

REPORT

Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion

Jorge M. Vivanco,^{1,2*} Harsh P. Bais,¹ Frank R. Stermitz,³ Giles C. Thelen⁴ and Ragan M. Callaway⁴

¹Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, USA

²Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA

³Department of Chemistry, Colorado State University, Fort Collins, CO, USA

⁴Department of Biological Sciences, University of Montana, Missoula, MO, USA

*Correspondence: E-mail: jvivanco@lamar.colostate.edu

Abstract

Centaurea diffusa is one of the most destructive invasive weeds in the western USA and allelopathy appears to contribute to its invasiveness (Callaway & Aschehoug 2000). Here we identify a chemical from the root exudates of *C. diffusa*, 8-hydroxyquinoline, not previously reported as a natural product, and find that it varies biogeographically in its natural concentration and its effect as an allelochemical. 8-Hydroxyquinoline is at least three times more concentrated in *C. diffusa*-invaded North American soils than in this weed's native Eurasian soils and has stronger phytotoxic effects on grass species from North America than on grass species from Eurasia. Furthermore, experimental communities built from North American plant species are far more susceptible to invasion by *C. diffusa* than communities built from Eurasian species, regardless of the biogeographical origin of the soil biota. Sterilization of North American soils suppressed *C. diffusa* more than sterilization of Eurasian soils, indicating that North American soil biota may also promote invasion by *C. diffusa*. Eurasian plants and soil microbes may have evolved natural resistance to 8-hydroxyquinoline while North American plants have not, suggesting a remarkable potential for evolutionary compatibility and homeostasis among plants within natural communities and a mechanism by which exotic weeds destroy these communities.

Keywords

8-Hydroxyquinoline, allelopathy, biota, *Centaurea diffusa*, microbes, soil.

Ecology Letters (2004) 7: 285–292

INTRODUCTION

Many exotic and invasive plants are a major economic problem and a threat to ecological diversity throughout the world (Callaway 2002). Many invasive species are uncommon in their native range, but become very abundant in their new habitats (Louda *et al.* 1990). The remarkable success of many exotic and invasive plant species is thought to be due to a release from natural enemies (Darwin 1859; Maron & Vila 2001; Keane & Crawley 2002; Van der Putten 2002; Mitchell & Power 2003), which allows more effective resource competition (Pattison *et al.* 1998; Grotkopp *et al.* 2002), rather than to unique interactions in their new plant communities. Another non-mutually exclusive explanation for the success of invaders is the 'novel weapons' hypothesis, where mechanisms evolved elsewhere have stronger impacts than other plausible invasion hypothesis on native species in invaded communities (Baldwin 2003).

The chemical suppression of competing plant species through allelopathy has been classified as one of these mechanisms. Some plant species have been shown to release allelopathic chemicals into their surroundings that may have deleterious or even deadly effects on neighbouring plants (Whittaker & Feeney 1971; Callaway 2002), thus allowing the allelopathic plant less impeded access to natural resources.

A related group of invasive plants called knapweeds [*Centaurea maculosa* Lam., *C. diffusa* Lam., and *Acroptilon repens* (L.) DC] are some of the most destructive invasive plants in Western North America (Muir *et al.*, 1987; Roché 1994; Hirsch & Leitch 1996; Sheley *et al.* 1998). These knapweed species, introduced from Eurasia, are a major concern for land managers because of their ability to rapidly establish themselves in disturbed areas and displace native plant communities. As is true for most exotic invasive plants, knapweeds are not nearly as common in

their Eurasian native range where they co-exist more evenly with their neighbours. One species, *C. maculosa* Lam. (spotted knapweed), has been demonstrated to engage in allelopathy at the rhizosphere level, which most likely contributes to the invasiveness of this knapweed (Callaway & Aschehoug 2000; Bais *et al.* 2002). Since 1842, ecologists have believed that knapweeds have achieved a competitive advantage through the secretion of phytotoxic allelochemicals (Darwin 1859). However, no such chemical had been identified until our laboratories determined that an exudate of *C. maculosa* roots, identified as (-)-catechin, is indeed phytotoxic at concentrations found in the soil. Additionally, (-)-catechin inhibits seed germination and root growth of some North American species by triggering a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca^{2+} signalling cascade triggering genome-wide changes in gene expression and ultimately death of the root system (Bais *et al.* 2002, 2003a). Chemical and ecological observations suggest that other knapweeds may have similar allelochemical properties. We recently reported that *A. repens* (Russian knapweed) roots exude 7,8-benzoflavone (α -naphthoflavone) as a potent phytotoxin (Stermitz *et al.* 2003). *Centaurea diffusa* (diffuse knapweed), another noxious weed in North America (14, 16), has much stronger negative effects on grass species from North America than on closely related grass species from communities to which *Centaurea* is native (Callaway & Aschehoug 2000) and these effects could be mediated by chemicals exuded from its roots. Although a highly aggressive invader in North America, *C. diffusa*, like many invasive plants (Louda *et al.* 1990), is not as aggressive in its native habitat. Previously, a study of *C. diffusa*, which focused on a variety of possible allelochemicals (phenolic acids, sesquiterpene lactones), plant litter and root exudates found no evidence for allelopathy against native American grasses (Muir *et al.*, 1987).

