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# Microstegium vimineum Spread Rate in Relation to Two Different Leaf Litter Disturbances and an Evaluation of Aboveground Biomass Accumulation and Photosynthetic Efficiency in Response to Four Light Treatments

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To the Graduate Council:

I am submitting herewith a thesis written by John Andrew Hull entitled "Microstegium vimineum Spread Rate in Relation to Two Different Leaf Litter Disturbances and an Evaluation of Aboveground Biomass Accumulation and Photosynthetic Efficiency in Response to Four Light Treatments." I have examined the final electronic 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 Forestry.

Wayne K. Clatterbuck, Major Professor

We have read this thesis and recommend its acceptance:

Christopher M. Oswalt; Gregory R. Armel

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Carolyn R. Hodges  
Vice Provost and Dean of the Graduate School

**(Original Signatures are on file with official student records.)**

***MICROSTEGIUM VIMINEUM* SPREAD RATE IN RELATION  
TO TWO DIFFERENT LEAF LITTER DISTURBANCES AND  
AN EVALUATION OF ABOVEGROUND BIOMASS  
ACCUMULATION AND PHOTOSYNTHETIC EFFICIENCY IN  
RESPONSE TO FOUR LIGHT TREATMENTS**

A Thesis  
Presented for the  
Master of Science  
Degree  
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John Andrew Hull  
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## ABSTRACT

*Microstegium vimineum* is a non-native invasive plant species classified as an annual, shade-tolerant C<sub>4</sub> grass. There is limited research regarding variables affecting the spread of *M. vimineum*. Two studies were conducted to investigate the spread of *M. vimineum*. A field study was undertaken in 2009 to determine how *M. vimineum* spreads in relation to litter disturbance. In 2010, a greenhouse study was conducted to determine the impact light has on *M. vimineum* aboveground biomass, height growth, and photosynthetic efficiency.

The field study consisted of three treatments, Undisturbed (Control), Stirring, and Removal of leaf litter, employed along the boundary of existing *M. vimineum* populations in 1/2-meter by 2-meter plots. Distance of spread from the existing population and percent cover were documented for one growing season. Plants were counted at the end of the study. Neither stirring nor removal of leaf litter had a significant impact on spread rate, percent cover, or the number of plants in a given treatment suggesting pre-growing season leaf litter disturbance does not influence *M. vimineum* spread, percent cover, or number of plants.

The greenhouse study consisted of growing *M. vimineum* under four light treatments: 100, 70, 45, and 20 percent of full light. Heights were measured weekly while minimum, maximum, and variable fluorescence emission, non-photochemical and photochemical quenching, and maximum quantum yield of Photosystem II photochemistry ( $QY_{max}$ ) were measured every 10 days. Aboveground biomass accumulation was calculated at the end of the study. Results indicate that *M. vimineum* aboveground biomass accumulation is highest in 70 percent to 100 percent light while photosynthetic efficiency is highest between 45 percent and 70 percent light.

This research indicates that *M. vimineum* does not spread appreciably at low light levels (closed canopies) in areas with litter disturbances that do not change the light regime. *M. vimineum* has greater aboveground biomass and photosynthetic efficiencies at higher levels of light. Thus,

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## INTRODUCTION

Exotic invasive plants are the greatest threat to endangered species in the United States excluding habitat loss and are a consistent threat to ecosystems worldwide (Flather et al. 1994; Wilcove et al. 1998; Stein et al. 2000). Infestations can cause undesirable changes in ecosystems via displacement of native plant and animal species, the alteration of nutrient cycling and successional recovery of a community following disturbance (Walker and Smith 1997). The lack of natural control mechanisms such as insects, predators, plant fungi, competing vegetation, and herbivory allow many of these exotics to thrive in North America (Sheley et al. 1999). Silvicultural practices and the activities associated with them, such as harvesting timber, road construction, and burning, inevitably result in some degree of habitat disturbance. The opening of the canopy and exposure of bare mineral soil are commonly caused by these activities which can stimulate the germination and growth of some exotic species (Honnay et al. 2002).

*Microstegium vimineum*, or Japanese stiltgrass, is one of many exotic invasive plant species in the United States. Classified as an annual, shade-tolerant C<sub>4</sub> grass, *M. vimineum* is native to Asia, particularly the lowland and lower mountain forests of Japan, Korea, China, India, Nepal, and Malaysia (Fairbrothers and Gray 1972; Sur 1985; Osada 1989; Hunt and Zaremba 1992). First documented in Tennessee in 1919, *M. vimineum* is now present throughout much of the eastern United States (excluding Maine, Vermont, and Connecticut) and reaches as far west as Texas (USDA 2010). The plant's colonization in Tennessee was probably a result of its use as a packaging material for porcelain (Swearingen and Adams 2008).

*M. vimineum* is a monocot in the Poaceae family with a decumbent and branched growth form. Stems are prostrate with multiple branches. Leaves are 4 to 8 cm long and 5 to 8 mm wide with a white midrib. Flowers, appearing from August to November, are produced in racemes with two to six glabrous spikelets. Fruits are oblong caryopses (Zheng et al. 2004).

*M. vimineum* spreads by means of tiny seeds while rooting of stem nodes allows the plant to exploit resources and aids in the process of seed distribution by allowing seed dispersal farther from the parent plant (Williams 1998; Mehrhoff 2000). In optimal conditions, the plant can reach up to 3 ½ feet in height and can self- or cross-fertilize producing up to 1000 seeds per plant (Virginia Department of Conservation and Recreation 2006; Swearingen and Adams 2008). Dense populations can produce anywhere from 16,000 to 50,000 seeds/m<sup>2</sup> (Williams 1998). Seeds germinate readily in the spring and can remain viable for 3 – 5 years (Barden 1987; Tu 2000; Miller 2003; Swearingen and Adams 2008). Seeds typically fall close to the parent plant, but can be further dispersed by streams and runoff from heavy rain. Other means of seed movement include, but are not limited to, animals, contaminated hay, transported soil, vehicles, and other human activities such as hiking, and road maintenance/construction.

Common habitats associated with *M. vimineum* are moist areas such as flood plains and stream sides, forests, lawns, roadsides, ditches, and disturbed areas; however, water availability is not a limiting factor for *M. vimineum* distribution (Touchette and Romanello 2010). *M. vimineum* growth rate is considerably affected by light availability and it can respond vigorously to high light conditions (Claridge and Franklin 2002) while having the ability to tolerate low light conditions as well (Barden 1987). The tolerance of such a broad range of light and soil moisture regimes allows it to adapt to a wide range of site conditions. Although *Microstegium* may not thrive in some conditions, it can still carry out its life cycle and thus slowly spread. This accounts for the invasion and spread of this species throughout the eastern United States in many different ecosystems.

## OBJECTIVES

Limited research is available regarding what impacts the spread of *M. vimineum*. Light, moisture, and nutrient levels all play roles in the rate of spread; however, the effect of disturbances around existing *M. vimineum* populations is unclear. The degree to which light influences the photosynthetic efficiency and the growth in height and aboveground biomass accumulation of *M. vimineum* is also unclear.

The objectives of this study are (1) to determine if pre-growing season leaf litter disturbance has an effect on the overall spread rate of *M. vimineum* and (2) to evaluate *M. vimineum* aboveground biomass accumulation and photosynthetic efficiency under different levels of light. To meet these objectives, two separate studies were conducted to address the following questions and hypotheses:

- 1) Does pre-growing season leaf litter disturbance affect the distance of *M. vimineum* spread?
- 2) To what degree does pre-growing season leaf litter disturbance affect the density of spread?
- 3) In what manner is growth in height and aboveground biomass accumulation of *M. vimineum* influenced by light?
- 4) How is the photosynthetic efficiency of *M. vimineum* influenced by light?

Hypotheses:

- 1) The spread rate of *M. vimineum* differs significantly when leaf litter is disturbed.
- 2) Percent cover and density of *M. vimineum* differs significantly when leaf litter is disturbed.
- 3) The amount of available light has a significant effect on the aboveground biomass accumulation of *M. vimineum*.
- 4) Photosynthetic efficiency differs significantly in different light levels.

Because greater overall growth (in the form of height and aboveground biomass) typically results in a greater amount of seed production for *M. vimineum* which would increase the potential for spread (Williams 1998), a better understanding of how light influences growth and how ground disturbances facilitate the spread of seed will aid in the control of this non-native, invasive species.

## LITERATURE REVIEW

### Natural Spread

While rooting of the nodes allows for an increase in both seed production and the distance at which seeds are shed, *M. vimineum* spread is generally dependent upon the dispersal of its seed from the parent plant. The primary method of *M. vimineum* seed dispersal is gravity (Cheplick 2005) and in some cases, water movement (Mehroff 2000). Seeds have no known mechanisms allowing for animal or wind dispersal despite an individual seed weighing only  $1.14 \pm 0.01$  mg (Cheplick 2005; 2010). Rauschert et al. (2010) investigated the natural spatial spread and population dynamics of *M. vimineum* in four different habitats over four growing seasons using planted populations. Results indicated that the natural spread of *M. vimineum* is rather slow and other factors, such as human disturbances, play a major role in its spread (Rauschert et al. 2010) and may facilitate invasion (Marshall and Buckley 2008). In a study by Oswald and Oswald (2007), undisturbed study plots also had a low mean rate of spread, as plants only emerged 0.37m away from existing populations in one growing season. Cheplick (2010) also investigated the spatial spread of *M. vimineum* and determined that dispersal of *M. vimineum* seed is relatively poor without human assistance. Therefore, disturbances, particularly those caused by humans, play a key role in the successful invasion and spread of *M. vimineum*.

