Testing for nativity, competitive, or allelopathic effects in interspecific interactions of *Centaurea pratensis* (meadow knapweed)

2011 Report

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Institute for Applied Ecology

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Preface

IAE is a non-profit organization dedicated to natural resource conservation, research, and education. Our aim is to provide a service to public and private agencies and individuals by developing and communicating information on ecosystems, species, and effective management strategies and by conducting research, monitoring, and experiments. IAE offers educational opportunities through 3-4 month internships. Our current activities are concentrated on rare and endangered plants and invasive species.

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Introduction

Invasive species pose a threat to native ecosystems worldwide (Wilcove *et al.* 1998). Many non-native invaders can alter fundamental ecosystem properties such as nutrient cycling and plant community composition (Vitousek 1990, Mack *et al.* 2000). Along with threats to ecosystem properties and native plant diversity, exotic species invasion can inflict severe economic costs from control and habitat losses (Pimentel *et al.* 2000).

Comparisons of invasive species in their native and introduced ranges are rare (Maron *et al.* 2004, Hierro *et al.* 2006), but offer insights into which mechanisms may contribute to their invasion potential in novel environments. While invasive species often dominate communities and show potential to form monocultures in their introduced range, they are often less prolific in their native range. Though competition is considered a major mechanism for invasive species success in novel environments, other mechanisms could also contribute to the success of the invader. Allelopathy, the negative effect of one plant on another through the release of chemical compounds, has been suggested as a mechanism which may promote success of the exotic invader (Inderjit and Callaway 2003, Inderjit *et al.* 2008a). The novel weapons hypothesis suggests that invasive species produce unique allelopathic chemicals which enable success against native species which have not evolved tolerance to the novel biochemicals (Rabotonov 1982, Callaway and Aschehoug 2000, Mallik and Pellissier 2000).

Figure 1. *Centaurea pratensis* capitula. Photo: Wes Messinger/USACE

Some of the best examples of evidence for the novel weapons hypothesis come from research on *Centaurea* species. Several *Centaurea* species are among the most devastating noxious plants in North America (Vivanco *et al.* 2004). For example, *Centaurea stoebe* (nee *maculosa;* spotted knapweed), has invaded 3 million ha in the U.S. and two Canadian provinces (Story *et al.* 2006). *Centaurea stoebe* produces the biochemical (±)-catechin (Bais *et al.* 2003), which has been found to negatively affect growth and survival of neighboring species in laboratory, greenhouse, and field experiments (Callaway *et al.* 2005b, Inderjit *et al*. 2008b; He *et al*. 2009, Thorpe *et al.* 2009a). Studies indicate that *C. stoebe* has stronger negative effects on species grown in its introduced range than closely related species grown in its native range (Inderjit *et al.* 2008b, He *et al.* 2009, Thorpe *et al.* 2009a). Other species of *Centaurea* have also shown evidence of producing novel weapons. Callaway and Aschehoug (2000) found *Centaurea diffusa* (diffuse knapweed) also had stronger negative effects on North American grass species than on closely related grasses from its native range. 8-hydroxyquinoline was found to be three times more concentrated in invaded soils than in soils of its native range (Vivanco *et al.* 2004), and is thought to be the allelopathic chemical enabling *C. diffusa*'s success over native species.

Meadow knapweed (Figure 1), *Centaurea pratensis*, is a noxious perennial forb classified by the Oregon Department of Agriculture as a category B non-native: "a weed of economic importance which is regionally abundant, but which may have limited distribution in some counties" (Pacific Northwest Invasive Plant Council 2011). This species is a the fertile hybrid between black knapweed (*C. nigra*) and brown knapweed (*C. jaceae*). *Centaurea pratensis* was introduced to Lane County around 1910 as a potential forage species (King County 2004). It's range has since expanded throughout western Oregon (Figure 2), Washington, and California. It has been noted to invade moist areas such as forested edges, meadows, and dominate disturbed sites with full sun. It is known to outcompete grasses and has shown potential to invade native ecosystems such as native prairie and oak savannah. *Centaurea pratensis* reproduces mostly by seed, which can spread by wind, water, animals, and with human activities such as recreation (King County 2004, Thorpe *et al.* 2009b).

