

Simulated Fire Season and Temperature Affect *Centaurea stoebe* Control, Native Plant Growth, and Soil (\pm)-catechin

Zachery T. Pitman and Todd A. Aschenbach

ABSTRACT

Invasive species, including the non-native forb *Centaurea stoebe* (spotted knapweed), constitute a threat to degraded and restored native prairies. Considering the threat that *C. stoebe* poses to prairie ecosystems, we examined the effectiveness of fire as a control for *C. stoebe* and (\pm)-catechin, a known allelopathic compound. We conducted an experiment in a reconstructed tallgrass prairie community at Pierce Cedar Creek Institute in Barry County, Michigan starting in May 2016. Our experiment consisted of individually burning 60 1-m² plots with a propane torch to achieve high (316°C) and low (103°C) temperatures across spring and summer seasons over two years, then planting and seeding six native prairie plant species to monitor their establishment after burning. We compared the effects of the different burn treatments on the plant community by estimating percent cover and biomass of all species within each plot in August 2017. We also examined the effects of the simulated burn treatments on soil (\pm)-catechin levels, which we quantified using High Performance Liquid Chromatography. *Centaurea stoebe* was less dominant in burned plots than unburned plots, with summer-burned plots having the lowest biomass and cover. Differences in burn temperature failed to produce significantly different results. Planted native grasses increased more after spring burns than after summer burns. Preliminary findings suggest that high-temperature spring burns may indirectly reduce soil (\pm)-catechin levels. Overall, these results indicate that prescribed burning is an effective tool for controlling *C. stoebe* and promoting native species establishment in restored tallgrass prairies.

Keywords: allelopathy, catechin, grassland, prescribed burn, restoration

Restoration Recap

- Burn season affects *C. stoebe* and native species establishment more than burn temperature.
- Mid-spring and summer burns reduce *C. stoebe* dominance.
- Summer burns are more effective at reducing *C. stoebe* dominance than spring burns, but may hinder native warm season grass establishment.
- High temperature spring burns may reduce soil (\pm)-catechin levels.

Conservation and restoration of valuable or imperiled ecosystems are major foci of restoration ecology. Native grassland ecosystems have suffered serious declines in Midwestern North America since European settlement (Samson et al. 2004, Savage 2011). Despite their rarity, these grasslands provide important habitat for many plant and animal species. Nearly 260 bird species use grasslands as nesting habitat in the North American Great Plains (Savage 2011). In Michigan, nearly one-third of the state's

threatened, endangered, or special concern species find their primary habitat in grasslands (O'Connor et al. 2009). Many of these grassland species are in decline due to habitat loss and fragmentation from agricultural development (Herkert et al. 2003, Savage 2011), which strengthens the case for grassland conservation and restoration. Therefore, developing techniques to restore and manage grassland communities should be a primary concern for both ecologists and land managers. In Michigan, some communities have experienced statewide declines of nearly 99.99 percent (O'Connor et al. 2009), leading to designation of all of the state's prairie communities as either imperiled or critically imperiled (Cohen et al. 2015). Although the majority of lost

grassland communities can be associated with conversion to agriculture, invasive species threaten what little remains (D'Antonio and Meyerson 2002, Grant et al. 2009).

Centaurea stoebe (spotted knapweed) is a non-native, invasive, Eurasian forb that has infested over 2.9 million hectares of degraded and remnant grassland communities in North America (DiTomaso 2000). *C. stoebe* forms dense monotypic stands and may outcompete some native plant species (Tyser and Key 1988). *C. stoebe* succeeds as an invasive plant due to high seed production and germination (Schirman 1981), effective use of abundant resources (Knoche et al. 2010), and production of (\pm)-catechin (hereafter catechin). Catechin is an allelopathic chemical which *C. stoebe* excretes into the soil and has been shown to decrease growth of other plants in both lab and field studies (Perry et al. 2005, Thorpe et al. 2009). Catechin is thought to be a novel weapon (Callaway and Ridenour 2004, Inderjit et al. 2011). However, some studies doubt the influence of catechin in *C. stoebe* invasion due to low levels of catechin found in *C. stoebe* soils and a lack of evidence for catechin as a cause of oxidative stress in affected plants (Blair et al. 2006, Duke et al. 2009). Recent studies have answered some of this criticism by demonstrating the potential for catechin to harm beneficial soil biota as well as interact with and amplify phytotoxic metals present in the soil (Pollock et al. 2009, 2011, Wang et al. 2013). Additionally, the effect of catechin may be variable within soils at a site and catechin retention may depend on site-specific conditions such as soil type and companion compounds (Perry et al. 2007, Tharayil et al. 2008, Pollock et al. 2009).

Naturally occurring frequent fires were an important force in shaping North American grassland communities prior to European settlement (Samson et al. 2004, Allen and Palmer 2011). As such, prescribed fire is a tool used in the restoration of grassland systems and often employed to suppress invasive species (Kyser and DiTomaso 2002, DiTomaso et al. 2006, Bowles and Jones 2013). Fire can reduce the dominance of *C. stoebe* and recruitment by seed in infested areas (Emery and Gross 2005, MacDonald et al. 2007, Vermeire and Rinella 2009). Research has also shown that infested areas subjected to fire saw increased establishment of native prairie plants (MacDonald et al. 2007, Martin et al. 2014). Emery and Gross (2005) found burning *C. stoebe* in mid-summer to be most effective in reducing its biomass and number of flowering individuals, compared to early spring and mid-fall burns, although fuel loadings were quite low during some burn dates due to low productivity and warm-season grass cover. MacDonald et al. (2007) observed significant reductions in *C. stoebe* densities and biomass as a result of mid-spring burning in an area with high fuel loadings and dominated by warm-season grasses.

Studies have shown wide-ranging effects on the chemical properties of soil in systems that are managed with prescribed fire (Gómez-Rey et al. 2013, Pereira et al. 2017).

Therefore, fire may also degrade or alter the allelopathic chemical catechin in the soil, although no research on the topic has been performed to our knowledge. Additionally, the effects of fire temperature on *C. stoebe* infestations is unknown, as is the optimal timing of burns for the restoration of *C. stoebe*-infested communities. Both mid-spring and summer burns are potentially effective control methods for *C. stoebe* in tallgrass prairies, but a direct comparison has yet to occur. Moreover, the response of the native plant community to summer burns in *C. stoebe* infestations is an important component of restoration that requires further study.