### Spread and Disturbance

To quantify the effect of disturbance on *M. vimineum* spread, Marshall and Buckley (2008) examined the influence of litter removal and mineral soil disturbance. Three treatments (leaf litter removal, mineral soil disturbance, and leaf litter removal and mineral soil disturbance) and a control were implemented on existing populations of *M. vimineum* and compared. No statistically significant differences in mean plants per plot were found between control plots and treatment plots. However,



differences were present in mean plant distance from the existing population boundary, indicating that leaf litter disturbances may increase *M. vimineum* spread rate, while undisturbed populations may exhibit slower rates of spread in comparison (Marshall and Buckley 2008).

Oswalt and Oswalt (2007) studied the effect of winter leaf litter removal on *M. vimineum* spread rate from existing populations. They documented linear spread and cover expansion away from the existing population after one full growing season; in fact, both percent cover and linear spread outward from the existing population were different in the treatment versus the control plots. *M. vimineum* spread was 4.5 times greater in disturbed populations (1.66m) compared to undisturbed populations (0.37m) (Oswalt and Oswalt 2007).

In greenhouse studies conducted by Judge (2005), germination rates after 90 days of storage, regardless of storage type and incubation conditions (light, dark, alternating light levels, consistent temperature, alternating temperature, etc.), exceeded 95 percent. Field stratification in leaf litter of freshly harvested *M. vimineum* seeds yielded germination rates of 93 percent after 90 days and 98 percent after 120 days (Judge 2005). No freshly harvested seeds germinated and less than 1 percent germinated after 15 days of storage regardless of storage and incubation conditions. When stored for 30 or 60 days, storage conditions and incubation conditions both impacted germination rates. Germination rates were highest when seeds were stored dry, at room temperature and seeds were incubated in alternating temperatures. Seeds stored 30 days exhibited over 22 percent germination while seeds stored 60 days exhibited over 88 percent germination (Judge 2005). A field study by Barden (1987) also reported germination rates of 77 percent on one site and 92 percent on another. Judge (2005) also determined that *M. vimineum* has no light requirements for germination once dormancy is broken. Ninety days of dry storage at room temperature completely satisfies the dormancy requirements for *M. vimineum* (Judge and Neal 2004; Judge 2005).

Previous studies have shown that leaf litter removal and mineral soil disturbance both have a statistically significant impact on the linear spread (distance of farthest plant) of *M. vimineum* from the existing population (Oswalt and Oswalt 2007; Marshall and Buckley 2008) and that natural dispersal of seed results in a relatively slow spread rate (Oswalt and Oswalt 2007; Marshall and Buckley 2008; Cheplick 2010; Rauschert et al. 2010). Similar to the findings of Judge (2005), the actual spread of *M. vimineum* in relation to disturbance appears dependent on the physical movement of the seed rather than factors associated with germination such as light levels; however, successful germination does not guarantee successful reproduction.

### **Growth and Light Levels**

While *M. vimineum* is classified as a C<sub>4</sub> species, it reacts differently to various light levels than typical C<sub>4</sub> species. Most C<sub>4</sub> species require high levels of light for greater growth (Winter et al. 1982); however, *M. vimineum* is capable of growing in more shaded areas. Winter et al. (1982) documented the aboveground dry weight of *M. vimineum* when grown in a greenhouse under four different light levels ranging from 5 percent to 100 percent of full light. Dry weight after 45 days of growth was similar from 18 percent to 100 percent of full sunlight indicating high shade adaptability, while aboveground biomass was still substantial even at 5 percent light (Winter et al 1982). Horton and Neufeld (1998) conducted a greenhouse study on the effects of light levels on the photosynthetic responses of *M. vimineum* with an emphasis on the plant's ability to use sun-flecks. Plants were grown under 50 percent and 25 percent of full light. Gas exchange, steady-state light response, induction response, and induction loss were measured. Results indicated that the C<sub>4</sub> pathway did not create a barrier for the plant's ability to respond to sun-flecks and that in long periods of low light it is able to maintain a positive, while small, carbon gain (Horton and Neufeld 1998). Therefore, although *M. vimineum* utilizes the C<sub>4</sub> pathway, the pathway itself does not appear to

negatively affect growth when *M. vimineum* is growing in an environment with substantial shade (25 percent light).

Cole and Weltzin (2005) conducted a study to investigate the influence of light, soil characteristics, and moisture levels on *M. vimineum* distribution. The hypotheses investigated were formulated based upon prior observations of *M. vimineum* being absent beneath midstory trees (Cole 2003; Cole and Weltzin 2004; 2005). Results indicated that soil characteristics and moisture levels were not responsible for the discontinuity of *M. vimineum* that was observed in the study area before the study was initiated (Cole and Weltzin 2005). Touchette and Romanello (2010) also found that *M. vimineum* can persist in a wide range of hydrological conditions indicating water availability is not a limiting factor for distribution. In both field and greenhouse studies by Cole and Weltzin (2005), survival decreased as available light decreased suggesting that light has the most notable effect on the distribution of *M. vimineum* with exceedingly low (less than 20 percent) light levels being the limiting factor.

### **Spread and Light Levels**

The spread and invasion of *M. vimineum* is most likely dependent on a combination of disturbance and light levels of at least around 20 percent of full sunlight (Winter et al 1982; Horton and Neufeld 1998; Cole and Weltzin 2005; Oswalt and Oswalt 2007; Marshall and Buckley 2008; Cheplick 2010; Rauschert et al. 2010). Thus, the spread rate would be more rapid in areas influenced by disturbance in which light is not a limiting factor, as has been demonstrated by previous studies (Oswalt and Oswalt 2007; Marshall and Buckley 2008). Many disturbances (particularly those associated with timber harvests) not only result in partial or full removal of the canopy, which increases light levels in the understory, but they also disturb leaf litter to varying degrees. Because higher light levels typically result in a larger crop of seed (Williams 1998), the litter disturbance may

cause this elevated amount of seed to be moved further away from the plant of first introduction than natural dispersal allows. The inevitable consequence would be a more rapid spread rate than exhibited by natural dispersal.

## Competition

In a controlled greenhouse study by Leicht et al. (2005), the competitive ability of *M. vimineum* with *Lolium multiflorum* and *Muhlenbergian mexicana* was assessed. Differences were observed in both full and low light conditions as *M. vimineum* exhibited a greater aboveground biomass, relative growth rate, and reproductive output than both competitors (Leicht et al. 2005). Previous studies have shown that *M. vimineum* can impact species diversity, density, and soil moisture availability (Marshall 2007; Oswalt et al. 2007; Sheherezade and Englehardt 2009). Sheherezade and Englehardt (2009) demonstrated that *M. vimineum* is associated with local declines in species richness and cover of native species by studying adjacent pairs of invaded and uninvaded plots. Oswalt et al. (2007) investigated the effects of *M. vimineum* on native woody species density and diversity in a productive mixed-hardwood forest in Tennessee. As *M. vimineum* percent cover increased, there was a significant decrease in stems per hectare of native woody species (Oswalt et al. 2007). Declines in simple species richness of native woody species and Shannon's and Simpson's diversity indices were also observed with increasing *M. vimineum* percent cover (Oswalt et al. 2007). Marshall (2007) examined competitive interactions between *M. vimineum* and native hardwood seedlings. *Acer rubrum* and *Liriodendron tulipifera* both exhibited reduced leaf area due to soil moisture competition with *M. vimineum*; however, no measureable competitive impacts were observed on *M. vimineum* aboveground weight, seed mass, or seed count, when grown in the presence of those hardwood species (Marshall 2007). *M. vimineum*'s ability to successfully compete with native plant species is another factor that aids in its spread and persistence.

## Management/Control Recommendations

*M. vimineum* can be controlled manually or with use of herbicides, however, currently there are no forms of biological control available for this species (Swearingen and Adams 2008). *M. vimineum* is a summer annual that is dependent on annual recruitment from its seed bank for continued survival (Radford et al. 1968; Fairbrothers and Gray 1972; Mehrhoff 2000). In a study by Barden (1987), seed survivability in the soil was examined. After stopping seed production in two separate populations of *M. vimineum* for three consecutive years by hand removing plants before seeds were produced, no plants emerged in the fourth year suggesting seeds remain viable for at least three years under natural conditions (Barden 1987). In a similar study, Woods (1989) determined seeds stay viable for a minimum of two years. Thus successful management of this species requires a multi-year elimination of seed production to stop annual inputs into the seed bank until it is exhausted (Woods 1989; Tu 2000; Gibson et al. 2002).

