REPORT

Compensatory growth and competitive ability of an invasive weed are enhanced by soil fungi and native neighbours

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Abstract

Compensatory responses to herbivory by invasive weeds may foil attempts to arrest their spread with biological controls. We conducted an experiment to study the effects of defoliation and soil fungi on interactions between *Centaurea melitensis*, an invasive annual from Eurasia, and *Nassella pulchra*, a native Californian bunchgrass. Defoliation of *C. melitensis* reduced its final biomass in all species–fungicide treatments, except when *C. melitensis* was grown with both *Nassella* and non-treated soil fungi at the same time. In this treatment, the biomass of clipped *C. melitensis* plants was equal to that of unclipped plants, indicating that soil fungi and *Nassella* promoted a compensatory response in the weed. Overall, the biomass of *C. melitensis* was 44% lower when soil fungi were reduced. However, in soil not treated with fungicide, the total biomass of *C. melitensis* increased in the presence of *Nassella*, but decreased when it was grown alone. When stressed by defoliation, *C. melitensis* may benefit from a form of mycorrhizae-mediated parasitism through a common mycorrhizal network, or *Nassella* may alter the fungal community in a way that enhances the positive direct effects of soil fungi on *Centaurea*.

INTRODUCTION

The use of biological control herbivores to suppress invasive exotic plants is based on the crucial, but mostly untested, assumption that they will provide competitive release for natives. However, plants commonly increase growth, or "compensate", after herbivory (McNaughton 1983; Paige 1992; Trumble *et al.* 1993), and evidence indicates that moderate herbivory on one of North America's most noxious invasive weeds, *Centaurea maculosa* (Asteraceae), may have weak effects on the weed and perhaps stimulate compensatory growth responses and increase the invader's competitive ability (Müller-Schärer & Schroeder 1991; Steinger & Müller-Schärer 1992; Callaway *et al.* 1999). Other research has shown that soil fungi enhance the ability of *C. maculosa* to outcompete native grasses (Marler *et al.* 1999). In this paper, we link these results and report on the effect of soil fungi on the response of *Centaurea melitensis*, an invasive annual from Eurasia (Rice 2000), to defoliation, and its interactions

with *Nassella pulchra*, a native Californian perennial bunchgrass.

MATERIALS AND METHODS

Plants were grown in a naturally lit greenhouse at the University of California, Santa Barbara, from February to June 1999. *Nassella* and *Centaurea* were grown alone and in interspecifically paired combinations, both with and without the fungicide Benomyl® added to the soil. Plants were grown in 4 L pots filled with washed blasting grade, 20/30 grit, Aeolian sand. To each pot, we added an inoculum consisting of 200 mL of whole field soil collected at the University of California Natural Reserve System's Sedgwick Ranch, located in the Santa Ynez Valley of Santa Barbara County, California. Soil was collected from the upper 15 cm of the profile at several locations in grasslands where both of the experimental species were common, and soil from all locations was sifted free from rocks and thoroughly mixed before using

it as inoculum. Sifting did not remove all seeds and roots. The inoculum was then mixed thoroughly with sand to produce a 20 : 1 sand : soil growth medium. *Nassella* was germinated in the pots and thinned to one individual per pot. Seven weeks after the *Nassella* had been planted, pregerminated *C. melitensis* seedlings were planted in the pots with an individual grass or alone, and thinned to one individual per pot. *Centaurea* seeds were pregerminated on sterilized sponges using distilled water free from mycorrhizal inoculum. Three weeks after *C. melitensis* seeds had been planted, soil fungi were reduced in half the pots with Benomyl applied in 100 mL of water per pot at a concentration of 50 mg Benomyl/kg soil (Hetrick *et al.* 1989). Benomyl was added every 3 weeks for the duration of the experiment. Benomyl has been shown to have minimal direct effects on plants (Paul *et al.* 1989), and is a recommended method for fungal experiments (Fitter &Nichols 1988; Smith *et al.* 2000); however, Benomyl kills other fungi as well as arbuscular mycorrhizal (AM) fungi (West *et al.* 1993; Newsham *et al.* 1994). Plants were watered four times per week until water drained from the pots. All treatments received 100 mL of a one-eighth strength Hoagland's solution every 3 weeks. Seven weeks after planting *Centaurea*, we clipped off all above-ground biomass from *Centaurea* plants except for one leaf. This constituted removing ≈ 30–90% of the total above-ground biomass at the time of clipping. Final replication was *n* = 17–20 for each of the 10 species–treatment combinations. The final experimental design was a fully factorial comparison of defoliation, fungicide and *Nassella* neighbour effects on *Centaurea* total biomass.

