INVASIVE SUCCESS OF *LESPEDEZA CUNEATA*: ALLELOPATHY AND COMPETITION

A Thesis by

Katherine Coykendall

Bachelors of Science, University of Kansas, 2008

Submitted to the Department of Biology
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Science

December 2011

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The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Biological Sciences.

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Gregory R. Houseman, Committee Chair

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Leland Russell, Committee Member

_____
Mark Schneegurt, Committee Member

_____
David Hughes, Committee Member

ACKNOWLEDGEMENTS

This research has been greatly influenced by those who have helped me realize it. First and foremost, I would like to thank my advisor, Dr. Gregory R. Houseman, Ph. D, for the guidance, advice and support at every stage of this project. The freedom I have been afforded to pursue this avenue of research, and the encouragement and interest in this work is something for which I am extremely appreciative and grateful.

Additionally, I would like to thank Dr. Mark Schneegurt for his valuable input and advice throughout this project for the microbial aspects of this work.

I would also like to thank Dr. Leland Russell for allowing me use of his lab and drying oven, as well as his thorough input on my written work. I greatly appreciate it.

The graduate school has been very supportive of my work as well, and I thank them for the funding support both for my research and for my conference travel stipend.

All the members of the community ecology lab have been integral to my research progress, and I am very grateful for their willingness to help me in the less glamorous, and more physically arduous, aspects of scientific inquiry.

The staff and students in the Department of Biological Sciences have been an amazing support network, and I could not have been able to conduct this research without their assistance.

Finally, I am grateful to my family and friends for being there for me during the course of my graduate school process.

ABSTRACT

Lespedeza cuneata is an Asian legume introduced to the U.S. in the 1930s as erosion control and forage, but it can also have strong negative effects on native species and in some cases form dense monocultures. One proposed explanation for this invasive success is that *L. cuneata* produces allelopathic chemicals that either directly suppresses native species or indirectly alters soil chemistry or microbial communities in ways that give *L. cuneata* a competitive advantage. Additionally, *L. cuneata* may have competitive advantages over native species that operate independently or in conjunction with these allelopathic mechanisms. To test these hypotheses, I collected soil from a previous three-year field experiment in which *L. cuneata* was established in or excluded from randomly selected plots in a field with a common soil type and site history. A series of greenhouse experiments were designed to isolate putative allelopathic effects, resource competition and effects of neighbor identity on native plants.   Invaded soil had positive effects on *L. cuneata* biomass while native biomass decreased for several native species. Additionally, water manipulation resulted in significant interactions with soil history or neighbor identity for a subset of the native species, indicating that resource competition may impact invasive success of sericea. These results support the hypothesis that *L. cuneata* can create a positive feedback that may increase invasion potential, as well as directly impacting growth of natives, and these effects may be intensified by low water conditions.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1

Background

Introduced non-native species can have strong, negative effects on the native

ecosystems.   One of the best-known examples is that of zebra mussels, *Dreissena*

polymorpha. Originally introduced in 1988, these mussels have spread across much of

North America. Zebra mussels are a problematic invader, because they can disrupt

planktonic food webs, which can lead to a domino effect of negative changes.   Dissolved

oxygen levels can drop when large numbers of zebra mussels grow, which alters the

suitability of that habitat for the native species, leading to a loss of biodiversity. Zebra

mussels can also have direct economic effects by clogging water intake pipes, and

disrupting recreational fish stocks (Strayer 2009). This is but one example of the potential

detrimental ecological and economical impact of invasive species.

With increased international trade and travel, the potential for exotic species

introduction remains high. Some exotic species are introduced intentionally for

agricultural or aesthetic reasons or as a biological control.   For example, recreational

divers will introduce zebra mussels as one of the side effects of zebra mussel growth is

increased water clarity (Strayer 2009).   Another intentionally introduced species was the

garlic mustard plant (*Allaria petiolata)*, which was introduced as a medicinal and culinary

herb to North America in the mid 1800s, but now dominates the understory of many North

American forests (Meekins and McCarthy 1999).   Other exotic species can be introduced

unintentionally through ballast water in ships, hitchhikers in seeds shipments, and with

other international trade. An example of this may be *Centaurea maculosa*, a highly invasive

knapweed in North America that is thought to have been introduced with European alfalfa seeds (Callaway and Ridenour 2004).

After introduction of a non-native species, there are three possible outcomes. The species may not establish at all, with the individual or group failing to reproduce. The species may survive, and form a limited population, and have limited impact on the habitat (Reinhart and Callaway 2006). Some species, however, are able to spread widely in the new habitat and change native species composition, as well as the functioning of the ecosystem. These are known as invasive species, and can be of great ecological concern particularly when one species destabilizes or modifies an ecosystem.

The spread of invasive species requires more than increased movement by humans among geographic regions. In addition to reaching the new habitat, the plant must also be able to establish, survive, reproduce, and spread in the new habitat (Mitchell et al. 2006). Invasive species are very successful at these steps, and are able to spread widely in the new range and suppress the native species (Callaway et al. 2008). Understanding how these steps contribute to invasive success may lead to methods to control the spread of invasive species. For this reason, determining how such factors influence invasion is an active area of research. Below I discuss several of the key hypotheses that have been proposed to explain the success of invasive species.

The first hypothesis is the *enemy release hypothesis* or ERH which postulates that invasive species have escaped from natural predators or pathogens by moving to a novel habitat (Torchin and Mitchell 2004). In their original environment, the invasive species have limits to their population growth due to either generalist or specific natural predators. When an invader colonizes a new habitat, the specialist predators found in the

native range may be absent in the new geographic region. Additionally, generalist

predators in the new habitat may be more likely to target the native species leading to an

even greater benefit to the invasive species (Keane and Crawley 2002). Resource

availability may be a contributing factor when the species has been released from an

enemy, as there may be less allocation to defensive mechanisms. This release may allow

more resource allocation to growth or reproduction than the native species. Consequently,

the invasive species may have a reproductive advantage over the native species

(Blumenthal 2005).

An extension of the ERH is the *evolution of increased competitive ability*, or EICA.

The EICA hypothesis suggests that when an organism invades a new community, it loses its

natural enemies; therefore there may no longer be a selective advantage in defensive traits.

Over time, individuals that allocate resources away from unneeded defense traits may be

more successful (Mitchell et al. 2006).   This depends on natural selection acting on the

genotype frequency of the species rather than the phenotypic plasticity alterations seen in

the ERH.   EICA takes the premise of enemy release hypothesis and extends it to include

natural selection.

A second hypothesis is the *empty niche hypothesis,* which proposes that invasive

success depends on the invader being able to use an unoccupied niche in the new

ecosystem.   Such empty niches provide unexploited resources that the invader can exploit

leading to high population growth rates (Shea and Chesson 2002). An example of this may

be a new predator arriving on an island where the prey had not historically encountered

predation, such as the brown tree snake (*Boiga irregularis)* in Guam.   Prior to the snake's

arrival, the community did not have a tree climbing predator, thus there was an open niche

for the snake to occupy (Facon et al. 2006). This may be especially effective when combined with the enemy release scenario, in which resources that would normally be allocated to enemy defense may be directed toward growth or resource acquisition (Blumenthal 2005).

A third hypothesis of invasion is that of *biotic resistance*. Biotic resistance is the ability of a community to withstand invasion. A community may resist invaders because of abiotic factors, competitive effects of other plant species, or the presence of an enemy or disease that reduce the success of invaders (Levine et al. 2004). A community with low biotic resistance would be more readily invaded than a community with high biotic resistance. Biotic resistance differs from empty niche, as it includes a negative pressure on the invader, rather than a simply as yet unexploited ecosystem niche. This interaction may be seen through competitive effects for light, nutrients, space or water.   Weak competitive effects among natives may lead to invasions if the invasive plant is a stronger competitor for these resources than the natives.   Likewise, other aspects of biotic resistance may affect the ability of a plant to colonize the habitat. For example, soil microbial communities may be beneficial for the invader by creating symbiotic relationships between the invader, or they may be detrimental, if soil pathogens are able to infect the invasive species (Callaway et al. 2004).

Finally, the *novel weapon hypothesis* predicts that invasive species have chemical traits that create a competitive advantage in their novel range, as the native species did not evolve any adaptive mechanisms for these new traits (Callaway and Ridenour 2004). Like the ERH and EICA, the novel weapons hypothesis depends on a lack of shared evolutionary history between the invasive plant and the environment. The new community is naïve to

the chemicals produced by the invader so that the lack of adaptive mechanisms by the native plants in the invaded range gives the invasive plant a competitive advantage over the native plants. Because the natives are susceptible to the new chemicals, they may allocate more resources to surviving in the presence of these compounds, and less to growth, which would give the invasive plant better access to shared resources. This kind of chemical interaction among plants is also known as allelopathy. Studies have demonstrated possible allelopathic pathways in which chemicals produced by the plant have altered interactions between microbes and other plants, or deterred herbivory. For example, garlic mustard can alter fungal associations made by surrounding trees (Stinson et al. 2006; Wolfe et al. 2008). This may give garlic mustard a competitive advantage by using a novel weapon in the invaded range. The trees are less able to grow as rapidly, leaving more resources for the garlic mustard, resources that include space and light as well as water and nutrients (Ridenour and Callaway 2001).

Another application of the novel weapon hypothesis can be seen in conjunction with the ERH when there is different selectivity in predation between the native and invasive species. This selectivity of the enemies may be due to the native predators specializing on native plants, or it may be due to allelopathic chemicals released by the invasive species, which may reduce pathogens or herbivory. Consequently, the effects of the ERH are more pronounced when a novel weapon is involved.

An important component of invasive success is the interactions of the invasive plant with microbes in the new environment. These interactions can be grouped under the concepts of plant-soil feedbacks, PSF (Bever et al. 1997). These interactions may be considered positive PSF, when the presence of the plant alters the soil community such that

it encourages its own growth, or it may be a negative PSF, when there are more negative effects on intraspecific interactions than interspecific interactions (Bever et al. 2010). Plant-soil feedback mechanisms are also considered as integral or complementary mechanisms to invasive success along with the theories outlined above. From an enemy release perspective the emphasis would be on escaping the soil pathogens in the invasive species' native habitat. This lack of specific pathogens in the invaded range may allow the invasive to cultivate a more beneficial microbial community, or escape some of the deleterious effects of pathogens (Reinhart et al. 2010). When looking at these interactions through a biotic resistance perspective, the microbes present may alter the ability of the invader to colonize the habitat, which would impact the overall invasibility of the community. Alternately, the novel weapon hypothesis would place the emphasis on the invasive plant altering the interactions of the native soil microbes and native plants, potentially by disruption below-ground mutualisms and interactions (Stinson et al. 2006).

