

## LETTER

# Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives

Giles C. Thelen,<sup>1</sup> Jorge M. Vivanco,<sup>2</sup> Beth Newingham,<sup>3</sup> William Good,<sup>4</sup> Harsh P. Bais,<sup>2</sup> Peter Landres,<sup>5</sup> Anthony Caesar<sup>6</sup> and Ragan M. Callaway<sup>1\*</sup>

## Abstract

Exotic invasive plants are often subjected to attack from imported insects as a method of biological control. A fundamental, but rarely explicitly tested, assumption of biological control is that damaged plants are less fit and compete poorly. In contrast, we find that one of the most destructive invasive plants in North America, *Centaurea maculosa*, exudes far higher amounts of ( $\pm$ )-catechin, an allelopathic chemical known to have deleterious effects on native plants, when attacked by larvae of two different root boring biocontrol insects and a parasitic fungus. We also demonstrate that *C. maculosa* plants experimentally attacked by one of these biocontrols exhibit more intense negative effects on natives.

## Keywords

Allelopathy, biocontrol insects, exotic invasion, herbivory, noxious weed, phytotoxic exudates, root exudates, spotted knapweed.

*Ecology Letters* (2005) 8: 209–217

## INTRODUCTION

Scientists, land managers and policy makers have been stymied by the inexorable success of highly invasive exotic plants around the world. These invaders threaten the biological diversity and ecological integrity of natural ecosystems and may cause more than US\$34 billion damage a year in the United States alone (Pimentel *et al.* 2000). The remarkable success of some invasive plant species is thought to be the result of the lack of specialist consumers in the regions they invade; known as the ‘enemy release hypothesis’ (Maron & Vilà 2001; Keane & Crawley 2002; Mitchell & Power 2003). Based on this hypothesis, biological control insects have become important tools used to combat exotic invasive plants. Biological control is a powerful management tool that has proved effective at controlling some invasive species (DeLoach 1991; McFadyen 1998) and so far is the only realistic option for controlling invaders that are widely

naturalized. However, despite the importance of biocontrols we know little about how they affect fundamental ecological processes (McEvoy & Coombs 1999; Pearson & Callaway 2003).

Plants damaged by herbivores are typically at a competitive disadvantage, which provides the logical and theoretical basis for biocontrol. However, plant response to herbivory is not a simple zero sum game for carbon and nutrients. Plants may compensate for biomass lost to herbivory by growing faster via mechanisms not yet understood (Trumble *et al.* 1993; Agrawal 2000), increase reproductive output (Paige & Whitham 1987; Paige 1999), and undergo complex biochemical changes in response to herbivory. For instance, plants can actively respond to insect herbivory by emitting volatile chemicals (Baldwin & Schultz 1983; Thaler 1999; Farmer 2001) and the induction of chemical defences. Some induced defence chemicals may enhance the competitive ability of attacked plants by

<sup>1</sup>Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA

<sup>2</sup>Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO 80523-1173, USA

<sup>3</sup>Department of Biological Sciences, 4505 Maryland Parkway, University of Nevada, Las Vegas, NV 89154, USA

<sup>4</sup>Department of Biology, Montana Tech of the University of Montana, Butte, MT 59701, USA

<sup>5</sup>Aldo Leopold Wilderness Research Institute, PO Box 8089, Missoula, MT 59807, USA

<sup>6</sup>USDA-ARS-Northern Plains Agricultural Research Laboratory, Sidney, MT 59270, USA

\*Correspondence: E-mail: ray.callaway@mso.umt.edu

acting as allelopathic or phytotoxic agents (Siemens *et al.* 2002).

Allelopathy may play a role in some exotic plant invasions (Rabotnov 1982; Hierro & Callaway 2003; Callaway & Ridenour 2004; Vivanco *et al.* 2004) and the exceptional competitive and invasive success of *Centaurea maculosa* (spotted knapweed) appears to be in part the result of allelopathic chemicals exuded from its roots (Ridenour & Callaway 2001; Bais *et al.* 2003) and its ability to compensate for herbivore damage. *Centaurea maculosa* was introduced from Eurasia, where it is not common, to North America, where it can form virtual monocultures (Ridenour & Callaway 2001). It now occupies over 7 million acres in the US (<http://www.fs.fed.us/database/feis/plants/forb/cenmac/all.html>). *Centaurea maculosa* roots produce an enantiomeric compound, ( $\pm$ )-catechin, with clearly documented phytotoxic properties (Bais *et al.* 2003). Greenhouse experiments have demonstrated inhibitory effects of *C. maculosa* roots on the roots and overall growth of a native North American grass, and activated carbon added as a purification agent ameliorates these inhibitory effects (Ridenour & Callaway 2001), and experiments show the inhibition of the growth and germination of native species in field soils at natural concentrations of the allelochemical (Bais *et al.* 2003). (-)-Catechin shows cell-specific targeting against meristematic and elongation zone cells in the roots of target plants, induction of reactive oxygen species (ROS)-related signalling that leads to rhizotoxicity in susceptible plants, and allelochemical-induced genome-wide changes in gene expression patterns. The concentrations of catechin reported as phytotoxic on naïve species did not have an effect on *C. maculosa* plants (Bais *et al.* 2003).