Seed production can be prevented by simple hand removal, mechanically (i.e., mowing), or chemically using a nonselective post emergent herbicide, such as glyphosate, mid to late summer before flowering occurs (Tu 2000; Swearingen and Adams 2008). Selective post-emergence herbicides such as fenoxaprop-P, imazapic, and sethoxydim can also be applied for control in areas where damage to desirable plants is a concern (Judge et al. 2005b). Although fenoxaprop-P, imazapic, and sethoxydim will control *M. vimineum* when applied any time throughout the growing season before flowering occurs, their selectivity varies (Judge et al. 2005b). This makes it possible to selectively control *M. vimineum* in areas with different species compositions. Studies by Judge et al. (2005a) also determined that pre-emergent herbicides are capable of successfully controlling *M. vimineum*. Eight herbicides (dithiopyr, metolachlor, napropamide, oryzalin, oxadiazon, pendimethalin, prodiamine, trifluralin) and 3 combinations of herbicides (benefin plus oryzalin, benefin plus trifluralin, and isoxaben plus trifluralin) were tested. Except for metolachlor and napropamide, all

provided control of 78 percent or greater of *M. vimineum* eight weeks after application (Judge et al. 2005a).

## STUDY SITE

Located in Oak Ridge, Tennessee in Anderson County, the University of Tennessee Forest Resources Research and Education Center (FRREC) is a 2,260-acre (915-ha) tract comprised of primarily forested land (Figure 1). The FRREC is within the ridge and valley physiographic province, distinguished by long and parallel ridges that run in a northeast-southwest direction (Moneymaker 1981). Average temperatures range between 37.3° F (2.9° C) and 77.1° F (25.1° C) with an average annual precipitation of 55 inches (140 cm) (SERCC 2010).

Soils at study Site 1 are in the Fullerton series found on gentle to steep slopes and consists of well drained, deep, cherty soils that formed in residuum weathered from cherty limestone or dolomite (NRCS 2010). Soils at study Sites 2 and 3 are in the Armuchee series. Typically found on slopes ranging from 5 to 60 percent, these soils are fairly deep, well drained, and formed in bedrock of acid shale on uplands. Slope across all study sites was 5 percent or less with a southeastern aspect.

Each study site was between 150-and 200-square meters while treatment areas covered a total of 27-square meters. Dominant overstory species were *L. tulipifera*, *A. rubrum*, and mixed *Quercus* species. *Cornus florida* was present in the midstory while *Liquidambar styraciflua* seedlings and saplings were in the understory. Herbaceous vegetation was comprised of a mixture of species (Table 1). Sites 1 and 3 had not been disturbed within the past 5 to 10 years, however, a disturbance had occurred at Site 2 when timber was thinned in 2004.

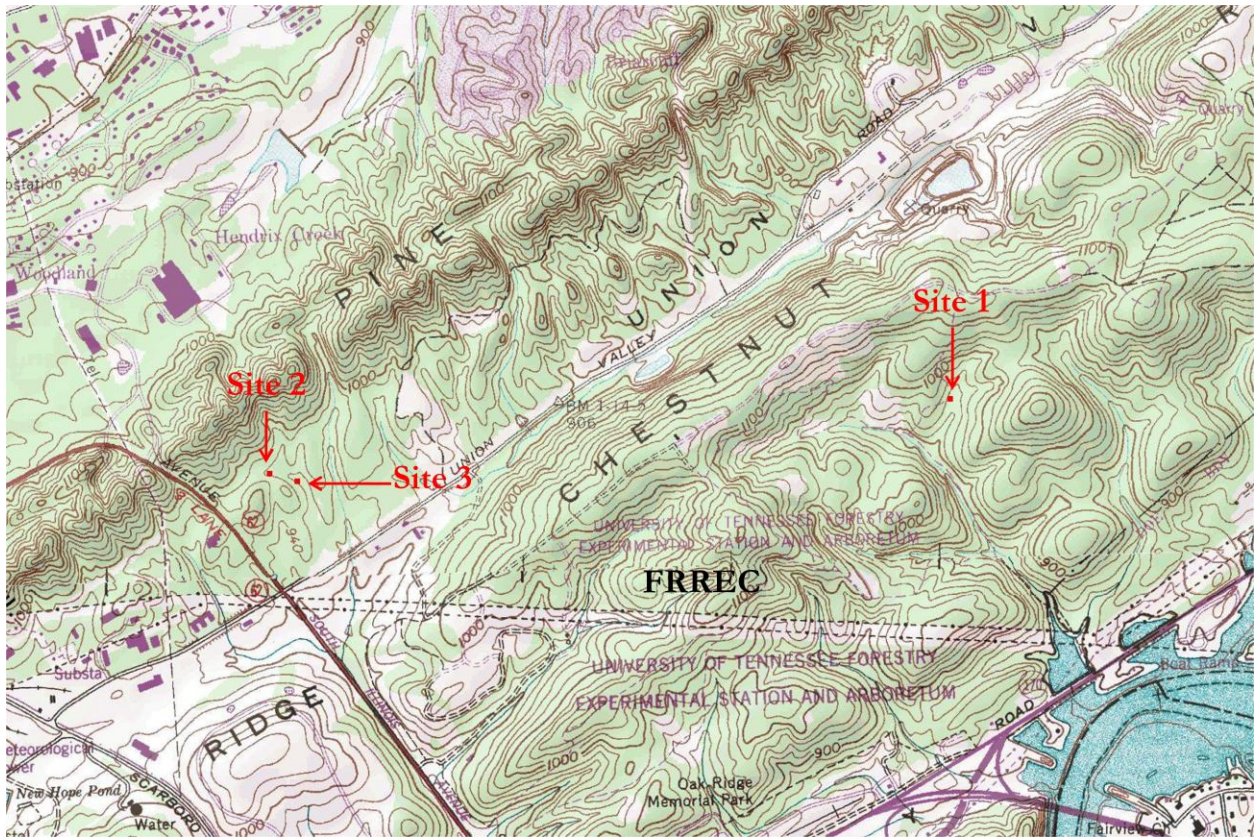


Figure 1: Study site locations for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee.



Table 1: Cumulative list of plants for all study sites for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Plants listed were found in the herbaceous layer at one or more study sites within treatment plots.

Scientific Names	Common Names
<i>Acer rubrum</i>	red maple
<i>Acer saccharum</i>	sugar maple
<i>Amphicarpaea bracteata</i>	hog peanut
<i>Anemone quinquefolia</i>	wood anemone
<i>Cercis canadensis</i>	redbud
<i>Cimicifuga racemosa</i>	black cohosh
<i>Elaeagnus umbellata</i>	autumn olive
<i>Liquidambar styraciflua</i>	sweet gum
<i>Liriodendron tulipifera</i>	yellow poplar
<i>Lonicera japonica</i>	Japanese honeysuckle
<i>Nyssa sylvatica</i>	black gum
<i>Parthenocissus quinquefolia</i>	Virginia creeper
<i>Pinus virginiana</i>	Virginia pine
<i>Polystichum acrostichoides</i>	christmas fern
<i>Prunus serotina</i>	black cherry
<i>Quercus alba</i>	white oak
<i>Quercus rubra</i>	northern red oak
<i>Quercus velutina</i>	black oak
<i>Rubus allegheniensis</i>	common blackberry
<i>Smilax rotundifolia</i>	common greenbriar
<i>Toxicodendron radicans</i>	poison ivy
<i>Uvularia perfoliata</i>	bellwort
<i>Viburnum acerifolium</i>	mapleleaf viburnum
<i>Vitis aestivalis</i>	summer grape

## **METHODS**

In this research, the spread rate of *M. vimineum* in relation to two different leaf litter disturbances and the influence of light on aboveground biomass accumulation were evaluated. Two separate studies were conducted. A field study was implemented to evaluate leaf litter disturbance on the rate of spread while a greenhouse study was implemented to evaluate photosynthetic efficiency and growth based on height and aboveground biomass accumulation in relation to available light.

Field study treatments consisted of:

Treatment 1 – No disturbance (Control)

Treatment 2 – Stirring of leaf litter

Treatment 3 – Complete removal of leaf litter

Greenhouse study treatments consisted of:

Treatment 1 – 100 percent of ambient light

Treatment 2 – 70 percent of ambient light

Treatment 3 – 45 percent of ambient light

Treatment 4 – 20 percent of ambient light

### **Field Methods**

This study was conducted at the University of Tennessee Forest Resources Research and Education Center (FRREC) in Oak Ridge, Tennessee. On March 9<sup>th</sup> of 2009 before bud burst, the study was initiated and 3 replicates of 3 different treatments were implemented consisting of 9 treatment plots per study site with a total of 27 treatment plots.

## Sample Design and Installation

In the September of 2008, existing *M. vimineum* populations were located and evaluated.

Three study sites were selected based on five criteria:

1. Land area occupied by population,
2. Coverage of area by *M. vimineum*,
3. Coverage of *M. vimineum* compared to other vegetation
4. Site location physiographic similarities, and
5. Definition of the population boundary in relation to the surrounding vegetation.

All selected populations had other plant species present throughout. An ideal population would be a dense, monoculture of adequate size<sup>1</sup> with a definitive edge at which the population ceases and would have similar site characteristics to any previously selected site(s) (i.e. soil moisture and light conditions). However, because this study was conducted in mixed hardwood stands, no selected populations were ideal, each one meeting only 3 or 4 of the 5 criteria. A total of four populations were selected on three sites with one site containing two separate populations.