Many hypotheses have been constructed to attempt to explain biological invasions. The empty niche hypothesis and biotic resistance hypothesis emphasize characteristics of the community, either through the suitability of the community for a specific invader, or through the ability of the community to withstand the invasion. The biotic resistance hypothesis may predict that invasive species have a greater advantage in nutrient rich environments. However, such predictions may not apply to legumes, as they are able to fix their own nitrogen and thus have an advantage even in nitrogen poor soil (Ritchie and Tilman 1995). A deeper understanding of this relationship as it applies to invasions would not only help predict invasive success, but may also indicate how invasions might be slowed or reversed.

The novel weapon hypothesis attempts to address increased success in the new environment due to biochemical weapons produced by the species. These results can be confounded by other factors, especially when resource competition comes into play as mentioned with the biotic resistance hypothesis. Additionally, there is little work done with a focus on allelopathy, PSFs, and invasions into the tallgrass prairie. The interactions and communities present in a prairie ecosystem may alter the sorts of invasion dynamics that are seen. It is helpful to understand if one mechanism has a more profound effect on the success of the invasion. This may be especially true when multiple hypotheses are implied, such as enemy release and empty niche, or novel weapon and competition via biotic resistance, as the effects may be synergistic rather than cumulative. Despite the large amount of work done on biological invasions there is a paucity of research on invasive legumes in a grassland ecosystem. A focused look at the effect of a nitrogen-fixing invasive species on native species under variable competitive conditions may be a valuable contribution to our understanding of plant invasions.

Study system

Sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don) is an invasive species found throughout tallgrass prairie of North America. Originally introduced in the 1930's as forage and erosion control, sericea lespedeza has low palatability for cattle, and has been declared a noxious weed in Kansas (Dudley and Fick 2003). Several hypothesis discussed above may explain the invasive success of sericea, including allelopathy as discussed in relation to the novel weapon hypothesis, resource competition as in the biotic resistance

theory, life history traits which may be either empty niche hypothesis or EICA, and enemy

release (Allred et al. 2010).

There is some evidence that sericea has an allelopathic effect on germination of

grasses. For example, Kalburtji and Mosjidis (1993a) found that germination of the

perennial Bermudagrass (*Cynodon dactylon* L. Pers), was decreased when a sericea extract

was applied to the seeds, as well as decreased growth in some tall fescue cultivars (*Festuca*

arundinacea Schreb.) and bahiagrass (*Paspalum notatum* Flugge) (Kalburtji and Mosjidis

1993b). Additionally, high tannin strains of sericea had a negative effect on the cool-season

rye germination and a decrease in biomass of rye (*Secale cereale* L.), tall fescue and

ryegrass (*Lolium multiflorum* Lam.) (Kalburtji and Mosjidis 1993a). Other research

indicates that extracts from sericea decrease germination of indiangrass (*Sorghastrum*

nutans L.), big bluestem (*Andropogon gerardii* Vitman) and Kentucky bluegrass (*Poa*

pratensis L.), and reduce biomass accumulation for big bluestem and Kentucky bluegrass

(Dudley and Fick 2003). By decreasing the rates of germination, over time sericea can

decrease the recruitment of the native plants. These effects by sericea on native grass

germination indicate that there may be an allelopathic interaction, but it is unclear if such

allelopathic effects explain invasion success.

In addition to potential allelopathic explanations for the invasiveness of sericea,

increased competitive ability may also be a reason for its success. This may be especially

true under stressful abiotic conditions. Allred et al. (2010) compared the morphological

and physiological parameters of sericea with two native tallgrass plants, big bluestem, a C_4

grass, and *Ambrosia psilostachya,* a C_3 forb. They found higher specific leaf area in sericea

compared to the native plants, as well as a relatively constant level of gas exchange

throughout the day, which is indicative of a stress-tolerant plant, including drought stress. The higher specific leaf area may also aid in competition by shading competitors once a monoculture of sericea has been established. Additionally, since sericea is a nitrogen-fixing legume, it may hold a competitive advantage over the grasses and forbs, especially in nitrogen-poor soil. When inorganic nitrogen is added to the soil, however, sericea responds poorly, decreasing the competitive advantage (Brandon et al. 2004). These studies suggest that the success of sericea may be due other factors or a low level of biotic resistance in the native population.

Many of the studies that have looked at interactions between sericea and native species in the lab have focused primarily on germination (Dudley and Fick 2003; Kalburtji and Mosjidis 1992; Kalburtji and Mosjidis 1993a; Kalburtji and Mosjidis 1993b). It would be beneficial to test whether the effects on germination persist through later life stages. Also, chemicals potentially produced by sericea may have direct effects on native plant species when released or accumulate in the soil (Callaway et al. 2008). Such chemicals may also operate indirectly by modifying soil microbial communities in ways that favor growth of sericea. Currently, there are no studies that test whether sericea plant extracts have direct or indirect effects on native species or test how competitive effects vary with water or nutrients. The objective of this project is to test whether allelopathic effects or resource competition drives the competitive advantage of sericea in North American prairie.

In the first study, I focus on the interactions of *L. cuneata* and *S. nutans*, a common dominant in the Great Plains and known to decrease in abundance following introduction of *L. cuneata* (Houseman, unpublished data). This experiment was a split plot factorial design with two soil history treatments, one with a history of *L. cuneata* and one with only

a history of native species as the nested factor. The factorial treatments included an extract made from *L. cuneata*, autoclaving the soil and neighbor identity. After the experiment was concluded, a nutrient analysis was conducted on a subset of the soil samples from the experimental pots.

The second set of experiments were designed to test the effects of resource competition on the interactions of *L. cuneata* on either *S. nutans* or on a suite of native species that have demonstrated some growth inhibition by *L. cuneata* (Houseman, unpublished data).   Both experiments used the same soil histories as the first experiment, and a similar factorial design. In the first of the two experiments, *Sorghastrum nutans* was grown either with itself or with *L. cuneata*, and nutrient and water levels were manipulated in a factorial fashion. The second experiment was a multi-species experiment, in which five different natives were grown either with themselves or with *L. cuneata*. Water levels were also manipulated in this experiment. Together, these studies are intended to provide some experimental support for the mechanisms involved in *L. cuneata* invasion.

CHAPTER 2

Introduction

Invasive plant species can have profound and detrimental impacts on the

ecosystems they invade by directly competing with native species or indirectly by altering

ecosystem processes such as fire regimes (Brooks et al. 2004; Meekins and McCarthy

1999). Despite these important consequences for ecosystems, it remains unclear why only

a subset of the potentially invasive species become problematic in their introduced range

(Mack et al. 2000). The negative effect of plant invaders on native species may operate

directly through competitive interactions or indirectly by altering interactions between

native species and other organisms. For direct effects, invaders may be strong competitors

for resources in the introduced environment. One framework for these is the *novel*

weapons hypothesis, which predicts that invasive species produce chemicals in the roots or

leaves that create a competitive advantage in their introduced range, where the native

species have not had opportunity to adaptive to these traits. This chemical interaction may

operate directly through allelopathic effects on native plant species, or indirectly through

alterations in soil microbial communities (Callaway and Ridenour 2004).

Indirect negative effects of invasive on native species can also occur when invaders

alter soil communities in ways that increase invasive success through plant-soil feedbacks

(Kulmatiski et al. 2008). Negative plant-soil feedbacks (PSF) occur where the presence of

the plant results in an increase in pathogens or chemicals leading to decreased plant

growth. These negative PSF may affect some invasive species through generalist

pathogens, but may have more acute impacts on native species (Mangla and Callaway

2008). These interactions may be a form of novels weapons, where a chemical produced

by the invader disrupts the symbiotic microbes, and negatively impacts the growth of the native plants (Stinson et al. 2006).

Positive PSF, however, result in an increase in beneficial soil microbes that may increase plant growth (Sanon et al. 2009). Invasive plants may experience more positive PSF leading to dominance, while native plants may experience more negative PSF, which may lead to extinction or coexistence, depending on the degree of PSF and other competitive factors (Bever et al. 2010). The novel weapons hypothesis has some overlap with PSF where the allelochemicals produced alter the litter decomposition rates by microbes. Additionally, there may be variable rates of allelochemical degradation by native plants due to changes in the microbial community (Inderjit and van der Putten 2010).

Despite the growing interest in PSF, the link between PSF and plant abundance is limited by methodological shortcomings. Plant-soil feedback is typically studied by culturing either field or greenhouse soil in the lab and then sowing a target species. Alternatively soil is collected from below naturally occurring plant populations from multiple study sites and then target species are used as an assay of plant-soil feedbacks (Kulmatiski et al. 2008). However, there are few studies in invasive biology that use field soil that had previously been experimentally controlled for presence or absence of the invader. Consequently, it is difficult to separate the effect of the soil on invader establishment from the effect of the invader on the soil. Here, I directly test whether growth of *Lespedeza cuneata* is associated with changes in PSF in plots where the invader has been experimentally controlled.

Lespedeza cuneata is an invasive legume found throughout tallgrass prairie of North America. Originally introduced in the 1930's as forage and erosion control, *L. cuneata* has

low palatability for cattle, and has been declared a noxious weed in multiple states (Dudley and Fick 2003). There is some evidence that *L. cuneata* potentially may have allelopathic effects on native grassland species. For example, Kalburtji and Mosjidis (1992; 1993a; 1993b) found that tissue leachate reduced germination of grasses including dominant native indiangrass (*Sorghastrum nutans).* Although allelopathic effects on germination may decrease recruitment of the native plants over time, such recruitment is a small component of population growth in intact grasslands (Benson and Hartnett 2006). These effects by *L. cuneata* on native grass germination indicate that there may be an allelopathic interaction, but it is unclear if such allelopathic effects on germination are sufficient to explain invasion success.

Many of the studies that have looked at interactions between *L. cuneata* and native species in the lab have focused primarily on germination (Kalburtji and Mosjidis 1992, 1993b, a, Dudley and Fick 2003), but it is unclear if such interactions persist through later lifestages. Chemicals potentially produced by *L. cuneata* may have direct effects on native plant species when released or accumulate in the soil (Callaway et al. 2004). Such chemicals may also operate indirectly by modifying soil microbial communities in ways that favor growth of *L. cuneata*, such as with positive PSF. Here I test whether allelopathic effects give *L. cuneata* a competitive advantage over *S. nutans*, a common dominant in the Great Plains and known to decrease in abundance following introduction of *L. cuneata* (Houseman, unpublished data).

To address this issues, two research questions were proposed 1) is there an allelopathic effect of *L. cuneata* on the growth of *S. nutans*, and 2) if so, is the interaction directly plant-to-plant, or microbially mediated.

Materials and Methods

To determine the potential allelopathic interactions of *L. cuneata*, a factorial experiment was designed consisting of soil history, soil autoclaving, plant leachate and neighbor identity.