Our experiment takes into account both fire season and temperature to identify how different prescribed burning techniques affect invasive weeds, native plant communities, and catechin in the soil. Understanding these relationships can help managers better address *C. stoebe* control, native species establishment, and soil catechin degradation. We address several questions: 1) how does fire season and temperature affect *C. stoebe* dominance? 2) how does fire season and temperature affect native species establishment? and 3) does the application of prescribed fire reduce the amount of catechin present in soils? Answering these questions will advance the field of restoration ecology and inform future restoration of grassland communities.

Methods

Study Site

Our study took place at Pierce Cedar Creek Institute (PCCI) in Barry County, Michigan. PCCI operates as an environmental education center and biological field station while the 742 acres of land is managed as a public nature reserve. Soils at the site are classified as Perrinton Loam and average annual rainfall is 95.15 centimeters (Natural Resources Conservation Service 2017). The specific study area was historically farmed but was taken out of production in the 1950s. The area has been reconstructed and is now classified as mesic prairie, which is considered critically imperiled in Michigan (Cohen et al. 2015). PCCI has engaged in prairie restoration activities since 1998, but our site has received little attention aside from occasional mowing, leading to continued infestation by *C. stoebe*. Prior to any treatments, average *C. stoebe* cover at the site was consistent with greater than 20% cover observed at sites used by Perry et al. (2007) in a similar examination of *C. stoebe* and catechin. Other common species at the site included *Bromus inermis*, *Poa pratensis*, *Rumex acetosella*, *Achillea millefolium*, and *Solidago* spp.

We established 60 1-m² plots in seven parallel rows at the site, with 5–13 plots in each row, and a 0.5-m buffer between each plot. The shape and size of the study area prohibited an even number of plots in each row. We incorporated six burn treatments: spring burn/high temperature

(SPHT), spring burn/low temperature (SPLT), spring control (no burning; SPC), summer burn/high temperature (SUHT), summer burn/low temperature (SULT), and a summer control (SUC). We subjected each burn plot to its specific treatment twice over the course of the study, once in 2016 and once in 2017. Treatments were randomly assigned to individual plots throughout the study area and each treatment was replicated 10 times for a total of 60 plots. We felt that a block design was unnecessary, as our study occurred in a small area with little variation in soils, topography, or plant composition.

Burn Treatments, Native Species Plantings, and Vegetation Sampling

To simulate prescribed fire, we used a propane torch to burn each plot individually. We chose the low (103°C) and high (316°C) temperatures to reflect the range of typical tallgrass prairie fire temperatures at the soil surface (Vermeire and Roth 2011, Ohrtman et al. 2015). We used Tempilaq G heat-sensitive paint (LA-CO Industries, Grove Village IL) applied to small sheets of aluminum to determine when the plots reached the specified temperature. This paint turns to liquid when it is heated to the correct temperature. Low temperature plots required five seconds of burning to reach 103°C and high temperature plots required 15 seconds of burning to reach 316°C. Spring burns were conducted on May 19, 2016, while summer burns were conducted on June 29, 2016. We removed plant biomass in control plots using a gas-powered weed trimmer in conjunction with the 2016 burns. This removed the influence of remaining aboveground biomass on planted species establishment, without the added effects of burning. MacDonald et al. (2013) demonstrated that such single-application mowing treatments did not significantly reduce *C. stoebe* densities or biomass, so we feel that these plots represented an appropriate control.

Following each burn, we seeded and planted plugs of a suite of native genotype grassland species (from Hidden Savanna Nursery, Kalamazoo, Michigan) in the burned plots and their associated control plots. Seeded species included three forbs: *Lupinus perennis* (wild Lupine), *Asclepias tuberosa* (butterfly-weed), and *Anemone cylindrica* (thimbleweed) and three grasses: *Sorghastrum nutans* (Indian grass), *Schizocyrium scorparium* (little bluestem), and *Panicum virgatum* (switchgrass). We included an equal number of seeds for each species. We raked seeds into the soil at a relative abundance of 600 total seeds/m² to a depth of approximately ¼ inch immediately after seeding in half of each plot. Prior to planting, we appropriately scarified and/or thermally stratified seeds for each species. We planted container grown plugs on the remaining side of each plot at a rate of two plugs per species for a total of 10 plugs per plot (n = 600 for experiment; 300 per burn season). Plug species included all seeded species, with the

exception of *A. cylindrica*, which could not be obtained from the supplier. At the time of planting, seedlings were well developed with heights between 10 cm and 20 cm, depending on the species. We irrigated seeds and plugs daily during the first week following seeding and planting. When rainfall fell at least 20 percent below the weekly average (2.36 centimeters from May to August), we irrigated all plots with enough water to achieve the average when combined with observed precipitation.

We collected vegetation data for all 60 plots on May 15 and 16, 2017. Within each plot, we sampled species richness, vegetative cover, and above-ground biomass. We determined vegetative, bare ground, and litter cover using point-intercept sampling. For the point-intercept sampling, we placed a 1 × 1-m PVC frame over each plot. We used permanent marker to place equally spaced hashes on the frame (six hashes on two opposite sides, nine hashes on the other two), which lined up to create a sampling grid of 54 points. At each point, we dropped a survey pin and recorded each plant species touching the pin, with the amount of touches for each species corresponding to total percent cover of that species within the plot. After estimating cover for each plant species, we harvested all aboveground biomass in a 10 cm × 1 m strip from each sampled plot, sorted to species, and dried all biomass at 65°C to a constant mass in a drying oven. We then weighed and recorded the biomass for each species in each plot.

We then burned the SPLT and SPHT plots a second time on May 19, 2017, following the same burn procedure from 2016. On June 30, 2017, we burned the SULT and SUHT plots a second time. We watered plots whenever weekly precipitation fell below average using the same procedures described for 2016. We did not seed or plant any new species following the 2017 burns. In August 2017, we collected species richness, cover, and biomass data for all 60 plots, avoiding the previous strip of biomass collection when collecting biomass for the second time. We calculated change in *C. stoebe*, planted grass cover, and biomass by comparing May and August vegetation sampling results.

