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NO EVIDENCE FOR TRADE-OFFS: *CENTAUREA* PLANTS FROM AMERICA ARE BETTER COMPETITORS AND DEFENDERS

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Abstract. The natural enemies hypothesis has led to a number of ideas by which invaders might evolve superior competitive ability. In this context, we compared growth, reproduction, competitive effect, competitive response, and defense capabilities between invasive North American populations of *Centaurea maculosa* and populations in Europe, where the species is native. We found that *Centaurea* from North America were larger than plants from European populations. North American *Centaurea* also demonstrated stronger competitive effects and responses than European *Centaurea*. However, competitive superiority did not come at a cost to herbivore defense. North American plants were much better defended against generalist insect herbivores and slightly better defended against specialists. North Americans showed a stronger inhibitory effect on the consumers (resistance) and a better ability to regrow after attack by herbivores (tolerance). Better defense by North Americans corresponded with higher constitutive levels of a biochemical defense compound precursor, tougher leaves, and more leaf trichomes than Europeans. North American F₁ progeny of field collected lines retained the traits of larger size and greater leaf toughness suggesting that genetic differences, rather than maternal effects, may be the cause of intercontinental differences, but these sample sizes were small. Our results suggest that the evolution of increased competitive ability may not always be driven by physiological trade-offs between the allocation of energy or resources to growth or to defense. Instead, we hypothesize that *Centaurea maculosa* experiences strong directional selection on novel competitive and defense traits in its new range.

Key words: allelopathy; *Centaurea maculosa*; competition; defense; EICA, evolution of increased competitive ability; herbivory; invasion; novel weapons.

INTRODUCTION

One of ecology's most perplexing questions is why human introduction of some plant species to new regions results in large increases in their abundance and competitive effects. The "natural enemies" hypothesis posits that exotic invaders explode in abundance because they are no longer suppressed by the specialist herbivore consumers they evolved with (Maron and Vilá 2001). Janzen (1975) hypothesized that populations freed from herbivory may lose adaptations to resist or tolerate herbivory over time. This idea has led to a number of hypotheses for how the competitive ability of invaders might evolve. Blossey and Nötzold (1995) proposed that such a release from natural enemies and loss of defense capabilities would allow plants to reallocate energy and resources from "defensive weapons" to growth—the "evolution of increased competitive ability" (EICA)—and would therefore make these new genotypes more competitive than their predecessors in the native range. Bossdorf et al. (2004) hypothesized that

if competition were less in the invaded range and competitive ability involves traits with a fitness cost, then selection might act against competitive ability: the "evolutionary reduced competitive ability" or ERCA hypothesis. Alternatively, if invaders have an "allelopathic advantage against resident species" (the AARS hypothesis, described later) then stronger allelopathic effects may be selected for in invaded ranges (Callaway and Ridenour 2004).

Studies have shown that exotic genotypes can be larger or more reproductive than the genotypes from which they appear to have originated (Schierenbeck et al. 1994), and some experiments have demonstrated one or both predictions of the EICA hypothesis (Siemann and Rogers 2001, 2003, Maron et al. 2004, Wolfe et al. 2004), whereas other studies of invasive plant species have not found evidence for changes in defense or growth (Vilá et al. 2003, Bossdorf et al. 2004). Bossdorf et al. (2005) suggested that comparisons of growth and defense characteristics among native and introduced populations are limited by focusing on defense and growth, and recommended that other characteristics be measured. For example, only two studies have explicitly and experimentally addressed competition (see Vilá and Weiner 2004). Leger and Rice (2003) found that individuals of *Eschscholzia californica* from the species'

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invasive range in Chile grew larger and produced more flowers than native individuals when released from competition from other plants. Bossdorf et al. (2004) measured competition between invasive and native genotypes of *Alliaria petiolata*, but did not measure competition with other species. However, no study has reported direct measurements of the competitive effects and competitive responses of an invasive plant comparing populations from the native and invaded range.

The “novel weapons” hypothesis posits that exotic invaders explode in abundance because invaders possess unique biochemicals that are more effective against evolutionarily naïve plants, microbes, and generalist herbivores than against those species that have evolved tolerance in their communities of origin (Rabotnov 1982, Callaway and Aschehoug 2000, Mallik and Pelliser 2000, Callaway and Ridenour 2004, Vivanco et al. 2004, Callaway et al. 2005, Cappuccino and Arnason 2006, Thorpe 2006, Callaway et al. 2008). If invaders possess competitive traits such as allelochemicals that provide greater competitive advantages in their new habitats than in their regions of origin, or “defensive weapons” such as unique defense compounds, then selection pressure for the traits conferring competitive advantages may be much greater for invasive genotypes than conspecific genotypes remaining at home: the AARS hypothesis (Callaway and Ridenour 2004). These evolutionary implications suggest that invasive genotypes may evolve superior competitive or defense ability without costs to other ecological functions.

There is evidence that *Centaurea maculosa* Lam. (spotted knapweed, Asteraceae, recently suggested to be *C. stoebe micranthos* L.), one of North America’s most devastating European invaders, possesses biochemicals that may function in a way predicted by the novel weapons hypothesis (species classification information available online).⁵ *Centaurea* exudes the compound (\pm)-catechin, a biochemical “offensive weapon,” from its roots (Bais et al. 2003, Weir 2003, Blair et al. 2005), which inhibits the growth of neighboring competing plants in laboratory experiments (Bais et al. 2003, Weir et al. 2003, 2006, Callaway et al. 2005, Perry et al. 2005a, b, D’Abrosca et al. 2006; Simões et al., *in press*) and can be phytotoxic in soils within the range of naturally occurring concentrations (Thelen et al. 2005, Thorpe 2006; Inderjit et al., *in press*; Perry et al., *in press*; J. Pollock, unpublished data). However, other research indicates that natural soil concentrations rarely if ever reach phytotoxic levels (Blair et al. 2005, 2006). Importantly, (\pm)-catechin and *Centaurea* may be more allelopathic to North American species than congeneric European species (Bais et al. 2003, Thorpe 2006; W. He and R. M. Callaway, unpublished data). In addition to a potential novel offensive weapon, glandular trichomes covering the epidermal surfaces of *C. maculosa* produce

cnicin, a biologically active sesquiterpene lactone which may function as a novel defensive weapon and appears to only occur in some *Centaurea* species (Olson and Kelsey 1997).

Because *Centaurea maculosa* is a strong invader (Lesica and Shelley 1996) and appears to be allelopathic, we compared the growth, reproduction, and competitive effect and response of invasive North American populations of *Centaurea* to populations from Europe, where the species is native. We also examined the effects of North American and European populations of *Centaurea* on specialist and generalist herbivores and the response of the plants to herbivory.

METHODS

We conducted a series of comparative greenhouse experiments using North American and European populations of *Centaurea*. Abiotic conditions were controlled so that growing conditions were identical for plants from both regions, thus allowing us to test for genetic variation among North American and European populations and rule out phenotypic plasticity (Reznick and Ghalambor 2001). All experiments except a comparison of (\pm)-catechin exudation were conducted at the University of Montana (Missoula, Montana, USA) in two different greenhouses. Each individual experiment was conducted in a single greenhouse. In this section we describe the general growing conditions for these experiments. For both greenhouses temperatures were between 15° and 30°C, corresponding with natural early summer temperatures. Natural light in the greenhouses was supplemented by metal halide bulbs, and total photosynthetically active radiation (PAR) during the day remained above 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. All plants were grown in 2.4-L pots (18 cm diameter and 22 cm depth), and with the exception of the soil comparison experiment we used a mixture of 20% local grassland soil (Missoula, Montana, USA; Haploxerolls and Argixerolls) and 80% 20/30 grit silica sand. These soils have a pH of 6.2 and contain approximately 7% organic matter. We chose this soil mix to be consistent with a number of previous experiments, to facilitate harvest of the fragile fine-root systems of *C. maculosa*, and to provide microbiota from the invaded range. Plants in our experiments were watered approximately every other day until the soil was saturated, and fertilized with 500 mL of Miracle-Gro at 0.34 g/L every two to three weeks. Over the course of a 200-day experiment (a typical length) this provided approximately 225 mg of total N, 450 mg of available P (P_2O_5), and 225 mg of potash (K_2O) to each pot. In all experiments, plants from different populations and regions were randomly spatially arranged on benches and rotated periodically. In all experiments in which two plants were in the same pot, they were planted so that the central stems were 10 cm apart. We conducted greenhouse rather than common garden experiments to avoid introducing novel European genotypes (via pollen and seeds) to North

⁵ (<http://plants.usda.gov/java/profile?symbol=CESTM>)

America. Consequently, our design does not take into account conditionality in the responses of North American and European populations, as demonstrated by Maron et al. (2004) for *Hypericum perforatum* grown in multiple common gardens in different parts of the world. We collected seeds from 22 European *Centaurea* populations and 23 North American *Centaurea* populations over the period of time we conducted these experiments, but the number of populations used in a single experiment ranged from 11 to 14 in the original growth and competition experiment to five and five in one of the herbivore experiments (Appendix A, Table 1). For each experiment populations were chosen randomly from the available pool; however the available pool of populations varied as seeds from new populations were acquired and as we ran out of seeds for some populations as experiments progressed. For each population (with the exception of three European populations, denoted in Appendix A, for which we did not know the collection protocol) we collected 300–3000 seeds from 30–100 parent plants. Seeds from a single population were mixed and selected randomly for each experiment.

