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Effects of fire season and temperature on a spotted knapweed (Centaurea stoebe) infested grassland

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Abstract

Invasive species, including the non-native forb *Centaurea stoebe* (spotted knapweed), constitute an imminent threat to degraded and restored native prairies. Considering the major threat that *C. stoebe* poses to imperiled prairie ecosystems, I examined the effectiveness of fire as a control agent of *C. stoebe* and (±)-catechin. I conducted a 2-year experiment in part of a restored tallgrass prairie community at Pierce Cedar Creek Institute in Barry County, Michigan between May and August of 2016 and 2017. My experiment consisted of individually burning 60 1-m^2 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. I compared the effects of the different burn treatments on the plant community by estimating percent cover and biomass of all species within each plot at the end of the field season in August 2017. I also examined the effects of the simulated burn treatments on (±)-catechin levels in the soil, which I quantified using High Performance Liquid Chromatography. On average, burned plots had 22 percent less *C. stoebe* cover and only one-fifth as much *C. stoebe* biomass when compared to unburned control plots. Summer-burned plots had 16 percent less *C. stoebe* cover and less than one-third as much *C. stoebe* biomass when compared to spring-burned plots. Differences in burn temperature failed to produce significantly different results. Planted native grass biomass increased almost three grams more on average after spring burns than after summer burns. Preliminary findings also suggest that burning at high temperatures in spring may indirectly reduce soil (±)-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.

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Chapter 1: Introduction

Introduction

Conservation and restoration of valuable or imperiled ecosystems is a major focus 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, which is mainly a product of 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, the threat to native grassland communities is amplified by their rarity. 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). Most past loss of grassland communities can be associated with conversion to agriculture, but 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 (Knochel et al. 2010), and production of (±)-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. 2005a, 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). Recently, studies have answered some of this criticism by demonstrating the potential for catechin to harm beneficial soil biota and the interaction between soil catechin and phytotoxic metals (Pollock et al. 2009, 2011, Wang et al. 2013). Additionally, the impact 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 an invasive species (Kyser and DiTomaso 2002, DiTomaso et al. 2006, Bowles and Jones 2013). Fire has been shown to 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 spotted knapweed in mid-summer to be most effective in reducing spotted knapweed 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 low productivity and warm-season grass cover. MacDonald et al. (2007) observed significant reductions in spotted knapweed densities and biomass as a result of mid-spring burning in an area with high fuel loadings and dominated by warm-season grasses.

Plants that are stressed by external factors may limit the amount of energy and resources devoted to the production of secondary chemicals in order to focus on growth (Herms and Mattson 1992, Fine et al. 2006). Therefore, fire has the potential to reduce catechin production by *C. stoebe*, although no research on the topic has been performed to our knowledge. Additionally, the effects of fire temperature on *C. stoebe* infestations is unknown, and further questions exist regarding the optimal timing of burns for the restoration of *C. stoebe*-infested communities. Both mid-spring and summer burns have been identified as 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.

Purpose

The purpose of this research is to gain a further understanding of the role of prescribed fire in grassland restoration. Specifically, my study examined how both fire season and temperature influenced *C. stoebe* control, native species establishment, and soil catechin levels. Ultimately, the knowledge gained from my study will be disseminated to the ecological restoration community with the goal of informing future grassland management.

Scope

C. stoebe is a non-native invasive plant throughout North America. This study took place in a restored Michigan tallgrass prairie, so the findings of my experiments are limited to eastern tallgrass prairies in Midwestern North America. My study observes the responses of both native and invasive plant species to the effects of different prescribed fire treatments. This allows me to make conclusions regarding the entire plant community, rather than just one species. Planting and seeding native plant species also allows me to observe the effects of burn treatments on newly established prairie restorations, although these effects could be different in previously established native plant restorations. Exact levels of catechin in the soil can be attributed to site-level conditions, so those data are only informative at my specific study site. However, any trends in the response of catechin to different burn treatments should be applicable outside of the study site.

Assumptions

In conducting this research, I made the following assumptions:

1. Prescribed fire is an effective tool for invasive plant management and ecosystem restoration.
2. Simulated burning via a propane torch produces comparable effects to burning with an actual fire.
3. Infestation by *C. stoebe* is the main cause of degradation in the plant community at my study site.
4. Reducing the dominance of *C. stoebe* at the site will aid the establishment of native plant species.

Hypotheses

I hypothesize the following:

1. Burning *C. stoebe* in mid-spring or in summer will reduce its dominance when compared to control treatments.

2. Summer burns will be a more effective control method for *C. stoebe* than mid-spring burns.
3. High temperature burns will be a more effective control method for *C. stoebe* than low temperature burns.
4. Burning *C. stoebe* will lead to a reduction in soil catechin levels.
5. Mid-spring burns will be more beneficial to seeded and planted native species than summer burns or the control treatment.

Significance

This study adds to the established literature regarding prescribed fire as a tool for restoration of plant communities. Due to the rarity and ecological value of grassland plant communities, particularly in Michigan, the restoration of grasslands is a major concern for conservationists and land managers. My study will help to inform the management and restoration of a rare and ecologically significant plant community, while also contributing to the body of scientific knowledge in the field of restoration ecology.

More specifically, my study provides a more complete understanding of the role of prescribed fire for the management of a prevalent non-native invasive species. Although past research has identified some of the nuances of *C. stoebe* control with fire, questions remain regarding the optimal timing of burning in infested areas. Additionally, I found no existing literature on the relationship between fire temperature and *C. stoebe* control. Fire temperature will vary from site to site and is partially dependent on fuel loads. Therefore, understanding how fire temperature impacts *C. stoebe* infestations is important to managers in determining the efficacy of prescribed fire at sites which lack the fuels required for hotter fires. My research investigates both fire season and fire temperatures as variables which influence *C. stoebe* control and native species establishment.

Finally, my study represents the first which examines the influence of fire on soil catechin levels. As a candidate novel allelopathic weapon, catechin may play a major role in structuring plant communities which have been invaded by *C. stoebe.* Reducing soil catechin levels could further assist in the establishment of native plant species, so identifying ways to do so would be beneficial to land managers. My research attempts to establish the possible relationships between prescribed fire and soil catechin levels.

Definitions

Allelopathy: Production of secondary chemicals by plants which they release into the environment in order to inhibit the growth of other nearby plant species.

Ecological Restoration: Encouraging the succession of a degraded ecosystem towards a more desirable plant community on a human timescale.

Fine Fuels: Fast-drying fuels which constitute the main driver of a fire across a landscape.

Invasive Species: Any non-native species which causes harm to the environment, the economy, or human health.

Prescribed Fire: Intentionally lit fires used as a tool to achieve ecological goals.