In this communication, we expand our mechanistic knowledge of allelochemistry as a driving force in the success of some exotic invasive weeds by the identification of a new and potent phytotoxin from the root exudates of *C. diffusa*. We have unraveled the deleterious chemical ecological communication process mediated by *C. diffusa* in the rhizosphere, and provide evidence that novel allelochemistry in conjunction with soil microbial ecology contributes to this species' invasive behaviour. Furthermore, we provide evidence of the effect of allelochemistry on North American vs. European native plants. More importantly, we report that the allelochemicals from *C. maculosa* and *C. diffusa* are very different and do not belong to similar chemical groups, which argues for possible allelochemical specificity in plants.

METHODS

Plant material and culture conditions

Seeds of *C. diffusa*, *C. maculosa*, *Festuca idahoensis*, *Koeleria micrantha* and *A. repens* were obtained from natural populations in Larimer and Routt Counties, CO, USA, except for the *C. diffusa* seeds used in the native/foreign soil experiments, which are described below. A voucher specimen is held at the Herbarium, in Colorado State University, Fort Collins, CO, USA (ref. 211/2001).

Seeds of *Lithospermum esculentum* and *Triticum aestivum* were obtained from Quality Seeds (The Rocky Mountain Seed Co., Denver, CO, USA). Seeds of *Arabidopsis thaliana* (wild type Columbia) were obtained from Lehle Seeds. Seeds were washed in running tap water and were surface sterilized using sodium hypochlorite ($0.3\% \text{ v v}^{-1}$) for 10–15 min, followed by three to four washes in sterile distilled water. Surface sterilized seeds were inoculated on static MS (Murashige & Skoog 1962) basal media in Petri dishes for germination. Seeds were allowed to germinate for 10 days until roots and shoots emerged. The light intensity within the growth chamber was $4.4117 \text{ J m}^{-2} \text{ s}^{-1}$. Ten-day-old seedlings were transferred to 50 ml culture tubes with 5 ml of liquid MS basal media. Plant cultures were maintained on an orbital platform shaker set at 90 rpm (Lab-Line Instruments, Inc., Melrose Park, IL, USA).

Extraction

The root exudates were collected from the liquid media in which *C. diffusa* plants were grown after 30 days of culture, excess cell debris was filtered using a $0.45 \mu\text{m}$ syringe filter and were subsequently lyophilized, concentrated, and extracted using 5 ml of ethyl acetate (Fisher Co., Fairlawn NJ, USA). The extracts were vortexed and stored for 24 h at $4 \text{ }^\circ\text{C}$. The method for collection and processing of root exudates were adapted from Bais *et al.* (2002). The supernatant was transferred with a Pasteur pipette to a separate test tube, and 1 mL of ethyl acetate (Fisher Co.) was added. The supernatant was further concentrated by freeze-drying (Virtis, Genesis), and the weighed powder was re-dissolved in $500 \mu\text{l}$ of absolute methanol (Fisher Co.) for HPLC analyses. The extracts were centrifuged at 10 000 rpm (5600 g) for 10 min; supernatants were concentrated under vacuum and were re-suspended in $500 \mu\text{l}$ of ethyl acetate for HPLC analyses.

HPLC-MS analysis

Compounds in the root exudates were chromatographed by gradient elution on a reverse phase $5 \mu\text{m}$, C_{18} column (25 cm \times 4.6 mm) (Supelco Co., Bellefonte, PA, USA). The

chromatographic system used in this study is described elsewhere (Bais *et al.* 2002, 2003a). Mobile phase Solution A consisted of double distilled water and solution B (ethyl acetate) (Fisher Co.). A multi-step gradient was used for all separations with an initial injection volume of 15 μl and a flow rate of 1 ml min^{-1} . The multi-step 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. Different peaks eluted in a chromatographic profile were collected for the bioassay against various other invasive weeds and crop plants. Various peak eluants were concentrated under vacuum at 30 °C and further purified by injecting them back into HPLC under similar conditions and were collected at similar retentions. The eluant showing biological activity was dried under vacuum at 30 °C resulting in 2 mg of an amorphous powder. We checked whether this substance's occurrence could be ascribed to contamination by micro-organisms, but this was not found to be the case. The biological activity was detected in the whole fraction, but was missing in fractions collected before and after 41 min. The HPLC eluant passed through a UV detector with a flow rate of 0.25 ml min^{-1} was delivered into the electron spin mass spectrometer (ESI-MS) (Finnigan LQ Qizmo, Hewlett Packard 1100 series). The mass spectrometer (MS) parameters were optimized to maintain a high gas temperature (200 °C) and gas flow (50 psi). Ions were referred to both positive and negative splits. Scan ranges of 100–750 amu (milli absorbance units) were used for negative ions. A step size of 1 amu and dwell time of 1 ms was used during the analysis. The active eluant had m/z 145 ($\text{M}^+ - 1$), for $\text{C}_9\text{H}_7\text{NO}$.

Compound identification by NMR

The ^1H and ^{13}C NMR spectra of the HPLC-purified active exudate component from *in vitro* conditions were essentially identical to those of commercial (Sigma-Aldrich St. Louis, MO, USA) 8-hydroxyquinoline and literature values for the compounds (Pouchert & Behnke 1993).