Eight weeks after defoliation, plants were harvested, separated into roots and shoots, dried for 48 h at 60 °C, weighed for total biomass and checked for mycorrhizal infection under 100× magnification. We examined the roots of 50 plants from subsets of all treatments for AM fungi and non-AM fungi using the "magnified intersections" method described in McGonigle *et al.* (1990) and Marler *et al.* (1999) to determine the percentage of colonized root length. AM fungi were distinguished from non-AM fungi by the presence of arbuscules, vesicles, hyphal coils and septate hyphae. Non-AM fungi included melanized hyphae and spores, septate hyphae and aseptate hyphae associated with non-AM fungi structures including oospores.

The mass of the clipped leaves was not added to the final biomass for analyses. The effects of neighbour, fungal treatment and defoliation on the biomass of *Centaurea* were analysed with a three-way analysis of variance (ANOVA). We analysed *Nassella* biomass using a single ANOVA (neighbour treatments were not fully factorial for *Nassella*), and post-ANOVA Tukey tests were used to compare the six treatments independently.

RESULTS

With Benomyl, AM fungal colonization levels inside plant roots decreased from 22.3 ± 5.0% (1SE) to 1.9 ± 1.6% for *C. melitensis* and from 6.5 ± 2.6% to 2.7 ± 1.3% for *Nassella*. In an ANOVA, the overall effect of Benomyl was highly significant ($F = 8.287$; d.f. = 1.43; $P = 0.007$), but the effects of species or clipping were not significant. There were no significant effects of species, Benomyl or clipping on non-AM fungi colonization within the roots. Non-AM colonization in *Centaurea* roots was 4.0 ± 1.9% and 9.5 ± 8.5% in Benomyl and no-Benomyl treatments, respectively, vs. 9.5 ± 8.5% and 5.5 ± 3.4% for *Nassella* in Benomyl and no-Benomyl treatments, respectively.

There was no overall effect of *Nassella* neighbours on *C. melitensis* biomass ($F_{neighbour} = 2.539$; d.f. = 1.152; $P = 0.113$); however, the total biomass of *C. melitensis* was 44% lower when soil fungi were reduced with Benomyl. Soil fungi increased the total biomass of *C. melitensis* in the presence of *Nassella*, but decreased *C. melitensis* biomass when grown alone ($F_{fungi \times neighbour} = 10.497$; d.f. = 1.72; $P = 0.002$; Fig. 1). Across all treatments combined, defoliation decreased the total biomass of *C. melitensis* ($F_{defoliation} = 17.244$; d.f. = 1.152; $P < 0.001$). Most importantly, defoliation of *C. melitensis* reduced its final biomass in all species–fungicide combinations, except when *C. melitensis* was grown with both *Nassella* and without soil fungicide at the same time (Fig. 1). Thus, in the presence of *Nassella* and without soil fungicide, *C. melitensis* was able to fully compensate for the removal of 30–90% of its above-ground biomass (at the time of defoliation) in just 7 weeks.

Despite the fact that *Nassella* was planted 7 weeks before *C. melitensis*, the final biomass of *Nassella* was lower when grown with defoliated *C. melitensis* and without soil fungicide than when grown alone and without fungicide, or with fungicide with either defoliated or non-defoliated *C. melitensis* (Fig. 2). This compensatory response of *C. melitensis* in the presence of *Nassella* and without fungicide corresponded to a decrease in the total biomass of *Nassella* in the same pots.