Soil Catechin Analysis

In April 2017, we set up five additional plots adjacent to the original 60 plots to directly examine catechin levels at the site and determine the effect of the different burn treatments on soil catechin. Our catechin study only incorporated one replicate for each treatment due to the logistical constraints associated with processing a large number of soil samples, so interpretations of the data were treated with caution. We chose five mature *C. stoebe* plants of approximately the same size (canopy diameter roughly 21 cm) to serve as the center of each 90 cm diameter plot. We then hand-pulled all other *C. stoebe* individuals within one meter of each of the five center individuals in order to isolate the analysis to a single plant. When necessary, we

used a trowel to assist in taproot removal. We continued to weed the plots throughout the summer as needed. Due to the relatively quick degradation of catechin in soils (Tharayil et al. 2008) and the demonstrated effectiveness of hand-pulling as a control method for *C. stoebe* (MacDonald et al. 2013), we are confident that no residual catechin from the pulled plants affected our analyses.

To identify the relationship between *C. stoebe* density and soil catechin levels, we divided each plot into three zones of 15 cm increments. Zone One was 0–15 cm from the center plant, Zone Two was 16–30 cm from the center plant, and Zone Three was 31–45 cm from the center plant. We collected a standard amount of soil from each of the three zones by filling a cylinder with the top five centimeters of soil at four points for a total of 8.84 cm³ per zone. We took our first samples immediately before burning on May 19, 2017, and continued collection once each month in June, July, and August. We randomly subjected each *C. stoebe* plant to one of the different burn treatments from the vegetation survey (SPLT, SPHT, SULT, and SUHT) and included a single control plant which was not burned. We also collected soil samples for analysis immediately after each plot received its burn treatment in case there were any immediate impacts on soil catechin levels. Immediately after collection, we froze all soil samples in an on-site freezer in order to prevent catechin degradation.

To determine catechin levels in our soil samples, we used High Performance Liquid Chromatography (HPLC) available via the Grand Valley State University Chemistry Department. Our method for catechin extraction followed that of Blair et al. (2005), which identified a 75% acetone, 25% water, and 0.1% phosphoric acid extraction solvent as the most efficient for catechin recovery. We ran extracted catechin samples through a gradient system using a 90% water, 10% acetonitrile, 0.1% phosphoric acid mobile phase, which was increased after five minutes to 30% acetonitrile over 10 minutes and held at 30% for three minutes (18 minutes total). Using catechin standards, we determined that catechin appeared on the HPLC chromatograms at roughly 9.1 minutes. We quantified catechin in µg/mL by comparing peak area of soil extractions to peak areas of known concentrations of catechin in prepared standards.

Data Analysis

Our data did not meet the assumptions for parametric statistical analyses, even after using transformations. We used a non-parametric Sheirer-Ray-Hare (Scheirer et al. 1976) test to determine whether significant differences in *C. stoebe* and planted species biomass and cover occurred in response to our methods. We ran Sheirer-Ray-Hare tests on average *C. stoebe* and planted grass cover in August, change in cover, biomass in August, and change in biomass with burn season (spring, summer) and burn temperature (control, low, high) as independent factors. For

comparisons of individual treatments, we used a non-parametric Mann-Whitney U test. We also ran a Sheirer-Ray-Hare test on average soil catechin with burn treatment (Control, SPLT, SPHT, SULT, SUHT) and distance from plant (0–15, 16–30, 31–45 cm) as independent factors. We used SPSS statistical software to conduct all tests, (SPSS v. 22, IBM Analytics, Armonk, NY).

Results

Plant Community Response

Across all plots and sampling dates, we encountered 55 total plant species. Of these 55 species, 25 were species native to Michigan, and 30 were non-native. *C. stoebe* was among the most common species, occurring in all 60 plots prior to 2017 burn treatments. Seeded grasses established in all plots, and we encountered seedlings of each planted species except *A. cylindrica* throughout the study site. Within the first few weeks of planting, all forbs planted as plugs were eaten by herbivores. Seeded forbs established in low numbers in 2017. We observed slightly more *A. tuberosa* seedlings (25) than *L. perennis* seedlings (17) at the end of data collection. Due to the small sample size, we were unable to perform statistical analyses on the seeded forb results.

On average, control plots contained 22 percent more *C. stoebe* cover and roughly five times more *C. stoebe* biomass when compared to all burned plots. We observed significant differences in *C. stoebe* cover among plots according to burn season ($F_{1,58} = 11.01$, $p = 0.001$) and burn temperature ($F_{2,57} = 17.74$, $p < 0.001$) across all treatments. However, the differences in *C. stoebe* cover were rarely significant between individual burn treatments (Figure 1). We also observed significant differences in *C. stoebe* cover change between plots according to burn season ($F_{1,58} = 6.48$, $p = 0.011$) and burn temperature ($F_{2,57} = 28.24$, $p < 0.001$) across all treatments. Again, differences were rarely significant between individual burn treatments (Figure 1B). In August, *C. stoebe* cover was lower in summer-burned plots than in spring-burned plots, with the lowest cover found in SUHT plots and the highest cover found in control and SPLT plots (Figure 1A). *Centaurea stoebe* cover increased the most in control plots from May to August, with lower increases observed in spring burn plots, and decreases observed in summer burn plots (Figure 1B).

Burning at both temperatures resulted in significantly lower *C. stoebe* biomass in August ($F_{2,57} = 17.63$, $p < 0.001$), and biomass change between May and August ($F_{2,57} = 15.13$, $p = 0.001$) when comparing all treatments together, although these differences were not significant between individual treatments. Burn season did not significantly affect average *C. stoebe* biomass or change in biomass overall, although individual treatments did significantly affect both biomass and change in biomass when compared to