Phytotoxic bioassay

Ten-day-old seedlings of *C. maculosa*, *C. diffusa*, *F. idahoensis*, *K. micrantha*, *A. repens*, *L. esculentum*, *T. aestivum*, and *A. thaliana* were placed on MS basal medium in Petri dishes after initial surface sterilization. Petri dishes were kept under a 16 h light and 8 h dark photoperiod in an incubator (Lab-Line Co.). Root exudates collected from 30-days old cultures of *C. diffusa* were subjected to autoclaving at 120 °C for 30 min at 15 lb pressure and were then administered in different concentrations (1–3 ml v v^{-1}) over the surface sterilized seedlings to analyse their phytotoxic effects. Autoclaving was performed to denature macromolecules

and to help identify the effect as that of a secondary metabolite. Arabidopsis was used to assess the phytotoxic minimum inhibitory concentration (MIC) of 8-hydroxyquinoline. Phytotoxicity of 8-hydroxyquinoline was determined as described elsewhere (Bais *et al.* 2002, 2003a). 8-Hydroxyquinoline used for the phytotoxicity assay on different plants including Arabidopsis was obtained from Sigma Chemical Co. (St. Louis, MO, USA). After incubation with different concentrations of 8-hydroxyquinoline, growth parameters such as length of shoots, number of shoots and length of primary root of the treated and untreated plants were measured, which were referred to as root and shoot differentiation in the figures. Ten plants per species were used for analysis of percentage root/shoot differentiation and germination inhibition rates.

Antimicrobial bioassay

Both fungal and bacterial isolates from a broad phylogenetic range were tested for inhibition of growth by 8-hydroxyquinoline. Inhibition of hyphal growth in *Aspergillus niger*, *Rhizoglyphus solani*, *Phytophthora infestans* and *Fusarium oxysporum* was tested by a linear growth assay. Fungal isolates were maintained on PDA in the dark at 24 °C. 8-Hydroxyquinoline was applied to sterile filter discs and allowed to air dry before being arranged in a circle on a 35 mm Petri dish. A 4-mm plug of fungal hyphae was placed in the centre of the Petri dish and inhibition was observed on a daily basis for 7 days. Each fungal isolate was tested against 8-hydroxyquinoline concentrations (10–250 $\mu\text{g ml}^{-1}$) in two separate replicates. Bacterial assays were performed in 96-well, sterile, flat bottom microtiter plates (Nalge Nunc International, Roskilde, Denmark). Bacterial suspension cultures of *Xanthomonas campestris*, *Pseudomonas syringae* pv. tomato DC3000, *Agrobacterium radiobacter*, *Erwinia carotovora* and *E. amylovora* were grown overnight at 37 °C to $\text{OD}_{600} = 0.2$. Test wells contained 5 μl of the tested bacteria in combination with varying amounts (10–250 $\mu\text{g ml}^{-1}$) of standard 8-hydroxyquinoline. Control wells contained 5 μl (*c.* 7.5×10^5 cells) of bacteria alone with the highest volume of methanol used. The plates were covered with sterile lids and placed in polystyrene boxes lined with moistened filter paper to maintain high humidity, and incubated at 37 °C. The absorbance of each well was determined at OD_{600} nm with an Opsys MR microtiter plate reader (Dynex Technologies, Chantilly, Virginia, USA). Net bacterial growth was calculated by subtracting the initial OD_{600} from the OD_{600} after 24 h of incubation. Percent inhibition (I%) was calculated using net bacterial growth based on OD_{600} readings with the following formula: $(\text{untreated-treated}/\text{untreated}) \times 100$. Data for antifungal assays were presented as follows: (–)–no inhibition of fungal growth, (+)–slight inhibition, (++)–weak inhibition, (+++)–moderate inhibi-

tion, (++++)-strong inhibition, and are the average of two separate experiments with two replicates in each treatment. In the table, each (+) represents 5 mm from the filter disc. (-) Depicts no fungal inhibition. All antifungal experiments were performed with standard, commercially available 8-hydroxyquinoline. Control discs contained the highest volume of methanol used for each treatment.

Soil and greenhouse experiments

We collected soil directly beneath five to 10 individual *C. diffusa* plants in each of four populations in The Republic of Georgia (Lisi Lake, near Tblisi: N41°44.389' E044°44.229'; Ksani: N 41°54.687' E044°34.065'; Okzolkara N41°40.892' E044°46.299'; and Rustavi N 41°34.540' E044°57.445') and from six to 20 individuals in each of three populations in the northwestern USA (The Dalles, OR N45°37.433' W121°12.841; Spokane, WA N47°36.996' W117°30.331; and Superior, MT N47°11.770' W114°52.960'). These soils were measured directly for 8-hydroxyquinoline concentrations using HPLC as described above. Seeds of *F. idaboensis*, *K. micrantha*, *Pseudoroegneria spicata*, and *Stipa comata* were collected in Missoula County, Montana, and seeds of *Agropyron cristatum*, *Melica transsilvanica*, and *Phleum nuniculatum* were collected near Tbilisi, Georgia from plants intermixed with *C. diffusa*. *C. diffusa* seeds were collected in Upper Kittitas County, Washington. Individual plants were grown from these seeds in 100 cm³ tubes for 6 months and then transplanted into 525 cm³ pots containing an 80:20 silica sand: Montana field soil mixture. Twenty days after the transplant we applied 250 µl of 5 mg ml⁻¹ of 8-hydroxyquinoline and ethyl acetate around the base of each grass and added the same concentration again 42 days after the first one. The grasses were harvested 105 days after transplanting. We constructed microcosms using soil and grass species described above. A 20 l buckets were filled with 20 of 30 grit sand, except for the top 5 cm, which we filled with a 1:1 combination of sand and soil. For each soil type we included both a sterilized and a non-sterilized treatment. Sterilization was a triple-autoclave procedure, where 4 l of soil were autoclaved for 2 h, then left to cool for 24 h, and then autoclaved and cooled two more times. Grasses endemic to each country were grown in native soil and foreign soil, with a sterilized and non-sterilized treatment for each, for a total of 52 replications. Treatment combinations did not have equal replication because the quantity of soil collected was inadequate in some cases. The soil added to an individual pot was not bulked by region or site; each bucket contained the soil from the rhizospheres of two individual *C. diffusa* plants and then this soil source was not used in any other replication. Therefore, each of the seven sites provided soil for two