DISCUSSION

We propose three hypotheses that might explain our results. We suspect, and work with other *Centaurea* species suggests, that *C. melitensis* may benefit from a form of mycorrhizae-mediated parasitism through common mycorrhizal networks (Grime *et al.* 1987; Marler *et al.* 1999; Carey & Callaway 1999). *Centaurea melitensis* may have the ability to harness AM fungi that are also connected to *Nassella* as conduits by which reduced carbon may be transferred from *Nassella* to *C. melitensis*. This is supported by the correlation between the increase in *C. melitensis* mass and the corresponding
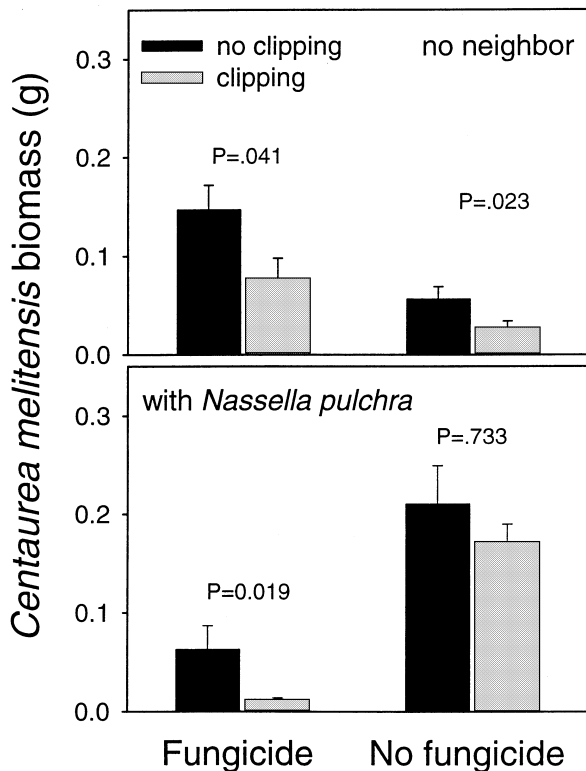
Figure 1 Total biomass of *Centaurea melitensis* grown alone or with *Nassella pulchra*, with or without soil fungicide, and with and without artificial defoliation. Error bars represent one standard error, and *P*-values are for *t*-tests between defoliation treatments within neighbour–soil fungi treatment combinations. Three-way ANOVA results are reported in the text.
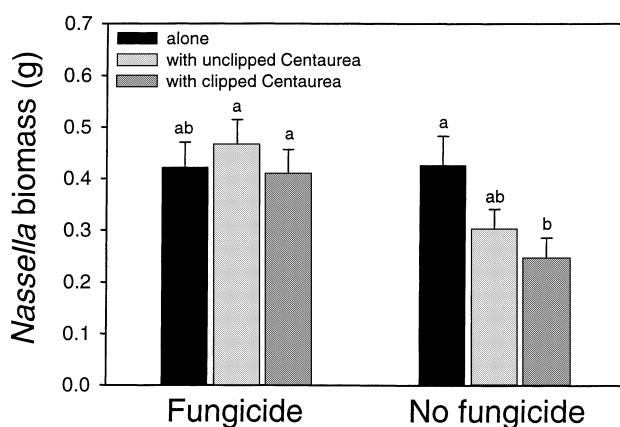

Figure 2 Total biomass of *Nassella pulchra* when grown alone with or without soil fungicide, and when grown with *Centaurea melitensis* that had been defoliated or had not been defoliated. Error bars represent one standard error; letters represent significant differences determined by post-ANOVA Tukey tests (one-way ANOVA, d.f. = 6.111; F = 3.635, d.f. = 5.111; P = 0.004).

decrease in *Nassella* mass in the presence of soil fungi. Carey & Callaway (in press) found that the stable carbon isotope concentration of *C. maculosa* shoot tissue was significantly more similar to that of *F. idahoensis* in the presence of mycorrhizae than without mycorrhizae, indicating that carbon was transferred from the *Festuca* to the *Centaurea*. However, the role of carbon transfer among plants via AM fungi remains controversial (Simard *et al.* 1997; Robinson & Fitter 1999). Our second hypothesis is that different combinations of plant species may alter microbial community composition (e.g. pathogenic or saprobic vs. mutualistic species), by shifts in the relative strengths of mutualistic vs. pathogenic effects of mycorrhizal species in the fungal community (see Bever 1994; Newsham *et al.* 1994; Bever *et al.* 1997; Johnson *et al.* 1997), or by shifts in the composition of AM fungal communities themselves (Egerton-Warburton & Allen 2000; Eom *et al.* 2000). A third possibility is that *Nassella* has strong positive effects on the overall growth of soil fungi, which in turn enhances *Centaurea* more than *Nassella*. This third hypothesis is unlikely, because we found no positive direct effects of soil fungi on *Centaurea*.