There is correlative evidence that biocontrol root herbivores have at least weak negative effects on *C. maculosa* populations (Story *et al.* 2000), but in general biocontrol insects have not yet been effective against the weed (Müller-Schärer & Schroeder 1993). Furthermore, some evidence indicates that biocontrol herbivory may actually stimulate compensatory growth and may have counterintuitive and unwanted effects. Müller (1989) found that *C. maculosa* plants increased fine root growth when infected by the biocontrol insect, *Agapeta zoegana*, and did not decrease in fecundity. Steinger & Müller-Schärer (1992) found that the biomass of *C. maculosa* seedlings grown in pots was not affected by *Agapeta* feeding, and attributed the lack of effect to compensatory root growth. In other experiments in the same study, however, the root-feeding weevil *Cyphocleonus achates* reduced whole-plant biomass. In other field experiments in Switzerland, Müller-Schärer (1991) found that low levels of *Agapeta* herbivory increased survival, shoot number and fecundity of *C. maculosa*, but the effects of herbivory were highly complex and were negative under other conditions. Ridenour & Callaway (2003) reported that

reproduction of *Centaurea* plants in plots infested with *Agapeta* was higher than in uninfested plots, suggesting a compensatory response, and Callaway *et al.* (1999) showed that infestation correlated with stronger competitive effects of *C. maculosa*. In addition to these effects of biocontrols, leaf herbivory by the generalist leaf feeder, *Trichoplusia ni* (cabbage looper), on *C. maculosa* appeared to enhance the invader's competitive effects against the native *Festuca idahoensis*. In artificial herbivory experiments, defoliation of potted *C. maculosa* (up to four times in *c.* 6 months and up to intensities of 75% of the leaves) had no effect on the final biomass of the defoliated plants (Kennett *et al.* 1992).

Many biological control insects are root feeders; however, most ecological research on herbivory has focused on insects that feed on aboveground tissues. For example, of 292 peer-reviewed articles recently published on insect herbivory only four focused on root-feeding insects (Hunter 2001). Despite the weak effects reported for *Agapeta* on *Centaurea*, root-feeding insects can have dramatic deleterious effects on their hosts (Reichman & Smith 1991; Strong *et al.* 1995; Grayston *et al.* 2001). Of the 16 species of biocontrol insects that have been introduced since the 1970s to limit the competitive effects of *C. maculosa* and its similar congener, *C. diffusa* (diffuse knapweed), five feed on the roots. The mechanisms by which moderate levels of root herbivory on *C. maculosa* can stimulate the weed's growth (Müller-Schärer 1991; Ridenour & Callaway 2003; Steinger & Müller-Schärer 1992) and more importantly, increase its competitive effects (Callaway *et al.* 1999) is not known.

Here, we ask if response of *C. maculosa* to herbivory is linked to its allelopathic effects, and explore the hypothesis that shoot herbivory, fungal infection and root herbivory by two widely established biocontrol insects on *C. maculosa* may increase the competitive effects of the weed by stimulating the exudation of the allelopathic chemical ( $\pm$ )-catechin.

## METHODS

### Experiment 1

*Centaurea maculosa* was planted from seed ( $n = 44$ ) in 2.4 L pots filled with 25% field soil from Montana and 75% 20/30 grit sand. After allowing the plants to grow for 40 days in the greenhouse we subjected them to two different biocontrol insect treatments, or to no herbivory. First, leaf damage was caused by or subjecting *C. maculosa* rosettes to herbivory by the cabbage looper moth (*T. ni*, family Lepidoptera; a generalist native to Eurasia, widespread in the US but not used as a biocontrol for *C. maculosa*). We removed *T. ni* after 50% of the leaf tissue was consumed, but if after 7 days plants had not lost 50% of their leaf tissue they were clipped to standardize 50% leaf loss. All plants in this treatment, however, experienced

substantial leaf herbivory. A second group of plants was subjected to herbivory by the Eurasian biological control agent, the root-boring weevil, *Cyphocleonus achates* (Coleoptera: Curculionidae). Adults were introduced to *C. maculosa* rosettes, which laid eggs near the root crown, and larvae hatched and fed in the taproot for 20 weeks. All roots were examined to confirm damage by *Cyphocleonus*. Soils from each pot were collected, and stored in a cool, dark, dry environment. Eighteen months later ( $\pm$ )-catechin was extracted from soils. A 500 mg of soil per sample was extracted in 1 mL of ACS grade methanol (Fisher Co., Pittsburgh, PA, USA). Extracts were thoroughly vortexed, concentrated under  $N_2$ , and resuspended in 200  $\mu$ L methanol. Methanol extracts were chromatographed (Dionex Co., Sunnyvale, CA, USA) on a reverse phase 5  $\mu$ m,  $C_{18}$  column (25 cm  $\times$  4.6 mm) (Supelco Co., Bellefonte, PA, USA) using a multistep gradient. The absorbance at the reference wavelength  $\lambda_{\max}$ -280 nm was measured by a PDA-100 Photodiode array variable UV/VIS detector (Dionex Co.). Mobile phase solution A consisted of double distilled water and solution B consisted of ACS grade methanol (Fisher Co.). A multistep gradient was used for all separations with an initial injection volume of 15  $\mu$ L and a flow rate of 1 mL  $\text{min}^{-1}$ . The multistep gradient was as follows: 0–5 min 5.0% B, 5–10 min 20.0% B, 15–20 min 20.0% B, 20–40 min 80.0% B, 40–60 min 100% B, 60–70 min 100% B, 70–80 min 5.0% B. ( $\pm$ )-Catechin concentrations in each sample were determined by comparison with 15  $\mu$ L injections from a 1 mg  $\text{mL}^{-1}$  catechin standard stock.