Statistical analysis

We took the same two-step approach to analyzing data collected in different greenhouse experiments for plant biomass, flower head production, and most measurements of insect growth response and effect on plants. First, we used nested ANOVA to explore how trait variation differed among populations and between continents of population origin. We performed these tests using the PROC GLM module within SAS using Type III sum of squares (SAS version 9.1, SAS Institute, Cary, North Carolina, USA), where continent of population origin (Europe vs. North America) was treated as a fixed factor and population nested within continent was treated as a random factor. Second, we performed ANCOVA (in SPSS 11.5; SPSS, Chicago, Illinois, USA) on population means, using continent of population origin as a fixed factor and latitude as a covariate. Since preliminary analyses revealed no continent \times latitude interaction for any trait, this term was not used in any further analyses. In a few cases we used other independent variables as covariates in the basic model described above, and these cases are described in the section relevant to the particular dependent variable tested.

There were a few cases in which there was no replication within populations (germination, competitive effects, and responses) or this replication was reduced to levels that precluded analysis of variation within populations. The statistical analyses of these cases are discussed in the specific methods sections below. Finally, transformation of most data was not required to meet ANOVA assumptions, but germination and all data for leaf number or proportional responses to herbivores were log-transformed.

Germination, growth, fitness, and competitive ability

To measure germination rates we planted *Centaurea* seeds from eight European populations and eight North American populations (chosen randomly from available populations, Table 1) in flats in the greenhouse on 8 May 2004. Germination was recorded over three weeks.

In a second experiment, we compared the growth, flower head number, and the competitive effects of North American and European *Centaurea* on two native grass species and the competitive response of North American and European *Centaurea* to native grasses. This experiment used 14 European populations and 11 North American populations chosen randomly from those available (Table 1). On 16 April 2004, *Centaurea* seeds from both regions were planted either alone or with *Pseudoroegneria spicata* Pursh., or *Festuca idahoensis* Elmer, two common native species in the Northern Rocky Mountains. For each *Centaurea* population from each continent, eight individuals were grown in pots with *Pseudoroegneria*, and eight individuals from each *Centaurea* population were grown in pots with *Festuca*. The biomass and flower head production of these *Centaurea* plants grown with native grasses was compared to that of eight other individual *Centaurea* plants from each of the same populations grown alone. Twenty individuals of *Pseudoroegneria* and *Festuca* were grown alone and the mean total biomass of these individuals was compared to the mean total biomass of the *Pseudoroegneria* and *Festuca* individuals grown with *Centaurea* to determine the competitive effect of the invader. At the end of the experiment, 27 October 2004, after 193 days, the total aboveground and belowground biomass of all *Centaurea*, *Pseudoroegneria*, and *Festuca* was harvested, dried at 100°C for three days, and weighed. Flower heads on all *Centaurea* plants were also counted at this time. Approximately 65% of *Centaurea* plants produced flower heads during the experiment, and only these plants were used for the analyses of flower heads.

Continental differences in biomass, flower head number, and photosynthesis were analyzed with the nested ANOVA procedure described above. However, continental differences in germination were tested with a single ANOVA (in SPSS 11.5) with seed age as a covariate (dates of collection differed), and population means as independent samples. To calculate competitive responses of *Centaurea*, the mean biomass of plants from a particular population grown in competition was calculated as a percentage of the biomass of plants from the same population grown alone. Similarly, the competitive effect of *Centaurea* was measured as the mean population effect on each of the two native grasses as a percentage of the biomass of the grasses attained when grown alone. Therefore, these measurements provide no replication at the population level and differences between continents were determined with a *t* test on the population means from each continent (in SPSS 11.5).



PLATE 1. First author, Wendy Ridenour, collecting insects for laboratory experiments. Photo credit: Thomas Bassett.

European populations of *C. maculosa* can be either tetraploid or diploid (H. Müller-Schärer, *personal communication*), and we did not know the ploidy of our original populations. Therefore, in a later experiment we used seeds provided from H. Müller-Schärer and U. Trier at Fribourg University of eight tetraploid European populations and nine tetraploid North American populations not used in any other experiment described here. We grew these to maturity in the conditions described above for the other experiments for 170 days and then measured the diameter of the rosettes. Diameter was compared between regions and among populations statistically as described in *Statistical analysis*.

Biogeographic differences in the effects of herbivores

The following experiments were designed to compare the effects of North American and European populations of *Centaurea* on herbivore survival, growth, and maturation rates, and the response of North American and European populations of *Centaurea* to herbivory. We experimented with consumers that we have observed on *Centaurea* in the field in sites in western Montana (see Plate 1). These included two specialist root herbivores (both from Europe), one of which is also a shoot herbivore as an adult, and two generalist shoot

herbivores (one naturalized in North America from Europe and one from North America).

The specialist herbivores *Cyphocleonus achates* (Fahraeus) (Insecta: Coleoptera: Curculionidae) and *Agapeta zoegana* (Linnaeus) (Insecta: Lepidoptera: Cochylidae) have been introduced as biocontrols for *Centaurea* and exist now in patchy populations throughout much of the weed's range. The European root boring weevil, *C. achates*, was first released in the United States in 1987. *Cyphocleonus* is a large, (approximately 20 mm long) brown-gray mottled, flightless weevil. *Agapeta zoegana*, a moth with root boring larvae from Europe, was first released in the United States in 1984. The larvae of *Cyphocleonus* and *Agapeta* overwinter in the roots of *Centaurea* and do substantial physical damage to the roots. Adult *Cyphocleonus* eat the leaves of *Centaurea*.

The naturalized generalist herbivore *Trichoplusia ni* (Hubner) (Insecta: Lepidoptera: Noctuidae) is not used as a biocontrol for *Centaurea*; it occurs at very low abundances on the weed in natural communities (T. Bassett and R. M. Callaway, *unpublished data*), but in experimental conditions larvae can do substantial damage to the leaves of *Centaurea* (Callaway et al. 1999). Larvae measure approximately 3–4 cm in length at maturity. The native generalist herbivore *Melanoplus sanguinipes* (Fabricius) (Insecta: Orthoptera: Acrididae), the lesser migratory grasshopper, is a broad generalist

native to North America. *Melanoplus* has been periodically observed on *Centaurea* stems in the field (W. M. Ridenour, *personal observation*). We incorporated generalists into our experiments to test hypotheses suggested by Müller-Schärer et al. (2004). They proposed that the most important change in herbivory experienced by introduced plant species is a “shift in the composition in the enemy complex towards an assemblage dominated by generalists” and argued that this may result in differential selection on plant resistance traits.

Prior to introduction of insects in each experiment, all *Centaurea* plants were grown in the general conditions described above and were exposed to direct sunlight outside of the greenhouse in order to induce the production of flavonoids and other plant secondary metabolites.

Cyphocleonus achaetes.—On 21 April 2004, *C. maculosa* was started from seed for this herbivore treatment. On 15 August 2004, two adult *Cyphocleonus* weevils were introduced to each of 50 2.4-L pots, each pot containing one European and one North American *Centaurea*. As the plants matured during the experiment, the leaves of the two individuals consistently intermixed. We used eight populations of *Centaurea* from each continent for this experiment (Table 1), chosen randomly from the total set of available populations, and then each pair was established through random choices from the North American and European populations. For these 50 paired combinations, specific pairs of populations were only used once. Thus we did not use every possible combination of the 16 populations but pairs were evenly and objectively spread among possible combinations. Prior to adding *Cyphocleonus*, all *Centaurea* plant basal rosette diameters were measured in order to evaluate initial size as a potential determinant of herbivore choice. Adult *Cyphocleonus* were obtained from “Weedbusters” of Missoula, Montana. To keep the flightless *Cyphocleonus* weevils in their pots, circular cages were constructed of 30 cm tall sheets of clear mylar. The cages were open at the top, but the upper 3 cm of mylar was covered with a thin layer of petroleum jelly which was too slippery for *Cyphocleonus* to climb. Adult *Cyphocleonus* were allowed to selectively graze on their choice of European or North American *Centaurea* leaves and to lay their eggs in their choice of hosts until 21 October 2004, when all adult weevils were removed (183 days after planting, and 67 days after applying *Cyphocleonus*). At this time, all leaves of each *Centaurea* plant were counted and each leaf was categorized into the following classes: (1) no sign of herbivory, (2) damage to the leaf but portions of blade remaining, and (3) leaf blade totally consumed, only leaf rachis remaining. On 28 October 2004, one week following adult *Cyphocleonus* removal, all *Centaurea* leaves, damaged and undamaged, were counted again as a measure of compensatory response, or “tolerance” (Strauss and Agrawal 1999, Willis et al. 1999, Müller-Schärer et al. 2004) as recommended by Bossdorf et al.

(2005). *Cyphocleonus* larvae were allowed to grow and mature within *Centaurea* taproots until 11 November 2004 (205 days after planting), when plants were harvested. Taproots were then dissected using 5–30 \times magnification and all *Cyphocleonus* larvae were collected. The number of *Cyphocleonus* larvae within each *Centaurea* taproot was recorded, and each *Cyphocleonus* larva was weighed, measured in length, and preserved in a 90% ethanol solution. *Centaurea* plants were dried at 100°C for three days and weighed.

