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**NO SIMPLE TRADEOFFS: *CENTAUREA* PLANTS FROM AMERICA ARE BETTER
COMPETITORS AND DEFENDERS THAN PLANTS FROM THE NATIVE RANGE**

BY

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Dissertation

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NO SIMPLE TRADEOFFS: *CENTAUREA* PLANTS FROM AMERICA ARE BETTER COMPETITORS AND DEFENDERS THAN PLANTS FROM THE NATIVE RANGE

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Two non-mutually exclusive hypotheses for invasive success have important evolutionary implications. The ‘natural enemies’ hypothesis posits that exotic invaders explode in abundance because they are not suppressed by specialist herbivore consumers in their invaded range. The ‘novel weapons’ hypothesis posits that exotic invaders explode in abundance because they possess 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. I explored the potential for novel allelopathic or herbivore defense biochemicals as a potential alternative mechanism to tradeoff-driven evolution of increased competitive ability in invasive plants by comparing growth, reproduction, competitive effect and response, and defense capabilities of invasive North American populations of *Centaurea maculosa* to populations in Europe, where the species is native. I found that *Centaurea* from North America were larger, but produced fewer flowers than plants from European populations. North American *Centaurea* demonstrated much stronger competitive effects and responses than European *Centaurea* against North American grasses. Importantly, competitive superiority did not appear to come at a cost to herbivore defense. North American *Centaurea* genotypes were better defended against specialist and generalist consumers, and showed both a stronger inhibitory effect on the consumers (resistance) and a better ability to grow in response to attack by herbivores (tolerance). Better defense by North Americans corresponded with higher constitutive levels of biochemical defense compound precursors, 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, caused the intercontinental differences. My results indicate that the evolution of increased competitive ability may not always be driven by simple physiological tradeoffs between the allocation of energy or resources to growth or to defense. Instead, I hypothesize that new plant neighbors and generalist herbivores encountered by *Centaurea* in its invaded range appear to exert strong directional selection on the weed’s competitive and defense traits.

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This work is dedicated to the memory of my grandmother, Margaree Ardelle Miller Adams, August 11, 1904 – January 22, 2006, who recently implored me to, “Get it done!”

I am thankful for the support of my entire family, without whom it would be impossible to be where I am today. I am especially thankful that my grandmother, Margaree Adams, my grandfather, Kenneth Adams, and my mother, Carolyn Ridenour, cultivated my love for and appreciation of the natural world from a very early age. Special thanks are also due to my mother for encouraging and directing my development as a teacher. Particular thanks also go out to my father, Harry Ridenour, my brother Paul Ridenour, and my sister in law Amy Ridenour for their loving support and expert advice during this exceptionally challenging and interesting period of my life.

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Chapter 1

**NO SIMPLE TRADEOFFS: *CENTAUREA* PLANTS FROM AMERICA ARE BETTER
COMPETITORS AND DEFENDERS THAN PLANTS FROM THE NATIVE RANGE**

Abstract

Two non-mutually exclusive hypotheses for invasive success have important evolutionary implications. The ‘natural enemies’ hypothesis posits that exotic invaders explode in abundance because they are not suppressed by specialist herbivore consumers in their invaded range. The ‘novel weapons’ hypothesis posits that exotic invaders explode in abundance because they possess biochemicals that are more effective against evolutionarily naïve plants, microbes, and herbivores than against organisms that have evolved tolerance in the invader’s communities of origin. I compared growth, reproduction, competitive effect, competitive response, and defense capabilities of invasive North American populations of *Centaurea maculosa* to populations in Europe, where the species is native. I found that *Centaurea* from North America were larger, but produced fewer flowers than plants from European populations. North American *Centaurea* demonstrated much stronger competitive effects and responses than European *Centaurea* against North American grasses. Competitive superiority did not appear to come at a cost to herbivore defense. Plants from North American populations were better defended against specialist and generalist consumers, and showed a stronger inhibitory effect on the consumers (resistance) and a better ability to grow in response to attack by herbivores (tolerance). Better defense by North Americans corresponded with higher constitutive levels of biochemical defense compound precursors, 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, caused the intercontinental differences. My results suggest that the evolution of increased competitive ability may not always be

driven by simple physiological tradeoffs between the allocation of energy or resources to growth or to defense. Instead, I hypothesize that *Centaurea maculosa* experiences strong directional selection on novel competitive and defense traits in its new range.

Key words: allelopathy, *Centaurea*, 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. Several non-mutually exclusive hypotheses have been proposed for this phenomenon, including two with important evolutionary implications. 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 (Elton 1958, Crawley 1987, Mack et al. 2000, Maron and Vilà 2001, Torchin et al., 2003, DeWalt et al. 2004). 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 (Callaway and Aschehoug 2000, Vivanco et al. 2004, Callaway and Ridenour 2004; Callaway et al. 2005). Interactions among specialist herbivores and their hosts are also determined by biochemistry, but specialists cannot be naïve to the biochemistry of their hosts. Not only are these two theories grounded in the past evolutionary history of communities, they prompt specific predictions about rapid contemporary evolution in invaders and the species they affect.

Janzen (1975) hypothesized that populations freed from herbivory may lose adaptations to resist or tolerate herbivory over time. 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. The EICA hypothesis explicitly predicts that invasive genotypes will be more poorly defended, but better competitors, or exhibit greater growth rates and reproductive outputs than genotypes in their native regions. A number of studies have shown that invasive genotypes are larger or more reproductive than the genotypes from which they appear to have originated (Elton 1958, Crawley 1987, Blossey and Notzold 1995, Mack et al. 2000; Schierenbeck et al. 1994, Wolfe 2002, Jakobs et al. 2004). For example, invasive populations of *Lythrum salicaria* have been shown to have greater fitness than native genotypes (Willis and Blossey 1999) and the invasive tree *Sapium sebiriferum* grows faster in its invasive range in the Southeastern United States and is preferentially consumed by the grasshopper *Melanoplus angustipennis* when given a choice between *Sapium* plants from the invaded range and plants from its native China (Siemann and Rogers 2001, 2003abc). *Sapium* is also avoided by herbivores in the field in its invasive range, a combination of results which supports herbivore-driven evolution of increased competitive ability. Maron et al. (2004) found that *Hypericum perforatum* has lost enemy resistance in its invaded range, but this change was not associated with an increase in plant size or fecundity. Other studies of invasive plant species have not found evidence for EICA (Agrawal and Kotanen 2003, Vilá et al. 2003, Bossdorf et al. 2004). In a literature review of invasive plants and evolution, Bossdorf et al. (2005, also see Willis et al. 2000) found that increased growth and reduced herbivory were common for plants in non-native habitats, but only a few studies have provided a full test of the EICA hypothesis by simultaneously addressing growth and defense, *and* finding directional shifts in both as predicted by EICA.

Bossdorf et al. (2005) suggested that studies of EICA are limited by focusing on defense and growth, and recommended that other characteristics be measured. For example, despite the emphasis on “competition” in the context of EICA, only two EICA studies have explicitly and experimentally addressed competition. Leger and Rice (2003) found that individuals of *Eschscholzia californica* from the species’ 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, neither of these studies reported direct measurements of the competitive effects and competitive responses of an invasive species in its native and invaded range.

There are also explicit evolutionary predictions in the novel weapons hypothesis. If invaders possess competitive traits such as allelochemical ‘offensive weapons’ that provide greater competitive advantages in their new habitats than in their regions of origin, then selection pressure for the traits conferring competitive advantages may be much greater for invasive genotypes than conspecific genotypes remaining at home (Callaway and Ridenour 2004). In other words, individuals that release a lot of effective toxins (i.e., effective if natives in the invaded range are not adapted) should grow and reproduce more than individuals that do not. The evolutionary implications of the novel weapons hypothesis suggest that invasive genotypes may evolve superior competitive ability for reasons in addition to those which were part of the original EICA hypothesis; effective competitive mechanisms may be selected for, but without easily predictable costs to other ecological functions. If a novel plant biochemical has extraordinarily

strong effects on other plants, microbes, or generalist herbivores in invaded communities by virtue of the evolutionary naïveté of the natives, then the benefit of this chemical would be disproportionately high relative to its cost. Several studies have now shown that invasive exotic species have more unique (or novel) biochemicals in their tissues than exotic species that have not become invasive (Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005, Cappuccino and Arnason 2006).

There is good evidence that *Centaurea maculosa* Lam. (spotted knapweed), one of North America's most devastating European invaders, is allelopathic and that allelopathy is a highly effective competitive mechanism (Ridenour and Callaway 2001, Bais et al. 2003, Baldwin 2003, Fitter 2003, Perry et al. 2005ab, Weir et al. 2006).

Centaurea exudes the compound (\pm)-catechin, a biochemical 'offensive weapon', from its roots into its rhizospheres, which inhibits the root growth of neighboring competing plants (Ridenour and Callaway 2001, Bais et al. 2003, Weir et al. 2003, Callaway et al. 2005, Perry et al. 2005ab, but see Blair et al. 2005). This gives the weed a competitive advantage (Ridenour and Callaway 2001, Thelen et al. 2005), and *Centaurea* is more allelopathic to North American neighbor species than congeneric European neighbor species (Bais et al. 2003, also see Callaway and Aschehoug 2000, Vivanco et al. 2004). For this racemic form of catechin, (-)-catechin appears to be the most phytotoxic, whereas (+)-catechin has weak phytotoxic effects and strong antimicrobial properties (Bias et al. 2003, Perry et al. 2005b). However, there are conflicting results about the amount of catechin naturally present in field soils in *C. maculosa* rhizospheres (Blair et al. 2005; Perry et al. 2005b).

If (\pm)-catechin allows *Centaurea* to more successfully compete for limited resources with its new neighbors in the invaded range than its original neighbors in the home range, any competitive advantage gained from (\pm)-catechin in North America may not depend on clear, zero-sum tradeoffs. In other words, if a novel allelochemical provides a competitive advantage highly disproportionate to its physiological cost, the benefit to cost “tradeoff” may not be easily quantified in terms of simple energy or resource budgets. Furthermore, *Centaurea* possesses what appears to be a novel defense chemical, cnicin, which has not been identified in other species (Landau et al. 1994, Kelsey and Locken 1987). The same ideas described above for novel allelopathic biochemicals also apply to defense biochemicals.

Here I explore the possibility that that direct selection on novel allelopathic or herbivore defense biochemicals represents a potential alternative mechanism to tradeoff-driven evolution of increased competitive ability in invasive plants. Specifically, I compared the growth, reproduction, and competitive effect and response of invasive North American populations of *Centaurea maculosa* to populations in Europe, where the species is native. I also examined the effects of North American and European populations of *Centaurea* on specialist and generalist herbivores and the response of the plants to these herbivores.

Methods

I 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 compare traits among North American and European populations of *Centaurea* while ruling out phenotypic plasticity (Reznick and Ghalambor 2001). Greenhouse experiments were conducted at the University of Montana, Missoula, Montana USA. Greenhouse temperatures were maintained between 15-30°C, corresponding roughly with ambient summer temperatures. Natural light was supplemented by Metal Halide bulbs, and total PAR during the day remained above 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. I chose greenhouse rather than common garden experiments to avoid introducing novel European genotypes (via pollen and seeds) to North America. Consequently, my 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. I collected seeds from 22 European *Centaurea* populations and 23 North American *Centaurea* populations (each separated by at least 50 km), but the number of populations used in an experiment ranged from 11-14 in the original growth and competition experiment to 5 in one of the herbivore experiments (Table 1, Supplementary Data). For each population (with the exception of three European populations denoted with asterisks) I collected 300-3000 seeds from 30-100 parent plants. Seeds from a single population were mixed and selected randomly for each experiment.

Germination, growth, fitness, competitive ability, and photosynthetic rates

To measure germination rates I planted *Centaurea* seeds from eight European populations and eight North American populations (chosen randomly from my collection of populations) in a mixture of 20% local grassland soil (Missoula, Montana; Haploxerolls and Argixerolls) and 80% 20/30 grit silica sand in flats in the greenhouse in early May 2004. Germination was recorded.

I used 14 European populations and 11 North American populations in an experiment designed to compare growth, flower number, and the competitive effect of *Centaurea* on two native grass species and the competitive response of *Centaurea* to these native grasses. *Centaurea* seeds were planted in 2.4 L pots, either alone or with *Pseudoroegneria spicata* Pursh., or *Festuca idahoensis* Elmer, two common native species in the intermountain prairie of the Northern Rocky Mountains. I chose *Pseudoroegneria* because previous experiments suggested it was a good competitor against *Centaurea*, and *Festuca* because previous experiments suggested it was a weak competitor (Callaway et al. 2004, Ridenour and Callaway 2001, 2003). For each *Centaurea* population, eight individuals were grown in competition with *Pseudoroegneria*, and eight individuals with *Festuca*, and the performance of these plants was compared to the performance of eight individuals from the same population grown alone. Twenty individuals of *Pseudoroegneria* and *Festuca* were grown alone, and the final biomass of these plants was compared to that of the *Pseudoroegneria* and *Festuca* grown with *Centaurea* to determine the competitive effect of the invader. All *Centaurea* plants were also monitored for flower production, and I estimated the average fecundity of all North American and European populations of *Centaurea* by measuring flower

number. At the end of the experiment, all *Centaurea*, *Pseudoroegneria*, and *Festuca* were dried at 100°C for 3 days and weighed, and the effects of region (fixed), population (random), and competitor (fixed) on *Centaurea* were compared using 3-way ANOVA in SPSS 11.5 (2002). The effects of region and population on the biomass of *Pseudoroegneria* and *Festuca* were compared using similar 2-way ANOVAs.

During the last week of November 2004, I measured the photosynthetic rates of 50 European and 50 North American *Centaurea*. Prior to photosynthesis measurements these plants were grown for 180 days in a greenhouse in 2.4 L pots filled with a mixture of 20% local grassland soil and 80% 20/30 grit silica sand. All *Centaurea* plants were watered every other day until the soil was saturated, and well-fertilized with 500 ml of Miracle-Gro at 0.34 g/L⁻¹ every two weeks. Photosynthesis measurements were made using a LiCor 6200 CO₂ gas analyzer, and while measurements were taken plants were well watered and kept at 22-24°C. Photosynthesis was measured at 1200 PAR, well above the light saturation point of *Centaurea*.

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. The theoretical prediction for species freed from herbivory is a loss of defense capability over generations (Janzen 1975), from which follows the prediction that herbivore performance will be greater when consuming plants from

invasive populations. Furthermore, EICA theory is based on a loss of defense capability in plants from invasive populations (Blossey and Nötzold 1995), ability.

I experimented with consumers that I have observed on *Centaurea* in the field in sites in western Montana. 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). These experiments focused on the palatability of *Centaurea* to consumers and the response of *Centaurea* populations to attack. In total, I conducted five experiments with these consumers.

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, *Cyphocleonus 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. *Agapeta* is a small (approximately 10 mm in length) bright yellow moth. 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*, but larvae can do substantial damage to the leaves of *Centaurea* (Callaway et al. 1999, Newingham 2002).

Trichoplusia is naturalized throughout Canada, Mexico, and the United States. 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 polyphagous (very broad) generalist native to North America and not a *Centaurea* biocontrol. *Melanoplus* host plants include a mixed diet of many kinds of forbs and grasses, and *Melanoplus* is commonly seen on *Centaurea* stems in the field (W.M. Ridenour, *pers. obs.*). *Melanoplus* is 20 -29 mm in length at maturity.

Prior to introduction of insects, all *Centaurea* plants were grown in 2.4 L pots filled with a mixture of 20% local grassland soil and 80% 20/30 grit silica sand, and were exposed to direct sunlight for 60 days in order to induce the production of flavonoids and other plant secondary metabolites. All plants were watered every other day until the soil was saturated, and fertilized with 500 ml of Miracle-Gro at 0.34 g/L⁻¹ every two weeks.

Cyphocleonus achates

On August 15, 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*, with the central stems 10 centimeters apart. As the plants matured during the experiment, the leaves of the two individuals consistently intermixed. I used 8 populations from each continent for this experiment (Supplementary Information, Table 1), chosen randomly from the total set of available populations, and then established pairs in equal proportions among the populations. 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, a local biocontrol supplier. 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 October 21, 2004, when all adult weevils were removed. 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 October 28, 2004, one week following adult *Cyphocleonus* removal, all *Centaurea* leaves, damaged and undamaged, were counted again as a measure of compensatory response, or “tolerance” (Müller-Schärer et al. 2004, Strauss and Agrawal 1999, Willis et al. 1999) as recommended by Bossdorf et al. (2005). *Cyphocleonus* larvae were allowed to grow and mature within *Centaurea* taproots until November 11, 2004 when plants were harvested. Taproots were then dissected using 5-30 x 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 3 days and weighed.

I statistically analyzed the damage done to *Centaurea* plants and the final biomass by conducting separate nested ANOVA on each of the three damage categories and biomass using region as a fixed factor and population as a random factor nested within regions, and included the initial size of individual plants as a covariate. I statistically

analyzed the final size of *Cyphocleonus* larvae with a nested ANOVA using region as a fixed factor and population as a random factor nested within regions, and included the size of individual plants as a covariate.

Agapeta zoegana

I collected *Agapeta* moths in the field, within 5 km of Missoula, Montana, in early September 2004. Eggs were obtained by identifying female moths (which were gravid at this time of year) and confining them in a paper cage so that they would lay eggs on the paper. Fifty 2.4-L pots were prepared so that each contained one European *Centaurea* and one North American *Centaurea* growing 10 centimeters apart as described above for the *Cyphocleonus* experiment. On September 17, 2004, small pieces $<1 \text{ cm}^2$ of the paper containing 2-3 *Agapeta* eggs each were cut out by hand and pinned precisely in the middle between the European and North American *Centaurea* plants (see Thelen et al. (2005) for detailed description). 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. All *Centaurea* pairs were promptly covered with paper enclosures so that emerging *Agapeta* larvae would be required to choose between the two *Centaurea* plants in their pot, and paper enclosures were also applied to *Centaurea* without insects as a methodological control. Paper covers were removed from the pots on September 27, 2004, after all *Agapeta* larvae had emerged and tunneled into *Centaurea* roots. *Agapeta* larvae were allowed to grow and mature within *Centaurea* taproots for over two months. On December 7, 2004, *Centaurea* taproots were dissected using 5-30 x magnification and all

Agapeta larvae were collected. The number of *Agapeta* larvae within each *Centaurea* taproot was recorded, and larvae were weighed, measured in length, and preserved in a solution of 90% ethanol. The number of flower heads and unopened buds on each *Centaurea* plant was recorded, and root caudex diameter measurements were taken with calipers so the effect of plant taproot size on herbivore choice could be evaluated. *Centaurea* plants were then dried at 100°C for three days and weighed. Initial *Centaurea* rosette width, and final *Centaurea* mass, bud number, and flower number, and total *Agapeta* mass were statistically analyzed with ANOVAs using region as a fixed factor with populations nested as random factors. *Centaurea* root caudex diameter was used as a covariate.

Trichoplusia ni

I obtained *Trichoplusia* eggs from the New York State Agricultural Experiment Station, Geneva, New York. Eggs hatched September 19, 2004, and on that day one caterpillar was introduced to each of 30 2.4 L pots containing pairs of European and North American *Centaurea* growing 10 centimeters apart, and 40 2.4 L pots containing a single European (n=20) or North American (N=20) *Centaurea* plant. I used 8 populations from each continent for this experiment (Table 1, Supplementary Information), chosen randomly from the total set of populations available, and then established pairs in equal proportions among the populations. 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 experiment I measured *Trichoplusia* growth rate, final mass prior to pupation, pupation rate, and adult emergence. As soon as all caterpillars on single European or North American plants were large enough to handle, each caterpillar was weighed daily until pupation. Once all *Trichoplusia* had pupated, 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, only leaf rachis remaining. As adult moths emerged on the single *Centaurea* treatments, they were collected and single male and female *Trichoplusia* moths reared on the same continental *Centaurea* source were confined in paper enclosures until they laid eggs on the paper's surface. While they were in these containers they were fed with sugar water. Eggs produced by pairs of *Trichoplusia* moths were counted to provide another measure of fitness.