### Experiment 2

In August 2002, immature *C. maculosa* rosettes ( $n = 104$ ) were collected from an invaded prairie near Missoula, Montana and transplanted into 2.4-L pots filled with 50% local soil and 50% 20/30 grit sand. Two weeks later we applied *A. zoegana* (Lepidoptera: Cochyliidae; a widespread root boring biocontrol moth from Europe) eggs to 52 of the plants. Eggs were obtained by collecting females in the field and confining them in a paper cage so that they would lay eggs on the paper. Small pieces  $<1 \text{ cm}^2$  of the paper containing two to three eggs were cut out by hand and, when the eggs were about to hatch, were pinned to the inside of paper cylinders enclosing the shoots of the target *C. maculosa*. As combined effects of consumers can have powerful effects on targets, we also experimented with a pathogenic fungus. Two weeks after applying *Agapeta* we added an inocula (provided by the USDA Northern Plains Agricultural Research Lab) of a North American isolate of the soil born fungus, *Rhizoctonia solani*, to half of the *Agapeta*-infested plants ( $n = 26$ ) and half of the plants without *Agapeta* ( $n = 26$ ). The fungus was grown as a liquid culture on a suspension of soya bean hulls and this liquid culture

was poured on the soil around *C. maculosa*. After 205 days the plants were harvested, sampled to confirm *Agapeta* infestation, and the soils were extracted and analysed for ( $\pm$ )-catechin as described above. If there was no evidence of *Agapeta* in the harvested roots of plants originally designated to the infested treatment, we assumed that the insect died after initial infection and we re-assigned these individuals to the uninfested treatment. We also confirmed the absence of *Agapeta* root infection in the control plants.

### Experiment 3

In an experiment designed to examine herbivore-induced chemical exudation in the field, we transplanted 240 immature rosettes of *C. maculosa* collected in the field into 525-mL containers, brought them into the greenhouse, and infested half of them with *A. zoegana* after 5 weeks of acclimatization to greenhouse conditions. To infest *C. maculosa* we followed the protocol described in experiment 2. *Agapeta* were applied once, between late July and early September, and in late autumn of 2001 *C. maculosa* were transplanted into the field and left until the following November 2002. We transplanted *C. maculosa* into the field at two natural intermountain prairie grasslands near Hamilton, Montana: Brennan Gulch (46°10'6.0" N, 113°59'42.0" W), and Sleeping Child (46°7'49.7" N, 114°2'13.0" W). These sites were lightly invaded by *C. maculosa*. At each of the locations we planted *C. maculosa* at three positions: (i) at least 10 cm away from any neighbours, (ii) within 5 cm of *Koeleria micrantha*, a small native grass, and (iii) within 5 cm of *Pseudoroegneria spicata*, a large native grass. This design allowed us to test the competitive effect of *Agapeta*-infested *Centaurea* and uninfested *Centaurea* on native species. In December 2001, when *C. maculosa* was planted, we measured the basal area composed of living culms of each neighbouring bunchgrass, and in September 2002, we re-measured the bunchgrass basal areas. When we harvested each *C. maculosa* in November 2002 we collected 1 L of soil from each *C. maculosa* rhizosphere by excavating the plants and collecting the soil shaken loose from the root systems. These soils were thoroughly mixed and analysed for ( $\pm$ )-catechin concentrations as described above. We also dissected *C. maculosa* taproots and recorded evidence of *Agapeta*. If there was evidence of *Agapeta* in the uninfected treatment or no evidence of *Agapeta* in the infected treatment we removed the replication from the analysis. We analysed the proportional change in the diameter of bunchgrasses in a three-way ANOVA using *Agapeta* infestation (fixed), bunchgrass species (fixed), and site (random) as factors, and the diameter of *C. maculosa* taproots as a covariate.

We also collected soil from this field experiment for a greenhouse experiment in which we tested the effects of

soil from rhizospheres of *Agapeta*-infested and non-*Agapeta*-infested *C. maculosa* plants on the growth of two native grasses. Rhizosphere soil collected from beneath 40 individual *C. maculosa* plants infested with *Agapeta* and 40 uninfested plants was placed in 350 cm<sup>2</sup> pots, and used to grow either *Koeleria micrantha* or *Festuca idaboensis* ( $n = 20$  for each species and each rhizosphere condition). Soils collected from individual rhizospheres in the field were matched with individual pots in this greenhouse experiment.

#### Experiment 4

In an experiment designed to test the effects of *Agapeta*-induced increases in ( $\pm$ )-catechin on the growth of North American native plants, in May 2003 we applied ( $\pm$ )-catechin to the rhizospheres of four common grass species, *Danthonia unispicata*, *Poa sanbergii*, *Festuca idaboensis* and *Pseudoroegneria spicata*, and four native forb species, *Balsamorhiza sagittata*, *Lupinus sericeus*, *Achillea millefolium*, and *Delphinium bicolor*. We conducted this experiment in a native grassland, not invaded by *C. maculosa*, on Moccasin Ridge near Clinton, Montana (46°45'43.5"N, 113°45'38.2"W). For each species we chose triplets ( $n = 10$  triplets for each species) of nearby individual of similar sizes and randomly assigned one of three treatments to each individual of the triplet. Using a micropipette, we applied either a methanol control or one of two different concentrations of ( $\pm$ )-catechin (0.188 mg mL<sup>-1</sup> and 0.020 mg mL<sup>-1</sup>). For each target individual we injected 800  $\mu$ L of solution into the rhizosphere. One concentration (low) was estimated to represent soil concentrations in the rhizospheres of *C. maculosa* not attacked by *Agapeta*, while the other concentration (high) was estimated to represent ( $\pm$ )-catechin concentrations in the rhizospheres of *C. maculosa* with *Agapeta* in their roots. Based on preliminary tests, we estimated that 800  $\mu$ L of solution would spread through *c.* 100 g of soil, and thus we calculated that the high and low concentrations used in this study correspond to *c.* 1500  $\mu$ g g<sup>-1</sup> and *c.* 250  $\mu$ g g<sup>-1</sup> soil of racemic catechin respectively. The allelopathically active (-)-catechin would have been present at one half of this concentration. However, it should be noted that we have recently found that (+)-catechin also holds some level of phytotoxic activity, although it was *c.* 1.5–2.0-fold less active compared with (-)-catechin in the model species *Arabidopsis thaliana*. Thus, (+)-catechin may have contributed to the overall phytotoxic effect of racemic catechin. Initial measurements of leaf number and length were taken for all plants prior to treatment, and 3 weeks after catechin application we returned to measure the relative change in leaf number and growth. We analysed the proportional change in the leaf number of the target plants in a three-way ANOVA using catechin concentration (fixed), and native species (fixed), as