Chapter 2: Manuscript

Simulated fire season and temperature affect spotted knapweed (*Centaurea stoebe*) dominance, native species establishment, and soil (±)-catechin levels in a Michigan tallgrass prairie

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Abstract

Invasive species, including the non-native forb *Centaurea stoebe* (spotted knapweed), constitute an imminent threat to degraded and restored native prairies. Considering the major threat that *C. stoebe* poses to imperiled prairie ecosystems, we examined the effectiveness of fire as a control agent of *C. stoebe* and (±)-catechin. We conducted a 2-year experiment in part of a restored tallgrass prairie community at Pierce Cedar Creek Institute in Barry County, Michigan between May and August of 2016 and 2017. Our experiment consisted of individually burning 60 1-m^2 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 at the end of the field season in August 2017. We also examined the effects of the simulated burn treatments on (±)-catechin levels in the soil, which we quantified using High Performance Liquid Chromatography. On average, burned plots had 22 percent less *C. stoebe* cover and only one-fifth as much *C. stoebe* biomass when compared to unburned control plots. Summer-burned plots had 16 percent less *C. stoebe* cover and less than one-third as much *C. stoebe* biomass when compared to spring-burned plots. Differences in burn temperature failed to produce significantly different results. Planted native grass biomass increased almost three grams more on average after spring burns than after summer burns. Preliminary findings also suggest that burning at high temperatures in spring may indirectly reduce soil (±)-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.

Key words: allelopathy, catechin, grassland, prescribed burn, restoration

Implications for Practice:

- Burn season impacts spotted knapweed and native species establishment more than burn temperature.
- Both mid-spring and summer burns reduce spotted knapweed dominance.
- Summer burns are more effective at reducing spotted knapweed dominance than spring burns but may hinder native warm season grass establishment.
- High temperature spring burns may reduce soil (±)-catechin levels.

Introduction

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Fire may also degrade 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, and further questions exist regarding the optimal timing of burns for the restoration of *C. stoebe*-infested communities. Both mid-spring and summer burns have been identified as 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 the relationship between prescribed burning techniques and invasive weeds, native plant communities, and allelopathic chemicals for *C. stoebe* control, soil catechin degradation, and native species establishment. We address several questions: (1) how does fire season and temperature affect *C. stoebe* abundance? (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 is an environmental education center and biological field station and 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 37.46 inches (Natural Resources Conservation Service 2017). The specific study area was historically farmed and was taken out of production in the 1950s. The area has been restored, 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*.

We established 60 1-m^2 plots in parallel rows at the site, with a 0.5-m buffer between each plot. 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 (Fig. 2).

Vegetation Response to Burn Treatments

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 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 5 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 on the same day as the 2016 burns in order to remove the influence of remaining aboveground biomass on planted species establishment, without the added effects of burning. MacDonald et al. (2013) demonstrated that single-application mowing treatments such as this did not significantly reduce *C. stoebe* densities or biomass, so these plots represent an appropriate control.

Following each treatment, 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 forb species: *Lupinus perennis, Asclepias tuberosa,* and *Anemone cylindrica* and three grass species: *Sorghastrum nutans, Schizacyrium scorparium,* and *Pancium virgatum*. Prior to planting, we appropriately scarified and/or thermally stratified seeds as appropriate for each species. We raked seeds into the soil at a rate of 600 seeds/m^2 to a depth of approximately ¼ inch immediately after seeding in half of each plot. 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 ($\sum$= 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. We irrigated seeds and plugs daily during the first week following seeding/planting. When rainfall was more than 20 percent below the weekly average (0.93 inches 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-m × 1-m frame over each plot, which created 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 percent cover. 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 the 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, 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* and 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 in the study area 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 spotted knapweed 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 spotted knapweed 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 impacted our analyses.

To identify the relationship between spotted knapweed density and soil catechin levels, we divided each plot into three zones of 15 cm concentric circle 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 8.84 cm^3 of soil from each of the three zones in all plots before burning on May 19, 2017, and continued collection once each month in June, July, and August. We randomly subjected each spotted knapweed plant to one of the different treatments from the vegetation survey: SPLT, SPHT, SULT, SUHT, and a control. 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 GVSU 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

We used a non-parametric Sheirer-Ray-Hare (Scheirer et al. 1976) test to determine whether we achieved significant differences in spotted knapweed and planted species biomass and cover 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 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 burn treatments. Planted 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 species 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.

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=11.01, *df*=1, p=0.001) and burn temperature (F=17.74, *df*=2, p<0.001) across all treatments. However, the differences in *C. stoebe* cover were rarely significant between individual burn treatments (Fig. 1). We also observed significant differences in *C. stoebe* cover change between plots according to burn season (F=6.48, *df*=1, p=0.011) and burn temperature (F=28.24, *df*=2, p<0.001) across all treatments. Again, differences were rarely significant between individual burn treatments (Fig. 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 (Fig. 1A). *C. 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 (Fig. 1B).

Burning at both temperatures resulted in significantly lower *C. stoebe* biomass in August (F=17.63, *df*=2, p<0.001), and biomass change between May and August (F=15.13, *df*=2, p=0.001) when comparing all treatments together, although these differences were not significant between individual treatments. Burn season variation did not significantly affect average *C. stoebe* biomass or change in biomass overall, although individual treatments did significantly impact both biomass and change in biomass when compared to their respective controls in most cases (Fig. 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 (Fig. 1C). *C. stoebe* biomass increased in control plots but decreased in all burn plots from May to August, with the largest decreases observed in SUHT plots (Fig. 1D).

Variation in burn season accounted for significant differences in planted grass cover (F=9.97, *df*=1, p=0.002), change in cover (F=8.21, *df*=1, p=0.004), biomass (F=6.59, *df*=1, p=0.010), and change in biomass (F=8.69, *df*=1, p=0.003). However, these overall differences seldom showed up between individual treatments (Fig. 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 (Fig. 2A). Planted grass cover increased when exposed to all treatments, although the increases were more substantial in spring, specifically SPHT plots (Fig. 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 (Fig. 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 (Fig. 2D).

Soil Catechin Results

We detected catechin at least once in all five plots throughout the season, although none of our catechin results proved statistically significant. We found the highest levels of catechin in June for all distance zones and 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 spotted knapweed plant, and the lowest levels in the 0-15cm zone (Table 1). Catechin levels in the soil never exceeded 1µg/mL.

Discussion

C. stoebe Dominance

Simulated 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, it should be noted that grassy fuel loadings were much 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 on the impact 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 fine fuels required for high temperature fires (Bidwell and Engle 1992). Our results indicate that burning in such areas can still be an effective tool for *C. stoebe* management, despite their lacking the 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 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 of 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 1µg/mL), which is lower than levels observed to inhibit growth in nearby plants (Perry et al. 2005a, Thorpe et al. 2009, May and Baldwin 2011). However, it is important to note that our study reflected low densities of *C. stoebe*, which 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 are considered to 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 impact 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 spotted knapweed 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.

Conclusions

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.

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

Treatment	Catechin (µg/mL)	Standard Error
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

Table 2. Average soil (±)-catechin with standard error (SE) 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.

