Effects of spotted knapweed (*Centaurea maculosa*) density, biomass, and removal on the growth of blueberry (*Vaccinium angustifolium*) and strawberry (*Fragaria virginiana*)

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Abstract

*Centaurea maculosa*, commonly known as spotted knapweed, is an allelopathic invasive plant species rapidly increasing across rangelands and meadows throughout North America. Such invasions have notable impacts on soil chemistry and biodiversity loss, though the differential responses of native plant species to increasing *C. maculosa* densities or to the removal of *C. maculosa* following population establishment remain unknown. This study sought to determine (1) relationships between the density of *C. maculosa* and those of two native plant species: *Vaccinium angustifolium* (low-bush blueberry) and *Fragaria virginiana* (strawberry); and (2) whether the removal of *C. maculosa* differentially affects the growth of *V. angustifolium* and *F. virginiana* by comparing density and percent cover of the species of interest along with other commonly found species in the plots. In order to measure the difference in the growth of *V. angustifolium* and *F. virginiana* in the absence of *C. maculosa*, manual removal of *C. maculosa* was performed on a series of paired plots. Density and percent cover of *C. maculosa* had no influence on the density and percent cover of *V. angustifolium* or *F. virginiana*. The results suggest that *C. maculosa* density does not affect *V. angustifolium* or *F. virginiana* density within the study site. The removal of *C. maculosa* did not affect the growth of either *V. angustifolium* or *F. virginiana*, indicating that *C. maculosa* does not have short-term effects on either native species, and that manual removal of *C. maculosa* may not be an effective removal method.
**Introduction**

Invasive species are those introduced outside of their native ranges that adversely influence the diversity and density of native species in a given area due to increased competition, having greater rates of growth and reproduction (Callaway & Ridenour 2004, Hejda et. al. 2009). Native species, that have not evolved ways to survive in the presence of invasive species, are consequently vulnerable to competition from invasive species and reduced fitness (Perry et. al. 2005). As invasive species use soil resources, native species diversity may decrease because these organisms cannot obtain resources necessary for proper growth (DiTomaso 2000). Further, invasive species may deter native species from inhabiting an area after foraging and living habitats have been physically or chemically altered (DiTomaso 2000). In addition to out-competing native species for space and nutrients, invasive species can alter the structure of environments as well. Many invasive plants use allelopathy to secrete chemicals into the soil, such that the chemistry of the habitat becomes altered (Perry et. al. 2005). Due to these potential effects, invasive species can be very costly to prevent and properly remove (DiTomaso 2000). Characterization of these impacts at both the population and community level is imperative, as such work may contribute to improved approaches of preventing biological invasions.

Densities of *Centaurea maculosa*, commonly known as spotted knapweed, are rapidly increasing across rangelands and meadows throughout North America (Knochel & Seastedt 2009). Native to Eurasia, the native range of *C. maculosa* spans from the Mediterranean to eastern Russia (Sheley et. al. 1998). Shortly after the introduction of *C. maculosa* in North America in the late nineteenth century, the plant established dominance in many habitats, most notably causing soil erosion, biodiversity loss, and reduction of grazing area in rangelands in the west (Sheley et. al. 1998). The mechanisms through which *C. maculosa* and its *Centaurea* relatives outcompete native species remain unknown and controversial, though several hypotheses have been proposed. These hypotheses fall into the following categories: direct plant-plant interactions, indirect plant-soil interactions and plant-herbivore interactions (Knochel & Seastedt 2009). The plant-plant hypothesis focuses on the allelopathic mechanism by which *C. maculosa* alters soil chemistry. The plant secretes a phytotoxin known as catechin, which causes cell death in the roots of plants that have not evolved resistance to it (Callaway & Ridenour 2004, Perry et. al. 2005). Most North American plants that have recently been exposed to *C. maculosa*
have yet to evolve resistance to catechin, and as such, are vulnerable to the effects of the toxin (Perry et al. 2005). The plant-soil hypothesis proposes that *C. maculosa*’s main competitive advantage in non-native habitats comes from its “selective inhibition of mycorrhizal fungi that benefit competing plants,” causing the roots of these plants to be less effective at uptaking nutrients (Knochel & Seastedt 2009). The plant-herbivore hypothesis maintains that *C. maculosa* populations benefit from low to intermediate levels of insect herbivory, as it increases the amount of catechin produced by individuals (Perry et al. 2005, Knochel & Seastedt 2009, Nilsson et al. 1993, Callaway & Ridenour 2004).