The findings of Grime *et al.* (1987) support the carbon transfer hypothesis. They found that *Centaurea nigra* plants were much larger in species-rich microcosms (that included *Festuca ovina*) when mycorrhizae were present than when mycorrhizae were absent, and that large amounts of ^{14}C were transferred to *C. nigra* from *F. ovina* when mycorrhizae were present. However, Grime *et al.* did not manipulate the presence or absence of the putative "donor" species nor specifically defoliate "target" species. Mycorrhizal transfer of carbon in Grime's system, composed of plants from natural communities, appeared to promote inferior competitors, constrain superior competitors and increase diversity; in contrast, in our invaded system, mycorrhizae appear to promote a superior competitor and decrease diversity.

Our results provide evidence for a compensatory biomass response, but not over-compensation. Over-compensatory growth is controversial, in part due to the difficulty in finding a plausible mechanism by which a plant that has experienced severe herbivory ultimately grows as large or larger than a plant that has remained intact (McNaughton 1983; Crawley 1987; Paige 1992; Trumble *et al.* 1993). Our results suggest that soil fungi and perhaps common mycorrhizal connections among plant species may provide a mechanism. Furthermore, substantial variation occurs among plant species in the nature of the mycorrhizal mutualism (Hartnett *et al.* 1993), environmental effects on mycorrhizal colonization (Johnson *et al.* 1992; Johnson 1993; Gehring & Whitham 1995) and the effects of different mycorrhizal fungal species on plants (van der Heijden *et al.* 1998). Such variation may help to explain the

contradictory results that have been reported for compensatory responses to herbivory.

The response of *Nassella* to the severe defoliation of neighbouring *Centaurea* plants suggests that defoliation of highly competitive species may have detrimental, indirect effects on neighbouring species. Artificial defoliation does not always elicit the same results as true herbivory (Crawley 1987), and our single bout of intense defoliation is not the same as chronic insect herbivory. Other experiments with *Centaurea* species and insect herbivores with long-term, chronic effects have produced results similar to ours (Müller-Schärer & Schroeder 1991; Callaway *et al.* 1999), but further experimentation with actual biocontrol herbivory and mycorrhizae is necessary. We chose to artificially defoliate *Centaurea* plants instead of using insects because it allowed us to precisely control the amount of defoliation. To our knowledge, this is the first evidence that implicates mycorrhizae as a mechanism for compensatory growth, and the first that suggests that compensatory responses to herbivory may increase a plant's competitive ability. However, we do not know if the important mechanisms in our highly controlled environment would have the same effects when embedded within a complex natural community. Our results could be altered by the presence of other plant species, spatio-temporal variation in plant patterns and different resource levels.

Biological control herbivores are imported with the expectation that they will reduce the invasive plant's competitive advantage over native species (Driesche & Bellows 1996). Biocontrols have been successful in reducing the spread of invasive plant species in some cases (Driesche & Bellows 1996). However, despite the crucial importance of understanding how biocontrols indirectly affect native plants and the potential ecological threat of biocontrols (Simberloff & Stiling 1996; Louda *et al.* 1997), most studies have been limited to measuring the direct effects of biocontrols on target weeds, or the indirect effects of biocontrols on native species taxonomically related to the target weed. Our finding that soil fungi can reverse the predicted competitive effect of defoliating an exotic plant suggests that the indirect effects of herbivory may be more complicated than currently thought, and understanding the indirect effects of biocontrols before their release is crucial.

ACKNOWLEDGEMENTS

REFERENCES

Bever, J.D. (1994). Feedback between plants and their soil communities in an old field community. *Ecology*, 75, 1965–1977.

Bever, J.D., Westover, K.M. & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J. Ecol.*, 85, 561–573.

Callaway, R.M., DeLuca, T. & Belliveau, W.M. (1999). Herbivores used for biological control may increase the competitive ability of the noxious weed *Centaurea maculosa*. *Ecology*, 80, 1196–1201.

Carey, E.V. & Callaway, R.M. (1999). Stable isotopes provide evidence for carbon transfer from the invasive weed *Centaurea maculosa* to a native bunchgrass. *Can J. Bot.*, in press.

Crawley, M.J. (1987). Benevolent herbivores? *Trends Ecol. Evol.*, 2, 167–168.