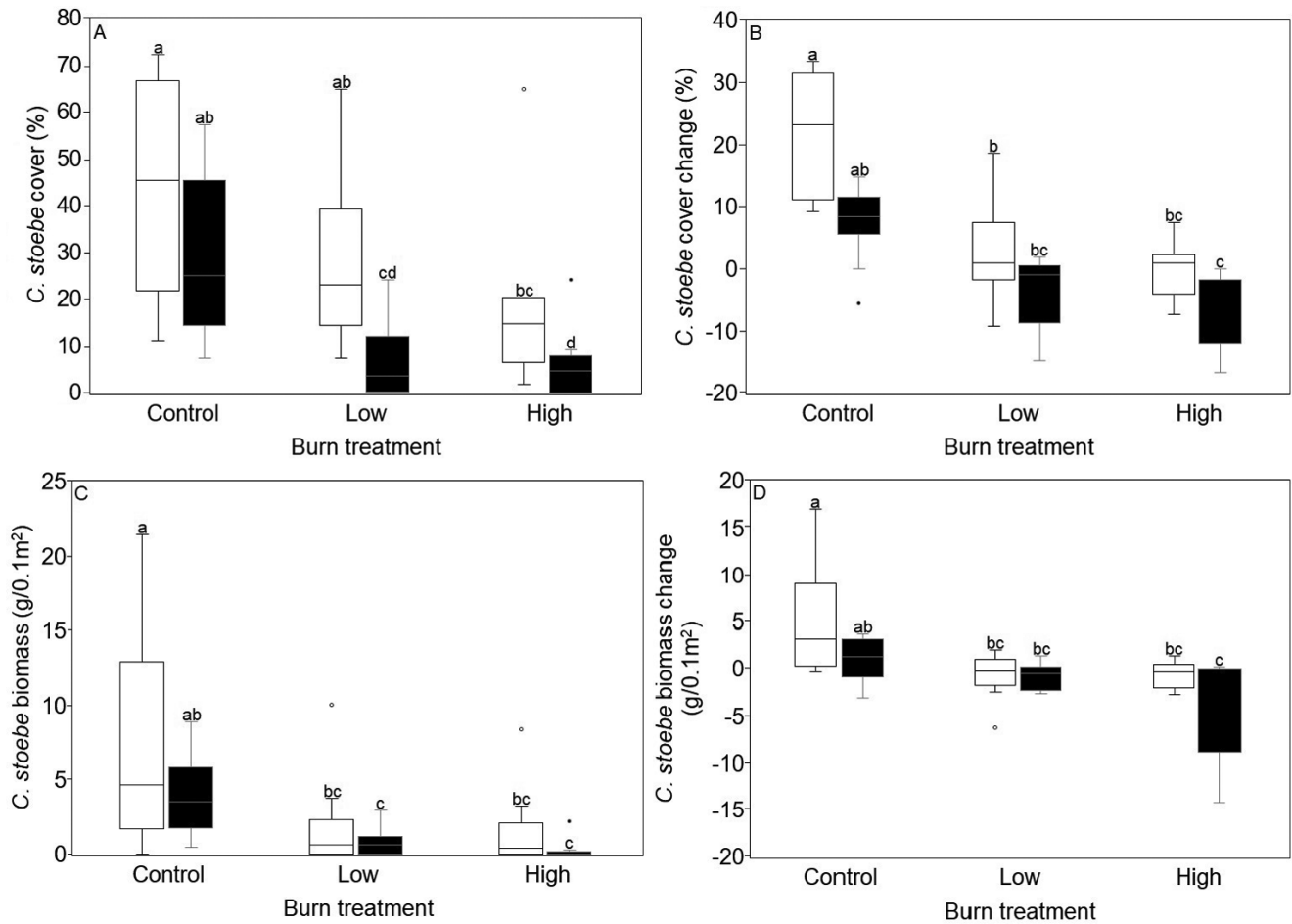


Figure 1. Median *C. stoebe* cover at the end of the growing season in August (A), median *C. stoebe* cover change between May and August (B), median *C. stoebe* biomass in August (C), and median *C. stoebe* biomass change from May to August (D) for each of the six burn treatments conducted at Pierce Cedar Creek Institute, Barry County, Michigan in spring and summer 2017. Spring treatments are displayed in white and summer treatments are displayed in black. Low temperature treatment plots were burned individually with a propane torch to reach 103°C and high temperature treatment plots were burned individually with a propane torch to reach 316°C in mid-May (spring) or late June (summer) of both 2016 and 2017. Control plots were mowed in either mid-May (spring) or late June (summer) of 2016 in order to coincide with the first round of burning. Different letters above treatments denote statistically significant differences (Mann-Whitney $p \leq 0.05$).

their respective controls in most cases (Figure 1). All burn treatments resulted in lower *C. stoebe* biomass in August when compared to control plots, with the lowest biomass found in summer burn plots (Figure 1C). *Centaurea stoebe* biomass increased in control plots but decreased in all burn plots from May to August, with the largest decreases observed in SUHT plots (Figure 1D).

Burn season affected planted grass cover ($F_{1,58} = 9.97, p = 0.002$), change in cover ($F_{1,58} = 8.21, p = 0.004$), biomass ($F_{1,58} = 6.59, p = 0.010$), and change in biomass ($F_{1,58} = 8.69, p = 0.003$). However, these overall differences seldom showed up between individual treatments (Figure 2). We did not observe any differences in planted grass response variables as a result of burn temperature. Planted grass cover was higher in spring-burned plots than in summer-burned plots, with the highest planted grass cover in SPHT

plots (Figure 2A). Planted grass cover increased when exposed to all treatments, although the increases were more substantial in spring, specifically SPHT plots (Figure 2B). Planted grass biomass was higher in spring-burned plots and lower in summer-burned plots when compared to control plots at the end of the season, and biomass was again highest in SPLT plots (Figure 2C). Planted grass biomass increased slightly in control plots, with larger increases observed in spring burn plots, and almost no increases observed in summer burn plots (Figure 2D).

Soil Catechin Results

We detected catechin at least once in all five plots throughout the season, although none of our treatments significantly affected soil catechin levels. We found the highest levels of catechin in June for all distance zones and