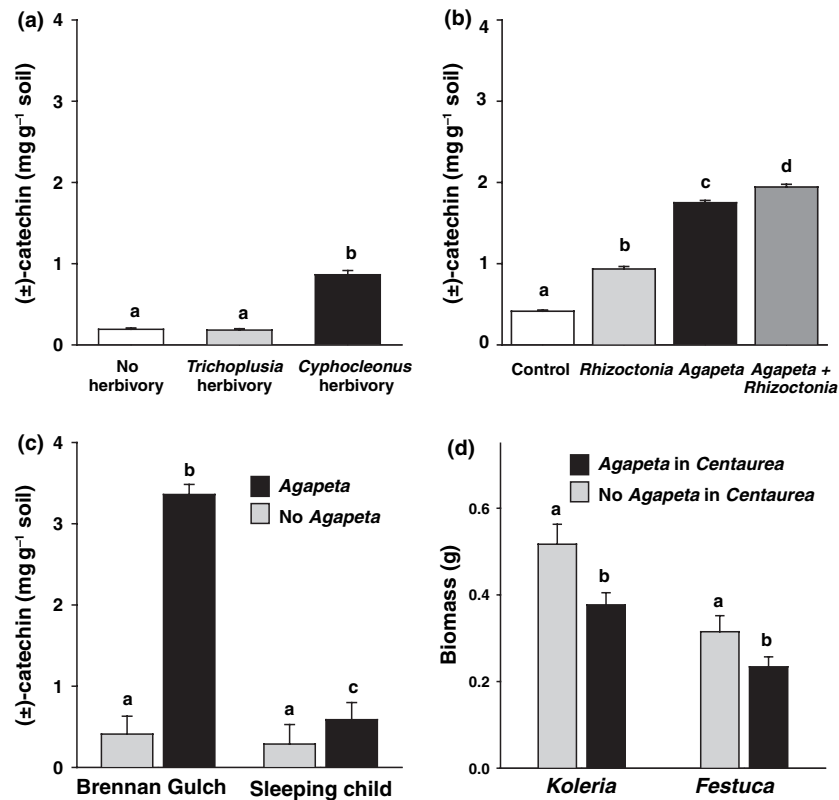
factors, and followed the 2-way ANOVA with single ANOVAS and Tukey analyses for each species.

#### RESULTS

In the first experiment, leaf herbivory by the generalist *T. ni* on *C. maculosa* grown alone had no effect on rhizosphere concentrations of ( $\pm$ )-catechin, but root herbivory by *Cyphocleonus* increased ( $\pm$ )-catechin levels to more than four times that of the control or the *T. ni* treatment (Fig. 1a). In the second experiment, application of a North American isolate of the globally distributed parasitic fungus (*Rhizoctonia solani*), to the rhizosphere of *C. maculosa* more than doubled the soil concentration of ( $\pm$ )-catechin relative to control plants (Fig. 1b). Fungal cell walls are known to elicit ( $\pm$ )-catechin exudation from *C. maculosa* roots (Bais *et al.* 2002). In this experiment, *Agapeta* root herbivory more than quadrupled ( $\pm$ )-catechin output, and adding the fungal pathogen to *Agapeta* infestation elicited higher catechin exudation than any of the other treatments (Fig. 1b). In this experiment, neither the fungal pathogen *Rhizoctonia* nor the herbivore *Agapeta* reduced the total biomass or flower production of *C. maculosa* (G.C. Thelen, unpublished data).

In the third experiment, the rhizospheres of *C. maculosa* plants infested with *Agapeta* and then transplanted into the field had two to nine times greater concentrations of ( $\pm$ )-catechin than uninfested plants after a single growing season (Fig. 1c). The mortality of *C. maculosa* infested with *Agapeta* (48%) was slightly higher than uninfested *C. maculosa* (41%), but, as in the greenhouse experiments *Agapeta* did not reduce the size or reproductive output of *C. maculosa* (G. C. Thelen, unpublished data). In the greenhouse experiment using soils collected from the field experiment, the native grasses *Festuca idaboensis* and *Koeleria micrantha*, were smaller when planted in soils collected from the rhizospheres of *Agapeta*-infested *Centaurea* plants at Brennan Gulch than in uninfested *Centaurea* plants (Fig. 1d, soils from the experiment described in Fig. 1c). In the field, *Agapeta* infection also appeared to enhance the competitive effect of *C. maculosa*. Across both sites and species, the basal area of the native grasses decreased (i.e. grass culms died) by 17.8% when *C. maculosa* was planted next to them (Fig. 2). However, the basal area of the native grasses decreased by an average of 28.5% when next to *C. maculosa* infested with *Agapeta* vs. 7.2% decrease for grasses next to uninfested controls, corresponding to the *Agapeta*-induced increase in ( $\pm$ )-catechin exudation. *Agapeta* did not have this indirect effect on *Pseudoroegneria spicata* at one site.