Centaurea plants from the same populations used for the paired experiment were also planted alone ($n = 50$ for each continent) to assess the performance of *Cyphocleonus*. Plants were grown in the same conditions as in the paired experiment, and on 14 November 2004, the number of *Cyphocleonus* larvae within each *Centaurea* taproot was recorded, larvae were weighed and measured in length, and preserved in a solution of 90% ethanol. However, not enough *Cyphocleonus* larvae developed and survived to test the effect of populations nested within regions, and therefore these results were tested with a *t* test for the effect of region.

Agapeta zoegana.—We collected *Agapeta* moths in the field, within 5 km of Missoula, Montana, from 12 to 14 September 2004. On 15 May 2004, 50 2.4-L pots were prepared so that each contained one European *Centaurea* and one North American *Centaurea*. We used the same populations and the same pairs of populations in this experiment as in the paired *Cyphocleonus* experiment (Table 1). On 17 September 2004 (125 days after planting) small pieces (<1 cm²) of paper containing two to three *Agapeta* eggs from moths collected in the field were cut out by hand and pinned precisely in the middle between the European and North American *Centaurea* plants (see Thelen et al. 2005). As in the *Cyphocleonus* experiment, *Centaurea* plant basal rosette diameter was measured immediately prior to the introduction of *Agapeta* eggs to each pot so the effect of plant size on herbivore choice could be evaluated. *Agapeta* larvae were allowed to grow and mature within *Centaurea* taproots for over two months. On 7 December 2004 (206 days after planting) *Agapeta* larvae were weighed, measured in length, and preserved in a solution of 90% ethanol. *Agapeta* preferentially attack plants with larger taproots (Story et al. 2000), but *Centaurea* root caudex diameter was not a significant covariate.

At the same time the *Centaurea* plants were planted in pairs, individuals from the same populations were planted alone ($n = 50$ for each continent) to assess the performance of *Agapeta*. Plants were grown in the same conditions as in the paired experiment, and on 8 December 2004, the number of *Agapeta* larvae within each *Centaurea* taproot was recorded; larvae were weighed and measured in length, and preserved in a solution of 90% ethanol.

Trichoplusia ni.—We obtained *Trichoplusia* eggs from the New York State Agricultural Experiment Station in Geneva, New York. Eggs hatched 19 September 2004,

TABLE 1. Summary of experimental results for *Centaurea maculosa*.

Experiment	European and North American replication	Plant growth	Plant reproduction	Insect development
Growth	14, 11	NA↑	NA↓	
Competition	11, 11	NA↑	NA↑	
<i>Cyphocleonus</i>	8, 8	NA↑		NA↓
<i>Agapeta</i>	8, 8	NA↑		NA↓
<i>Trichoplusia</i>	8, 8			NA↓
<i>Melanoplus</i>	5, 5			NA↓
Maternal effects	4, 5	NA↑		

Notes: Replication shows the number of populations used from each region, respectively. Upward-pointing arrows indicate that plants from North American (NA) populations grew larger, produced more flower heads, or inhibited insect growth more than the European populations; downward arrows indicate the reverse. For growth, the results for two of the four herbivory experiments are presented in Appendix D. The four species in column 1 are insect herbivores with which experiments were conducted. Empty cells indicate that no information is available.

and on that day one caterpillar was introduced to each of 30 2.4-L pots containing pairs of European and North American *Centaurea*, and 40 2.4-L pots containing a single European ($n = 20$) or North American ($n = 20$) *Centaurea* plant. These plants were started from seed on 8 May 2004. We used eight populations from each continent for this experiment (Appendix A, Table 1), and these were chosen randomly from the total set of populations available. Each pair was established through random choices from the North American and European populations. For these paired combinations, specific pairs of populations were only used once. The paired *Trichoplusia* caterpillars were kept in their pots with cages made of transparent mesh, "bridal veil" fabric, and this mesh was also applied to *Centaurea* without insects as a methodological control. In the paired experiment, *Trichoplusia* could select a European or North American *Centaurea*, and this experiment was used to quantify host choice. In the single-host-plant experiment, we measured *Trichoplusia* growth rate, final mass prior to pupation, pupation rate, and adult emergence. Each caterpillar was weighed daily until pupation. Once pupation was no longer occurring (42 days after application and 184 days after planting), all leaves of each *Centaurea* plant (paired and single) were counted and placed into the following classes: (1) no sign of herbivory; (2) damage to the leaf but portions of blade remaining; and (3) leaf blade totally consumed, with only the leaf rachis remaining.

We statistically analyzed the damage done to *Centaurea* plants by *Trichoplusia* and the final mass and growth rate of *Trichoplusia* caterpillars using the nested ANOVA procedure. Differences in proportion emerged and proportion pupated among *Trichoplusia* on *Centaurea* from different regions were compared by fitting nontransformed survival numbers to log-normal distribution curves and testing the whole model and paired treatments with chi-square analyses. We did not include population as a factor in these analyses.

Melanoplus sanguinipes.—Beginning on 3 December 2004 we grew individual *Centaurea* plants from seed from five different populations of European *Centaurea*

and five different populations of North American *Centaurea* (Table 1, chosen randomly from available populations). On 17 February 2005 *Centaurea* root caudex diameters were measured using calipers as an initial metric of plant performance, prior to herbivore application. We used 10 replicates of each population for the herbivore treatment and another 10 replicates for controls, for a total of 100 plants per region.

On 18 February 2005 (71 days after planting), third-instar *Melanoplus* were obtained from Stefan Jaronski at the USDA Agricultural Research Station (ARS) in Sidney, Montana and on 19 February 2005 these insects were introduced to *Centaurea* plants. Two *Melanoplus* were applied to each of the 100 *Centaurea* plants. Pots were covered with "bridal veil" fabric. All *Melanoplus* treatment pots were monitored daily for insect mortality. Initially, *Melanoplus* mortality was high (possibly due to stress related to shipping), and on 23 February 2005 all dead *Melanoplus* were removed from pots, and herbivore density was adjusted (either dead insects replaced with live ones or insects removed) so that each *Melanoplus* treatment pot contained one insect. At this time, all *Melanoplus* were weighed, body length measured, and current instar recorded for initial pretreatment metrics. Over the next 30 days, insect body length, mortality, and instar were measured four more times, and the experiment ended on 22 March 2005.

We analyzed the effects of North American and European *Centaurea* on mass and length growth rates of *Melanoplus* using the nested ANOVA procedure. However, differences in the numbers of *Melanoplus* at the fourth instar, fifth instar, or adult stage were tested using chi-square analysis.

Biogeographic differences in defense traits and plant nutrition

We measured leaf chemistry and toughness (see Coley 1983) for all European and North American *Centaurea* used in the *Agapeta* experiment, described above. On 29 November 2004, one week prior to *Agapeta* larval harvest, one leaf that showed no signs of physical damage or discoloration from each *Centaurea* plant was

removed, and preserved in plastic tubes on dry ice until analysis of volatile herbivore defense compounds was completed. Sample leaves were ground with ethyl acetate (1 mL/0.1 g sample mass) at room temperature, and then transferred to a glass tube. The glass tube was shaken for 10 min at 200 rpm [2550 m/s²], and centrifuged for 10 min (2000 rpm). Supernatant fluids were filtrated (Superclean Envi-carb tubes; SUPELCO, Bellefonte, Pennsylvania, USA) and 1 μ L of the supernatant was analyzed using a gas chromatograph/mass spectrometer (Hewlett-Packard 5890 series II, Avondale, Pennsylvania, USA) equipped with a 30 m \times 0.25 mm capillary column (DB-5.625; J&W Scientific, Folsom, California, USA) with helium as the carrier gas. The initial oven temperature was maintained at 0°C for eight minutes by cryogenic cooling. The oven temperature was increased to 70°C at rate of 7°C/min, and then to the final temperature of 300°C at the rate of 20°C/min, which was maintained for 10.5 min. The injection port temperature was 250°C, and the helium carrier gas linear velocity was maintained at 35 cm/s with automated pressure control. Detection was achieved by mass-selective detection (Hewlett-Packard 5972) in the scan mode (mass to charge ratio: 33–500). The chemical structures were identified by using a Wiley 138-K mass spectral database (John Wiley and Sons, New York, New York, USA), and by comparing the mass spectra and the retention time with those of authentic chemical samples. Quantitative analysis is possible by measuring the relative “ion intensities” produced by the mass spectra (see Arimura et al. 2000).

On 6 December 2004, immediately prior to *Agapeta* larval harvest, two healthy leaves from each *Centaurea* plant were also analyzed for leaf toughness (a potential anti-herbivore defense mechanism) using a penetrometer (John Chatillon and Sons, Scales and Force Measuring Instruments, New York, New York, USA), which measures the grams of force required to punch through a particular leaf. Punches were taken 1 cm from the leaf tip and equally distant from the mid-vein and the edge of the leaf.