Site 1 contained a large population along the side of an unpaved dirt road. While *M. vimineum* dominated, other species were present within the population. Site 2 was a large population located on an old logging road and was interspersed with sweet gum (*Liquidambar styraciflua*) saplings. Site 3 contained two populations, neither large enough to support all replicates. One population was a monoculture while the other, dominated by *M. vimineum*, contained multiple plant species.

The following plot establishment procedure was conducted at each site:

A ½-meter by 2-meter rectangle was laid out with the rectangle extending outward from the population edge into the existing surroundings (Figures 2 and 3). Plots were marked using colored

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<sup>1</sup> Adequate size meaning the population was large enough to support all replicates of each treatment, approximately 16m<sup>2</sup> minimum.



Figure 2: Photograph of Stir (yellow flags) and Removal (red flags) treatments for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Subplot 1 for the Stir treatment is closest to the bottom of the photograph.

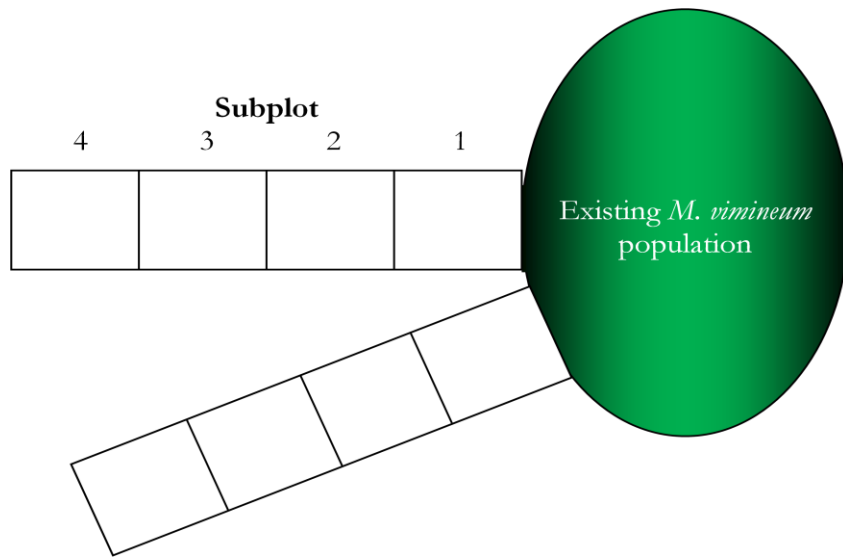


Figure 3: Diagram of example study plot for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee.

wire flags, each color denoting a different treatment. The plots were designated by placing a wire flag at each corner and at each ½-meter lengthwise. This resulted in four 0.5- x 0.5-meter subplots per 0.5- x 2-meter plot. The process was repeated 9 times per study site. Treatments were randomly implemented on each plot. Control treatments were left untouched. On Treatment 2, leaf litter was stirred rigorously while all leaf litter was completely removed from Treatment 3 and taken offsite. Stirring and removal of litter was only conducted once. Stirring was done by hand. Litter was grabbed by the handful, lifted from the ground approximately 20 centimeters, and then dropped back into the treatment plot. This process was conducted repeatedly at a rapid pace throughout each Stir treatment plot so as to displace all litter material without allowing any litter to be removed from the plot. The process increased the overall volume occupied by the litter due to an increase in the volume of air space between leaves. Stirring the litter for a given treatment plot took about 1 to 2 minutes. Removal of litter consisted of hand removal of all litter and any other debris present, leaving bare mineral soil exposed. Photographs were taken throughout the entire course of the study at all sites for purpose of illustration.

On April 23<sup>rd</sup>, 2010, and each month thereafter until September 23<sup>rd</sup>, the following measurements were taken for each treatment plot:

A percent cover estimation based on seven categories was taken for each 0.5- x 0.5-meter subplot of each treatment plot (Table 2). Percent cover estimation was made by a visual examination of the subplot. Accuracy of estimations was tested by laying wire flags across a randomly chosen subplot at intervals of 0.1-meters to create a 5- x 5-grid, each square representing 4 percent of the total area. The linear distance of the farthest plant from the previous delineated boundary between the existing population and the treatment plot was measured in meters. Upon study termination, all plants in all treatment plots, regardless of size, were counted.

Table 2: Percent cover by cover class and corresponding midpoint used for percent cover classification for each subplot for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee.

<b>Cover Class</b>	<b>Percent Cover</b>	<b>Midpoint</b>
1	0	0
2	1 to 5	3
3	11 to 25	18
4	26 to 50	38
5	51 to 75	63
6	76 to 95	86
7	96 to 100	98

To determine whether light and moisture levels on the study sites were similar, photosynthetically active radiation (PAR) and soil moisture were measured. An AccuPAR LP-8 Ceptometer was used to measure PAR. Measurements were taken simultaneously at each site and in open fields adjacent to the sites. A percentage was then calculated (hereby referred to as “percent total PAR”). Three soil samples were taken randomly from each site, weighed, oven-dried at 70° Celsius for 48 hours, then weighed again to determine percent soil moisture. Soil samples were only taken one time on June 23<sup>rd</sup>, 2010 between 2 and 3 o’clock P.M. to quantify the characteristics of each site for comparison.

### **Greenhouse Methods**

In November of 2009, *M. vimineum* seeds were collected from a large monoculture along the wood’s edge at the University of Tennessee Forest Resources and Research Center in Oak Ridge, Tennessee. The population was approximately 5-meters long by 3-meters wide with an average plant height of approximately 1-meter. Entire plants were collected, placed in large plastic bags, and then transported to the lab. The plants were placed on a table to air dry for 72 hours. After covering a large table with a white sheet, the plants were shaken vigorously over the table to detach seeds. Large debris, such as plant stems and leaves, were removed by hand. Small debris, such as leaf and stem fragments, were removed using a sifter. The seeds were then placed into a plastic bag and stored dry, indoors, at room temperature for 90 days in order to break dormancy. Previous studies by Judge and Neal (2004) and Judge (2005) have reported germination rates exceeding 90 percent after 90 days of storage regardless of storage conditions or photoperiod.

On March 15<sup>th</sup> of 2010, a greenhouse study was initiated (Figure 4). Using ½-inch PVC (polyvinyl chloride) pipe, three 0.66- x 1.22- x 0.30-meter boxes were constructed. Three 1.68- x 2-meter custom fabricated pieces of black, woven commercial shade cloth were fastened to the boxes,





Figure 4: Photograph of second greenhouse experiment for the *M. vimineum* greenhouse study at the University of Tennessee in Knoxville.

covering all sides excluding the bottom. Each individual shade cloth provided a different amount of shade. Sixteen 25- x 50-centimeter plastic trays with drainage holes, four per treatment, were then filled with 6.35 centimeters of Premier Pro-Mix Bx General Purpose Growing Media and watered until saturated. One level tablespoon of *M. vimineum* seed was evenly distributed over each of the 16 trays by scooping them out of the plastic bag and gently shaking the tablespoon over the tray allowing the seeds to fall in a diffuse manner. The seeds in a representative tablespoon were individually counted, yielding 2,780 seeds. Based on the volume of 100 seeds, a given tablespoon could have 2,600 – 3,000 seeds. This amount of seed was used to create a seed density similar to that exhibited by dense natural populations where seed density ranges from 16,000 to 50,000 seeds/m<sup>2</sup> (Williams 1998). Seed densities on each tray were approximately 20,000 to 23,100 seeds/m<sup>2</sup>.

In order to maintain consistent soil moisture across all treatments, soil moisture was measured weekly. This was done to eliminate any effects water might have had on growth. Soil moisture levels across all treatments were kept relatively high ( $\geq 74$  percent) so moisture was not a limited resource. No soil moisture measurements were taken on the same day water was applied. The actual amount of water applied to each treatment varied, as did watering frequency. The full light treatment required more water at a higher frequency while the 20 percent light treatment required notably less. This was due to the differences in the amount of light received by those two treatments. Higher amounts of light inevitably result in higher rates of soil moisture depletion. Soil moisture was monitored 4-5 times per week to determine if watering was necessary.

Fertilizer was applied equally to all treatments after seeds germinated. Each treatment received 5 tablespoons of 20 percent nitrogen 20 percent phosphorus and 20 percent potassium (20-20-20) all purpose fertilizer dissolved in two gallons of water. Fertilization was done to help maintain plant health; however, plants began turning yellow in all treatments about 2 weeks after germination. Fertilizer was then applied again using the same application rate and method. Plant condition

continued to deteriorate as the study progressed, eventually resulting in some notable damage on some plants by study termination on April 14<sup>th</sup>. The cause for this condition was determined to be toxicity from excessive fertilizer application. The study was then repeated beginning on May 15<sup>th</sup> using identical methods, however, fertilizer application was reduced to 3 tablespoons of 20-20-20 all purpose fertilizer dissolved in 5 gallons of water. Fertilizer was applied equally to all treatments after germination. All plants across all treatments maintained a healthy condition throughout the study.