	Pre-Burn		Post-Burn	
Treatment	Catechin (µg/mL)	SE	Catechin (µg/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

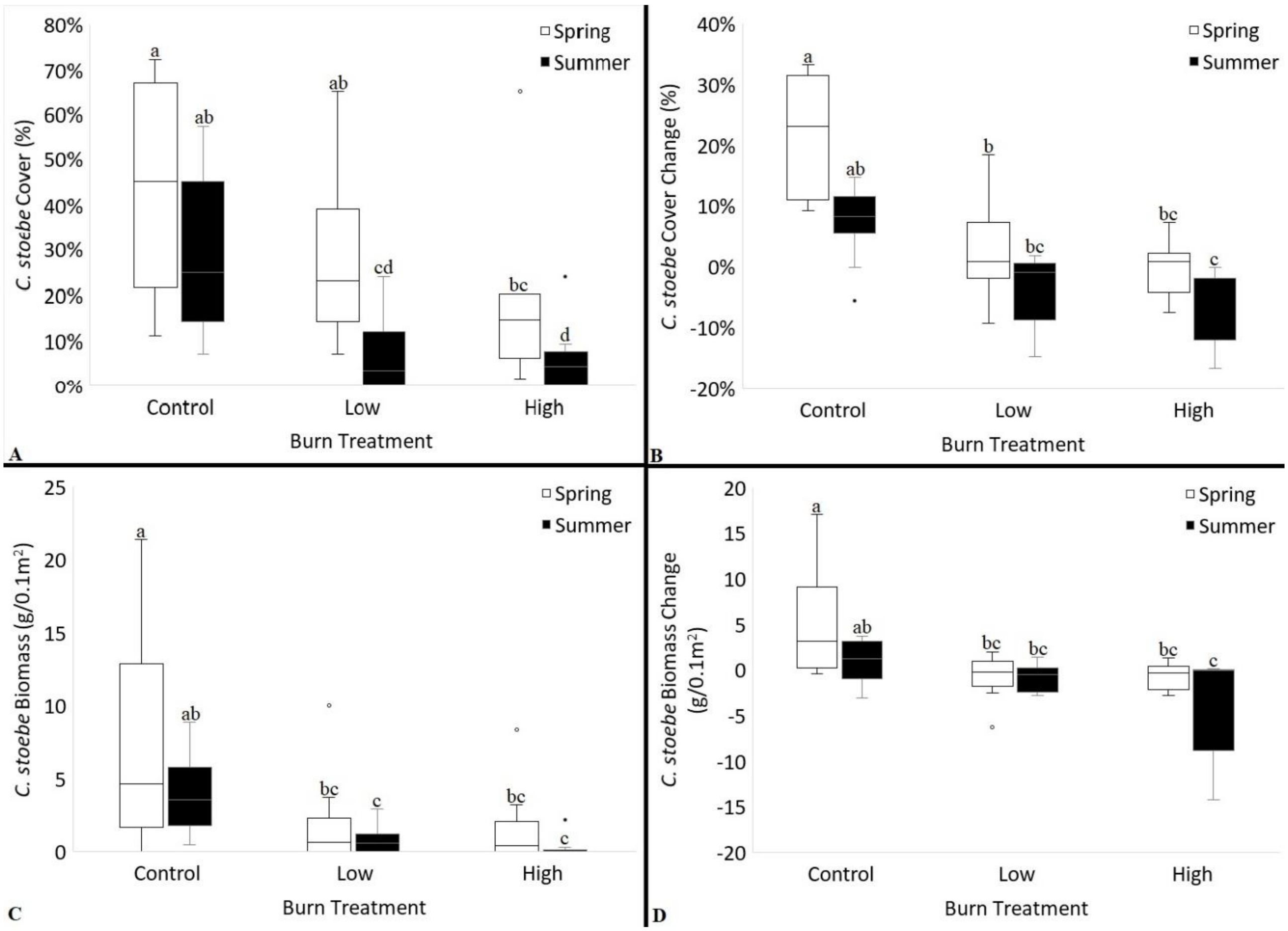

Figure 1. Median *C. stoebe* cover at the end of the growing season in August (A), median *C. stoebe* cover change between May and August (B), median *C. stoebe* biomass in August (C), and median *C. stoebe* biomass change from May to August (D) for each of the six burn treatments conducted at Pierce Cedar Creek Institute, Barry County, Michigan in spring and summer 2017. 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 $p \leq 0.05$).

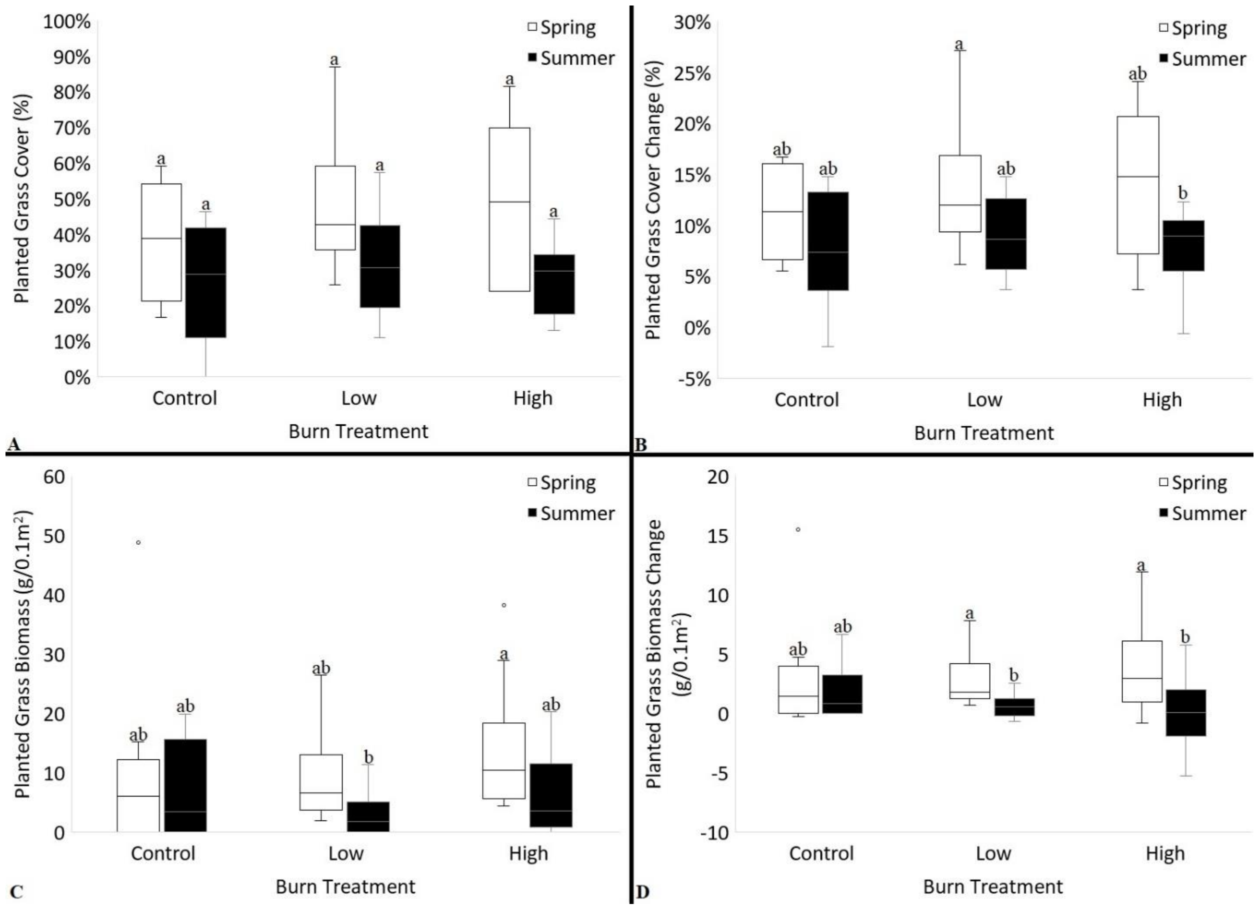

Figure 2. Median planted grass cover at the end of the growing season in August (A), median planted grass cover change between May and August (B), median planted grass biomass in August (C), and median planted grass biomass change from May to August (D) for each of the six burn treatments conducted at Pierce Cedar Creek Institute, Barry County, Michigan in spring and summer 2017. 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 $p \leq 0.05$).