As part of a *L. cuneata* invasion experiment, *L. cuneata* seed addition and unsown control plots were established in a restored grassland located in northeast Kansas (Nelson Environmental Research Station (39.05N, 95.19W))that was dominated by *S. nutans* (Houseman unpublished data). Each treatment was also crossed with simulated haying, simulated grazing or no disturbance and replicated three times. Plots were arrayed in a grid and treatments assigned in randomized block design. After three years the mean *L. cuneata* plant density was 57 plants/0.5m^2 (SE=6.67) in *L. cuneata* seed addition plots and 0.4 plants/0.5m^2 (SE=0.14) in unsown controls. Only three of the nine unsown controls had any *L. cuneata*, containing either one or two plants/plot. After the third year, adult *L. cuneata* plants were killed using Escort herbicide (DuPont, Wilmington) and the entire site was burned. In the following spring, soil was collected from 9 *L. cuneata* addition or unsown controls. The soil was collected from the upper 15cm of each plot, and was not pooled (Stinson et al. 2006). The soil was sieved to remove roots, rocks, seeds and other debris and allowed to air dry.

Soil from each group was then autoclaved at 121 degrees Celsius for 30 minutes on three consecutive days to sterilize the soil (Mangla and Callaway 2008). This treatment was to determine if the PSF is positive or negative in nature. The soil was then distributed into 262 mL sterile pots.

An additional treatment included an extract from ten adult *L. cuneata* plants also collected from plants occurring at the field site. This treatment was intended to detect allelopathic effects. Leaves and roots from each plant were rinsed with distilled water to remove the attached soil. Leaves and roots from each plant were incubated at amounts of 1 g of plant for every 20 mL of distilled water (Cipollini and Darning 2008). The ratio of 1 g of plant mater to 20mL of water was selected to ensure that the plants were fully covered by water during the incubation period. Plants soaked at room temperature for 48 hours, and the extract was then filtered to remove the particulates and stored below 0° C until needed. The extract receiving plants received 2 mL of extract applied to the base of each plant once a week (Cipollini and Darning 2008). The plants not receiving extract received 2 mL of water applied to the base of each plant in place of the extract.

Neighbor identity consisted of two possible species combinations. In each pot, there were either two individuals of *S. nutans* or one *S. nutans* and one *L. cuneata* individual in each pot (Cipollini and Darning 2008). Because legume seeds often germinate at low rates in the lab, the *L. cuneata* seeds underwent a sulfuric acid scarification before sowing. Seeds were soaked in undiluted sulfuric acid for 20 minutes, then rinsed with deionized water and allowed to air dry (Bentley 1933). The seeds were sown into the pots, covered with a thin layer of soil, and thinned one week after germination to two plants per pot. There were 9 replicates of each treatment, and the pots were kept in the greenhouse under natural lighting and watered daily.

After 12 weeks, plants were removed from the pots, and nodules of *L. cuneata* were counted. The plants were then separated into root and shoot mass, and dried at 60° C for 48 hours. After collection of the plants from this experiment, the soil was saved and frozen,

15

then a subset of the treatments were submitted for soil analysis, including total nitrogen, total carbon, organic matter, pH and phosphorus. Total nitrogen and carbon was determined using dry combustion. Organic matter was determined using a modified Walkley-Black method, while phosphorus was determined using the Mehlich-3 procedure. Testing was conducted at the Kansas State Soil Testing facility.

I tested for effects on *S. nutans* and *L. cuneata* biomass and *L. cuneata* nodules using mixed-model ANOVA. The treatments compared in the ANOVA were species sown, autoclaving history and extract, with soil history as a nested factor. Where significant interactions occurred, significance was determined by slicing of main effects.

Results and Discussion

Although *L. cuneata* commonly suppresses *S. nutans* under field conditions (Houseman unpublished data), in our controlled experiment *S. nutans* biomass was higher when grown with an individual of *L. cuneata* than with a conspecific ($F_{1, 112}$=4.08, P=0.046, figure 1.1). This apparent facilitative effect may have been due to nitrogen fixation capability of *L. cuneata*. Although we found no difference in N among treatments ($F_{1, 18}$=0.00, P=1.000), N availability may have been higher in the presence of *L. cuneata* over the course of the experiment, but was not detectable when measured at the conclusion of the experiment. Although our results suggest that *L. cuneata* can have positive effects on native grass seedlings, presumably this is a short-term effect that will be overshadowed by competitive suppression at later life stages due to decreases seen under field conditions (Brandon et al. 2004).

One way that an invader may negatively affect a native plant species or positively influence its own growth is through chemicals released from root or stem tissue. We tested this possibility by the addition of extract from *L. cuneata*, but found no positive or negative effect on the mass of either species (*L. cuneata*: $F_{1,48}$=0.14, P=0.71, *S. nutans*: $F_{1,112}$=0.45, P=0.50). Although we did not detect allelopathic effects, our extraction method may not have obtained compounds typically delivered from living roots or such chemicals were at insufficient quantities to illicit a detectable effect on neighboring species. Given that previous research has found allelopathic effects of *L. cuneata* compounds on germination of native species (Dudley and Fick 2003; Kalburtji and Mosjidis 1992; Kalburtji and Mosjidis 1993a; Kalburtji and Mosjidis 1993b), it would be premature to conclude that allelopathic interactions are unimportant in this system without additional testing of both additional extraction methods and a broader range of leachate concentration.

In contrast to the direct effects of *L. cuneata* on *S. nutans*, soil history significantly altered plant growth rates. *Lespedeza cuneata* biomass increased when grown in soil with a *L. cuneata* history as opposed to a soil with a history of native species alone ($F_{1,16}$=4.79, P=0.044, figure 2.1). This result suggests that *L. cuneata* may facilitate its own growth through a positive PSF mechanism rather than or in addition to direct effects on native species. A facilitative effect may occur through establishing favorable microbial communities in the soil that enhance N availability through N-fixation or by decreasing harmful soil organisms, such as generalist herbivores or pathogens (Klironomos 2002). Positive PSFs would allow for more successful growth in non-native ranges for the invasive plant, thus furthering invasive success, which may also result in a decrease in native success.

In addition to changes to plant mass, I found treatment effects on nodulation rates of the invasive species indicating differences between soil treatments. The soils were autoclaved to isolate the potential effects of microbial community changes in the soil from other factors by decreasing the microbial communities. Only recently has invasion biology begun to determining the nature of the impact of invaders on the native species ability to interact with soil microbes. Other research in different systems has indicated that invasive species may impact the recruitment of microbes to the native species (Stinson et al. 2006). One methodology used to try to isolate out these impacts from those of the putative allelochemical directly on the native plants has been to autoclave the soil to decrease microorganisms present and viable (Mangla and Callaway 2008). Autoclaving increased the biomass of *S. nutans* regardless of soil history ($F_{1, 44.9}$=11.09, *P*=0.0017, figure 3.1), but this increase was higher ($F_{1, 16}$=7.22, *P*= 0.016) in soils with a *L. cuneata* history. Furthermore, the biomass of *S. nutans* increased more in the autoclaved soil with a history of *L. cuneata* than in the autoclaved soil with a history of native species ($F_{1, 112}$=3.93, *P*=0.05). This increase by the native plant may be due to a reduction in soil pathogens in the invaded soil relative to the native soils. Total nitrogen was analyzed, and no significant difference was found between the soil histories ($F_{1, 6}$=2.19, *P*=0.189). This indicates that the difference in indiangrass biomass was not due simply to an increased nitrogen amount, but rather some other mechanism.

In contrast to the native grass, *Lespedeza cuneata* biomass was not affected by autoclaving the soil ($F_{1, 48}$=0.37, *P*=0.5481). Soil that had been autoclaved displayed higher phosphorus levels compared to non-autoclaved soil as well ($F_{1, 18}$=235.84, *P*=<0.0001, figure 4.1). This is consistent with studies that report an increase in

phosphorus availability after heating the soil (Serrasolses et al. 2008). Phosphorus

increases after autoclaving due to the death of soil microbes, and results in a release of

phosphorus and other nutrients into the soil. This increase in phosphorus may help

explain the increased grass biomass in autoclaved soil as native grasses are commonly

phosphorus limited in this region(Hartnett et al. 1993).

Nodulation of *L. cuneata* was higher with a soil history of *L. cuneata* than compared

to soil with a history of native species ($F_{1, 16}$=6.01, *P*=0.026, figure 5.1). This supports the

hypothesis that there are alterations in the microbial communities due to the presence of

the invasive plant. Increased nodulation in invaded soil also indicates that the differences

in biomass may not simply be a result of increased nitrogen in the soil due to fixation.

Nodulation was also higher in soil that had not been autoclaved relative to soil that was

autoclaved ($F_{1, 48}$=75.2, *P*=<0.0001). This suggests that the PSF present may be due to an

alteration in soil microbial community after *L. cuneata* invasion, as it may enable the

invasive plant to nodulate more frequently and rapidly potentially increasing invasibility.

In this system, there is support for multiple mechanisms of invasive success.   The

invader, in this case, demonstrates increased nodulation in soil with a history in invasion,

indicating increased density of symbiotic microbes. At the same time, there may also be a

decrease in pathogens, as indicated by increased growth rates of *S. nutans* in the soil with

an invasive history. The lack of differences in the nutrients between the soil histories

supports the indirect interaction via soil microbes. This alteration in soil microbes to *L.

cuneata's* benefit may provide an advantage at earlier life stages, thus facilitating the

invasion's progression into an already established ecosystem.   While the decrease in soil

pathogens may benefit the native species (in this case, *S. nutans*) in the earlier life stages, over time, *L. cuneata* may accumulate more competitive advantage.