The following measurements described herein were taken weekly for four weeks beginning on March 26<sup>th</sup> on the first study and May 25<sup>th</sup> on the second study, one week after seed germination. Plant height was measured with a ruler. Each replicate exhibited a height fairly consistent with the other replicates in that particular treatment (Figure 5). Therefore, average height was determined based on the general height of the canopy of *M. vimineum* in a given treatment. Soil moisture was measured by taking a random soil sample from 3 of the 4 replicates per treatment. The sample was then weighed, oven dried at 70° Celsius for 48 hours, then weighed again to determine percent soil moisture. Using an open FluorCam FC 800-O/1010 chlorophyll fluorescence meter the following variables were measured to help determine photosynthetic efficiency under the different lighting conditions:

Minimum fluorescence emission – ( $F_0$ )

Maximum fluorescence emission – ( $F_M$ )

Variable fluorescence emission – ( $F_V$ ) which is equal to ( $F_M - F_0$ )

Non-photochemical quenching – ( $NPQ$ )

Photochemical quenching – ( $Qp$ )

Maximum quantum yield of Photosystem II photochemistry – ( $QY_{max}$ ) which is equal to ( $F_V/F_M$ )



Figure 5: Second greenhouse study, 100 percent light treatment height measurement for the *M. vimineum* greenhouse study at the University of Tennessee in Knoxville.

Measurements of chlorophyll fluorescence were performed 2 times, once ten days into the study and again at thirty days into the study. The measurements were taken by removing three random samples from each treatment. One sample consisted of the soil and plants on an approximately 1-inch diameter area. Samples were removed from 3 of the 4 trays in a given treatment by neatly cutting the soil using a sharp knife taking care not to damage any plants. The samples were dark adapted (placed in darkness) for one hour in order to shut down Photosystem II before measurements were taken. All samples were discarded after the measurements were taken. Because an equal proportion of each treatment was removed for measuring, aboveground biomass was also reduced equally among treatments causing no influence on aboveground biomass measurements.

All aboveground biomass was harvested upon study completion and placed into paper bags by treatment by replicate (i.e., 100 percent light, replicate 1 – 100 percent light replicate 2 – etc.) for weighing. Harvesting was done by cutting plant stems level with the soil. Plants were then oven dried at 70° Celsius for 72 hours and then weighed again to determine the dry weight of aboveground biomass. Photographs were taken throughout the study for the purpose of illustration.

## STATISTICAL ANALYSIS

### Field Study

To determine if there were statistically significant differences among the three study sites, the percent soil moisture and percent total PAR of each study site was compared using a one-way analysis of variance (ANOVA). Because there were no statistical differences in percent total PAR ( $p=0.3498$ ) and percent soil moisture ( $p=0.1849$ ) between sites, sites were considered similar and data from all study sites were combined for statistical analysis (Figures 6 and 7). A one way ANOVA was conducted to test for significant differences among percent cover and treatments for each month, April through September. To better quantify percent cover, the midpoint of each cover category (hereby referred to as “percent cover”) was used for analysis rather than the cover category itself. Analysis was conducted on the treatments as a whole (2 meters) and on the treatments divided into ½ -meter subplots (i.e. percent cover of Control, subplot 1 in August – percent cover of Control, subplot 2 in August etc. compared to percent cover Stir treatment, subplot 1 in August – percent cover Stir treatment, subplot 2 in August and so forth for all treatments and all subplots by month). A one-way ANOVA was conducted on percent cover for all treatments by subplot. To determine if significant differences existed among distance to the farthest plant and treatments, a one-way ANOVA was conducted for each month. A one-way ANOVA was also conducted on the number of plants per treatment by subplot. To determine if there were significant increases in percent cover and distance to the farthest plant through time, a one-way ANOVA was conducted based on each month, month being the x-axis and percent cover or distance to the farthest plant being the y-axis. If a significant difference was found in any scenario, a Tukey’s HSD post-hoc test was conducted to determine which groups differed.

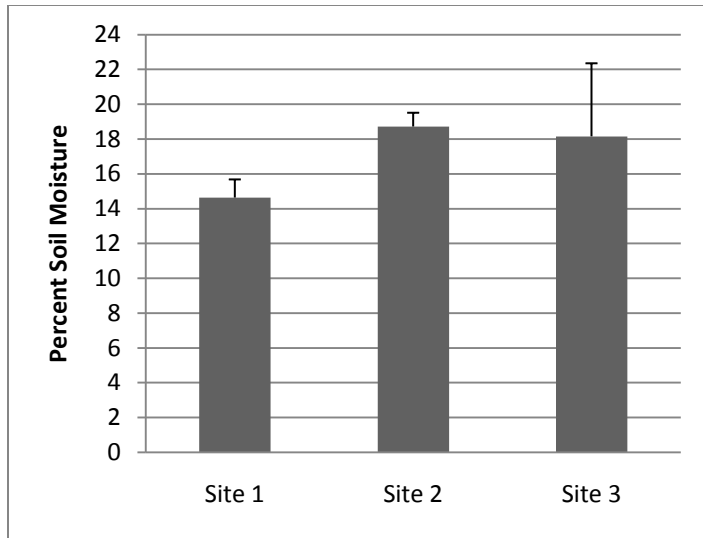


Figure 6: Mean percent soil moisture by site for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Error bars represent one standard deviation.

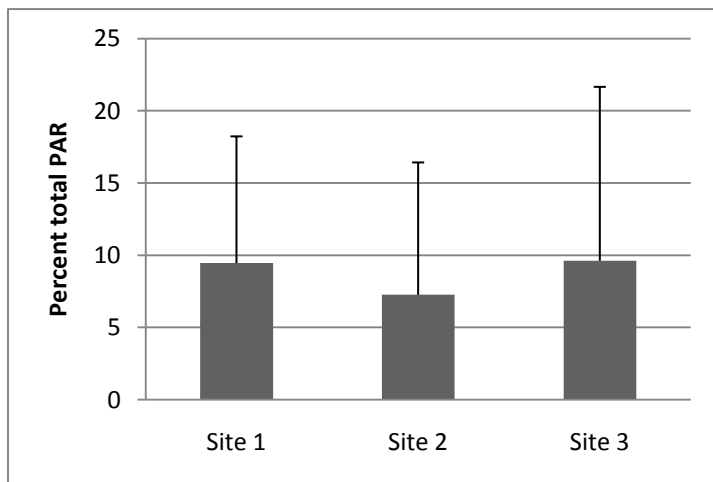


Figure 7: Mean percent total PAR by site for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Error bars represent one standard deviation.

## Greenhouse Study

Weekly average height measurements were used to calculate plant growth rate for all treatments. No other analysis was performed on height measurements. To determine if there were any significant differences in dry aboveground biomass (hereby referred to as “aboveground biomass) between treatments, a one-way ANOVA was conducted. If significant differences were found, a Tukey’s post-hoc HSD test was used to determine which groups differed. The variables  $F_o$ ,  $F_M$ ,  $F_V$ ,  $NPQ$ ,  $Qp$ , and  $QY_{max}$ , were analyzed using an ANOVA and means separated with Fisher’s protected LSD test at the 0.05 significance level to determine photosynthetic efficiency. If no significant interactions were found between studies by the ANOVA, the variables from both studies were pooled for analysis.



## **RESULTS and DISCUSSION**

### **Results – Field Study**

While significant differences did not exist between the percent cover of treatments in any specific month, there was a significant difference between the mean percent cover of the Control and the Removal treatment when all percent covers from all months were combined and analyzed (Tables 3 and 4). Percent cover means in the Stir and Removal treatments were consistently higher than in the Control throughout all months (Figure 8). Further analysis of percent cover for each treatment by month and by subplot yielded no statistically significant differences for a specific subplot in a specific month (Table 5). There were significant differences among percent cover regardless of month in each treatment with Subplot 1 having a greater average than Subplots 2, 3, and 4 (Tables 6, 7, and 8). Differences in mean percent cover among subplots were greatest in the Removal treatment. Average distance of the farthest plant was similar in each month across all treatments with no significant differences present (Figure 9). Stirring the litter yielded the highest overall (all months combined) mean distance to the farthest plant. There were no significant differences in number of plants per  $\frac{1}{2}$  square meter subplot between treatments (Table 9).

### **Discussion – Field Study**

#### **Study Sites**

While percent soil moisture was similar across all study sites (Figure 6), no soil tests were conducted to determine other variables such as the pH level and soil nutrient content. The differences between sites are assumed not to be large enough to have a notable impact on the variables being measured. Percent soil moisture measurements for all study sites had relatively

Table 3: Mean percent cover for each treatment by month with corresponding p-value for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 1 = Control, Treatment 2 = Stirring of litter, and Treatment 3 = Removal of litter.

Month	Treatment			p-value
	1	2	3	
April	3.33	4.08	4.81	0.6417
May	3.92	6.56	6.33	0.4795
June	4.47	7.36	7.97	0.4378
July	5.03	8.14	10.67	0.2836
August	8.36	10	12.78	0.614
September	9	11.86	14.28	0.5887

Table 4: Mean percent cover for each treatment across all months for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 1 = Control, Treatment 2 = Stirring of litter, and Treatment 3 = Removal of litter. Treatments not connected by the same letter are significantly different at the 0.05 level.