I statistically analyzed the damage done to *Centaurea* plants by *Trichoplusia* in the paired-plant experiment by conducting separate nested ANOVA on each of the three damage categories using region as a fixed factor and population as a random factor nested within regions, and included the size of individual plants as a covariate. I analyzed the damage done to *Centaurea* plants by *Trichoplusia* in the isolated-plant experiment by conducting an ANOVA on the number of leaves damaged per plant using region as a fixed factor and population as a random factor nested within regions. I included the size of individual plants as a covariate. I statistically analyzed the final size and growth rate of *Trichoplusia* with nested ANOVAs using region as a fixed factor and population as a random factor nested within regions, including the size of individual plants as a covariate.

Differences in proportion emerged and proportion pupated among *Trichoplusia* on *Centaurea* from different regions were compared by fitting non-transformed survival numbers to log-normal distribution curves and testing the whole model and paired treatments with Chi-square analyses. I did not include population as a factor in these analyses.

Melanoplus sanguinipes

Beginning on December 3, 2004, I grew individual *Centaurea* plants from five different populations of European *Centaurea* and five different populations of North American *Centaurea* (Table 1, Supplementary Information) in 2.4 L pots in a mixture of 20% local soil and 80% 20/30 grit silica sand in 2.4 L pots. On February 17, 2005, *Centaurea* root caudex diameters were measured using calipers as an initial metric of plant performance, prior to herbivore application. I used 10 replicates of each population for the herbivore treatment and another 10 replicates for no-herbivory controls, for a total of 100 plants per region.

On February 18, 2005, third-instar *Melanoplus* were obtained from Stefan Jaronski at the USDA Agricultural Research Station (ARS) in Sidney, Montana and on February 19, 2005 these insects were introduced to *Centaurea* plants. Two *Melanoplus* were applied to each of the 100 *Centaurea* plants. In order to contain *Melanoplus* within their respective pots, pots were covered with transparent mesh “bridal veil” fabric. All 100 control plants and pots were covered with mesh fabric at the same time. All *Melanoplus* treatment pots were monitored daily for insect mortality. Initially, *Melanoplus* mortality was high (possibly due to stress related to shipping), and on

February 23, 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 March 22, 2005. Once *Melanoplus* reached adulthood, they were sexed. *Melanoplus* did not damage *Centaurea* extensively, with minor damage limited to the stem epidermis. Therefore, I did not measure the effect of *Melanoplus* on *Centaurea*.

I analyzed the effects of North American and European *Centaurea* on growth rates of *Melanoplus* larvae with a nested ANOVA using region as a fixed factor and population as a random factor nested within regions, including the size of individual plants as a covariate. Differences in the proportions of *Melanoplus* surviving the third, fourth, and fifth instars and as adults were compared with a nested ANOVA using region of origin for *Centaurea* as a fixed factor and population as a random factor nested within region.

Biogeographic differences in defense traits and plant nutrition

I measured leaf chemistry and toughness for all European and North American *Centaurea* used in the *Agapeta* experiment, described above. On November 29, 2004, one week prior to *Agapeta* larval harvest, one healthy leaf 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 (1ml

/0.1 g sample weight) at room temperature, and then transferred to a glass tube. The glass tube was shaken for 10 min at 200 rpm, and centrifuged for 10 min (2000 rpm). Supernatant fluids were filtrated (SUPELCO, Superclean Envi-carb tubes) and 1 μ l of the supernatant was analyzed using a GC/MS (Hewlett-Packard 5890 series II, Avondale, PA) equipped with a 30 m x 0.25 mm capillary column (DB-5.625, J&W Scientific, Folsom, CA) with helium as the carrier gas. The initial oven temperature was maintained at 0°C for eight min by cryogenic cooling. The oven temperature was increased to 70°C at rate of 7°C min⁻¹, and then to the final temperature of 300°C at the rate of 20°C min⁻¹, 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⁻¹ with automated pressure control. Detection was achieved by mass selective detection (Hewlett-Packard 5972, Avondale, PA) in the scan mode (m/z 33-500). The chemical structures were identified by using a Wiley 138K mass spectral database (John Wiley and Sons, New York), and by comparing the mass spectra and the retention time with those of authentic chemical samples (Horiuchi et al. 2005).

On December 6, 2004, immediately prior to *Agapeta* larval harvest, two healthy leaves from each *Centaurea* plant were also analyzed for leaf toughness (another potential anti-herbivore defense mechanism) using a penetrometer (John Chatillon and Sons, Scales and Force Measuring Instruments, NY), which measures the grams of force required to punch through a particular leaf.

I conducted an additional greenhouse experiment to ascertain if leaf nutrition and structural defenses play a role in differences between the defense capabilities of North American and European *Centaurea*. I measured leaf trichome density and leaf carbon

and nitrogen concentration on 11 North American populations and 11 European populations of *Centaurea*. On December 20, 2005, I planted 6 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. Pots contained a mixture of 20% local soil and 80% 20/30 grit silica sand. Plants were watered every other day to the point of soil saturation and fertilized with 500 ml of Miracle-Gro at 0.34 g L⁻¹ every two weeks. Due to greenhouse malfunction and plant mortality, my final replication was 49 North American individuals from 10 populations and 50 European individuals from 9 populations. On March 13-15, 2006, leaf trichome density was measured on two healthy leaves taken from each plant. Trichome density was measured by removing a disc, 28.3 mm² in area, with a paper punch and counting the number of trichomes at 5 – 30X magnification. All plants were dried at 60° C for 3 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. The effects of region (fixed) and population (random), on trichome density and leaf C:N ratios were compared using 2-way ANOVAs in SPSS 11.5 (2002).

Catechin production

I measured *Centaurea* catechin production in 4 populations of European *Centaurea* and 11 populations of North American *Centaurea*. Replication was limited for European *Centaurea* because I could not successfully sterilize the seeds from many of the populations I used, and therefore could not rule out the microbial contaminant as a source of catechin. For each population I initially processed 5 replications, consisting of

3 individuals grown in 3 ml of Murashige and Skoog (MS) medium. However, due to poor germination the final replication of 5 populations was reduced to 3. 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 3x using an equal volume of acidified ethyl acetate (1% acetic acid). Samples were concentrated under N₂, and re-suspended in 500 µl methanol. Methanol extracts were chromatographed (Dionex Co.) on a reverse phase 5 µm, C₁₈ column (25 cm x 4.6 mm) (Supelco Co.) using a multi-step gradient. The absorbance at the reference wavelength λ_{max} -280 nm was measured by a PDA-100 Photodiode array variable UV/VIS detector (Dionex Co.). Mobile phase solution A consisted of double distilled water containing 0.1% acetic acid and solution B consisted of ACS grade methanol (Fisher Co). A multi-step gradient was used for all separations with an initial injection volume of 20 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. (±)-Catechin concentrations in each sample were determined by comparison to 20 µL injections from a 1 mg ml⁻¹ catechin standard stock.

Maternal effects

Single *Centaurea* plants from the same eight European populations and eight North American populations used in the *Cyphocleonus*, *Agapeta*, and *Trichoplusia* herbivore treatment experiments were allowed to flower, and were hand pollinated

among plants from the same populations 3 consecutive times between December 6th and December 8th, 2004. Flower heads were collected and counted, and their seeds were removed and counted. However, I were only able to obtain viable seeds from four European and five North American populations, and only 2 to 19 seeds per population. On December 21st, 2005, all of these maternal line seeds were planted individually in the greenhouse in 2.8 liter pots containing a mixture of 20% soil and 80% 20/30 grit sand 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 March 15, 2006, two healthy leaves from each maternal line *Centaurea* plant were analyzed for leaf toughness using a penetrometer (John Chatillon and Sons, Scales and Force Measuring Instruments, NY), which measures the grams of force required to punch through a particular leaf. On March 16, 2006, all plants were harvested, dried at 100°C for 3 days, and weighed (g). The effects of region (fixed) and population (random), on plant mass and leaf toughness were compared using 2-way ANOVAs in SPSS 11.5 (2002).

Results

Germination, growth, fitness, competitive ability, and photosynthetic rates

The mean germination rate of *Centaurea* from North American populations was 81% higher than that of European populations (Figure 1). The age of the collected seed did not affect germination (age as a covariate; $F_{\text{age}}=1.55$; $df=1,15$; $F=0.236$). I did not estimate within-region variation for germination, but only one of the eight North

American populations germinated at the mean rate of the European populations (Kellogg Biological Station, Michigan, 35%), and only one European population (Roman, Romania, 68.3%) germinated above the mean rate of the North Americans.

In a second experiment, the total biomass of *Centaurea* plants from North American populations averaged 30% greater than that of European populations (Figure 2A). Biomass differed significantly among populations as well, but the effect of region was three times greater than the effect of population. The mean biomass of North American populations (7.43 g) was significantly higher than the grand mean (6.58 g), whereas the mean biomass of the European populations (5.73 g) was significantly lower than the grand mean. Only two of the 14 European populations exceeded the grand mean, whereas all of the 11 North American populations equaled or exceeded the grand mean. In contrast, the average flower number of European populations was 62% higher than that of North American populations (Figure 3A).

Interestingly, comparisons of competitive responses of North American and European *Centaurea* to *Pseudoroegneria* and the competitive effects of *Centaurea* on both grass species showed much stronger and more consistent regional differences than measurements of growth and fecundity on isolated *Centaurea* plants (Figure 2B, Figure 3B). When grown alone, North American *Centaurea* were an average of 30% greater in total mass than European *Centaurea*. Competition with *Pseudoroegneria*, however, reduced the mass of plants from North American populations by 25%, but *Pseudoroegneria* competition reduced the mass of plants from Europe by 58%. *Festuca* did not have a significant effect on *Centaurea* (data not shown). The most notable biogeographical effect of competition, by far, was observed for the effect of

Pseudoroegneria on *Centaurea* flower production. Even though European *Centaurea* populations had more flowers than North American populations when grown alone (Figure 3A), European flower production was reduced 159% more than that of North Americans when competing with *Pseudoroegneria* (Figure 3B). There was no effect of *Festuca* on *Centaurea* flower production. For *Centaurea* in competition, there was no overlap in flower number between the means of North American and European populations, with the proportional reduction of all North American populations by competition less than the grand mean (dashed line on figure) for competitive response and the proportional reduction of all European populations greater than the grand mean.

The competitive effect of *Centaurea* on native grasses was strong, but as for competitive response, competitive effect also showed significant biogeographical differences (Figure 4). When competing against plants from North American populations, the total mass of both *Pseudoroegneria* and *Festuca* decreased significantly more than when competing against European populations.

Corresponding with the general pattern of growth, *Centaurea* plants from eight North American populations showed 27% higher photosynthetic rates (based on leaf area) than *Centaurea* from eight European populations (10.9 ± 0.70 1 SE versus 8.6 ± 0.60 $\mu\text{mol m}^{-2} \text{s}^{-1}$; $F_{\text{region}}=17.60$; $df=1,59$; $P=0.019$; $F_{\text{population}}=2.30$; $df=7,59$; $P=0.179$).

Plant-herbivore interactions

Cyphocleonus achates

Even though I attempted to introduce *Cyphocleonus* to paired North American and European *Centaurea* plants of similar sizes in the regional comparison experiment,

North American rosettes still tended to be larger than European rosettes at the beginning of the herbivory experiment (19.5 ± 0.6 versus 18.0 ± 0.8 ; $T_{\text{region}} = 0.133$). Therefore, rosette size was always included as a covariate. *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 (Figure 5). My measurements of *Cyphocleonus* effect was only for adult leaf herbivory (too few larvae were found to measure larval effect). Similar numbers of *Cyphocleonus* larvae were discovered in the taproots of European and North American plants (12 versus 13, respectively), indicating that host choice was not affected by the region of population origin, but individual larvae consuming taproots of European *Centaurea* plants were 57% larger than those recovered from North American plants (Figure 6). However, this result should be considered with caution because of the small sample size and my inability to conduct the appropriate statistical analysis of populations nested within regions. Overall, *Cyphocleonus* treatment plant mortality was high, with 66% of plants from both continents 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). Moreover, North American plants that survived appeared to be more tolerant to herbivory than European plants, as North American *Centaurea* regrew faster following removal of adult *Cyphocleonus*. One week after removing *Cyphocleonus* adults, North American plants had produced 5.7 ± 0.3 new leaves per plant versus 4.0 ± 0.5 for European plants (ANOVA with larval mass as a covariate, $F_{\text{region}} = 13.95$; $df = 1, 15$; $P < 0.001$, $F_{\text{population}} = 0.91$; $df = 7, 47$; $P = 0.512$, data not shown). Of

those plants living at the end of the experiment, North American *Centaurea* mass was 35% larger than that of European plants (Supplementary Information, Figure 1B).

Agapeta zoegana

Prior to insect treatments, 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 (data not shown). But even though North American plants were larger, and *Agapeta* prefers to infest larger plants (Story et al. 2000, Ridenour and Callaway 2003), *Agapeta* larvae preferentially chose European plants (Figure 7). The mortality of plants treated with *Agapeta* was low (15% of all *Centaurea* exposed to *Agapeta* died), but 33% more European plants died than North American plants. Importantly, *Agapeta* larvae were 2.3 times heavier when feeding on the taproots of European *Centaurea* plants than when feeding on North American plants (Figure 7). Supporting my comparisons of *Centaurea* growth in other experiments, the final mass of North American *Centaurea* plants was 30% greater than that of European plants (Supplementary Information, Figure 1A).

Trichoplusia ni

When reared on isolated European or North American *Centaurea* plants, *Trichoplusia* severely damaged (left nothing but the leaf rachis) European *Centaurea* leaves five times more frequently than North American leaves (Figure 8A). When given a choice between North American and European *Centaurea* planted in the same pot, *Trichoplusia* preferentially grazed upon European *Centaurea* leaves, heavily damaging

(consuming the entire leaf down to the rachis) 12% of the leaves of European *Centaurea* plants compared to only 1% of North American leaves (Figure 8B). This preference for the leaves of European *Centaurea* was particularly striking given that North American *Centaurea* plants averaged 40% more leaves than European plants. Correspondingly, 40% more leaves of North American *Centaurea* plants remained completely undamaged by these generalist herbivores than leaves on European plants (Figure 8B).

This feeding preference corresponded with faster caterpillar growth; *Trichoplusia* caterpillars that were fed only European *Centaurea* grew more than twice as fast as caterpillars feeding on North American conspecifics (Figure 9A). Moreover, *Trichoplusia* caterpillars averaged 28% larger final mass prior to pupation when consuming European *Centaurea* leaves (Figure 9C). When reared on European *Centaurea*, *Trichoplusia* also pupated more rapidly. 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 (Figure 9B). All *Trichoplusia* reared on European *Centaurea* emerged as adults, whereas only 80% of *Trichoplusia* reared on North American conspecifics survived to adulthood. 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 (Figure 9D). *Trichoplusia* reared on European plants tended to lay more eggs than those reared on North American plants, but this difference was not significant (data not shown).

Melanoplus sanguinipes

Total mortality of the North American generalist, *Melanoplus*, was much higher than that of the European generalist, *Trichoplusia*, but mortality was similar for *Melanoplus* reared on North American and European populations (data not shown). However, *Melanoplus* grew 127% faster in length and acquired mass 156% faster when feeding on European plants than on North American plants. Moreover, 56% more *Melanoplus* reached maturity prior to senescence when feeding on European plants than when feeding on North American *Centaurea* (Figure 10).

Mechanisms of Centaurea resistance to herbivores

For all three metrics of plant defense measured, North American *Centaurea* populations were better defended, corresponding with the consistent pattern of superior herbivore resistance by North American populations against the insect herbivores I tested. North American populations of *Centaurea* contained approximately 2-3 times the volatile defense compounds germacrene D and phytol in their leaves than European populations (Figure 11A). Furthermore, North American *Centaurea* leaves were also 166% tougher when tested with a leaf penetrometer than European conspecifics (Figure 11B). Finally, North American *Centaurea* leaves had 42% more trichomes than leaves on European plants (Figure 11C). European *Centaurea* tended to have a higher percent N than North American *Centaurea* (Figure 11D), but these differences were not significant and leaf C:N ratios were almost identical between biogeographic regions (data not shown).

Biogeographic differences in Centaurea catechin production

The catechin concentration in North American plant rhizospheres was 0.127 ± 0.033 (1SE) mg ml^{-1} , compared to 0.071 ± 0.023 mg ml^{-1} within the rhizospheres of European conspecifics, but this difference was not significant ($P=0.118$, Figure 12).

Maternal effects

The F_1 lines derived from hand crossed pollination among maternal lines and raised in identical conditions suggest that the greater mass and leaf toughness observed for North American populations is based on genetic differences rather than maternal inheritance (Figure 13). The average biomass of North American *Centaurea* was 152% greater than the average biomass of European *Centaurea*. There was statistical overlap between some North American and European populations, but the mean biomass of all North American populations was greater than the grand mean, and the mean biomass of all European populations was below the grand mean. 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*. The mean leaf toughness of all North American populations was greater than the grand mean, and the mean leaf toughness of all European populations was below the grand mean.

Discussion

I 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 measurements of increased

competitive ability have been inferred from growth, but my direct measurements of competitive effect and response indicate the possibility of much stronger evolutionary changes in North American *Centaurea* populations than measurements of plant size. On average, European *Centaurea* populations produced more flower heads, but only when grown alone. When grown with competitors, all North American populations produced more flower heads than all European populations. Furthermore, total biomass remained substantially larger for North American populations even after growing populations in common conditions for a generation, reducing the possibility of maternal effects (Rossiter 1996). However, my experiment on maternal effects is limited because of very low replication among and within populations.

In an experiment with smaller subsets of the populations, plants from North America photosynthesized at 27% higher rates (per unit leaf area) than plants from Europe. This corresponds with the greater size of North American plants, but conflicts with leaf %N, which tended towards higher levels in European populations. High leaf %N is consistently correlated with high photosynthetic rates (Field and Mooney 1986). However, since North American populations had far “tougher” leaves than European populations, I suspected that European populations would have equal or higher photosynthetic rates if calculated on a leaf mass basis. Therefore I grew another subset of nine populations (total n=30 per region) from each region and found that North American populations consistently had higher Leaf Specific Mass ($42.9 \pm 2.1 \text{ g}^{-1} \text{ m}^{-2}$) than European populations ($25.0 \pm 1.4 \text{ g}^{-1} \text{ m}^{-2}$; $F_{\text{region}}=51.7$; $df=1,10$; $P<0.001$; $F_{\text{population}}=1.4$; $df=7,44$; $P<0.001$). Correspondingly, the mean photosynthetic rate of European plants per unit leaf mass was 35% higher than that of North American plants.

All differences in traits I measured 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, my design did not take into account the possibility that the comparative responses of North American and European populations are conditional. In other words, the regional differences I found may vary in different abiotic conditions, as demonstrated by Maron et al. (2004) for *Hypericum perforatum* grown in multiple common gardens in different parts of the world. Furthermore, even though my experiments suggest that North American *Centaurea* populations have evolved to be better competitors, as for virtually all studies of the evolution of invasives, I cannot rule out founder effects, which is the weakest link in my interpretation of regional population differences as evolution. In other words, perhaps the larger, more competitive, and better defended North American populations were founded by a European population that shared these traits. However, some traits showed no overlap at all among North American and European populations and I know of no *Centaurea maculosa* population in Europe that forms dense, near monospecific stands like populations often do in North America.

If indeed *Centaurea* has evolved to be larger and more competitive in North America, as my results suggest, 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 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 contradicts the predictions of the EICA hypothesis and questions it as a mechanistic basis

for *C. maculosa*. I note that the number of populations used in my herbivory experiments was lower than for growth and competition; however, all five experiments consistently showed the same pattern; herbivores performed worse on North American populations. My results suggest that the evolution of increased competitive ability may not always require simple physiological tradeoffs between the allocation of energy or resources to growth or to defense. My results also suggest that the broader “grow or defend” conceptual foundation of EICA theory, developed by Hermes and Matson (1992), may not always constrain the evolution of competitive and defensive traits. However, my results suggest the possibility of a tradeoff between reproduction and defense, as European *Centaurea* produced significantly more flowers than North American *Centaurea* in the absence of competition and herbivory. In other words, *Centaurea* plants from European populations appear to behave like true ruderals, allocating large amounts of resources to dispersal, whereas North American *Centaurea* appear to have shifted allocation towards growth and defense, as would be expected of a “good competitor” (Grime 1977).