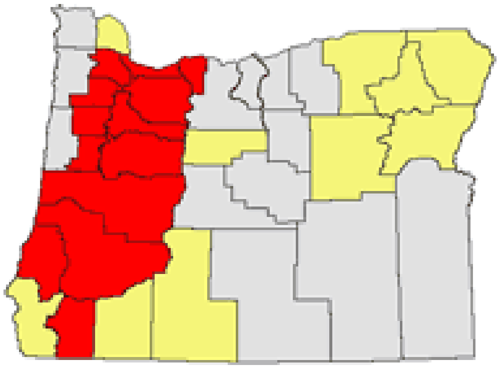

Figure 2. 2011 distribution of *Centaurea pratensis* in Oregon. Red indicates known abundance, yellow indicates limited distribution, and grey indicates no known presence. Image: www.weedmapper.org.

While *C. pratensis* is known to be invading many habitats throughout the Pacific Northwest, no studies have addressed the mechanism of this invasion. Evidence of allelopathy in other species of *Centaurea* suggest that this species could also exude novel biochemicals that are harmful to native species. A preliminary laboratory study using fragments of roots from *C. pratensis* collected from several sites in the Willamette Valley suggested that this species may have more allelopathic potential than either *C. stoebe* or *C. diffusa* (R.M. Callaway, *personal communication*). A quantitative study is needed to increase understanding of *C. pratensis* and potential interactions with native species in the Pacific Northwest.

We used two greenhouse experiments to explore the roles of allelopathy, competition, and nativity on *C. pratensis* success. First, we tested for evidence of allelopathy in the interactions between *C. pratensis* and *Elymus glaucus*, a native grass common to the Pacific Northwest. We grew *E. glaucus* and *C. pratensis* in pairs to test for interspecific interactions, as well as pairs of each species to test for intraspecific effects on growth. To test for allelopathic interactions, we added activated carbon to half of the pots; activated carbon neutralizes organic allelochemicals and has been used in many studies to identify allelopathic effects (e.g. Callaway and Aschehoug 2000). Second, we investigated if nativity affected interspecific interactions with *C. pratensis.* We grew *C. pratensis*, with three species native to the Pacific Northwest, three closely related species native to Eurasia, and with itself to test for intraspecific interactions. Similar to the first study, we grew species pairs with and without activated carbon to test for allelopathy. Additionally, we grew species pairs in pots with two different levels of fertilization to investigate whether competitive effects differ with varied levels of nutrients.

Methods

Experiment 1: C. pratensis and E. glaucus

To investigate the role of allelopathy between *C. pratensis* and *E. glaucus*, individual *E. glaucus* test plants were planted in 60 Deepots (6.4 cm diameter x 36cm deep; 983 ml) filled with sintered clay (non-clumping kitty letter) containing either conspecific (n=30) or *C. pratensis* (n=30) neighbors. In order to test for conspecific interactions, we also planted two *C. pratensis* plants in 30 pots. For each of the three neighbor treatments, half of the pots (15) contained finely ground activated carbon (AC) that had been mixed into the sand at a rate of 20 ml AC to l L clay, and half were planted in clay only. All plants were provided with plentiful quantities of water 3 times per week, and Scotts Miracle Gro (8 % N, 7 % P, 6 % K) once every two weeks for the duration of the experiment. After 120 days, plants of both species were harvested (Figure 3), divided into aboveground and belowground biomass, dried at 60°C, and weighed.

Figure 3. *Centaurea pratensis* (A.) and *Elymus glaucus* (B.) grown for experiment 1.

Table 1. Characteristics of experimental species seeded in experiment 2.

Scientific name	Common name	Family	Longevity
Exotic species			
Centaurea pratensis[1]	meadow knapweed	Asteraceae	perennial
Festuca arundinceae & *F. rubra*[2]	tall & red fescues	Poaceae	perennial
Chrysanthemum maximum[2]	Shasta daisy	Asteraceae	annual
Phaseolus coccineus[2]	scarlet runner bean	Fabaceae	annual
Native species			
Elymus glaucus[1]	blue wild rye	Poaceae	perennial
Echinacea purpurea[2]	Purple coneflower	Asteracae	annual
Lupinus bicolor[1]	small-flowered lupine	Fabaceae	annual

[1]local collection or production; [2]cultivar

We used 2-factor ANOVA (R Development Core Team 2009) for mean aboveground biomass of *E. glaucus* and *C. pratensis* separately, to test for differences between means using neighbor and treatment (with or without AC) as fixed factors. Mean aboveground biomass for *C. pratensis* was log-transformed in order to meet assumptions of normality. When significant treatment effects were found, pairwise comparisons between each treatment were made using the Tukey Kramer HSD method, basing the mean square error off of the 2-way ANOVA results. Mean belowground biomass indicated similar trends as mean aboveground biomass and was not analyzed statistically.