Treatment			Mean
3	A		9.47
2	A	B	8.00
1		B	5.69

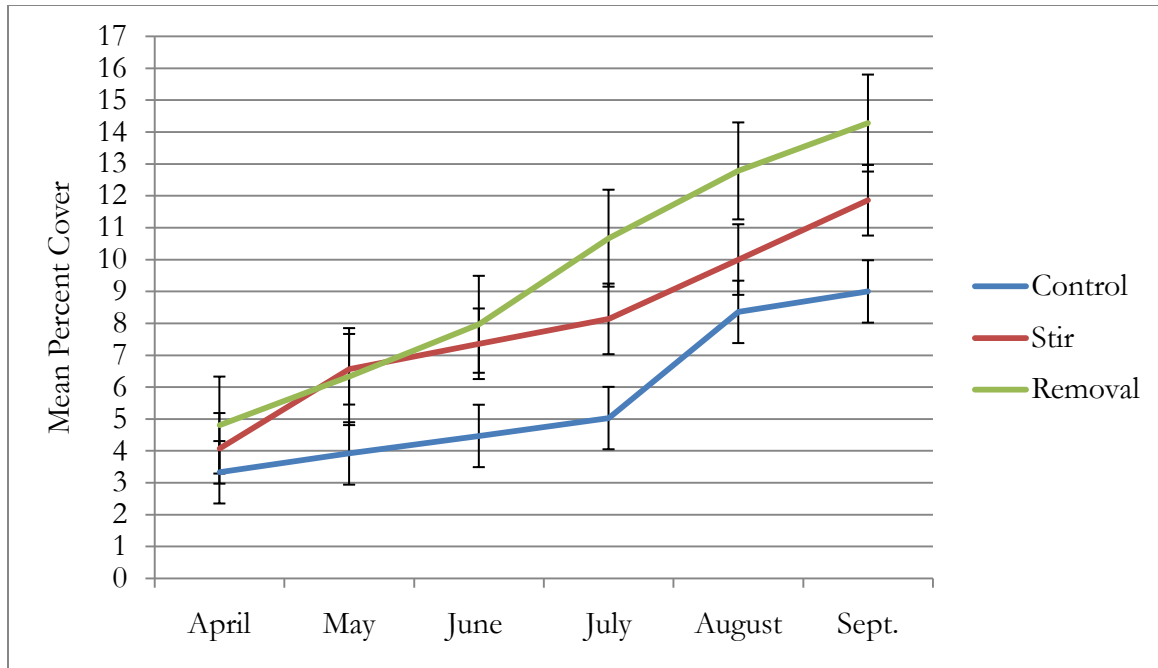


Figure 8: Mean percent cover for each treatment (Control, Stir, and Removal) by month for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Error bars represent mean percent cover  $\pm 1$  standard error.

Table 5: Mean percent cover for each treatment by month by subplot with corresponding p-value for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 1 = Control, Treatment 2 = Stirring of litter, and Treatment 3 = Removal of litter. Subplot numbering is based on distance from the existing population with Subplot 1 being closest and Subplot 4 being farthest away. Subplots represent 1/2-meter intervals.

<b>SUBPLOT 1</b>				
	<b>Treatment</b>			
<b>Month</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>p-value</b>
April	7.67	13	13.56	0.3464
May	8	20.22	18	0.2487
June	10.22	22.78	23.56	0.2824
July	12.44	25.56	28.33	0.3294
August	23.56	31.33	38.56	0.5395
September	26.11	37.44	43.56	0.5095
<b>SUBPLOT 2</b>				
	<b>Treatment</b>			
<b>Month</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>p-value</b>
April	2.67	1	3.67	0.2588
May	2.67	2.33	4.33	0.3711
June	2.67	2.33	4.33	0.3711
July	2.67	3	8.22	0.201
August	2.67	4.67	6.56	0.5489
September	2.67	6.33	6.56	0.5079
<b>SUBPLOT 3</b>				
	<b>Treatment</b>			
<b>Month</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>p-value</b>
April	2	1	1.33	0.3827
May	2	1.67	2	0.8676
June	2	2	2.33	0.853
July	2	2	4.33	0.2341
August	2	2	4.33	0.2341
September	1.67	2	5.67	0.1115
<b>SUBPLOT 4</b>				
	<b>Treatment</b>			
<b>Month</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>p-value</b>
April	1	1.33	0.67	0.6358
May	3	2	1	0.5036
June	3	2.33	1.67	0.7322
July	3	2	1.67	0.7168
August	5.22	2	1.67	0.5264
September	5.56	1.67	1.33	0.3978

Table 6: Difference in mean percent cover for Treatment 1 by subplot for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 1 = Control. Subplot numbering is based on distance from the existing population with Subplot 1 being closest and Subplot 4 being farthest away. Subplots represent 1/2-meter intervals (\* signifies  $p \leq 0.05$ ).

Treatment 1			
Subplot	Subplot	Difference	p-Value
1	2	12.00	<.0001*
1	3	12.72	<.0001*
1	4	11.20	<.0001*
2	3	0.72	0.9839
2	4	-0.80	0.9786
3	4	-1.52	0.8729

Table 7: Difference in mean percent cover for Treatment 2 by subplot for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 2 = Stirring of litter. Subplot numbering is based on distance from the existing population with Subplot 1 being closest and Subplot 4 being farthest away. Subplots represent 1/2-meter intervals (\* signifies  $p \leq 0.05$ ).

Treatment 2			
Subplot	Subplot	Difference	p-Value
1	2	21.78	0.0000*
1	3	23.28	0.0000*
1	4	23.17	0.0000*
2	3	1.50	0.9410
2	4	1.39	0.9523
3	4	-0.11	1.0000

Table 8: Difference in mean percent cover for Treatment 3 by subplot for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 3 = Removal of litter. Subplot numbering is based on distance from the existing population with Subplot 1 being closest and Subplot 4 being farthest away. Subplots represent 1/2-meter intervals (\* signifies  $p \leq 0.05$ ).

Treatment 3			
Subplot	Subplot	Difference	p-Value
1	2	21.98	0.0000*
1	3	24.26	0.0000*
1	4	26.26	0.0000*
2	3	2.28	0.7865
2	4	4.28	0.2983
3	4	2.00	0.8448

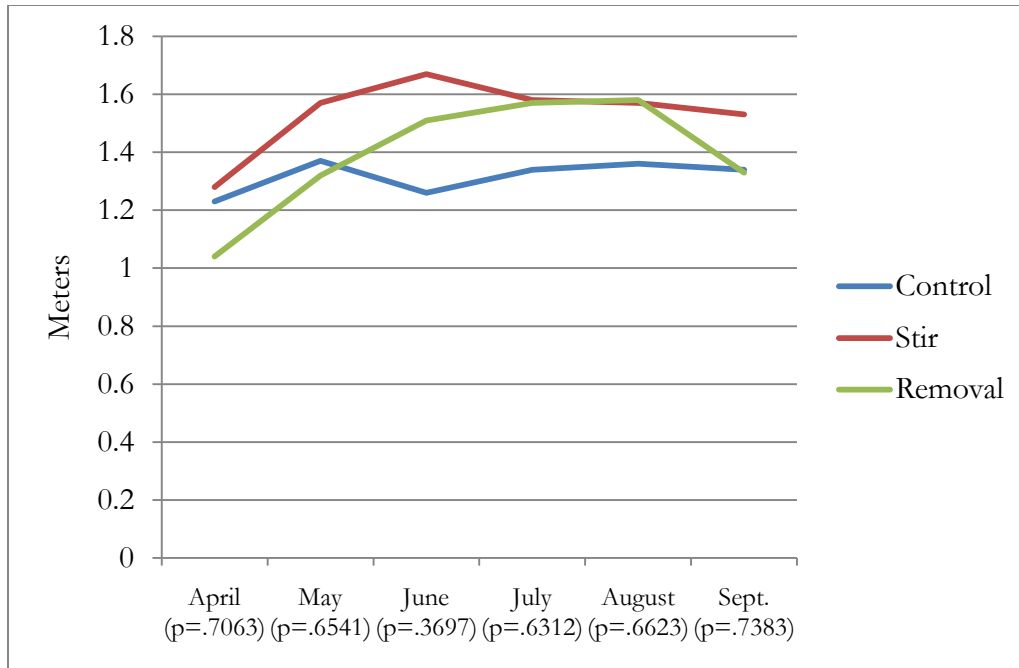


Figure 9: Mean distance to the farthest plant in meters for each treatment (Control, Stir, and Removal) by month with corresponding p-value for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee.

Table 9: Mean number of plants per 1/2-square meter subplot for each treatment and corresponding p-value for the *M. vimineum* study at the FRREC in Oak Ridge, Tennessee. Treatment 1 = Control, Treatment 2 = Stirring of litter, and Treatment 3 = Removal of litter.

Subplot	Treatment			p-value
	1	2	3	
1	28.2	49.2	62.0	0.4005
2	1.9	3.8	9.8	0.2704
3	1.4	2.7	4.9	0.2954
4	4.4	3.2	3.0	0.8892

low variability (Figure 6), while percent total PAR measurements exhibited high variability (Figure 7). Measurements ranged from 1.30 percent total PAR to 54.71 percent for a single measurement while the mean of all measurements across all sites was 8.78 percent total PAR. Shifts in canopy due to wind, time of day, and percent cloud cover can result in a marked increase or decrease in light in these environments. While the overall mean percent PAR was relatively low, all study sites most likely experienced a wide range of light levels throughout the course of a given day. However, based on the PAR readings taken, all sites had about the same variability in light levels in a given day (Figure 7).