What might be an alternative to tradeoff-based evolutionary changes in competitive ability and defense for invasives? First, it is clear that herbivore defense must come at some *physiological cost* (McKey 1974, Agren and Schemske 1992), and physiological tradeoffs in the evolution of different traits are ultimately inescapable. However, I suggest that selection for effective competitive or defense traits may not be easily coupled to resource or energetic tradeoffs 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 substantial evidence that *Centaurea* is allelopathic and (\pm)-catechin is an active biochemical agent of phytotoxicity (Ridenour and Callaway 2001, Bais et al. 2002, 2003, Weir et al. 2003, Thelen et al. 2005, Callaway et al. 2005, Perry et al. 2005b, but see Blair et al. 2005, 2006). Furthermore, there is evidence that (\pm)-catechin, and other allelopathic chemicals, are more toxic to naïve North American species than to European species in their native communities (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 proposed by Callaway and Ridenour (2004). I found support for this in higher amounts of defense precursors produced by North American populations (Figure 11) and a trend in this direction for (\pm)-catechin (Figure 12) but North American and European populations did not differ significantly in the amount of (\pm)-catechin produced. However, this measurement was severely hampered because I could not successfully sterilize seeds from several European populations of *Centaurea*, and rule out microbial contaminants as a potential (\pm)-catechin source. Therefore my replication of European *Centaurea* in this experiment was very low. Regardless, a biochemistry-focused perspective on the evolution of increased competitive ability is quite different than perspectives based on zero-sum-game measurements of caloric content or resource concentrations.

Maternal effects are important to consider in evolutionary studies of invasive organisms (Maron et al. 2004, Conner 2004, Hierro et al. 2005). Because the female cytoplasm contributes mitochondria and chloroplasts, and these two organelles contain DNA that controls some traits in offspring, differences in phenotypic expression among populations may be produced by maternal inheritance rather than by genetic differences. The recommended practice to eliminate or reduce maternal inheritance effects is to compare traits of the offspring from maternal lines grown in a common environment. If differences in the traits in question disappear in the second generation, then the original differences are less likely to be genetically based. My experiment with a second generation of *Centaurea* from North American and European populations grown in a common greenhouse environment was limited by the small number of seeds produced by hand pollination, but I found highly significant differences in total biomass and leaf

toughness among regions even in the second generation. In fact, the biogeographical differences between F_1 's were stronger than those in the initial experiment with the parental genotypes. These results suggest that the differences I describe among regions are genetically based.

If indeed the regional differences described here are genetically based, I do not know the genetic processes that may have produced them. One possibility of course is that selection for greater size, competitive ability, and defense is due to the different biotic and abiotic conditions encountered in the new North American range, resulting in contemporary evolution. Additionally, genetic drift, including genetic bottlenecks and founder effects (see above), is an important mechanism for evolutionary change. It is also quite possible that introduction of multiple European *Centaurea* populations into North America has brought together genotypes that had been geographically isolated in Europe, resulting in post-colonization hybridization and novel genotypes as was found for *Tamarix ramosissima*, an invasive plant in North America (Gaskin and Schaal 2002). Such hybridization would result in increased genetic variation upon which selection may act (Blair and Wolfe 2004, Gaskin and Schaal 2002). For example, the invasive weed *Sorghum halepense* readily hybridizes with the crop plant *S. bicolor*. In the southern United States, plants classified as *S. halepense* may actually be stable *S. halepense* \times *S. bicolor* hybrids (Arriola and Ellstrand 1996). Root exudates of "*S. halepense*" contain over ten times more sorgoleone, a highly allelopathic root exudate of *Sorghum*, than any other *Sorghum* species (Czarnota et al. 2001).

The superior performance of North American populations 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 exclusively tetraploid whereas in Europe both tetraploid and diploid populations are common (H. Müller-Schärer, *personal communication*). I do not know the ploidy of my original populations; however, H. Müller-Schärer and U. Trier at Fribourg University provided me with seeds of 8 tetraploid European populations and 9 tetraploid North American populations. I grew these to maturity in the conditions described above for the other experiments and found again that plants from the North American populations were much larger than European tetraploid plants. The mean diameter of the rosette for North American plants was 33.3 ± 1.0 cm ($n=30$ individuals) versus 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, $F_{\text{region}}=38.38$; $df=1,7$; $P<0.001$; $F_{\text{population}}=1.78$; $df=8,43$; $P=0.235$). These results suggest that greater mean ploidy is not the reason North American plants are larger. Furthermore, even the tetraploid populations are not “invasive” in their native Europe and 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).

Even if differences in some traits between invasive and native populations of a species are due to evolutionary changes, it is very hard to know what selective factors might drive such evolution. EICA-based theory poses that changes are due to the release from natural enemies, but to distinguish genetic changes due to enemy release from those due to any other novel factor in invaded regions is difficult. For example, latitude has been shown to correlate with herbivore defense (Coley and Barone 1996, Pennings et al. 2001) and plant size (Maron et al. 2004). However, I found no correlation between latitude and the mean size of individual plants in a population for all populations

combined (taken from Figure 1; $r^2 = 0.037$, $P=0.357$), European populations ($r^2 = 0.079$, $P=0.333$), or North American populations ($r^2 = 0.017$, $P=0.703$). Furthermore, using the mean responses of *Centaurea* populations to *Cyphocleonus* herbivory, there was no significant correlation between response to herbivory and latitude within regions or for regions combined. This does not prove that selection for larger, more competitive, and better defended genotypes evolved in North America through interactions with other species, but suggests that abiotic factors associated with latitude are unlikely to explain my results.

Another limitation of my results is that all common experimental conditions were in greenhouses in Montana. Although I found regional biomass differences in two different greenhouses, Maron et al. (2004) demonstrated that both introduced and native European populations of *Hypericum perforatum* exhibited variation in plant size depending on the latitude of origin and the latitude of the experiment. In common garden sites used for population comparisons of *Hypericum perforatum*, they found that plants originating from more northern latitudes outperformed those from southern latitudes in relatively northerly common gardens, while the reverse was true in more southern latitude common gardens in both Europe and North America. My results cannot address the possibility of such conditional latitudinal responses.

A third important limitation of my experimental design was that I added only North American soils to the sand used in my experiments. My intention was to provide AM fungi and other soil biota so that *C. maculosa* was grown in a more realistic environment. Only later did I realize that if North American populations had adapted in some way to North American soil biota I could have biased my results. Therefore I

conducted a follow up experiment in which I selected six North American and six European populations (for which I had soil from the same sites where I collected seeds) and grew each population in soil from its native home site and in a randomly selected soil from a site in the non-native range. The idea was that if my finding of larger North American plants was biased by growing all populations in a North American soil/sand mixture, then I would observe populations growing larger in their “home” soil. I did not find this. Again I found that plants from North American populations were larger (0.53 ± 0.18 g versus $.38 \pm .015$; $F_{\text{region}} < 0.001$), but all populations grew best in European soil, suggesting that European soil was more fertile. Moreover, there was no interaction 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, I found no evidence that plants from North American populations were larger than plants from European populations because they were growing in soil from North America.

I have suggested that the higher amounts of herbivore defense compounds and trend toward more allelopathic catechin produced by North American *Centaurea* populations may be an evolved response to novel selection pressures in the invaded region. However, this interpretation would be improved by a better understanding of the heritability of defense biochemistry and allelopathic catechin exudation. The heritability of these biochemical traits has not been quantified, but it is unlikely that heritable variance in these *Centaurea* traits limits selection considering that natural populations almost always show enough variation in morphological, defensive, and life history characteristics to result in evolutionary change (Phillips and Shine 2004, Conner 2004, Roff 1997). Furthermore, the observed changes in traits between North American and

European *Centaurea* genotypes occurred under controlled conditions in the greenhouse, so it is possible to rule out phenotypic plasticity and conclude that my results provide reasonable evidence of a genetic basis for the documented trait changes (Reznick and Ghalambor 2001).

Other studies of invasive plants have also found higher concentrations of biologically active chemicals in invasive populations than in native populations. Stastny et al. (2005) found that *Senecio jacobaea* from invasive populations in New Zealand and North America grew larger and had greater reproductive output than plants from the native range in Europe. North American plants also experienced more feeding damage, consistent with the EICA hypothesis. However, North American populations had *higher* levels of pyrrolizidine alkaloid defense compounds, suggesting the absence of a simple tradeoff between competition and allocation to defense. Siemens et al. (2002) also found that plants do not necessarily either allocate limited resources to growth and competitive ability *or* allocate these resources to defense. They found that *Brassica rapa* plants may have evolved to compete *and* defend when the biochemicals involved in plant defenses also benefit plants in competitive interactions. Additionally, Daehler and Strong (1997) reported an absence of tradeoffs between allocation to biomass and allocation to resistance to herbivory in invasive populations of *Spartina alterniflora* on the west coast of North America. In contrast, they found that plants with faster growth rates also had higher resistance to herbivory.

If the higher levels of defense compounds found in North American *Centaurea* populations, and the more negative effects of North American *Centaurea* on herbivores are the products of selection, then it is interesting to speculate on the particular aspects of

herbivory that might have driven such selection. The most compelling and accepted explanation of invasive success is the “natural enemies hypothesis”, the idea that invasive plants explode in abundance and community dominance because they have been moved to a new place without the *specialist* herbivores that eat them (Elton 1958, Crawley 1987, Maron and Vilà 2001), although generalists are predicted to have a greater impact on native competitors and release invaders (Keane and Crawley 2002). The reason for the overwhelming focus on specialists is that generalist herbivores are found both in the old range and in the new range, therefore making escape from generalists impossible. My finding that North American *Centaurea* populations are better defended than European *Centaurea* populations is not likely to be explained by specialist herbivory. Thirteen species of specialists have been introduced to North America as biological control agents, and their abundance on *Centaurea* plants is comparable in Europe and North America (Lang et al. 2000, Müller-Schärer, H., and D. Schroeder. 1993). Furthermore, specialist enemies have not arrested the growth of *Centaurea maculosa* populations (Müller-Schärer and Schroeder 1993, Ridenour and Callaway 2003, Pearson and Callaway 2006). Therefore, it is unlikely that specialists have driven the increase in the constitutive defense capability observed for North American *Centaurea* populations.

If specialist herbivores are not the likely cause of increased defense in North American *Centaurea* populations, that leaves generalists, and a disproportionately strong effect of generalists on invasive plants aligns partially with hypotheses suggested by Müller-Schärer et al. (2004). They argued that selection pressures exerted by herbivores differ between the native and introduced ranges of a plant species, that the invasion process affects the amount of genetic variation expressed in a population, and that this

may result in differential selection on plant resistance traits in native and introduced ranges, primarily due to a lack of specialist enemies. However, 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”. 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”, as quantitative defense compounds deter herbivory by both specialist and generalist herbivores, but that qualitative defenses that are toxic to generalist herbivores and non-adapted specialists may increase in the invaded range (Müller-Schärer et al. 2004). 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. They presented empirical results that support this view, with some specialist herbivores increasing survival or growth on populations collected in introduced ranges, but no such effects for generalists feeding on the same plants. In other words, invasive plants appeared to reduce quantitative defense costs in the absence of specialists, but were still able to defend against the new generalist complex.

My case for *Centaurea* is similar to that proposed by Müller-Schärer et al. (2004), but with a new twist. I found that both specialist and generalist herbivores demonstrated superior performance on plants from native European *Centaurea* populations. These results suggest that selection for increased defense against generalists can have negative consequences for specialists as well, despite the prospect that some adapted specialists

may use defense compounds to locate their prey (Müller-Schärer et al. 2004). In fact, Landau et al. (1994) found that the specialist *Centaurea* root-boring moth, *Pterolonche inspersa*, was attracted to cnicin, a *Centaurea* defense compound. I propose that the increased defense capabilities of North American *Centaurea* may be due to a more powerful effect of *Centaurea*'s defense compounds (including cnicin, which has not been reported from other species in the North American systems *Centaurea* invades) on North American generalist herbivores than on European generalists. In other words, naïve North American generalist herbivores that have never experienced cnicin may well be more susceptible to this compound than European generalists that have a shared evolutionary history with *Centaurea maculosa*, and consequently may have adapted to cnicin or other *Centaurea* defense compounds (Callaway and Ridenour 2004). The increased efficacy of novel chemical defenses against “naïve” native North American generalist herbivores may have resulted in strong selection for increased quantities of these compounds in North America, a parallel to the AARS hypothesis discussed above.

The differences described here for North American and European populations of *C. maculosa* suggest that the fundamental life history strategy of this species may be shifting from “ruderal” in Europe to “competitor” in North America (after Grime 1977). First, plants from North American populations are better competitors than plants from European populations and *C. maculosa* is far more dominant in North America. Second, European populations allocate more to reproduction in the first year than North American populations. Third, greater leaf toughness, (most likely due to more lignin) represents an increase in “quantitative” defense in North American *Centaurea* populations. This is consistent with a shift in life history strategy towards the “competitor” end of the

spectrum. Interestingly, apparency theory (Feeny 1976) predicts that plants that are apparent, or “bound to be found” (such as dominant *Centaurea* in North America) will employ constitutive chemical defenses more than unapparent plants (such as the less common *Centaurea* in Europe). The situation with increasing chemical defense in North American populations is less clear, as cnicin, a sesquiterpene lactone, is a so-called “quantitative” defense compound. However, since cnicin apparently first appeared in North America in *Centaurea* leaves, it is clearly “qualitative” in its invaded range. Fourth, there is some evidence that plants live longer in North America than in Europe (Muller 1989), consistent with a competitive life history approach.

These speculative interpretations of my results for the importance of generalist herbivores in plant invasions are controversial because generalists have not been considered as potential drivers of invasion in the past (Keane and Crawley 2002, Müller-Schärer et al. 2004) and studies have shown that exotic plants can be harmed by herbivores native to the invaded range (Maron and Vila 2001, Levine et al. 2004, Parker and Hay 2005, Parker et al. 2006). Furthermore, if generalists in North America are more “naïve” to the defenses produced by European native North American invasive plants than generalists in Europe, then at least some diffuse evolutionary relationships among generalists and their hosts in native communities must exist. But I now know that diffuse evolutionary relationships among species are common and that fully obligate relationships among co-evolved species are not the rule (Thompson 2005, also see Colautti et al 2004). Therefore it is reasonable to suspect that generalist herbivores might co-adapt in loose ways with the species they live with. This co-adaptation would likely take the form of tolerance to the defense chemicals used by particular plant species.

Interestingly, I find far fewer generalist insects on *Centaurea* plants in Montana than in Romania, where *Centaurea maculosa* is native (T. Bassett, W.M. Ridenour, A. Diaconu, and R.M. Callaway *unpublished data*). My results, although very limited at this point, also show that the European generalist cabbage looper (*Trichoplusia ni*), performs very well on *Centaurea*, suggesting adaptation, whereas the native North American generalist, the lesser migratory grasshopper (*Melanoplus sanguinipes*), performs very poorly, suggesting a lack of adaptation. Furthermore, a recent study of native (California) and invasive (Chile) populations of *Eschscholzia californica* tested the performance of two generalist insect herbivores (one native to coastal California and one native to Eurasia but naturalized in North America) and found that these generalists performed better when reared on native populations of *Eschscholzia* from California than on invasive populations from Chile (Leger and Forister 2005). They interpreted these results as evidence for escape from the tradeoffs associated with specialist herbivores and the evolution of increased production of defensive compounds “effective at deterring generalist herbivores in the introduced range”.

Although I have speculated that *C. maculosa* is less susceptible to non-adapted and naïve generalist herbivores in its invaded range because of its novel defense system, the alternative may certainly be true for other species. Exotic species may be more susceptible due to their naiveté to new enemies in invaded regions, which can result in very strong consumer pressure, the ‘new associations principle’ hypothesized by Hokkanen and Pimentel 1989 (Colautti et al. 2004). Colautti et al. (2004) called this the ‘increased susceptibility hypothesis’, suggesting that introduced populations could be subjected to greater enemy effects than the source population (see Parker et al. 2006). A

deeper understanding of the role of enemies in invaded regions will provide crucial insight into plant invasions, but there is no reason to expect unanimity for all species. Some exotics may be suppressed by their naiveté to the local herbivore community and other exotics may be released due to the naiveté of the local herbivore community. Either way, such powerful shifts in the relationships among species suggests that loose evolutionary relationships among species are powerful organizers of natural communities (Callaway et al. 2005, Hallett 2006).

Why might generalists avoid novel defense compounds? Contemporary diet selection theory indicates that food preferences and aversions are based on experiences within the life of the generalist herbivore and on inherited traits (Launchbaugh et al. 1999). They argued that herbivores reject foods when consumption is followed by negative gastro-intestinal consequences. Such learning has been demonstrated in many herbivores, including generalist insects (Bernays and Lee 1988). However, Launchbaugh et al. (1999) argued that digestive feedback, which determines whether or not a plant is palatable, is inherited.

Based on my evidence and theory proposed by Müller-Schärer et al. (2004), generalists in invaded ranges are not likely to have inherited preferences for an invasive species, because invaders often possess defense chemicals that are novel in the invaded range (Cappuccino and Carpenter 2005, Carpenter and Cappuccino 2005, Cappuccino and Arnason 2006). Thus, an invader might be more likely to be avoided by generalists in invaded ranges than in native ranges (Siemann and Rogers 2003). In contrast, generalists in natural ranges of a species would be more likely to have inherited preferences or tolerances to compounds produced by that species. This sets a clear

conceptual framework for the possibility that generalist herbivores may fail to suppress invasive species in their invaded ranges, but have strong effects in the native ranges of invasive species.

Conversely, Parker and Hay (2005) proposed that “as exotic prey share no evolutionary history with native enemies, they will not have experienced selection from these consumers and may lack effective defences”. Thus, exotic invasive plants may be preferentially consumed by generalist herbivores in the plant’s invaded range. They tested the feeding preferences of two North American native generalist herbivores (crayfish, *Procambarus* species), and one non-native naturalized generalist (grass carp, *Ctenopharyngodon idella*) when offered native and non-native freshwater plants. They found that “both native crayfish showed a significant, 3-fold preference for exotic over native species” and that exotic generalists did not exhibit a preference. Parker et al. (2006) followed up on these experimental results by conducting a meta-analysis of 63 published studies assessing generalist herbivore effects on exotic plants. Based upon this analysis, they concluded that “native herbivores suppressed the abundance of exotic plants, whereas exotic herbivores suppressed the abundance of native plants”. They further posed that these results, which support the biotic resistance hypothesis (Elton 1958, Maron and Villa 2001), suggest that exotic plants are *less adapted* to deter native herbivores than native plants. However, based upon this logic, it is equally likely that native consumers may be naïve (due to their lack of adaptation) to the novel defense compounds that some invasive plants, or “exotic prey”, possess, or put another way, the lack of a shared evolutionary history.

Invasive organisms have the potential to evolve rapidly because of strong directional selection exerted by new abiotic or biotic factors. Climate, edaphic factors, and new competitors, enemies and mutualists often differ between the native and introduced ranges. In a review of studies documenting rapid contemporary adaptive evolution, Reznick and Ghalambor (2001) found a strong association between rapid evolution and colonization events. Of the 47 total studies of contemporary adaptation reviewed, all but six were characterized as the product of colonization, and of these, 18 were cases of establishment of new populations in novel environments. Each of the many organisms a plant interacts with has its “own agenda”, and all of these interactions are “potential selective forces” on the plant (Harrison and Baldwin 2004, Thompson 2005). Changes in species assemblages due to biological invasion result in geographic differences that have the potential to create “coevolutionary hotspots” (Thompson 2005) as novel species interactions occur. Novel competitors and generalist herbivores in *Centaurea*'s introduced range may exert very different selection pressures on this species than species in *Centaurea*'s native range, for example. Differences in abiotic factors in different habitats may also influence the interactions between a given set of organisms such that reciprocal selection, or coevolution, only occurs in some habitats (Thompson 2005). Thus, interactions between a host plant and its specialist herbivores may also differ between a plant's native and introduced ranges, where specialist biological control herbivores have been recently introduced. In the case of *Centaurea*, such differences in selection pressures between its native and introduced ranges may potentially have resulted in directional selection that has produced larger, more competitive, and better defended plants than conspecifics in its native range.

