stress and allelopathy. Pages 142–157 in Inderjit, K. M. M., Dakshini, and F. A. Einhellig, editors. *Allelopathy*. American Chemical Society, Washington, D.C., USA.
- Tate, R. L. 1995. *Soil microbiology*. Wiley, New York, New York, USA.
- Varin, L. 1992. Flavonoid sulfation: phytochemistry, enzymology, and molecular biology. Pages 233–254 in H. A. Stafford and R. K. Ibrahim, editors. *Phenolic metabolism in plants. Recent advances in phytochemistry*. Plenum Press, New York, New York, USA.
- Weeden, C. R., A. M. Sheldon, and M. P. Hoffman. 1997. *Biological control: a guide to natural enemies in North America*. Cornell University, New York. (<http://www.nysaes.cornell.edu/ent/biocontrol/weedfeeders/aga-peta.html>).
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics* **25**:443–466.