Chapter 3: Extended Literature Review

Introduction to Grasslands and Spotted Knapweed

Conservation and restoration of valuable or imperiled ecosystems is a major focus of natural resource managers. Grassland ecosystems, usually dominated by warm-season grasses and forbs, typically occur in areas that have well-drained soils, low precipitation, or both (Cohen et al. 2015). Fire is a major component of structuring grassland plant communities by introducing disturbance to delay succession towards a forest community, and fires historically occurred in North American tallgrass prairies at regular intervals (Samson et al. 2004, Allen and Palmer 2011). Grassland ecosystems provide important habitat for many plant and animal species. Nearly 260 bird species use grasslands as nesting habitat in the North American Great Plains, although nesting bird populations are currently in decline due to prairie loss and fragmentation (Herkert et al. 2003, Savage 2011). In Michigan, nearly one-third of the state's threatened, endangered, or special concern plants and animals find their primary habitat in grasslands (O'Connor et al. 2009). Unfortunately, many of these species are in decline, which strengthens the case for grassland conservation and restoration. Grassland ecosystems in Michigan have declined by about 99.99 percent since European settlement, and now all grassland natural communities in Michigan are considered either imperiled or critically imperiled (O'Connor et al. 2009, Cohen et al. 2015). Although conversion to agriculture is primarily responsible for loss of grassland ecosystems, invasive species threaten what little remains (D'Antonio and Meyerson 2002, Grant et al. 2009).

Spotted knapweed (*Centaurea stoebe*) is an invasive forb from eastern Europe, and since its introduction to North America has infested over 2.9 million hectares of land (DiTomaso 2000). Areas invaded by *C. stoebe* may have reduced species richness and due increased competition (Tyser and Key 1988, May and Baldwin 2011). May and Baldwin (2011) found that *C. stoebe* altered grassland communities in British Columbia, Canada, and that native species abundance was negatively correlated to *C. stoebe* presence at research sites. *C. stoebe* succeeds as an invasive plant due to high reproductive capacity, effective use of resources, and production of an allelopathic chemical, (±)-catechin (Schirman 1981, Perry et al. 2005a, Knochel et al. 2010). Schirman (1981) described the high seed output of the species in detail, finding that a square meter patch of *C. stoebe* can produce nearly 30,000 seeds in a single growing season – over 1,000 times more than required to maintain the population. He also found seed viability to exceed 95% in a laboratory setting, although significantly fewer viable seeds are produced in natural settings.

Reinhart and Rinella (2011) doubted the invasive potential of *C. stoebe* in eastern North American grasslands. In an observation of a single population of sotted knapweed in Virginia, USA, they found no evidence that increasing *C. stoebe* density lead to a decrease in native species density. They also found that *C. stoebe* seedlings did not outcompete the seedlings of typical eastern grassland species in a greenhouse experiment, suggesting that eastern grassland plant communities could resist invasion by *C. stoebe* (Reinhart and Rinella 2011). Contrary to their expectations, *C. stoebe* invasions in Michigan grasslands are well documented, with many studies attempting to find the proper control method to prevent further invasions (Emery and Gross 2005, MacDonald et al. 2007, 2013).

Allelopathy and (±)-catechin

Allelopathy refers to the production of secondary chemicals, by plants, which they introduce to their environment in order to harm other plants and gain a competitive advantage. Many invasive plant species use allelopathy to gain a competitive advantage in their new environment, where they may quickly take over and establish monocultures (Meiners et al. 2012). However, these same plant species do not enjoy any major competitive advantages in their native ranges, where they exist as typical members of the plant community (Callaway and Ridenour 2004, Thorpe et al. 2009, Inderjit et al. 2011). Through the "novel weapons hypothesis", Callaway and Ridenour (2004) explain how species may become invasive when exposed to a new, naïve plant community.

Within a species' natural range, the native plant community may have existed alongside the species for millennia. The plant community would have evolved certain traits to contend with any secondary chemicals a plant may produce, rendering the "weapon" ineffective, as it is a familiar weapon to the plant community. The plant may also face increased pressure to spend energy on defenses against common herbivores for which the plant is a source of food, thereby decreasing the amount of energy available for the production of allelopathic chemicals. When introduced to an entirely new and naïve plant community, the allelopathic chemical may be extremely effective because the naïve plant community has not evolved to cope with the chemical – it is a novel weapon (Callaway and Ridenour 2004). The potency of the allelopathic chemical may also be magnified by "enemy release;" without natural herbivores to contend with, the plant can spend more energy on production of the chemical (Meiners et al. 2012).

Thorpe et al. (2009) tested the "novel weapons hypothesis" by conducting a field experiment on the effects of catechin in the native (Romania) and non-native (Montana, USA) ranges of *C. stoebe*. They exposed plant communities in Romania and Montana to catechin in the soil and quantified the effects of the chemical on shoot and leaf growth over two growing seasons. The plant community in Montana experienced significantly reduced stem and leaf growth when exposed to catechin. However, the plant community in Romania, within the native range of *C. stoebe*, did not experience significantly reduced growth after exposure to catechin, which supports the tenets of the "novel weapons hypothesis" (Thorpe et al. 2009).

The "novel weapons hypothesis" is often applied to *C. stoebe* and the allelopathic chemical it produces, called (±)-catechin (hereafter catechin). However, scientific debate rages over the effectiveness of catechin and its ecological role as an allelopathic chemical. In many instances, field and laboratory data indicate that catechin produced by *C. stoebe* persists in the soil and impedes the growth of native plant species (Perry et al. 2005a, 2007, Thorpe et al. 2009, May and Baldwin 2011) as well as other members of the *C. stoebe* population (Perry et al. 2005b). Perry et al. (2005a) found compelling results, with catechin reducing root growth by at least 55 percent in many native prairie species. May and Baldwin (2011) also demonstrated that exposure to catechin can reduce root growth and even result in death for native plant species during a greenhouse experiment. Other studies disagree with the conclusions reached in such experiments, specifically regarding the presence of soil catechin in quantities required to adversely affect other plants (Blair et al. 2005, 2006). Blair et al. (2005) argued that the results of previous studies could not be replicated, and that *C. stoebe* does not naturally produce enough catechin to elicit reduced growth in nearby plants. Blair et al. (2006) noted that catechin degrades quickly in soils with high moisture or high pH. Additionally, further research argued that catechin is a strong antioxidant, and therefore cannot damage plants via oxidative stress, as was previously suggested (Duke et al. 2009).

Differences in findings regarding the concentration of catechin in *C. stoebe* infested soils could possibly be attributed to site-specific environmental factors (Pollock et al. 2009, Inderjit et al. 2011). Inderjit et al. (2011) conducted a review of catechin research and suggested that factors including soil nitrification, soil biota, light, and other variables could dictate the amount of the allelopathic chemical present in the soil. Therefore, the discrepancies in observed soil catechin levels could be a result of site-specific differences. Pollock et al. (2009) found that the presence of different metals in the soil can affect the behavior of catechin. Most of the metals tested led to increased oxidation of soil catechin into different forms, although the presence of calcium in the soil reduced auto-oxidation of soil catechin. Additionally, Pollock et al. (2009) found that catechin may interact with and amplify phytotoxic metals in the soil, which could be a mechanism for catechin's allelopathic activity. Other research produced similar conclusions, with catechin persistence and phytotoxicity strongly influenced by the presence of other soil compounds (Tharayil et al. 2008). Further research has proposed that catechin may be bacteriostatic, meaning that soil biota lose function in the presence of catechin (Pollock et al. 2011, Wang et al. 2013). If catechin inhibits the activity of symbiotic soil biota, that could also account for some of its observed allelopathic effects.