Strong directional selection can work rapidly; Thompson (1998) 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; Hendry and Kinnison 1999; Siemann and Rogers 2001, 2003; Phillips and Shine 2004) and my 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 Callaway and Ridenour 2004, Hallett 2006) may explain why some plant species are such successful invaders.

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Table 1. Summary of experimental results. Replication shows the number of populations used from each region, and arrows designate whether or not North American populations performed significantly better or worse with respect to the trait listed. For growth, the results for two of the four herbivory experiments are presented in Figure 1 in the Supplementary Information.

Experiment	European & North American replication	Plant Growth	Plant Repro.	Insect Devel.
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↑	-	-

Figure Legends

Figure 1. Germination rates (percent) for North American and European populations of *Centaurea maculosa*. Means for each population are presented in the narrow bars, and means and 1 SE for each region using the means of each population as replicates are presented in the two thicker bars in the center of the figure. In an ANOVA with seed age as a covariate (dates of collection differed), and region as the factor, $F_{\text{region}} = 8.90$, $df = 1, 15$; $P = 0.011$, $F_{\text{age}} = 1.55$; $df = 1, 15$; $F = 0.236$.

Figure 2. A) Total biomass of *Centaurea maculosa* from North American and European populations. Means and 1 SE for each population are presented in the narrow bars, means and 1 SE for each region using the means of each population as replicates are presented in the two thicker bars in the center of the figure. In a t-test using the means of each population as independent replicates $T_{\text{region}} = 6.51$, $df = 1, 24$, $P < 0.001$. In a two-way ANOVA, $F_{\text{region}} = 12.12$; $df = 1, 24$; $P < 0.001$, $F_{\text{population}} = 2.44$, $df = 24, 171$; $P = 0.084$. B) The response of *Centaurea* to competition from *Pseudoroegneria* as percent decrease in biomass. The mean for each population is presented in the narrow bars, means and 1 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, $T_{\text{region}} = 7.70$, $df = 1, 21$, $P < 0.001$. There are fewer populations shown in this panel because mortality with competition reduced the sample size in some populations below an analytical level.

Figure 3. A) Flower head production of *Centaurea maculosa* from North American and European populations. Means and 1 SE for each population are presented in the narrow bars, means and 1 SE for each region using the means of each population as replicates are presented in the two thicker bars in the center of the figure. In a t-test using the means of each population as independent replicates $T_{\text{region}}=11.12$, $df=1,17$, $P<0.001$. In a two-way ANOVA, $F_{\text{region}}=10.07$; $df=1,17$; $P=0.002$, $F_{\text{population}}=3.44$, $df=17,163$; $P=0.028$. B) The response of *Centaurea* to competition from *Pseudoroegneria* as percent decrease in flower head production. The mean for each population is presented in the narrow bars, means and 1 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, $T_{\text{region}}=9.44$, $df=1,16$; $P<0.001$.

Figure 4. The response of two native North American grasses, *Pseudoroegneria spicata* and *Festuca idahoensis*, to competition from *Centaurea*, as percent decrease in grass biomass when grown with *Centaurea* versus alone. The mean for each population is presented in the narrow bars, and means and 1 SE for each region are presented in the two thicker bars in the center of the figures. In t-tests with populations as independent samples, for *Pseudoroegneria*, $T_{\text{region}}=16.04$; $df=1,21$; $P=0.001$, and for *Festuca*, $T_{\text{region}}=13.50$; $df=1,21$; $P=0.002$.±

Figure 5. Damage rankings for North American and European *Centaurea maculosa* plants subjected to leaf herbivory by the adults of the specialist biological control weevil, *Cyphocleonus achates*. Error bars show 1 SE and shared letters represent no significant difference between regions within a damage class. In an ANOVA with region as a fixed factor and populations nested within regions; for the “no damage” class, $F_{\text{region}}=10.17$; $df=1,15$; $P=0.002$; $F_{\text{population}}=0.22$; $df=15,84$, $P=0.979$. For the “some damage” class $F_{\text{region}}=1.10$; $df=1,15$; $P=0.299$; $F_{\text{population}}=0.653$; $df=15,84$, $P=0.710$. For the “only rachis remaining” class, $F_{\text{region}}=7.47$; $df=1,15$; $P=0.008$; $F_{\text{population}}=0.636$; $df=15,84$, $P=0.724$.

Figure 6. Mass of *Cyphocleonus achates* larvae consuming the taproots of European and North American *Centaurea maculosa* plants. 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. $T_{\text{region}}=2.11$; $df=1,23$; $P=0.045$.

Figure 7. A) Mean number of *Agapeta zoegana* larvae infesting taproots of individual North American and European *Centaurea maculosa* plants planted in NA-Europe pairs. In an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}}=5.25$; $df=1,15$; $P=0.025$; $F_{\text{population}}=1.35$, $df=15,82$; $P=0.240$. B) Total mass of *Agapeta* larvae per individual North American and European *C. maculosa* plant. $F_{\text{region}}=6.31$; $df=1,15$; $P=0.014$; $F_{\text{population}}=1.35$, $df=15,82$; $P=0.241$. For both figures, error bars represent 1 SE.

Figure 8. A) Effect of the European generalist herbivore, *Trichoplusia ni* caterpillars, on North American and European *Centaurea maculosa* grown in NA-European pairs. Error bars show 1 SE and shared letters represent no significant difference between regions within a damage class. In an ANOVA with region as a fixed factor and populations nested within regions; for the “no damage” class, $F_{\text{region}}=6.31$; $df=1,15$; $P=0.009$; $F_{\text{population}}=1.26$; $df=15,58$, $P=0.518$. For the “some damage” class $F_{\text{region}}=3.77$; $df=1,15$; $P=0.035$; $F_{\text{population}}=2.72$; $df=15,58$, $P=0.046$. For the “only rachis remaining” class, $F_{\text{region}}=14.01$; $df=1,15$; $P<0.001$; $F_{\text{population}}=0.800$; $df=15,58$, $P=0.872$. B) Effect of *T. ni* on North American and European *C. maculosa* grown as individuals, $F_{\text{region}}=9.22$; $df=1,15$; $P<0.001$; $F_{\text{population}}=5.35$, $df=15,38$; $P=0.007$.

Figure 9. The effect of North American or European *Centaurea maculosa* plants on the performance of the generalist European herbivore, *Trichoplusia ni*. In all figures, error bars show 1 SE and shared letters represent no significant difference between regions. A) Growth rates of *T. ni* on North American or European *Centaurea*. In an ANOVA with region as a fixed factor and populations nested within regions, caterpillar gender as a fixed factor, and initial mass as a covariate, $F_{\text{region}}=7.35$; $df=1,15$; $P=0.012$; $F_{\text{population}}=0.443$, $df=15,45$; $P=0.690$; $F_{\text{initial mass}}=8.12$; $df=1,45$; $P=0.009$. B) Pupation rates of *T. ni* on North American or European *Centaurea*. C) Final *T. ni* mass on North American or European *Centaurea*. In an ANOVA with region as a fixed factor and populations nested

within regions, caterpillar gender as a fixed factor, and initial mass as a covariate, $F_{\text{region}}=4.01$; $df=1,15$; $P=0.053$; $F_{\text{population}}=0.955$, $df=7,45$; $P=0.477$; $F_{\text{initial mass}}=40.62$; $df=1,45$; $P<0.001$. D) Adult emergence rates of *T. ni* on North American or European *Centaurea*. The effect of gender was never significant.

Figure 10. The effect of North American or European *Centaurea maculosa* plants on the performance of the generalist North American herbivore, *Melanoplus sanguinipes*. In all figures, error bars show 1 SE and shared letters represent no significant difference between regions. A) Growth rates in length of *Melanoplus* on North American or European *Centaurea*. In an ANOVA with region as a fixed factor and populations nested within regions, and initial length as a covariate, $F_{\text{region}}=5.09$; $df=1,9$; $P=0.030$; $F_{\text{population}}=0.88$, $df=9,39$; $P=0.733$; $F_{\text{initial length}}=4.00$; $df=1,39$; $P=0.028$. B) Growth rates in mass of *Melanoplus* on North American or European *Centaurea*. In an ANOVA with region as a fixed factor and populations nested within regions, and initial mass as a covariate, $F_{\text{region}}=10.65$; $df=1,9$; $P=0.005$; $F_{\text{population}}=1.22$, $df=9,39$; $P=0.119$; $F_{\text{initial mass}}=11.06$; $df=1,39$; $P<0.001$. C) The proportion of surviving *Melanoplus* in the 4th (instar at application), and 5th instars and reaching adulthood on North American or European *Centaurea* plants.

Figure 11. 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 1 SE and shared letters designate no significant difference A) leaf concentrations of phytol and germacrene D, defense compound precursors. In a one-way ANOVA with region as a fixed factor (population effects were not tested due to low replication) and for the log of the dependent variable, for phytol $F_{\text{region}} = 3.51$, $df = 1,64$; $P = 0.035$; for germacrene D, $F_{\text{region}} = 2.58$, $df = 1,68$; $P = 0.061$. B) Leaf “toughness” as measured by the pressure needed to force the pin of a penetrometer through the leaf blade. In an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}} = 32.44$; $df = 15,99$; $P < 0.001$; $F_{\text{population}} = 0.75$; $df = 15,99$; $P = 0.614$. C) Trichome number on the abaxial (underneath) surface of mature *C. maculosa* leaves. In an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}} = 5.29$; $df = 1,18$; $P = 0.045$; $F_{\text{population}} = 0.15$; $df = 18,98$; $P = 0.995$. D) Leaf nitrogen concentration in mature *C. maculosa* leaves. In an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}} = 2.39$; $df = 1,19$; $P = 0.166$; $F_{\text{population}} = 0.21$; $df = 19,98$; $P = 0.982$.

Figure 12. Mean (\pm)-catechin concentration produced *in vitro* by North American and European *Centaurea maculosa* seedlings. In an ANOVA with region as a fixed factor and population as a random factor, $F_{\text{region}} = 4.71$; $df = 1,9$; $P = 0.118$; $F_{\text{population}} = 2.60$; $df = 9,46$; $P = 0.233$.

Figure 13. Biomass and leaf “toughness” (the pressure needed to force the pin of a penetrometer through the leaf blade) for the F_1 generations of plants from North American and European populations of *Centaurea maculosa*. All hand pollination crosses were made within populations. For biomass, in an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}}=6.79$; $df=1,8$; $P=0.051$; $F_{\text{population}}=0.738$; $df=8,60$; $P=0.653$. For leaf toughness, in an ANOVA with region as a fixed factor and population nested within region, $F_{\text{region}}=27.15$; $df=1,8$; $P<0.001$; $F_{\text{population}}=0.191$; $df=9,60$; $P=0.991$.

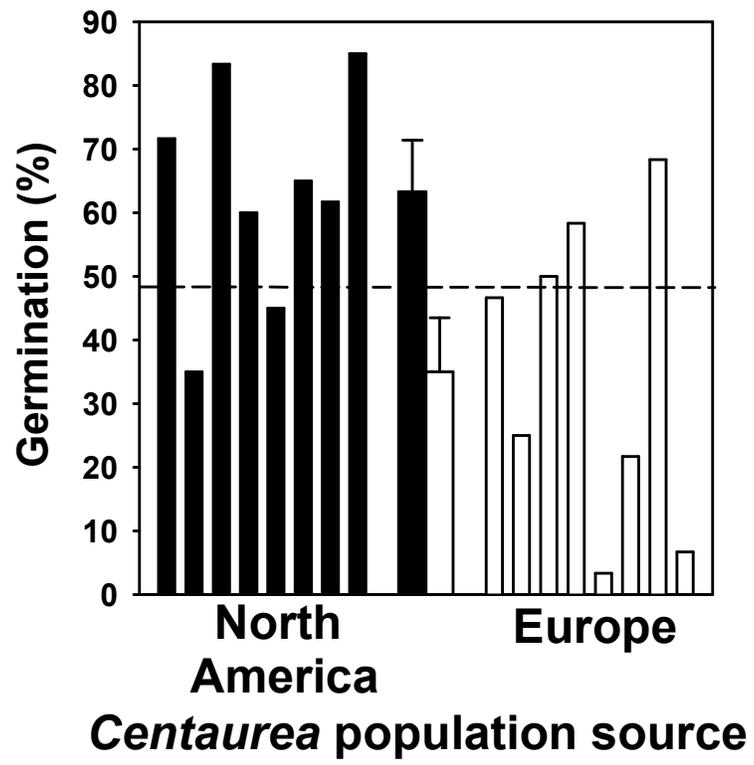


Figure 1.

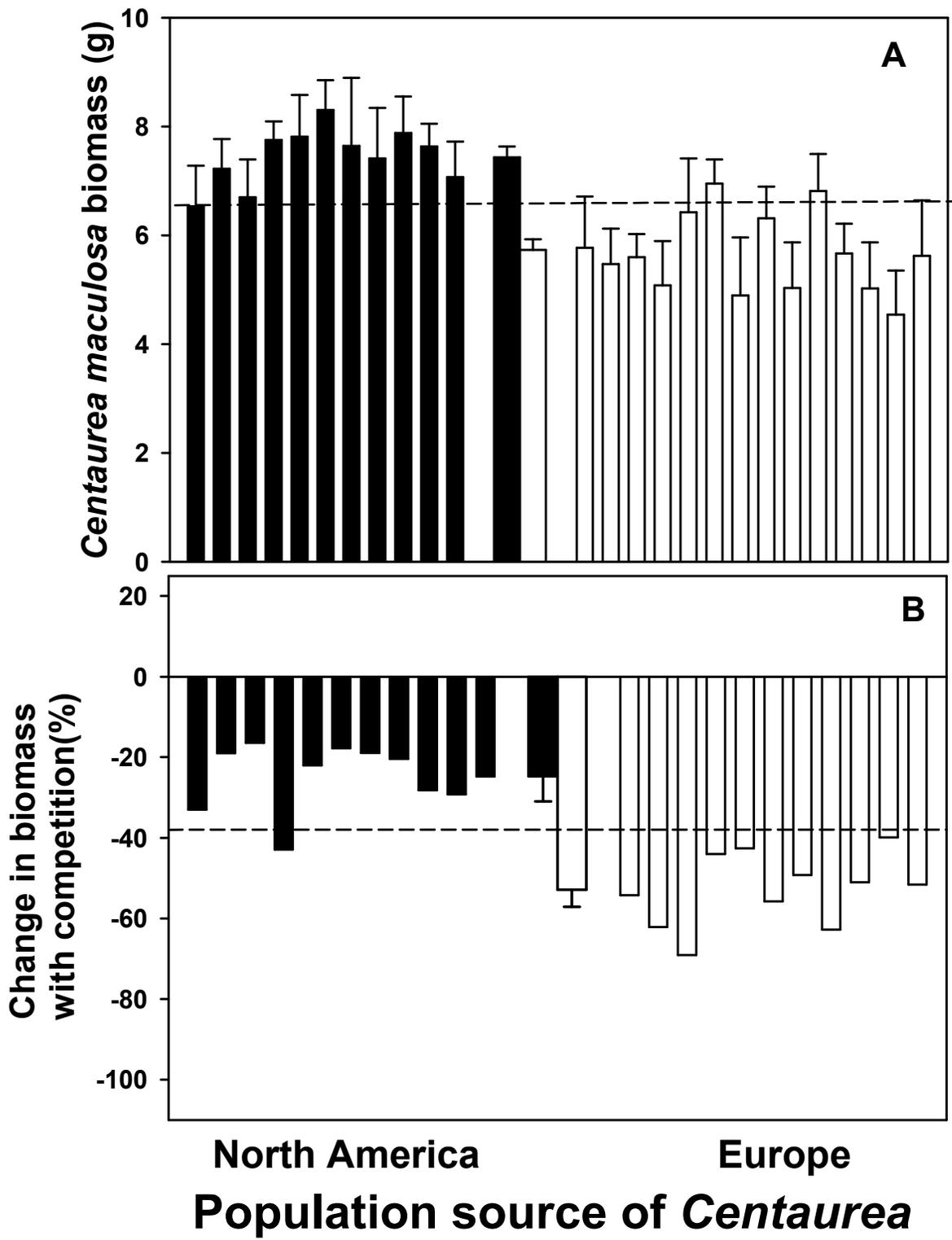


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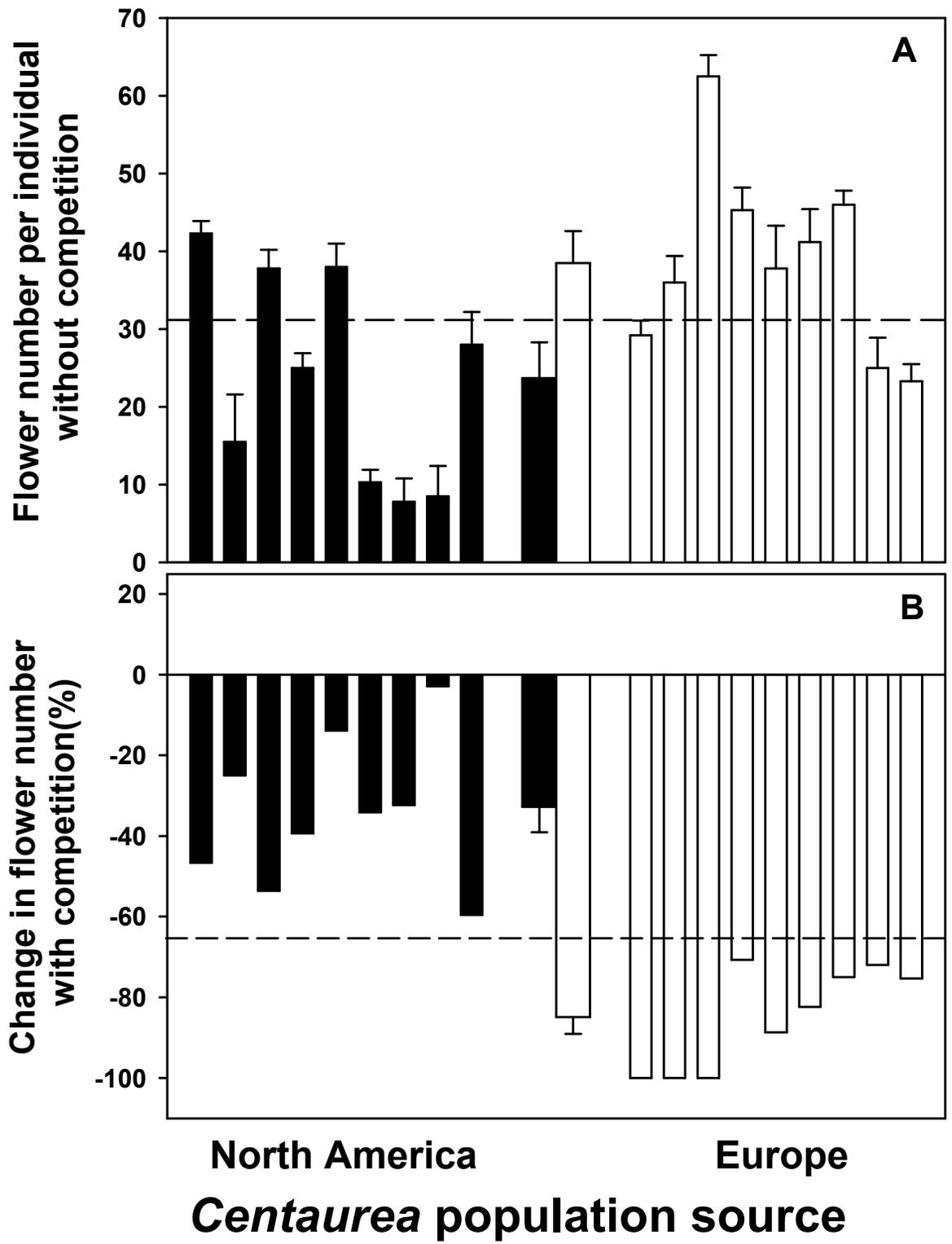