buckets, but each bucket represented soil from different rhizospheres. With a replication of two per site we could not test for the effect of site, and we treated each of the seven sites as independent replicates. Four buckets, all in different treatment combinations, were removed from the analysis because of early grass mortality. A single individual of the larger grass species, *Agropyron* (Eurasia) and *Pseudoroegneria* (N.A.), was planted in the center of the buckets, with two individuals of each of the other three species from the regional communities planted in a circle around it, alternating the species, for a total of seven individual grasses and four species in each community. The grasses were allowed to establish for 136 days under aseptic conditions for sterilized soil treatments before the communities were seeded with 50 seeds of *C. diffusa*. This establishment period allowed for the development of 50–75% cover of aboveground grass tissue. After seeding, the invasions were allowed to proceed for another 186 days under aseptic conditions for sterilized soil treatments before harvesting the total biomass of all plants. Nutrients (Miracle-Gro Excel 0.34 g l⁻¹) were applied every 3 weeks, with each community receiving 400 ml each week. The total biomass of all *C. diffusa* plants was analysed using the region of grass origin, the region of soil origin, and the sterilization of the soil as fixed factors in a three way ANOVA with the total grass biomass as a covariate. The concentration of 8-hydroxyquinoline in the mesocosms was measured and compared among treatments using a three-way ANOVA using *C. diffusa* biomass as a covariate.

Addition of 8-hydroxyquinoline in North American natives rhizosphere

To compare the susceptibility of North American natives grown under natural conditions against 8-hydroxyquinoline, two North American natives' monocultures (*Artemisia tridentata* and *Achillea millefolium*) in *C. diffusa*-invaded regions were identified at Walker Ranch, Boulder County, CO, USA. 8-Hydroxyquinoline dissolved in methanol was added at the low concentration (*c.* 80 µg g⁻¹ soil) found in Eurasian soil to the rhizosphere of natural populations of *A. tridentata* and *A. millefolium*. Controls received only methanol. Similar concentration of 8-hydroxyquinoline (*c.* 80 µg g⁻¹ soil) was also added in the *C. diffusa*'s rhizosphere to evaluate percentage mortality. Ten plants per species were used for analysis of percentage mortality rates. The 8-hydroxyquinoline-treated natives along with the untreated controls were allowed to grow for 14 more days, and were subsequently photographed and analysed for mortality counts. To evaluate the proximal localization of 8-hydroxyquinoline in *C. diffusa*'s habitat, soil samplings was performed both horizontally (10–15 cm) and vertically in depth (10–40 cm) around the rosette and was further extracted and subjected

to HPLC analysis as described previously (Bais *et al.* 2002, 2003a).

RESULTS AND DISCUSSION

Root exudates of *in vitro*-grown *C. diffusa* plants were collected in sterile media (Bais *et al.* 2002, 2003a) and biologically active chemical constituents were assayed for their effect on the growth and germination efficiency of a suite of species, including the model plant *A. thaliana*, native North American species such as *F. idahoensis* and *K. micrantha*, crops such as wheat (*T. aestivum*) and tomato (*Lycopersicon esculentum*), and other invasive species such as *C. maculosa* (spotted knapweed) and *A. repens* (Russian knapweed) (Fig. 1a–c; Fig. S1). Root exudates were also tested against *C. diffusa* itself. All species except *C. diffusa* showed 80–100% mortality by the 14th day after addition of root exudates from *C. diffusa*. Plants showed wilting symptoms prior to senescence with reduced shoot and root differentiation (Fig. 1a, b). All species except *C. diffusa* showed reduced germination in response to *C. diffusa* root exudates (Fig. 1c).

Extracts of freeze-dried medium in which *C. diffusa* had been grown were then subjected to HPLC analysis and the collected fractions were used for a second phytotoxic bioassay. The only phytotoxic fraction of the exudate was found to be 8-hydroxyquinoline. 8-Hydroxyquinoline is a common, commercially available compound well known as an analytical reagent for its metal chelating properties and as a fungistat and antiseptic (The Merck Index 1996). Although a few simple 2- and 4-hydroxyquinolines are produced by microbes (Luckner 1984), 8-hydroxyquinoline does not appear to have previously been reported as a natural product. The quinoline ring system occurs as part of more complex plant alkaloid structures, where anthranilic acid has been identified as a biosynthetic precursor (Luckner 1984; Grundon 1988). 8-Hydroxyquinoline is not structurally related to (–)-catechin, an allelochemical previously isolated from *C. maculosa* root exudates (Bais *et al.* 2002, 2003a,b) or to 7,8-benzoflavone, a phytotoxin exuded from the roots of *A. repens* (Stermitz *et al.* 2003). These results suggest allelochemical specificity in the knapweed clade. If closely related species such as *C. diffusa* and *C. maculosa* have this diversity in their allelochemicals, the diversity in less closely related invasive plants (with allelochemical properties) could be even larger. Although 8-hydroxyquinoline was phytotoxic to all other plant species tested, including *C. maculosa*, *C. diffusa* itself was resistant to 8-hydroxyquinoline (Fig. S1).