This study provides a novel approach to studying PSF. Few studies have focused on the response of the invader in its invaded soil, and those that do use soil cultured in the greenhouse, rather than experimentally manipulated field soil (Callaway et al. 2004; Klironomos 2002). Those that have utilized field soil have collected from extant populations of an invader, and because these locations are not experimentally controlled, run the risk of confounding inherent soil properties with the effect of the invader on the soil (de la Pena et al. 2010). These studies, while providing valuable insight into PSF in invaded systems, are unable to fully distinguish between physical differences in the soil and microbially induced differences that lead to changes in final plant biomass, especially without an analysis of microbial community. Because we experimentally controlled initial plant establishment of the invader, we can clearly ascribe the effect of *L. cuneata* history on subsequent plant growth rates. Furthermore, because the invasion was experimentally controlled, we know the timeline of invasion as three years, indicating that this invasive is able to change the soil conditions in a significant fashion in that time. This helps illustrate the importance of understanding and controlling the spread of invasive species into novel habitats.
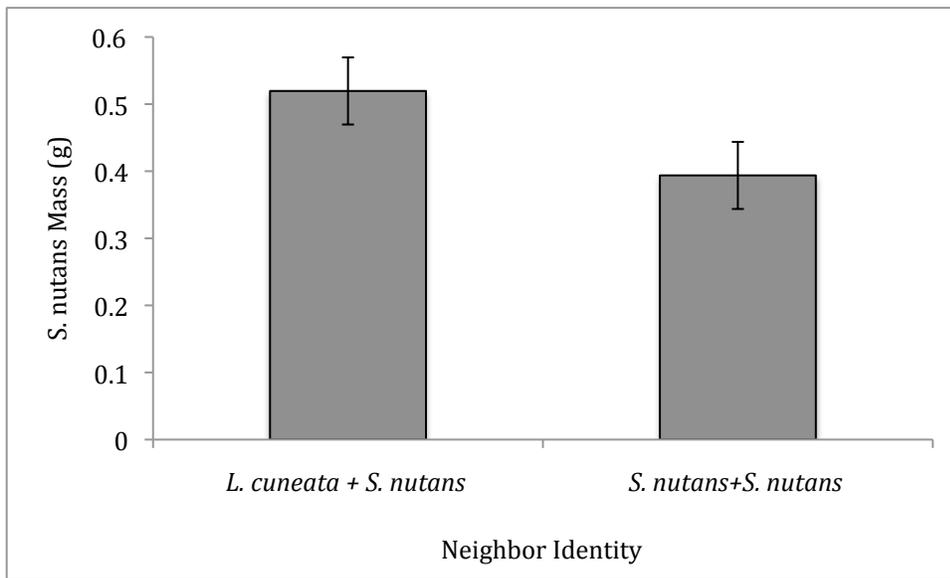
Figure 1.1: Biomass of *S. nutans* in response to the presence of the invader or a conspecific. (means ±1 standard error; $F_{1, 112}$=4.08, *P*=0.046).
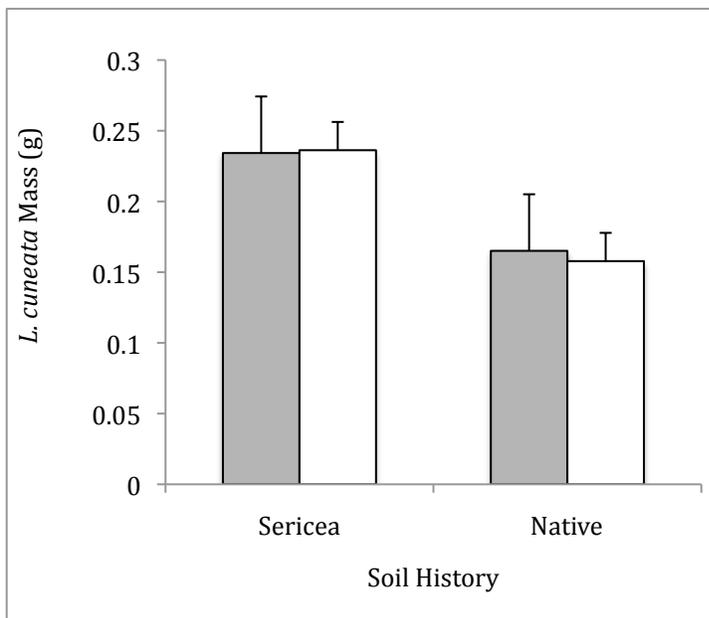

Figure 2.1: Effect of soil history on *L. cuneata* biomass (means ±1 standard error). Shaded bars indicate autoclaved soil, white bars indicate non-autoclaved soil. Positive effect of *L. cuneata* soil history was significant ($F_{1, 16}$=4.79, *P*=0.044)
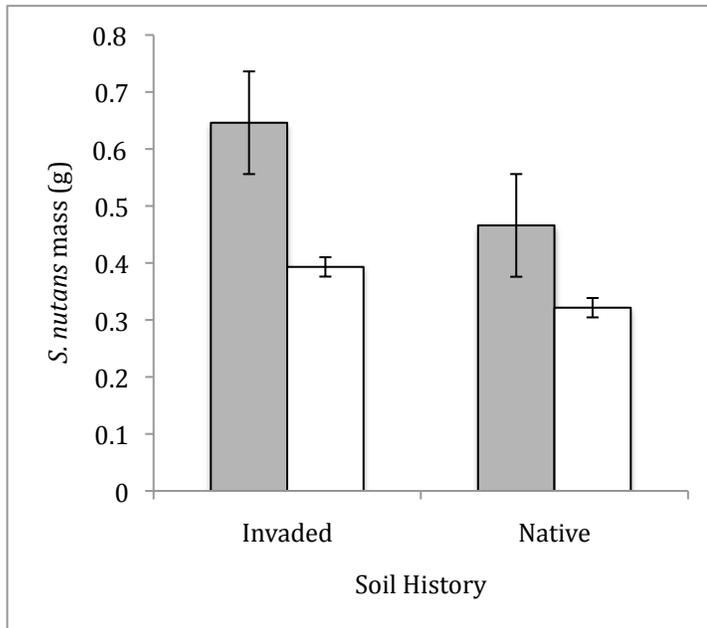
Figure 3.1: Effects of soil history on *S. nutans* biomass (means ± 1 standard error). Shaded bars indicate autoclaved soil, white bars indicate non-autoclaved soil. Autoclaving had a significant positive effect on biomass ($F_{1, 112}$=101.19, P=<0.001), and there was a significant autoclaving by soil history interaction ($F_{1, 112}$=3.93, P=0.05).
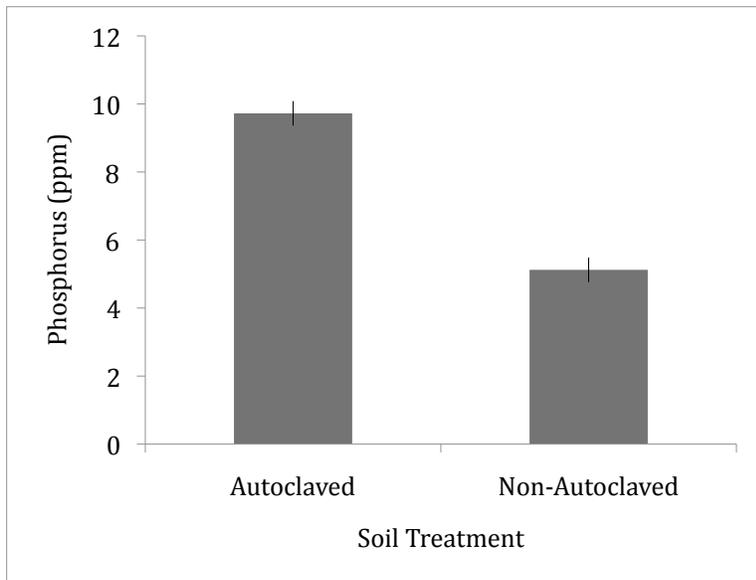

Figure 4.1: Phosphorus present in autoclaved versus non-autoclaved soil (means ± 1 standard error; $F_{1, 18}$=235.84, P=<0.0001)
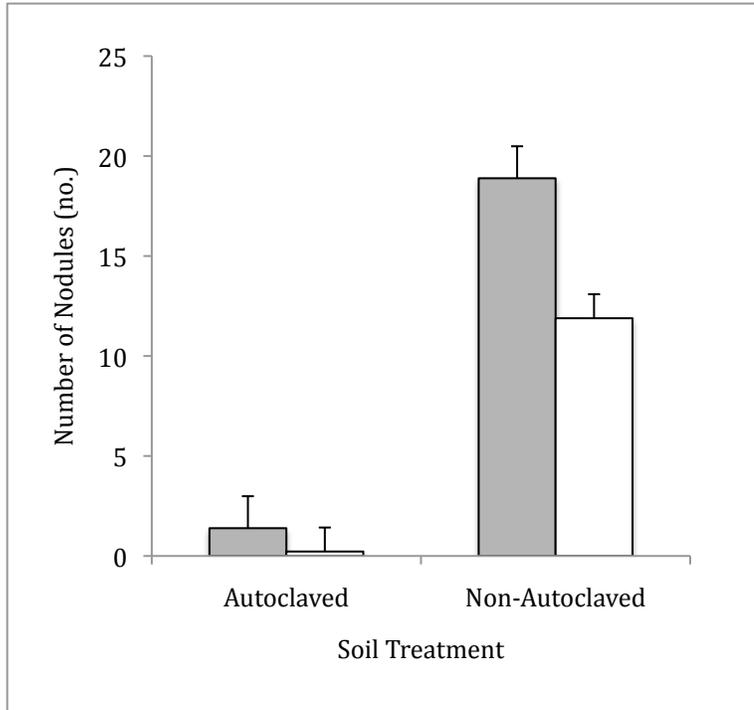
Figure 5.1: Effects of soil history and autoclaving on nodulation of *L. cuneata* (means ± 1 standard error)*.* Shaded bars indicate soil with a history of *L. cuneata*, clear bars indicate a soil history of native plants. Nodulation was higher with a soil history of *L. cuneata* than compared to soil history of native species ($F_{1, 16}=6.01$, $P=0.026$), and nodulation was significantly higher in soil that had been autoclaved ($F_{1, 48}=75.2$, $P=<0.0001$).

CHAPTER 3

Introduction

Invasive species can have strong impacts on the communities they invade. Because of this, several hypotheses have been proposed to explain why some species become problematic invaders while other species appear relatively benign. Of the hypotheses that are used to explain invasive success, empty niche hypothesis and biotic resistance hypothesis focus more on the invaded system (Levine et al. 2004; Shea and Chesson 2002) while others, such as the novel weapons hypothesis, place the emphasis on traits present in the invasive plant (Callaway and Ridenour 2004; MacDougall et al. 2009).

The novel weapons hypothesis predicts that invasion success in a novel environment is due to chemical traits possessed by the invasive plant (Callaway and Ridenour 2004). The allelopathic chemicals produced by the invader have a detrimental effect on native species, either by directly altering growth rates or by indirectly interrupting below-ground symbioses between the plant and soil microbes (Stinson et al. 2006). However, invaders may also create positive soil feedbacks that enhance subsequent invader growth. For example, Coykendall and Houseman (unpublished data) report that the invasive species *Lespedeza cuneata*, is able to alter the soil to facilitate its own growth.

In contrast to invader traits, the biotic resistance hypothesis suggests that the ability of a community to withstand invasion determines invasive success. A system with high biotic resistance will more successfully resist invasion by a novel species than a system with low biotic resistance. A community may resist invaders because of abiotic factors, strong competitive effects of other plant species, or the presence of an enemy or disease that reduce the success of invaders (Levine et al. 2004). These interactions may be seen

through competitive effects for light, nutrients, space or water. Weak competitive effects among natives may lead to invasions if the invasive plant is a stronger competitor for these resources than the natives. Likewise, other aspects of biotic resistance may affect the ability of plants to colonize the habitat. For example, soil microbe communities may be beneficial for the invader by creating mutualistic relationships between the invader, or they may be detrimental, if soil pathogens are able to infect the invasive species (Callaway et al. 2004).