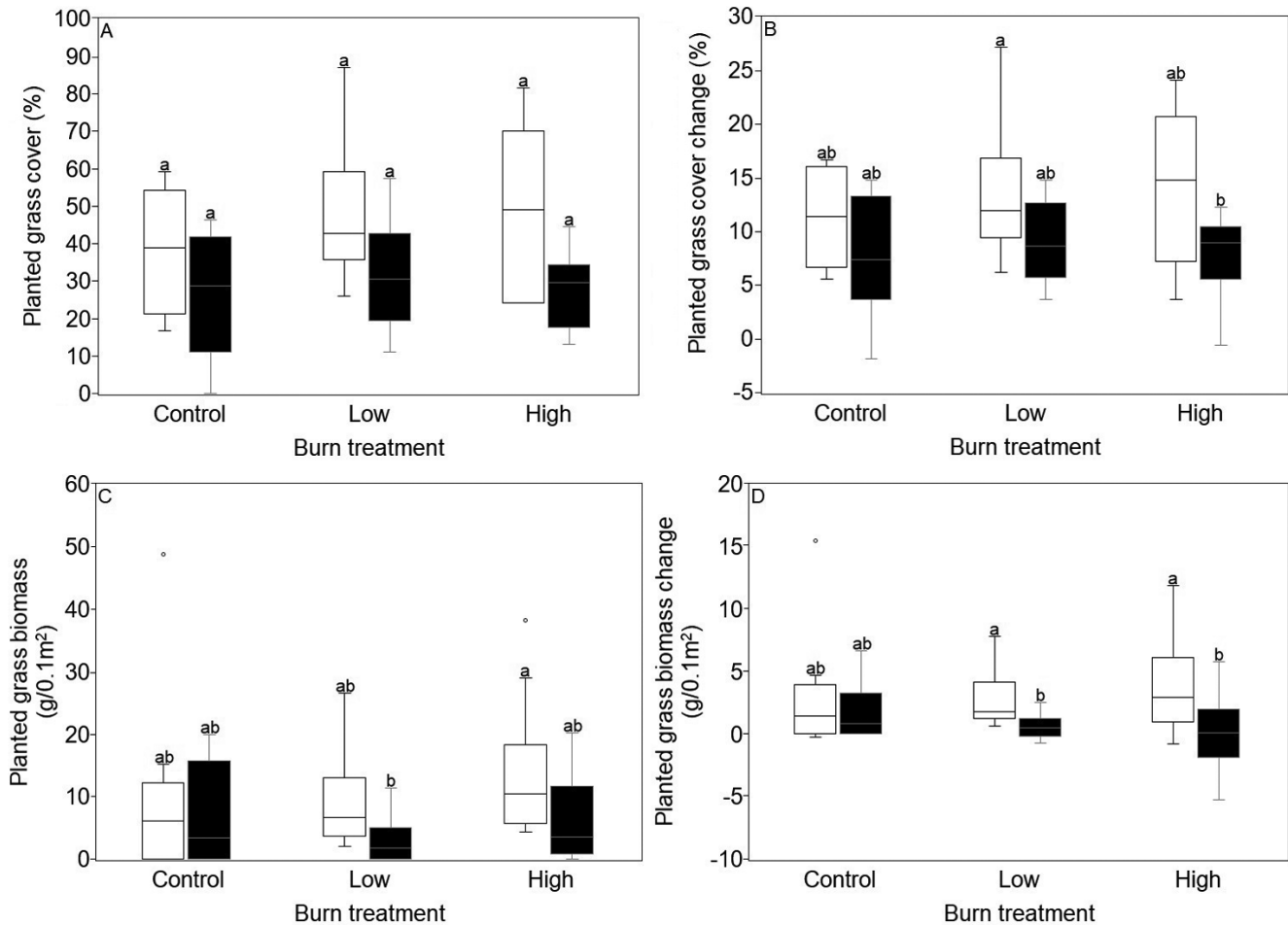


Figure 2. Median planted grass cover at the end of the growing season in August (A), median planted grass cover change between May and August (B), median planted grass biomass in August (C), and median planted grass biomass change from May to August (D) for each of the six burn treatments conducted at Pierce Cedar Creek Institute, Barry County, Michigan in spring and summer 2017. Spring treatments are displayed in white and summer treatments are displayed in black. Low temperature treatment plots were burned individually with a propane torch to reach 103°C and high temperature treatment plots were burned individually with a propane torch to reach 316°C in mid-May (spring) or late June (summer) of both 2016 and 2017. Control plots were mowed in either mid-May (spring) or late June (summer) of 2016 in order to remove the influence of aboveground biomass on grass seedling establishment. Seeds and plugs of three grass species, *Panicum virgatum*, *Schizachyrium scoparium*, and *Sorghastrum nutans* were planted following burning and mowing in spring and summer of 2016. Different letters above treatments denote statistically significant differences (Mann-Whitney $p \leq 0.05$).

treatments, with the exception of SPHT, in which we detected no catechin in June even though catechin was present in samples taken from this plot in May. Catechin was typically present in lower levels in May, and completely absent from our soil samples in July and August (Table 1). We generally found more soil catechin in the SPLT plots, although the differences between treatments were not significant. Samples taken immediately before and after burning revealed no differences in soil catechin levels (Table 2). We found highest soil catechin levels in the zone 15–30cm away from the *C. stoebe* plant, and the lowest levels in the 0–15cm zone (Table 1). Catechin levels in the soil never exceeded 1µg/mL.

Discussion

Centaurea stoebe Dominance

Fire reduced *C. stoebe* dominance in all burn plots relative to control plots, although individual burn treatments differed in overall success. Both our study, and that of MacDonald et al. (2007) show that mid-spring burning can be an effective control for *C. stoebe*. Although generally effective, mid-spring burns were less successful at reducing *C. stoebe* cover, biomass, and growth than summer burns. Emery and Gross (2005) also found summer burns to be most successful for *C. stoebe* control, and they concluded that early-spring burns did not significantly reduce recruitment or biomass. However, grassy fuel loadings were much

Table 1. Average soil (\pm)-catechin for each burn treatment, for each month of the sampling season, and for each distance from *C. stoebe* individuals. Samples were taken in mid-May, mid-June, mid-July, and mid-August 2017 at Pierce Cedar Creek Institute in Barry County, Michigan. Collection of May and June soil samples occurred before spring and summer burn dates, respectively.

Treatment	Catechin ($\mu\text{g/mL}$)	SE
CTRL	0.12	0.05
SPLT	0.18	0.08
SPHT	0.06	0.04
SULT	0.09	0.05
SUHT	0.06	0.03
Date		
May	0.11	0.03
June	0.29	0.07
July	0.00	0
August	0.00	0
Zone		
0–15 cm	0.07	0.03
16–30 cm	0.14	0.05
31–45 cm	0.10	0.04

higher in the study conducted by MacDonald et al. (2007), and the study area utilized by Emery and Gross (2005) was not always able to sustain a fire. Therefore, it is important to consider the effects of both fuels and burn timing when considering the results of past studies. Summer burns are likely most effective due to the phenology of *C. stoebe*, which had bolted and was beginning to flower around the time of our summer burns, but was still in rosette form during spring burns at our site. Repeated burns that coincide with a target plant's growing season may reduce root carbon reserves, thereby limiting future growth (Schutz et al. 2011). Additionally, defoliating *C. stoebe* during the flowering stage severely limits seed production and viability, thereby limiting reproductive capacity and contributions to the seedbank (Benzel et al. 2009). Such benefits relating to seed reduction likely were not observed during our study and may become more evident over time. Overall, summer burns were more effective for reducing *C. stoebe* dominance in invaded communities than spring burns.