8-Hydroxyquinoline also displayed strong antibacterial and antifungal activity against important plant pathogenic microbes such as *X. campestris*, *P. syringae*, *A. radiobacter*, *E. carotovora*, *E. amylovora* and fungi viz., *A. niger*, *R. solani*, *P. infestans* and *F. oxysporum* (Fig. S2, Table S1). Thus, it is

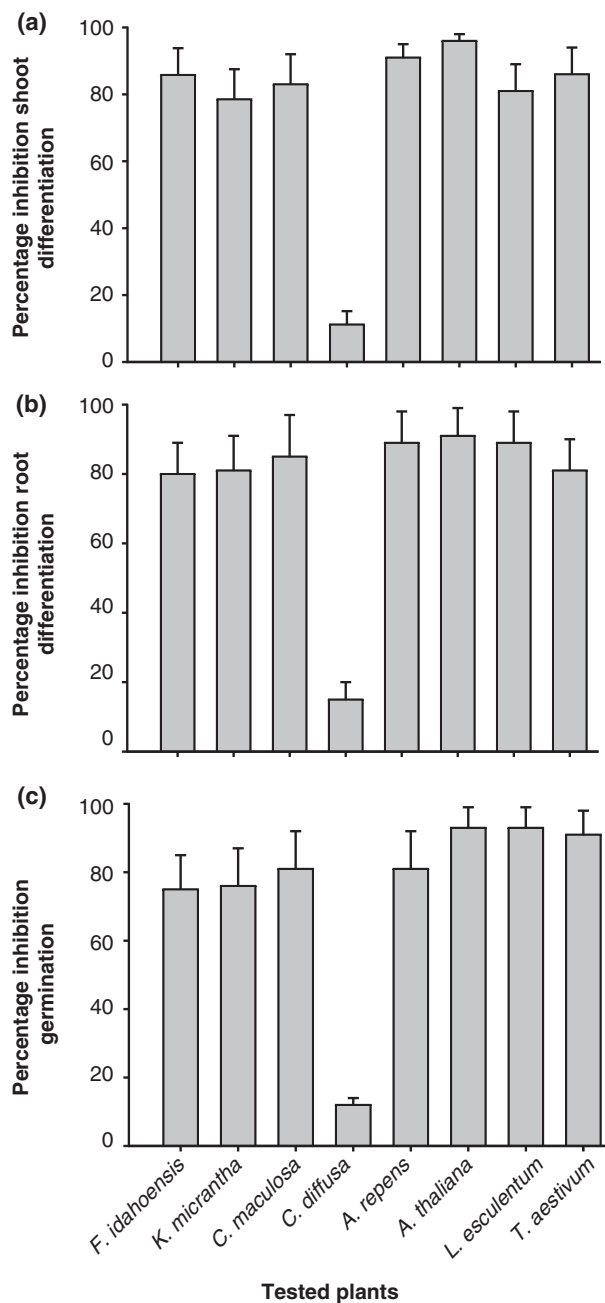


Figure 1 Effect of root exudates of *C. diffusa* (3 ml v v^{-1}) on shoot (a) and root (b) differentiation of 10-day-old *in vitro*-grown weeds and crop plants on the 14th day after treatment. The data represent the percent inhibition relative to the untreated control in shooting and rooting efficiency response in various tested seedlings (values are mean \pm SD, $n = 10$). (c) Effect of root exudates of *C. diffusa* (3 ml v v^{-1}) on percent inhibition in germination of different weeds and crop plants on the 14th day after treatment. The data represent the percent inhibition relative to the untreated control in shooting and rooting efficiency response in various tested seedlings (values are mean \pm SD, $n = 10$).

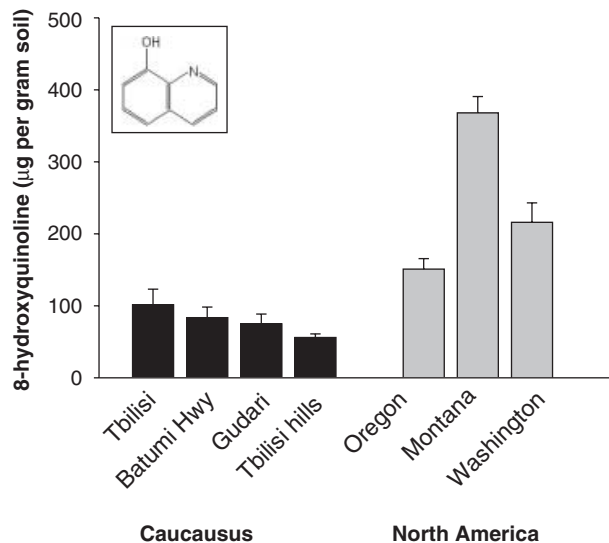


Figure 2 Concentrations of 8-hydroxyquinoline (chemical structure in the inset) in natural soils supporting populations of *Centaurea diffusa* in native habitats (the Caucasus) and in invaded habitats (North America). Each bar represents one local population and error bars show one SE. For statistical analysis sites were nested under region (Eurasia vs. NA) with site analysed as a random effect and region as a fixed effect; $F_{\text{continent}} = 7.72$, d.f. = 1,3, $P = 0.004$).

possible that *C. diffusa* benefits from this allelochemical both as a phytotoxin and antimicrobial.