Driesche, R.G. & Bellows, T.S. Jr (1996). *Biological Control*. New York: Chapman & Hall.

Egerton-Warburton, L.M. & Allen, E.B. (2000). Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecol. Appl.*, 10, 484–496.

Eom, A.H., Hartnett, D.C. & Wilson, G.W.T. (2000). Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia*, 122, 435–444.

Fitter, A.H. & Nichols, R. (1988). The use of benomyl to control infection by vesicular-arbuscular mycorrhizal fungi. *New Phytol.*, 110, 210–206.

Gehring, C.A. & Whitham, T.G. (1995). Duration of herbivore removal and environmental stress affect the ectomycorrhiza of pinyon pines. *Ecology*, 76, 2118–2123.

Grime, J.P., Mackey, J.M.L., Hillier, S.H. & Read, D.J. (1987). Floristic diversity in a model system using experimental microcosms. *Nature*, 328, 420–422.

Hartnett, D.C., Hetrick, B.A.D., Wilson, G.W.T. & Gibson, D.J. (1993). Mycorrhizal influence of intra- and interspecific neighbor interactions among co-occurring prairie grasses. *J. Ecol.*, 81, 787–795.

Hetrick, B.A.D., Wilson, G.T. & Hartnett, D.C. (1989). Relationship between mycorrhizal dependence and competitive ability of two tallgrass prairie grasses. *Can J. Bot.*, 67, 2608–2615.

Johnson, N.C. (1993). Can fertilization of soil select less mutualistic mycorrhizae? *Ecol. Appl.*, 3, 749–757.

Johnson, N.C., Graham, J.H. & Smith, F.A. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol.*, 135, 575–585.

Johnson, N.C., Tilman, D. & Wedin, D. (1992). Plant and soil controls on mycorrhizal fungal communities. *Ecology*, 73, 2034–2042.

Louda, S.M., Kendall, D., Connor, J. & Simberloff, D. (1997). Ecological effects of an insect introduced for the biological control of weeds. *Science*, 277, 1088–1090.

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.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.*, 115, 495–501.

McNaughton, S.J. (1983). Compensatory plant growth as a response to herbivory. *Oikos*, 40, 329–336.

Müller-Schärer, H. & Schroeder, D. (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.

Newsham, K.K., Fitter, A.H. & Watkinson, A.R. (1994). Root pathogenic and arbuscular mycorrhizal fungi determine fecundity of asymptomatic plants in the field. *J. Ecol.*, 82, 805–814.

Paige, K.N. (1992). Overcompensation in response to mammalian herbivory: from mutualistic to antagonistic interactions. *Ecology*, 73, 2076–2085.

Paul, N.D., Ayres, P.G. & Wyness, L.E. (1989). On the use of fungicides for experimentation in natural vegetation. *Func. Ecol.*, 3, 759–769.

Rice, P. (2000). INVADERS Database. [WWW document] URL http://invader.dbs.umt.edu.

Robinson, D. & Fitter, A. (1999). The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. *J. Exp. Bot.*, 50, 9–13.

Simard, S.W., Perry, D.S., Jones, M.D., Myrold, D.D., Durall, D.M. & Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388, 579–582.

Simberloff, D. & Stiling, P. (1996). How risky is biocontrol? *Ecology*, 77, 1965–1974.

Smith, M.D., Hartnett, D.C. & Rice, C.W. (2000). Effects of long-term fungicide applications on microbial properties in tallgrass soil. *Soil Biol. Biochem.*, 32, 935–946.

Steinger, T. & Müller-Schärer, H. (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.

Trumble, J.T., Kolodny-Hirsch, D.M. & Ting, I.P. (1993). Plant compensation for herbivory. *Annu. Rev. Entomol.*, 38, 93–119.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engle, R., Boller, T., Wiemken, A. & Sanders, I.R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.

West, H.M., Fitter, A.H. & Watkinson, A.R. (1993). The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* spp. *ambigua* under natural conditions. *J. Ecol.*, 81, 345–350.

BIOSKETCH

Ragan M. Callaway's research interests include positive interactions among plants, mechanisms by which invasive weeds interact with natives, how invasive plants alter ecosystems, indirect interaction among plants, mycorrhizal effects on interactions, biological control, effects of parasitic plants on hosts and interactions among old-growth trees. This work is linked by the common thread of a fundamental interest in plant communities and how they are organized.