

No evidence for root-mediated allelopathy in *Centaurea solstitialis*, a species in a commonly allelopathic genus

Bo Qin · Jennifer A. Lau · Joseph Kopshever · Ragan M. Callaway · Heather McGray · Laura G. Perry · Tiffany L. Weir · Mark W. Paschke · Jose L. Hierro · John Yoder · Jorge M. Vivanco · Sharon Strauss

Received: 14 August 2006 / Accepted: 3 January 2007 / Published online: 1 March 2007
© Springer Science+Business Media B.V. 2007

Abstract Phytotoxicity bioassays and pot experiments using activated carbon both suggest that *Centaurea solstitialis* (yellow star-thistle) does not rely on phytotoxic root exudates for invasion of California grasslands. Pot experiments in which five native species were grown in the presence/absence of *C. solstitialis* and in the presence/absence of activated carbon (fully crossed design) showed that *C. solstitialis* competitively suppressed native species, but did not

inhibit them through allelochemicals. In separate experiments examining the role of root exudates in invasion success, treatment with crude root exudates and chloroform-extracted root exudates from *C. solstitialis* reduced growth of the model plant *Arabidopsis thaliana*. However, high concentrations of the exudates (50%, v/v or 500 $\mu\text{g mL}^{-1}$) were required to inhibit *A. thaliana* growth and did not result in *A. thaliana* mortality, suggesting the presence of only a weak growth inhibitor. Moreover, high concentrations of *C. solstitialis* crude root exudates did not affect the growth of five native grass species often displaced by *C. solstitialis* invasions in California grasslands. Finally, root exudates collected from *C. solstitialis* had weaker effects on a native California root parasite, *Triphysaria versicolor*, than root exudates collected from *Zea mays*, a species not renowned for its competitive or invasive capabilities. Our results suggest that, while *C. solstitialis* might possibly “be persuaded to yield a product that is toxic to one species or another” (Population biology of plants, Academic, 1977), we find no evidence that allelopathic root exudates play a role in the competitive success of this invasive.

B. Qin · L. G. Perry · T. L. Weir · M. W. Paschke · J. M. Vivanco
Center for Rhizosphere Biology, Colorado State University, Fort Collins, CO 80521, USA

J. A. Lau · J. Kopshever · S. Strauss
Center for Population Biology, University of California, Davis, CA 95616, USA

R. M. Callaway (✉) · J. L. Hierro
Division of Biological Sciences, The University of Montana, Missoula, MT 59812, USA
e-mail: ray.callaway@mso.umt.edu

H. McGray
Department of Plant Sciences, University of California, Davis, CA 95616, USA

Present Address:
H. McGray · J. Yoder
Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA

Keywords Allelopathy · Invasion · Competition · Exudates · Activated carbon · *Centaurea*

Introduction

The genus *Centaurea* includes a large number of invasive species. The allelopathic, or phytotoxic, effects of root exudates appear to play an important role in the invasive success of several *Centaurea* species. *Centaurea maculosa* (spotted knapweed) and *Centaurea diffusa* (diffuse knapweed) appear to use root exudates to gain a competitive advantage over North American species based on experiments using activated carbon to minimize effects of soil allelochemicals (Callaway and Aschehoug 2000; Ridenour and Callaway 2001). *C. maculosa* exudes (\pm)-catechin from its roots, which possesses phytotoxic, antimicrobial, and chelation properties (Bais et al. 2002, 2003; Weir et al. 2003, 2006; Perry et al. 2005a, b; Thelen et al. 2005; Thorpe 2006), although the importance of (\pm)-catechin in *C. maculosa* invasion has recently been questioned (Blair et al. 2005) and other compounds from *C. maculosa* could also be important. *C. diffusa* roots appear to exude 8-hydroxyquinoline, another compound with antibacterial, antifungal, and phytotoxic attributes (Vivanco et al. 2004). Roots of *Acroptilon* (nee *Centaurea*) *repens* may exude 7,8-benzoflavone (α -naphthoflavone), a phytotoxin not previously known as a natural product (Stermitz et al. 2003). Interestingly, these chemicals are not similar to each other. Each chemical has negative effects on *Centaurea* species that do not produce them, but little or no autotoxicity (Bais et al. 2003; Vivanco et al. 2004; but see Perry et al. 2005b).

Some evidence suggests that *C. maculosa* and *C. diffusa*, and their respective phytotoxins, have stronger effects on species in invaded communities in North America than on species in the Eurasian communities where the invaders are native (Callaway and Aschehoug 2000; Bais et al. 2003; Vivanco et al. 2004). This has led to the “novel weapons” hypothesis for plant invasions (Callaway and Aschehoug 2000; Callaway and Ridenour 2004), which proposes that these biogeographic differences are due to the evolution of tolerance to chemicals produced by species that have coexisted for centuries, and the lack of evolutionary relationships between invaders and their new neighbors. In other words, Eurasian plants present in communities with these *Centaurea* species may have adapted to

(\pm)-catechin and 8-hydroxyquinoline, but North American species may not yet have adapted, resulting in susceptibility to the novel allelochemicals.

Here, we explore the allelopathic potential of another member of the *Centaurea* genus, *C. solstitialis* L. (yellow star-thistle). *C. solstitialis* is an exotic annual that is native to the Balkans, Turkey, the Caucasus region and Iran. It is often considered to be native to southern Europe; however, Prodan (1930, as cited in Maddox 1981; Maddox et al. 1985) argued that southern Europe was invaded by *C. solstitialis* from eastern Eurasia. The genus *Centaurea* exhibits its greatest diversity in eastern Eurasia (Wagenitz 1955, as cited in Maddox et al. 1985; Davis 1975). *C. solstitialis* has invaded large areas of native grassland and rangeland around the world, usually as a contaminant of alfalfa seeds (Roché and Thill 2001), with some of the more dramatic invasions occurring in California, USA, where it occupies over 5 million ha and is continuing to spread (Uyger et al. 2004). The mechanisms of *C. solstitialis* invasion are not known, and to our knowledge, allelopathy in *C. solstitialis* has not been studied previously.