Prescribed Fire and Management of *C. stoebe*

As previously mentioned, fire is an important agent for disturbance of the plant community of grassland ecosystems (Samson et al. 2004, Hillhouse and Zedler 2011). Therefore, prescribed fire is a tool used in the restoration of grassland systems and often employed to suppress an invasive species (Kyser and DiTomaso 2002, DiTomaso et al. 2006, Bowles and Jones 2013). In a study involving removal of invasive yellow star thistle (*Centaurea solstitial*), Kyser and DiTomaso (2002) observed that periodic prescribed burns are required for the maintenance of a vibrant native plant community and for defense against further invasions, thus demonstrating the importance of prescribed fires in grassland conservation. Continued exposure to fire may weaken plants by depleting root reserves, as plants which are defoliated can lose photosynthetic potential and must expend extra energy and resources on regrowth (Schutz et al.

2011). DiTomaso et al. (2006) note that fire is a generally effective tool for managing grassland invasive plants. However, they also note that certain perennial species with deep roots and high resprouting capacity may be less amenable to treatment by fire. Additionally, some invasive weeds may lessen the ability of some systems to carry fire by reducing fine fuel loads typically provided by native grass species (DiTomaso et al. 2006). Bowles and Jones (2013) found that prescribed fires have broad positive impacts on the plant community by increasing overall richness and diversity and assisting forbs, legumes, and warm season grasses, while also discouraging establishment of woody species and accumulation of litter.

Depending on management goals, the temperatures achieved by prescribed fires can be quite important. Grassland community fires exhibit predictable behavior patterns, with typical temperatures ranging between 100 and 400 degrees Celsius (Vermeire and Roth 2011, Ohrtman et al. 2015). Fire temperature is largely dependent on fuel availability, which may be influenced by burn frequency or changes in community composition due to invasive species (Bidwell and Engle 1992, McGranahan et al. 2013, Ohrtman et al. 2015). Estimating fire temperature may be done using a variety of techniques including use of pyrometers, calorimeters, or thermocouples; although, thermocouples are typically most accurate (Kennard et al. 2005).

C. stoebe is a prime candidate for control by prescribed fire, and past research has sought to determine the efficacy of prescribed fire as a control agent. Emery and Gross (2005), observed the effects of burn timing on *C. stoebe* recruitment and dominance in a Michigan grassland. Both early spring (April) and fall (October) burns elicited minimal responses in *C. stoebe* biomass and recruitment when compared to a control, although early spring burns did slightly reduce the number of flowering individuals later in the season. However, summer (July) burns did reduce *C. stoebe* biomass, recruitment, and number of flowering individuals when compared to a control.

MacDonald et al. (2007), also found that burning in mid-spring (May) was an effective tool for reducing *C. stoebe* dominance in an invaded grassland. It should be noted that fire temperatures in both studies were relatively low, and Emery and Gross (2005) had some difficulty getting their study plots to carry fire due to low occurrence of native grasses at the site. To date, there has not been a direct comparison of mid-spring and summer burning effects on *C. stoebe*.

Prescribed fire may also be an effective control agent for *C. stoebe* by reducing reproductive capacity (Benzel et al. 2009, Vermeire and Rinella 2009). Research by Benzel et al. (2009) in Montana, USA found that defoliating *C. stoebe* individuals during the flowering and seeding stages in their phenology led to complete reductions in the production of viable seeds by the end of the growing season. Although this research was meant to mimic the effects of herbivore grazing, defoliation by fire should produce similar effects. Additionally, Vermeire and Rinella (2009) examined the effects of fire temperatures on seed emergence of several invasive species, including *C. stoebe*. 97 percent of soil deposited seeds of *C. stoebe* failed to germinate after exposure to fire temperatures of 143 degrees Celsius. They concluded that fire may be an effective agent for invasive species control by reducing germination of seeds in the soil. Other studies have identified hand-pulling of *C.* stoebe as an effective control measure, especially when combined with other treatments such as fire or herbicide (MacDonald et al. 2013, Martin et al. 2014). However, hand-pulling of *C. stoebe* is labor intensive and is not generally feasible in areas of large infestation.

Native Plant Establishment

In addition to controlling invasive plant species, restorations attempt to promote the establishment of native plants in the formerly degraded areas. The effects of fire on these native plant species must also be taken into consideration, and some plants will respond differently to fire than others (Towne and Kemp 2008, Pavlovic et al. 2011). Towne and Kemp (2008) observed the response of different prairie plant species to frequent spring or summer burns during a long-term study in the Konza Prairie of Kansas. They found that summer-burned prairies had higher species diversity and richness at the end of the study than those which were burned in spring. When observing specific plant groups, they found that both annual and perennial forb species responded more positively to summer burns than spring burns, and native warm season grasses did not experience any significant declines as a result of summer burning. However, Pavlovic et al. (2011) did find that summer burns may result in fewer flowering warm-season grasses, which could lead to negative impacts on the native plant community in the long term. They also found that too many recurring burns in the same season may inhibit reproduction and recruitment of some plant species, suggesting that restoration sites should not be continually burned in the same season.

Past studies have also examined the effects of fire on native species within the context of *C. stoebe* invasion and suggest that prescribed fire can promote the dominance of native grass species in areas of *C. stoebe* infestation (MacDonald et al. 2007, Martin et al. 2014). MacDonald et al. (2007) observed increasing dominance of previously seeded native warm season grasses in a *C. stoebe* infestation after it was exposed to several mid-spring burns. Throughout the study, *C. stoebe* dominance decreased while the biomass and dominance of warm season grasses increased. These results indicate that prescribed burning is an effective tool for managers to shift the competitive advantage in a system from non-native invasives to native plant species. In a continuation of that study, Martin et al. (2014) also found a shift towards native species in burned areas, however, they also noted that burning was most effective in promoting native species when combined with other control methods for *C. stoebe*, particularly hand-pulling.

It is important to establish these native species, because healthy plant communities may also be more resistant to invasion (Stevens and Fehmi 2011). In a greenhouse experiment, Stevens and Fehmi (2011) examined the effects of invasive buffelgrass (*Pennisetum ciliare*) on well-established and unestablished native Arizona cottontop (*Digitaria californica*), a bunchgrass. They found that well-established native grasses were resistant to and outcompeted the invasive buffelgrass; however, native Arizona cottontop grass of the same age or younger than the competing buffelgrass experienced major mortalities. These results suggest that establishment of native plant communities may help grassland ecosystems resist invasion. Additionally, warm-season grasses may be less susceptible to the allelopathic effects of catechin, further suggesting that the establishment of such species may increase the resistance of grassland communities to *C. stoebe* invasion (Perry et al. 2005a). Native grasses also provide the necessary fine fuels to carry fire through an ecosystem, while non-native species often impede the spread and intensity of fires in grassland ecosystems (Bidwell and Engle 1992, McGranahan et al. 2013). Therefore, an established native plant community may also promote the very management techniques required to sustain it.