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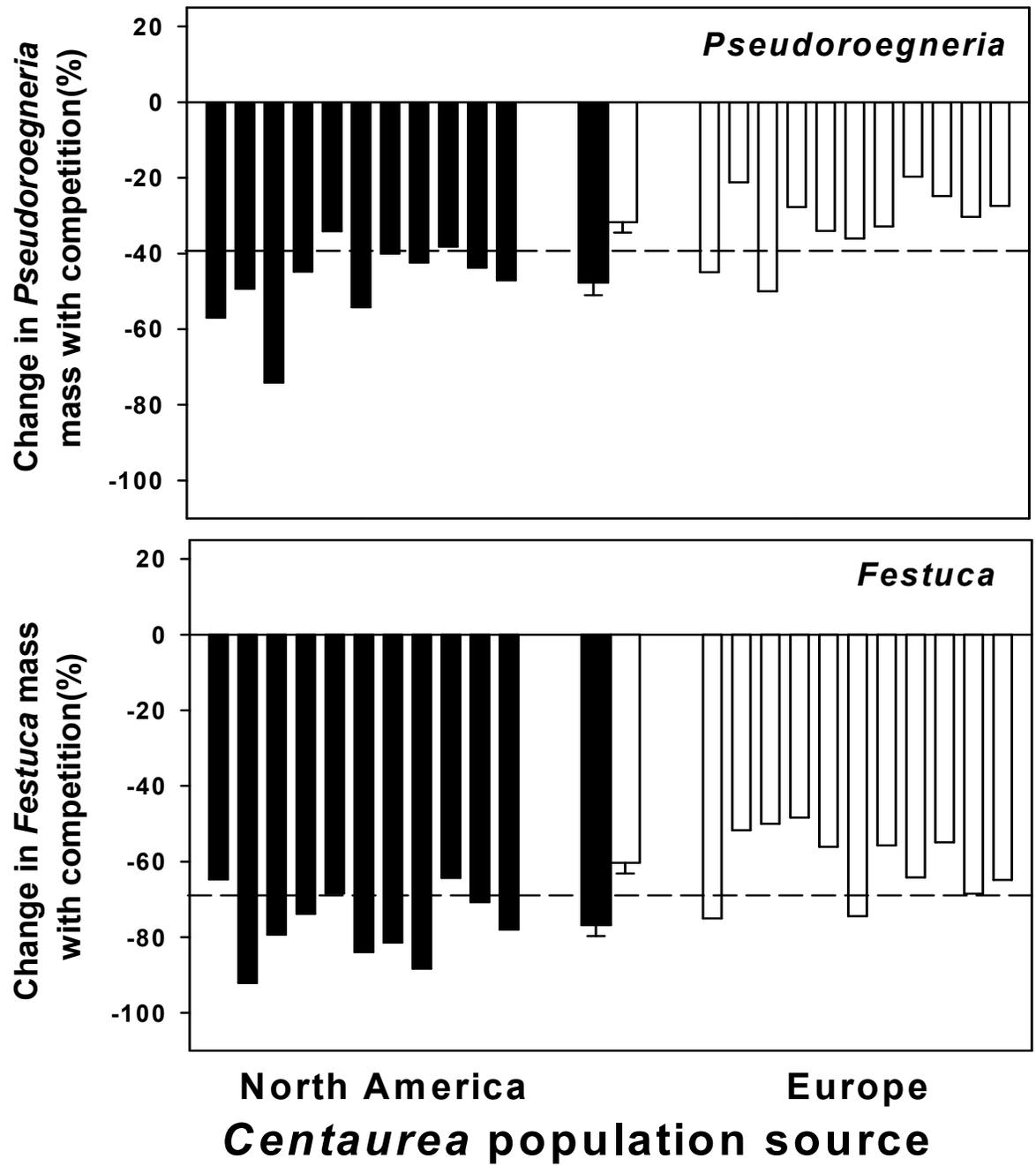


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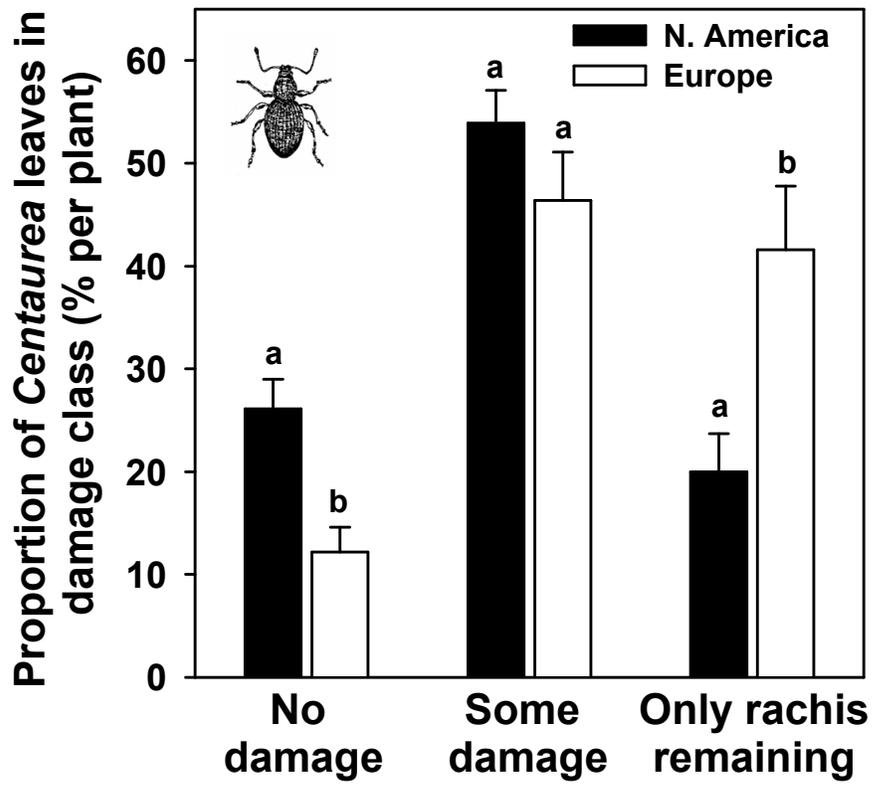


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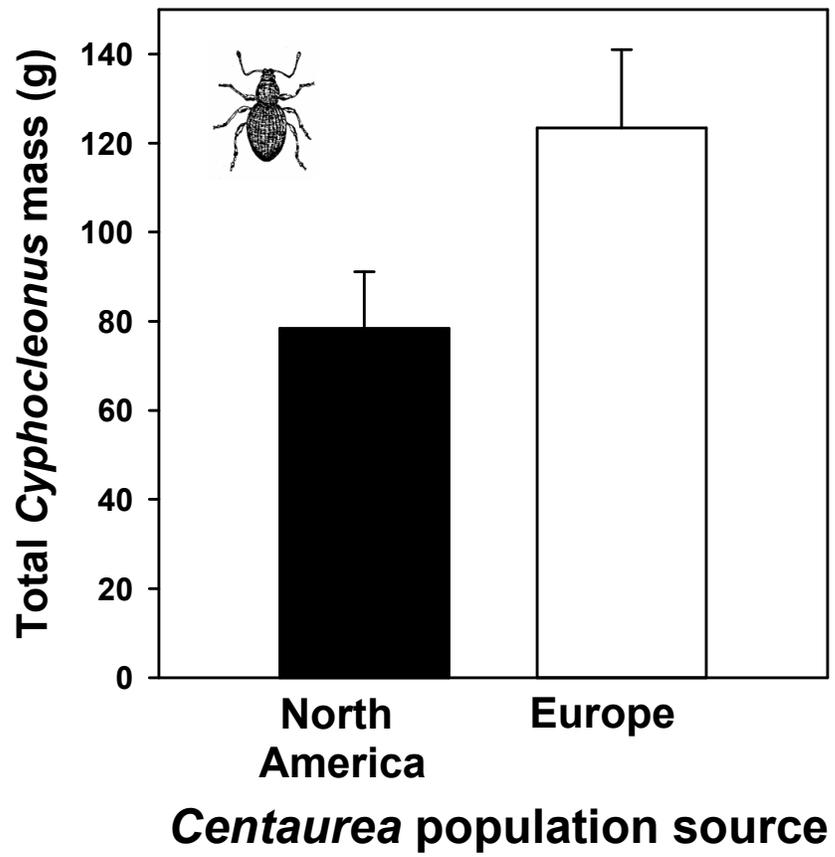


