A Study of Invasive Spotted Knapweed (*Centaurea stoebe*: Asteraceae) at Pierce Cedar Creek Institute

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ABSTRACT

Centaurea stoebe (spotted knapweed) is a noxious weed known for outcompeting species in their natural habitats. With its ability to reproduce quickly, grow rapidly, and secrete allelopathic root exudates, *C. stoebe* is a major threat to the biological diversity of habitats within which it becomes established. More specifically, *C. stoebe* is a noxious invasive weed at Pierce Cedar Creek Institute (PCCI). In recent years, PCCI has been working to construct native prairie habitat on its property and spotted knapweed is a potential threat to those efforts. We evaluated the age structure of spotted knapweed populations at PCCI in three fields with differing histories of disturbance and management in an effort to shed light on the stability of these populations and the effectiveness of the management efforts at PCCI. We used eight native prairie species (*Andorpogon gerardii* Vitman (Big Bluestem), *Schizachyrium scoparium* (Michx.) Nash (Little Bluestem), *Panicum virgatum* L. (Switch Grass), and *Elymus canadensis* L. (Canada Wild Rye), *Lupinus perennis* L. (Wild Lupine), *Ratibida pinnata* (Vent.) Barnhart (Yellow Coneflower), *Monarda fistulosa* L. (Wild-bergamot), and *Penstemon hirsutus* (L.) Willd. (Hairy Beard-tongue)), used by PCCI in their prairie construction efforts and studied the effects of *C. stoebe* root exudate on seed germination, and radicle /shoot growth. Our results indicate that for the eight species studied, seed germination and seedling growth of *Lupinus perennis, Ratibida pinnata*, and *Panicum virgatum* were unaffected by the allelopathic root exudate of *C. stoebe.* In addition, we evaluated the effects of competition by *C. stoebe* on the eight species by performing a common garden growth experiment. The results of this experiment indicate that, within the timeframe of this study, all eight species were capable of surviving in the presence of *C. stoebe* but that each experienced decreased growth.

INTRODUCTION

Invasive plant species pose an enormous threat to natural habitats. According to Pimentel et al. (2000), it is estimated that invasive plant species cause roughly $34 billion in damages in the United States annually. However, it is not only the economy that suffers from spreading populations of invasive species but fragile ecosystems also suffer as invasive species put stress on native species often leading to their complete eradication (Pimentel et al. 2000).

Centaurea stoebe L. subsp. *micranthos* (Gugler) Hayek (Asteraceae; =*C. maculosa* and =*C. biebersteinii*; spotted knapweed) is a highly invasive herbaceous perennial plant species. Depending on soil fertility and moisture conditions spotted knapweed plants are typically between 30- to 125-cm tall and produce 1 to 10 flowering stems annually. Each stem typically contains between 10 and 60 capitula (flower heads of the Asteraceae) that are enclosed by black-tipped bracts; the character for which the species received its common name. Spotted knapweed plants typically bolt (produce floral stems) in June, flower in July, and set seed in August (Watson and Renney 1974). Sheley et al. (1998) showed that annual seed production ranged from 5,000 to 40,000 seeds/m^2. The plants reproduce only from seed (Davis et al. 1993), although taproots damaged at their crown typically branch with each branch producing a basal rosette of leaves and flowering stems, thus appearing as two or more separate individuals (P. Laureto personal observation). Spotted knapweed has a stout taproot that can live an average of 3 – 5 years but which frequently lives up to 9 years (Story et al. 2001). Similar to secondary xylem growth in woody plants, the taproot of *C. stoebe* deposits one ring of xylem annually (Boggs and Story 1987). Boggs and Story (1987) determined that even in years of unusual weather conditions, where aboveground growth of spotted knapweed occurred in spring and then again in fall but not during summer due to extreme drought conditions, plants of known age only

deposited one ring of new xylem tissue annually. This allows for spotted knapweed plants to be aged by counting the growth rings.

Centaurea stoebe is native to Europe and western Asia. It is believed to have been introduced to the Pacific Northwest in the early 1890's through contaminated alfalfa, *Medicago sativa* L., seed (Mauer et al. 1987) and it has now spread into 46 of the 50 US states and is found in Canada from Nova Scotia to British Columbia (USDA, NRCS 2013). The presence of spotted knapweed often leads to imbalanced ecosystems and reduced plant diversity (Neu 2000). In western North America it has invaded nearly 7 million ha of rangeland and pasture (Sheley et al. 1998) where it has often resulted in the complete competitive exclusion of native plants and the development of dense monospecific stands (Ridenour and Callaway 2001). The plant preferentially invades old fields and remnant prairies throughout the mid-western US where it reduces the number of native species (Emery and Gross 2005) and it is a major threat to the fragile sand dune ecosystems within the Great Lakes region (Marshall 2011).

For a plant to be biologically efficient and ecologically effective, it must interfere with other surrounding species. Interference includes both competition and allelopathy (Ridenour and Callaway 2001 and references therein). Competition is the removal of essential resources from the environment whereas allelopathy is the addition of plant-produced secondary chemical compounds into the soil environment which enhances survival and reproduction. Allelopathy can describe any positive or negative effect one plant has on another; however, it is most often used to refer to chemical-mediated negative interference between plants. Allelochemicals are released into the soil by a variety of mechanisms, including decomposition of dead plant material, volatilization, and root exudation. The chemical structures and modes of action for allelochemicals are diverse but research has shown that some function to inhibit growth of

competing species (Weston and Duke 2003). As early as 1962, allelopathic interactions were hypothesized to explain the aggressive behavior of *C. stoebe* (Suchy and Herout 1962). A number of studies have confirmed that *C. stoebe* produces root exudates that have inhibitory effects on several native plant species (Ridenour and Callaway 2001; Bais et al. 2003; Vivanco et al. 2004; Callaway et al. 2008; He et al. 2009).

While *Centaurea stoebe* has been identified as a noxious weed capable of creating monospecific stands by excluding almost all native plant species (Ridenour and Callaway 2001), there are some North American native plants that exhibit varying degrees of resistance. For example, *Lupinus sericeus* Pursh and *Gaillardia grandiflora* Van Houtte, both native to the grasslands of western North America, show resistance to the allelopathic root exudates of *C. stoebe* (Weir et al. 2006). Furthermore, field experiments demonstrated that in *C. stoebe*-infested fields, species which are sensitive to the allelochemicals of *C. stoebe* are more successful when grown near *L. sericeus* plants (Weir et al. 2006). *Ammophila breviligulata* Fern., a dominant native grass of the Great Lakes sand dune community, is also unaffected by *C. stoebe* (Emery and Rudgers 2012). When *C. stoebe* and *A. breviligulata* were grown in competition experiments, the growth of *C. stoebe* was unexpectedly reduced, although this may have been due in part to the low nutrient and drought conditions of the sand dune environment (Emery and Rudgers 2012). Additionally, a recent study by Reinhart and Rinella (2011) demonstrated that eastern North American species may be more tolerant of interference from *C. stoebe* than species from western North America.

Land managers throughout the US are concerned about the presence of *C. stoebe* and are actively managing habitat to eliminate and/or slow its spread. The Natural Area Management Plan for PCCI lists spotted knapweed as one of the most threatening non-native and invasive

plants on the property (Howell 2010). Over the past ten years, PCCI stewardship staff has converted old field areas into 30 ha of short grass and tall grass prairie habitat. These constructed prairies are actively managed through the use of controlled burns and the pulling of mature spotted knapweed plants.