Summary

Grassland ecosystems provide immense ecological value, despite their extreme rarity in Michigan (Samson et al. 2004, O'Connor et al. 2009, Savage 2011). Invasive plants such as *C. stoebe* constitute a major threat to grasslands, and have already infested large areas of the United States (Tyser and Key 1988, DiTomaso 2000). *C. stoebe*'s success as an invasive plant comes in part from production of the allelopathic chemical, catechin (Callaway and Ridenour 2004, Perry et al. 2005b, Thorpe et al. 2009), although some studies disagree (Blair et al. 2006, Duke et al. 2009). Prescribed fires can be an effective tool for manipulation of plant communities (Kyser and DiTomaso 2002, Bowles and Jones 2013), including removal of *C. stoebe* (Emery and Gross 2005, MacDonald et al. 2007, Martin et al. 2014). Prescribed fires may also provide an opening for the establishment of native plants in infested areas (MacDonald et al. 2007, Martin et al. 2014), which may strengthen the resistance of the community to future invasions, while also providing the necessary fine fuel required for future prescribed burns (Bidwell and Engle 1992, Stevens and Fehmi 2011, McGranahan et al. 2013). Fully understanding the ecological role of catechin in *C. stoebe* invasions, as well as subsequent methods for *C. stoebe* control and native species establishment are crucial for the conservation and restoration of grassland ecosystems.

Appendix

Additional Plant Community Data

According to holistic plant community analyses such as Alpha diversity and Floral Quality Index (FQI), there were few differences in the plant community at the end of sampling as a result of our burn treatments. Species diversity was highest in control plots, and lowest in high temperature plots across both treatment seasons (Table 3). However, non-native plant cover was highest in spring and summer control plots (Figure 3), so increased prevalence of non-native species could explain the higher diversity observed in control plots. FQI values were highest in SPLT plots, and lowest in SUHT plots (Table 4).

Among the planted and seeded grass species, *Panicum virgatum* and *Sorghastrum nutans* exhibited similar responses to the burn treatments. Both species had generally higher average cover in August when exposed to spring burns than when exposed to summer burns (Figures 4 & 5). This trend is reflected in the combined analysis of all three planted grass species. *Schizachyrium scoparium* generally did not exhibit different reactions to the burn treatments, although August cover was lowest in high temperature burn plots, regardless of season (Figure 6). By the end of the summer, just over half of the plots contained at least one flowering *P. virgatum* individual and almost all plots contained at least one flowering *S. scoparium* individual (Table 5).

Within the first few weeks of planting, all forbs planted as plugs were eaten by herbivores. Seedlings established in low numbers the following season. I observed slightly more *Asclepias tuberosa* seedlings (25) than *Lupinus perennis* seedlings (17). I did not observe any *Anenome cylidrica* seedlings during the study. *L. perennis* seedlings established in much greater numbers in all summer plots compared to all spring plots (Figure 7). I observed more *A. tuberosa* seedlings in spring plots overall, although this trend did not occur across all treatments (Figure 8). Future research involving forb plugs should take some measures to discourage herbivory after planting.

Table 3. Average alpha diversity of all treatment plots according to vegetation data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI.

Average Alpha Diversity		
	Season	
Treatment	Spring	Summer
Control	16.9	16.5
Low Temperature	15.7	16.1
High Temperature	13.6	15.5

Table 4. Average Floristic Quality Index of all treatment plots according to vegetation data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI.

FQI		
	Season	
Treatment	Spring	Summer
Control	12.73	14.76
Low Temperature	15.21	14.79
High Temperature	14.2	12.56

Table 5. All catechin concentrations for each sample taken between May and August 2017 at Pierce Cedar Creek Institute, Hastings, MI.

Date	**Zone**	**Burn Season**	**Treatment**	**Catechin (μg/mL)**
May	1	N/A	Control	0.00
May	2	N/A	Control	0.19
May	3	N/A	Control	0.09
May	1	Spring	Low	0.13
May	2	Spring	Low	0.18
May	3	Spring	Low	0.13
May	1	Spring	High	0.07
May	2	Spring	High	0.34
May	3	Spring	High	0.33

May	1	Summer	Low	0.11
May	2	Summer	Low	0.12
May	3	Summer	Low	0.00
May	1	Summer	High	0.00
May	2	Summer	High	0.00
May	3	Summer	High	0.00
June	1	N/A	Control	0.31
June	2	N/A	Control	0.44
June	3	N/A	Control	0.40
June	1	Spring	Low	0.39
June	2	Spring	Low	0.98
June	3	Spring	Low	0.30
June	1	Spring	High	0.00
June	2	Spring	High	0.00
June	3	Spring	High	0.00
June	1	Summer	Low	0.00
June	2	Summer	Low	0.29
June	3	Summer	Low	0.54
June	1	Summer	High	0.29
June	2	Summer	High	0.22
June	3	Summer	High	0.24
July	1	N/A	Control	0.00
July	2	N/A	Control	0.00
July	3	N/A	Control	0.00
July	1	Spring	Low	0.00
July	2	Spring	Low	0.00
July	3	Spring	Low	0.00
July	1	Spring	High	0.00
July	2	Spring	High	0.00
July	3	Spring	High	0.00
July	1	Summer	Low	0.00
July	2	Summer	Low	0.00
July	3	Summer	Low	0.00
July	1	Summer	High	0.00
July	2	Summer	High	0.00
July	3	Summer	High	0.00
August	1	N/A	Control	0.00
August	2	N/A	Control	0.00
August	3	N/A	Control	0.00
August	1	Spring	Low	0.00
August	2	Spring	Low	0.00

August	3	Spring	Low	0.00
August	1	Spring	High	0.00
August	2	Spring	High	0.00
August	3	Spring	High	0.00
August	1	Summer	Low	0.00
August	2	Summer	Low	0.00
August	3	Summer	Low	0.00
August	1	Summer	High	0.00
August	2	Summer	High	0.00
August	3	Summer	High	0.00

Figure 3. Map indicating position of Barry County, Michigan, where PCCI is located.