Experiment 2: C. pratensis with native and exotic competitors

We planted seeds of *C. pratensis* with either conspecific (n=20) neighbors, exotic neighbors (*Festuca arundinaceae* n=20, *Chrysanthemum maximum* n=20, *Phaseolus coccineus* n=20), or native neighbors (*Elymus glaucus* n=20, *Echinacea purpurea* n=20, *Lupinus bicolor* n=20). Species were chosen to represent a wide variety of growth forms (annual/perennial, forb, grass, or legume; Table 1). For each neighbor combination, 3 seeds of each species were sown in pots containing 300 mL vermiculite or vermiculite/AC mix (20mL AC to 1L vermiculite). Pots within each species x AC combination were randomly assigned to receive normal (0.032mL N, 0.028 mL P, 0.024 mL K) or dilute (0.0032mL N, 0.0028 mL P, 0.0024 mL K) fertilizer. 100 mL of fertilizer was added to each pot weekly, and pots were watered every other day. After germination, plants were thinned to one of each species. After 49 days, whole plants were harvested, dried at 65° C until at a constant weight, and weighed.

Mean biomass was calculated for all plants. We used a multi-factor ANOVA (R Development Core Team 2009) to test for differences between means of *C. pratensis* biomass using competitor and treatment (+/- AC, high/low fertilizer) as fixed factors. To meet assumptions of normality, mean biomass of *C. pratensis* was square-root transformed. When a significant main factor effect was found, we used a single factor ANOVA to test for differences in mean *C. pratensis* biomass for that factor. Pairwise comparisons between factors were made using the Tukey Kramer HSD method, basing the mean square error off of the single factor ANOVA results.

Results

Experiment 1: C. pratensis and E. glaucus

Mean aboveground biomass of *E. glaucus* did not differ significantly between treatments with and without activated carbon (Figure 4). Mean aboveground *E. glaucus* biomass was greater when grown with *C. pratensis* as its neighbor ($p < 0.0005$). In a 2-factor ANOVA, no neighbor:activated carbon interaction was found.

Mean aboveground biomass of *C. pratensis* did not differ significantly between those with and without activated carbon (Figure 5). However, a neighbor:activated carbon interaction was detected ($p < 0.0005$; Appendix A). Biomass of *C. pratensis* was lower when grown with *E. glaucus* and activated carbon, compared to other treatments.