Invasive species, such as spotted knapweed, often threaten the establishment of desirable plants in vegetation restoration projects (Emery and Gross 2005). Therefore, we undertook this study to provide PCCI with baseline data that could be useful to their prairie reconstruction and management efforts. The objectives of this study were to 1) characterize the functional relationship between plant community composition and spotted knapweed in three fields with differing disturbance and management regimes, 2) determine the age structure of spotted knapweed populations within three fields that differ in their disturbance and management history, 3) investigate the effects of spotted knapweed root exudates on the germination and seedling growth of native prairie seeds, and 4) evaluate the degree of interference by spotted knapweed plants on the growth of native plant species used in the prairie reconstruction efforts at PCCI.

METHODS

Study Site

This study was conducted at Pierce Cedar Creek Institute (PCCI) near Hastings, Michigan, USA, from May through August of 2013. PCCI is a biological field station and nature center whose 267 ha property consists of forest, old-field, constructed prairie, open water, and a variety of wetland habitats. Much of the property was previously disturbed through farming activities. These abandoned fields are prime habitat for many invasive species including *Centaurea stoebe* which is identified in the PCCI management plan as one of their more invasive

species on the property. Of the 267 ha site approximately 30 ha consists of reconstructed short-grass and tall-grass prairies. In an effort to control *C. stoebe,* and other invasive species, PCCI regularly burn these constructed prairies and organizes volunteer days where mature *C. stoebe* plants are pulled.

Diversity in Three Fields of Varied Disturbance at PCCI

Patterns of species diversity were evaluated and compared among three fields each known to contain a population of *Centaurea stoebe* but each differing in their disturbance and management histories (Fig. 1). The Lupine Trail (LT) site is a constructed short-grass prairie that is managed by the PCCI staff. Prior to the onset of this study, the most recent management efforts included a controlled burn in April of 2013, and organized pulls of mature plants in July of 2012. Burning is expected to kill spotted knapweed seedlings and seeds lying on the surface while pulling should remove a large portion of the adult plants.

A second study site was located in an old-field south of the PCCI Education Building (EB). This site was most recently disturbed in 2000 when the building was undergoing construction. The area has remained relatively undisturbed since that time and management efforts have included hand pulling or mowing to control spotted knapweed. The EB site has not been planted, but is undergoing succession of herbaceous plants. The third study site was a field along the entrance trail to the Little Grand Canyon (LGC). This field is progressing through secondary succession with woody species moving in from the edges. While the exact date of last disturbance is unknown, inspection of historic aerial photos reveals that it was open until about 25 years ago. At each of these three study sites we established a sampling grid with ten 50 × 50 cm plots. The corner of each grid was randomly established and then three transects were evenly spaced from that corner and run the length of the field. Each of the two outer transects had three

evenly spaced plots and the center transect had four evenly spaced plots. We identified all species growing within each plot and counted the number of individuals for each species. The data were used to estimate *C. stoebe* percent cover for the field, and the overall species richness, evenness, and diversity of the field using Shannon-Weaver's diversity index (Shannon and Weaver 1949).

Age Structure of Three Centaurea stoebe Populations

The age structure of *Centaurea stoebe* populations from each of the three fields described above was determined by root ring counts. Following bolting all *C. stoebe* plants from within each plot (see above) were harvested and taken to the laboratory for analysis. The number of floral stems, the root crown diameter, and the number of root rings were recorded for each plant. Root crown diameter was measured using 0 – 150 mm digital calipers (Marathon Watch Co., Richmond Hill Ontario CA). Following the methods of Boggs and Story (1987) root cross sections were made with a razor blade immediately below the root crown and the annual xylem rings were counted using a dissecting microscope. Each root was sectioned twice and ring counts were made from each cross section to ensure accuracy.

Root ring counts that could not be determined because of root rot were estimated from linear regressions of root crown diameter against number of root rings (Boggs and Story 1987). Separate linear regressions were made for each study site in EXCEL and R^2 values were calculated.

The mean (±SD) root diameter of all spotted knapweed plants at each study site was calculated. The difference in mean root diameter between the populations for each age class was analyzed with one way ANOVAs and compared at the level $\alpha < 0.05$. All analyses were carried out via Analysis of Variance (ANOVA) Calculator - One-Way ANOVA from Summary Data

[Software] (Soper, D.S. 2013). Additionally, the mean age (±SD) was calculated for All Plants, Flowering Plants, and Vegetative Rosettes for each of the three study sites and used to evaluate the percentage of flowering plants in each age class. In order to gain insight into the potential for reproductive success of each age class the total number of flowering stems was compared to the total number of plants across the three study sites.

Annual age-specific survivorship of *Centaurea stoebe* plants was estimated for each study site using direct counts of plants. Therefore, the survivorship between age 3 and 4 was calculated by the number of plants of age 4 divided by the number of plants of age 3. Similar calculations were made for all *C. stoebe* plants by using the average number of plants in each age class across all three study sites.

Germination Study

Plant Material ─ Roots of *Centaurea stoebe* were collected from the old fields at PCCI and used in evaluating the effects of various concentrations of root exudate on seed germination and seedling growth of four native grass species and four native forb species. The native grass species used in this study were *Andropogon gerardii* Vitman (Big Bluestem), *Schizachyrium scoparium* (Michx.) Nash (Little Bluestem), *Panicum virgatum* L. (Switch Grass), and *Elymus canadensis* L. (Canada Wild Rye). The native forb species were *Lupinus perennis* L. (Wild Lupine), *Ratibida pinnata* (Vent.) Barnhart (Yellow Coneflower), *Monarda fistulosa* L. (Wild-bergamot), and *Penstemon hirsutus* (L.)Willd. (Hairy Beard-tongue). We chose these eight species because they have all been used by PCCI in the construction of the various prairie habitats (Howell 2010). We obtained seed from Michigan Wildflower Farm (Portland, MI, USA) because seed source populations used by this company come from native Michigan populations.

Preparation of Root Extracts ─Following Turk et al. (2005), fresh root tissue was

ground in a blender and soaked in deionized water for 24 h at 24 ºC in a lighted room with continuous stirring to produce concentrations of 1, 4, 8, and 12% w/v (i.e., g of tissue per 100 mL of water). Each solution was filtered through cheesecloth to remove the tissue debris and then centrifuged (1500 g) for 30 min. The supernatant was filtered again through filter paper (Whatman No. 1) to produce the final extract.

Seed Bioassay ─Seeds were first surface sterilized with 5.25 w/v sodium hypochlorite (10:1 water/bleach) for 30 min at room temperature and then rinsed 3 times in deionized water to remove any trace of bleach. Sterile 9 cm petri plates were labeled with species name and extract concentration (1, 4, 8, and 12%). Distilled water (0% root extract) was used as the control. The four extract treatments and the control were arranged in a Completely Randomized Design (CRD) with four replicates. For each plant species, 15 seeds were then evenly placed on the filter paper and 10 mL of extract solution or distilled water was added as indicated by the CRD. Petri plates for all treatments were sealed with Parafilm® (Pechiney Plastic Packaging Company, Chicago, IL) and placed in a continuously lighted room at 20 – 24 ºC to germinate. Seeds were checked for germination every 12 h for the first 3 days, and then on a daily basis for 15 days. Seed germination was defined as the radicle or plumule had extended 1 mm beyond the seed coat or caryopsis (the fused seed and fruit of grass). Percent germination was determined by counting the number of germinated seeds in each treatment and expressing it as a percent of the total seeds treated (Turk et al. 2005). A *Z*-test ($\alpha < 0.05$) comparing the percent germination with water vs. the percent germination with extract treatment was performed for each species and each extract concentration. Following the germination period, plumule and radicle lengths were measured for all germinated seeds. The mean radical lengths and mean plumule lengths for all treatments were analyzed with one-way ANOVA and compared at the level $\alpha < 0.05$. All analyses were

carried out via Analysis of Variance (ANOVA) Calculator - One-Way ANOVA from Summary Data [Software] (Soper, D.S. 2013).