The mediation of invasive success by resource competition may be especially important under stressful abiotic conditions. For example, Allred et al (2010) compared the morphological and physiological traits of the invasive species *Lespedeza cuneata* with two native tallgrass plants, big bluestem, a C_4 grass, and *Ambrosia psilostachya,* a C_3 forb. They found higher specific leaf area in invasive *L. cuneata* compared to the native plants, as well as a relatively constant level of gas exchange throughout the day, which is indicative of a stress-tolerant plant, including drought stress. The higher specific leaf area may also aid in competition by shading competitors once a monoculture of *L. cuneata* has been established. This response does not explain, however, how the invader is able to successfully establish a new population by progressing from seedling to reproductive stages.

In addition to competition under drought stress, *L. cuneata*, which is a nitrogen-fixing legume, may have a competitive advantage over grasses and forbs in nitrogen-poor soil. When inorganic nitrogen is added to the soil, however, *L. cuneata* responds poorly, decreasing the competitive advantage (Brandon et al. 2004). Consequently, the competitive effects of *L. cuneata* on natives may be dependent water or nutrient status, as

well as changes in soils that may develop following establishment, however, there is currently no experimental evidence to support these potential interactions. Here I present two competition experiments designed to address the following questions: 1) Does resource competition in the form of nutrients or water alter the interaction of *L. cuneata* and a dominant prairie grass (*Sorghastrum nutans*)? 2) Does water limitation alter the interaction between *L. cuneata* and a suite of native prairie species? 3) Does a history of *L. cuneata* in the soil have any impact on these interactions?

Materials and Methods

Study System

As part of a *L. cuneata* invasion experiment, *L. cuneata* seed addition and unsown control plots were established in a restored grassland located in northeast Kansas that was dominated by *Sorghastrum nutans* (Houseman unpublished data). Each treatment was also crossed with simulated haying, simulated grazing or no disturbance and replicated three times. Plots were arrayed in a grid and treatments assigned in randomized block design. After three years the mean *L. cuneata* plant density was 57 plants/0.5m^2 (SE=6.67) in *L. cuneata* seed addition plots and 0.4 plants/0.5m^2 (SE=0.14) in unsown controls. Only three of the nine unsown controls had any *L. cuneata*, containing either one or two plants/plot. After the third year, adult *L. cuneata* plants were killed using Escort herbicide (DuPont, Wilmington) and the entire site was burned. In the following spring, soil was collected from nine *L. cuneata* addition or unsown controls. The soil was collected from the upper 15 cm of each plot, and was not pooled (Stinson et al. 2006). The soil was sieved to remove roots, rocks, seeds and other debris and allowed to air dry. For both

experiments, soil was distributed to 262 mL pots.   Due to low germination rates, *L. cuneata*

seeds underwent an acid scarification process using sulfuric acid prior to sowing the seeds

(Bentley 1933).

Single Species Competition

The first experiment focused on resource competition in the form of water and

nutrients. The experiment was a factorial design, with two water treatments, two fertilizer

treatments, two soil histories as described above, and two plant species combinations. A

single *S. nutans* plant was used as the target species, and was grown with either one *S.*

nutans or one *L. cuneata* plant per pot. The water treatment consisted of either high or low

water. The high water treatments received 20 mL of water daily, while the lower water

treatment received 10 mL daily. After plants had established at four weeks, these rates

were decreased to 15mL and 7.5mL per day for the high and low water treatments,

respectively. These levels had been established in a pilot study in the greenhouse as levels

that allowed for growth, but did result in some symptoms of water stress for the low water

treatment.

To determine how nutrient availability influences competition with *L. cuneata*

under variable soil types and neighboring plants, I also manipulated soil fertility by

addition of fertilizer or used an unfertilized control. For the fertilized treatment, a 2mL

aliquot of 20-20-20 fertilizer (Ferti-lome, Voluntary Purchasing Group, Bonham, TX) at a

concentration of 0.4g/L water was added to the base of the each plant once per week

(Cipollini and Darning 2008). When fertilizer was applied, the water given to those

containers was decreased by 4mL to account for fertilizer dose.

Plants were grown in the greenhouse for 12 weeks. As natural light decreased in late summer, light was supplemented with metal halide bulbs under a 12/12 h light/dark cycle. At harvest, above and belowground biomass were separated and soil removed from roots by suspension and hand-agitation in water. Plant material was, dried at 60 °C for 48 hours and weighed. Results were analyzed with a mixed model ANOVA, with soil history as a nested factor and water, fertilizer, and plant identity as fixed factors, with response variables being *S. nutans* or *L. cuneata* biomass, as well as a nodule to root mass ratio for *L. cuneata,* using SAS v9.2 (SAS Institute Inc., Cary, NC, USA).

Multi-species competition

To test for allelopathic or legacy effects of *L. cuneata* on species interactions, a second factorial greenhouse experiment was designed in which native species identity, soil history and water availability were manipulated. The target species selected were *Rudbeckia hirta, Helianthus maximilliani, Desmanthus illinoensis, Lespedeza virginica*, and *Schizachyrium scoparium*. These plants were selected based on prior research indicating that these species are susceptible to *Lespedeza cuneata* (Houseman, unpublished data) and because they represent the dominant plant families of forbs, grasses and legumes in grasslands. Each species was sown in 262 mL pots with either a conspecific or *L. cuneata*. The soil representing two different soil histories was the same as for the single species competition experiment. The high water treatment received 15mL and the low 7.5 mL per pot each day with levels increasing to 20mL, or 10mL per day, respectively during weeks when temperatures in the greenhouse increased above normal levels. The pots were thinned one week after germination to two plants per pot.

28

After 16 weeks, plants were removed from the pots, separated into root and shoot, and soil removed by floating in water. Biomass was dried at 60 $^{\circ}$C for 48 hours. Results were analyzed with a mixed model ANOVA with soil history as a nested factor, and fertilizer, water, and neighbor identity as fixed effects, with response variables being native species or *L. cuneata* biomass or nodulation to *L. cuneata* root mass ratio using SAS v9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Single Species Competition

There was a significant species by water level interaction for *S. nutans* biomass ($F_{1, 256}$=5.67, *P*=0.018). This demonstrated that under high water conditions, biomass of *S. nutans* was similar regardless of neighbor identity, while under low water conditions, the biomass of *S. nutans* was reduced in the presence of *L. cuneata* compared to a conspecific ($F_{1, 256}$=12.07, *P*=0.0006 Figure 1.2). Other interactions between water, fertilizer and neighbor identity were non-significant for *S. nutans* biomass (Table 1).

Water manipulation produced significant effects on the biomass of indiangrass ($F_{1, 256}$=6.62, *P*=0.011, figure 2.2), with high water levels resulting in higher biomass than low water levels. Additionally, fertilizer resulted in increased *S. nutans* biomass ($F_{1, 256}$=13.88, *P*=0.0002, figure 3.2). There was also a significant effect of neighbor plant identity so that the biomass of *S. nutans* grown with *L. cuneata* was lower than when grown with *S. nutans* ($F_{1, 256}$=6.41, *P*=0.012, Figure 4.2).

For *L. cuneata*, increased water availability led to increased biomass but this effect was marginally significant ($F_{1, 128}$=3.14, *P*=0.079, figure 2.2). As expected, fertilizer had

strongly positive effects on *L. cuneata* biomass ($F_{1, 120}$=37.7 P<0.0001, figure 3.2). There was also a significant increase in number of nodules on *L. cuneata* with presence of fertilizer (F$_{1, 120}$=27.5, P=<0.0001), although when the number of nodules was scaled to plant size, this relationship was no longer significant (F$_{1, 120}$=1.64, P=0.20). This ratio did, however, produce a marginally significant result for soil history (F$_{1, 16}$=3.96, P=0.064, figure 5.2), indicating that the nodule to root mass ratio was higher in soil with a history of *L. cuneata* relative to soil without that history.

Multi-Species Competition

Neighbor Identity

Neighbor identity was found to significantly affect most of the species studied. Only *D. illinoensis* did not demonstrate a significant change in biomass when grown with *L. cuneata* compared to a conspecific ($F_{1, 47.7}$=0.02, P=0.89). Conversely, *H. maximilliani* was the only species to exhibit increased biomass when grown with *L. cuneata* compared to a conspecific ($F_{1, 48}$=13.18, P=0.001). The remaining species displayed depressed growth when grown with *L. cuneata* (*L. virginica*: ($F_{1, 46.9}$=10.92, P=0.002), *R. hirta:* ($F_{1, 22.4}$=12.85, P=0.002) and *S. scoparium* ($F_{1, 48}$=4.29, P=0.044), figure 6.2). *Lespedeza virginica* also had a three-way interaction between soil history, species sown and water ($F_{1, 46.9}$=4.33, P=0.043, figure 7.2), in which *L. virginica* biomass was higher when grown at high water with *L. cuneata* compared to a conspecific regardless of soil type. Under low water, there was no difference between soil types when *L. virginica* was grown with a conspecific. However, biomass of *L. virginica* was reduced when grown with *L. cuneata* in soil with a history of invasion under low water conditions.

Soil History

Soil with a history of *L. cuneata* significantly increased biomass of *D. illinoensis* ($F_{1, 16.3}$=5.43, P=0.033, figure 8.2) and *H. maximilliani* ($F_{1, 16}$=15.32, P=0.001, figure 7.2), but had no effect on *L. virginica* ($F_{1, 16.4}$=2.74, P=0.117), *R. hirta*, ($F_{1, 18.8}$=0.68, P=0.42) and *S. scoparium* ($F_{1, 16}$=0.06, P=0.82). *Lespedeza virginica* also had a three-way interaction between soil history, neighbor identity and water availability ($F_{1, 46.9}$=4.33, P=0.043, figure 7.2) discussed above.

Lespedeza cuneata displayed a soil history by water interaction when grown with *L. virginica,* displaying much higher growth in invaded soils under low water conditions, relative to uninvaded soil ($F_{1, 31.9}$=10.85, P=0.002, figure 10.2).

Water

For all native species, water had a significant effect on biomass ($F_{1, 296}$=93.61 P=<0.0001). While most species demonstrated the expected increase in biomass under higher water conditions, biomass of *R. hirta* increased under low water conditions ($F_{1, 20.2}$=8.41, P=0.009). *Helianthus maximilliani* also displayed a soil history by water level interaction where biomass was higher under low water conditions in invaded soil relative to native soil ($F_{1, 43.5}$=21.17, P=<0.0001, figure 11.2). *Lespedeza cuneata* increased in biomass when grown under high water conditions ($F_{1, 158}$=172.42, P=<0.0001). As mentioned previously, *Lespedeza cuneata* displayed a soil history by water interaction when grown with *L. virginica,* displaying much higher growth in invaded soil under low water conditions, relative to native soil ($F_{1, 31.9}$=10.85, P=0.002, figure 10.2). The biomass of

31

L. virginica did not have the same response to soil history by water interaction ($F_{1, 46.4}$=0.09, *P*=0.763). *Lespedeza virginica* also had a three-way interaction between soil history, species sown and water ($F_{1, 46.9}$=4.33, *P*=0.043, figure 7.2), where the biomass when grown with *L. cuneata* in soil with a history of invasion under low water conditions was lower than *L. virginica* grown under other conditions in invaded soil.