We did not find an overall trend for the effect of burn temperature on the success of *C. stoebe* control. Communities that are invaded by *C. stoebe* often lack large amounts of native grasses, which provide the fine fuels required for high temperature fires (Bidwell and Engle 1992). Our results indicate that burning in such areas can be an effective tool for *C. stoebe* management, despite the lack of necessary fuels for more intense fires. However, when considering both season and temperature, high temperature summer burns (SUHT) were consistently more successful at reducing *C. stoebe* cover, biomass, and growth than any other burn treatment. Although successful

Table 2. Average soil (\pm)-catechin immediately before and after burn treatment at Pierce Cedar Creek Institute in Barry County, Michigan. Spring burns were conducted May 15, 2017 and summer burns were conducted June 30, 2017. All soil samples for this analysis were collected separately from the samples collected at monthly intervals, which are displayed in Table 1.

Treatment	Pre-Burn		Post-Burn	
	Catechin ($\mu\text{g/mL}$)	SE	Catechin ($\mu\text{g/mL}$)	SE
SPLT	0.15	0.02	0.17	0.05
SPHT	0.25	0.09	0.25	0.03
SULT	0.00	0.00	0.07	0.13
SUHT	0.00	0.00	0.00	0.00

overall, low temperature spring burns (SPLT) were the least effective treatment for reducing *C. stoebe* cover, biomass, and growth. Spring burns effectively reduced *C. stoebe* cover in high temperature burn plots only, suggesting that spring burns for *C. stoebe* management should be conducted at high temperatures if possible. This could explain why Emery and Gross (2005) found spring burns to be ineffective for *C. stoebe* control, since all their burns were reported to be of low intensity. High temperature burns are not necessary for *C. stoebe* cover and biomass reduction, indicating that burning can still be an effective management tool in areas with high *C. stoebe* densities and relatively little fine fuels. However, managers should attempt high temperature burns when feasible, either by manipulating fuels or through burn techniques.

Planted Species

We found no impact of burn temperature on patterns of planted grass establishment. We also found little impact of burn season on planted grass species, with some exceptions. Spring burn plots were very similar to control plots when measuring cover, biomass, and growth. Conversely, both MacDonald et al. (2007) and Martin et al. (2014) found increased growth of warm season grasses in *C. stoebe*-infested areas that were treated with mid-spring burns. However, both studies conducted their burns in areas with established warm-season grasses, while our study burned newly planted grasses that were still establishing. It is possible that our grasses would have responded more positively to mid-spring burns had they been given time to establish themselves. We also found that planted grass cover and biomass were generally higher in spring burn treatments than in summer burn treatments, although the differences were negligible. Despite similarities in final biomass levels at the end of the season, increases in planted grass biomass were reduced in summer burn plots when compared to spring burn plots, but not when compared to control plots. Our results suggest that summer burns at high or low temperatures can reduce the growth of warm season grasses as compared to spring burns. However,

burning in the summer did not seem to meaningfully harm warm season grasses overall in our study. This is consistent with past research (Towne and Kemp 2008), although other studies indicate that growing season burns may reduce the flowering potential of warm season grasses in prairie restorations (Pavlovic et al. 2011). It is likely that the positive effects on our planted grasses of summer burns from removing *C. stoebe* outweighed the negative effects from reduced growth. Therefore, summer burns in areas of *C. stoebe* with establishing warm season grasses are still beneficial to the community overall and should be considered by managers.

Planted forb species did not make meaningful contributions to planted species cover or biomass. This is likely related to herbivory that occurred in our plots immediately after planting in 2016. We observed herbivory of every planted forb plug within one week of planting in both spring and summer plots, although grasses remained mostly untouched. Past research indicates that planted prairie forbs exposed to herbivory for the duration of the growing season suffer detrimental reductions to growth and reproduction (Sullivan and Howe 2009). The herbivory that we observed suggests that planting forb plugs may not be effective in the first year of planting without substantial herbivore controls. Native forbs also take a longer time to establish from seed than grasses (Hillhouse and Zedler 2011), so the effects of our burn treatments on the planted forb species may not be evident for several more growing seasons. However, past research by Towne and Kemp (2008) indicates that summer burns may benefit perennial forb species, with inconsistent effects on both annual and biennial forbs.

Soil Catechin

Our study of fire effects on soil catechin was limited, and results should be considered preliminary. However, the results do reveal interesting trends that warrant discussion. We only found catechin at very low levels during our experiment (never exceeding 1 µg/mL), which is lower than levels observed to inhibit growth in nearby plants (Perry et al. 2005, Thorpe et al. 2009, May and Baldwin 2011). It is important to note that our catechin study reflected low densities of *C. stoebe* due to removal of all but one individual in the five experiment plots. These low densities could account for the observed low catechin levels. Perry et al. (2007) found that soil catechin levels may be highly variable within an invasion site. This variation may occur due to differences in soil pH or moisture (Blair et al. 2006), or due to the presence of certain metals in the soil (Pollock et al. 2009). Blair et al. (2006) found that catechin persisted longer in dry, acidic soils. The loamy soils at our site have high moisture-holding capacity and are very slightly acidic (pH = 6.7) (Natural Resources Conservation Service 2017), which is consistent with the low amount of catechin found in our soils. Further monitoring of soil catechin at our site

could help determine the exact impact of catechin on the plant community.

Absence of soil catechin in July and August samples suggests that catechin production ceased after mid-June at our site. As a result, summer burns likely did not influence soil catechin. Total loss of soil catechin in the SPHT plot between the time of burning in May and sampling in June indicates that high temperature spring burns could reduce soil catechin levels even though we did not observe an immediate reduction in catechin after burning. None of the *C. stoebe* individuals in the catechin study died immediately after burning, and all survived until at least August. Therefore, any changes in soil catechin levels cannot simply be attributed to *C. stoebe* removal. While burning did not directly affect soil catechin, it may have indirectly lowered catechin levels over time by physiologically stressing *C. stoebe*. Stressed plants with limited energy and resource access often exhibit trade-offs between growth and secondary chemical production (Herms and Mattson 1992, Fine et al. 2006). Significant reductions in *C. stoebe* cover, biomass, and growth as a result of high temperature spring burns could have forced the plant to use energy for growth that would otherwise go towards catechin production. Therefore, in addition to reducing *C. stoebe* dominance, high temperature spring burns may also limit the influence of catechin in systems where it plays a major role in *C. stoebe* invasion. This could, in turn, promote establishment of native species by reducing the allelopathic advantage of *C. stoebe*. However, a more extensive study is required to further elucidate the effects of prescribed burns on soil catechin.