The purpose of the study was to determine (1) relationships between the density of *C. maculosa* and those of two native plant species: *Vaccinium angustifolium* (low-bush blueberry) and *Fragaria virginiana* (strawberry); and (2) whether the removal of *C. maculosa* differentially affects the growth of *V. angustifolium* and *F. virginiana*. Primary literature on *C. maculosa* indicates a strong correlation between *C. maculosa* density and catechin levels in soil (Perry et al. 2005), allowing *C. maculosa* density to be used as a proxy for potential allelopathic effects on co-occurring native plant species. Some plant species’ root systems contain long, deep roots, while other species specialize in shallower, more angled roots (Fitter 2008). *C. maculosa* exhibits a long taproot system, composed of a single long root extending deep into the soil and many small roots extending laterally along its sides (Fitter 2008). This taproot system allows *C. maculosa* to reach underground water resources more successfully than native species with shallower root systems, which could hinder the growth of these native plants (Fitter 2008, United States 2006). Both *V. angustifolium* and *F. virginiana*, co-occur with *C. maculosa*, while differing in root systems. *V. angustifolium* has a small tap root system, while *F. virginiana* has a shallow root system. The manual removal of *C. maculosa* on the short-term growth rates of the two native species were characterized in plots where *C. maculosa* was removed, and in paired control plots where *C. maculosa* densities were maintained. We hypothesized that increasing density of *C. maculosa* would be negatively associated with the densities of the two native species. We also expected results that *C. maculosa* density would have more adverse effects on the growth of *F. virginiana* due to its shallow root system and possible increased uptake of catechin.

**Materials and Methods**

Site Description
The study was conducted on the UVB field, a meadow located on the University of Michigan Biological Station (UMBS) property in Pellston, Michigan. The study site measures approximately 100 m x 175 m and is located to the west of Douglas Lake’s South Fishtail Bay. The UVB field is surrounded by a hardwood forest dominated by *Acer rubrum*, *Quercus rubra*, and *Populus grandidentata*. Native species found within the UVB Field include: *Pteridium aquilinum*, *Verbascum thapsus*, *Asclepias syriaca*, *Fragaria virginiana*, *Vaccinium angustifolium*, *Centaurea maculosa*, *Hieracium pratense*, *Cladonia rangiferina*, *Cladonia cristatella*, *Taraxacum officinale*, and a species in the *Lamiaceae* family.

Plant Diversity Field Studies

To determine whether *C. maculosa* density affects growth of native *V. angustifolium* and *F. virginiana*, thirty-one 1 m x 1 m plots were set up along parallel linear transects in the UVB field (Figure 1). Each plot was placed more than 10 meters from the forest edge, to minimize any edge effects. The plots were established to represent the full range of densities of *C. maculosa*, *V. angustifolium*, and *F. virginiana* within the study site. The density (individuals/m²) of each target species and other native species (excluding grasses) were recorded. The main stem of each plant was measured from the tip to the base. Percent cover of each plant species (including grasses) was taken and recorded, as well. Percent cover was measured by dividing a 1 m x 1 m grid into forty-nine smaller cross sections. At each cross-section where two or more lines intersected, the plant species was recorded. The number of species occurrences was divided by total cross-section number to estimate percent cover. Following field measurements, *C. maculosa* density and percent cover were related to the density and percent cover of *V. angustifolium*, and *F. virginiana*.

Short-term Response of *V. angustifolium* and *F. virginiana* to *C. maculosa* Removal

Plot-level plant community similarity was determined by pairwise comparisons using Chi square tests, for which $\alpha > 0.01$. To minimize variability in other environmental properties not measured across the field (e.g. amounts of sunlight, soil moisture, soil nutrient content, and soil pH), the study site was partitioned into quadrants, and pairs of plots were chosen within each section. *C. maculosa* was manually removed from one plot of each pair, and maintained in the second plot as a control. To measure the effects of *C. maculosa* density on growth of *V*. 
angustifolium and F. virginiana, three individuals of each species were measured in each plot. Three growth metrics measured three times over nine days following C. maculosa removal included: height of the plant stem, the length of one leaf, and the width of the same leaf. Total C. maculosa biomass (fresh-weight) collected by plot was recorded. A subset of the C. maculosa biomass was then dried in a 60°F oven for approximately 40 hours. A fresh weight to dry weight ratio was calculated and used to determine the dry weight biomass (g) of C. maculosa for each plot.