8-Hydroxyquinoline was abundant in soil extracts from *C. diffusa*-invaded fields in North America, which validates our *in vitro* results and strongly supports the idea that *C. diffusa*'s invasiveness is in part due to the exudation of 8-hydroxyquinoline in the soil (Fig. 2; Fig. 1). Natural soil concentrations of 8-hydroxyquinoline were far higher than those that caused mortality in greenhouse experiments although the effects of the exudate might be much different in natural conditions. The concentration of 8-hydroxyquinoline the soil used in the greenhouse experiment was *c.* 40 µg g⁻¹ soil DW basis but soil from North American fields varied with the soil extract's proximity to a taproot of *C. diffusa* from 264.12 ± 21.2 µg g⁻¹ soil DW basis *c.* 10 cm from the taproot to 256.21 ± 12.5 µg g⁻¹ soil DW basis at 15 cm from the taproot. Concentrations of 8HQ varied with the depth of the soil sampled from 259.81 ± 14.8 µg g⁻¹ soil DW basis 10 cm from the topsoil vertically near the rosette, to 221.61 ± 12.4 µg g⁻¹ soil DW basis 40 cm below the rosette. Clearly, 8-hydroxyquinoline is naturally exuded, occurs in and is stable in the soil and, because of its phytotoxic and antimicrobial activity, may be a major factor in the invasive success of *C. diffusa*. To further investigate the potential role of this chemical in the invasion process we explored

biogeographical differences in its abundance and effects, and conducted more realistic ecological experiments.

The concentration of 8-hydroxyquinoline was over three times higher in soils supporting *C. diffusa* in North America than in similar soils in Eurasia (Fig. 2). This difference may have been due to greater exudation from North American plants, longer persistence in North American soils due to slower microbial breakdown (see below), or higher densities of *C. diffusa* in North American plant communities. However, these regional differences in 8-hydroxyquinoline were not the only possible explanation for *C. diffusa*'s exceptional success in North America. North American plant species were much more susceptible than Eurasian species to identical concentrations of 8-hydroxyquinoline added to field soil (Fig. 3a), supporting the hypothesis that the success of some exotic invasive plants may be due to competitive mechanisms that do not occur in the natural communities they invade and which disrupt inherent and co-evolved interactions among long-associated native species (Callaway & Aschehoug 2000).

We then tested how whole communities resist *C. diffusa*, and investigated the antibacterial and antifungal activity of 8-hydroxyquinoline in more realistic conditions by establishing microcosms in which we constructed North American and Eurasian plant communities in both North American and Eurasian soils (As described in methods). The regional source of the plant community was a highly significant factor in the resistance to *C. diffusa* (Fig. 3B, $F_{\text{region of plant origin}} = 8.209$, d.f. = 1,51, $P = 0.006$), with North American plant communities showing far less resistance than Eurasian communities. Importantly, sterilization of North American soils resulted in much stronger suppression of *C. diffusa* growth than sterilization of Eurasian soils, suggesting that differences in the soil microbial communities in Eurasia and North America may also play a major role in *C. diffusa* invasions (Fig. 3b, $F_{\text{soil origin} \times \text{soil sterilization}} = 4.863$, d.f. = 1,51, $P = 0.033$). One important difference in the function of the Eurasian and North American microbial communities may be how they break down or even utilize 8-hydroxyquinoline. Even when the biomass of *C. diffusa* was incorporated as a covariate (to account for large differences in the biomass of *C. diffusa* in different treatments), Eurasian soils accumulated much less 8-hydroxyquinoline than North American soils (Fig. 4). More importantly, sterilization of Eurasian soils resulted in a 134% decrease in 8-hydroxyquinoline concentrations, whereas sterilization of North American soils resulted in only a 41% increase ($F_{\text{soil origin} \times \text{soil sterilization}} = 23.798$, d.f. = 1,51, $P < 0.001$). These results suggest that microbes in Eurasian soils supporting populations of *C. diffusa* may be better adapted to 8-hydroxyquinoline and perhaps even use the exudate as an organic carbon source.