To test whether root-mediated allelopathy may play a role in *C. solstitialis* invasions, we conducted experiments in three different laboratories, with each laboratory taking a different approach. First, we grew native plants in the presence versus absence of *C. solstitialis* and with and without activated carbon, a substance that minimizes allelopathic effects when added to the substrate. Second, we collected *C. solstitialis* root exudates and tested their effects on the growth *Arabidopsis thaliana* L. seedlings and five native California grasses often displaced by *C. solstitialis*. Finally, we used a different protocol to collect *C. solstitialis* root exudates and compared the effects of these exudates on root necrosis to the effects of root exudates from *Zea mays* (corn), a species not commonly thought to be allelopathic.

Methods

Activated carbon experiment

We used a factorial design in which we grew native plants in the presence versus absence of

C. solstitialis and in the presence versus absence of activated carbon. Because activated carbon adsorbs too many different organic compounds (Cheremisinoff and Ellerbusch 1978), it often reduces or eliminates allelopathic effects (Mahall and Callaway 1992; Nilsson 1994; Ridenour and Callaway 2001). Activated carbon does not directly reduce the activity of phytotoxins but acts by trapping or binding phytotoxins, particularly if the compounds are large structurally. If phytotoxins are small in size, or very polar, activated carbon may not be effective.

Activated carbon (grade SA-30 steam activated wood, Carbochem Inc., Ardmore, PA, USA) was added to potting soil by hand-mixing 20 mL of carbon per 1 L soil (University of California Research Mix). *C. solstitialis* presence was manipulated by planting three *C. solstitialis* seeds into half of the treatment pots. Four native seeds (all of a single species) were added to each pot. Natives used as test species included native grasses *Festuca idahoensis*, *Nassella lepida*, *Elymus glaucus*, and *Vulpia microstachys*, and a native composite, *Grindelia camperum*, that grows and flowers at the same time as *C. solstitialis*. *C. solstitialis* seeds were collected from Yolo and Santa Clara Co., California, and *F. idahoensis*, *N. lepida*, *E. glaucus*, *V. microstachys*, and *G. camperum* were purchased from Hedgerow Farms (Winters, CA, USA); seed source populations used by this company came from local provenances in Yolo and adjoining Solano counties. There were 25 replicates per treatment for each of the five native test species, yielding a total of 500 experimental pots. Because no seeds germinated in some pots, final sample sizes ranged from 20 to 25 per species per treatment. All pots were placed into pre-determined, fully randomized positions in the greenhouse.

The samples were initially misted with fertilizer water to increase germination. At the seedling stage, we switched to bottom-watering the plants with deionized water to prevent a loss of any allelochemicals that may have been produced. Both natives and *C. solstitialis* were thinned at the seedling stage so that only one individual of each species grew in each pot. All native individuals and *C. solstitialis* were harvested approximately 2.5 months after

planting, and the aboveground biomass of each plant was dried at 60°C for a minimum of 5 days before weighing.

To test for the effects of carbon and *C. solstitialis* on native growth, we used an ANOVA that included the log-transformed aboveground biomass of the native test species as a response variable (PROC GLM, SAS Institute 2000). Carbon treatment, *C. solstitialis* treatment, and their interaction were included as fixed factors, and greenhouse tray was included as a random blocking factor. Statistical interactions between the effect of carbon and the effect of *C. solstitialis* treatments, where *C. solstitialis* decreases native growth more in the absence of carbon than in the presence of carbon, would indicate that *C. solstitialis* is allelopathic. To determine whether the effects of *C. solstitialis* on native growth were greater in the presence or absence of carbon, pairwise comparisons between all treatment combinations were performed. Separate tests were run for each native target species. All comparisons between treatments were corrected for multiple comparisons within tests (species) with a Tukey correction and across tests with a Bonferroni correction.

Root exudate collection

Experiment 1

Centaurea solstitialis seeds, collected from six populations (Table 1) in California in August 2002, were surface-sterilized with 50% bleach for 30 min and germinated on solid MS medium (Murashige and Skoog 1962) in a 25°C incubator with a 16-h/8-h day/night schedule. Fifty seedlings were transferred into 400 mL of liquid MS medium in a single 1-L Erlenmeyer flask. After 6 weeks, the plants were treated with chitosan, a root exudate elicitor (Walker et al. 2003). Chitosan was dissolved in 0.1 N acetic acid and adjusted to pH 5.8 with 1 M NaOH. The chitosan was then applied to the MS medium to create a 0.012% chitosan solution. Three days after chitosan treatment, the MS medium and root exudates were collected from the flask. Crude root exudates were collected and extracted with an equal volume of chloroform, which results in an easily

Table 1 ANOVA of the effects of activated carbon, *Centaurea solstitialis* competition, and their interaction on the biomass of five test species

Source	df	<i>Vulpia</i>		<i>Grindelia</i>		<i>Elymus</i>		<i>Festuca</i>		<i>Nassella</i>	
		F	P	F	P	F	P	F	P	F	P
<i>C. solstitialis</i>	1	30.56	<0.0001	22.09	<0.0001	1.41	0.2378	23.57	<0.0001	30.20	<0.0001
Carbon	1	6.12	0.0153	7.76	0.0067	0.51	0.4787	1.09	0.2991	5.28	0.0241
Carbon × <i>C. solstitialis</i>	1	0.91	0.3425	0.10	0.7559	0.10	0.7479	0.06	0.8012	2.63	0.1088
Tray	6	6.40	<0.0001	1.73	0.1247	6.36	<0.0001	1.56	0.1699	1.53	0.1768

Significant carbon and competitor effects are shown in bold ($P < 0.01$, Bonferroni adjustments for experiment-wide significance at $P < 0.05$)

separable liquid/liquid partition. The chloroform layer containing non-polar compounds from the crude exudates was removed and concentrated under vacuum to evaporate the chloroform. The remaining water layer was then extracted with an equal volume of ethyl acetate, another organic solvent that results in a liquid/liquid partition and extracts moderately polar compounds from the medium. Again the ethyl acetate layer was collected and concentrated under vacuum, removing the ethyl acetate. The remaining water phase was then collected and lyophilized. The two extracted fractions, the water phase, and the remaining crude root exudates (total of four fractions) were stored at -20°C prior to use in phytotoxicity assays.