Interference Study

To investigate *Centaurea stoebe* interference on native plant species used in the construction of prairie habitat at PCCI, seedlings of eight native species (see above) were purchased from Wildtype Native Plant Nursery (Mason, MI). Seedlings of *C. stoebe* individuals (defined as plants with root diameters < 1.0 mm [Story et al. 2001]) were dug from the wild in early March, transplanted to 2" pots with potting soil, and grown in greenhouse cultivation at Grand Rapids Community College for approximately 8 weeks to ensure both viability and growth conditions comparable to the seedlings obtained from the nursery. Three plant combinations were used: *C. stoebe* and each of the 8 native species were grown alone as controls, one *C. stoebe* individual was grown in competition with one of each of the native species, and two individuals of each native species were grown in competition with each other. Each plant combination was replicated 10 times.

In early May, a 10 m × 7 m fenced garden plot was treated with Roundup® (Scotts Company, Marysville, OH) and after several days the topsoil was thoroughly rototilled. Ten rows, each with 25 3-L pots, were laid out. Pots were filled with topsoil and buried so the rim was approximately 3 cm above the surface of the ground. Within rows the pots were separated by approximately 20 cm and rows were separated by approximately 0.5 m. A Randomized Complete Block Design (RCBD) was established for the nine plant species (8 natives and *C. stoebe*) and the three treatment combinations described above where each row represented a block and the various plant combinations (treatments) were randomly assigned to one of the 25 pots. The seedlings were then transplanted into the prepared pots according to the RCBD and

watered. Five rain gauges were set throughout the garden (4 corners and center) and the garden was watered as necessary. Height for each plant was measured at planting to serve as a baseline for plant growth. We estimated initial biomass for each plant species by separating the roots and shoots of 10 seedlings, drying the shoots at 60 ºC for three days, and using the calculated average weight as the estimate of initial biomass for the species (Emery and Rudgers 2012). Plants were allowed to grow in the garden plot for two months. At the beginning of August we measured final plant height, harvested the shoots and placed them in paper bags for drying. Plants were dried at room temperature for a minimum of three days then moved to a drying oven set at 60 ºC for three additional days. Plants were then weighed to obtain final biomass. Total plant growth was calculated by subtracting initial height and estimated biomass from the final height and actual biomass (Emery and Rudgers 2012). The effects of competition on plant biomass and above-ground growth were analyzed with one-way ANOVAs and compared at the level $\alpha < 0.05$. The mean biomass for each species grown alone was compared to the mean biomass of individuals grown in competition with a conspecific and grown in competition with spotted knapweed. Then the means for the two competition treatments were compared. Similar analyses were done for height. All analyses were carried out via Analysis of Variance (ANOVA) Calculator - One-Way ANOVA from Summary Data [Software] (Soper, D.S. 2013).

RESULTS

Diversity in Three Fields of Varied Disturbance at PCCI

The percent cover of spotted knapweed varied with the disturbance history. In the LP site, the most recently disturbed site, the percent cover was 1.3%. At the EB site, which was last disturbed about ten years ago, the percent cover was 12.4% and at LGC it was 22.9%. At the LGC site both species diversity (H - Shannon-Weaver diversity index) and evenness (E) of distribution are higher than at the EB site (LGC – H = 2.17, E = 0.71; EB – H = 1.33, E = 0.44). Typically a high diversity value indicates there is less competition between species and a low diversity value indicates that competition has reduced the number of species able to make a living in that community. The species diversity and evenness of distribution at the LT site, the most recently disturbed site, was intermediate between the LGC and EB sites (LT – H = 1.81, E = 0.63).

Age Structure of Three Centaurea stoebe Populations

A total of 292 *Centaurea stoebe* plants were harvested from three study sites; 125 from site EB, 163 from site LGC, and 9 from the LT site. Of these, 252 were readily aged from root ring counts. Due to rotten or difficult to distinguish rings, 40 could not be aged (Table 1). Of the 40 plants that could not be aged by root ring counts, 29 were harvested from the LGC site and 11 were harvested from the ED site. No root rot was observed from the LT site. The proportion of harvested plants with root rot increased with age (Table 2). For those plants whose age could not be directly determined we estimated age from regressions of root crown diameter against number of root rings. The high R^2 values (0.91calculated for EB and 0.89 for LGC) suggest the reliability of these age estimates (Table 1).

The mean root diameter of spotted knapweed plants by age class is depicted in Figure 2.

There was no significant difference in mean root diameter between *C. stoebe* populations from each of the three study sites ($P_{age\ class\ 1} = 0.769$; $P_{age\ class\ 2} = 0.976$; $P_{age\ class\ 3} = 0.998$; $P_{age\ class\ 4} = 0.997$; $P_{age\ class\ 5} = 0.999$ as calculated by ANOVA). This suggests that the root ring age estimates are reliable.

Root ring counts of plants harvested from the three sites indicate that spotted knapweed at Pierce Cedar Creek Institute can live for up to 8 years (Table 3; Figure 3). The maximum age class at each of the three sites was 5, 6 and 8 years for LT, LGC, and EB respectively. The mean age (±SD) of all plants per site ranged from 4.01 ± 2.21 years to 5.62 ± 2.29 years at LGC and LT respectively (Table 3).

The total number of spotted knapweed plants per age class harvested from the three study sites is shown in Figure 3. Annual age-specific survivorship of *C. stoebe* plants from the EB site was 82, 61, 21, 75, 66, and 50% for survivorship to age 3, 4, 5, 6, 7, and 8 respectively. No plants survived into the 9th year. Similarly, the annual age-specific survivorship for plants from the LGC site was 55, 56, 37, and 14% for survivorship to age 3, 4, 5, and 6 respectively. No plants older than 6 years were harvested from the LGC site. Because of the small number of plants harvested from the LT site survivorship was not individually calculated. The LT plants were included in the average number of plants from across all three study sites. Annual age-specific survivorship for the pooled data was 67, 60, 29, 33, 50, and 49% for survivorship to age 3, 4, 5, 6, 7, and 8 respectively. Survivorship was not calculated for age 0 to age 1, age 1 to 2, or age 2 to 3 because the plants in those age classes were underestimated.

We also evaluated the relationship between *Centaurea stoebe* age and flowering. Spotted knapweed plants with floral stalks were consistently older than plants that had only vegetative rosettes (Table 3). The mean age (±SD) of flowering plants per site ranged from 5.27 ± 1.87

years to 6.06 ± 1.99 years at LGC and LT respectively compared to plants with only vegetative rosettes whose mean ages ranged from 1.65 ± 0.81 years to 2.33 ± 1.35 years at ED and LGC respectively (Table 3). The mean ages of flowering plants and plants with only vegetative rosettes were significantly different for each of the study sites (*t*-test, P<0.0001 for EB and LGC; P = 0.01 for LT).

