EVALUATION OF LANDOWNER ACCESSIBLE CONTROL METHODS FOR JAPANESE STILTGRASS (*MICROSTEGIUM VIMINEUM*)

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ABSTRACT

Japanese stiltgrass (*Microstegium vimineum*) is an invasive, annual C_4 grass that has spread extensively throughout riparian areas and upland forests in the United States. *Microstegium vimineum* can establish a dense monoculture in the understory of forest stands and inhibit the regeneration of native herbaceous and woody plant species. Controlling the occurrence and spread of this species is of great concern to both land managers and private landowners, but there are few direct comparisons of the effectiveness or application timing of different treatments for controlling *M. vimineum*. To fill this knowledge gap, a randomized complete block design was established in the Piedmont region of North Carolina to compare four potential control treatments (glyphosate, mechanical removal, manual removal, and vinegar) and three application timings (spring, summer, and fall). Prior to treatment and 6 weeks post-treatment, plots were measured for *M. vimineum* percent coverage and species richness, using a method of ocular estimation and Shannon's index, respectively. Results showed that 6 weeks post application, glyphosate was highly effective in removing *M. vimineum* regardless of timing (95-100 percent reduction) and vinegar was highly effective in the summer and fall applications (90-100 percent reduction). Species richness was higher in all treatment plots as opposed to control plots. These results provide preliminary evidence of the effectiveness of several common treatments on controlling *M. vimineum* that are accessible to landowners and have implications for plant diversity.

INTRODUCTION

Globalization has increased the spread of invasive species and is of particular concern in the Southeastern United States. One invasive plant species is Japanese stiltgrass (*Microstegium vimineum* [Trin.] A. Camus), an annual, C_4 grass physiologically suited for dry, open, sunlit conditions (Flory 2010, Hall and others 2014, Judge and others 2005). Native to Southeast Asia, *M. vimineum* was unintentionally introduced to the United States in Tennessee during the early 1900s (Barkworth and others 2003, Fryer 2011, Hall and others 2014, Judge and others 2005) when it was used as packaging material for Chinese porcelain (Culpepper and others 2018). Currently, *M. vimineum* is found in 27 states and Puerto Rico; however, it is most noted as a serious problem in the Eastern United States (EPPO 2016, North and Torzilli 2017). Unlike many C_4 grasses, this species has high habitat plasticity and can be found in a variety of low light, mesic conditions with moderate soil moisture and in soils with varying nutrient levels across upland and riparian areas (Hall and others 2014, Judge and others 2005).

Microstegium vimineum can produce up to 1,000 viable seeds during the growing season, and seeds can remain viable for 2 to 5 years in the soil seedbank (Gibson and others 2002, Redwood and others 2018). Seeds are easily disseminated by a variety of vectors (e.g., vehicles, clothing, animal fur) (Manee and others 2015), including natural and anthropogenic disturbances (Hall and others 2014, Ward and Mervosh 2012). One example of seed dispersal through natural disturbance is flooding, because seeds are light-weight, buoyant, and therefore easily dispersed by water (Flory 2010, Nees 2016). Anthropogenic methods of transport include the transport of soil containing *M. vimineum* seeds (Christen and Matlack 2009, Rauschert and others 2010), timber harvesting using equipment that has worked in prior invaded areas and not been cleaned (Nees 2016), and attachment to human clothing and animal fur (EPPO 2016).

Microstegium vimineum has a competitive advantage among some native vegetation due to its ability to grow in a variety of soil and light conditions (Cutway 2017). *Microstegium vimineum* can occur in dry, sunlit locations such as open areas, blowdowns, hillsides, and manicured lawns, in addition to invading more heavily shaded areas like closed canopy forests (Flory 2010). Even at light levels as low as 18 percent, *M. vimineum* experiences little reduction in growth and often outcompetes and displaces other understory vegetation (Judge and others 2005, Ward and Mervosh 2012).

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Because *M. vimineum* is widely dispersed and can establish in mesic conditions, this species is often found along roadsides and riparian areas (Cole and Weltzin 2004, Hall and others 2014, Manee and others 2015, North and Torzilli 2017).

Once established, *M. vimineum* forms dense monocultures that slowly decompose at the end of the growing season, creating a mat of dense plant material inhibiting the regeneration of native species (Hall and others 2014, Ward and Mervosh 2012). Even after a disturbance, *M. vimineum*'s direct competition for resources and hypothesized allelopathy lead to decreased regeneration of native herbaceous plants and trees (EPPO 2016, North and Torzilli 2017, Pisula and Mieners 2010, Ward and Mervosh 2012). Pisula and Mieners (2010) found *M. vimineum* has an ability to inhibit germination through allelopathy of coexisting species within an ecosystem.