Our results suggest that prescribed burning can be an effective tool for restoring native grasslands by helping to control *C. stoebe* and by shifting the competitive advantage to native grass species. Both mid-spring and summer burns reduced *C. stoebe* dominance, although summer burns were clearly more effective in our study. When combined with the findings of past studies, our research indicates that prescribed fire increases in *C. stoebe* control effectiveness from early-spring (not effective), to mid-spring (somewhat effective), to summer (most effective). Burn season is more influential than burn temperature, but higher temperature burns typically increase the effectiveness of fires, especially in spring. Moreover, burning may have the added benefit of reducing soil catechin levels, although more study is required. While slightly less beneficial than spring burns for native grass establishment, summer burns still provide net benefit for establishing warm season grasses that are competing with *C. stoebe*, and overall did not prohibit their establishment. However, if establishment of warm season grasses is of more importance than *C. stoebe* removal, a spring burn may be more appropriate. Ultimately, management goals and site-specific conditions will determine the best management strategy for impaired grassland communities.

Acknowledgements

We thank Pierce Cedar Creek Institute for funding this project, lending equipment, and providing logistical support. We also thank the staff at PCCI for their help with various aspects of the project. Additionally, thank you to Katie Walker and members of the Aschenbach Lab at GVSU for assistance in data collection. Finally, thank you to Dr. Blair Miller and the GVSU Chemistry Department for assistance with soil analyses.

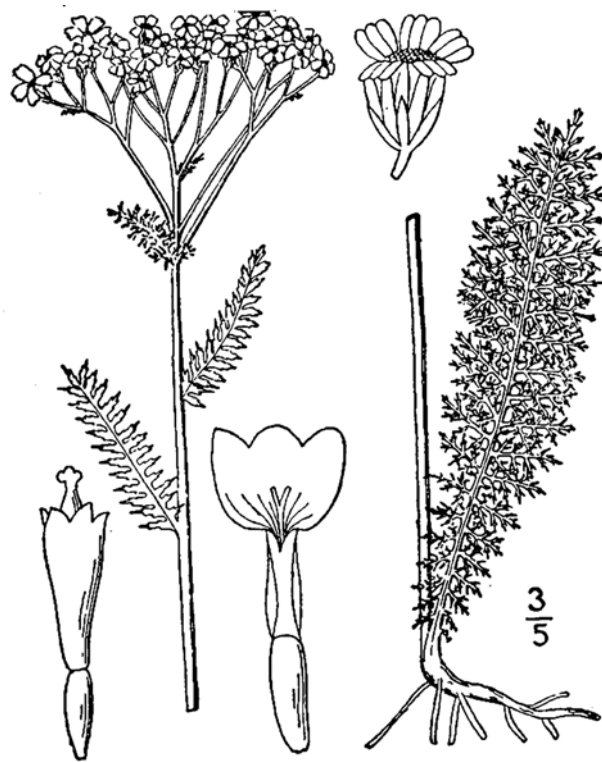
References

- Allen, M.S. and M.W. Palmer. 2011. Fire history of a prairie/forest boundary: More than 250 years of frequent fire in a North American tallgrass prairie. *Journal of Vegetation Science* 22:436–444.
- Benzel, K.R., T.K. Mosley and J.C. Mosley. 2009. Defoliation timing effects on spotted knapweed seed production and viability. *Rangeland Ecology & Management* 62:550–556.
- Bidwell, T.G. and D.M. Engle. 1992. Relationship of fire behavior to tallgrass prairie herbage production. *Journal of Range Management* 45:579–584.
- Blair, A.C., S.J. Nissen, G.R. Brunk and R.A. Hufbauer. 2006. A lack of evidence for an ecological role of the putative allelochemical (\pm)-catechin in spotted knapweed invasion success. *Journal of Chemical Ecology* 32:2327–2331.
- Bowles, M.L. and M.D. Jones. 2013. Repeated burning of eastern tallgrass prairie increases richness and diversity, stabilizing late successional vegetation. *Ecological Applications* 23:464–478.
- Callaway, R.M. and W.M. Ridenour. 2004. Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436–443.
- Cohen, J.G., M.A. Kost, B.S. Slaughter and D.A. Albert. 2015. *A Field Guide to the Natural Communities of Michigan*. East Lansing, MI: Michigan State University Press.
- D'Antonio, C. and L.A. Meyerson. 2002. Exotic plant species as problems and solutions in ecological restoration: a synthesis. *Restoration Ecology* 10:703–713.
- DiTomaso, J.M. 2000. Invasive weeds in rangelands: species, impacts, and management. *Weed Science* 48:255–265.
- DiTomaso, J.M., M.L. Brooks, E.B. Allen, R. Minnich, P.M. Rice and G.B. Kyser. 2006. Control of invasive weeds with prescribed burning. *Weed Technology* 20:535–548.
- Duke, S.O., A.C. Blair, F.E. Dayan, R.D. Johnson, K.M. Meepagala, D. Cook, et al. 2009. Is ($-$)-catechin a novel weapon of spotted knapweed (*Centaurea stoebe*)? *Journal of Chemical Ecology* 35:141–153.
- Emery, S.M. and K.L. Gross. 2005. Effects of timing of prescribed fire on the demography of an invasive plant, spotted knapweed *Centaurea maculosa*. *Journal of Applied Ecology* 42:60–69.
- Fine, P.V.A., Z.J. Miller, I. Mesones, S. Irazuzta, H.M. Appel, M.H.H. Stevens, et al. 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.
- Gómez-Rey, M.X., A. Couto-Vázquez, S. García-Marco and S.J. González-Prieto. 2013. Impact of fire and post-fire management techniques on soil chemical properties. *Geoderma* 195–196:155–164.
- Grant, T.A., B. Flanders-Wanner, T.L. Shaffer, R.K. Murphy and G.A. Knutsen. 2009. An emerging crisis across northern prairie refuges: Prevalence of invasive plants and a plan for adaptive management. *Ecological Restoration* 27:58–65.
- Herkert, J.R., D.L. Reinking, D.A. Wiedenfeld, M. Winter, J.L. Zimmerman, W.E. Jensen, et al. 2003. Effects of prairie fragmentation on the nest success of breeding birds in the Midcontinental United States. *Conservation Biology* 17:587–594.
- Hermes, D.A. and W.J. Mattson. 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* 67:283–335.
- Hillhouse, H.L. and P.H. Zedler. 2011. Native species establishment in tallgrass prairie plantings. *The American Midland Naturalist* 166:292–308.
- Inderjit, I., D.A. Wardle, R. Karban and R.M. Callaway. 2011. The ecosystem and evolutionary contexts of allelopathy. *Trends in Ecology & Evolution* 26:655–662.
- Knochel, D.G., C. Flagg and T.R. Seastedt. 2010. Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). *Biological Invasions* 12:3771–3784.
- Kyser, G.B. and J.M. DiTomaso. 2002. Instability in a grassland community after the control of yellow starthistle (*Centaurea solstitialis*) with prescribed burning. *Weed Science* 50:648–657.
- MacDonald, N.W., L.M. Martin, C.K. Kopolka, T.F. Botting and T.E. Brown. 2013. Hand pulling following mowing and herbicide treatments increases control of spotted knapweed (*Centaurea stoebe*). *Invasive Plant Science and Management* 6:470–479.
- MacDonald, N.W., B.T. Scull and S.R. Abella. 2007. Mid-spring burning reduces spotted knapweed and increases native grasses during a Michigan experimental grassland establishment. *Restoration Ecology* 15:118–128.
- Martin, L.M., N.W. MacDonald and T.E. Brown. 2014. Native plant establishment success influenced by spotted knapweed (*Centaurea stoebe*) control method. *Ecological Restoration* 32:282–294.
- May, L. and L.K. Baldwin. 2011. Linking field based studies with greenhouse experiments: the impact of *Centaurea stoebe* (= *C. maculosa*) in British Columbia grasslands. *Biological Invasions* 13:919–931.
- Natural Resources Conservation Service. 2017. Web Soil Survey. websoilsurvey.sc.egov.usda.gov/App/HomePage.htm.
- O'Connor, R., M.A. Kost and J. Cohen. 2009. *Prairies and Savannas in Michigan: Rediscovering Our Natural Heritage*. East Lansing, MI: Michigan State University Press.
- Ohrtmann, M.K., S.A. Clay and A.J. Smart. 2015. Surface temperatures and durations associated with spring prescribed fires in Eastern South Dakota tallgrass prairies. *The American Midland Naturalist* 173:88–98.
- Pavlovic, N.B., S.A. Leicht-Young and R. Grundel. 2011. Short-term effects of burn season on flowering phenology of savanna plants. *Plant Ecology* 212:611–625.
- Pereira, P., A. Cerda, D. Martin, X. Úbeda, D. Depellegrin, A. Novara, et al. 2017. Short-term low-severity spring grassland fire impacts on soil extractable elements and soil ratios in Lithuania. *Science of the Total Environment* 578:469–475.
- Perry, L.G., C. Johnson, E.R. Alford, J.M. Vivanco and M.W. Paschke. 2005. Screening of grassland plants for restoration after spotted knapweed invasion. *Restoration Ecology* 13:725–735.
- Perry, L.G., G.C. Thelen, W.M. Ridenour, R.M. Callaway, M.W. Paschke and J.M. Vivanco. 2007. Concentrations of the allelochemical (\pm)-catechin in *Centaurea maculosa* soils. *Journal of Chemical Ecology* 33:2337–2344.
- Pollock, J.L., R.M. Callaway, G.C. Thelen and W.E. Holben. 2009. Catechin-metal interactions as a mechanism for conditional allelopathy by the invasive plant (*Centaurea maculosa*). *Journal of Ecology* 97:1234–1242.
- Pollock, J.L., L.A. Kogan, A.S. Thorpe and W.E. Holben. 2011. (\pm)-Catechin, a root exudate of the invasive *Centaurea stoebe* Lam. (spotted knapweed) exhibits bacteriostatic activity against