The percentage of flowering spotted knapweed plants across all study sites increased with age to a peak of 100% in the fifth year (Fig. 4). No flowering occurred in age class 0 (seedlings) and only 8% of plants in age class 1 flowered. The percentage of plants flowering increased to 64% in age class 2. Even though we found high incidences of root rot in plants five years of age or older (Table 2) plants of these age classes exhibited nearly 100% flowering (Fig. 4). We also compared the total number of flowering stems across all study sites to the total number of plants per age class. Figure 5 shows that there is a greater number of total plants as compared to flowering stems in the young age classes and that this trend reversed in age class 3 with relatively fewer and older plants producing a proportionately larger number of flowering stems as compared to the actual number of plants.

Germination Study

Seed germination of *Penstemon hirsutus* was significantly affected at all extract concentrations (Table 4). The degree of inhibition increased with extract concentration (1 g/L, P = 0.027; 4 and 8 g/L, P = 0.003; 12 g/L, P = 0.000). At higher root extract concentrations the allelochemicals of *Centaurea stoebe* appeared to be autotoxic (8 g/L, P = 0.000 and 12 g/L, P = 0.006). Germination of *Ratibida pinnata*, *Lupinus perennis*, and *Monarda fistulosa* seed was not significantly affected by exposure to spotted knapweed (*Centaurea stoebe*) root extracts. All grass species had a low proportion of seeds germinate in both the water and extract treatments.

Centaurea stoebe root extract appeared to have no significant effect on the germination of all grass species regardless of concentration.

Because growth in plants normally reflects an increase in both cell number and cell size, the effects on root/shoot growth (measured as the radical emerging 1mm past the seed coat) may be a better indicator of allelopathic effects than seed germination. Shoot length (plumule) of *Penstemon hirsutus*, *Lupinus perennis*, and *Monarda fistulosa* were unaffected by all concentrations of *C. stoebe* root extract (Figure 6a). The mean shoot lengths of *Ratibida pinnata* were significantly longer than the control plants at 4, 8 and 12 g/L concentrations and shoot lengths of *Centaurea stoebe* were significantly longer at all extract concentrations. The overall shoot (coleoptile) length results for the prairie grasses were similar with *Andropogon gerardii* and *Panicum virgatum* being unaffected at all concentrations. *Schizachyrium scoparium* had significantly longer shoots at the 8 g/L concentration and the shoots of *Elymus canadensis* were significantly reduced at 4, 8 and 12 g/L concentrations, however, these results may be skewed by the low percent germination of *Schizachyrium scoparium* and *Elymus canadensis*.

Radicle length may have been more sensitive to the allelochemicals of *Centaurea stoebe* root extract than was shoot length (Figure 6b). The mean radicle lengths of *Penstemon hirsutus* were significantly longer at 1 g/L but were significantly reduced at 4, 8 and 12 g/L concentrations. Likewise, radicle lengths of *Monarda fistulosa* were significantly reduced at 4, 8 and 12 g/L concentrations. Radicle length of *Lupinus perennis* was unaffected by all concentrations of *C. stoebe* root extract and *Ratibida pinnata* had significantly longer radicles at the 1 g/L concentration but was unaffected at the higher concentrations. Spotted knapweed also had significantly longer radicles at the 1 g/L concentration but was unaffected at the higher concentrations.

Interference Study

There was little difference in plant height in response to *Centaurea stoebe* (Fig. 7). The height of *Lupinus perennis* was significantly taller when grown in competition with a conspecific and there was a significant difference between the two competition treatments with *L. perennis* being shorter when grown with spotted knapweed than when grown with a conspecific. However there was not a significant difference between *L. perennis* grown with spotted knapweed and grown alone ($P = 0.088$). *Elymus canadensis* did not display a significant difference when grown in competition with a conspecific or in competition with spotted knapweed but there was a significant difference ($P = 0.088$) between the two competition treatments with *E. canadensis* being taller in the presence of spotted knapweed than in the presence of a conspecific.

Biomass was significantly reduced from the control plants (grown alone) for *Ratibida pinnata*, *Lupinus perennis*, *Monarda fistulosa*, *Schizachyrium scoparium*, and *Panicum virgatum* (Fig. 8). *Ratibida pinnata*, *S. scoparium*, *A. gerardii*, *E. canadensis*, and *P. virgatum* all displayed a significant decrease in biomass when grown with a conspecific, *L. perennis* had significantly increased biomass when grown with a conspecific. There was a significant depression in biomass for plants grown with spotted knapweed for both *L. perennis* and *M. fistulosa.*

DISCUSSION

Diversity in Three Fields of Varied Disturbance at PCCI—The results of this study show that the unmanaged fields at PCCI have high densities of spotted knapweed plants as compared to the planted and managed fields; an indication that management efforts are effectively controlling spotted knapweed in the constructed prairies. When comparing the two unmanaged fields, EB and LGC, the time since disturbance appears to be a major difference between them. Even though both fields had the same number of species, 21 at LGC and 21 at ED, both species diversity and evenness were higher at LGC than at the EB site. This indicates that there is less competition among species at LGC. Species richness is generally not considered to be a good measure of diversity. This was evident in comparing the two unmanaged fields. While both had the same number of species, 16 of the 21 species present at EB were represented by only a few individuals (<10) while there was 706 individuals of *Poa* sp. (a grass) and 132 individuals of spotted knapweed. At the LGC site the species that contributed the greatest number of individuals to our sample was spotted knapweed with 99 individuals. Timothy grass (*Dactylis glomerata* L.) contributed the next greatest number with 48 individuals. As expected, evenness is a better measure of diversity in these two fields.

While we could calculate species richness, diversity, and evenness for the LT site it seems unreasonable to compare a constructed and managed prairie to fields with natural succession and less management. Diversity at the LT site is a function of what species were planted and the management efforts to remove unwanted species. Only 9 spotted knapweed individuals were counted at this site which points to the success of the PCCI prairie construction and management efforts.

Age Structure of Three Centaurea stoebe Populations—If the recruitment of seedlings is constant from year to year, one would expect an age distribution curve to be monotonically decreasing as deaths are accumulated in each successive age class. The annual age-specific survivorship curves (Fig. 3) for knapweed populations at the EB and LGC sites indicate there was low recruitment of seedlings (none at EB) in 2012 and that survivorship to age 1 was also low. The underestimation of individuals in these age classes could be due to the researcher's inability to accurately identify juvenile plants; however we are confident that was not the case. It seems more likely that recruitment and survivorship of juvenile plants may have been diminished by weather conditions. The summer of 2012 was noteworthy, in the state of Michigan and elsewhere, as one of extreme heat and drought. It is possible that these weather extremes led to low recruitment of seedlings and poor seed set which could account for our underestimation of individuals in the seedling (age class 0) and age class 1 groups in 2013. Unlike the EB site, the LGC site is a field surrounded by trees. These trees may have provided young plants with some shading, thereby reducing heat and increasing moisture. Interestingly, we harvested the majority of the seedlings and 1 year plants at the LGC site from the outer transect plots for which partial shading would have been likely. There was no shading at the EB site. Of course, seed and root pathogens could also account for low seed set and low recruitment and survival of one year plants, but this study did not examine pathogens of spotted knapweed.

Beginning with age class 2, the age distribution curves resemble the expected monotonic decrease in number of individuals with age. The similar trends in the curves for the EB and LGC sites suggest that the estimates were accurate and that recruitment and age-specific survivorship were effectively constant from year to year prior to 2012. According to Carey

(1993), this indicates that the populations at the EB and LGC sites have reached a stable age distribution.