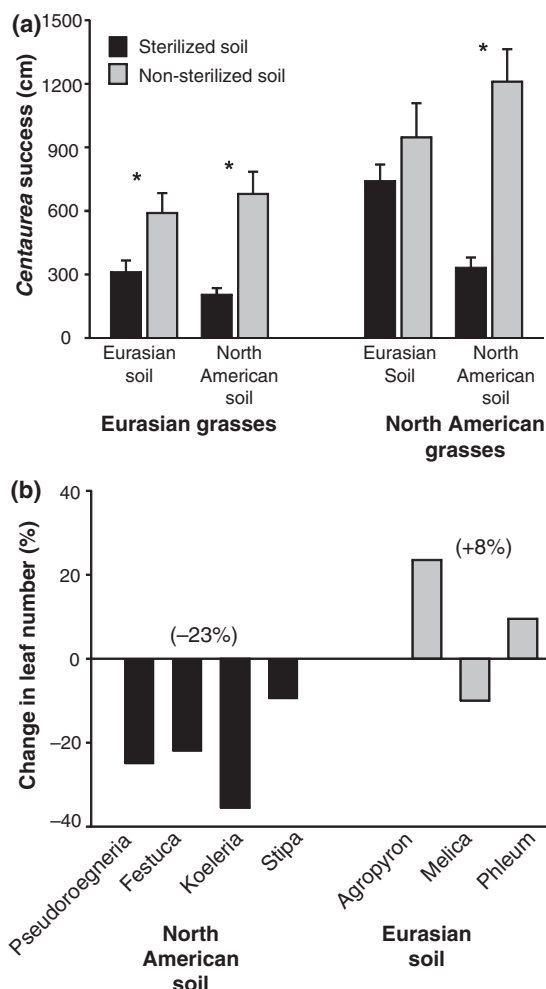


Figure 3 (a) Total biomass of *Centaurea diffusa* invading experimental microcosms (as described in Methods) containing plant communities from either the native or invaded region of *C. diffusa* (the Caucasus or North America, respectively), planted in substrate with soil inocula from the native or invaded regions, and with soil from each region either sterilized or unsterilized. Error bars show one SE. *Significant effects of sterilization. In a three-way ANOVA with all factors fixed $F_{\text{region of plant origin}} = 8.209$, d.f. = 1,51; $P = 0.006$; $F_{\text{region of soil origin}} = 1.721$, d.f. = 1,51, $P = 0.197$; $F_{\text{soil sterilization}} = 22.769$, d.f. = 1,51, $P < 0.0001$; $F_{\text{soil origin} \times \text{soil sterilization}} = 4.863$, d.f. = 1,51, $P = 0.033$; $F_{\text{grass origin} \times \text{soil sterilization}} = 7.421$, d.f. = 1,51, $P = 0.010$, no other significant interactions. Total grass biomass as covariate, $F = 1.243$, d.f. = 1,51, $P = 0.271$; there were no significant interactions between the covariate and treatments. (b) Proportional differences in the change in leaf number of grass species from the native region (Caucasus) vs. the invaded region (North America) of *C. diffusa* treated with 8-hydroxyquinoline, a chemical exuded from the roots of the invasive weed. In an ANOVA testing the effects of 8-hydroxyquinoline and using the region of grass origin, and grass species as fixed factors nested within region $F_{8\text{-hydroxyquinoline}} = 0.997$, d.f. = 1,3, $P = 0.320$; $F_{\text{region of grass origin}} = 17.997$, d.f. = 1,3, $P < 0.001$ (grasses from the Caucasus were larger); $F_{8\text{-hydroxyquinoline} \times \text{region}} = 8.69$, d.f. = 1,3, $P = 0.004$.

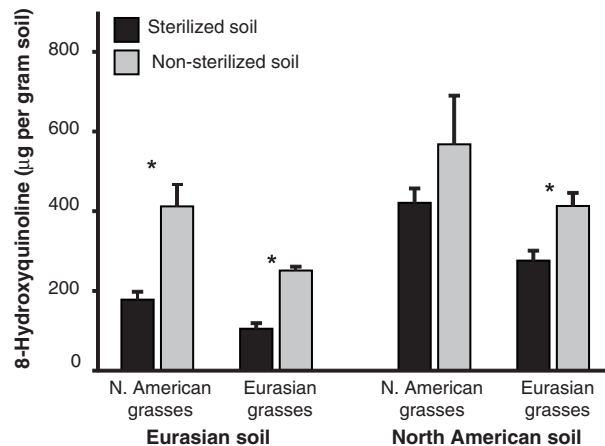


Figure 4 8-Hydroxyquinoline concentrations in experimental microcosms containing plant communities from either the native or invaded region of *C. diffusa* (the Caucasus or North America, respectively), planted in substrate with soil inoculum from the native or invaded regions, with soil from each region either sterilized or unsterilized, and invaded with *C. diffusa*. Error bars show one SE. *Significant effects of sterilization. In a three-way ANOVA with all factors fixed, $F_{\text{region of plant origin}} = 0.002$, d.f. = 1,51, $P = 0.963$; $F_{\text{region of soil origin}} = 73.206$, d.f. = 1,51, $P < 0.001$; $F_{\text{soil sterilization}} = 1.330$, d.f. = 1,51, $P = 0.255$; $F_{\text{soil origin} \times \text{soil sterilization}} = 23.798$, d.f. = 1,51, $P < 0.001$; no other significant interactions. Total *C. diffusa* biomass as covariate, $F = 4.134$, d.f. = 1,51, $P = 0.048$.

To address the effects of root-secreted 8-hydroxyquinoline on naturally growing North American natives in fully realistic field conditions, we added a low concentration of 8-hydroxyquinoline (*c.* $80 \mu\text{g g}^{-1}$ soil, the concentration found in the soil of Eurasian *C. diffusa* communities) to the rhizosphere of populations of two North American natives growing naturally in the field (*A. tridentata* and *A. millefolium*) (as described in methods). Similar to the findings of our greenhouse experiments, the field results clearly indicated that North American plant species were susceptible (*c.* 90% mortality) to low concentrations of 8-hydroxyquinoline (Fig. S3). However, this concentration of 8-hydroxyquinoline did not affect Eurasian species under greenhouse conditions (Fig. 3a).

Our results have several important implications for community ecology. First, the indication that plant species may be selected to tolerate specific characteristics of their neighbours suggests that natural plant communities may be less individualistic than generally thought. Second, our results imply that natural biological communities may evolve in some functional manner (Goodnight 1990; Wilson 1997), and third some exotic invasive plants may use aggressive mechanisms that are not present in the natural communities they invade to disrupt inherent, co-evolved functions.

ACKNOWLEDGEMENTS

This work was supported by grants from Colorado State University Agricultural Experiment Station (to J.M.V. and F.R.S.), NSF-CAREER (MCB 0093014 to J.M.V.), Invasive Weeds Initiative of the State of Colorado (to J.M.V. and F.R.S.) and USDA-WRIPM (2003–05060 to J.M.V.) and NSF (DEB-9726829 to R.M.C.), Andrew W. Mellon Foundation (R.M.C.), and USDA-NRI (2003–02433 to R.M.C. and J.M.V.).