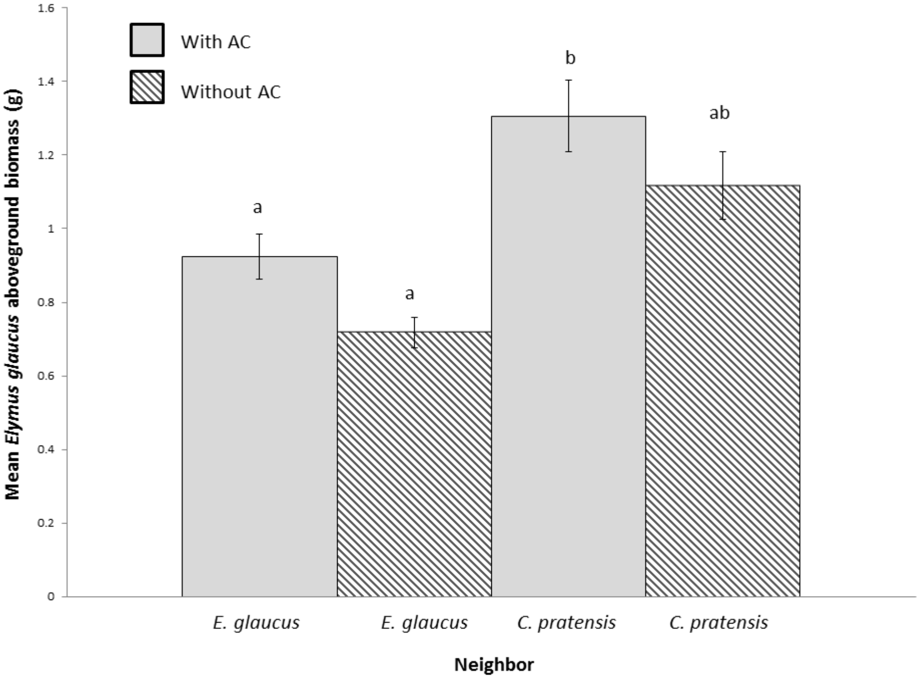

Figure 4. Mean aboveground biomass for *Elymus glaucus* grown with conspecific or *Centaurea pratensis* neighbors, with or without activated carbon (AC) in the soil. Error bars indicate 1 standard error of the mean (SEM). Means with different letters differed in pairwise comparisons ($p < 0.01$).

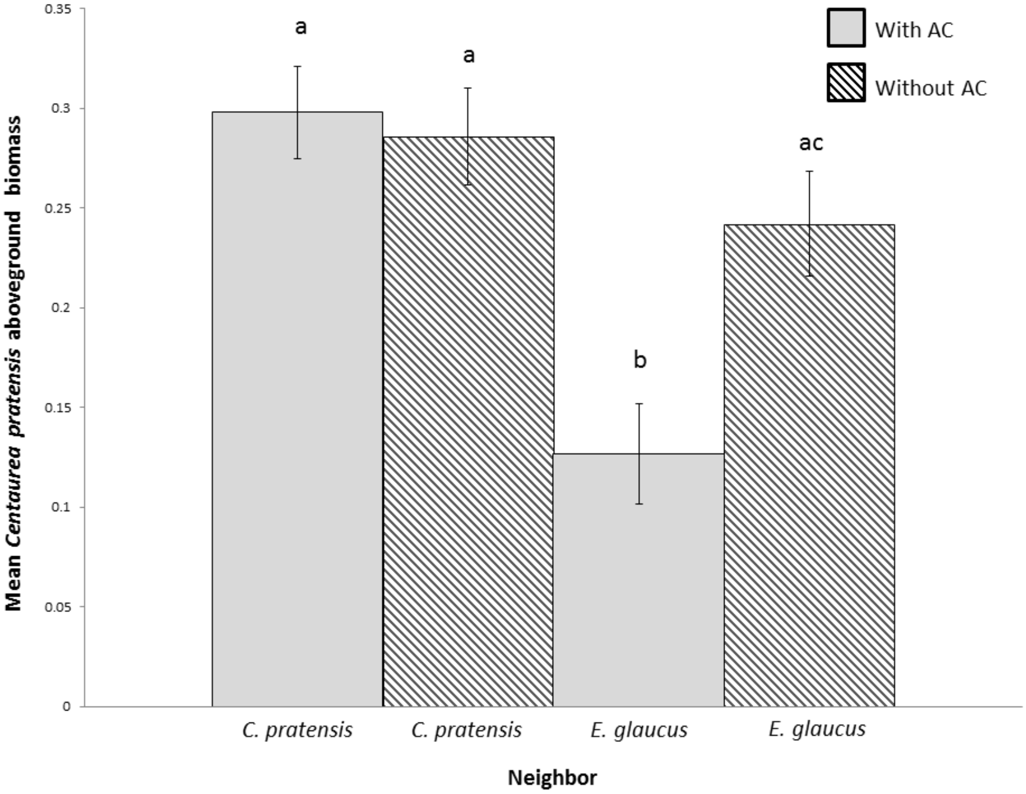

Figure 5. Mean aboveground biomass for *C. pratensis* grown with conspecific or *E. glaucus* neighbors, with or without activated carbon (AC) in the soil. Error bars indicate 1 standard error of the mean (SEM). Means with different letters differed in pairwise comparisons ($p < 0.01$).

Experiment 2: Centaurea pratensis with native and exotic competitors

Mean biomass of *C. pratensis* did not differ with either treatment (activated carbon or two levels of fertilizer). Mean biomass of *C. pratensis* was less when grown with *Phaseolus coccineus* (scarlet runner-bean) ($p < 0.0005$; Appendix B). There was also an effect of nativity; mean *C. pratensis* biomass was less when grown with Eurasian competitors than US competitors ($p < 0.0005$, Appendix C; Figure 6). These effects appeared to be driven by *P. coccineus* (scarlet runner-bean), an exotic legume. Mean biomass of *C. pratensis* was significantly less ($p < 0.0005$; Figure 7) when grown with this species, but did not differ significantly when grown with any other species.