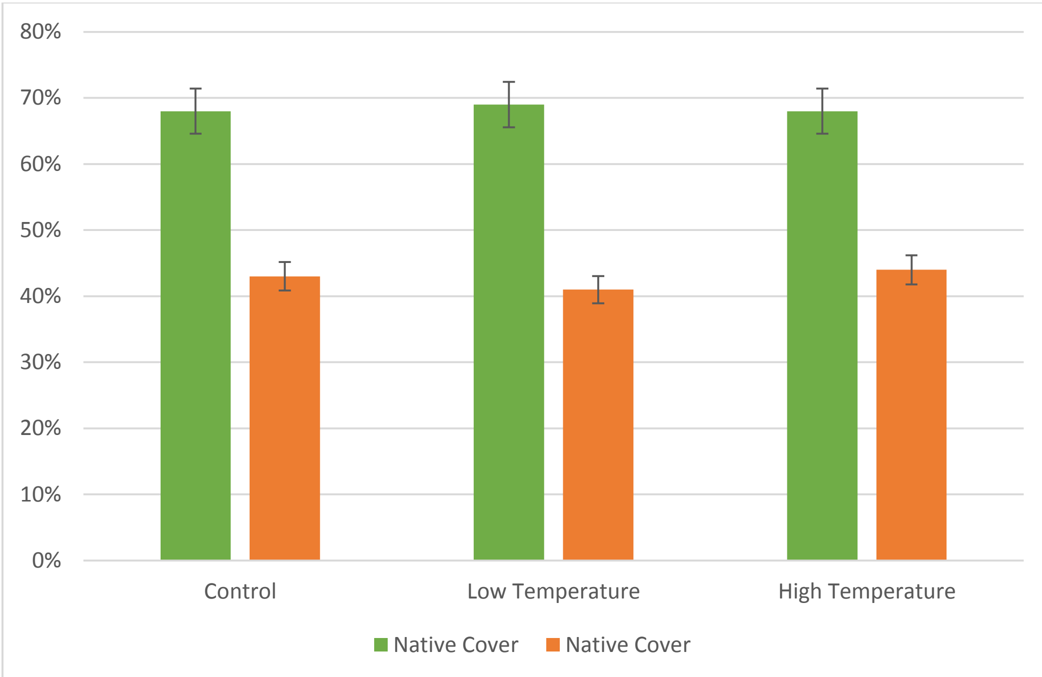

Figure 4. Average cover of native species in all treatment plots according to vegetation data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI +/- 1 SE.

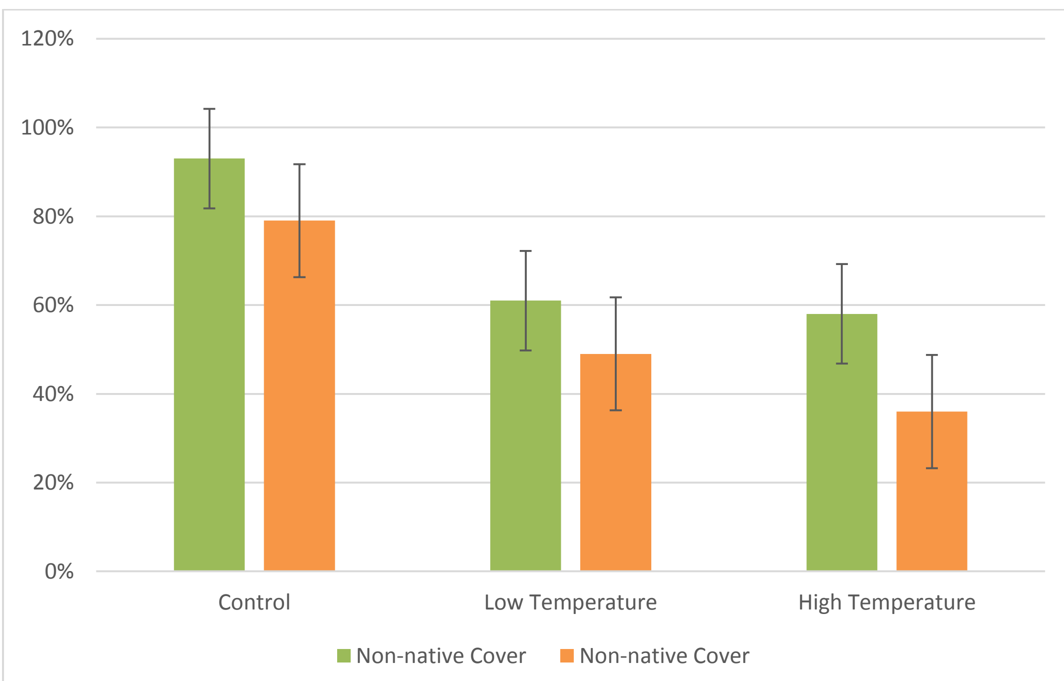

Figure 5. Average cover of non-native species in all treatment plots according to vegetation data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI +/- 1 SE.

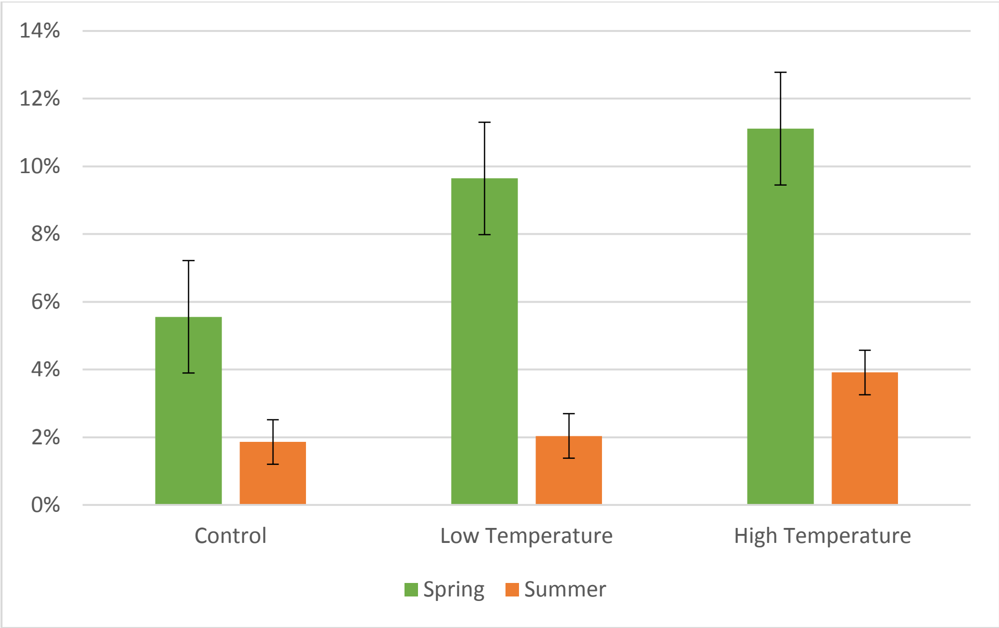

Figure 6. Average cover of *P. vigatum* in all treatment plots according to data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI +/- 1 SE.

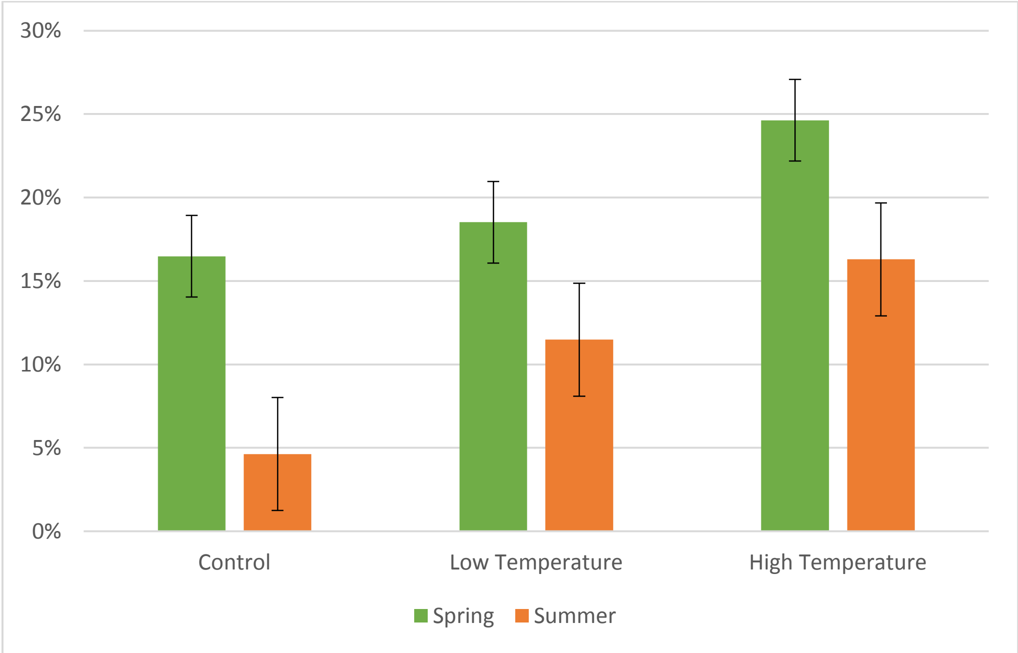

Figure 7. Average cover of *S. nutans* in all treatment plots according to data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI +/- 1 SE.