SUPPLEMENTARY MATERIAL

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/ELE/ELE576/ELE576sm.htm>

Figure S1 Phytotoxic effect of 8-hydroxyquinoline on seedlings of *C. maclosa* and *A. repens* on 14th day.

Figure S2 Antibacterial activity of 8-hydroxyquinoline on different bacterial pathogens.

Figure S3 Effect of low concentration of 8-hydroxyquinoline found in Eurasian soil applied to the rhizosphere of natural populations of two North American natives (*Artemisia tridentata* and *A. millefolium*), and *C. diffusa*.

Table S1 Antifungal activity of 8-hydroxyquinoline against various plant pathogens.

REFERENCES

- Bais, H.P., Walker, T.S., Stermitz, F.R., Hufbauer, R.A. & Vivanco, J.M. (2002). Enantiomeric-dependent phytotoxic and antimicrobial activity of (\pm)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol.*, 128, 1173–1179.
- Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. & Vivanco, J.M. (2003a). Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, 301, 1377–1380.
- Bais, H.P., Walker, T.S., Kennan, A.J., Stermitz, F.R. & Vivanco, J.M. (2003b). Structure-dependent phytotoxicity of catechins and other flavonoids: flavanoid conversions by cell-free protein extracts of *Centaurea maculosa* (spotted knapweed) roots. *J. Agri. Food. Chem.*, 51, 897–901.
- Baldwin, T. (2003). Finally, proof of weapons of mass destruction. *Science* STKE 2003, 42.
- Callaway, R.M. (2002). The detection of neighbors by plants. *Trends Ecol. Evol.*, 17, 104–105
- Callaway, R.M. & Aschehoug, A.T. (2000). Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science*, 290, 521–523.
- Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection*. John Murray, London.
- Goodnight, C.J. (1990). Experimental studies of community evolution. 2. The ecological basis of the response to community selection. *Evolution*, 44, 1625–1636.
- Grotkopp, E., Rejmanek, M. & Rost, R.L. (2002). Toward a causal explanation of plant invasiveness: seedling growth and life-history strategies of 29 pine (*Pinus*) species. *Am. Nat.*, 159, 396–419.
- Grundon, M.F. (1988). Quinoline, acridone and quinazoline alkaloids: chemistry, biosynthesis, and biological properties. *Alkaloids: Chemical and Biological Perspectives*, 6, 339–345.
- Hirsch, S.A. & Leitch, J.A. (1996). *The Impact of Knapweed on Montana's Economy*. North Dakota State University, Fargo. Agricultural Econ. Rep. No. 355. 43. p.
- Keane, R.M. & Crawley, C.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends Ecol. Evol.*, 17, 164–170.
- Louda, S.M., Keeler, K.M. & Holt, R.D. (1990). Herbivore influences on plant performance and competitive interactions. In: *Perspectives on Plant Competition* (eds Grace, J.B. & Tilman, D.). Academic Press, Elsevier, Amsterdam, pp. 414–444.
- Luckner, M. (1984). *Secondary Metabolism in Microorganisms, Plants, and Animals*. Springer Verlag, Berlin.
- Maron, J.L. & Vila, M. (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, 95, 361–373.
- Mitchell, C.E. & Power, A.G. (2003). Release of invasive plants from fungal and viral pathogens. *Nature*, 421, 625–627.
- Muir, D., Muir, A.D., Maja, K.W., Balza, F. & Towers, G.H.N. (1987). A search for the allelopathic agents in diffuse knapweed. In: *Allelochemicals: Role in Agriculture and Forestry. ACS Symposium Series 330* (ed. Waller, G.R.). American Chemical Society, Washington DC, pp. 238–246.
- Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 18, 473–497.
- Pattison, R.R., Goldstein, G. & Ares, A. (1998). Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. *Oecologia*, 117, 449–459.
- Pouchert, C.J. & Behnke, J. (1993). *The Aldrich Library of ¹³C and ¹H FT NMR Spectra*, 1st edn, Vol. 1–3. Aldrich Chemical Co., Milwaukee, WI.
- Roché, B.F., Jr (1994). Status of knapweeds in Washington. *Wash. State Univ. Coop. Ext. Serv. Knapweed Newslett.*, 8, 2–4.
- Sheley, R.L., Jacobs, J. & Carpinelli, M.F. (1998). Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). *Weed Tech.*, 12, 353–362.
- Stermitz, F.R., Bais, H.P., Foderaro, T.A. & Vivanco, J.M. (2003). 7,8-Benzoflavone: a phytotoxin from root exudates of invasive Russian knapweed. *Phytochemistry*, 64, 493–497.
- The Merck Index (1996). *8-Hydroxyquinoline*. Merck & Co. Inc., Whitehouse, NJ, USA, pp. 4890.
- Van der Putten, W.H. (2002). How to be invasive. *Nature*, 417, 32–33.
- Whittaker, R.H. & Feeney, P.P. (1971). Allelochemicals – chemical interactions between species. *Science*, 171, 757–770.
- Wilson, D.F. (1997). Biological Communities as Functionally Organized Units. *Ecology*, 78, 2018–2031.

Editor, Mark Schwartz

Manuscript received 17 November 2004

First decision made 23 December 2004

Manuscript accepted 12 January 2004