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

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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 (±)-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.

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 & Vila 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; Thaler 1999) and the induction of chemical defences. Some induced defence chemicals may enhance the competitive ability of attacked plants by

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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, (+)-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 meristmatic 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 Cypholeonous 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 (2)-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 (+)-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 N2, and resuspended in 200 µL methanol. Methanol extracts were chromatographed (Dionex Co., Sunnyvale, CA, USA) on a reverse phase 5 µm, C18 column (25 cm × 4.6 mm) (Supelco Co., Bellefonte, PA, USA) using a multistep gradient. The absorbance at the reference wavelength λmax=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 µL and a flow rate of 1 mL 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. (+)-Catechin concentrations in each sample were determined by comparison with 15 µL injections from a 1 mg 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: Coehylidae), 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 cm2 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 (+)-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°57′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 (+)-catechin concentrations as described above. We also dissected C. maculosa taproots and recorded evidence of Agapeta. If there was evidence of Agapeta in the uninfested 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$^2$ pots, and used to grow either *Koeleria microstoa* or *Festuca idahoensis* (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 (±)-catechin on the growth of North American native plants, in May 2003 we applied (±)-catechin to the rhizospheres of four common grass species, *Dactylis glomerata*, *Agapeta agapetoides*, 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 (±)-catechin (0.188 mg mL$^{-1}$ and 0.020 mg mL$^{-1}$). For each target individual we injected 800 µL 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 (±)-catechin concentrations in the rhizospheres of *C. maculosa* with *Agapeta* in their roots. Based on preliminary tests, we estimated that 800 µL 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 µg g$^{-1}$ and c. 250 µg g$^{-1}$ 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 (±)-catechin, but root herbivory by *Cyphocleonus* increased (±)-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 (±)-catechin relative to control plants (Fig. 1b). Fungal cell walls are known to elicit (±)-catechin exudation from *C. maculosa* roots (Bais et al. 2002). In this experiment, *Agapeta* root herbivory more than quadrupled (±)-catechin output, and adding the fungal pathogen to *Agapeta* infestation elicited higher catechin exudation that 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 (±)-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 idahoensis* 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 (±)-catechin exudation. *Agapeta* did not have this indirect effect on *Pseudoroegneria spicata* at one site.

We found that the higher soil concentrations of (±)-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
native species exposed to (±)-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 (±)-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 (±)-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 (±)-catechin. For all eight native species combined, mortality was zero for the controls, 15.7% for natives exposed to low doses of (±)-catechin, and 23.6% of the individuals exposed to high doses of (±)-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 (±)-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 (±)-catechin mimicking the effects of biocontrol-stimulated exudation had effects comparable with those of the biocontrol-infested plants themselves. Higher rates of root

Figure 1 Concentrations of the root exudate, (±)-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 (±)-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 idahoensis* 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, $P = 0.110$. ©2005 Blackwell Publishing Ltd/CNRS
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 (Chermisnoff & 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 mycorrhizal-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 (±)-catechin, but costs...
associated with higher (±)-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 & Vila 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

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, (±)-catechin. Native species are Balsamorhiza sagitaria, Festuca idahoensis, Lupinus sericeus, Achillea millefolia, Delphinium bicolor, Poa sandbergii, Danthonia unispicata, Pseudoregneria 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, $F_{dose} = 13.022$, 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, $F_{dose} = 4.022$, 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.
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.

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REFERENCES


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