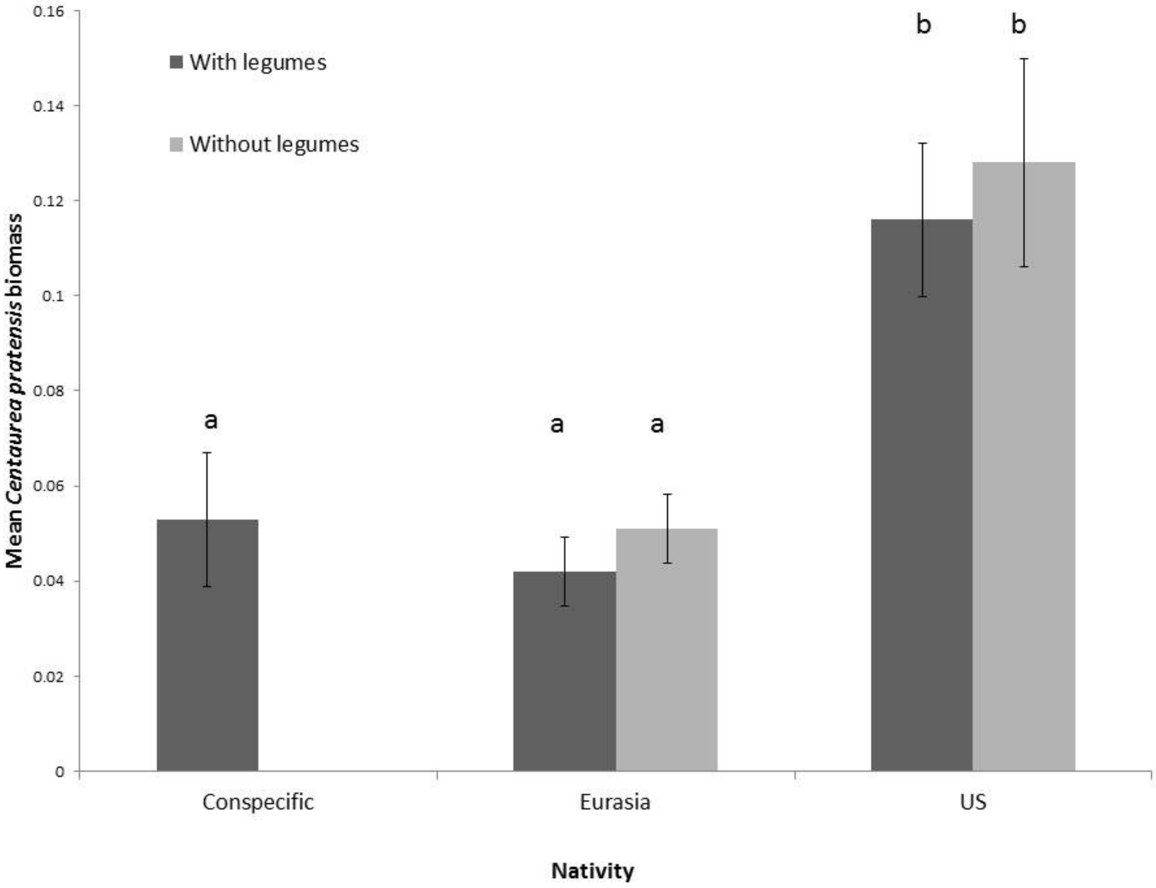

Figure 6. Mean *C. pratensis* biomass grown either conspecific, Eurasian, or US competitors. Error bars represent 1 standard error of the mean (SEM). Means with different letters differed in pairwise comparisons ($p < 0.05$).