We found that the higher soil concentrations of ( $\pm$ )-catechin associated with *Agapeta*-infested *C. maculosa* are more harmful to native plants than lower concentrations associated with uninfested *C. maculosa* (Fig. 3). Of eight



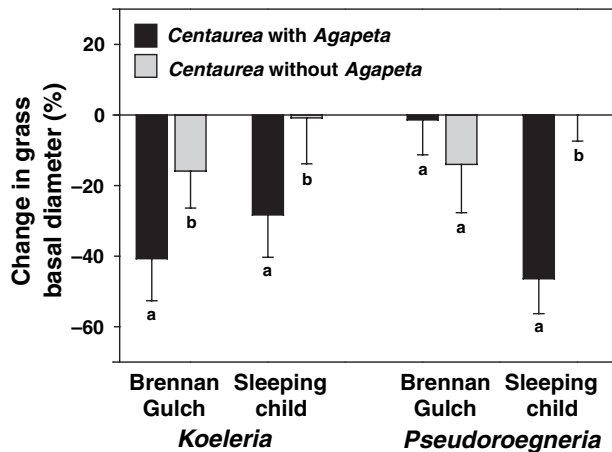
**Figure 1** Concentrations of the root exudate, ( $\pm$ )-catechin, in the rhizospheres of *Centaurea maculosa* in experiments where *Agapeta zoegana* or *Cyphocleonus achates* (a specialist insect biocontrol root herbivore), *Trichoplusia ni* (a naturalized generalist leaf herbivore native to Europe), and *Rhizoctonia solani* (a soil born fungus isolated from *C. maculosa* in North America) were applied to *C. maculosa*. (a) Effects of *Trichoplusia* and *Cyphocleonus* in a greenhouse experiment. (b) Effects of the fungal parasite *Rhizoctonia* and *Agapeta* in a greenhouse experiment. (c) Concentrations of ( $\pm$ )-catechin in the rhizospheres of *Agapeta*-infested and uninfested *Centaurea* transplanted next to native grasses. Error bars represent 1 SE. Mean values with different letters were significantly different in pairwise Student's *t*-test comparisons. (d) Biomass of *Festuca idaboensis* and *Koeleria micrantha* when grown in soil collected from the rhizospheres of *C. maculosa* either infested with *A. zoegana* or not infested with *Agapeta* (see Fig. 1c). Soil was collected from the Brennan Gulch site. Error bars represent 1 SE. Different letters above the bars designate significant differences between the mean values for a species, as determined with separate ANOVAs with site and *Agapeta* infestation as factors for each species. For *Koeleria*,  $F_{Agapeta \text{ infestation}} = 8.05$ , d.f. = 1,50,  $P = 0.007$ . For *Festuca*,  $F_{Agapeta \text{ infestation}} = 4.06$ , d.f. = 1,46,  $P = 0.110$ .

native species exposed to ( $\pm$ )-catechin only one, the large perennial (and deeply taprooted) herb *Balsamorhiza sagittata*, was not affected by either the low or high dose. Six species had lower relative leaf growth when a low dose of ( $\pm$ )-catechin (approximating the rhizosphere concentrations of *C. maculosa* without *Agapeta* herbivory) was applied to their rhizospheres than in the controls. Another species, *Pseudoroegneria spicata*, did not respond to the low dose of ( $\pm$ )-catechin, but decreased significantly when a high dose (approximating the rhizosphere concentrations of *C. maculosa* with *Agapeta* herbivory) was applied. The bunchgrass *Danthonia unispicata* and the perennial herb *Achillea millefolium*, significantly decreased in growth with the low dose, but significantly more with the high dose of ( $\pm$ )-catechin. For all eight native species combined, mortality was zero for the controls, 15.7% for natives exposed to

low doses of ( $\pm$ )-catechin, and 23.6% of the individuals exposed to high doses of ( $\pm$ )-catechin.

## DISCUSSION

Our results indicate that the counterintuitive, root herbivory-stimulated, competitive effects previously reported for *C. maculosa* (Callaway *et al.* 1999) may be caused by increased exudation of allelopathic chemicals. Biocontrol root herbivory caused more exudation of the phytotoxin ( $\pm$ )-catechin in the greenhouse and in the field, native plants in the field associated with biocontrol-infested *C. maculosa* were smaller than those with uninfested *C. maculosa*, and doses of ( $\pm$ )-catechin mimicking the effects of biocontrol-stimulated exudation had effects comparable with those of the biocontrol-infested plants themselves. Higher rates of root



**Figure 2** Proportional growth of two native bunchgrass species adjacent to experimentally planted *C. maculosa* plants either infested with the biocontrol root herbivore, *Agapeta zoegana* or not infested with *Agapeta*. Error bars represent 1 SE. In an ANOVA with site, grass species and *Agapeta* as factors,  $F_{Agapeta \text{ infestation}} = 6.987$ , d.f. = 1,133,  $P = 0.009$ . Different letters for a species at a given site designate means that were significantly different in a *t*-test ( $P < 0.05$ ).

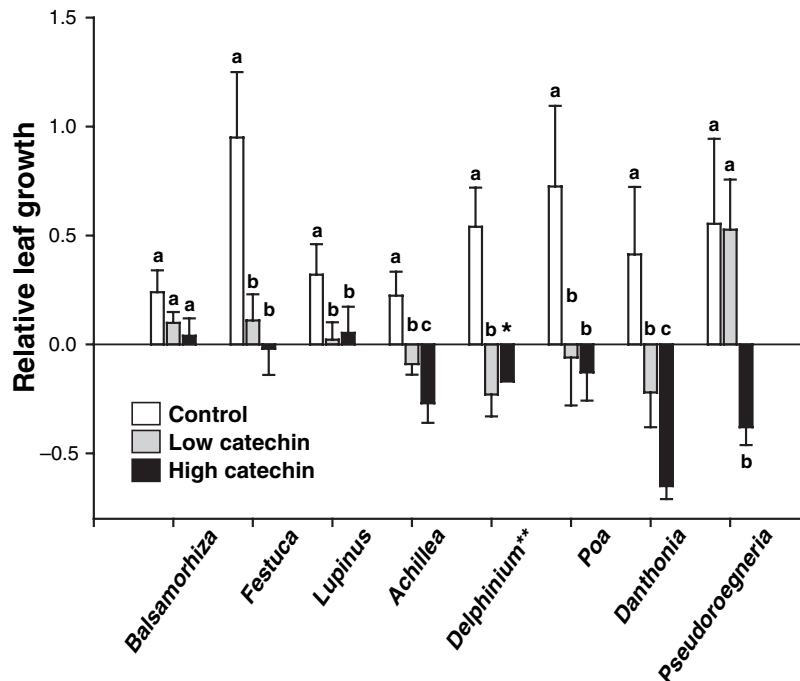
exudation after herbivory are not uncommon (Bardgett *et al.* 1998), but to our knowledge our study is the first to link increased exudation to stronger competitive effects and allelopathy.