Centaurea solstitialis seeds, collected from six populations (Table 1) in California in August 2002, were surface-sterilized with 50% bleach for 30 min and germinated on solid MS medium (Murashige and Skoog 1962) in a 25°C incubator with a 16-h/8-h day/night schedule. Fifty seedlings were transferred into 400 mL of liquid MS medium in a single 1-L Erlenmeyer flask. After 6 weeks, the plants were treated with chitosan, a root exudate elicitor (Walker et al. 2003). Chitosan was dissolved in 0.1 N acetic acid and adjusted to pH 5.8 with 1 M NaOH. The chitosan was then applied to the MS medium to create a 0.012% chitosan solution. Three days after chitosan treatment, the MS medium and root exudates were collected from the flask. The contents of the flask were used to obtain four different solutions of root exudates: (1) crude exudates, (2) chloroform-extracted exudates, (3) ethyl acetate extracted exudates, and (4) the remaining aqueous phase. To obtain the chloroform-extracted exudates,

a subsample of the crude root exudates was extracted with an equal volume of chloroform. The organic layer was removed and concentrated under vacuum, removing the chloroform. The remaining aqueous layer was then extracted with an equal volume of ethyl acetate to obtain the ethyl acetate extracted exudates. Again the organic layer was collected and concentrated under vacuum, removing the ethyl acetate. The remaining aqueous phase was then collected and lyophilized. The extracted fractions, the aqueous phase, and the remaining crude root exudates were stored at -20°C .

Arabidopsis thaliana seeds obtained from Lehle Seeds (Cat. No. WT-02-36-01) were surface-sterilized with 50% bleach for 20 min, and germinated on solid MS medium in a 25°C incubator with a 16-h/8-h day/night schedule. Seven-day-old plants were transferred into 1 mL of liquid MS medium in 12- and 24-well plates (VWR Scientific). After 24 h, the plants were treated with five concentrations of *C. solstitialis* crude root exudates (0, 10, 20, 50, and 100%, v/v) or with six concentrations (0, 20, 50, 100, 200, and $500\ \mu\text{g mL}^{-1}$) of the chloroform ethyl acetate, and aqueous extracts, with four replicates per treatment. The crude root exudates and the aqueous extract were applied directly to the liquid medium containing the seedlings. The chloroform and ethyl acetate extracts were re-suspended in 100% methanol, filtered with a $0.2\text{-}\mu\text{m}$ acrodisc syringe filter (VWR Scientific), and applied to fresh 12-well plates. The methanol was then allowed to evaporate to avoid effects on the plants. Once the methanol had evaporated, 1 mL of liquid MS medium was added to each well and *A. thaliana* plants were transferred to the wells (see Perry et al. 2005a, b). Control plants were

grown in liquid MS medium alone. Because the chloroform and ethyl acetate used for the extractions were removed under vacuum and the methanol used as a solvent was removed by evaporation, the treated plants only could have been exposed to small quantities of the solvents, which would be insufficient to influence plant growth. Plants were blotted dry and weighed 7 days after treatment. Relationships between root exudate or extract concentrations and plant weight were examined with linear regression analysis using SAS statistical software.

Seeds of five grass species commonly displaced by *C. solstitialis* (*Achnatherum coronatum*, *N. lepida*, *Nassella pulchra*, *V. microstachys*, and *Vulpia myuros*) were purchased from S&S Seeds in Santa Barbara, CA in September 2000. The seeds were surface-sterilized for 15 min in 100 mL of 30% bleach with two drops of Tween 20 and germinated on solid MS medium. Plants between 7 and 10 days old were transferred to liquid medium and tested and for responses to *C. solstitialis* crude root exudates as described above. Treatments were replicated four times for *A. coronatum*, and three times for *N. lepida*, *N. pulchra*, *V. microstachys*, and *V. myuros*.

Experiment 2

In a second experiment, we compared the effects of root exudates collected from *C. solstitialis* to those collected from *Z. mays* (corn) on the root parasite *Triphysaria versicolor*. *Triphysaria* is a small genus of five hemiparasitic species that are common in grassland stands throughout the Pacific Coast (Hickman 1993). In the field and in vitro, *Triphysaria* will invade a broad spectrum of hosts, including maize, clover, and *Arabidopsis* (Estabrook and Yoder 1998). We used *T. versicolor* because parasitic plants can detect chemicals in root exudates and may show particular sensitivities to species-specific differences in the chemical composition of exudates. Exudates were collected in hydroponic nutrient cycling systems comprising five pots of a 2:1 sterilized sand:Vermiculite mixture for *Z. mays* and another five pots for *C. solstitialis*. In each pot, 3 L of 10% strength Hoaglands nutrient solution, pH 6.1 (Hoaglands

and Arnon 1950), were cycled through the system by pumping solution from a collection reservoir across the surface sand of each pot. The solution then filtered through the sand and drained back into the collection reservoir. This cycling allowed for fertilization of plant roots by nutrient solution as well as flushing of exudates from plant roots into solution. Root exudates were collected once weekly by removing solution from the collection reservoir and replacing it with fresh solution. *C. solstitialis* seeds were received from Dr Joseph DiTomaso (University of California, Davis) and grown at a density of 20 seeds per pot in the nutrient cycling system. Pots were planted in succession, one pot per week for 5 weeks, to ensure a range of ages of plants growing in the system. *Z. mays* seeds were received from Dr John Yoder (University of California, Davis) and planted and grown in the same manner as described above at a density of ten seeds per pot.