Figure 6

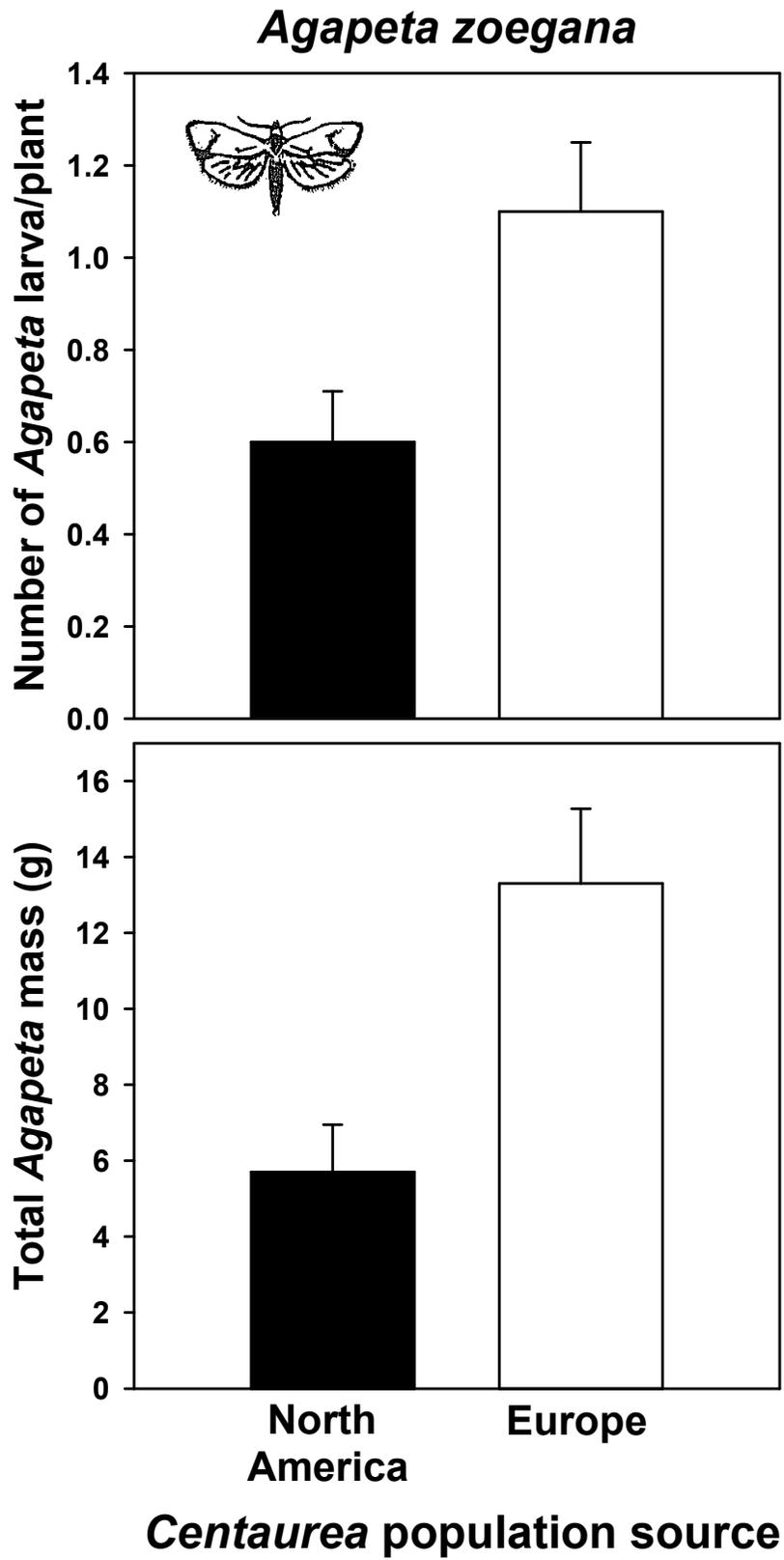


Figure 7

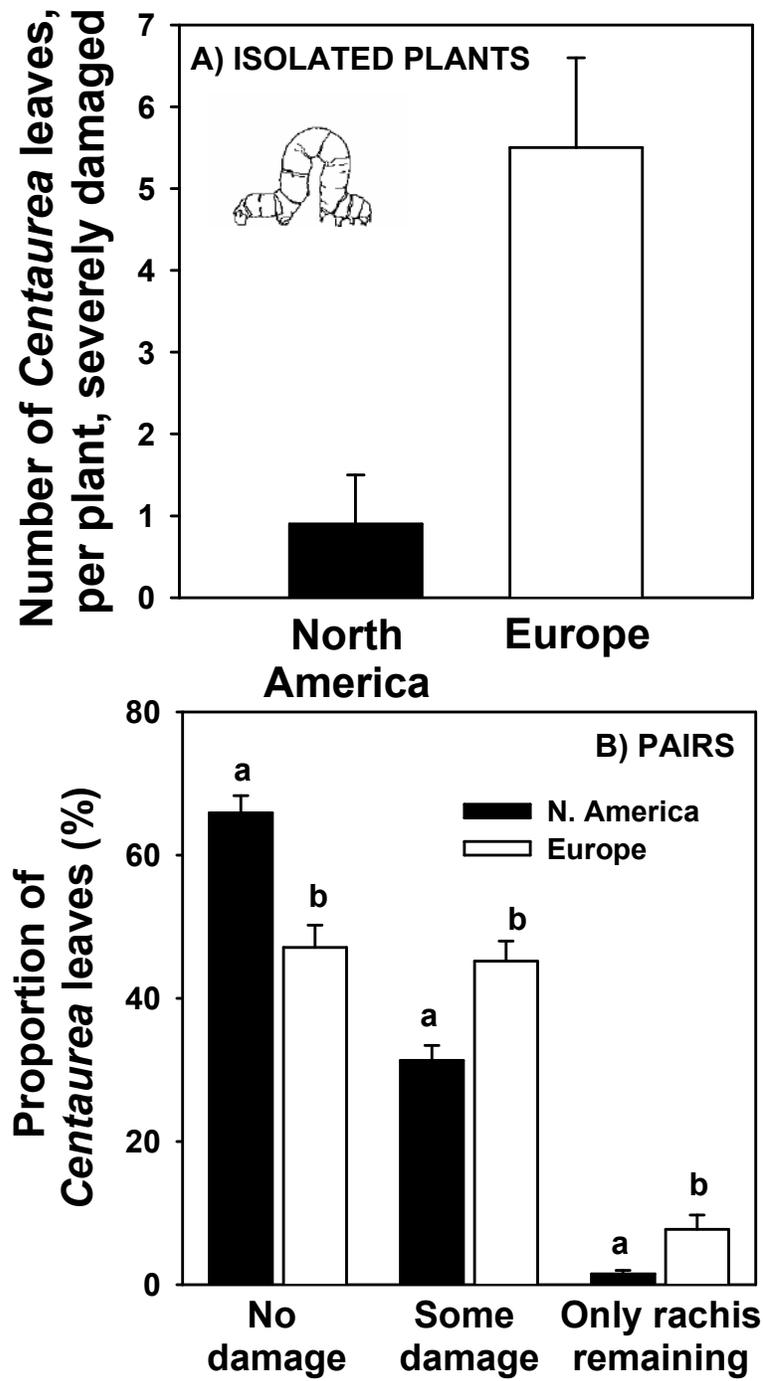


Figure 8

Trichoplusia ni

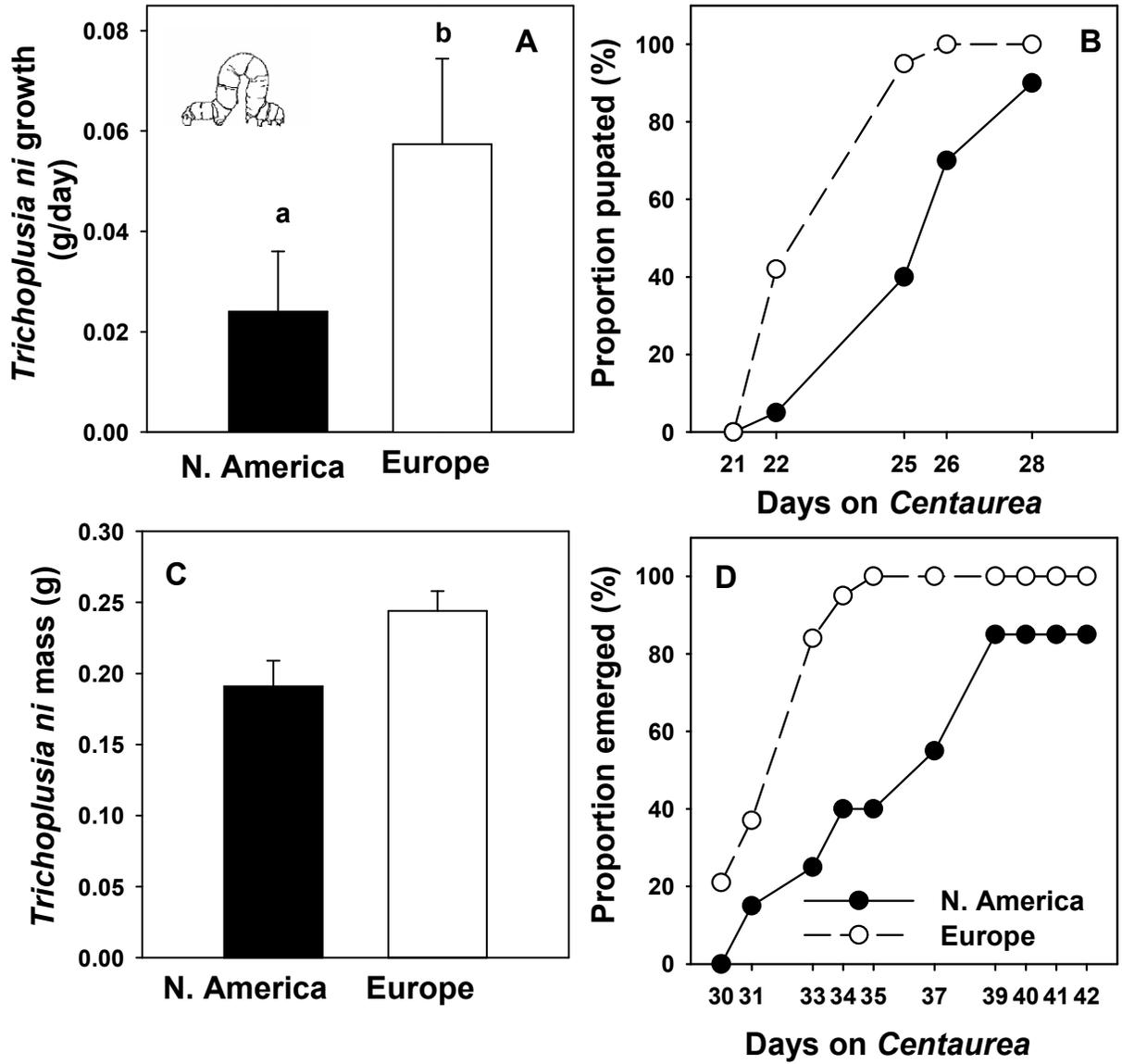


Figure 9

Melanoplus sanguinipes

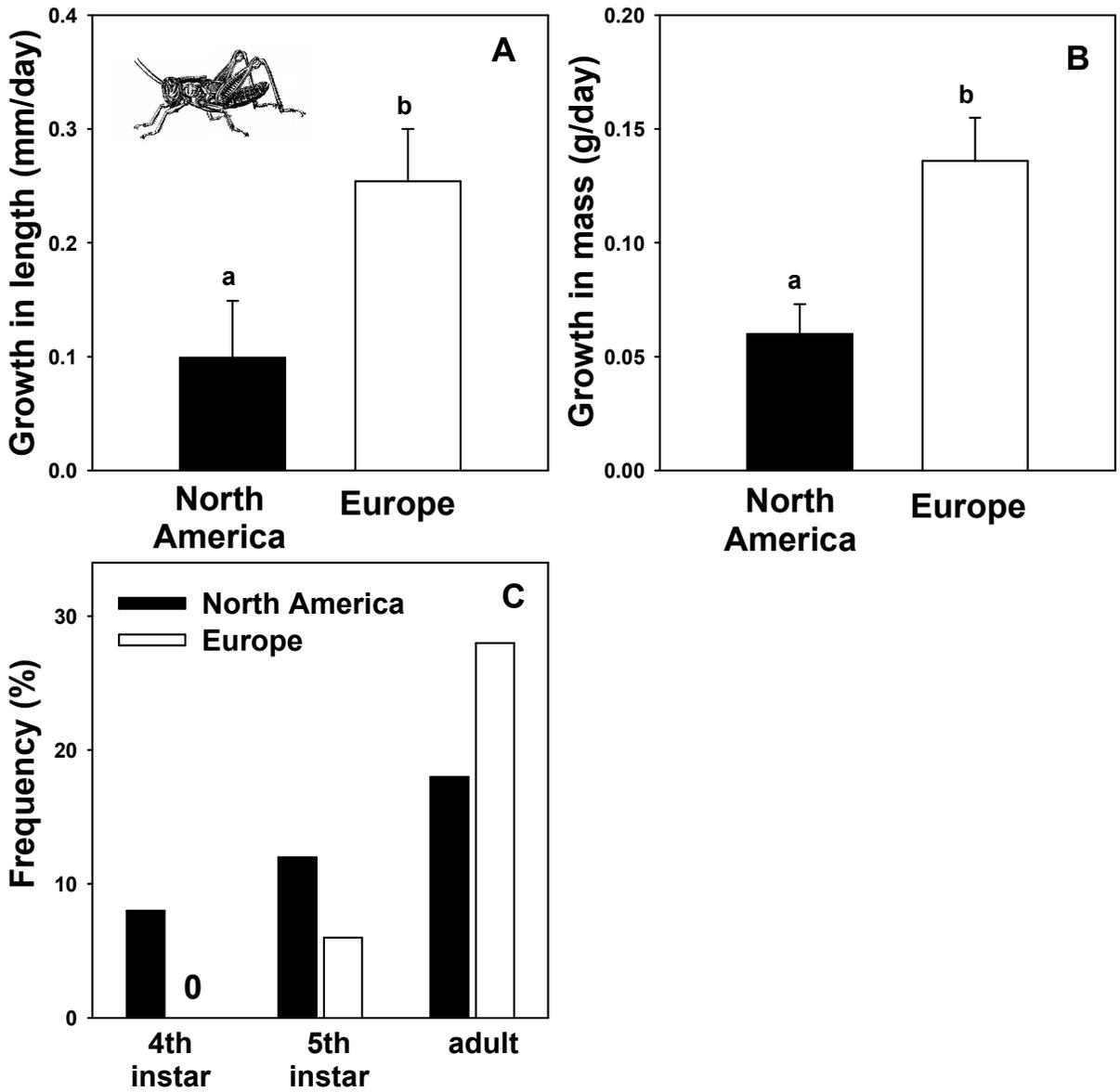


Figure 10

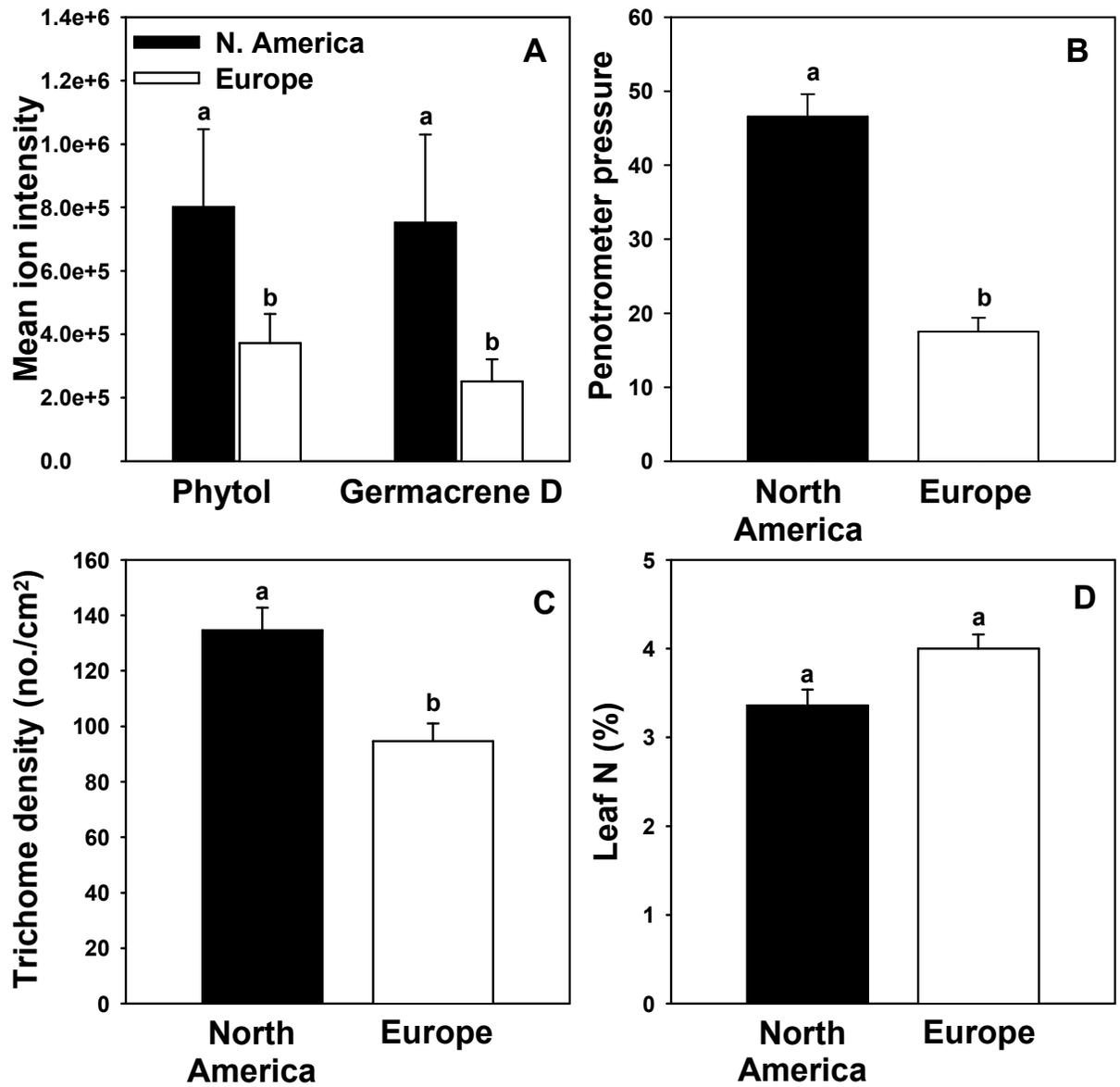


Figure 11

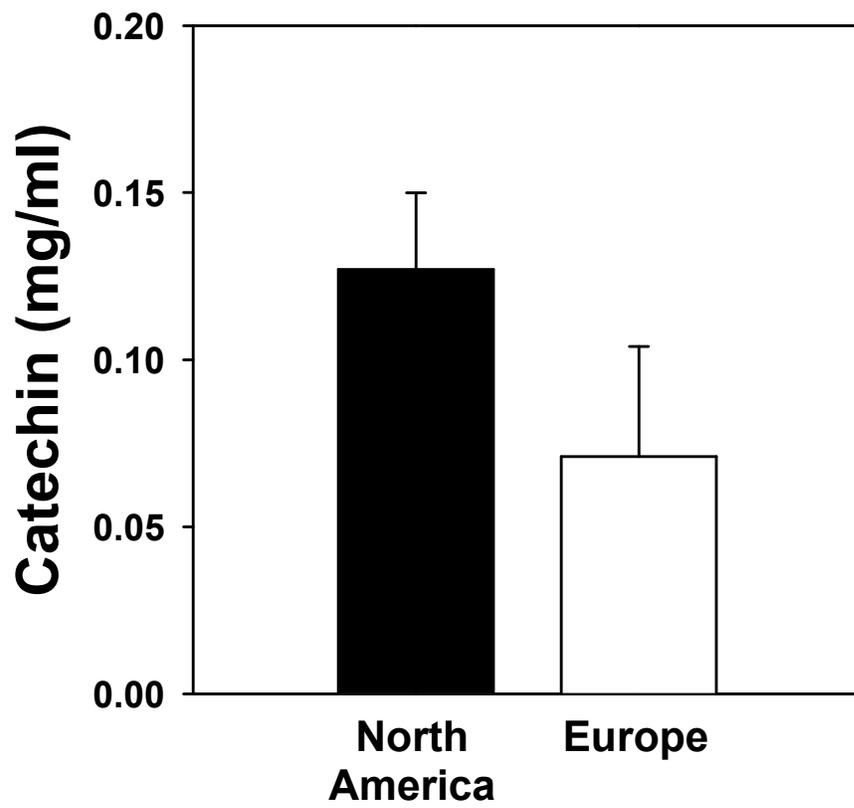


Figure 12

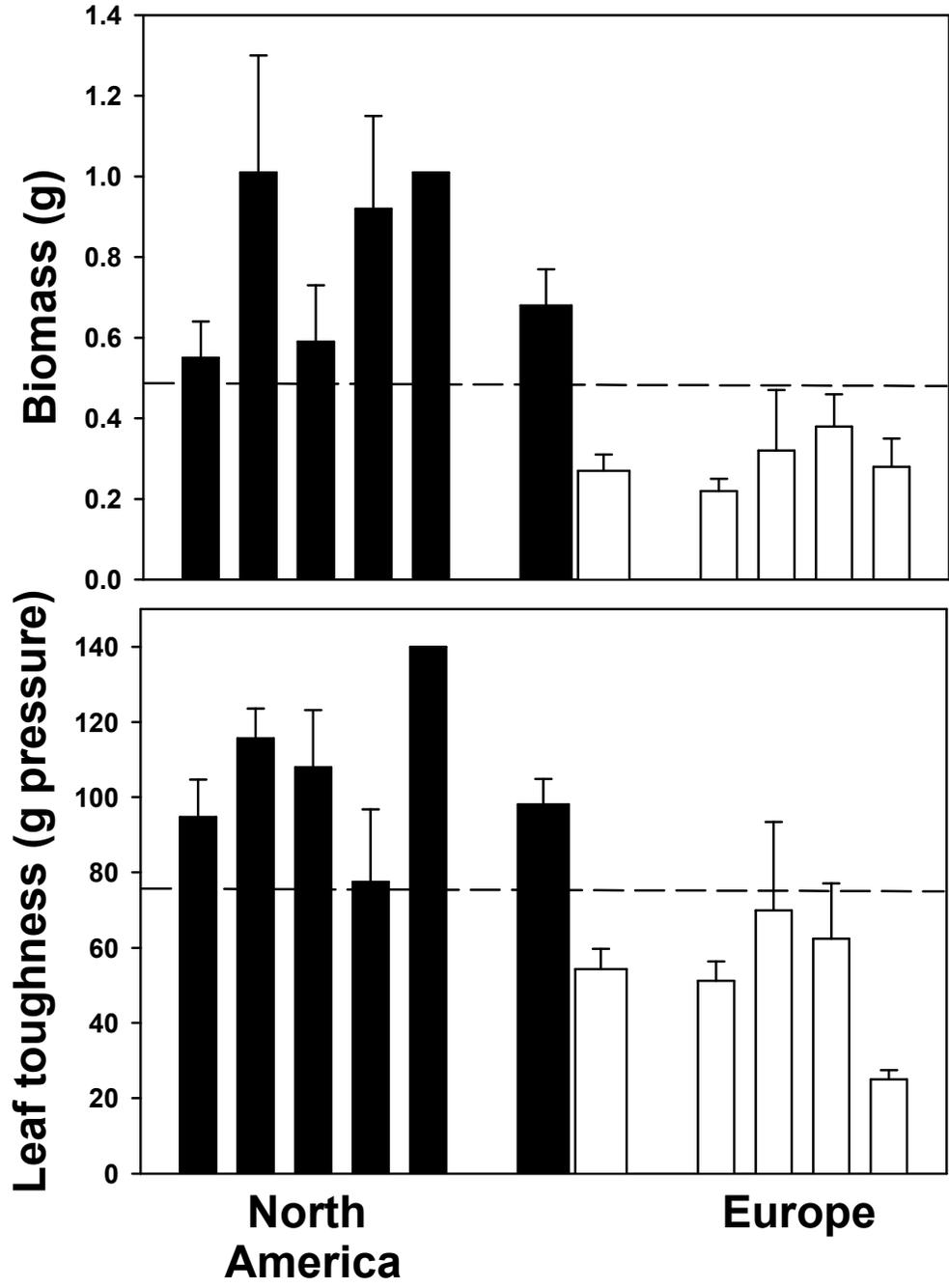


Figure 13

Supplementary Information

Supplementary Table 1. Populations used in growth and herbivory experiments.

<i>Centaurea</i> population	Latitude/longitude	Growth & competition	<i>Cyphocleonus</i>	<i>Agapeta</i>	<i>Trichoplusia</i>	<i>Melanoplus</i>
Lk.Voronezh (RUS)	51.48°/39.16°	*	*	*	*	*
Liski (RUS)	51.00°/39.33°	*				
Putlava (UKR)	49°00'/34.34°		*	*	*	*
Khotyn (UKR)	48.29°/26.29°					
Zolochiv (UKR)	49.48°/24.53°	*				
Czortova (UKR)	49.40°/24.66°					
Poltava (UKR)	49.34°/34.33°					
Novoarknanhel (UKR)	49.65°/30.78°					
Vienna (AUS)	48.11°/16.21°	*	*	*	*	*
Hornstein (AUS)	47.52°/16.26°	*				
Deutschkreuz (AUS)	47.59°/16.63°	*				
Timisesti (ROM)	47.13°/26.29°	*				
Roman (ROM)	46.55°/26.56°	*	*	*	*	*
Iasi (ROM)	47.09°/27.35°	*				
Falticeni (ROM)	47.27°/26.14°	*				
Crasna (ROM)	47.10°/22.52°	*	*	*	*	
Tecuci (ROM)	45.51° /27.26°	*	*	*	*	*
Fenestrelle (ITA)	45.02°/7.02°	*				
Vallouise (FRA)	44.50°/26.26°	*				*
Pontamafrey (FRA)	45.18°/6.20°	*				
Kembs (FRA)	47.41°/7.30°		*	*	*	*

Halle (GER)	51.28°/11.58°	*	*	*	*	
Bearmouth (MT)	46.43°/113.43°					
Bison Range (MT)	47.17°/114.15°	*				*
Blacktail Point (MT)	48.01°/133°					
Bozeman (MT)	45.40°/111.02°					
Butler Cr. (MT)	46.57°/NA	*				
Clearwater (MT)	47.00°/NA	*	*	*	*	
Eureka (MT)	48.52°/115.03°	*	*	*	*	
Glacier (MT)	48.40°/113.35°	*	*	*	*	*
Many Glacier (MT)	48.81°/113.60°					
Missoula (MT)	46.51°/113.59°	*	*	*	*	*
Mt. Jumbo (MT)	46.60°/113.57°	*				
Petty Mtn. (MT)	46.97°/114.38°					
Skalkaho Pass (MT)	46.10°/113.46°					
Sula (MT)	45.50°/113.58°					
St. Mary's (MT)	48.44°/113.25°	*				
Spokane (WA)	47.40°/117.29°					
Roanoke (VA)	37.24°/79.56°					*
Kellogg Biol. Station. (MI)	42.23°/85.23°	*	*	*	*	*
Hwy. 93 (ID)	45.18°/113.53°	*				
Elko (NV)	40.49°/115.45°	*	*	*	*	*
Hwy. 90 (WA)	47.30°/116.00°	*	*	*	*	*
Hwy. 93 (Canada)	49.01°/115.02°	*				
Salmon (ID)	45.10°/113.53°		*	*	*	

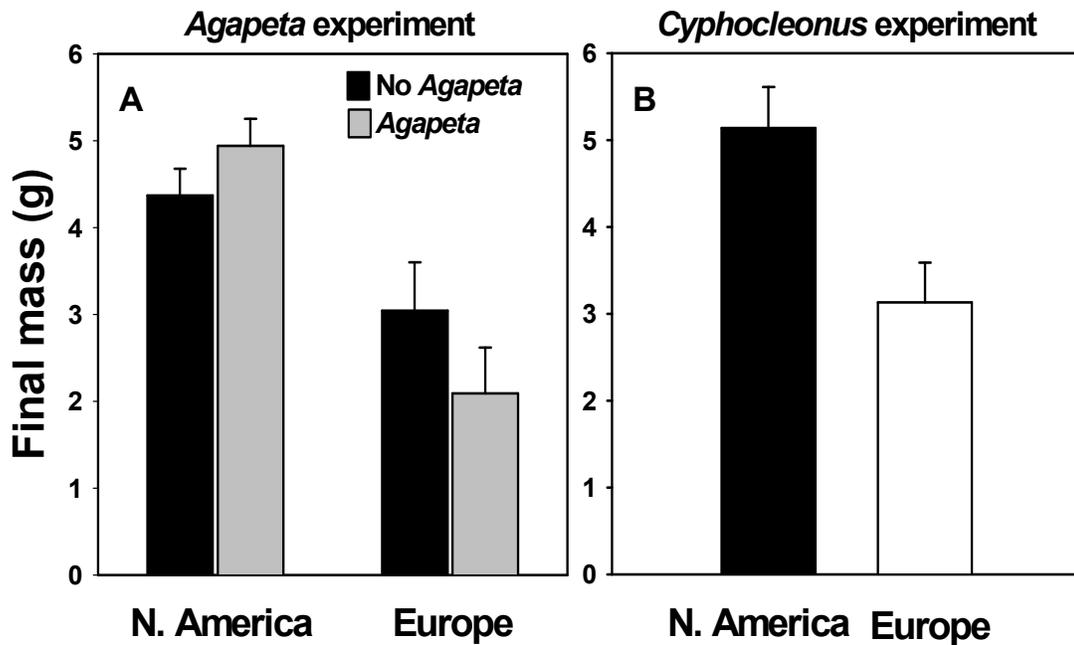


Figure 1. A) Mean total biomass of *Centaurea maculosa* from European and North American populations, and with and without *Agapeta zoegana* infecting roots; mass was measured at the end of the experiment. In an ANOVA model with region and *Agapeta* as fixed factors, and populations as a random factor ($F_{\text{region}}=2.82$; $df=1,15$; $P=0.035$; $F_{\text{Agapeta}}=0.338$, $df=1,15$; $P=0.568$; $F_{\text{population}}=5.40$, $df=15,250$; $P<0.001$). B) Mean total biomass of *Centaurea maculosa* from European and North American populations, with *Cyphocleonus achates* controls and treatment combined; mass was measured at the end of the experiment. ANOVA with larval mass as a covariate, ($F_{\text{region}}=10.11$; $df=1,15$; $P<0.001$, $F_{\text{population}}=1.44$; $df=15,84$; $P=0.277$).