Annual age-specific survivorship was not calculated for the LT site due to the small sample of harvested plants. The LT site is a constructed prairie, which is managed by periodic burning and the organized removal of invasive species such as spotted knapweed. The site was burned prior to the onset of this study. In addition, for the last several years bolted spotted knapweed plants have been pulled by hand prior to their setting seed. It appears that the combination of these management strategies is effectively controlling the recruitment and survival of juvenile spotted knapweed plants. Only 5 of the 10 plots harvested at the LT site were found to contain spotted knapweed and from those plots only nine individuals ranging in age from two to five years were harvested. These mature individuals may have been either overlooked or topped (viable roots survived) in the previous year's removal effort.

The relationship between spotted knapweed age and flowering indicated that most plants do not produce flowering stems until age two but then continue to produce floral stems until senescence. This was in contrast to Boggs and Story (1987) who showed that floral stem production by spotted knapweed from western Montana peaked at ages 5 – 7 and then declined with age. The difference between our results and those of Boggs and Story (1987) could be due to 1) variation between Western and Midwestern plants, 2) a consequence of collecting data from a static cohort, or 3) the small sample size for age classes 6 – 8.

We were particularly intrigued by the comparison of the total number of flowering stems to the total number of plants in each age class. It appears that just a few plants in the oldest age classes have the potential to contribute a large amount of seed to the seed bank. For example, there were 103 2-year old plants harvested and between them they produced 81 flowering stems.

If each stem produced only 10 capitula and each capitula produced 20 seeds then this cohort of 2 year plants could contribute 16,200 seeds to the seed bank. By comparison, there were only two 8-year old plants harvested that collectively produced 41 flowering stems. Again, if each stem produced only 10 capitula with 20 seeds per capitula then two plants had the potential to contribute 8,200 seeds to the seed bank. The taproot of each of the two 8-year old plants had forked multiple times and each fork had produced a basal rosette of leaves and multiple flower stems. Therefore it is critical to remove entire plants and not just the vegetative growth because "topping" a spotted knapweed plant apparently leads to increased growth and reproductive efforts. We believe this example could be useful to land management efforts at PCCI as volunteers should be instructed on the importance of removing the entire plant.

Our age structure data for the unmanaged fields at PCCI indicate that those populations reached a stable age distribution prior to 2012. Because the number of seedlings and one year old plants were fewer than the number of two year old plants it would indicate that populations in those fields are in decline. However our data provided only a snapshot of age structure in 2013. The question of population age structure should be evaluated over a multiple year period in order to obtain an accurate indication of whether populations are stable, expanding, or declining in the fields at PCCI.

Germination Study—We choose to use root tissue in our preparations of spotted knapweed extracts because the chances for the roots of competing plants to be in the proximity of an allelochemical is greater with root exudates than through surface deposition from leaf and stem tissue. However, Turk et al. (2005) reported that for many allelopathic plants, leaf extract treatments have a greater inhibitory effect than root extract treatments. This may be the case for

the allelopathic spotted knapweed as seed germination for all of the prairie grass species was unimpaired.

Germination of three of the four prairie forb species used in this study was not significantly affected by *Centaurea stoebe* root extracts at any concentration. Alternatively, the effect of extract concentration may vary by the age of plant roots used in the preparation. We harvested all mature spotted knapweed plants (5 years of age and older) because they had a greater amount of root tissue per plant than younger plants. If the production of allelochemicals by *C. stoebe* is greater in younger plants then this could account for our results. The amount of root exudate produced by *C. stoebe* at different ages is unknown to us but we suspect that allelochemical production is greater at younger ages when plants are trying to become established and that production of allelochemicals may be less important to mature plants which are already established.

It has been reported that the effects of water extracts of allelopathic plants were more pronounced on radicle growth than shoot growth (Turk et al. 2003; Turk et al. 2005); a result that may be expected because roots would be the first to absorb the allelochemicals from the environment. Our results were consistent with this observation as 5 of the 8 prairie species used in this study had decreased radicle growth in the presence of spotted knapweed extract as compared to shoot growth for which only 1 of the 8 species showed decreased growth. We believe the measure of radicle/shoot growth may be a better measure of the allelopathic ability of spotted knapweed than percent seed germination because it is the recruitment of seedlings into a population which determines whether that population will be increasing or decreasing in numbers. While none of our 8 prairies species exhibited reduced germination in the presence of spotted knapweed, 5 of them had reduced growth of the radicle which may equate to weak

seedlings, low recruitment, and eventual population decline in the presence of spotted knapweed.

The low percent germination for the prairie grasses *Schizachyrium scoparium* and *Elymus canadensis* has affected our ability to accurately assess knapweed root extracts on these species. The low percent germination may be attributed to the age or quality of the seeds. Alternatively, germination for grass species may require a day/night regime and/or germination under a specific temperature regime. We suggest reevaluating the effect of *C. stoebe* allelochemicals on the germination of *E. canadensis* and *S. scoparium* under a variety of light and temperature regimes.

Taken together, the effects of *Centaurea stoebe* on seed germination and root/shoot growth indicate that *Penstemon hirsutus* will be most affected by the presence of spotted knapweed in the constructed prairies at PCCI. Additionally, the results indicate that *Lupinus perennis*, *Ratibida pinnata*, and *Panicum virgatum* will do well in the presence of spotted knapweed.

Interference Study—In this portion of the study we compared growth in terms of initial and final biomass and initial and final height of eight native species grown in competition with spotted knapweed or in competition with a conspecific to those grown alone. We hypothesized that the introduction of competition, from knapweed or a conspecific, would decrease the average growth of plants. Our results indicate that biomass is a better indicator of plant growth than height as none of the eight species showed a significant decrease in height in either of the competition treatments. In contrast, all species except *Penstemon hirsutus* showed a significant decrease in biomass when grown in competition, either with a conspecific or with spotted knapweed.

It is important to note that we measured final biomass and not growth rates. This is important because very small differences in initial seedling size can produce substantial differences in final size. Additionally, if growth rate is more important in determining the competitive ability of a plant than size, we may have missed important information about the competitive ability of these eight species in the presence of spotted knapweed.

We had expected that *Lupinus perennis* would not have a significant decrease in biomass in the presence of spotted knapweed because growth of *L. sericeus*, a congeneric species, was unaffected by *C. stoebe* (Weir et al. 2006) and because the results of our germination study indicated it was unaffected by spotted knapweed. We suspect the decreased growth of *L. perennis* is a result of the very small and immature seedlings we obtained for use in this study. Four of the ten seedlings planted with knapweed died early in the study and the remainder were shaded significantly by the rapidly growing *C. stoebe* plants. We suggest that interactions between *L. perennis* and *C. stoebe* be further studied.

In general, the results of the interference study produced different results than the germination study. Where *Penstemon hirsutus* did not perform well in the germination study its growth was not significantly affected by *C. stoebe.* Conversely *Ratibida pinnata*, *Lupinus perennis*, and *Panicum virgatum* all performed well in the germination study but showed a significant decrease in biomass when grown in competition with *C. stoebe*. Except for the immature seedlings of *L. perennis*, no other seedlings experienced death in the presence of spotted knapweed indicating that mature plants of all tested species are capable of successful growth in the prairies at PCCI even if spotted knapweed is present.

Conclusions—Based on the results of this study, it appears that the management efforts in the constructed prairies at PCCI are effectively controlling spotted knapweed and we suggest

that the same management strategies be applied to additional fields. We recommend that *Lupinus perennis, Ratibida pinnata*, and *Panicum virgatum* be used to plant any additional prairies at PCCI as they appear to be able to germinate well in the presence of spotted knapweed. We also feel that it would be beneficial to evaluate additional species for success in the presence of spotted knapweed and that this evaluation should be done prior to the use in these species in the prairie reconstruction efforts at PCCI.