The collected exudate solution was vacuum filtered through Whatman 2 filter paper to remove large particles of sand and plant material. One liter of filtered exudate solution was reserved and stored at 4°C for use in bioassays. Phenolic compounds were extracted from solution using resin chromatography. Five grams of BioRad SM-2 Biobeads were added to 2 L filtered exudate solution and stirred for 5 h. Resin was separated from exudate solution by vacuum filtration through Whatman 2 filter paper then transferred to a BioRad PolyPrep column for elution. Filtration flow through was reserved and stored at 4°C for use in bioassay. Phenolic compounds were eluted from the resin column using 500 mL methanol. Methanol was removed from the phenolic fraction by vacuum evaporation at 40°C. The remaining solution (<1 mL) was diluted to 5 mL with DI H₂O. The overall phenolic concentration of this solution was quantified by the Folin-Denis assay using a catechin standard (Swain and Goldstein 1964).

Triphysaria versicolor seeds were surface-sterilized, germinated, and transferred to bioassay plates following procedures developed by Albrecht et al. (1999). To assay for growth inhibition of *T. versicolor* roots by *C. solstitialis* and *Z. mays* root exudate phenolics, bioassay plates ($n = 2$; these two trials are not completely independent as the exudates collected were from the same plants growing

in the same system) were treated with 2 mL of one of the following treatments: filtered exudate, 1:10 filtered exudate, extraction flow through, and 100, 50, 20, 10, and 2 μM phenolic solutions. Plates were stored horizontally for 1 h to allow absorption of treatment solution then stored vertically at 25°C. Each root tip was scored for necrosis.

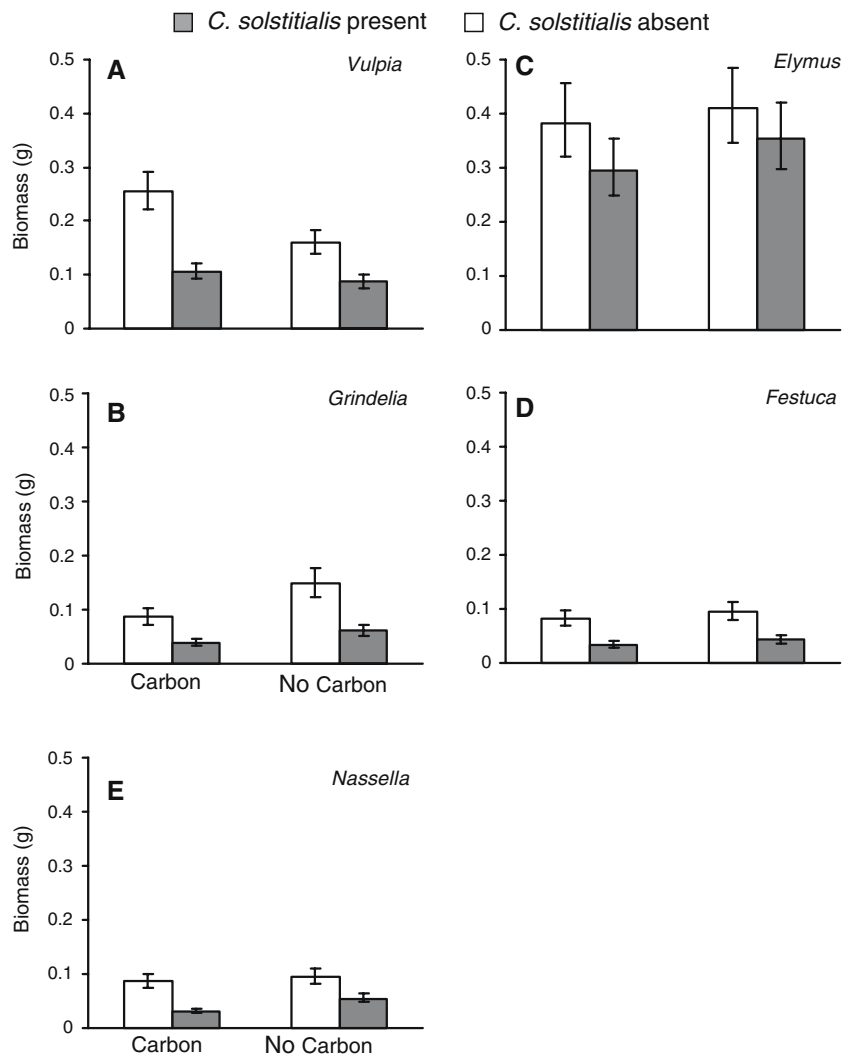
Results

Activated carbon experiment

When using activated carbon to test for allelopathic effects of a focal species on a target

species, allelopathy is inferred if there is an interaction between carbon and competitor treatments, such that the focal competitor decreases the fitness of the target species more in the absence of carbon than in the presence of carbon. There were no significant interactions between activated carbon and competitor for any of the five native species tested (Table 1). However, *C. solstitialis* was highly competitive; with the exception of *E. glaucus*, *C. solstitialis* significantly decreased the biomasses of all native Californian species. The presence of activated carbon did not significantly influence the magnitude of this competitive effect, suggesting that the effect of *C. solstitialis* on these native species was due to