Discussion

Species Interactions

The biomass response of native species grown with *L. cuneata* indicates that the effect of this invader on native plants is likely context-dependent both in terms of soil history and water availability. Clearly, *L. cuneata* negatively impacted the growth of *S. nutans, L. virginica, R. hirta,* and *S. scoparium*, but the strength and conditions under which these negative effects occurred varied by species. Although natives were selected representing several families typically associated with grassland functional groups, these are single examples of each so that generalizations cannot be made to the groups as a whole without further experiments.

Interpreting these results through the lens of novel weapons would suggest that *L. cuneata* may produce an allelochemical early in its life stage that is able to inhibit nearby plants, and the species impacted may simply be susceptible at early life stages themselves. Prior experimentation on this topic did not find any negative effect of leachate, however methodological limitations prevent allelopathy from being eliminated as a possible explanation (Coykendall and Houseman, unpublished data). However, these interactions may also be the result of competitive effects, where *L. cuneata* is a better competitor for

32

limited resources than the native plants, which may manifest in the field as a lower biotic

resistance to invasion.   The manipulation of resources in the form of nutrients and water

is intended to start to address this hypothesis, however disentangling the hypotheses will

require additional work to identify specific mechanisms, such as nutrient use efficiency, or

drought tolerance resulting in improved nutrient acquisition.

Soil History

A soil history that includes the invasion by *L. cuneata* displayed either no effect or a

positive effect on native biomass.   In some cases, this positive effect was dependent on

water availability in the multispecies competition experiment. The results indicate that the

changes in the soil made by *L. cuneata* have a stronger impact under low water conditions,

although for both of the species affected (*H. maximilliani* and *L. cuneata*), these effects were

positive in the invaded soil.

Although there were no significant differences found in the soil nutrients of the two

soil histories in a related experiment (Coykendall and Houseman, unpublished data), there

may be other factors that differ between the soils.   For example, a decrease in native

pathogens in the soil with a history of *L. cuneata* may enable increased growth rate in the

native species grown in that soil.   This positive effect may also be related to the difference

in nodulation between *L. cuneata* when grown with *L. virginica* and when grown with the

other species. In this case, both species are able to benefit from the same nodule forming

microbes, however *L. virginica* may be more sensitive to pathogenic microbes than the

invasive species, resulting in an advantage for the invasive, and potentially a disadvantage

for the native species. Additionally, these changes may also lead to increased nodulation in

the invasive species by more rapid or more effective root colonization by nodule forming microbes. Further analysis including soil microbial community analysis and comprehensive soil nutrient analysis are needed to provide a clearer view of the effect of this invader on soil conditions.

Lespedeza cuneata displayed increased growth when grown in invaded soil with native *L. virginica*, although these benefits were only evident under low water conditions. This supports prior work done in this system that indicates that *L. cuneata* is working through soil alterations (Coykendall and Houseman, unpublished data). Both the present and former study use soils that have experienced an experimentally controlled invasion. Although the differences in *L. cuneata* response to soil history between the different experiments may be due to the time lapse between soil collection and the final experiment, results from all experiments suggest that there is a species effect that persists in the soil. Because these effects were seen only with a congeneric species (*L. virginica*), it also supports the hypothesis that *L. cuneata* is creating a positive plant soil feedback mechanism. In this instance, the native species may be able to help further facilitate or reinforce that feedback.

Water and Nutrient Competition

Resource competition, such as water or nutrient competition, can alter the biotic resistance of an ecosystem to invasive species. In this system, water availability was manipulated as morphological data suggests that *L. cuneata* may be particularly drought tolerant (Allred et al. 2010). As expected, most plants had higher growth rates in high water conditions, with the exception of *R. hirta*, which increased in biomass under low

water, indicating drought tolerance. It was under low water conditions, however, that some interactions became apparent. For example, biomass of *L. cuneata* increased under low water conditions in the soil with a history of invasion relative to soil with a history of native species. As this interaction was only found when *L. cuneata* was grown with *L. virginica*, this effect may be due to facilitation of *L. virginica* on *L. cuneata*. Likewise, the effects of *L. cuneata* tended to increase under low water availability. For example in the single species competition experiment, there was greater inhibition of *S. nutans* growth under low water conditions when grown with *L. cuneata* than either high water conditions, or when grown with a conspecific. The fact that some of these interactions occur through changes in soil conditions in the invaded soils suggests a potential role of chemical or microbial changes fostered through invasion by *L. cuneata* particularly at low water availability. However, increased water use efficiency may also contribute to these interactions.

Other experiments have manipulated resource levels in a controlled setting (Cipollini and Darning 2008; Mangla et al. 2011), and while these have shown interactions of nutrient addition and soil history or competition, there are as yet few studies of resource competition from a system invaded by a legume.

While field experiments indicate that *L. cuneata* performs less well compared to native species when nitrogen addition is used, the fertilizer used for the single species competition experiment contained phosphorus and potassium as well as the nitrogen, and may have altered that interaction by maintaining the nutrient benefit to nodulation (Brandon et al. 2004). Other field experiments have found similar increased benefits to native plants with a nitrogen, phosphorous and potassium fertilizer (Houseman,

unpublished data). The differences between the field experiments and the greenhouse

work displayed here may be due to increased shading of *L. cuneata* in the field by native

species.

Conclusion

My experiments show that soil community alterations by invasive plants can be a

mechanism of invasive success depending on the identity of neighboring species and

abiotic conditions.   The possible dynamic between the invasive plant with potentially

novel weapons, and the ability of the ecosystem to resist the presence of the invader is

challenging to isolate, however, the changes seen in this system indicate that *L. cuneata* is

able to affect the system in two primary ways. Under some circumstances, it is able to alter

the soil in a way that creates a positive feedback on its own growth and direct effects on

neighboring plants either through resource competition or allelopathic effects.

Additionally, water limitation may increase the success of this invader, indicating that the

biotic resistance ability of the system to deter invasion may depend on resource

availability. Taken together, these results suggest that a single attribute may be insufficient

to explain the success of a particular invader among a suite of native species experiencing

differing environmental conditions.

Table 1: Table of ANOVA results for *S. nutans* for single species competition experiment . Species indicates neighbor identity.

Effect	DF	F Value	P value
Soil History	1, 16	2.86	0.1101
Water	1, 256	6.62	0.0107
Fertilizer	1, 256	13.88	0.0002
Species	1, 256	6.41	0.012
Soil History*Species	1, 256	0.03	0.8649
Water*Species	1, 256	5.67	0.018
Fertilizer*Species	1, 256	0.97	0.3246
Soil History*Water	1, 256	1.57	0.212
Soil History*Fertilizer	1, 256	0.15	0.6961
Water*Fertilizer	1, 256	3.52	0.0618
Soil History*Water*Fertilizer	1, 256	0.06	0.8058
Soil History*Water*Species	1, 256	3.15	0.0772
Water*Fertilizer*Species	1, 256	0.02	0.8895
Soil History*Fertilizer*Species	1, 256	1.55	0.2136
Soil History*Water*Fertilizer*Species	1, 256	0.05	0.8222
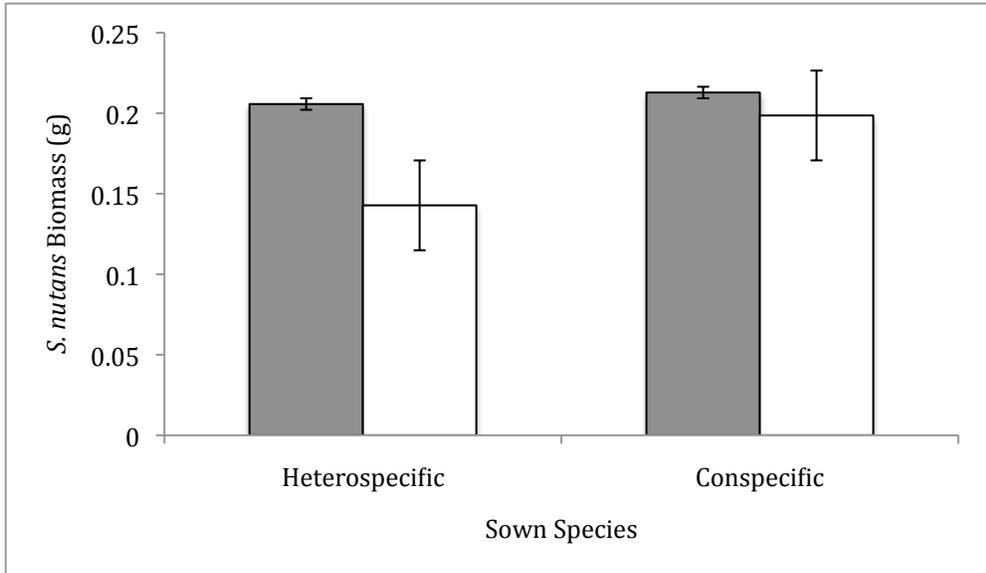
Figure 1.2: Response of *S. nutans* to neighbor identity (means ±1 standard error). Dark bars represent high water, white bars represent low water treatments. There was a significant water by species interaction on indiangrass biomass, sliced by low water treatment ($F_{1, 256}$=12.07, P=0.0006)
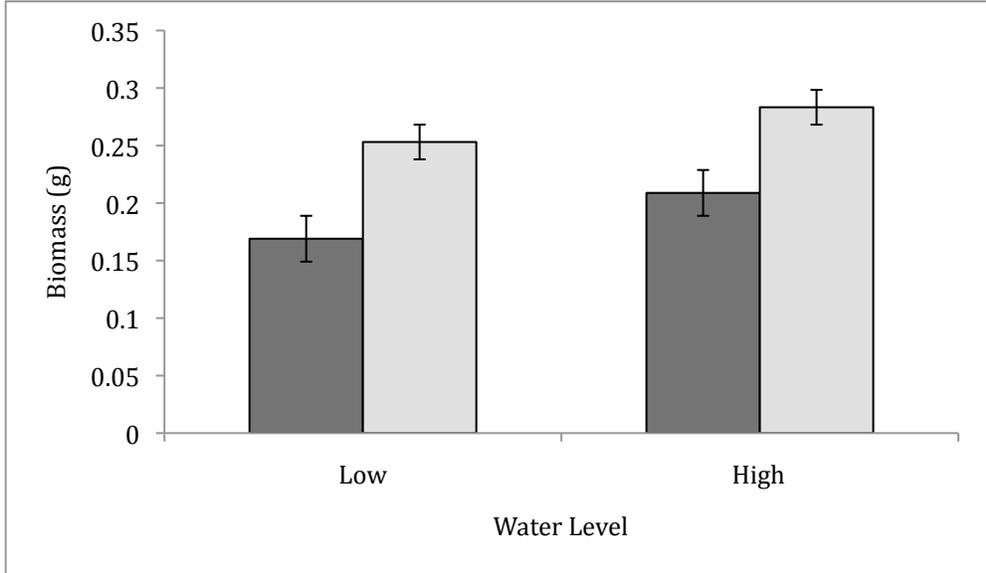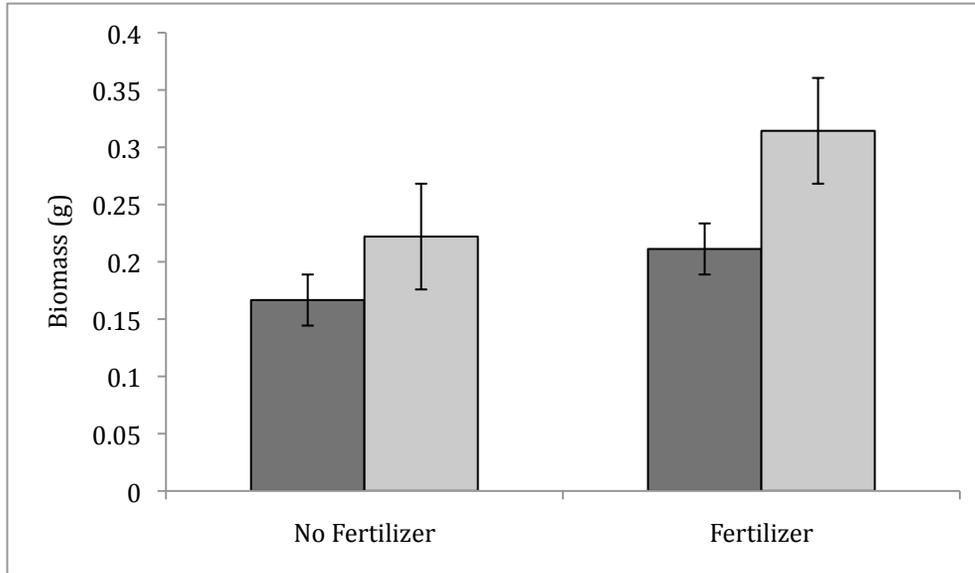