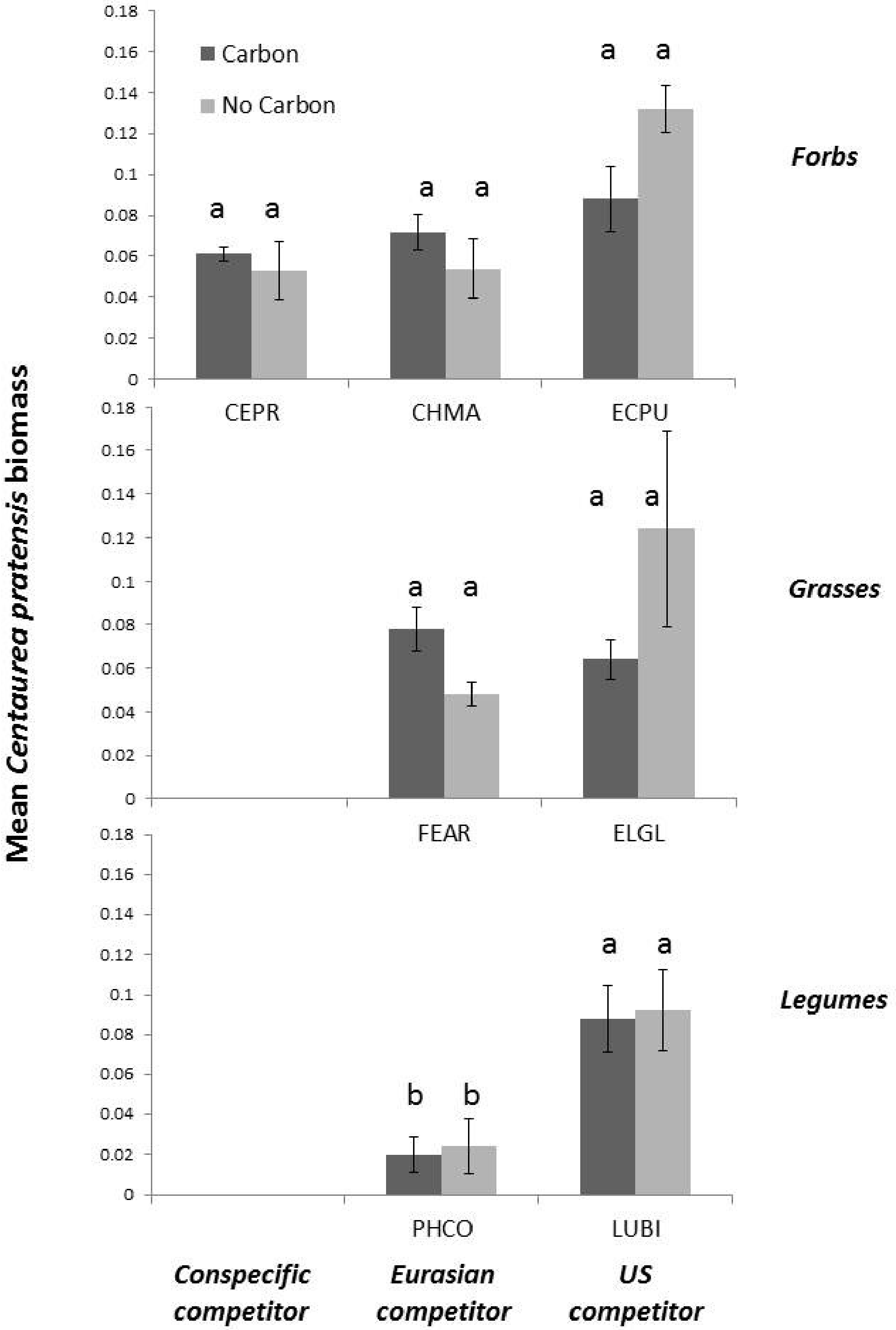

Figure 7. Mean *C. pratensis* biomass when grown in the presence of conspecific, Eurasian, or US competitors, grown with or without activated carbon. Species are identified by species code. Error bars represent 1 standard error of the mean (SEM). Means with different letters differed in pairwise comparisons ($p < 0.05$).

Discussion

We found no evidence of *C. pratensis* producing allelopathic chemicals at this growth stage. Though our study did not indicate production of allelopathic chemicals, there is potential that *C. pratensis* may create these chemicals at a later growth stage. Preliminary laboratory analyses using mature root fragments of *C. pratensis* suggested production of allelopathic chemicals (R.M. Callaway, *personal communication*), thus future studies of later growth stages are needed to increase understanding of potential interactions.