Fig. 1 Lack of an interaction between effects of activated carbon and *Centaurea solstitialis* on growth of five native plant species: *Vulpia microstachys* (A), *Grindelia camperum* (B), *Elymus glaucus* (C), *Festuca idahoensis* (D), *Nassella lepida* (E). Open bars represent plants grown without *C. solstitialis*; filled bars, with *C. solstitialis*. We failed to detect significant interactive effects of activated carbon and *C. solstitialis*, indicating that *C. solstitialis* is not allelopathic, although it does suppress native growth, presumably via resource competition. This lack of an interaction is especially noticeable for those test species that were not directly affected by activated carbon (right column, C and D). Error bars are one standard error of the mean



resource competition and not allelopathy (Fig. 1). While direct effects of carbon can obscure potential allelopathic effects (J.A. Lau et al., unpublished data), we did not detect any significant *C. solstitialis* by carbon treatment interactions even for the two species that were not directly affected by activated carbon (Fig. 1C, D).

Interestingly, no native Californian species significantly reduced the growth of *C. solstitialis*, indicating highly asymmetrical, or unequal, competitive relationships (Fig. 2).

Root exudate collection

Experiment 1

High concentrations of *C. solstitialis* crude root exudates (Fig. 3) significantly reduced the fresh weight of *A. thaliana* (linear regression, $F_{1,22} = 25.08$, $P < 0.0001$). However, the crude root exudates were applied in nutrient-depleted growing media previously used by *C. solstitialis* plants for 6 weeks. Therefore, the effect of the crude exudates when applied as 50 or 100% of the total growing media may be due to lower nutrient availability rather than to phytotoxic root exudates.

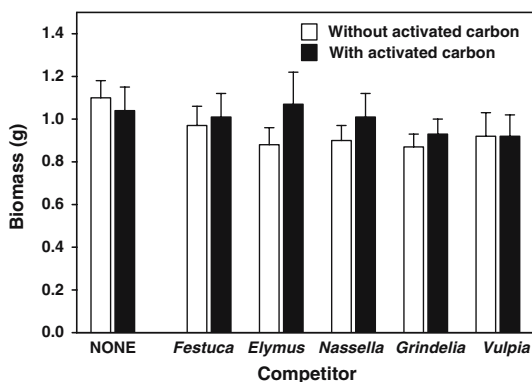


Fig. 2 Biomass of *Centaurea solstitialis* grown alone or in competition with five native California species, and either with or without activated carbon added to the soil. Open bars = without activated carbon, filled bars = with activated carbon. In an ANOVA with competitor and activated carbon as fixed treatments, neither effect was significant ($F_{\text{competitor}} = 0.793$; $df = 5, 264$; $P = 0.555$; $F_{\text{activated carbon}} = 1.013$; $df = 1, 264$; $P = 0.315$; $F_{\text{competitor} \times \text{activated carbon}} = 0.394$; $df = 5, 264$; $P = 0.893$). Note the difference between the competitive effects of *C. solstitialis* and the natives (compare Fig. 1 and Fig. 2). Error bars are one standard error of the mean

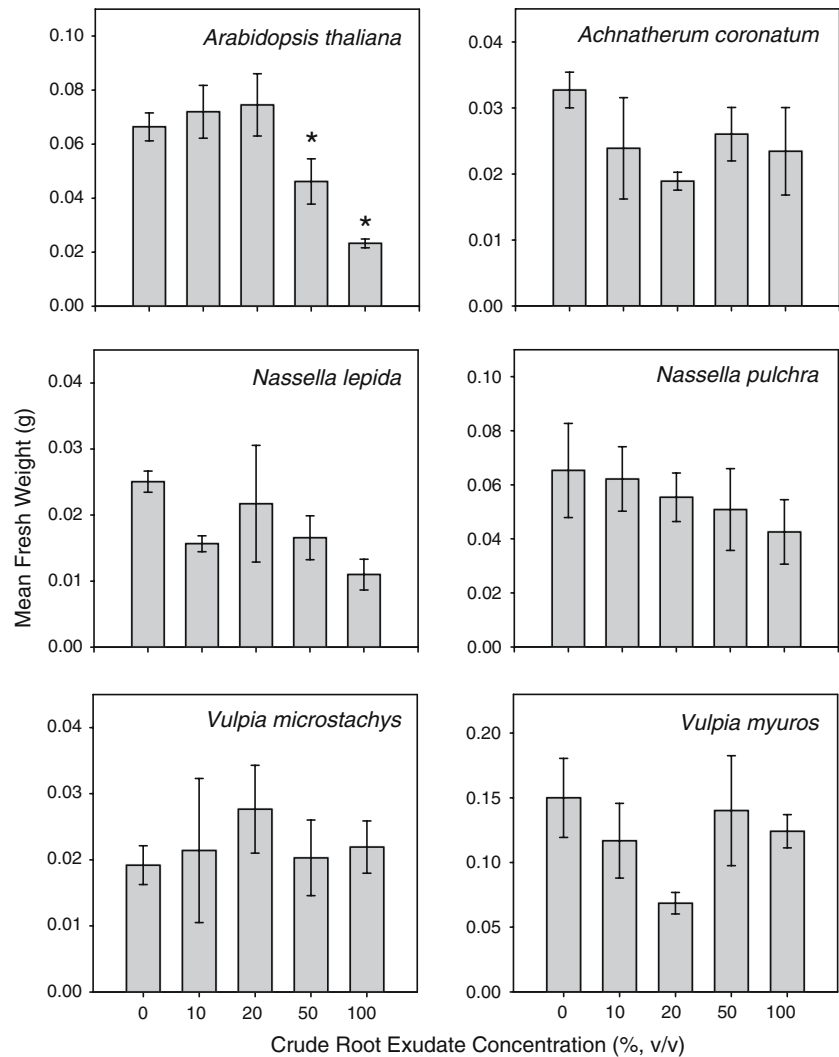
To alleviate this effect, concentrated fractions of chloroform and ethyl acetate extracts of the crude exudates were tested. The chloroform extract, containing the non-polar compounds from the crude exudates, reduced *A. thaliana* growth (linear regression, $F_{1,22} = 35.30$, $P < 0.0001$) by 66% at the highest treatment concentration (Fig. 4). Neither the ethyl acetate extract (moderately polar compounds) nor the aqueous phase (polar compounds) reduced *A. thaliana* growth (Fig. 4). A phytotoxin in *C. solstitialis* root exudates may account for the reduced growth of *A. thaliana* plants treated with *C. solstitialis* crude root exudates and chloroform-extracted exudates. However, high concentrations were required to have even minor effects on *A. thaliana* growth (Figs. 3, 4), and did not result in mortality, suggesting that any phytotoxin, if present, is relatively weak.