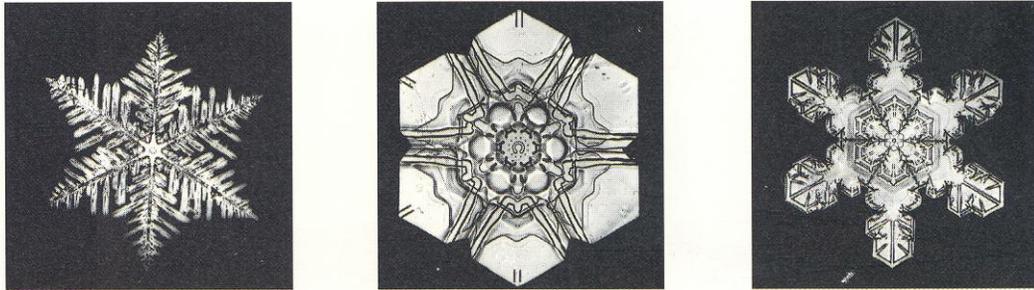
Chapter Two

**WINTER ECOLOGY:
NO CHILD LEFT INDOORS**

ABSTRACT/BROADER IMPACTS

In addition to conducting ecological research, I was a recipient of an NSF ECOS PhD Fellowship (2004-05), which provided me with training in the field of science education. This fellowship afforded me the opportunity to participate in research focused on improving science education in our local Missoula County K-12 schools, which will subsequently contribute to a national model of how authentic locally based ecological research can be introduced to improve the teaching and learning of science in K-16 learning environments. Specifically, as my ecological research generates exceptional interest in a lay audience, and because of the unusual ways that *C. maculosa* interacts with native North American species, it is an ideal subject to integrate with K-12 educational goals. To this end, I developed several ECOS web-published inquiries, including “Knapweed in the web” (Appendix 1), which were piloted at my assigned school, the Sussex School, grades K-8.

More generally, I believe that the basic thought processes that drive scientific inquiry, when integrated into K-12 educational curriculum improve the teaching and learning of science and help raise ecological awareness. Towards this goal, and as a requirement of the ECOS PhD Fellowship, a portion of my research focuses on effective methods in K-12 science education to the result of producing a place-based curriculum unit that was also tested in the classroom (and in the “outdoor classroom”). **Because I worked primarily with grades K-5 while at the Sussex School, I designed science curricula and inquiries for grades K-5.**



Photos by Wilson "Snowflake" Bentley

"Under the microscope, I found that snowflakes were miracles of beauty; and it seemed a shame that this beauty should not be seen and appreciated by others. Every crystal was a masterpiece of design and no one design was ever repeated. When a snowflake melted, that design was forever lost. Just that much beauty was gone, without leaving any record behind."



Wilson "Snowflake" Bentley 1925

Introduction

In much of North America, winter is part of our annual experience; most of the continent lies under snow and ice for portions of the year. Regardless, there is very little emphasis in traditional science education curricula on the problems faced by plants and animals wintering in North America. And in the snowy winter latitudes, practically no time is spent in the field during winter months. Some of my teacher colleagues believe that winter field trips or schoolyard activities would be unsafe, and

others report that they lack the expertise necessary to conduct winter ecology investigations.

I have found that concepts of winter ecology can easily be incorporated into K-12 science curricula in ways that address the National Science Education Standards (NCR 1996) using a combination of directed instruction and reading along with outdoor inquiries, all of which address multiple learning styles. In the investigations described here, I take an experiential approach to investigating winter ecology, because active inquiry-based learning allows students to construct their own meanings and understandings by connecting new information and concepts to what they already know or believe.

The following investigations were designed to span a month during the winter. In part one, I set the stage for subsequent outdoor investigations. This unit was designed for upper elementary school students, but it could be easily modified to be appropriate for older or younger students.

Week One: Indoor Preparation for Winter Fun and Safety

Part One: Staying Warm; The Art of Layering

The first step in a winter ecology unit is to provide students instruction on how to safely enjoy the winter world. This activity consists of a class discussion about how to stay warm when outdoor temperatures plummet followed by an inquiry about how

layering affects temperature. Finally, there is a demonstration and discussion to apply what was learned in the inquiry to appropriate winter clothing with the help of a student volunteer “model”.

I engaged students in a discussion of the preparations necessary to *safely* enjoy the winter outdoors (see Appendix A for an informational parent letter to send home with students). In northern latitudes many students have participated in outdoor winter activities such as skiing or sledding, and everyone has had to dress warmly when it is cold outside, so most students are familiar with the advantage of bundling up in warm layers of clothing.

Students investigate the effects of layers of clothing on heat retention using mugs of hot water (representing an organism), thermometers to measure heat loss over time, and different numbers of insulating layers (zero, one, and two). By way of introduction to this inquiry, describe the traditional British Isles use of a tea cozy; for the perfect cup of tea, the teapot is covered with a thick, insulating tea cozy, which will keep a pot of tea warm for hours.

I found that student volunteers enjoyed modeling the appropriate layers (purchased from a thrift store) needed to stay warm when it is cold outside. Some key concepts to be covered include the importance of avoiding cotton clothing, wearing a “wicking” synthetic thermal base layer next to the skin to increase winter comfort, demonstrating several additional insulating layers of synthetic fleece and warm wool, a wind and waterproof “shell” as an outermost layer, a warm hat, gloves, wool or synthetic socks and waterproof boots over extremities, which cool down very

quickly when exposed. From these introductory lessons, students learn how to dress to stay warm during outdoor investigations.

Part Two: Winter Survival Strategies; How Do They Do It? (Animals in Winter)

Through the following set of exercises, students learn the relative advantages and disadvantages of the three different common winter survival strategies described below (migration, hibernation, and resistance) via a combination of group discussion, book research, a game, and brief student presentations.

Migration

Migration is a strategy that allows escape from cold northern winters, but migration is a perilous journey. First, it is energetically “expensive” to travel long distances. Birds must accumulate reserve energy for migration of up to 50% of their total body weight as fat before departure! Even these energy reserves might not be enough for long migratory flights, however. Other perils of migration include strong winds and storms that may carry birds far off course, cold rains that may result in hypothermia, fog that may confuse their sense of direction, and light beams (over airports, for example) that also confuse their sense of direction. Birds often crash into tall lighted buildings and lighthouses, as the lights attract night migrating birds.

However, migration is a risk some birds must take. *Birds whose food source is unavailable during a northern winter (flying insects, small fishes found in shallow water, etc.) have no alternative but to migrate.* Because it is much more energetically “expensive” to travel overland than to fly, mammals generally do not migrate. An exception to this rule is the migration of whales along the entire distance of the Pacific and Atlantic coastlines of North America. Swimming, even for these great distances, provides an energetic savings even greater than flying.

Hibernation

Like migration, hibernation allows animals to avoid many of a northern winter’s stresses. Hibernation comes with its own set of associated risks and costs, however. Hibernating mammals are endotherms (“warm-blooded” animals) that have the ability to enter a state of reduced metabolic activity. When mammals hibernate, their body temperature tracks ambient air temperature, much like ectotherms, or “cold-blooded” animals. To avoid subfreezing temperatures and freezing to death, hibernating animals must seek refuge in places where the temperature is unlikely to drop below 0° Celsius, or 32° Fahrenheit. Generally, if the hibernating mammal’s body temperature approaches freezing, it must arouse itself and return its body temperature to normal before re-entering a hibernating state. Waking up like this is, of course, extremely energetically costly. Ectotherms such as reptiles and amphibians also hibernate as a means of surviving the winter season, and like hibernating mammals,

they too need to find refuge where they can avoid sub-freezing temperatures to assure their survival.

Resistance

Some animals and plants simply resist extreme cold stress and snowcover. Plants, as well as winter-active birds and mammals that do not migrate or hibernate must cope with cold temperatures, snow and ice cover. There are *many* different interesting plant and animal adaptations for coping with the cold and snow of winter. A few of the adaptations animals have to resisting the cold winter environment include fluffing fur or feathers to trap air warmed by the body, increasing insulative fat thickness, increasing the thickness of their fur or feathers, increasing food consumption, curling up to reduce exposed surface area, huddling together (communal nesting) to decrease exposed surface area, and creating a warmer microclimate via nest building or tunneling in the subnivean zone under the snow to reduce the difference between body and air temperatures. An interesting adaptation involves transferring heat between arteries carrying warm blood from the body core and parallel veins carrying cooler blood from the extremities. This process, called counter current exchange, decreases heat loss from long, thin legs (for example deer, wolves, wading birds) because arterial blood is pre-cooled on its way out and venous blood is pre-warmed as it returns to the body core. Some animals have more brown fat deposits, usually found between the shoulder blades, to produce heat through non-shivering thermogenesis. And some animals generate heat by involuntary shivering

as a last resort. For animals that have white winter coats, such as the ermine (shorttail weasel) and the arctic fox, the fur provides both insulation and camouflage in the snowy environment, keeping them warm and making them hard to spot by potential predators and prey. Both white fur and feathers lack the pigment melanin, and are hollow, leaving air spaces in the place of pigment granules which make them better insulation!

“What Would You Do?”

I introduced the background material on migration, hibernation, and resisting via direct instruction and group discussion. Next, students applied what they had learned in a simulation game on animal winter survival. Students were divided into groups of three or four. Each group received a printed description of an animal and its niche (“What Would You Do?” cards, Appendix B) and field guides to birds and mammals of North America (any field guides available in your classroom or school library can be used). Based upon the printed description, students attempted to discern their animal’s identity and then came to a group decision about which strategy (migration, hibernation, or resisting winter) was the best choice for their animal and why. Student groups completed the activity with short presentations to their classmates. After reading their “What Would You Do?” card to the rest of the class, each group explained what their animal’s best winter survival strategy was and why it seemed to be the best choice. I also allowed each group to call on their classmates to guess their animal’s identity. While the “What Would You Do?” cards in Appendix B are

typical of the Northern Rocky Mountain Region, this information can easily be adjusted for other regions as needed.

Week Two: Outdoor Winter Schoolyard Investigation

Taking students outside into their schoolyard in winter gives them a chance to apply what they have learned in their classroom investigations and learn some new ideas. All of the following exercises can be used in the schoolyard or on a field trip.

Activity One: “Track ID Relay”

One way to learn about animals that do not hibernate or migrate during winter is to look for their tracks and signs. Through a tracking game, students learn to identify our winter-active mammals by their tracks. I began this investigation by familiarizing students with some of the common tracks left by the mammals of our region.

Exceptional resources include the following: *Crinkleroot’s Book of Animal Tracks and Wildlife Signs*, *Track Watching*, *Footprints in the Snow*, or relevant chapters from *Follow the Trail: A young Person’s Guide to the Great Outdoors*. I read these to my students. This introduction was followed by a brief group discussion of the reading, and then students completed the introductory “Animal Tracks!” worksheet (Appendix C).

To play the “Track ID Relay” game, I divided students into two equal teams. I then asked them to form two separate lines facing each other, about 10 feet apart.

The laminated animal track drawings (copied and enlarged from animal track field guides or the “Animal Tracks!” worksheet) were placed in a row on the ground between the two teams. As I laid them out, students were asked to provide a little background information about each different type of track based on the previous readings and discussions. The last step was to assign a number to each student on each team (one through the highest number of players on each team). The goal of the game was for a team to collect the most correctly identified tracks. First I called out the name of an animal followed by a number. The two players on opposite teams that shared this number raced to be the first to find the animal track of the animal named. When there were an odd number of students in the group, I rotated students so that a different player sat out for a round, allowing everyone gets to play. Players had only one chance to correctly identify the track called; if they touched the wrong track, they went back to their place in line immediately. Ties were broken by placing a track back on the centerline and calling it again during a later round. Shuffle the order of the tracks between rounds. Finally, I extended this investigation by having students look around the schoolyard for evidence of animal tracks and signs, and write a story describing the local fauna.

Activity Two: “Snowpack Metamorphism and Snow Shelters”

This investigation requires some snow cover. Prior to this activity, and as an introduction to snowflakes (snow crystals), I read *Snowflake Bentley* to my class. Snowflakes are made of ice. The water molecules in an ice crystal freeze into a six-

sided form. The most basic snow crystal is a six-sided or hexagonal prism, but they may also branch to form elaborate, lacey shapes as water vapor in clouds freezes into ice. Differently shaped snow crystals form under different temperature and moisture conditions. During this investigation, students will learn about the natural deterioration of snow crystals to form rounded ice grains in the snowpack, a process called destructive metamorphism by scientists. As a result of destructive metamorphism, snowflake crystals pack together over time, increasing the density and the mechanical strength of the snow substantially even within a few hours after snowfall.

Students will build a snow house after the Athabaskin quin-zhee snow shelter traditionally used by native peoples of the boreal forest regions of North America to learn about snow metamorphosis. Snow shelters like the Athabaskin quin-zhee are thermally stable structures because snow provides good protection from the wind and is a good insulator; the snow functions much as a winter coat or a tea cozy works to prevent heat loss from your body or a pot of tea. A snow shelter warmed by body heat alone will be noticeably warmer than the outside temperature.

To construct a quin-zhee, snow is shoveled into a large pile. The weight of the overlying snow is sufficient to cause destructive metamorphism of the snowpack so that air spaces are reduced in size and individual ice grains pack together and bond. By piling up loose snow and letting it “set” a short while, destructive metamorphism increases the strength of the snow enough that the inside living quarters can be hollowed out, and the domed outside of the structure becomes self-supporting. The

interior of the quin-zhee can become as much as 25° warmer than the outside air temperature.

To make a quin-zhee, students first outlined a large circle, about 10 feet in diameter, with their footprints. Then they made a big pile of snow, about 6-8 feet high, within the circle. The snow was allowed to settle and compact for an hour or two while the students went back to their classrooms to other lessons. Later the students took turns hollowing out a small entrance, about 2 feet in diameter (small enough to keep drafts out), and an interior room just above the level of the entrance by throwing snow out the door. When completed, student groups took turns using thermometers to record temperatures measured in and outside of their quin-zhee. Finally I asked students to write a story about a family using the shelter as protection from a Montana blizzard.

Activity Three: “Bird Brain Tag”

This lesson builds upon concepts learned during the “What Would You Do?” activity. During an active game of tag, students are asked to “think like a bird” to survive the winter. To prepare for the outdoor part of this lesson, students review what they learned about migrating and resisting as winter strategies. Bird field guides were helpful during a review to prepare students for this activity.

Students role-played different bird species, and had to decide whether to migrate or stay and face (resist) the winter. Both strategies have their associated stresses and risks, and many birds do not live through the winter regardless of their overwintering

strategy. Each student received a “bird brain tag” game card (Appendix D, laminated and tied with yarn to hang as a necklace) printed with either the name of a bird species and what it eats, a migratory stressor (cause of stress), or an overwintering stressor. Students started this game on the midline of a field. The two “end zones” were designated as the “migratory home” and the “winter cafeteria”. Students with migratory stressors and overwintering stressors were asked to head out in opposite directions from the centerline towards one of two finish lines at opposite “end zones” of the field. Their mission was to attempt to tag any bird species before they could successfully make their round-trip annual migration OR make it through the winter (i.e., make it to their respective finish line in their designated “end zones”). Before the starting signal was given, students playing bird species had to decide whether to migrate or stay and resist the cold. This decision was based primarily on whether or not food needed by their species is available in North America during the winter, and their decisions were reviewed by the class as a group before proceeding. Once the starting signal was given, students playing birds tried to make it to the appropriate “end zone” finish line without getting tagged, which represented living through the winter. Students enjoyed playing several rounds of this game, switching roles in between games.

Week Three: Winter Ecology Field Trip

This field experience consists of a set of three investigation stations set up at a local park, field station, or recreation area. Student groups cycle through each for a glimpse of a slightly “wilder” winter world. Ideally, each station has a leader; if this

is not possible, stations can be set up ahead of time, and then the entire class can cycle through each station together. This set of investigations takes one and a half to two hours.

Field Trip Activity One: “Snow is a Blanket - the Subnivean Zone”

This investigation requires some snow cover. Students dig snow pits to examine a snowpack profile, allowing them to learn about snowpack layering from different snowfall events and the resulting subnivean zone. The subnivean zone is the gap between the snow and the ground beneath. This space is formed when the warmer unfrozen ground melts the snow above it. Water vapor condenses and freezes on the undersurface of the snow, forming a solid ceiling. Snow is a very good insulator; the air in the subnivean zone remains just above freezing, even when outside temperatures dip below zero. Snow cover also reduces daily temperature fluctuations under the snowpack. Under as little as approximately one and a half feet of snow, the temperature of the subnivean environment is almost constant.

The presence of adequate snow cover is of critical importance to the overwintering success of many small mammals. Small mammals such as mice and voles remain active throughout the winter in the subnivean zone, feeding on grasses, seeds, fungi, and tender sprouts. Some of these rodents are highly territorial in spring and summer, but during the winter they will tolerate members of their own species and may even form communal nests under the snow, benefiting from the effects of combined body heat (huddling). Although invisible from the surface, subnivean

rodents are still subject to predation from above and below. Weasels hunt beneath the snow, as does their larger relative, the American marten. Large predators such as coyotes, foxes, and owls use their keen hearing to detect activity under the snow, and then pounce through the surface to capture their prey.

At this station, students will examine a temperature profile from the snow surface down to the subnivian zone. Students can either make a data sheet or use one that is pre-made. First they use a thermometer to measure air temperature just above the snow surface. Then students measure the temperature of the snow one-third and two-thirds of the distance from the soil. Finally, they make a measurement at the base in the subnivean zone. Students need to allow the thermometers to stay at each measurement location for approximately three minutes before they record the temperatures at various depths in the snowpack. Students were asked to draw a picture of the snow pack and label the temperature recorded at each location. Then they wrote a summary of their observations and discussed what their data meant for the survival of small mammals. As an extension, students can look for signs of burrowing animals and their subnivian tunnels.

Field Trip Activity Two: “Tracks and Traces”

This exploration builds upon concepts learned during the “Track ID Relay”. During a short nature walk, students look for animal tracks and identify other signs of animal activity (scat, hairs, feathers, dens, nests, gnawed seeds or nuts, trails, beds, etc.). Using their track worksheet and with the help of animal track field guides (you

may use any field guides available in your classroom or school library), students compile a list of animals living nearby and using the trail.

As an extension, I constructed “track catchers” to determine what animals were actively using our schoolyard. To make “track catchers”, I fastened white contact paper sticky-side up to plywood boards. Students placed these contact paper-covered boards in several locations in the schoolyard. I surrounded the boards with a fine dusting of powdered charcoal, and placed a teaspoon of peanut butter or oatmeal in the center of half the boards, and a teaspoon of tuna in the center of the other half of the boards. “Track catchers” were left out overnight, and animals visiting them left exquisitely detailed tracks behind on the contact paper, which I preserved with a thin coating of spray lacquer. Students were then asked to identify the tracks they collected with the “track catchers”.

Field Trip Activity Three: “Blind Tree ID”

For this investigation I identified four to six trees and shrubs commonly found in our area, and collected twig samples. Each was identified to species, along with the common name. Field guides and local experts can be very helpful resources at this station. To prepare the students I shared two or three winter-identifying characteristics for each tree and shrub species sampled, and passed the twigs around for them to look at while I described them. After I looked carefully at all the samples, I divided the students into pairs, and asked each pair to stand two or three feet away from other pairs of students. Next each student pair was given a blindfold and asked

to decide which of them would wear the blindfold first. The blindfolded team member was given one of the twigs and asked to identify the twig sample *by touch* based on their memory of the characteristics I had described for each species. They were allowed to ask their partner yes or no questions about the winter-identifying characteristics of the twig until they determined the correct identity of their twig. When all students had guessed the correct tree or shrub name, I collected all samples and had them switch roles. It is important to hand out **different** samples to each pair of students for the second round. This activity, which made extensive use of the sense of touch in addition to sight, was quite effective, as many of my students were able to identify the local trees and shrubs used for this activity several months following the exercise.

Following the field trip, I kept our twig samples in water in the classroom, and as an extension, students were asked to draw their favorite twig. They used field guides and other available references to research the native American uses of the tree or shrub as well as local wildlife uses, and wrote a short story describing human and wildlife interactions with these plants to accompany their drawings.

Conclusion

This winter ecology unit is a very effective way to actively involve students in discovering the winter world around them while meeting many of the National Science Education Standards. Students learned about how to stay warm when it is cold outside, how layering affects temperature, the relative advantages and

disadvantages of three different common animal winter survival strategies (migration, hibernation, and resistance), to identify common winter-active mammals by their tracks, and to identify common local trees and shrubs during winter as well as their uses by local animals and native American peoples. Students also learned about snow crystals (snowflakes) and their natural deterioration to form rounded ice grains in the snowpack (destructive metamorphism), snowpack layering from different snowfall events and the resulting subnivean zone, and the insulative properties of snow. Students enjoyed the unit, and the quality of student understanding remained high several months following completion of the unit, based upon informal group discussions, question and answer sessions, and student portfolios.