We conducted an additional greenhouse experiment to ascertain if leaf nutrition and trichomes play a role in differences between the defense capabilities of North American and European *Centaurea*. We measured leaf trichome density and leaf carbon and nitrogen concentration on 11 North American populations and 11 European populations of *Centaurea*. On 20 December 2005 we planted six seeds per population in each of 10 2.4-L pots (220 total pots) and several weeks later thinned the germinants to one individual per pot. Our final replication was 49 North American individuals from 10 populations and 50 European individuals from nine populations. On 13–15 March 2006 (85–87 days after planting) leaf trichome density was measured on two apparently healthy leaves taken from each plant. We collected one disc, 28.3 mm² in area, from each of the two leaves with a metal paper punch, with discs collected 1

cm from the leaf tip and equally distant from the mid-vein and the edge of the leaf. Trichome density was counted at 5–30 \times magnification. All plants were then dried at 60°C for three days, weighed, and the shoots were ground and prepared for carbon and nitrogen content analysis. Carbon and nitrogen content analysis was measured using a CE Instruments EA 1100 CHNS-O Elemental Analyzer (CE Instruments, Wigan, UK).

Catechin production

We measured *Centaurea* catechin production in four populations of European *Centaurea* and 11 populations of North American *Centaurea*. Replication was limited to four populations for European *Centaurea* because we could not successfully sterilize the seeds from many of the populations we possessed, and therefore could not rule out microbial contaminants as a source of catechin. Results from these populations are not included here. For each population we initially processed five replications, consisting of three individuals grown in 3 mL of Murashige and Skoog (1962) medium (MS). However, due to poor germination the final replication for three of the populations was reduced to three. Fifteen-day-old *Centaurea* plants were grown in 50-mL glass tubes containing 3 mL sterile MS medium on an orbital platform shaker for seven days. After seven days, the medium was collected, filtered through Whatman filter paper to remove debris, and extracted in a separating funnel three times using an equal volume of acidified ethyl acetate (1% acetic acid). Samples were concentrated under N₂, and resuspended in 500 μ L methanol. Methanol extracts were chromatographed (High Performance Liquid Chromatography, Dionex Summit System, Sunnyvale, California, USA) on a reverse-phase 5- μ m, C₁₈ column (25 cm \times 4.6 mm) (Supelco) using a multistep gradient. The absorbance at the reference wavelength, λ_{max} , 280 nm was measured by a PDA-100 Photodiode array variable UV/VIS detector (Dionex Company). Mobile phase solution A consisted of double-distilled water containing 0.1% acetic acid and solution B consisted of ACS grade methanol (Fisher Scientific, Pittsburgh, Pennsylvania, USA). A multistep gradient was used for all separations with an initial injection volume of 20 μ L and a flow rate of 1 mL/min. The multistep gradient was as follows: 0–5 min 5.0% B, 5–10 min 20.0% B, 15–20 min 20.0% B, 20–40 min 80.0% B, 40–60 min 100% B, 60–70 min 100% B, 70–80 min 5.0% B. In each sample, (\pm)-catechin concentrations were determined by comparison to 20- μ L injections from a 1 mg/mL catechin standard stock.

Maternal effects

Eight *Centaurea* plants from each of the same eight European populations and eight North American populations used in the *Cyphocleonus*, *Agapeta*, and *Trichoplusia* herbivore treatment experiments were grown to maturity and allowed to flower. Seeds were planted on 9 May 2005. Pollen was transferred among

all plants within a population in three sessions between 6 and 8 November 2005, 180 days later. In each session, this was done by touching the stamens on a single flower on a randomly chosen plant with a size-7 watercolor paint brush (Rhapsody, Raleigh, North Carolina, USA) and then touching the paintbrush to a single flower on another randomly chosen plant, and doing this consecutively until 10 flowers on each of the eight plants had been touched. Flower heads were collected and counted, and their seeds were removed and counted. However, we were only able to obtain viable seeds from four European and five North American populations, and only 2–19 seeds per population. On 21 December 2005 all of these maternal line seeds were planted individually in the greenhouse in 2.4-L pots to determine if two of the more salient biogeographic trait differences, total plant mass and leaf toughness, could be explained by maternal effects rather than genetically based population differences. On 15 March 2006, two leaves that showed no sign of damage or discoloration from each maternal line *Centaurea* plant were analyzed for leaf toughness. On 16 March 2006 (85 days after planting) all plants were harvested, dried at 100°C for three days, and weighed (in grams).

Soil effects

An important limitation of our experimental design was that we added only North American soils to the sand used in the growth, competition, and herbivore experiments. Our intention was to provide AM fungi and other soil biota so that *C. maculosa* was grown in a more ecologically realistic environment (Callaway et al. 2004). Only later did we realize that if North American populations had adapted in some way to North American soil biota we could have biased our results. Therefore we conducted an experiment in which we selected six North American and six European populations (for which we had collected soil and seeds from the same sites) and grew each population in soil from its native home site and in soil from a randomly selected soil from the nonnative range. We used the same mix of 20% soil:80% sand. The idea was to determine if North American plants benefit more than European plants from North American soil. For each of the six populations we began with 16 replicates but after mortality the total was $n = 170$ for the experiment, with 12–16 replicates for each North American population and 10–16 for each European population.

RESULTS

Germination, growth, fitness, and competitive ability

The mean germination rate of *Centaurea* from North American populations was 81% higher than that of European populations (Appendix C, ANOVA with log-transformed data; age [covariate], $F_{1,15} = 1.55$, $P = 0.236$; region, $F_{1,15} = 8.90$, $P = 0.011$). The age of the collected seed did not affect germination.

In a second experiment with plants grown alone, the total biomass of *Centaurea* plants from North American populations averaged 30% greater than that of European populations (Fig. 1A; ANOVA table in Appendix B, region, $P > 0.001$). The average flower head number of European populations tended to be higher than that of North Americans but the effect of region was not significant ($P = 0.065$; Fig. 2A; ANOVA table in Appendix B).

Competition with *Pseudoroegneria* reduced the mass of plants from North American populations by 25%, but reduced the mass of plants from Europe by 58% (Fig. 1B). The flower head production of North American plants was reduced by less than 40% by competition, whereas European flower head production was reduced by over 80% (Fig. 2B).

When competing against *C. maculosa* plants from North American populations, both *Pseudoroegneria* and *Festuca* had significantly less total mass than when they were grown in competition with *Centaurea* from European populations (Fig. 3).

For the comparison of tetraploid populations from North America and Europe, the mean diameter of the rosette for North American plants was 33.3 ± 1.0 cm (mean \pm SE; $n = 30$ individuals) vs. 20.9 ± 1.0 cm ($n = 30$ individuals) for European plants (in an ANOVA with region as a fixed variable and population as a random variable: region, $F = 38.38$; $df = 1, 7$, $P < 0.001$; population, $F = 1.78$, $df = 8, 43$, $P = 0.235$).

Plant–herbivore interactions

Cyphocleonus achates.—In the experiment with paired plants, *Cyphocleonus* adults preferentially grazed upon European *Centaurea* leaves, heavily damaging (consuming the entire leaf down to the rachis) more than twice as many leaves of European *Centaurea* plants as leaves of North American plants (Fig. 4A, ANOVA table in Appendix B). Similar numbers of *Cyphocleonus* larvae were discovered in the taproots of European and North American plants in this experiment (12 vs. 13, respectively).

In the second experiment with solitary *Centaurea* plants, individual larvae consuming taproots of European *Centaurea* plants (123.4 ± 17.6 mg; mean \pm SE) were 57% larger than those recovered from North American plants (78.4 ± 12.7). However, not enough *Cyphocleonus* larvae developed and survived to test the effect of populations nested within regions, and therefore these results were tested with a *t*-test for the effect of region (region $t = 2.11$, $df = 1, 23$, $P = 0.045$).

For solitary *Centaurea* plants, plant mortality in the *Cyphocleonus* treatment was high, with 66% of all experimental plants in both experiments dying by the end of the experiment. However, nearly twice as many North American *Centaurea* plants survived *Cyphocleonus* herbivory as European plants (data not shown). One week after removing *Cyphocleonus* adults, North American plants had produced 5.7 ± 0.3 new leaves per plant