Centaurea solstitialis crude root exudates did not reduce the growth of any of the five native California grasses examined (Fig. 3), suggesting that *C. solstitialis* root exudates do not contain compounds phytotoxic to the native grasses commonly displaced by *C. solstitialis*. *A. coronatum*, *N. lepida*, and *N. pulchra* showed slight but insignificant (linear regression, $F_{1,22} = 0.33$, $P = 0.57$; $F_{1,13} = 4.36$, $P = 0.06$; $F_{1,19} = 2.01$, $P = 0.17$, respectively) reductions in growth with increasing exudate concentrations, which might have been significant with more statistical power. However, even if these trends were significant, they would indicate only that very high concentrations of *C. solstitialis* root exudates have small effects on native plant growth. These results suggest that *C. solstitialis* does not have strong phytotoxins in its root exudates, supporting the results of the activated carbon experiment.

Experiment 2

Filtered root exudates collected from *C. solstitialis* did not cause any necrosis on the roots of *T. versicolor* (Fig. 5). Further, dilution experiments indicated that the phenolic component of *C. solstitialis* was less toxic to *T. versicolor* than root exudates collected from *Z. mays*. At the 50- μm concentration, the extracted phenolics from *Z. mays* caused 100% necrosis of *T. versicolor* root tips, whereas 50 μm concentrations of the

Fig. 3 Fresh weight of *Arabidopsis thaliana* and five native California grasses treated for 7 days with *Centaurea solstitialis* crude root exudates. Asterisk indicates a mean significantly lower than the control for the marked species (Dunnett's one-tailed *t*-test, $P < 0.05$). Error bars are one standard error of the mean



extracted phenolics from *C. solstitialis* had no effect. At 100 μ molar concentrations, the root exudates of both *C. solstitialis* and *Z. mays* caused 100% necrosis of *T. versicolor* roots.

Discussion

We found no evidence for root-mediated allelopathy of *C. solstitialis* in three experiments conducted in three different labs; however, we emphasize that we have specifically addressed root exudates, not all potential allelopathic processes. We have not measured the toxicity of shoot tissues (Hierro and Callaway 2003) nor

have we addressed potential indirect allelopathic effects (Stinson et al. 2006). Shoot extracts and leachates from other members of the Asteraceae have been shown to be highly allelopathic (Hierro and Callaway 2003). Our experiments with root extracts and leachates employed techniques similar to those used to examine allelopathic effects of two other invasive *Centaurea* species and the related *A. repens* (Callaway and Aschehoug 2000; Bais et al. 2002, 2003; Stermitz et al. 2003; Vivanco et al. 2004; Perry et al. 2005a, b; Weir et al. 2006). Interestingly, *C. maculosa*, *C. diffusa*, and *A. repens* produce three different and chemically unrelated phytotoxins in their root exudates, indicating a surprising degree of variability in

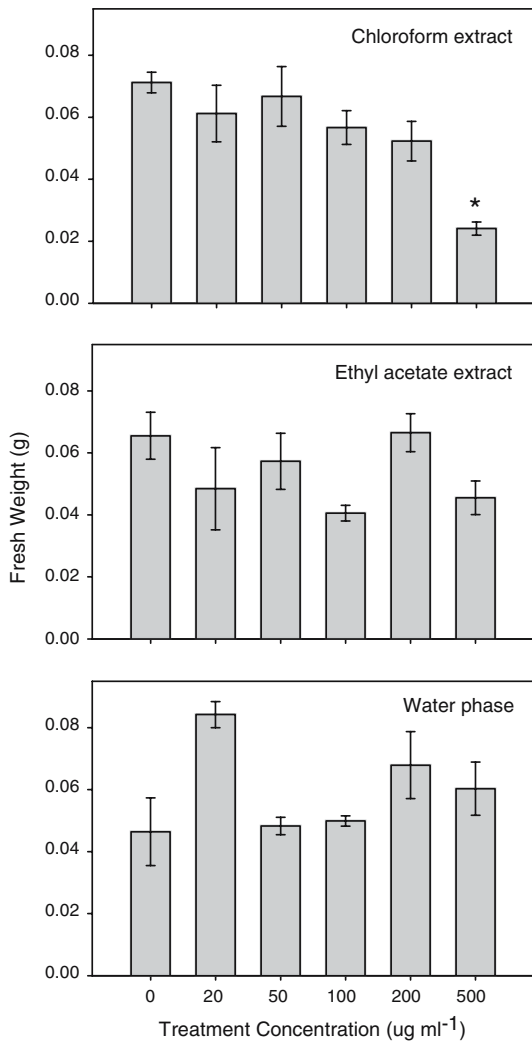


Fig. 4 Fresh weight of *Arabidopsis thaliana* treated for 7 days with *Centaurea solstitialis* root exudates extracted with chloroform and ethyl acetate, and the remaining water phase. *Asterisk* indicates a mean significantly lower than the control (Dunnett’s one-tailed *t*-test, $P < 0.05$). *Error bars* are one standard error of the mean

the phytochemistry of these closely related species. However, the results presented here suggest an even greater degree of variability in the chemical ecology of *Centaurea* species and the potential mechanisms by which they invade and dominate native communities. Each of the *Centaurea* species discussed here transmogrify from relatively minor components of their native communities to dominants that can form virtual monocultures where they invade, but this transmogrification