LITERATURE CITED

Bais, H. P., R. Vepachedu, S. Gilroy, R. M. Callaway, and J. M. Vivanco. (2003). Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science 301: 1377 – 1380.

Callaway, R. M., D. Cipollini, K. Barto, G. C. Thelen, S. G. Hallett, D. Prati, K. Stinson, and J. Klironomos. (2008). Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89: 1043 – 1055.

Carey, J. R. (1993). *Applied Demography for Biologists with Special Emphasis on Insects*. New York: Oxford University Press. 206 p.

Davis, E. S., P. K. Fay, T. K. Chicoine, and C. A. Lacey. (1993). Persistence of spotted knapweed (*Centaurea maculosa*) seed in soil. Weed Science 41: 57 – 61.

Emery, S. M., and K. L. Gross.(2005). Effects of timing on prescribed fire on the demography of an invasive plant, spotted knapweed *Centaurea maculosa*. Journal of Applied Ecology 42: 60 – 69.

Emery, S. M., and J. A. Rudgers.(2012). Impact of competition and mycorrhizal fungi on growth of Centaurea stoebe, an invasive plant of sand dunes. The American Midland Naturalist 167: 213 – 222.

He, W. M., Y. Feng, W. M. Ridenour, G. C. Thelen, J. L. Pollock, A. Diaconu, and R. M. Callaway. (2009). Novel weapons and invasion: biogeographic differences in the competitive effect of *Centaurea maculosa* and its root exudate (±)-catechin. Oecologia 159: 803 – 815.

Howell, J. (2010). Pierce Cedar Creek Institute Natural Area Management Plan.

Marshall, J. M. (2011). *Tanacetumbipinnatum* germination and competitive interaction with *Centaurea stoebe* seedlings. The Michigan Botanist 50: 139 – 145.

Mauer, T., M. J. Russo, and M. Evans.(1987). *Element Stewardship Abstract for Centaurea maculosa*.The Nature Conservancy.

Neu, Catherine. (2000). *Centaurea maculosa*: Invader of the Western U.S. Grasslands. Restoration and Reclamation Review Vol. 6, No. 1, Fall 2000.

Pimentel, D., L. Lach, R. Zuniga, andD. Morrison.(2000).Environmental and economic costs of nonindigenous species inthe United States.Bioscience: 50, 53–65.

Reinhart, K. O., and M. Rinella. (2011). Comparing susceptibility of eastern and western US grasslands to competition and Allelopathy from spotted knapweed [*Centaurea stoebe* L. subsp. *Micranthos* (Gugler) Hayek]. Plant Ecology 212: 821-828.

Ridenour, W. M., and R. M. Callaway. (2001). The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecologia 124: 444 – 450.

Shannon, C. E., and W. Weaver. (1949). *The mathematical theory of communication*. University of Illinois Press, Urbana, Ill.

Sheley, R. L., J. S. Jacobs, and M. F. Carpinelu.(1998). Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technology 12: 353 – 362.

Soper, D.S. (2013). Analysis of Variance (ANOVA) Calculator - One-Way ANOVA from Summary Data [Software]. Available from http://www.danielsoper.com/statcalc

Story, J. M., L. Smith, and W. R. Good. (2001). Relationship among growth attributes of spotted knapweed (*Centaurea maculosa*) in western Montana. Weed Technology 15: 750 – 761.

Suchy, M., and V. Herout. (1962). Onterpenes. CXXXVI. Identity of the bitter principle from *Centaurea stoebe* (L.) with cnicin. *Collection of Czechoslovak Chemical Commuinications*27: 765.

Turk, M. A., M. K. Shatnawi, and A. M. Tawaha. (2003). Inhibitory effects of aqueous extracts of black mustard on germination and growth of alfalfa. Weed Biological Management 3:

Turk, M. A., K. D. Lee, and A. M. Tawaha. (2005). Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. Research Journal of Agriculture and Biological Sciences 1: 227 – 231.

USDA, NRCS. 2013. The PLANTS Database (http://plants.usda.gov, 12 January 2013). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Vivanco, J. M., H. P. Bais, F. Stermitz, G. C. Thelen, and R. M. Callaway. (2004). Biogeographical variation in community response to root allelochemicistry: novel weapons and exotic invasion. Ecology Letters 7: 285 – 292.

Watson, A. K. and A. J. Renney. 1974. The biology of Canadian weeds *Centaurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science 54: 687 – 701.

Weir, T. L., H. P. Bais, V. J. Stull, R. M. Callaway, G. C. Thelen, W. M. Ridenour, S. Bhamidi, F. R. Stermitz, and J. M. Vivanco. (2006). Oxalate contributes to the resistance of *Gaillardia grandiflora* and *Lupinussericeus* to a phytotoxin produced by *Centaurea maculosa*. Planta 223: 785 – 795.

Weston, L. A., and S. O. Duke. (2003). Weed and crop Allelopathy. Critical Reviews in Plant Sciences 22: 367 – 390.

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Table 1. Age structure and mean root diameter of all spotted knapweed plants at three sites.

Site	Age[a]	N[b]	Mean Root Diameter (±SD)
Ed Bldg	1	24	1.33 ± 0.60
	2	39(1)	3.32 ± 0.65
	3	32(3)	4.58 ± 0.89
	4	19(1)	7.31 ±1.24
	5	4(2)	8.96 ± 0.88
	6	3(1)	12.8 ± 0.81
	7	2(1)	15.66 ± 0.98
	8	2(2)	18.20 ± 1.12
LGC	0	20	0.79 ± 0.30
	1	15(2)	2.08 ± 0.45
	2	62(2)	3.53 ± 0.59
	3	34(10)	4.79 ± 0.73
	4	19(9)	7.16 ± 1.17
	5	7(5)	8.76 ± 0.54
	6	1(1)	10.99 ± 0.00
Lupine	2	2	2.60 ± 0.77
	3	3	4.95 ± 0.20
	4	3	7.05 ± 0.80
	5	1	9.39 ± 0.00

[a] Age as determined by the number of annual rings of root secondary xylem

[b] The number of plants harvested in each age class. The numbers in parentheses are the number of plants for which root rings could not be counted due to root rot. The ages of these plants were estimated from regressions of root crown diameter against number of root rings for each site. Education Building $y = 0.4167x + 0.7791$, $R^2 = 0.9111$; Little Grand Canyon $y = 0.5646x - 0.0113$, $R^2 = 0.8904$

Table 2. Percentage of total spotted knapweed individuals with root rot by age class

Age[a]	% root rot
0	0
1	5
2	3
3	20
4	26
5	64
6	50
7	50
8	100

[a] Age as determined by the number of annual rings of root secondary xylem

Table 3. Maximum and mean age of all spotted knapweed plants, plants with floral stalks, and plants with only vegetative rosettes (including plants that are less than 1 year old) at three sites.

Site	N[a]	Max age[b]	Mean Age (±SD)		
			All Plants	Flowering Plants	Vegetative Rosettes
		(yr)		(yr)	
Education Building	124	8	4.64 ± 3.32	5.64 ± 3.24	1.65 ± 0.81
Little Grand Canyon	159	6	4.01 ± 2.21	5.27 ± 1.87	2.33 ± 1.35
Lupine Trail	9	5	5.62 ± 2.29	6.06 ± 1.99	2.05 ± 0.00

[a] Total number of plants harvested per site.
[b] Maximum age of plants per site.