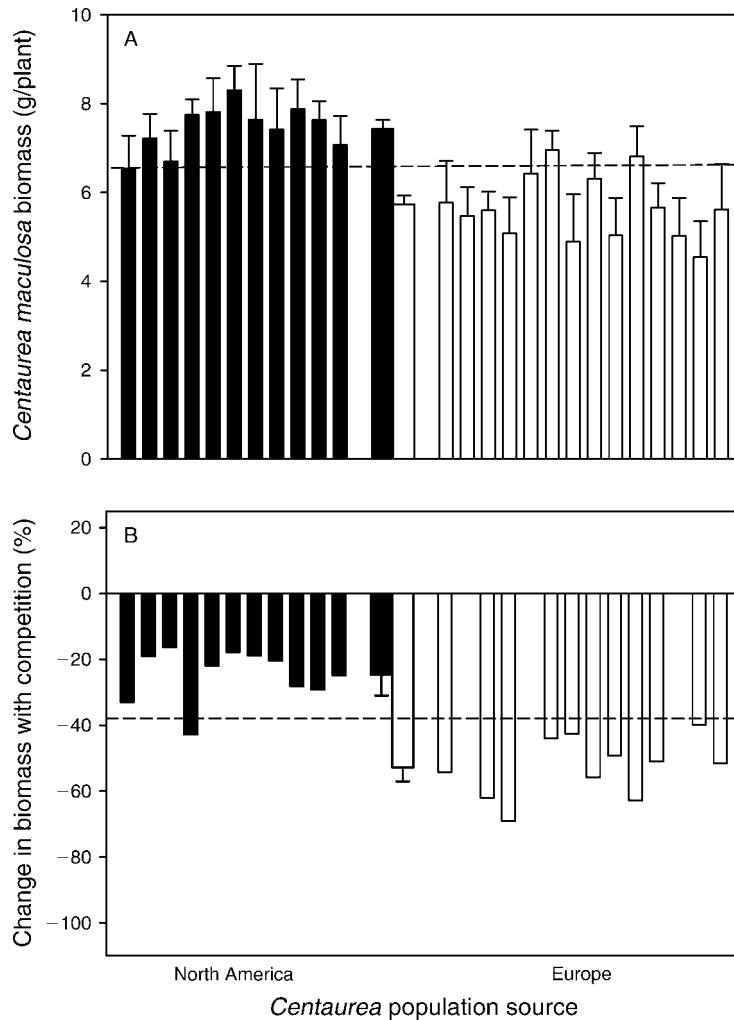


FIG. 1. (A) Total biomass of *Centaurea maculosa* from North American and European populations without competition. Narrow bars depict means and SE for each population; two thicker bars in the center are means and SE for each region using the means of each population as replicates. The dashed line shows the mean for all populations. Results from ANOVA: region, $F_{1,23} = 43.79$, $P < 0.001$; population, $F_{23,145} = 0.86$, $P = 0.657$ (ANOVA table is in Appendix B). (B) The response of *Centaurea* to competition from the native grass *Pseudoroegneria spicata* as the percentage decrease in biomass. The mean for each population is presented in the narrow bars; means and SE for each region are presented in the two thicker bars in the center of the figure. The dashed line shows the mean for all populations. In a t test with populations as independent samples, region $t_{1,21} = 7.70$, $P < 0.001$. Populations are matched in panels A and B, but there are fewer populations in panel B because mortality with competition reduced the sample size in some European populations to one or two individuals.

vs. 4.0 ± 0.5 for European plants (ANOVA with larval mass as a covariate; region $F_{1,14} = 13.95$, $P < 0.001$; population $F_{14,69} = 0.91$, $df = 7, 47$, $P = 0.512$). Of those plants living at the end of the experiment, North American *Centaurea* mass was 35% larger than that of European plants (Appendix D, Fig. 2B).

Agapeta zoegana.—Prior to application of insect treatments in the paired plant experiments, basal rosette diameter of North American plants was 14% larger than European plants and North American caudex diameters were nearly twice as large as those of European plants. But even though North American plants were larger, and *Agapeta* prefers to infest larger plants (Story et al. 2000), *Agapeta* larvae preferentially chose European

plants in the paired plant experiment (Fig. 5). However, in the experiment with *Centaurea* planted alone, the total mass of *Agapeta* larvae per individual *Centaurea* plant did not differ significantly among North American and European *Centaurea* populations (Fig. 5; region, $F_{1,59} = 2.22$, $P = 0.1498$, Appendix B). The total mass of North American *Centaurea* plants in the paired-plant experiment was 30% greater than that of European plants (Appendix D, Fig. 2A).

Trichoplusia ni.—When reared on paired European and North American *Centaurea* plants, *Trichoplusia* severely damaged (left nothing but the leaf rachis) European *Centaurea* leaves five times more frequently than North American leaves (data not shown; ANOVA

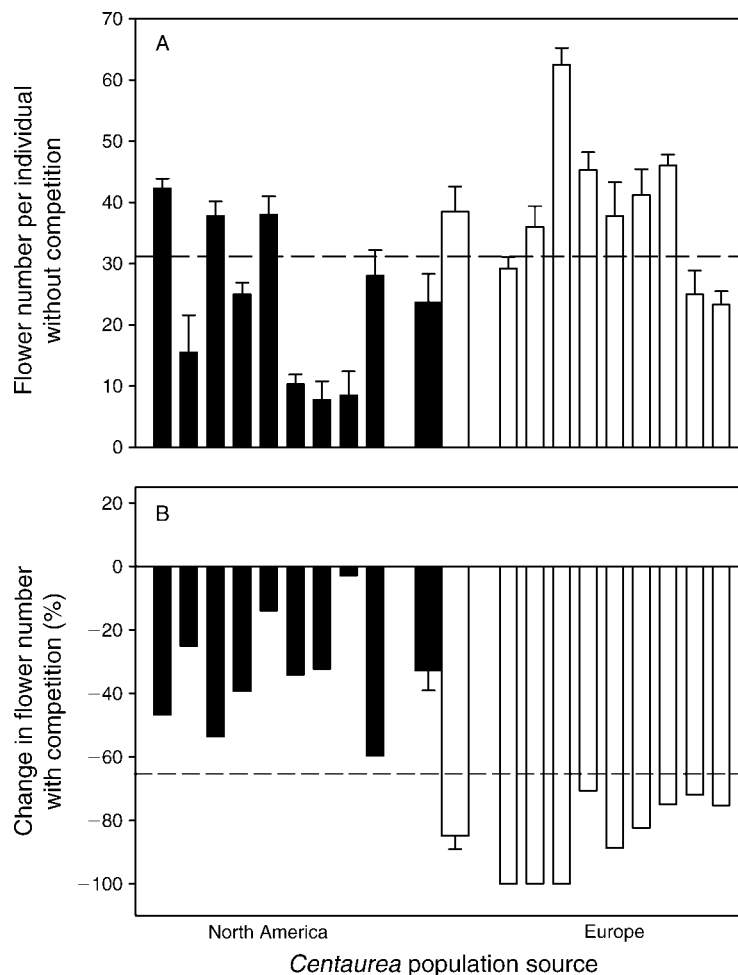


FIG. 2. (A) Flower head production of *Centaurea maculosa* from North American and European populations. The narrow bars show means and SE for each population; the two thicker bars in the center of the figure show means and SE for each region using the means of each population as replicates. The dashed line shows the mean for all populations. Results from ANOVA (full table is in Appendix B): region, $F_{1,16} = 3.91$, $P = 0.0652$; population, $F_{16,114} = 5.68$, $P < 0.001$. (B) The response of *Centaurea* to competition from *Pseudoroegneria* as the percentage decrease in flower head production. The mean for each population is presented in the narrow bars; means and SE for each region are presented in the two thicker bars in the center of the figure. In a t test with populations as independent samples, $t_{1,17} = 9.44$, $P < 0.001$. Populations are matched in panels A and B.

region, $F_{1,15} = 9.22$, $P < 0.001$; population $F_{1,15} = 5.35$, $P = 0.007$, Appendix B). *Trichoplusia* preferentially grazed upon European *Centaurea*, heavily damaging 12% of the leaves of European *Centaurea* compared to only 1% of North American *Centaurea* leaves (Fig. 4B). For North American plants, 20% more leaves remained completely undamaged by these generalist herbivores than leaves on European plants (Fig. 4B).

Trichoplusia caterpillars fed only European *Centaurea* grew more than twice as fast as caterpillars feeding on North American conspecifics (Fig. 6A; Appendix B). Moreover, *Trichoplusia* caterpillars averaged 28% larger final mass prior to pupation when consuming European *Centaurea* leaves (Fig. 6B). All *Trichoplusia* caterpillars pupated by 26 days on European *Centaurea*, whereas only 70% of those reared on North American plants had pupated by 26 days (Fig. 6C). All *Trichoplusia* reared on

European plants emerged as adult moths by 35 days, but only 40% of those reared on North American plants had emerged as adults by the 35th day (Fig. 6D).

Melanoplus sanguinipes.—Mortality of the native North American generalist, *Melanoplus*, was much higher than that of the naturalized European generalist, *Trichoplusia*, but mortality was similar for *Melanoplus* reared on North American and European populations in isolated plant experiments (data not shown). However, *Melanoplus* grew 127% faster in length (Fig. 7A) and acquired mass 156% faster when feeding on European plants than on North American plants (data not shown, ANOVA table in Appendix B). Moreover, 56% more *Melanoplus* reached maturity prior to senescence when feeding on European plants than when feeding on North American *Centaurea* (Fig. 7B; $\chi^2 = 13.2$, $df = 6$, $P < 0.05$).

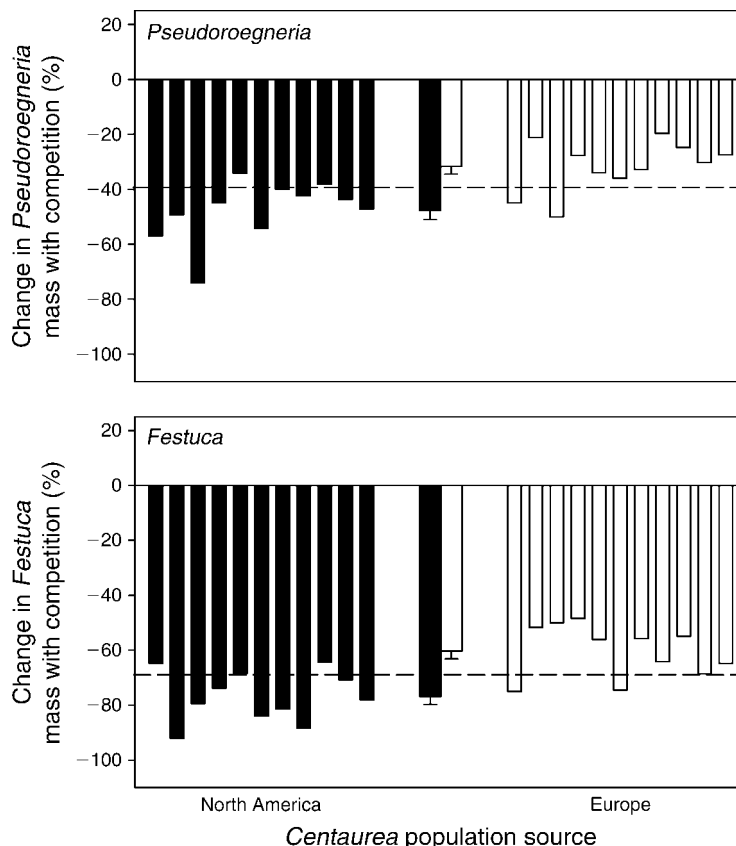


FIG. 3. The response of two native North American grasses, *Pseudoroegneria spicata* and *Festuca idahoensis*, to competition from *Centaurea*, as the percentage decrease in grass biomass when grown with *Centaurea* vs. alone. The mean for each population is presented in the narrow bars, and means and SE for each region are presented in the two thicker bars in the center of the panels. The dashed lines show the means for all populations. In *t* tests with populations as independent samples, for *Pseudoroegneria*, region $t_{1,21} = 16.04$, $P = 0.001$; for *Festuca*, region $t_{1,21} = 13.50$, $P = 0.002$.