Statistical Analysis

Relationships between the percent cover and density of C. maculosa and that of the two native species (V. angustifolium and F. virginiana) were analyzed using both linear regression and Mann-Whitney U tests. Research suggests that catechin levels in soil are directly correlated with C. maculosa biomass (Perry et. al. 2005), so we also tested whether target native plants were less abundant in areas with higher C. maculosa density using linear regression. The data was then split into three groups based on C. maculosa density (<10, 10-79, and >80), and Kruskal-Wallis tests were performed followed by Mann-Whitney U tests to determine pair-wise differences in native plant species responses to C. maculosa density classes. The significance of the Kruskal-Wallis test and Mann-Whitney U test outcomes were evaluated at $\alpha = 0.05$. Wilcoxon Rank Sum tests were used to determine the variation among means of native species growth in the paired plots. The significance of the Wilcoxon rank sum test outcomes were evaluated at $\alpha = 0.05$.

Results

Linear regressions indicated that C. maculosa density was positively associated with, but did not significantly explain, V. angustifolium density (Figure 2; $R^2 = 0.102$, $p = 0.553$). Similarly, C. maculosa density was negatively associated with, but did not significantly explain, F. virginiana density (Figure 3; $R^2 = 0.072$, $p = 0.445$). C. maculosa percent cover was positively associated with, but did not significantly explain, V. angustifolium percent cover (Figure 4; $R^2 = 0.063$, $p = 0.787$). C. maculosa percent cover was negatively associated with, but did not significantly explain, F. virginiana percent cover (Figure 5; $R^2 = 0.005$, $p = 0.240$).
Nonparametric Mann-Whitney U tests were performed to analyze the relationships between abundances and percent cover data of *C. maculosa* with *V. angustifolium* and *F. virginiana*, separately. No significant correlation was found between the density of *C. maculosa* and *V. angustifolium* ($p = 0.466$). The test between the density of *C. maculosa* and *F. virginiana* indicates no significant correlation between the densities of the two species ($p = 0.787$). No significant associations were found from the test between the percent cover of *C. maculosa* and *V. angustifolium* ($p = 0.492$) or from *C. maculosa* and *F. virginiana* ($p = 0.950$).

*C. maculosa* density was divided into three density classes based upon number of *C. maculosa* individuals per plot: <10, 10-79, and >80. Split into these three density classes, nonparametric Kruskal-Wallis tests were performed to determine whether the changes in native species density or percent cover were correlated with differing density groups (Table 1). No significant correlation was found between densities of *C. maculosa* and *V. angustifolium* density ($p = 0.525$). Similarly, no significant correlation was found between different densities of *C. maculosa* and *F. virginiana* density ($p = 0.366$). No significant correlation was found between different densities of *C. maculosa* and *V. angustifolium* percent cover ($p = 0.303$), or between different densities of *C. maculosa* and *F. virginiana* percent cover ($p = 0.630$).

Plots A2 and B5, C1 and E3, and E2 and Y3 were paired together based on Chi square tests, for which the significance value was set to $p = 0.01$. *C. maculosa* was removed from plots A2, C1, and E2, and was maintained in plots B5, Y3, and E3. The Wilcoxon Rank Sum test compared the overall change in stem height of *F. virginiana* in all pairs and the overall change in stem height of *V. angustifolium* in the pair of E2 and Y3 (the only statistically similar plots in which *V. angustifolium* was present). No significant difference was found between the change in *F. virginiana* stem height in plots in which *C. maculosa* was removed or maintained ($p = 0.342$). A similar result was found for growth rates in *V. angustifolium* ($p = 0.109$).

**Discussion**

Correlations between density and percent cover of both *V. angustifolium* and *F. virginiana* and *C. maculosa* density were compared to determine whether the density of either native species would be affected by *C. maculosa* differently. Given that neither the density nor the percent cover of *C. maculosa* were significantly associated with the density and percent
cover of either *V. angustifolium* or *F. virginiana*, the null hypothesis was retained that *C. maculosa* does not affect the density or percent cover of one native species more adversely than the density or percent cover of the other. It is unlikely that *C. maculosa* has no effect on either *V. angustifolium* or *F. virginiana*, as this invasive species has been known to alter the structure of similar plant communities and produce negative effects on the native species present (DiTomaso 2000).