Figure 2.2: Biomass response (means ±1 standard error) of *S. nutans* (dark grey bars) and *L. cuneata* (light grey bars). Water level was a significant main effect on indiangrass biomass ($F_{1, 256}$=6.62, P=0.0107). Water level was not significant for *L. cuneata* ($F_{1, 120}$=3.14, P=0.079).

Figure 3.2: Biomass response (means ±1 standard error) of *S. nutans* (dark grey bars) and *L. cuneata* (light grey bars). Fertilizer was also a significant main effect on *S. nutans* biomass ($F_{1, 256}$=13.88, *P*=0.0002), and on *L. cuneata* ($F_{1, 120}$=37.66, *P*=<0.0001)
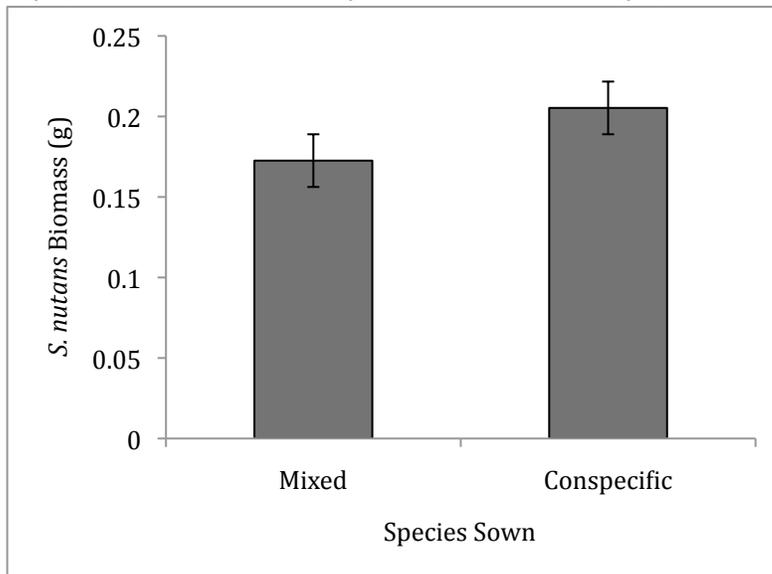
Figure 4.2: Response of *S. nutans* to neighbor identity (means ±1 standard error). Species had a main effect on *S. nutans* biomass, where *S. nutans* grown with *L. cuneata* had a lower biomass that when grown with another *S. nutans* individual ($F_{1, 256}$=6.41, *P*=0.012).
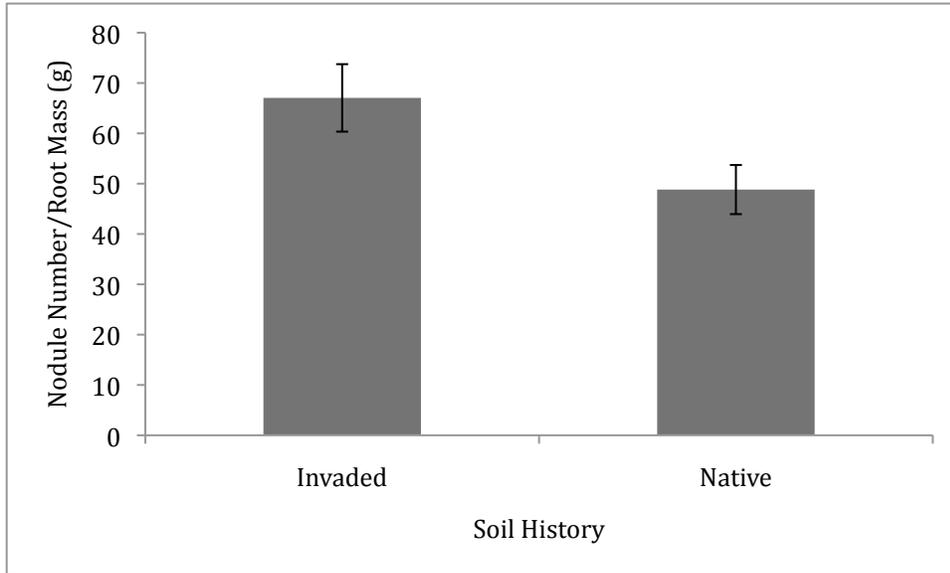
Figure 5.2: Nodule/Root Mass ratio for *L. cuneata* in single species competition (means ± 1 standard error; $F_{1, 16}=3.96$, $P=0.064$).
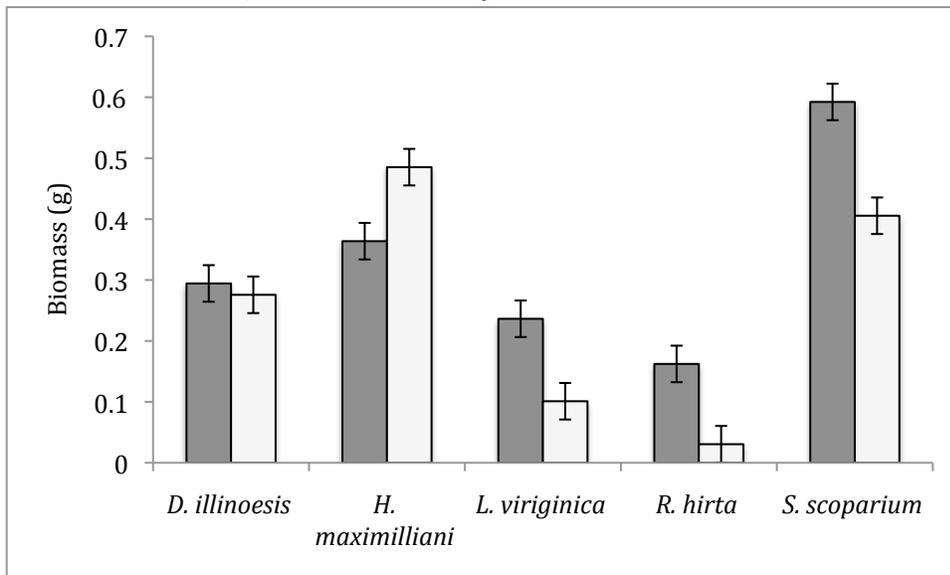

Figure 6.2: Effects of neighbor identity on target species (means ± 1 standard error). Dark bars represent conspecific pairings, white bars represent heterospecific pairings. Neighbor identity was not significant for *D. illinoensis* ($F_{1, 47.7}=0.02$, $P=0.89$), however it was significant for the remaining species (*H. maximilliani* ($F_{1, 48}=13.18$, $P=0.0007$), *L. virginica* ($F_{1, 46.9}=10.92$, $P=0.002$), *R. hirta* ($F_{1, 22.4}=12.85$, $P=0.002$) and *S. scorparium* ($F_{1, 48}=4.29$, $P=0.044$).
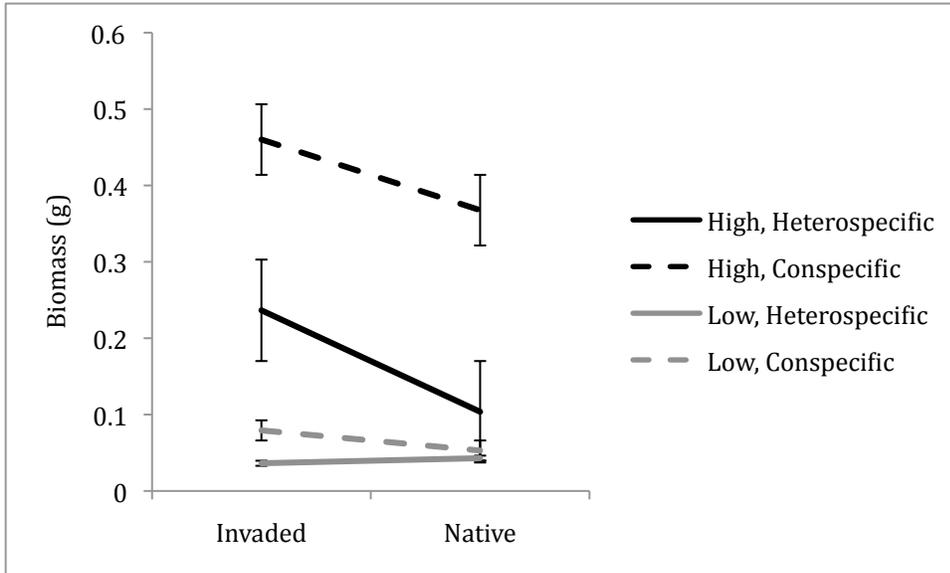
Figure 7.2: Three-way interaction among soil history, water and neighbor identity for *L. virginica* biomass (means $\pm$1 standard error; $F_{1,\,46.9}$=4.33, P=0.043).
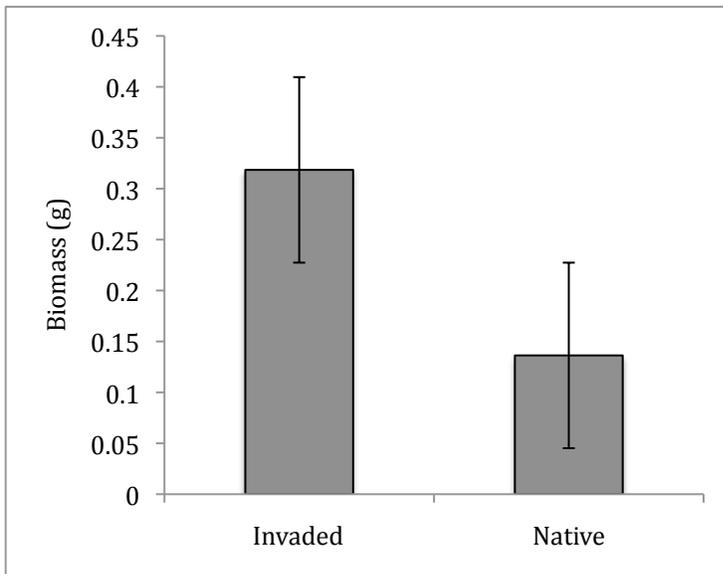