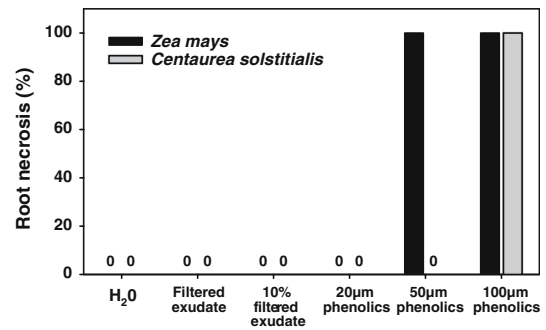


Fig. 5 Percentage of *Triphysaria versicolor* root tips showing signs of necrosis after application of deionized water, root exudates collected from *Centaurea solstitialis* and *Zea mays* (applied at the concentration collected and at 10% of that concentration), and different concentrations of the phenolic fraction of root exudates from *C. solstitialis* and *Z. mays*. $n = 11-20$ for each treatment

may be caused by very different processes, with phytotoxins potentially contributing to the invasive success of some species, but not others.

A trait that sets *C. solstitialis* apart from its allelopathic congeners is its annual life history. We know of no compelling reason why annual species should be inherently less allelopathic than perennial species, and there is strong evidence for the allelopathic effects of some annuals. However, the large majority of putatively allelopathic invaders discussed by Hierro and Callaway (2003) are perennials. Perhaps the physiological costs of allelopathy are too high for rapidly growing annuals or the role of allelochemicals as within-population regulators of germination (Perry et al. 2005b) is less important for annuals than for perennials.

Allelopathy is not alone in its failure to provide a convincing explanation for *C. solstitialis* invasiveness. In a common garden experiment the biomass and fecundity of *C. solstitialis* populations from invaded ranges were similar to those from the native range (J.L. Hierro and R.M. Callaway, unpublished data), suggesting that evolution of increased invasiveness (Blossey and Nötzold 1995; Bossdorf et al. 2005) is not responsible for the remarkable abundance of this species in non-native regions. Similarly, parallel field experiments in native and introduced ranges of *C. solstitialis* (Hierro et al. 2006) and the general failure of introduced biological control agents in Califor-

nia (DiTomaso and Gerlach 2000; Pitcairn et al. 2002; J. Garren and S. Strauss, unpublished data) suggest that release from aboveground specialist herbivores in non-native regions (Darwin 1859; Elton 1958) also may not underlie the remarkable invasive success of *C. solstitialis*.

Our study did show that *C. solstitialis* is a good competitor relative to native species, but the short time frame of the experiment probably exaggerated the competitive dominance of the very fast growing *C. solstitialis*. However, *C. solstitialis* is an exceptionally strong competitor in field experiments (Dukes 2001, 2002) and the competitive advantage due to deep rooting by *C. solstitialis* may explain in part *C. solstitialis*' success in California's Mediterranean climate (DiTomaso et al. 2003; Enloe et al. 2004; Morghan and Rice 2006). However, deep rooting could not have explained the strong competitive effects of *C. solstitialis* in our pot experiments.

In conclusion, the absence of evidence for a process is quite different than the presence of evidence. Failure to find evidence may occur because of inappropriate methodology, small sample sizes, or because of conditionality in the intensity of the process. For example, different extraction procedures may yield highly different effects. For example, our results suggest potential differences between the "aqueous phase" and "ethyl acetate" extractions and the "crude root exudates." Therefore, the absence of evidence for allelopathy for *C. solstitialis* cannot be taken as definitive rejection of allelopathic potential for the species. We cannot rule out the potential of litter or leaf leachates to be allelopathic, but our results, from multiple experiments employing a wide range of techniques, indicate that *C. solstitialis* is a good competitor but that it likely does not rely heavily on allelopathic compounds in root exudates to suppress native Californian species.