We do not know why *C. maculosa* might exude more ( $\pm$ )-catechin after root herbivory. One of the molecular forms may have unknown herbivore defence characteristics, but ( $\pm$ )-catechin does not occur within the root tissues of *C. maculosa* (Bais *et al.* 2002), thus its induction cannot suppress herbivores that have already penetrated into the root. However, catechin may protect the root from further attack from other consumers once the root has been damaged. (+)-Catechin has a potent antibacterial activity (Bais *et al.* 2002), and increased exudation in response to herbivory may be an adaptation to ward off bacterial infection in root tissue damaged by herbivores. Catechins are also chelators, making bound nutrients such as phosphorus available. It is possible that increased exudation is a strategy to obtain limiting nutrients once plants are damaged. An alternative explanation for increased exudation of catechin upon herbivory damage is that catechin is a non-specific stress response. Our *Agapeta* infestation densities were controlled and varied from one to two larvae per root. Exudation of ( $\pm$ )-catechin from roots infested with one *Agapeta* was virtually identical to exudation from roots with two *Agapeta* (data not shown), but we do not know if higher infestation rates will produce different responses in plant growth, death or root exudation.

The effects of *Agapeta* on root exudation were strikingly different between field sites. We do not know why, but the weak effects of *Agapeta* at the Sleeping Child site may have been the result of the deposition of charcoal by a recent wildfire. Charcoal can adsorb organic chemicals (Chermisinoff & Ellerbusch 1978) and may have ameliorated *Centaurea*'s allelopathic effects. However, variation in soil moisture, texture, pH, parent material, microbial communities and organic content could affect the accumulation of catechin, or our ability to measure it. Catechin is a relatively insoluble compound, which makes extraction from soil and other solutions difficult and variable. Despite the lower levels of catechin detected at the Sleeping Child site, *Agapeta* infestation had strong negative effects on *Pseudoroegneria*. However, this species tends to be a strong competitor with *Centaurea* (Callaway *et al.* 2004a) and responded only to the high catechin dose in experiment 4. These inconsistencies suggest that the increased exudation of catechin by plant experiencing root herbivory may not be the only mechanism driving herbivore-enhanced competitive ability of *Centaurea*, as discussed below.

We have not ruled out other mechanisms that might contribute to, or confound, our interpretation of allelopathy as the cause of *C. maculosa*'s herbivore-enhanced competitive response. Hamilton & Frank (2001) found that herbivory stimulated the production of root exudates by *Poa pratensis* which benefited associated mutualistic soil microorganisms, thereby enhancing the delivery of resources to the damaged plants. Several other studies have reported grazing-enhanced microbial biomass and changes in soil biota (see review by Bardgett *et al.* 1998). Soil microbes have strong effects on the interactions between *C. maculosa* and North American species (Marler *et al.* 1999; Callaway *et al.* 2004a,b) and exudation stimulated by herbivory may alter these interactions in ways that provide an advantage to *C. maculosa*. When stressed by defoliation, *C. melitensis* may benefit from a form of mycorrhizae-mediated parasitism through a common mycorrhizal network (Callaway *et al.* 2001, Callaway *et al.* 2003, also see Marler *et al.* 1999; Zabinski *et al.* 2002; Carey *et al.* 2004), and this phenomenon has also been observed in other plant systems (Waters & Borowicz 1994).

Another possible explanation for herbivore-enhanced competitive ability is compensatory growth (Paige & Whitham 1987; Trumble *et al.* 1993; Paige 1999; Agrawal 2000). Plants that respond to herbivory by increasing growth rates may also increase resource uptake. We do not know of any studies in which compensatory growth has been explicitly linked to greater resource uptake, but by stimulating root growth (Simberloff *et al.* 1978; Müller 1989) herbivores may generate unexpected responses in their hosts. We do not know how *C. maculosa* can tolerate herbivory and increase exudation of ( $\pm$ )-catechin, but costs



**Figure 3** Relative growth as measured by leaf number and plant height of eight native species in an uninvaded prairie when exposed to three concentrations of the *C. maculosa* root exudate, ( $\pm$ )-catechin. Native species are *Balsamorhiza sagittaria*, *Festuca idahoensis*, *Lupinus sericeus*, *Achillea millefolium*, *Delphinium bicolor*, *Poa sandbergii*, *Danthonia unispicata*, *Pseudoroegneria spicata*. Doses are described in the methods and were designed to approximate *C. maculosa* without (low concentrations) and with (high concentrations) *Agapeta zoegana* in their roots. Error bars represent 1 SE. Mean values with different letters within a species were significantly different in single ANOVAs followed by post-ANOVA Tukey tests. We compared dose levels in two different ANOVAs. In the first ANOVA, with species and dose as factors, the low dose of catechin had significant greater effects than the control across all species,  $F_{\text{dose}} = 13.022$ , d.f. = 1,102,  $P < 0.001$ . In the second ANOVA, with species and dose as factors, the high dose of catechin had significantly greater effects than the low dose across all species,  $F_{\text{dose}} = 4.022$ , d.f. = 1,93,  $P = 0.016$ . The asterisk denotes a species treatment that experienced very high mortality and was therefore not possible to include in the statistical analysis of growth.

associated with higher ( $\pm$ )-catechin output may be offset if catechin or other co-exuded compounds have other benefits to *C. maculosa*.