Table 4. The mean percent germination over time for seeds of *Centaurea stoebe* and 8 native prairie species at four concentrations of aqueous root extract made from *C. stoebe*. Distilled water (0% extract) was used as the control. *Significant *Z*-test comparisons for day 18 treatments within a species ($\alpha = 0.05$).

		Extract Concentration (g/L^{-1})				
		0	1	4	8	12
Species	Day	% Germination				
Centaurea stoebe (Spotted knapweed)	3	38	32	25	15	10
	6	52	40	37	25	17
	9	58	50	45	28	28
	12	60	50	45	28	32
	15	62	52	45	30	35
	18	62	57	45	30*	37*
Penstemon hirsutus	3	67	13	12	7	12
	6	72	60	43	45	40
	9	80	62	53	50	47
	12	80	62	55	52	47
	15	80	62	55	55	47
	18	80	62*	55*	55*	47*
Ratibida pinnata	3	58	82	68	70	63
	6	78	87	85	87	80
	9	88	93	87	92	85
	12	90	93	87	92	85
	15	90	93	87	92	85
	18	90	93	87	92	85
Lupinus perennis	3	10	8	5	10	7
	6	10	12	5	12	8
	9	10	12	5	15	8
	12	10	12	5	15	8
	15	10	12	5	15	8
	18	10	12	5	15	8
Monarda fistulosa	3	42	48	47	25	33
	6	58	55	55	45	45
	9	58	60	58	57	50
	12	58	60	58	60	53
	15	60	60	58	60	53
	18	60	60	58	60	53

Andropogon gerardii	3	3	2	0	0	0
	6	23	27	12	18	10
	9	33	38	18	40	20
	12	35	42	22	45	27
	15	35	43	23	47	27
	18	35	43	23	47	27
Schizachyrium scoparium	3	0	0	0	0	0
	6	5	3	5	2	0
	9	8	3	10	3	0
	12	8	3	10	3	2
	15	8	3	10	3	2
	18	8	3	10	3	2
Elymus canadensis	3	0	0	0	0	0
	6	0	0	0	0	0
	9	0	3	0	2	0
	12	2	5	0	2	0
	15	2	5	0	2	0
	18	2	5	0	2	0
Panicum virgatum	3	0	3	2	0	3
	6	5	7	12	7	10
	9	7	8	12	7	10
	12	8	8	12	7	10
	15	8	8	12	7	10
	18	8	8	12	7	10

- Lupine Trail
- Education Building
- Little Grand Canyon

Figure 1. Aerial photo of Pierce Cedar Creek Institute and study site locations.

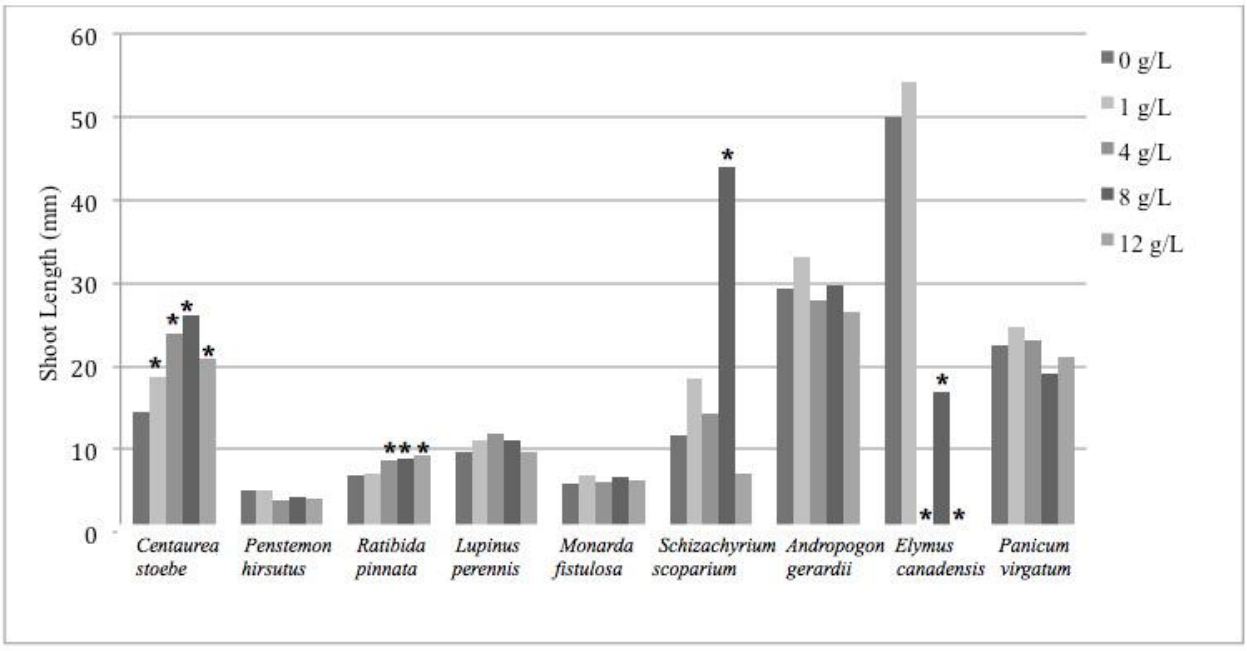
Figure 2. Mean root diameter of spotted knapweed plants by age class for each of the three study site.

Figure 3. Total number of plants in each age class at each of the three study sites.

Figure 4. Percentage of spotted knapweed plants with floral stalks per age class. The data from all sites were combined due to the low number of individuals in the older age classes.

Figure 5. The total number of flowering stems as compared to the total number of plants per age class.

(a)



(b)

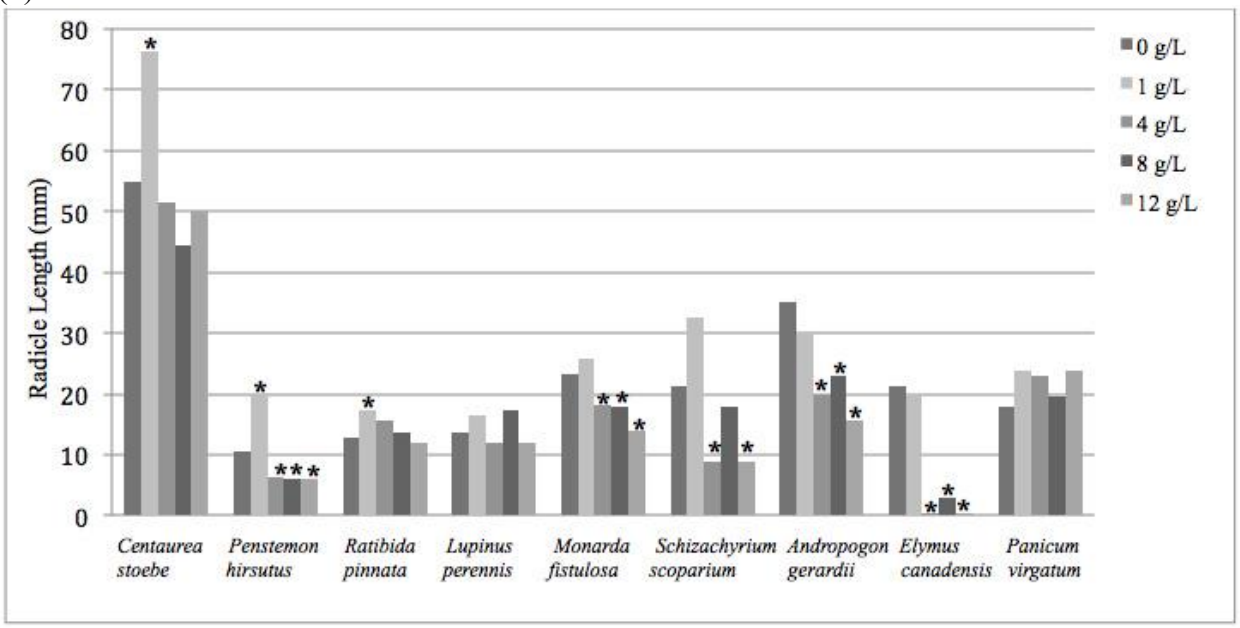


Figure 6. The pooled mean (a) shoot length and (b) radical length of 8 different native prairie species and spotted knapweed (*Centaurea stoebe*) after 18-days of treatment at various concentrations of *C. stoebe* root extract. * = significantly different from 0 g/L extract at $\alpha < 0.05$.