References

- Albrecht H, Yoder JI, Phillips DA (1999) Flavonoids promote haustoria formation in the root parasite *Triphysaria versicolor*. *Plant Physiol* 119:585–591
- Bais HP, Walker TS, Stermitz FR, Hufbauer RA, Vivanco JM (2002) Enantiomeric dependent phytotoxic and antimicrobial activity of (\pm)-catechin; a rhizosecreted racemic mixture from *Centaurea maculosa* (spotted knapweed). *Plant Physiol* 128:1173–1179
- Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plants: from genes to invasion. *Science* 301:1377–1380
- Blair AC, Hanson BD, Brunk GR, Marrs RA, Westra P, Hufbauer RA (2005) New techniques and findings in the study of a candidate allelochemical implicated in invasion success. *Ecol Lett* 8:1039–1047
- Blossey B, Nötzold T (1995) Evolution of increased competitive ability in invasive non-indigenous plants: a hypothesis. *J Ecol* 83:887–889
- Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144:1–11
- Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* 290:521–523
- Callaway RM, Ridenour WM (2004) Novel weapons: a biochemically based hypothesis for invasive success and the evolution of increased competitive ability. *Front Ecol Environ* 2:436–433
- Cheremisinoff PN, Ellerbusch F (1978) Carbon adsorption handbook. Ann Arbor Science Publishers, Ann Arbor
- Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London
- Davis PH (ed) (1975) Flora of Turkey and the east Aegean Islands, vol. V. Edinburgh Press, Edinburgh, Scotland
- DiTomaso JM, Gerlach JD (2000) *Centaurea solstitialis* L. In: Bossard CC, Randall JM, Hoshovsky MC (eds) Private invasive plants of California's wildlands. University of California Press, Davis, pp 101–105
- DiTomaso JM, Kyser GB, Piroso CB (2003) Effect of light and density on yellow starthistle (*Centaurea solstitialis*) root growth and soil moisture use. *Weed Sci* 51:334–341
- Dukes JS (2001) Biodiversity and invasibility in grassland microcosms. *Oecologia* 126:563–568
- Dukes JS (2002) Species composition and diversity affect grassland susceptibility and response to invasion. *Ecol Appl* 12:602–617
- Elton CS (1958) The ecology of invasions by animals and plants. Methuen, London
- Enloe SF, DiTomaso JM, Orloff SB, Drake DJ (2004) Soil water dynamics differ among rangeland plant communities dominated by yellow starthistle (*Centaurea solstitialis*), annual grasses, or perennial grasses. *Weed Sci* 52:929–935
- Estabrook EM, Yoder JI (1998) Plant–plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. *Plant Physiol* 116:1–7
- Harper JL (1977) Population biology of plants. Academic, New York
- Hickman JC (1993) The Jepson manual: higher plants of California. University of California Press, Berkeley

- Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. *Plant Soil* 256:29–39
- Hierro JL, Villarreal D, Eren O, Graham JM, Callaway RM (2006) Disturbance facilitates invasion, but effects are stronger abroad than at home. *Am Nat* 168:144–156
- Hoaglands DR, Arnon DJ (1950) The water culture method of growing plants without soil. *Calif Agric Exp Stn Bull* 347: 1–39
- Maddox DM (1981) Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. USDA ARS ARR-W-20
- Maddox DM, Mayfield A, Poritz NH (1985) Distribution of yellow starthistle (*Centaurea solstitialis*) and Russian knapweed (*Centaurea repens*). *Weed Sci* 33:315–327
- Mahall BE, Callaway RM (1992) Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. *Ecology* 73:2145–2151
- Morghan KJR, Rice KJ (2006) Variation in resource availability changes the impact of invasive of invasive thistles on native bunchgrasses. *Ecol Appl* 16:528–539
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tissue culture. *Physiol Plant* 15:473–476
- Nilsson MC (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum*. *Oecologia* 98:1–7
- Perry LG, Johnson C, Alford ER, Vivanco JM, Paschke MW (2005a) Screening of grassland plants for restoration after spotted knapweed invasion. *Restor Ecol* 13:725–735
- Perry LG, Thelen GC, Ridenour WM, Weir TL, Callaway RM, Paschke MW, Vivanco JM (2005b) Dual role for an allelochemical: (±)-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *J Ecol* 93:1126–1135
- Pitcairn MJ, Woods DM, Joley DB, Popescu V (2002) Seven-year population buildup and combined impact of biological control insects on yellow starthistle. In: Woods DM (ed) Biological control program annual summary, 2001. California Department of Food and Agriculture, Plant Health and Pest Preservation Services, Sacramento, pp 57–59
- Ridenour WM, Callaway RM (2001) The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. *Oecologia* 126:444–450
- Roché CT, Thill DC (2001) Biology of common crupina and yellow starthistle, two Mediterranean winter annual invaders in western North America. *Weed Sci* 49:439–447
- Stermitz FR, Bais HP, Foderaro TA, Vivanco JM (2003) 7,8-Benzoflavone: a phytotoxin from root exudates of invasive Russian knapweed. *Phytochemistry* 64:493–497
- Stinson KA, Campbell SA, Powell JR, Wolfe BE, Callaway RM, Thelma GC, Hallett SG, Prati D, Klironomos JN (2006) Invasive plant suppresses the growth of native tree seedlings by disrupting below-ground mutualisms. *Public Lib Sci Biol* 4:e140. doi 10.1371/journal.pbio.0040140
- Swain T, Goldstein JL (1964) The quantitative analysis of phenolic compounds. In: Pridham JD (ed) *Methods in polyphenol chemistry*. Pergamon, Oxford, pp 131–146
- Thelen GC, Vivanco JM, Newingham B, Good W, Bais HP, Landres P, Caesar A, Callaway RM (2005) Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecol Lett* 8:209–217
- Thorpe AS (2006) Biochemical effects of *Centaurea maculosa* on soil nutrient cycles and plant communities. Ph.D. Dissertation, The University of Montana, Missoula
- Uyger S, Smith L, Uyger FW, Cristofaro M, Balciunas J (2004) Population densities of yellow starthistle (*Centaurea solstitialis*) in Turkey. *Weed Sci* 52:746–753
- Vivanco JM, Bais HP, Stermitz FR, Thelen GC, Callaway RM (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecol Lett* 7:285–292
- Walker TS, Bais HP, Halligan KM, Stermitz FR, Vivanco JM (2003) Metabolic profiling of root exudates of *Arabidopsis thaliana*. *J Agric Food Chem* 51:2548–2554
- Weir TL, Bais HP, Vivanco JM (2003) Intraspecific and interspecific interactions mediated by a phytotoxin, (±)-catechin, secreted by the roots of *Centaurea maculosa* (spotted knapweed). *J Chem Ecol* 29:2397–2412
- Weir TL, Bais HP, Stull VJ, Callaway RM, Thelen GC, Ridenour WM, Bhamidi S, Stermitz FR, Vivanco JM (2006) Oxalate contributes to the resistance of *Gaillardia grandiflora* and *Lupinus sericeus* to a phytotoxin produced by *Centaurea maculosa*. *Planta* 223:785–795