Mechanisms of Centaurea resistance to herbivores

Leaves on plants from North American populations contained approximately two times higher concentrations of the defense compound precursor, phytol, in their leaves than European populations (Fig. 8A). For another defense compound precursor, germacrene D, the trend was the same but there was no significant difference for plants among regions. North American *Centaurea* leaves were also 166% tougher when tested with a leaf penetrometer than European conspecifics (Fig. 8B). North American *Centaurea* leaves had 42% more glandular trichomes than leaves on European plants (Fig. 8C). Leaves from European *Centaurea* had significantly higher percent N than North American *Centaurea* (Fig. 8D; region, $F = 6.21$, $P = 0.0214$), but leaf C:N ratios were almost identical between biogeographic regions (data not shown).

Biogeographic differences in Centaurea catechin production

Mean (\pm)-catechin concentrations in solutions containing North American *Centaurea* seedlings was $42 \pm$

$12 \mu\text{g/mL}$ (mean \pm SE), compared to $24 \pm 0.09 \mu\text{g/mL}$ in solutions containing European conspecifics, but there was no significant difference between regions (ANOVA; region, $F_{1,11} = 3.18$, $P = 0.1002$; population, $F_{11,46} = 1.98$, $P = 0.0527$).

Maternal effects

For F_1 lines, the average total biomass of North American *Centaurea* was 152% greater than the average total biomass of European *Centaurea* (Fig. 9; Appendix B). Leaf toughness followed the same pattern. The average leaf toughness of North American *Centaurea* was 81% greater than the average leaf toughness of European *Centaurea*. There was no overlap in the means of any North American and European population for either variable.

Soil effects

Again we found that plants from North American populations were larger (0.53 ± 0.18 g per plant vs. 0.38 ± 0.015 ; region $F < 0.001$), but all populations grew best in European soil, suggesting that European soil was more fertile. Moreover, there was no interaction

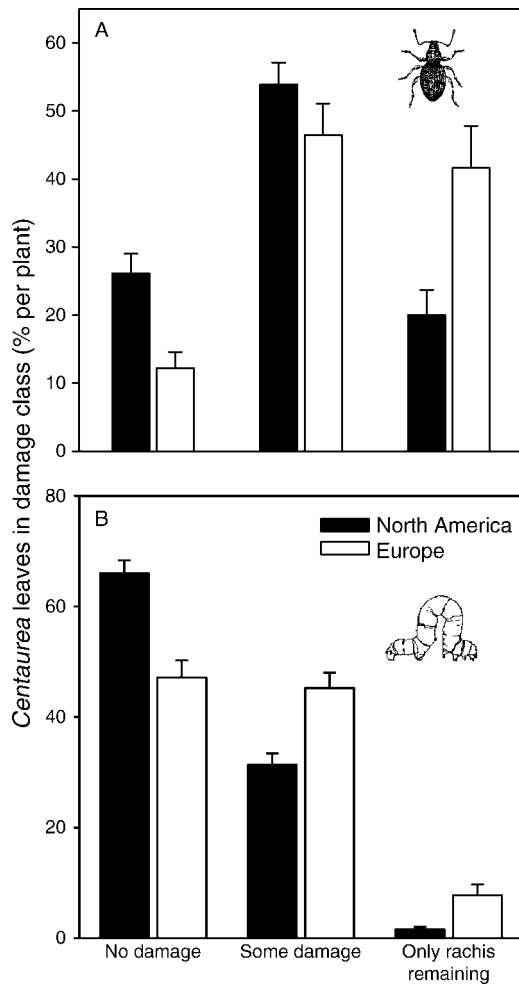


FIG. 4. (A) Damage rankings for North American and European *Centaurea maculosa* plant pairs subjected to leaf herbivory by the adults of the specialist weevil *Cyphocleonus achates*. Bars show percentage distribution (mean + SE) of leaves per plant in damage classes. ANOVA (Appendix B) for the “no damage” class: region, $F_{1,14} = 5.91$, $P = 0.0205$; population, $F_{14,69} = 0.27$, $P = 0.9874$. For the “some damage” class: region, $F_{1,14} = 1.32$, $P = 0.2580$; population, $F_{14,69} = 0.73$, $P = 0.7343$. For the “only rachis remaining” class: region, $F_{1,14} = 8.58$, $P = 0.0057$; population, $F_{14,69} = 0.79$, $P = 0.6733$. (B) Damage ranking for North American–European pairs, subjected to leaf herbivory by caterpillars of the naturalized European generalist herbivore, *Trichoplusia ni*. Bars show percentage distribution (mean + SE) of leaves per plant in damage classes. ANOVA (Appendix B) for the “no damage” class: region, $F_{1,14} = 125.86$, $P < 0.0001$; population, $F_{14,24} = 0.52$, $P = 0.8986$. For the “some damage” class: region, $F_{1,14} = 0.76$, $P = 0.3972$; population, $F_{14,24} = 1.23$, $P = 0.3169$. For the “only rachis remaining” class: region, $F_{1,14} = 17.35$, $P = 0.0008$; population, $F_{14,24} = 1.0$, $P = 0.4510$.

between the effects of the region of soil collection and the region of seed collection ($F = 1.04$, $df = 1$, 170 , $P = 0.308$). In other words, we found no evidence that plants from North American populations were larger than plants from European populations because they were

growing in substrate containing soil from North America.

DISCUSSION

We found that plants from North American *Centaurea* populations were bigger, elicited stronger competitive effects, and demonstrated stronger competitive responses than European populations (Table 1). Almost all previous similar studies of populations in the invaded and native ranges of an invasive species have compared growth or biomass, and inferred increased competitive ability from these metrics. We found that measurements of competitive effect and response show stronger biogeographical difference in comparisons of European and North American *Centaurea* populations than measurements of plant size or reproduction. For example, European *Centaurea* plants tended to produce more flower heads than North American plants when grown without competition, but when grown with

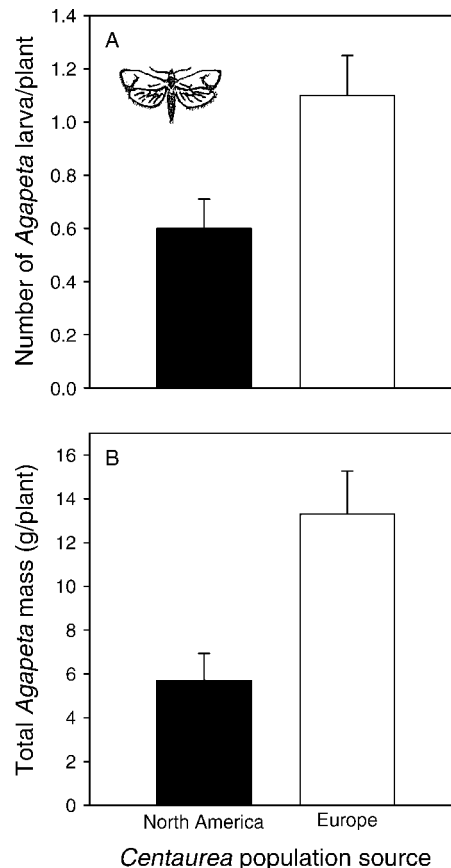


FIG. 5. (A) Mean number of *Agapeta zoegana* larvae infesting taproots of individual North American and European *Centaurea maculosa* plants planted in North-American–Europe pairs. ANOVA: region, $F_{1,15} = 5.25$, $P = 0.025$; population, $F_{15,82} = 1.35$, $P = 0.240$. (B) Total mass of *Agapeta* larvae per individual North American and European *C. maculosa* plant (full tables are in Appendix B). ANOVA: region, $F_{1,11} = 2.22$; $P = 0.1498$; population, $F_{11,59} = 0.95$, $P = 0.5017$. Error bars represent +SE.