## Spread

Pre-growing season leaf litter disturbance, whether complete litter removal or a vigorous stirring of the litter, did not increase the percent cover of *M. vimineum*. All treatments exhibited similar mean percent cover for all subplots suggesting that stirring or removing the leaf litter is no more effective at causing a *M. vimineum* infestation than when litter is left undisturbed. The same holds true for spread rate. Mean distance to the farthest plant was similar for all treatments, peaking between May (Control) and August (Removal treatment). In this study, the presence or absence of a single plant had the ability to alter this measurement. Mean number of plants per  $\frac{1}{2}$  square meter subplot was also similar across all treatments (Table 9). These results suggest that the type of disturbance implemented for this study, or the manner in which it was implemented, did not successfully transport the seed that was present outside the population boundary to a new location farther from the boundary. Litter removal had an influence on *M. vimineum* seed movement similar to that of litter stirring indicating that although the litter was removed, a negligible amount of seed was transported with it. Hand removal of litter may have allowed seeds to simply fall back into place

or avoid movement altogether. The manner in which the litter was stirred may have had similar effects, merely causing a slight amount of seed movement.

The timing of study implementation may have also influenced the degree to which seed was moved. In previous studies by Oswalt and Oswalt (2007) and Marshall and Buckley (2008) there were significant increases in percent cover and spread rate of *M. vimineum* when leaf litter was disturbed, however, the disturbances (treatments) were implemented in December and October, respectively. This study was implemented in early March. In previous studies, seeds had more time to become settled and secured in the seed bank following disturbance. Fall and winter disturbances may have a higher chance of causing an invasion of *M. vimineum* than disturbances in late winter/early spring.

The light level among study sites could impact spread of *M. vimineum*. However, PAR levels were relatively low (7 to 10 percent total PAR – Figure 7). As previous studies have shown (Oswalt and Oswalt 2007; Marshall and Buckley 2008), the spread rate would be more rapid in areas influenced by disturbance in which light is not a limiting factor because higher light levels typically result in larger crops of seed for *M. vimineum* (Williams 1998). Because all existing populations used for this study were located in areas where the canopy had been disturbed (particularly on or near forest roads), light levels in the population centers may have been higher than those at the edge of the population. Although light measurements were not taken within the center of the existing populations at any study site, light levels on the edges of the populations were most likely similar in all treatments at all sites. Thus, seed production by plants near the population boundary was possibly lower than those closer to the center due to lower light levels. A lower amount of seed production near the population boundary would result in a lower potential for spread.

The reason for a definitive patch edge when study sites were selected may have been light related. Cole and Weltzin (2005) observed that *M. vimineum* was not present beneath *Asimina triloba*



and determined the reason for this pattern to be low light levels. In this study all treatment plots radiated outward from the existing population into areas that had lower light levels due to increased canopy cover. Thus, spread into the treatment plots may have been limited by light whereas in other studies, light was not a limiting factor (Oswalt and Oswalt 2007; Marshall and Buckley 2008).

Mean percent cover for all treatments and all months was significantly higher in Subplot 1 than in Subplots 2 through 4 further indicating similarity among treatments, as there were no significant differences when Subplot 1 was compared by treatment (Tables 6, 7, and 8). The lack of difference in percent cover across treatments suggests that the rate of spread is limited to about 1/2-meter per growing season.

## **Results – Greenhouse Study**

Height data is only presented for the second greenhouse study as all heights were notably similar in the first study due to toxicity from excess nutrients (data not presented). All reference to cumulative height hereby regards the second greenhouse study only. One week after germination, plants receiving 100 percent light had the greatest mean cumulative height, however, at week two, mean cumulative height was greatest in plants grown under 70 and 45 percent light reaching a maximum of 28 centimeters after four weeks of growth (Figure 10). Seventy and 45 percent light treatments had nearly identical height measurements throughout the study while the 100 percent light treatment was notably shorter.

Significant differences in mean aboveground biomass were present in both greenhouse studies (Table 10). Aboveground biomass was substantially lower in the first greenhouse study than in the second greenhouse study. In both studies, mean aboveground biomass decreased as light availability decreased; however in the second greenhouse study the 100 and 70 percent light treatments had nearly identical means and mean aboveground biomass decreased significantly from

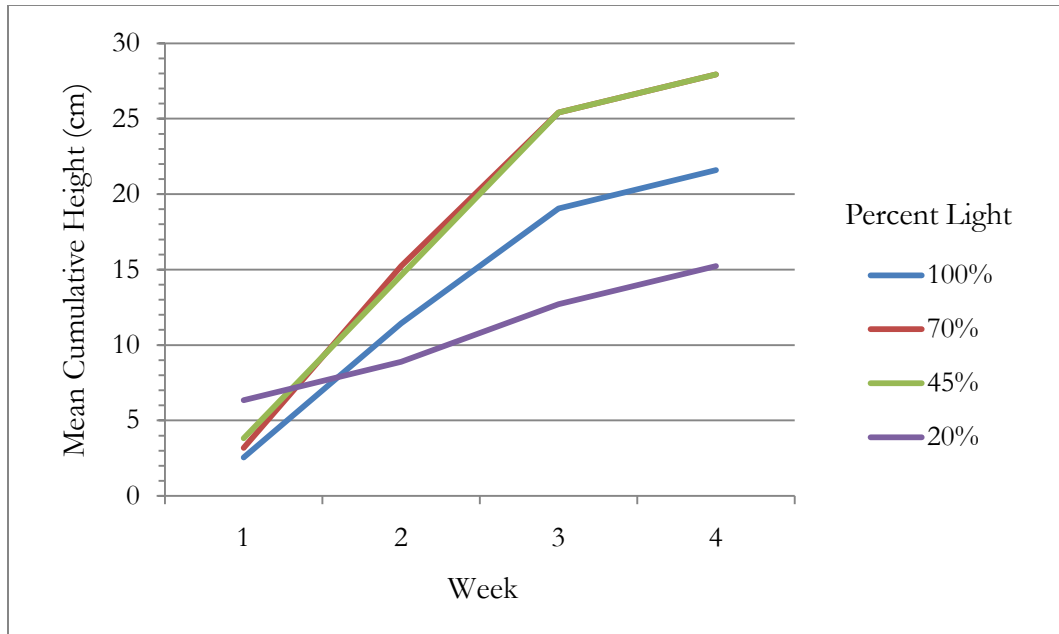


Figure 10: Mean cumulative growth in height (centimeters) of each treatment for the second greenhouse study for 4 weeks for the *M. vimineum* greenhouse study at the University of Tennessee in Knoxville.

Table 10: Mean aboveground biomass (grams) from both studies for each treatment from for the *M. vimineum* greenhouse study at the University of Tennessee in Knoxville. Treatments not connected by the same letter are significantly different ( $p < 0.0001$  for both studies).

Mean Aboveground Biomass (grams)								
First Greenhouse Study				Second Greenhouse Study				
Treatment			Mean	Treatment				Mean
100% Light	A		16.46	100% Light	A			31.43
70% Light		B	10.97	70% Light	A			31.35
45% Light		B	8.29	45% Light		B		21.68
20% Light		C	5.45	20% Light			C	7.03

70 percent light to 45 percent light and again from 45 percent light to 20 percent light.

There were no interactions between studies for any variables after 10 days so data for both studies was pooled. After 30 days, there were interactions between studies in  $F_o$ ,  $F_M$ , and  $QY_{max}$ , therefore, these variables were analyzed separately. After 10 days, differences were present in  $NPQ$  and  $QY_{max}$  (Table 11). Non-photochemical quenching and photosynthetic efficiency were highest when plants were grown under 100 percent light and lowest when grown under 45 and 70 percent light.  $F_o$ ,  $F_M$ ,  $F_V$ , and  $Qp$  differed after 30 days but  $NPQ$  and  $QY_{max}$  did not (Table 12). There were differences in  $F_o$  in the second greenhouse study after 30 days but not in the first study. On day 30 in the second greenhouse study, mean  $F_o$  for plants grown under 70 percent light was higher than that of plants grown under 20 percent light. No differences existed between mean  $F_M$  on day 30 in the first greenhouse study. Plants grown under 20 percent light in the second study had the lowest mean  $F_M$  while plants grown under 70 and 45 percent light had the highest.  $F_V$  was highest when plants were grown under 45 percent light and lowest when grown under 20 percent, after 30 days. Photochemical quenching at day 30 was lowest in the 45 percent light treatment and highest in the 100 percent light treatment.

## **Discussion – Greenhouse Study**

During the first greenhouse study, fertilizer was applied at equal rates across all treatments therefore the impact of toxicity due to excess nutrients should have been relatively equal for all plants. After 10 days, there were no variations in variables ( $F_o$ ,  $F_M$ , etc.) between treatments. After 30 days, the only variables to differ between studies were  $F_o$ ,  $F_M$ , and  $QY_{max}$ , further indicating the similarities between the studies. As would be expected, excess fertilizer impacted aboveground biomass accumulation in the first study causing it to be substantially reduced (Table 10); however, similar trends were observed as plants grown under 100 percent light had the highest aboveground

Table 11: Means of variables after 10 days for both studies combined for the *M. vimineum* greenhouse studies at the University of Tennessee in Knoxville. Both three-replication studies are presented together because no block by treatment interaction occurred.