Figure 8.2: Response of *D. illinoensis* to soil history (means $\pm$1 standard error; $F_{1,\,16.3}$=5.43 P=0.033).
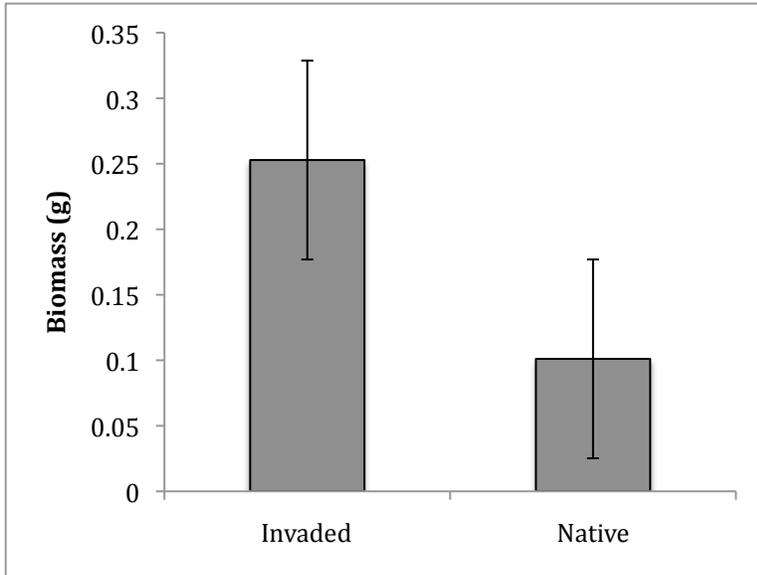
Figure 9.2: Response of *H. maximilliani* to soil history (means ± 1 standard error; $F_{1, 16}=15.32$ $P=0.0012$).
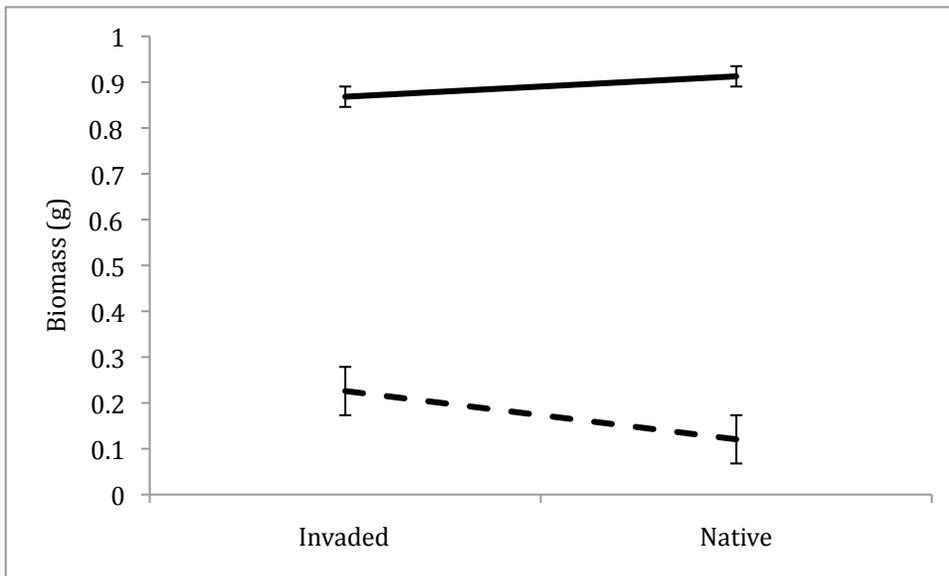

Figure 10.2: Effect of soil history and water on *L. cuneata* (means ± 1 standard error) when grown with *L. virginica* ($F_{1, 31.9}=10.85$, $P=0.002$) at high water (solid line) or low water (dashed line).
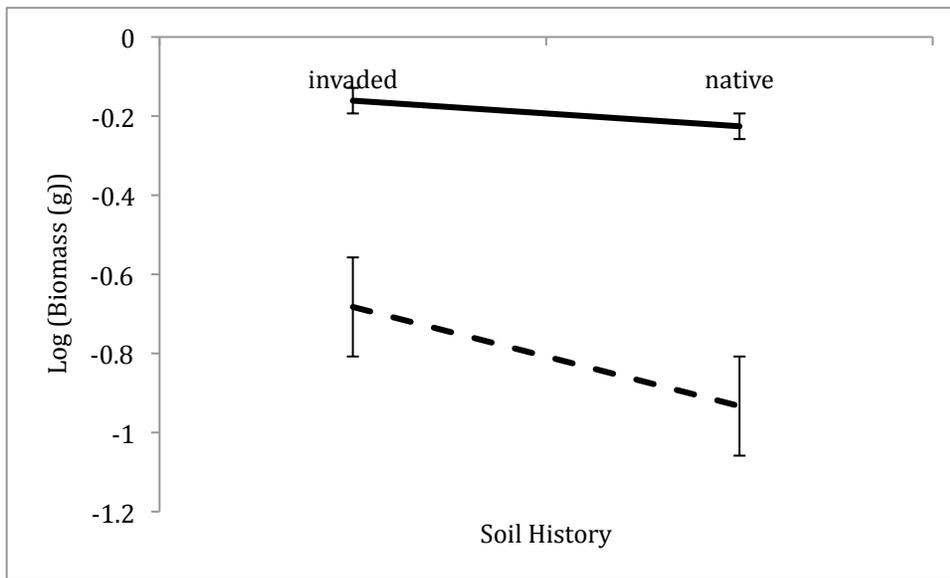
Figure 11.2: Interaction of soil history and water treatment on *H. maximilliani.* Solid line represents high water treatments, dashed represents low water (means ±1 standard error; $F_{1, 43.5}=21.17$, P=<.0001, low water slice).

CHAPTER 4

Lespedeza cuneata is able to alter the species abundance in prairie ecosystems and

create monocultures. The experiments discussed here were designed to provide some

evidence of the underlying mechanisms leading to invasion, and suggest new directions for

management options in the field.

The three primary frameworks for researching the invasion of *L. cuneata* are plant-

soil feedback or PSF, novel weapons, and biotic resistance (Bever et al. 2010; Callaway and

Ridenour 2004; Levine et al. 2004). These experiments have demonstrated how these

three hypotheses regarding invasive species success may work in concert, rather than

opposition in influencing the success in this study system.

Soil history, and as an extension, PSF, is an area of interest for this system, as all

three experiments revealed a connection between soil history and invader grow. While the

allelopathy experiment displayed a facilitative effect of *L. cuneata*, these results were not

consistently seen in the later experiments, but were observed under certain circumstances,

such as when grown with a native congeneric (*L. virginica*). Because the soil from the

same collection was used for all three experiments, it may be that the positive effects are

transient and dissipate over time. Further experimentation focusing on the effects of soil

culturing may provide a timeframe for the alterations in the soil. Additionally, the

experiments provided evidence for increased native growth in soil with a history of *L.*

cuneata.   This may be due to nutrient addition from legume activities, rather than a specific

benefit of the invasive.   The nutrient data presented in chapter two were taken after the

experiment had run, which may have allowed for initial differences in nitrogen levels to be

eliminated through plant use or microbial nitrogen cycling. More comprehensive soil

nutrient sampling at multiple stages before, during, and after the experiment would provide a more detailed understanding of the nutrient alterations. Additionally, microbial community analysis may provide evidence regarding alterations in soil communities due to the invasive species.

These results demonstrate some support for water competition as a factor in invasive success of *L. cuneata*. The single species competition revealed a water by neighbor identity interaction, and the multispecies competition experiment suggested a interaction between water and the soil history for one of these species. These results indicate that *L. cuneata* may have a competitive advantage over the native species under lower water conditions, which is consistent with morphological data indicating that *L. cuneata* is drought tolerant (Allred et al. 2010). While nutrient addition did not seem to affect the interactions or demonstrate any change in the biotic resistance, future experiments using multiple forms of nitrogen may alter the interactions by being more biologically available.

Additionally, both the single and multiple species competition results demonstrated decreased biomass of the native species when grown with the *L. cuneata* individuals. While the allelopathic extract treatment did not display significant results, there may still be allelopathic chemicals produced from living plant tissues that were not effective extracted in sufficient quantities, thus indicating that *L. cuneata* may produce a novel weapon. Also, the dosage present during the extract treatment may have been lower than the constant presence of chemicals from a living plant. A thorough assay of the dosage and extract methodologies would be a valuable next step in isolating the mechanisms of *L. cuneata's* invasive success, and obtain a more definitive answer regarding allelopathic capability of *L. cuneata.*

These experiments provide an important foundation for understanding invasive success of *L. cuneata*. Research into this invasion is relatively recent, and this work concentrates on a life stage that had not received a great deal of attention in the literature, and using these methodologies for studying this invasion. While the results obtained in this work may not apply to all systems, the methodology used and the data obtained are a valuable addition to the growing literature of invasive biology.

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