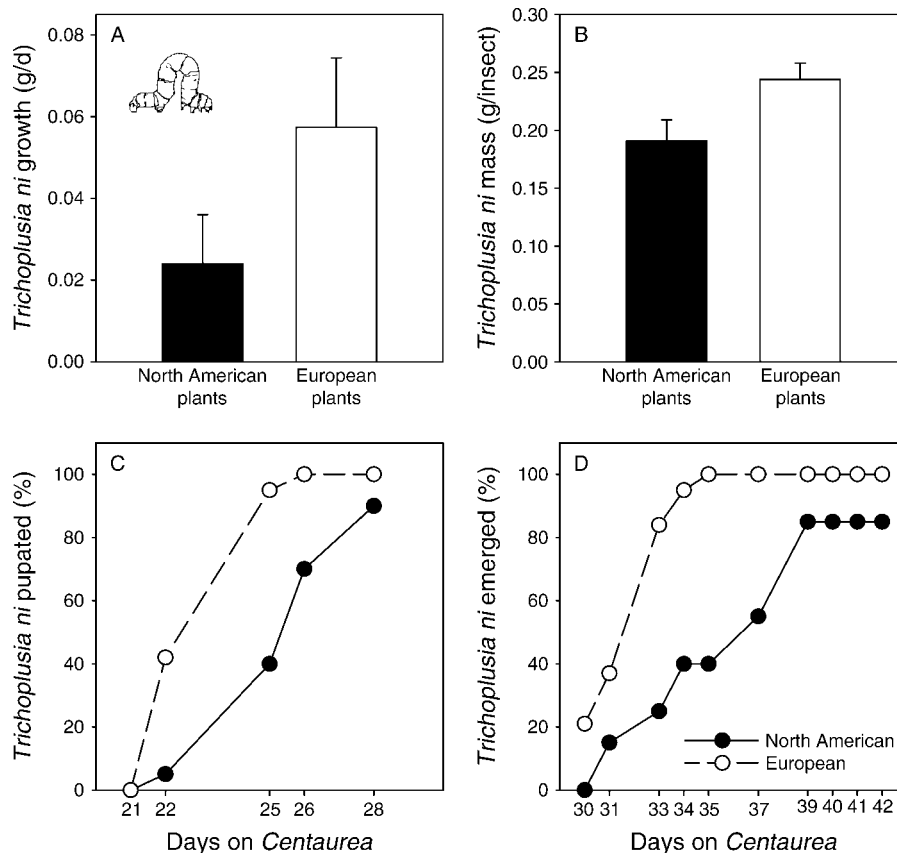


FIG. 6. The effect of North American or European *Centaurea maculosa* plants on the performance of the generalist European herbivore *Trichoplusia ni*. In all panels, error bars show SE. (A) Growth rates of *T. ni* on North American or European *Centaurea*. ANOVA (full tables are in Appendix B): region, $F_{1,14} = 5.62$, $P = 0.0313$; population, $F_{14,24} = 0.830$, $P = 0.6374$; initial mass[covariate], $F_{1,24} = 8.12$; $P = 0.009$. (B) Final *T. ni* mass on North American or European *Centaurea*. ANOVA with initial caterpillar mass as a covariate: region, $F_{1,14} = 4.05$, $P = 0.0628$; population, $F_{14,24} = 1.28$, $P = 0.2887$; initial mass[covariate], $F_{1,24} = 40.62$, $P < 0.001$. (C) Pupation rates of *T. ni* on North American or European *Centaurea*. (D) Adult emergence rates of *T. ni* on North American or European *Centaurea*.

competitors, European populations produced far fewer flower heads than North American populations.

All differences in traits between North American and European *Centaurea* populations occurred under identical conditions in greenhouses, so phenotypic plasticity can be ruled out as a cause (Reznick and Ghalambor 2001). However, our design did not take into account the likelihood that these regional differences will vary in different abiotic conditions, as demonstrated by Maron et al. (2004). Furthermore, even though our experiments suggest that North American *Centaurea* populations have evolved to be better competitors, as for virtually all studies of the evolution of invasives we cannot rule out founder effects. Total biomass remained substantially larger for North American populations even after growing populations in common conditions for a generation, reducing the potential of maternal effects to explain our results (Rossiter 1996). However, our experiment on maternal effects is limited because of very low replication among and within populations.

Trade-offs vs. direct selection

If indeed *Centaurea* has evolved to be larger and more competitive in North America, this supports one part of the “evolution of increased competitive ability” hypothesis (Blossey and Notzold 1995). However, North American *Centaurea* genotypes were also consistently better defended against (or avoided by) specialist and generalist consumers, demonstrating both a stronger inhibitory effect on the consumers (resistance) and a better ability to grow in response to herbivory (tolerance), which questions the trade-off based assumptions of EICA as a consistent mechanistic basis for the continental differences between populations of invaders. Our results suggest that the evolution of increased competitive ability may not always require physiological trade-offs between the allocation of energy or resources to growth or to defense. Our results also suggest that the broader “grow or defend” conceptual foundation of EICA theory, developed by Herms and Mattson (1992), may not always constrain the evolution of competitive

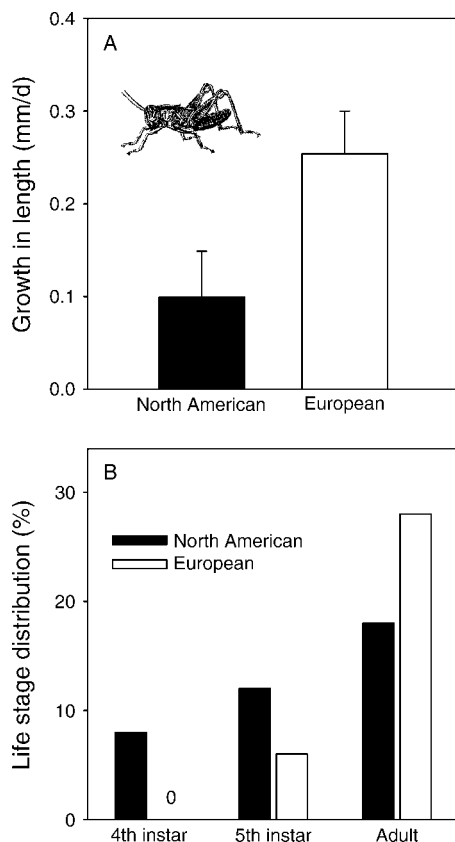


FIG. 7. The effect of North American or European *Centaurea maculosa* plants on the performance of the generalist North American herbivore, *Melanoplus sanguinipes*. (A) Growth rates in length of *Melanoplus* on North American or European *Centaurea*. ANOVA (Appendix B): region, $F_{1,8} = 26.88$, $P = 0.0001$; population, $F_{8,32} = 0.50$, $P = 0.8490$; initial length [covariate], $F_{1,32} = 4.00$, $P = 0.028$. Error bars show SE. (B) Percentages of surviving *Melanoplus* at different life stages on North American or European *Centaurea* plants at the time some host plants began to senesce ($\chi^2 = 13.2$, $df = 6$, $P < 0.05$). Percentages do not add to 100% for a region because they incorporate mortality.

and defensive traits. Using a model from the optimal defense literature, Zhang and Jiang (2006) argued that enemy release is neither sufficient nor necessary for evolution of reduced resistance in introduced plants to occur when habitat productivity covaries. However, there is a possibility of a trade-off between reproduction and defense, as European *Centaurea* tended (region $P = 0.0652$) to produce more flower heads than North American *Centaurea* in the absence of competition and herbivory. In other words, *Centaurea* plants from European populations may behave like ruderals, allocating more to dispersal, whereas North American *Centaurea* appear to have shifted allocation toward growth and defense, as would be expected of a “good competitor” (Grime 1977).

What might be an alternative to trade-off-based evolutionary changes in competitive ability and defense for invasives? First, defenses against herbivores gener-

ally come at some *physiological* cost (McKey 1974, Agren and Schemske 1992). However, others have not found defense costs and suggested costs may be minimal or that costs may only be manifest in terms of broader life history traits, “multiple costs” or “alternative defense strategies” (Agrawal and Karban 1999, Agrawal and Fishbein 2006). Along these lines we suggest that selection for effective competitive or defense traits may not be easily coupled to resource or energetic trade-offs for a simple reason; different defense or allelopathic chemicals may cost the same energetically or nutritionally, but differ a great deal in effectiveness. The effectiveness of a biochemical reduces its relative cost (Siemens et al. 2002). In other words, physiological costs of a biochemical may be trivial in an ecological context if the biochemical is exceptionally effective or performs more than one job.