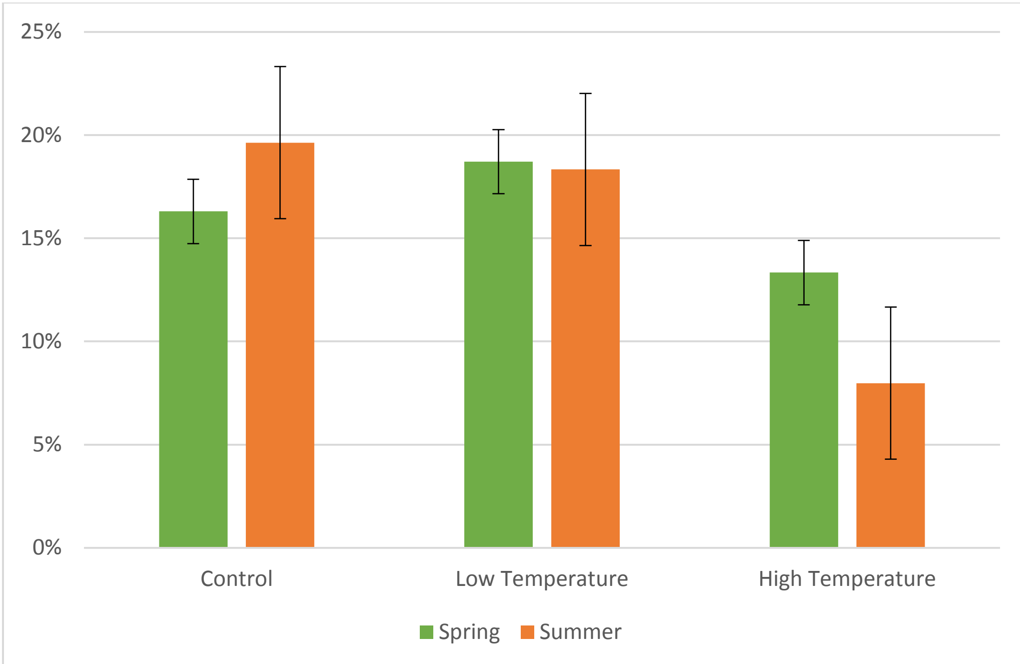

Figure 8. Average cover of *S. scoparium* in all treatment plots according to data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI +/- 1 SE.

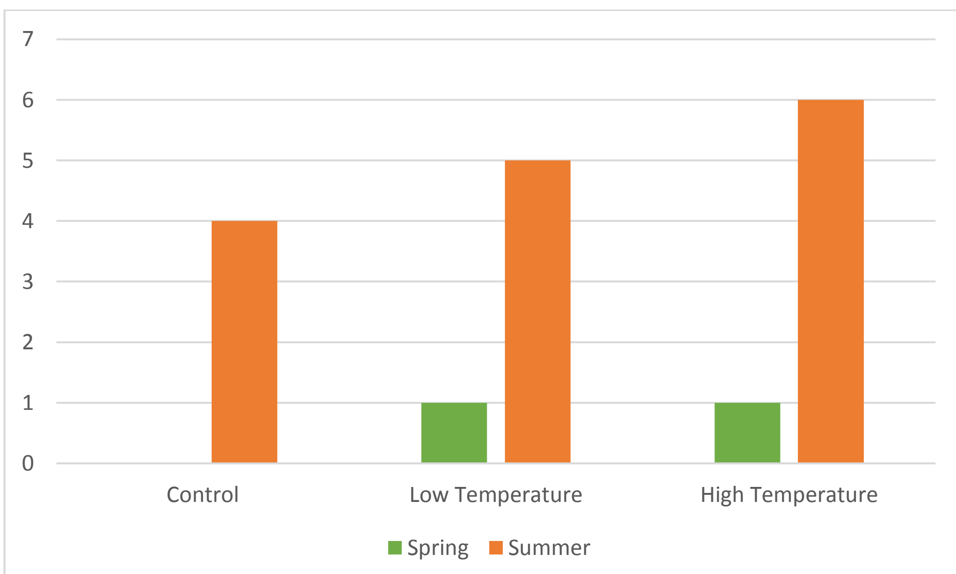

Figure 9. Total *L. perennis* seedlings found in all plots for each burn treatment according to data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI.

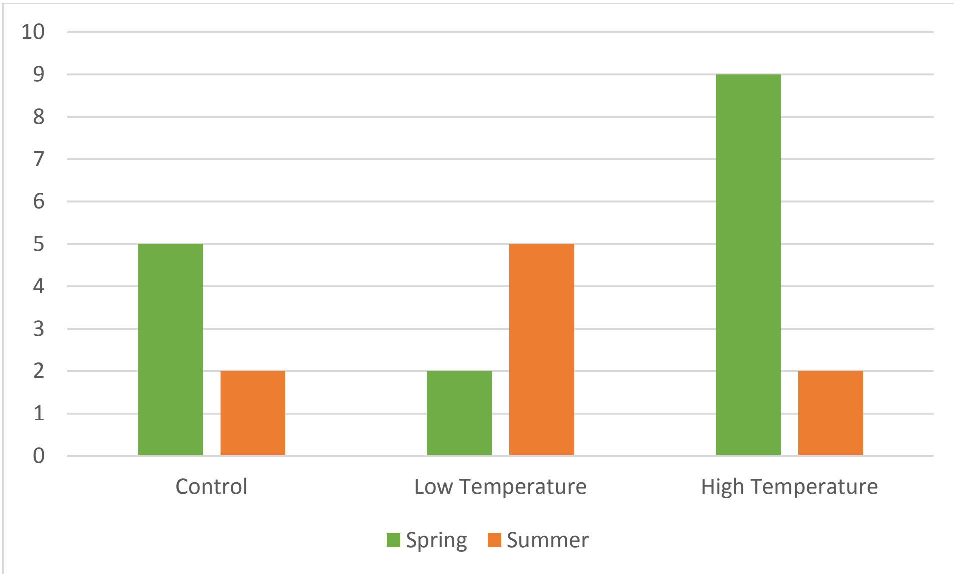

Figure 10. Total *A. tuberosa* seedlings found in all plots for each burn treatment according to data obtained in August 2017 at Pierce Cedar Creek Institute, Hastings, MI.

Figure 11. An individual high-temperature spring burn plot in May 2017. Also pictured is the survey pin and plot frame used for conducting point-intercept vegetation sampling.

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