For an herbivore to enhance the competitive effect of its target is odd, but perhaps only if the effects of herbivores are simplified to a zero sum game for energy and resources and the responses of plants are simplified to 'grow or defend' paradigms (Herms & Mattson 1992). Plants interact in far more complex ways than strict resource competition (Mallik & Pellissier 2000; Callaway 2002; Gruntman & Novoplansky 2004), and plants may be able to grow and defend (Siemans *et al.* 2000). The latter found that herbivory appears to increase allelopathic effects on neighbouring plants, apparently by inducing defence chemicals that are also allelopathic.

To our knowledge there are only three other studies suggesting herbivore-enhanced competitive ability. Ramsell *et al.* (1993) found that *Lolium perenne* grazed by *Tipula paludosa*, a root herbivore, appeared to compete more strongly against *Rumex obtusifolius* neighbours than ungrazed

*Lolium*. No mechanism was identified, but they hypothesized that herbivory-caused reallocation from roots to shoots may have stimulated the competitive effects of *Lolium*. Defoliation of another *Centaurea* invader, *C. melitensis*, increased its negative effects solely on the native *Nassella pulchra*, but only when soil fungi were abundant in the soil (Callaway *et al.* 2001).

The occasional successes of biocontrol insects can be astounding. However, these successes are rare (Maron & Vilà 2001; Agrawal & Kotanen 2003) and unexpected indirect effects (Pearson & Callaway 2003), and the stimulation of complex underground biochemical responses such as those demonstrated in this study indicate that the use of biocontrol insects should be accompanied by a detailed understanding of their basic ecology. Some understanding of how a target invasive species suppresses other plants would be helpful; for example, an invasive species that inhibits natives via unusually deep shade might be a more appropriate target for biological control than allelopathic invaders. However, we know little in general about



the mechanisms by which invaders competitively exclude natives and such knowledge is difficult to acquire. A more practical approach may be to require evidence of efficacy before introducing new biocontrol species. If a biocontrol kills its target, there can be no biochemical response, no compensatory growth and no indirect effects.

## ACKNOWLEDGEMENTS

This research was supported by grants from The Aldo Leopold Wilderness Research Institute, Rocky Mountain Research Station, USDA Forest Service to RMC; NSF (DEB-0236061, INT-0331964, and DEB-9726829 to RMC); Civilian Research and Development Foundation and USDA-NRI 2003-02433 to RMC (<http://www.plantecology.org>); NSF (IBN-0335203) and USDA-WRIPM (2003-05060) to JMV, and by USDOD-SERDP (CS1388) to JMV and RMC.

## REFERENCES

- Agrawal, A.A. (2000). Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends Plant Sci.*, 5, 309–313.
- Agrawal, A.A. & Kotanen, P.M. (2003). Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecol. Lett.*, 6, 712–715.
- Bais, H.P., Walker, T.S., Stermitz, F.R., Hufbauer, R.A. & Vivanco, J.M. (2002). Isomer-dependent phytotoxic and antimicrobial activity of ( $\pm$ )-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol.*, 128, 1173–1179.
- Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003). Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, 301, 1377–1380.
- Baldwin, I.T. & Schultz, J.C. (1983). Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science*, 221, 277–279.
- Bardgett, R.D., Wardle, D.A. & Yeates, G.W. (1998). Linking above-ground and belowground interactions: how plant responses to foliar herbivory influence soil organisms. *Soil Biol. Biochem.*, 30, 1867–1878.
- Callaway, R.M. (2002). The detection of neighbors by plants. *Trends Ecol. Evol.*, 17, 104–105.
- Callaway, R.M. & Ridenour, W.M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. *Front. Ecol. Environ.*, 2, 436–443.
- Callaway, R.M., DeLuca, T.H. & Ridenour, W.M. (1999). Herbivores used for biological control may increase the competitive ability of the noxious weed *Centaurea maculosa*. *Ecology*, 80, 1196–1201.
- Callaway, R.M., Mahall, B.E., Wicks, C., Pankey, J. & Zabinski, C. (2003). Soil fungi and the effects of an invasive forb on native versus naturalized grasses: neighbor identity matters. *Ecology*, 84, 129–135.
- Callaway, R.M., Newingham, B., Zabinski, C.A. & Mahall, B.E. (2001). Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbors. *Ecol. Lett.*, 4, 429–433.
- Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W. & Gannon, J.E. (2004a). Soil fungi alter interactions between North American plant species and the exotic invader *Centaurea maculosa* in the field. *Ecology*, 85, 1062–1071.
- Callaway, R.M., Thelen, G.C., Rodriguez, A. & Holben, W.E. (2004b). Soil biota and exotic plant invasion. *Nature*, 427, 731–733.
- Carey, E.V., Marler, M. & Callaway, R.M. (2004). Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecol.*, 172, 133–141.
- Chermisinoff, P.N. & Ellerbusch, F. (1978). *Carbon Adsorption Handbook*. Ann Arbor Science Publishers, Ann Arbor, MI.
- DeLoach, C.J. (1991). Past successes and current prospects in biological control of weeds in the United States and Canada. *Nat. Areas J.*, 11, 129–142.
- Farmer, E.E. (2001). Surface-to-air signals. *Nature*, 411, 854–856.
- Grayston, J.S., Dawson, L.A., Treonis, A.M., Murray, P.J., Ross, J., Reid, E.J. *et al.* (2001). Impact of root herbivory by insect larvae on soil microbial communities. *Eur. J. Soil Biol.*, 37, 277–280.
- Gruntman, M. & Novoplansky, A. (2004). Physiologically mediated self-non-self discrimination in roots. *Proc. Natl Acad. Sci.* 101, 3863–3867.
- Hamilton, E.W., III & Frank, D.A. (2001). Can plants stimulate soil microbes and their own nutrient supply? *Ecology*, 82, 2397–2402.
- Herms, D.A. & Mattson, W.J. (1992). The dilemma of plants: to grow or defend. *Quart. Rev. Biol.*, 67, 283–335.
- Hierro, J.L. & Callaway, R.M. (2003). Allelopathy and exotic plant invasion. *Plant Soil*, 256, 25–39.
- Hunter, M.D. (2001). Out of sight, out of mind; the impact of root – feeding insects in natural and managed systems. *Agri. Forest Ent.*, 3, 3–9.
- Keane, R.M. & Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.*, 17, 164–170.
- Mallik, A.U. & Pellissier F. (2000). Effects of *Vaccinium myrtillus* on spruce regeneration: testing the notion of coevolutionary significance of allelopathy. *J. Chem. Ecol.*, 26, 2197–2209.
- Marler, M.J., Zabinski, C.A. & Callaway, R.M. (1999). Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology*, 80, 1180–1186.
- Maron, J.L. & Vilà, M. (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypothesis. *Oikos*, 95, 361–373.
- McEvoy, P.B. & Coombs, E.M. (1999). Biological control of plant invaders: regional patterns, field experiments, and structured population models. *Ecol. Appl.*, 9, 387–401.
- McFadyen, R.E. (1998). Biological control of weeds. *Annu. Rev. Entomol.*, 43, 369–393.
- Mitchell, C.E. & Power, A.G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature*, 421, 625–627.
- Müller, H. (1989). Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae) and effects of the root mining moth *Agapeta zoegana* (L.) (Lepidoptera: Cochyliidae). *Weed Res.*, 29, 103–111.
- Müller-Schärer, H. (1991). The impact of root herbivory as a function of plant density and competition: survival, growth, and fecundity of *Centaurea maculosa* in field plots. *J. Appl. Ecol.*, 28, 759–776.