Trends suggest that competition may be a mechanism mediating interaction at this growth stage. Biomass of *C. pratensis* and *E. glaucus* was slightly less when grown with *E. glaucus*, indicating that this species may be a stronger competitor both with itself and with other species at the seedling stage. *Elymus glaucus,* a native perennial grass, is often used in restoration activities due to its ability to germinate quickly and compete with surrounding vegetation. It may be possible that under these conditions *E. glaucus* tends to be more competitive than *C. pratensis.* We found both a nativity and competitor effect when *C. pratensis* was grown with multiple competitors, however both of these relationships are likely the reflection of an interaction of *C. pratensis* with *Phaseolus coccineus,* an exotic legume. Mean biomass of *C. pratensis* decreased when grown with *P. coccineus,* which was much more prolific than all other species in the experiment. Due to this, the nativity and competitor effects could be a reflection of the clear differences between biomass grown with or without this highly competitive species. Futhermore, this species' seed was from a mass-produced cultivar. It is likely that there has been selection during cultivation for high competitive ability. Further investigations of different species combinations and with wild-collected or limited production seed would increase understanding of the interactions of *C. pratensis.*

Another factor limiting interpretation of our results is the time frame of the studies. These experiments were harvested while plants were in the seedling to young-rosette stages. Future studies of later growth stages would increase understanding of how these intra- and interspecific interactions may change over time.

Centaurea pratensis shows potential to invade a wide range of habitat types, from native prairies to roadsides. Our results suggest that different species assemblages might be useful in restoration after removal of *C. pratensis,* depending on management objectives. Many restoration efforts focus on increasing native species diversity to form a community more resistant to invasion (Naeem *et al.* 2000). When *C. pratensis* is invading areas where maintaining native assemblages is a restoration goal, our results suggest that *E. glaucus* might be a useful native species. However, establishment of *E. glaucus* can be slow when just seeded into plots where *C. pratensis* has been removed (Thorpe *et al.* 2009b). It is possible that better competitive effects of *E. glaucus* would be observed if seeded plots were watered or plots were planted with plugs. We found that Eurasian species tended to reduce *C. pratensis* biomass more than those from the US. The strong effects of *P. coccineus* on *C. pratensis* suggest that a highly competitive legume might be considered in restoration or rehabilitation in highly degraded areas such as roadsides where the goal is simply to establish a less noxious plant community. Use of any competitive non-native species should be justified, and only used if it has demonstrated inability to spread into native ecosystems and a lack of negative interactions with native species.

This study increases understanding of the mechanisms mediating interspecific interaction at the seedling stage. *Centaurea pratensis* poses a major threat to native and agricultural systems throughout Oregon. Though our study indicates that *C. pratensis* does not produce allelopathic chemicals at this growth stage, future studies focusing on interactions of mature plants would greatly increase understanding of potential interactions over time. The ability of *C. pratensis* to create monocultures in invaded areas demonstrates that this species is highly competitive, likely over a longer time frame than encompassed by our study.

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Appendices

Appendix A. Analysis of variance (ANOVA) table for the response of log (*C. pratensis* aboveground biomass) when grown with *E. glaucus* and itself.

	Df	SS	MS	F value	*P* value
Neighbor	1	6.520	6.520	27.77	<0.0005
AC	1	1.111	1.111	4.732	0.03
Neighbor: AC	1	3.345	3.345	14.248	<0.0005
Residuals	84	19.721	0.235		

Appendix B. Analysis of variance for the response of *C. pratensis* biomass (square-root transformed) when grown with native, exotic, and conspecific competitors.

	Df	SS	MS	F value	*P* value
Competitor	6	0.373	0.0622	10.942	< 0.0005
AC	1	0.0159	0.0159	2.805	0.099
Fertilizer	1	0.001	0.001	0.110	0.74
Competitor AC	6	0.028	0.0046	0.812	0.56
Competitor: Fertilizer	6	0.053	0.009	1.567	0.16
AC: Fertilizer	1	0.008	0.008	1.399	0.24
Competitor: AC: Fertilizer	6	0.048	0.008	1.415	0.21
Residuals	108	0.614	0.006		

Appendix C. One-way analysis of variance for the response of *C. pratensis* biomass (square-root transformed) in relation to nativity (native or exotic competitors).

	Df	SS	MS	F value	*P* value
Nativity	1	0.03	0.03	16.23	< 0.0005
Residuals	134	0.255	0.002		