The lack of significant relationships could have been caused by an inability of *C. maculosa* to affect the native species selected, the timing of the observations made, and noisy variables that were not properly standardized. *C. maculosa* populations have been established on UMBS property for a few years, such that the the remaining *V. angustifolium* and *F. virginiana* populations in the area likely contain phenotypes with the ability co-occur with *C. maculosa*. This possibility would also yield a non-significant response between the density and percent cover of *C. maculosa* and the two native species. This is a reasonable explanation, due to the fact that *C. maculosa* individuals begin producing catechin within the first few weeks after germination, and the toxin can be fatal to seeds of native species at low levels (Weir 2003, Inderjit et al. 2008). While noisy variables were standardized wherever possible, undetected noisy variables could have created no-correlation responses. All plots were placed at least 10 m from the forest to avoid edge effect; however, as no standard exists for where edge effect begins, and it remains unknown whether this effect altered the data collected. Possible edge effect could have contributed differences in soil composition, light availability, and species diversity in each plot. Soil composition and nutrients were also not measured and as such, location of differing amounts of invasive and native plants could have been caused by any of these factors, and relationships between the invasive and native species abundances may have been obscured. Last, a better representation of the effects of *C. maculosa* could have been obtained with a greater number of plots and knowledge of the species composition of the study site before the introduction of *C. maculosa*. Standardizing environmental conditions and establishing the composition of plant communities before *C. maculosa* invasions would have allowed for more rigorous tests of community responses to *C. maculosa* densities.

Herbivory may play a significant role in the ability of invasive species to spread in their respective environments, as native specialist herbivores do not prey on invasive species, and generalist herbivores tend to ignore the presence of the new invasive plants (Inderjit 2012).
Similarly, herbivory rates on invasive species are lower than those of non-invasive exotic species, which are non-native species that do not create the same risks of habitat invasion and biodiversity loss (Cappuccino & Carpenter 2005). Invasive species suffer “on average, 96% less leaf damage than non-invasive species,” likely due to the greater taxonomic gap between invasive species and native species than that of non-invasive and native species (Cappuccino & Carpenter 2005). Herbivory on the *F. virginiana* plants by *Emphytus maculatus* larvae (Scholtens, personal communication) could have functioned as a confounding variable; we attempted to avoid this when selecting paired plots for *C. maculosa* removal by pairing plots in the same quadrant that exhibited similar levels of herbivory. Entomologist, Brian Scholtens identified the larvae as “*Emphytus maculatus*, the strawberry sawfly in the family Tentredinidae,” based upon the placements of its eyespots and its particular host (Scholtens). Herbivory on native plants may still pose a problem, however, as the absence of leaves could altogether hinder potential plant growth. Additionally, leaf length and leaf width measurements on native plants could not be quantified in certain cases due to herbivory. Though not quantified in this study, higher levels of herbivory on native plant specie relative to that of herbivory on *C. maculosa*, could have long-term effects on the abundance of either native species in the field and indicates a competitive advantage of *C. maculosa* in that it lacks a natural herbivore in the study site.

Current removal techniques of *C. maculosa* include the introduction of co-evolved herbivores (e.g. natural enemy release), removal using chemicals or fire, addition of other native plant species, or manual removal (Knochel & Seastedt 2009). While the best method for eliminating *C. maculosa* in a habitat is “concurrent top-down (herbivory), and bottom-up (plant-mediated resource density) effects,” these methods require several years to become effective, due to pre-existing *C. maculosa* seed banks in many habitats (Knochel & Seastedt 2009). The possibility also exists that insect herbivores introduced for biocontrol could be one of the species that cause increased catechin dispersal by *C. maculosa*, thereby conferring this invasive a competitive advantage (Thelen *et al.* 2005). Most removal techniques, with the exception of manual removal, tend to have significant negative impacts on total biomass of native species in the area (Knochel & Seastedt 2009). While manual removal was the only removal method available for the study, it is not considered the best technique for permanently removing *C. maculosa* from a given area, and may lead to the colonization of the area by other weeds and the return of *C. maculosa* (Knochel & Seastedt 2009, Mauer 2001). The high density of *C. maculosa*
in certain plots also made it difficult to remove *C. maculosa* individuals without disturbing native species, and some invasive roots and seeds may have been left behind. This may have created a noisy variable of differential disturbance across plots, with the highest levels of disturbance in plots that exhibited higher densities of *C. maculosa*.