- multiple soil bacterial populations. *Journal of Chemical Ecology* 37:1044–1053.
- Samson, F.B., F.L. Knopf and W.R. Ostlie. 2004. Great plains ecosystems: Past, present, and future. *Wildlife Society Bulletin* 32:6–15.
- Savage, C. 2011. *Prairie: A Natural History*. Second. Vancouver, Canada: Greystone Books.
- Scheirer, C.J., W.S. Ray and N. Hare. 1976. The analysis of ranked data derived from completely randomized factorial designs. *Biometrics* 32:429.
- Schirman, R. 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. *Journal of Range Management* 34:45–47.
- Schutz, A.E.N., W.J. Bond and M.D. Cramer. 2011. Defoliation depletes the carbohydrate reserves of resprouting Acacia saplings in an African savanna. *Plant Ecology* 212:2047–2055.
- Sullivan, A.T. and H.F. Howe. 2009. Prairie forb response to timing of vole herbivory. *Ecology* 90:1346–1355.
- Tharayil, N., P.C. Bhowmik and B. Xing. 2008. Bioavailability of allelochemicals as affected by companion compounds in soil matrices. *Journal of Agricultural and Food Chemistry* 56:3706–3713.
- Thorpe, A.S., G.C. Thelen, A. Diaconu and R.M. Callaway. 2009. Root exudate is allelopathic in invaded community but not in native community: Field evidence for the novel weapons hypothesis. *Journal of Ecology* 97:641–645.
- Towne, E.G. and K.E. Kemp. 2008. Long-term response patterns of tallgrass prairie to frequent summer burning. *Rangeland Ecology & Management* 61:509–520.
- Tyser, R.W. and C.H. Key. 1988. Spotted knapweed in natural area fescue grasslands: an ecological assessment. *Northwest Science* 62:151–160.
- Vermeire, L.T. and M.J. Rinella. 2009. Fire alters emergence of invasive plant species from soil surface-deposited seeds. *Weed Science* 57:304–310.
- Vermeire, L.T. and A.D. Roth. 2011. Plains prickly pear response to fire: Effects of fuel load, heat, fire weather, and donor site soil. *Rangeland Ecology & Management* 64:404–413.
- Wang, C.M., T.C. Li, Y.L. Jhan, J.H. Weng and C.H. Chou. 2013. The impact of microbial biotransformation of catechin in enhancing the allelopathic effects of *Rhododendron formosanum*. *PLoS ONE* 8:e85162.

Zachery T. Pitman (corresponding author) Natural Resources Management Program, Biology Department, Grand Valley State University, 1 Campus Drive, Allendale, MI 49401, USA, pitmanz@mail.gvsu.edu.

Todd A. Aschenbach Natural Resources Management Program, Biology Department, Grand Valley State University, Allendale, MI, USA,



Achillea millefolium. USDA-NRCS PLANTS Database.
 Britton, N.L. and A. Brown. 1913. *An Illustrated Flora of the Northern United States, Canada and the British Possessions*. New York, NY: Charles Scribner's Sons.