Appendix A

Informational Parent Letter

Dear parent:

Your child has the opportunity to explore their winter world on a field trip to a local park or open space. With a little preparation, it is possible to *safely* enjoy the winter outdoors! You may already know the advantage of bundling up in a number of warm layers for safe and toasty winter fun, avoiding cotton clothing. Wearing “wicking” synthetic long johns as your base layer will increase winter comfort. Add several insulating layers of synthetic fleece and warm wool, a wind and waterproof “shell” as your outermost layer, a warm hat, gloves, wool and/or synthetic socks and warm boots and you’re set! It’s as simple as that. Remove layers as you begin to warm up, avoiding overheating, and add layers before you get cold. With that in mind, here are a few *suggestions* for things you may want your child to bring along on their winter adventure:

- ❖ **3-4 warm winter clothing layers**
- ❖ **Warm, preferably waterproof boots**
- ❖ **Warm wool or synthetic socks**
- ❖ **A warm hat**
- ❖ **Warm mittens or gloves**
- ❖ **A scarf or neck gaiter**
- ❖ **A daypack**
- ❖ **Sun block**
- ❖ **Sun glasses**
- ❖ **Water bottle, sports drink, or a thermos of hot soup or cocoa**
- ❖ **Bag lunch**
- ❖ **Snacks or energy bars**
- ❖ **Binoculars, if you have them**
- ❖ **A hand lens if you have one**

Your child’s teacher will bring the following:

- ❖ **A field notebook & pen or pencil for each student**
- ❖ **A first aid kit**

Appendix B

“What Would You Do?” Cards:

Descriptions of Animals and their Niches

- (1) **I am a tiny mammal. I eat mostly insects and their eggs and larvae, and I need to feed almost continuously to fuel my high metabolism!**
- (2) **Small flying insects make up most of my diet. I am a small mammal, but I may take a large number of flying insects such as mosquitoes in a single feeding bout, which makes me the perfect guest near homes and cabins!**
- (3) **I live in boulder fields at higher elevations. I am a small mammal and I eat plants only. I harvest plants during the summer months that I can store in “hay stacks” deposited under rock ledges in my territory.**
- (4) **You can tell I’m nearby when you see my runways along the ground in dense plant cover. I am a small mammal, but I eat over 70 kinds of plants, and I’ll even eat their seeds, roots, and fungi!**
- (5) **I am a medium-sized mammal and I eat enough plants during the summer months to put on some fat. I usually live in rocky outcroppings at high elevations, where I can burrow. I build my nest about 1 meter underground, where the temperature will remain just above freezing.**
- (6) **I eat most small mammals, birds, and even some plants. I sneak up on my prey and pounce on it! I’m a medium sized mammal with a thick coat and a bushy tail.**
- (7) **I am a large mammal that will eat practically anything! I forage primarily during the day, but am also sometimes active at night. I live in a variety of habitats world-wide.**
- (8) **I forage at night, and I’ll eat almost everything. I’m a medium-sized mammal, and I live in a variety of habitats. My dens can be found in rock crevices, hollow trees, the abandoned burrows of other animals, and caves.**
- (9) **I am a large mammal that eats both plants and animals. I must eat enough in the summer months to put on large amounts of body fat. My dens may be found at the base of a hollow tree, under fallen logs, in rock cavities, or excavated into a hillside.**
- (10) **I am a large animal that uses different habitats at varying elevations depending upon food availability. I need to be able to find enough of the plants I like to eat, such as grasses, forbs (leafy plants), grains, tree and shrub leaves, buds, bark, and twigs, and even lichens!**

- (11) **Fish are my only prey. I plunge into the water, taking fish at or just below the surface. I am a medium sized animal. I build my nests on platforms near the water.**
- (12) **I am a large carnivore. I like heavily wooded areas and have a large home range, where I can sneak up on my prey. I will actively stalk my prey anytime day or night, depending upon when available prey species are active. I prefer larger prey species, and I generally prey upon the weaker animals. When my preferred prey is unavailable, I will take whatever is available!**

What Would You Do?

Key to Descriptions of Animals and their Niches

- (1) Shrew / resists
- (2) Bat / hibernates
- (3) Pika / resists
- (4) Meadow vole / resists
- (5) Yellow-bellied marmot / hibernates
- (6) Red fox / resists
- (7) Human / resists
- (8) Raccoon / resists
- (9) Black bear / hibernates
- (10) Elk / vertically migrates
- (11) Osprey / migrates
- (12) Mountain lion / resists

Appendix C
“Animal Tracks!” worksheet

(Note to teachers: The track drawings below appear next to the name of the mammal that made them; please scramble them for student use).

Match the following drawings of mammal tracks with the appropriate mammal:
(mammal track drawings by Kim A. Cabrera, <http://www.bear-tracker.com/mammals.html>)

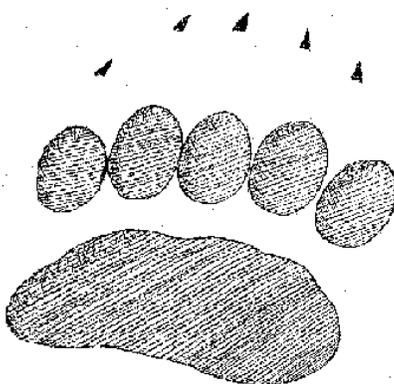


Hind Foot



Front Foot

Raccoon



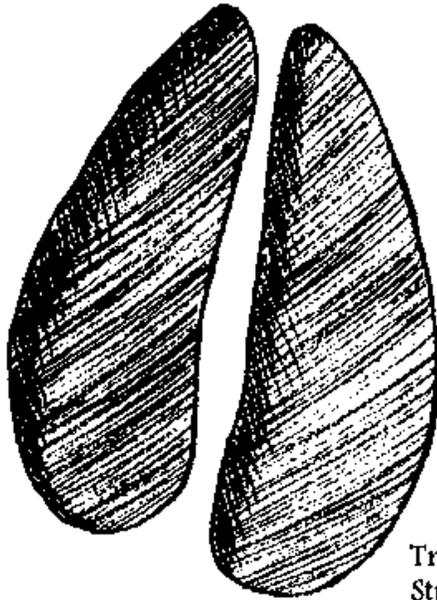
Front Foot



Hind Foot

Grizzly Bear

Elk



4¾" L x 3" W

Trail width: 8"
Stride length: 26"-28"



Trail Pattern

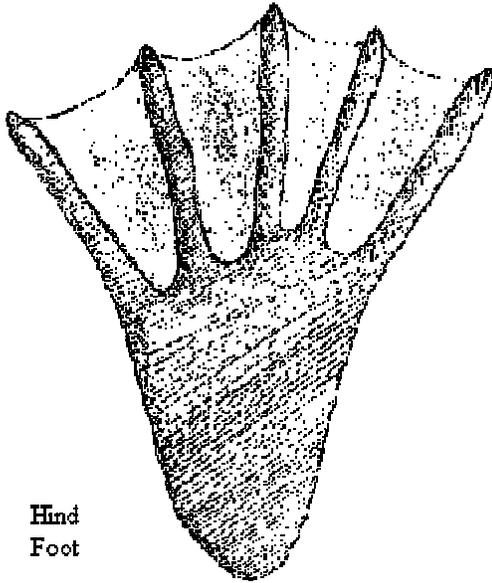


Hind feet

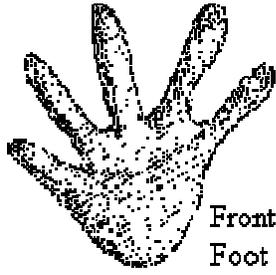


Front feet

Deer Mouse



Hind
Foot



Front
Foot

Beaver



Hind Foot



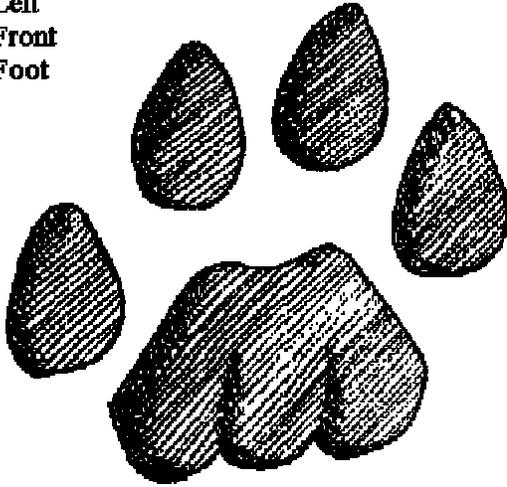
Front Foot



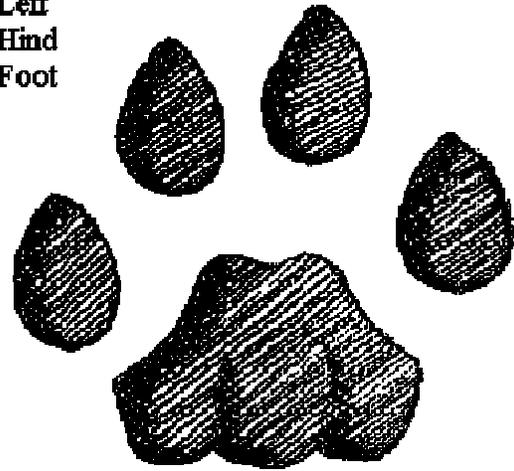
Trail Pattern

Red Fox

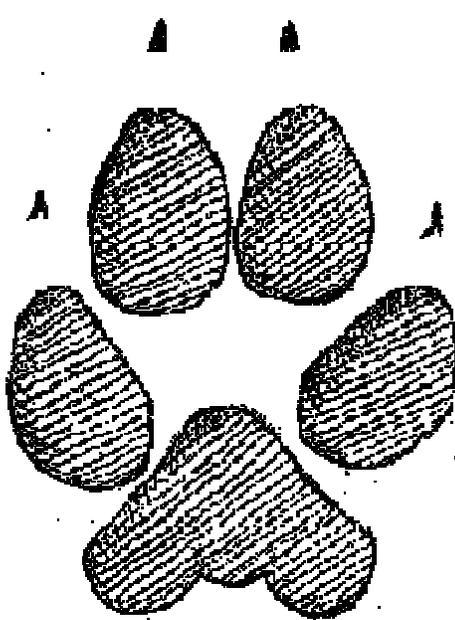
Left
Front
Foot



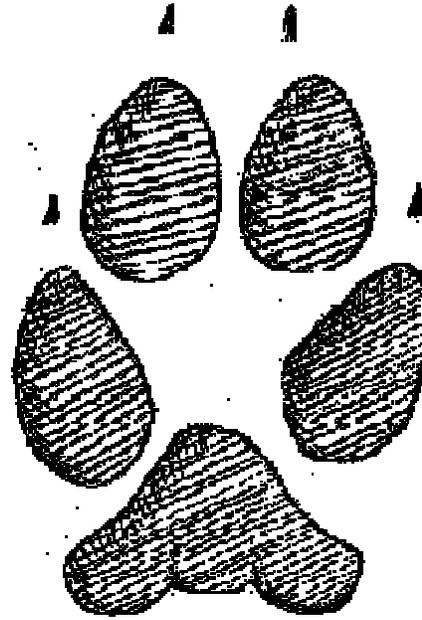
Left
Hind
Foot



Mountain Lion



Front Foot



Hind Foot

Coyote



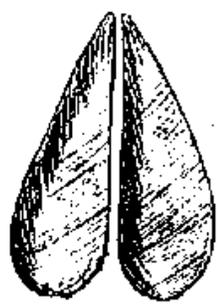
Hind feet



Front feet

Squirrel

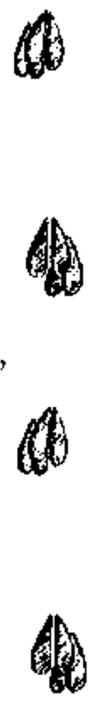
Black-tailed Deer



3 1/2" L x 2 1/4" W

Trail width: 6"
Stride length: 21"-24"

Toes spread when in soft mud or when the animal is running. Dewclaws show.



Trail Pattern

Appendix D

“Bird Brain Tag” Game Cards

- **Strong Winds and Storms**
(May carry birds far off course)
- **Cold Rain**
(May result in hypothermia)
- **Fog**
(May confuse birds’ sense of direction)
- **Light Beams**
(Over airports, for example; confuse birds’ sense of direction)
- **Tall Lighted Buildings and Lighthouses**
(Birds often crash into these structures as the lights attract night migrating birds)
- **Very Cold Temperatures**
(Staying warm during the winter requires lots of energy, and lots of high-energy food)
- **Snow and Ice**
(Can cover food sources)
- **Lack of High Energy Food**
(Large amounts of high-energy food are needed to make it through each cold winter day and night)
- **Black Capped Chickadee**/insect eggs and larvae, cached seeds, seed from bird feeders
- **Hummingbird**/ flower nectar, small insects, tree sap, visits hummingbird feeders
- **Downy Woodpecker**/insects, insect larvae and insect eggs in tree bark and plant stems, fruits, seeds, some tree sap, suet at birdfeeders
- **Yellow Warbler**/insects and other arthropods captured by flycatching, hovering, and gleaning (picking insects off tree leaves and branches); occasionally fruit
- **Osprey**/fish
- **Violet-green Swallow**/flying insects

- **Great Blue Heron**/mostly fish; also invertebrates, amphibians, reptiles, birds, and small mammals
- **American Robin**/invertebrates, especially earthworms, and fruit
- **White-breasted Nuthatch**/insects, insect larvae, and insect eggs from tree bark; nuts and seeds
- **Dark-eyed Junco**/seeds and insects, visits bird feeders

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Appendix 1: Knapweed in the web

1. CONTRIBUTOR'S NAME: **WENDY M. RIDENOUR**

2. NAME OF INQUIRY: **Knapweed in the Web!**

3. GOALS AND OBJECTIVES:

a. Inquiry Questions:

- 1) What are food webs?
- 2) Can you provide examples of local food webs?
- 3) Can food webs be altered by the introduction of non-native species?
- 4) Can you provide examples of non-native species?

b. Ecological Theme(s): Students learn about a local Missoula, Montana example of a food web altered dramatically by the introduction of exotic species.

c. General Goal: Students will become familiar with the concept of food webs and some of the ecological problems associated with invasive species and their control.

d. Specific Objectives: After completing this module, students will be able to:

- 1) Discuss food webs.
- 2) Identify the exotic weed species spotted knapweed.
- 3) Explain the possible effects of exotic species on native species distributions, including indirect effects such as food web subsidies.

e. Grade Level: 2nd – 5th

f. Duration/Time Required:

- Prep time: 15 – 30 minutes
- Implementing Exercise During Class: 30 minutes to 1 hour
- Assessment: 5 – 10 minute discussion/ question an answer session

4. ECOLOGICAL AND SCIENCE CONTEXT:

a. Background (for Teachers):

1) Food Webs: Food webs are representations of the feeding relationships that exist within a community. Briefly, food webs involve both species interactions and the transfer of energy and nutrients through an ecosystem. Food webs can also be simplistically represented as “food chains” or “food pyramids” (see below, Handout: Knapweed Food Pyramid).

Handout: Knapweed Food Pyramid



2) Spotted Knapweed: Spotted knapweed, *Centaurea maculosa*, was introduced into western North America from Eurasia early this century, and has since invaded millions of hectares of intermountain prairie, decreasing the abundance and productivity of native species and reducing local plant diversity. It is an **exotic** plant species because it did not evolve in the region where it has been introduced. *C. maculosa* invasion is often characterized by the almost complete competitive exclusion of native plants and the development of dense single species patches. Since its introduction into North America,

C. maculosa has also had a significant economic impact due to its negative effects on rangeland forage quality. *C. maculosa* occurs across most of Europe, but almost never at the densities achieved in North America and is never found as a landscape dominant.

3) Indirect Effects of Biocontrols: Exotic species removal is very expensive; it conservatively costs this country over \$8 billion annually to control exotic species. Removal of exotics is also a difficult practical problem. Hand-pulling weeds simply doesn't work in areas where many species of exotics occupy huge areas. A common approach is to kill these plants with herbicides. Yet while effective, this can also negatively influence native plant species as well as wildlife. It can also be prohibitively expensive. An alternative approach to remove weeds is **biological control**. Biological control involves introducing a **specialist** herbivore (or pathogen) from an exotic plant's native range to control the plant where it is introduced. A specialist biocontrol agent is an herbivore that only attacks the target weed. Screening biocontrol agents for host specificity prior to introduction is extremely important. There have been a few cases in which biocontrol agents, many years after introduction, have switched host plants and fed on native plants that were closely related to the target weed.

Biological control agents that are introduced into new regions are also exotic species, and they often establish high population sizes. Biocontrol agents can also maintain high population sizes because, at least initially, they have a super abundant food source (the weed!). At least in theory, large populations of biocontrol insects can severely reduce the population size of their host plants.

Thirteen species of insect herbivores have been introduced to control *C. maculosa* since the 1970's; however, the consensus is that these biocontrols have not yet been successful. Pearson and Callaway (2003) discovered that host-specific biocontrol agents can exhibit substantial nontarget effects through indirect interactions and food-web subsidies. Host-specific biocontrol agents can exhibit nontarget effects on native species and ecosystems.

b. Background (to present to Students):

Knapweed food pyramid game:

Begin by engaging the students in a brief (5-10 minute) discussion of food webs. Ask them if they can tell you what a food web is and provide examples.

Next have the class build a food pyramid. Without providing any background information, tell the students that they need to arrange themselves into a pyramid that will represent a generalized food pyramid. Have each student decide whether they want to role-play knapweed, gall fly larvae, deer mice, or great horned owls during the food pyramid game. At this time, have them write their species name on a slip of paper. Once they've decided on their roles, let them know that the knapweed plants will provide the base of the pyramid upon which all the other players are supported. Ask them why this is the case. Ask whether enough students are playing knapweed plants to support the weight of the rest of the class. If not, they can change roles, preferably with the bigger students assigned to the base of the pyramid. Have them guess the position of the rest of the species in this local food pyramid example and show them the food pyramid handout (from the bottom up: knapweed, gall flies, deer mice, and great horned owls). Give them a few minutes to build their pyramid and take a photo! Then lead a discussion/question and answer session on the exotic plant spotted knapweed and the indirect effects of the gall fly introduced for the biocontrol of knapweed.

5. MOTIVATION AND INCENTIVE FOR LEARNING: It's a fun game!

6. VOCABULARY:

Food Webs: Food webs are representations of the feeding relationships that exist within a community.

Spotted Knapweed: Spotted knapweed, *Centaurea maculosa*, is a non-native, or exotic weed that was introduced into western North America from Eurasia early this century.

Exotic plant species: A plant that did not evolve in the region where it has been introduced.

Biological control: Biological control involves introducing a **specialist** herbivore (or pathogen) from an exotic plant's native range to control the plant where it is introduced.

Specialist: A specialist biocontrol agent is an herbivore that only attacks the target weed.

7. SAFETY INFORMATION: N/A

8. MATERIALS LIST (including any handouts or transparency masters):

- 1) Several color copies of the knapweed food pyramid handout
- 2) Several slips of paper and pencils or pens for each student

9. ASSESSMENT:

Assess students' grasp of this lesson through a brief discussion/question and answer session following the game.

10. EXTENSION IDEAS:

Have students draw expanded prairie ecosystem food webs that include the exotic species they covered in this exercise. Ask students to hypothesize about how the exotic species spotted knapweed and the gall fly may affect the expanded prairie food web.

11. SCALABILITY

This exercise may be scaled down accordingly to be used with K/1st students.

12. REFERENCES:

Pearson, D.E. and R.M. Callaway. 2003. Indirect effects of host-specific biocontrol agents. *Trends in Ecology and Evolution* 18:456-461.

13. LIST OF EXPERTS AND CONSULTANTS

Dean E. Pearson
Wendy M. Ridenour

14. EVALUATION/REFLECTION BY FELLOWS AND TEACHERS OF HOW IT WENT:

Students and teachers had fun with this exercise, and students learned a lot. Good “sense of place” exercise as well.