Prior research posits that *C. maculosa* biomass is directly correlated with catechin levels in the soil, and as such, the assumption was made that plots in which *C. maculosa* was more abundant would also exhibit higher levels of catechin in the soil (Perry *et. al.* 2005). After quantifying *C. maculosa* biomass removed from the study site (Table 2), a significant positive relationship between *C. maculosa* density and biomass was verified by a linear regression (Figure 6). Catechin remains in the soil for an unknown period of time following the removal of *C. maculosa*, and that “North American soil biota may promote invasion” by allelopathy from the Centaurea family (Vivanco 2004). Thus, after the removal of *C. maculosa*, catechin may have remained in the soil and inhibited the growth of *V. angustifolium* and *F. virginiana*, thereby contributing a noisy variable to the experiment. *C. maculosa* also reappeared in the plots in which it was removed towards the end of experiment, thereby allowing individuals to secrete catechin again. *C. maculosa* seeds likely remained in the soil (Emery & Gross 2005). The most effective method of removing the viable seeds and reducing catechin levels would include annual burnings of the soil; however, while effectively removing *C. maculosa*, prescribed burns would do so at the expense of the native species abundances as well (Emery & Gross 2005).

**Future Directions**

The results of this study indicate that further research will be necessary to better understand the effects of *C. maculosa* on specific native plants. A possible topic for further study would be to explore the effects of juvenile *C. maculosa*, rather than that of established individuals, on native species. Also, this study could be replicated over a longer observation period, to more accurately define the time-scale over which *C. maculosa* may affect the density and growth of native plant species. The removal technique could also be evaluated over an extended period of time, as manual removal is the simplest and least habitat-damaging technique available. The ability to predict biomass from *C. maculosa* density could facilitate future studies linking population density to soil catechin levels.
Acknowledgments

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Literature Cited


Scholtens, B. (personal communication, June 7, 2013).


Table 1: Kruskal-Wallis tests describing significant differences in the density and percent cover of the native plants among different densities of *C. maculosa*.

<table>
<thead>
<tr>
<th>Transect</th>
<th>Plot Number</th>
<th><em>C. maculosa</em> Fresh Weight (g)</th>
<th>Subsample Fresh Weight (g)</th>
<th>Subsample Dry Weight (g)</th>
<th>Dry Weight to Fresh Weight Ratio</th>
<th><em>C. maculosa</em> Dry Weight (g)</th>
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</thead>
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<td>80.41</td>
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<td>0.1435</td>
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<td>129.87</td>
<td>20.78</td>
<td>0.1600</td>
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<td>36.63</td>
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<td>7.40</td>
<td>0.1599</td>
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<td>33.22</td>
<td>6.37</td>
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<td>17.7543</td>
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Table 2: Fresh weight (g) and dry weight (g) of *C. Maculosa* removed from experimental plots.
Figure 1: Google Maps’ image of the UVB field at UMBS. Parallel yellow lines represent five, 25 m transects, each with four to five 1x1 m plots. Yellow boxes represent areas each containing 3, 1x1 m plots. The study site contained 31 total plots. Blue circles represent plots A2 and B5, one experimental pair of plots. Additional pairs include C1 and E3 (orange circles) and E2 and Y3 (purple circles).
Figure 2: Linear regression between *V. angustifolium* density and *C. maculosa* density. $R^2$ value is 0.102. Regression equation is: $y = 19.37 + 0.18x$.

Figure 3: Linear regression between *F. virginiana* density and *C. maculosa* density. $R^2$ value is 0.072. Regression equation is: $y = 37.68 - 0.18x$. 
Figure 4: Linear regression between *V. angustifolium* percent cover and *C. maculosa* percent cover. $R^2$ value is 0.063. Regression equation is: $y = 0.16 + 0.21x$.

Figure 5: Linear regression between *F. virginiana* percent cover and *C. maculosa* percent cover. $R^2$ value is 0.005. Regression equation is: $y = 0.15 - 0.12x$. 
Figure 6: Linear regression describing *C. maculosa* density according to *C. maculosa* biomass. R² value is 0.959. Regression equation is: y = -8.91 + 1.85x.