	Day 10 Variables					
Treatment	$F_0$	$F_M$	$F_V$	$NPQ$	$Qp$	$QYmax$
<b>100 % Light</b>	269.00	1107.72	838.72	0.08	0.58	0.76
<b>70% Light</b>	302.27	1221.82	919.55	0.04	0.62	0.75
<b>45% light</b>	265.02	1046.03	781.01	0.04	0.58	0.74
<b>20% Light</b>	259.12	1050.31	791.19	0.05	0.59	0.75
<b>LSD<sub>0.05</sub></b>	49.62	203.38	154.31	0.03	2.27	0.0065

<sup>a</sup> Abbreviations: Minimum chlorophyll fluorescence ( $F_0$ ), maximum chlorophyll fluorescence ( $F_M$ ), variable chlorophyll fluorescence ( $F_V$ ), non-photochemical quenching ( $NPQ$ ), photochemical quenching ( $Qp$ ), and maximum quantum yield of Photosystem II photochemistry ( $QYmax$ ).

Table 12: Means of variables after 30 days for the *M. vimineum* greenhouse study at the University of Tennessee in Knoxville. Means for  $F_V$ ,  $NPQ$ , and  $Qp$  for both three-replication studies are presented together because no block by treatment interaction occurred in those variables. Test 1 = First greenhouse study, Test 2 = Second greenhouse study, and Both = Both studies combined.

	Day 30 Variables								
Treatment	$F_0$		$F_M$		$F_V$	$NPQ$	$Qp$	$QYmax$	
	Test 1	Test 2	Test 1	Test 2	Both	Both	Both	Test 1	Test 2
<b>100 % Light</b>	372.17	578.36	869.83	1452.43	723.50	0.23	0.68	0.55	0.60
<b>70% Light</b>	433.39	591.76	1057.94	1675.21	854.00	0.27	0.54	0.58	0.65
<b>45% Light</b>	877.13	494.78	1893.14	1537.05	1031.76	0.24	0.49	0.54	0.68
<b>20% Light</b>	472.14	389.58	991.01	1094.41	630.45	0.20	0.50	0.52	0.64
<b>LSD<sub>0.05</sub></b>	466.39	58.29	1053.95	272.78	200.54	0.19	0.09	0.12	0.04

<sup>a</sup> Abbreviations: Minimum chlorophyll fluorescence ( $F_0$ ), maximum chlorophyll fluorescence ( $F_M$ ), variable chlorophyll fluorescence ( $F_V$ ), non-photochemical quenching ( $NPQ$ ), photochemical quenching ( $Qp$ ), and maximum quantum yield of Photosystem II photochemistry ( $QYmax$ ).

biomass in both studies with biomass decreasing as available light decreased. Notable differences between greenhouse studies in aboveground biomass by treatment can be attributed to the toxicity that occurred in the first study.

Cumulative growth in height across all treatments indicates that *M. vimineum* is capable of growth in light conditions as low as 20 percent of full light for at least the first 4 weeks. Plants grown under 20 percent light were just over half the height of plants grown under 70 and 45 percent light suggesting light has a notable influence on the maximum growth for *M. vimineum*.

In the second greenhouse study, mean aboveground biomass accumulation under 100 and 70 percent light was nearly identical indicating a 30 percent reduction in sunlight had no influence on *M. vimineum* aboveground biomass accumulation. A significant decrease in aboveground biomass accumulation occurred when only 45 percent of full light was available. Further reduction of the amount of light to 20 percent of full light yielded another significant decrease. These data indicate that optimal aboveground biomass accumulation for *M. vimineum* can occur in light levels ranging from at least 70 percent of full light to 100 percent. *M. vimineum* also is capable of growth and survival at 20 percent of full light for at least 30 days.

Plants grown under 70 and 45 percent light had near identical mean heights at each 1-week interval, however significant differences in aboveground biomass accumulation between these treatments suggests that plants in the 45 percent light treatment had less biomass per plant than plants grown under 70 percent light. The same applies when comparing the 100 percent light treatment to the 70 percent light treatment. Although mean cumulative height was lower when plants were grown in full light, aboveground biomass accumulation was relatively equal to that of plants grown under 70 percent light. Though not quantified when the plants were harvested, the rigidity of the plants tended to decrease as the availability of light decreased. As larger plants (height and biomass) typically produce more seeds (Williams 1998), *M. vimineum* growing in areas receiving

around 70 percent of full light may have larger seed crops than those growing in full light. Thus, the potential for spread may be higher.

Differences in mean photosynthetic efficiency between plants grown under 100 and 45 percent light after 10 days, and no differences after 30 days, suggests that while photosynthetic efficiency may be reduced when light levels are at 45 percent for the first 10 days, after 30 days, the amount of light being received makes little difference for photosynthetic efficiency. Thus, *M. vimineum* is capable of maintaining a consistent photosynthetic efficiency throughout a wide range of light conditions (20 to 100 percent). The same is true when examining non-photochemical quenching (*NPQ*). The 100 percent light treatment differed from the 45 and 70 percent light treatments in *NPQ* after 10 days but after 30 days, all treatments were similar. This suggests that in the first 10 days, varying light levels influence non-photochemical quenching (*NPQ*) of the plant, but once 30 days have passed, *NPQ* is no longer influenced. Similarities in both photosynthetic efficiency and *NPQ* across all treatments after 30 days suggests *M. vimineum* is adapted to both high and low light levels consistent with the findings of previous studies (Winter et al. 1982; Horton and Neufeld 1998).

Most  $C_4$  plants thrive in high light environments while  $C_3$  plants are more adapted to low light levels. Though a  $C_4$  plant, *M. vimineum* is adapted to low light levels. Thus, *M. vimineum* can successfully compete with native  $C_3$  plants in shaded areas. Winter et al. (1982) determined that in light levels of 25 and 50 percent there were no differences in the ability of *M. vimineum* to respond to sun-flecks and even without sun-flecks, the plant could maintain positive carbon gain. As evidenced by aboveground biomass and photosynthetic efficiency measurements from greenhouse studies presented in this study, *M. vimineum* is a  $C_4$  species with some shade tolerance.

## SUMMARY/MANAGEMENT IMPLICATIONS

The research presented here was conducted to determine if pre-growing season leaf litter disturbance had an effect on the overall spread of *M. vimineum* (Field study) and to examine the influence of light on aboveground biomass accumulation and photosynthetic efficiency of *M. vimineum* (Greenhouse studies). In the Field study, no significant differences in spread between treatments were indicated in any of the data collected. Although these results suggest that pre-growing season leaf litter disturbance does not significantly impact *M. vimineum* spread, this study does not agree with similar previous studies by Oswalt and Oswalt 2007 and Marshall and Buckley 2008. The disturbances in those studies were implemented in October and December rather than early March as in this study suggesting disturbance has a higher impact on spread and percent cover when it occurs in the fall or mid winter. When examining data collected in the greenhouse studies, light possibly played a role in the lack of spread in the field study as mean percent PAR at all study sites was below 10 percent and greenhouse studies indicated a significant decrease in aboveground biomass accumulation at 45 percent of full light and below. Previous studies (Winter et. al 1982; Horton and Neufeld 1998; Cole and Weltzin 2005) have determined light is the most limiting factor on growth of *M. vimineum*. Less growth would result in less seed production therefore reducing the potential for spread regardless of disturbance. The physical movement of seed caused by disturbance may cause *M. vimineum* to spread, however, if light levels are insufficient, substantial growth and seed production may not occur or minimally occur. While *M. vimineum* is capable of surviving and reproducing in low light environments, it may take several growing seasons for the population to be considered invasive. This research suggests that in order for *M. vimineum* to spread rapidly, there must be a disturbance to physically move the seed away from the parent plant and light levels sufficient enough to allow for notable growth in height and aboveground biomass accumulation. Thus, in undisturbed closed canopy forests, *M. vimineum* is typically just another herbaceous plant

growing in the understory while along roadways, streams, and other areas with partial light, it becomes more competitive with native vegetation.

When considering the management implications of this research, *M. vimineum* may compete with native vegetation in areas with 45 to 100 percent of full light. Thus, populations that have the highest potential for spread would be those growing in areas of partial to full light. Preventing seed movement in these areas by minimizing disturbance, particularly during the fall and mid-winter months, should lessen the chances of rapid spread. However, even left undisturbed, *M. vimineum* is still capable of consistently spreading each growing season albeit in a much slower progression.



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## VITA

John Andrew Hull was born in 1985, in Bristol, Tennessee where he grew up with his parents and three brothers. His interest in forestry was spurred by the long hours he spent working outdoors with his father in Bristol and his experiences working with his grandfather on his grandparent's farm, in South Carolina. He graduated from Tennessee High School in May, 2004 and began attending the University of Tennessee in Knoxville the following fall. In 2008, he received his Bachelors in Forestry and immediately began pursuing his Master's in Forestry under Dr. Wayne K. Clatterbuck.

While in college he worked for 3 summers as a Biological Science Technician on the North Zone of the Cherokee National Forest conducting botanical surveys and assisting in other biological work. He was then offered a SCEP (Student Career Experience Program) position on the Daniel Boone National Forest in Kentucky where he worked intermittently while earning his Master's. Upon graduation in 2010, with a Master's in Forestry, he acquired full time employment with the Forest Service where he intends to work until retirement.