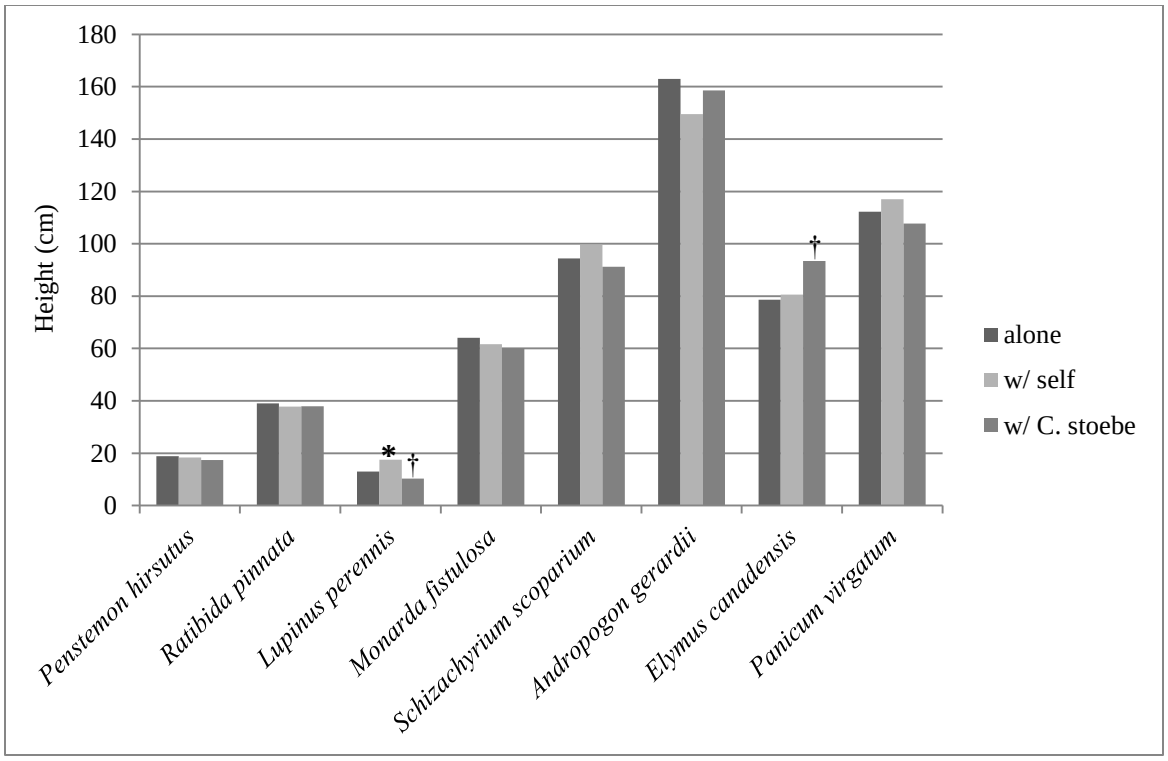


Figure 7. Mean plant height for eight native prairie species grown in competition with a conspecific or in competition with *Centaurea stoebe*. * = significantly different from control plant (alone) at $\alpha < 0.05$. † = significant difference between 2 competition treatments at $\alpha < 0.05$.

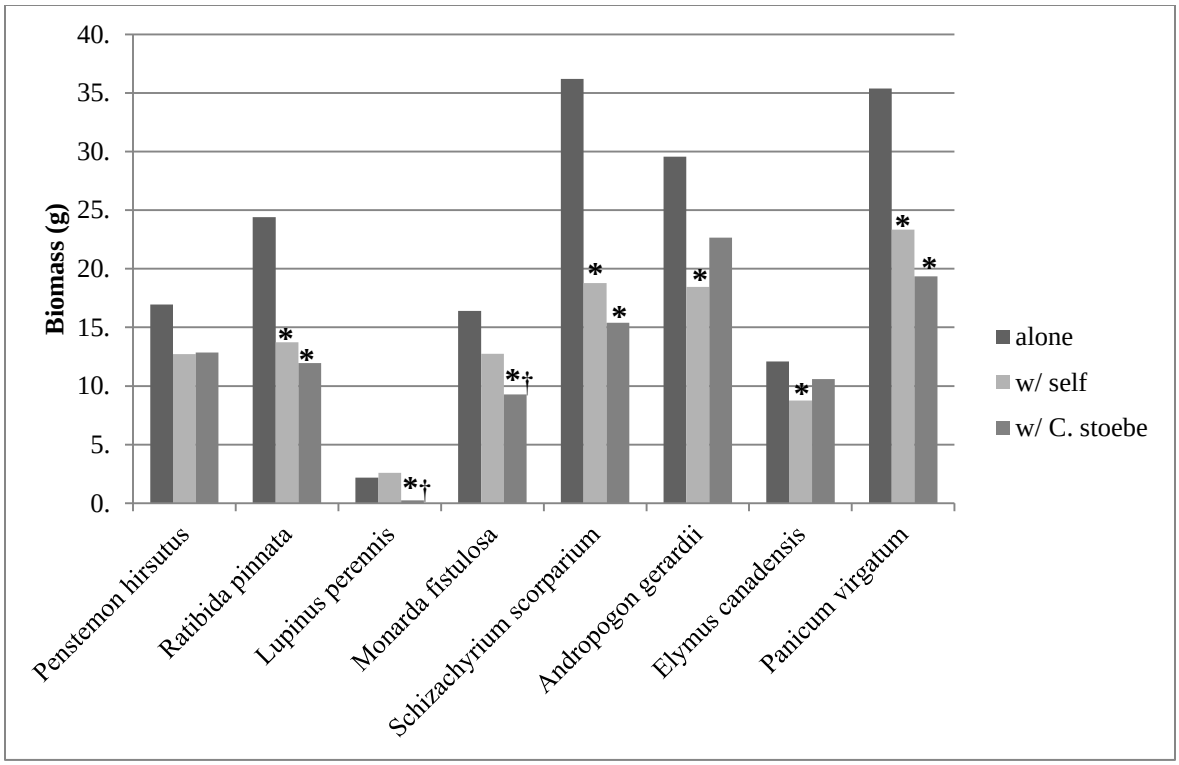


Figure 8. Mean plant biomass for eight native prairie species grown in competition with a conspecific or in competition with *Centaurea stoebe*. * = significantly different from control plant (alone) at $\alpha < 0.05$. † = significant difference between 2 competition treatments at $\alpha < 0.05$.