In this context, there is evidence that *Centaurea* is allelopathic and (\pm)-catechin is an active biochemical agent of phytotoxicity (Buta and Lusby 1986, Ridenour and Callaway 2001, Bais et al. 2003, Iqbal et al. 2003, Weir et al. 2003, 2006, Callaway et al. 2005, Perry et al. 2005b, Thelen et al. 2005, D’Abrosca et al. 2006; Simões et al., *in press*; Inderjit, *unpublished data*; but see Blair et al. 2005, 2006). Furthermore, there is evidence that (\pm)-catechin, and allelopathic chemicals from other *Centaurea* species, are more toxic to naïve North American species than to European species in their native communities; the “novel weapons hypothesis” (Callaway and Aschehoug 2000, Bais et al. 2003, Callaway and Ridenour 2004, Vivanco et al. 2004; W. He, Y. Feng, and R. M. Callaway, *unpublished data*). Regardless of the factors that originally select for the chemical composition of root exudates of a particular species (e.g., nutrient chelation, offense, defense, or microbial interaction), the novelty of a biochemical may correlate with its superior effectiveness because new and naïve neighbors would not have had the opportunity to evolve tolerance or resistance (see Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005, Cappuccino and Arnason 2006). If invaders possess traits, such as allelochemical weapons or defense chemicals, that provide greater competitive or defense advantages in their new habitats than in their original ranges, then selection pressure for the traits conferring competitive advantages may be greater on the genotypes in the invaded regions than on the conspecific genotypes remaining at home. In other words, individuals that produce larger amounts of unusually effective defense or allelopathic chemicals might grow and reproduce more than individuals that do not, resulting in adaptive evolution driven by selection on specific biochemistry, the “allelopathic advantage against resident species” or AARS hypothesis derived from the novel weapons hypothesis and proposed by Callaway and Ridenour (2004). We found support for such directional selection in higher amounts of defense precursors produced by North Americans (Fig. 9) and a trend (region $P =$

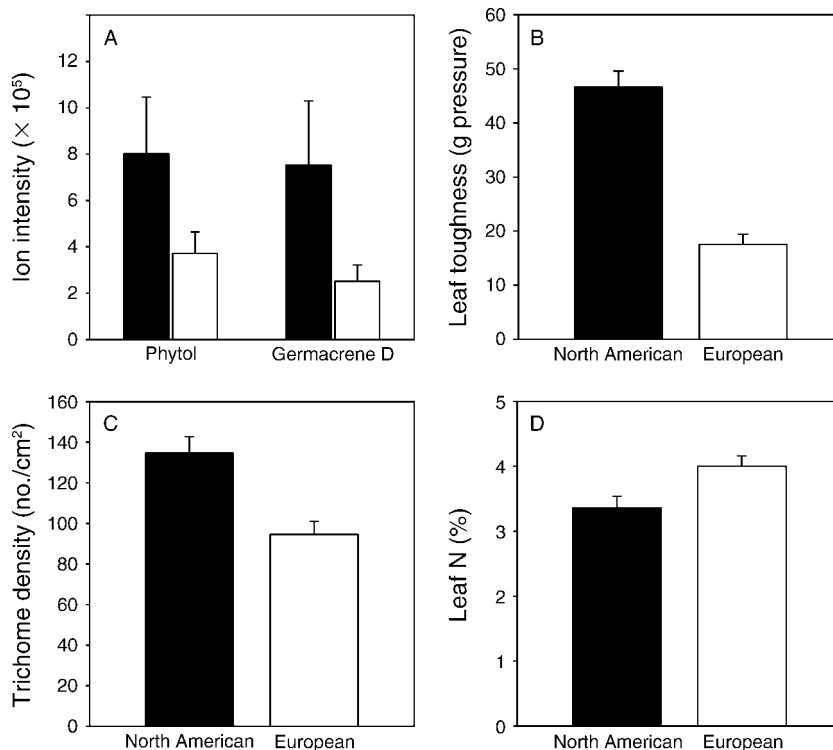


FIG. 8. Potential mechanisms by which North American populations of *Centaurea maculosa* could derive greater resistance to herbivory than European populations. For all measurements only *Centaurea* plants free from herbivory were used. Error bars show SE. (A) Leaf concentrations of the defense compound precursors phytol (ANOVA: region, $F_{1,13} = 4.64$, $P = 0.0451$) and germacrene D (ANOVA: region, $F_{1,13} = 2.48$, $P = 0.1330$); full tables are in Appendix B. (B) Leaf toughness (ANOVA: region, $F_{1,13} = 34.95$, $P < 0.0001$). (C) Trichome number on the abaxial (underneath) surface of mature *C. maculosa* leaves (ANOVA: region, $F_{1,18} = 8.42$, $P = 0.0084$; population, $F_{18,79} = 2.81$, $P = 0.0009$). (D) Leaf nitrogen concentration in mature *C. maculosa* leaves (ANOVA: region, $F_{1,18} = 6.21$, $P = 0.0214$).

0.1002) in this direction for (\pm)-catechin. Zhang and Jiang (2006) argued that if the invasive range is more nutrient-poor than the native range there could be selection for increased plant defense even if exotics experience enemy release.

Ploidy

The higher performance of North American populations in our experiments could be due to differences in ploidy. *Centaurea maculosa* populations can be either diploid or tetraploid (Müller 1989), and North American populations appear to be almost exclusively tetraploid whereas in Europe both tetraploid and diploid populations are common (H. Müller-Schärer, *personal communication*). However, in one experiment we found that North American tetraploids were larger than European tetraploids, suggesting that ploidy is not the reason North American plants are larger. An earlier comparison found that plants from a diploid population in Hungary were larger than plants from a tetraploid population in North America (Müller 1989).

Generalists vs. specialists

EICA makes predictions about specialist herbivores, not generalists. Müller-Schärer et al. (2004) proposed

that the most important change in herbivory experienced by introduced plant species is a “shift in the composition in the enemy complex towards an assemblage dominated by generalists.” Based on this perspective they reasoned that the evolution of increased competitive ability documented for many invasive species “is best explained by a reallocation of resources from costly quantitative defenses to growth,” because quantitative defense compounds deter herbivory by both specialist and generalist herbivores. Importantly, they also argue that qualitative defenses that are toxic to generalist herbivores may actually increase in the invaded range, as we found for *C. maculosa*. Thus, they predict that some invasive species might be able to evolve increased resistance to generalist herbivores without cost, as qualitative plant defense compounds effective at deterring generalists would confer an advantage in an environment containing generalists but lacking specialists.

Our case for *Centaurea* appears to be similar to the idea proposed by Müller-Schärer et al. (2004). We found that both specialist and generalist herbivores demonstrated superior performance on plants from native European *Centaurea* populations, but specialist insects showed a weaker response. Almost all metrics for the

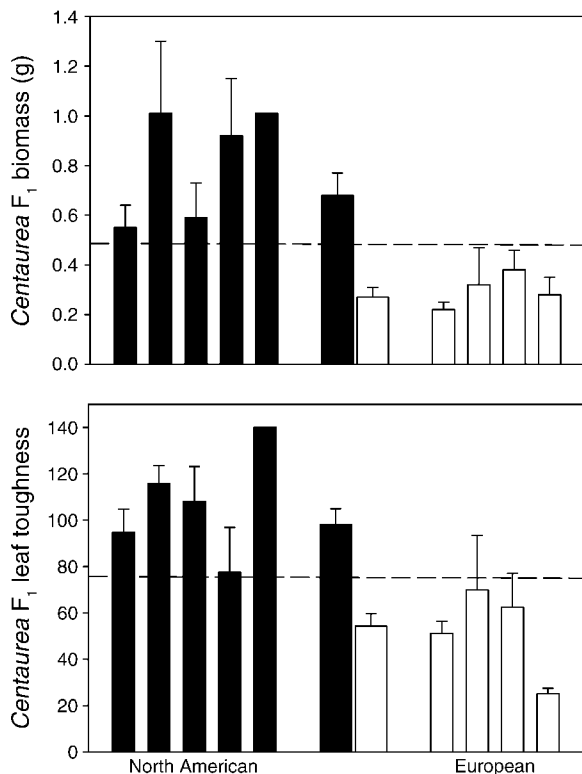


FIG. 9. Biomass and leaf "toughness" for the F_1 generations of plants from North American and European populations of *Centaurea maculosa*. All hand-pollination crosses were made within populations. Thinner bars represent means and SE for individual populations; thicker bars in the center represent means and SE for the two regions. ANOVA for biomass (Appendix B): region, $F_{1,7} = 13.01$, $P = 0.0045$. ANOVA for leaf toughness: region, $F_{1,7} = 8.96$, $P = 0.0171$. Toughness was measured in units of grams by a penetrometer.

two generalists showed strong and highly significant regional differences. Similarly, Joshi and Vrieling (2005) found that invasive populations of *Senecio jacobaea*, a noxious invasive weed worldwide, demonstrated higher biomass, but that pyrrolizidine alkaloids, primary anti-generalist herbivore compounds, were also higher in invasive populations. However, they observed that this shift resulted in lower protection against specialists.

Conclusions

Strong directional selection can work rapidly; Thompson (1998, also see 2005) argued that "interspecific interactions have now been shown to coevolve over the timescale of decades." Others have reported rapid evolution in natural populations (Reznick et al. 1997, Siemann and Rogers 2001, 2003) and our results suggest that the new plant and generalist herbivore neighbors encountered by *Centaurea maculosa* in its invaded range may exert strong directional selection on this invader's defensive and allelopathic biochemistry. If this is so, the disruption of such evolutionary relationships (see Cal-

laway and Ridenour 2004, Hallett 2006) may explain why some plant species are such successful invaders.

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APPENDIX A

Populations used in growth and herbivory experiments (*Ecological Archives* M078-014-A1).

APPENDIX B

ANOVA tables for growth, herbivory, palatability, (±)-catechin, and maternal effects experiments (*Ecological Archives* M078-014-A2).

APPENDIX C

Germination rates (percentage) for North American and European populations of *Centaurea maculosa* (*Ecological Archives* M078-014-A3).

APPENDIX D

Mean total biomass of *Centaurea maculosa* from European and North American populations: (A) with and without *Agapeta zoegana* infecting roots and (B) at the end of the experiment with *Cyphocleonus achates*; controls, and treatment combined (*Ecological Archives* M078-014-A4).