- Müller-Schärer, H.A. & Schroeder, D. (1993). The biological control of *Centaurea* spp. in North America: do insects solve the problem? *Pest. Sci.*, 37, 343–353.
- Paige, K.N. (1999). Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. *Oecologia*, 118, 316–323.
- Paige, K.N. & Whitham, T.G. (1987). Overcompensation in response to herbivory: the advantage of being eaten. *Am. Nat.*, 129, 407–416.
- Pearson, D.E. & Callaway, R.M. (2003). Indirect effects of host-specific biocontrol agents. *Trends Ecol. Evol.*, 18, 456–461.
- Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *Bioscience*, 50, 53–65.
- Rabotnov, T.A. (1982). Importance of the evolutionary approach to the study of allelopathy. Translated from *Ékologia*, 3, 5–8.
- Ramsell, J., Malloch, A.J.C. & Whittaker, J.B. (1993). When grazed by *Tipula paludosa*, *Lolium perenne* is a stronger competitor of *Rumex obtusifolius*. *J. Ecol.*, 81, 777–786.
- Reichman, O.J. & Smith, S.C. (1991). Responses to simulated leaf and root herbivory by a biennial *Tragopogon dubius*. *Ecology*, 72, 116–124.
- Ridenour, W.M. & Callaway, R.M. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia*, 126, 444–450.
- Siemens, D., Garner, S., Mitchell-Olds, T. & Callaway, R.M. (2002). The cost of defense in the context of competition. *Brassica rapa* may grow and defend. *Ecology*, 83, 505–517.
- Simberloff, D., Brown, B.J. & Lowrie, S. (1978). Isopod and insect root borers may benefit Florida mangroves. *Science*, 201, 630–632.
- Steinger, T. & Müller-Schärer, H. (1992). Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific competition and soil nitrogen availability. *Oecologia*, 91, 141–149.
- Story, J.M., Good, W.R., White, L.J. & Smith, L. 2000. Effects of the interaction of the biocontrol agent *Agapeta zoegana* (L.) (Lepidoptera:Cochyliidae) and grass competition on spotted knapweed. *Biol. Control*, 17, 182–190.
- Strong, D.R., Maron, J.L., Connors, P.G., Whipple, A., Harrison, S. & Jeffries, R.L. (1995). High mortality, fluctuation in numbers, and heavy subterranean insect herbivory in bush lupine, *Lupinus arboreus*. *Oecologia*, 104, 85–92.
- Thaler, J.S. (1999). Jasmonic-inducible plant defences cause increased parasitism of herbivores. *Nature*, 399, 686–688.
- Trumble, J.T., Kolodny-Hirsch, D.M. & Ting, I.P. (1993). Plant compensation for arthropod herbivory. *Annu. Rev. Entomol.*, 38, 93–119.
- Vivanco, J.M. Bais, H.P. Stermitz, F.R. Thelen, G.C. & Callaway, R.M. (2004). Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecol. Lett.*, 7, 285–292.
- Waters, J.R. & Borowicz, V.A. (1994). Effect of clipping, benomyl and genet on <sup>14</sup>C transfer between mycorrhizal plants. *Oikos*, 71, 246–252.
- Zabinski, C.A., Quinn, L. & Callaway, R.M. (2002). Phosphorus uptake, not carbon transfer, explains arbuscular mycorrhizal enhancement of *Centaurea maculosa* in the presence of native grassland species. *Funct. Ecol.*, 16, 758–765.

Editor, Ted Turlings

Manuscript received 14 September 2004

First decision made 19 October 2004

Manuscript accepted 10 November 2004