The ability of *M. vimineum* to outcompete native plant species and alter conditions conducive to native vegetation is concerning (Culpepper and others 2018, North and Torzilli 2017). Reductions in native plant diversity reduce habitat quality for wildlife and pollinators (Ward and Mervosh 2012). Frey and Schmit (2015) found that white-tailed deer (*Odocoileus virginianus*) avoid eating *M. vimineum*, as do other foragers. In addition, forests infested with *M. vimineum* can reduce aesthetic appeal and lead to decreased utilization of public parks and nature preserves (Ward and Mervosh 2012).

Methods of *M. vimineum* control include prescribed fire, application of pre- and/or post-emergent herbicides, hand pulling, and mowing. Prescribed burning can increase in *M. vimineum* growth post-fire (Wagner and Fraterrigo 2015, Ward and Mervosh 2012). Pre-emergent herbicides have been shown to have variable effects on *M. vimineum* emergence and reproduction. For example, Judge and others (2015) found that pre-emergent herbicides reduced *M. vimineum* biomass by 87 percent for at least 8 weeks. Post-emergent herbicides, including those registered for aquatic use, can be highly effective control methods. Studies show up to a 97 percent loss of *M. vimineum* biomass 8 weeks after application (Flory 2010) and a 75 to 100 percent loss, 6 weeks after application (Hall and others 2014). Ward and Mervosh (2012) showed that some post-emergent herbicides were equally effective at reducing *M. vimineum* biomass when applied at low doses as when applied at higher doses; however, using a low dosage of herbicide may require additional applications within the same season. Applications of post-emergent herbicides must be repeated annually, or *M. vimineum* will return to levels equivalent to untreated stands (Frey and Schmitt 2015, Hall and others 2014, Judge and others 2005, Ward and Mervosh 2012). The effectiveness of hand-pulling largely depends on the size of the treatment area (EPPO 2016, Ward and Mervosh 2012) and the care taken to completely remove all stems (Flory 2010). For smaller sites where hand pulling would be feasible, hand pulling is considered the best and most cost-effective method of control when completed at the end of the growing season before seed release (EPPO 2016). Mowing and trimming was found to lead to an 82-percent loss of *M. vimineum* biomass during treatment years when performed during July or August (Ward and Mervosh 2012). However, a similar study by Flory and Lewis (2009) found that September or October mowing and trimming treatments were more effective. The difference in timing between the two studies could be due to the different phenological timing of the study locations. Ward and Mervosh (2012) completed their study in Connecticut, whereas Flory and Lewis (2009) completed their study in Indiana, which is more southern and would have an extended growing season relative to Connecticut. Mowing and trimming must be repeated in subsequent years, or it is hypothesized that *M. vimineum* biomass will return to levels of biomass present prior to the treatment application (Ward and Mervosh 2012). Thus, the seasonal timing of treatment applications is emerging as an important consideration of *M. vimineum* control.

With the threat that *M. vimineum* poses as an invasive species in forests evident, effective treatments for reducing *M. vimineum* cover need further evaluation among more diverse landscapes. Information is also needed regarding native vegetation responses to treatment applications including treatment types, rates, and timing. Prior studies regarding *M. vimineum* removal have investigated several control methods but lacked side-by-side comparisons to assess treatment effectiveness. Therefore, the objectives of this study are to: (1) compare effectiveness of four control methods for reducing *M. vimineum* cover among spring, summer, and fall application periods that are readily available and cost-effective to landowners in the Southeastern United States, and (2) evaluate species richness response to treatments and *M. vimineum* removal by comparing Shannon's Index of baseline and post-treatment vegetation communities.

MATERIALS AND METHODS

Study Site

The study occurred in Denver, NC (35° 31' 31.19" N, -81° 01' 28.20" W), in the western Piedmont, at ~ 235 m above sea level with minimal changes in topography (0-2 percent slope; Lincoln County GIS, Lincolnton, NC). Summer temperatures average 27.5 °C, and winter temperatures average 8.6 °C (U.S. Department of Commerce, NOAA 2021). Average annual precipitation is 119.6 cm (U.S. Department of Commerce, NOAA 2021). The soil type present throughout the study site is Chewlaca loam (Lincoln County GIS, Lincolnton, NC). Chewlaca loam soils are somewhat poorly drained with moderate permeability (U.S.

Department of Agriculture, NRCS 2021). The study site is located within a bottomland hardwood forest that is ~ 100 contiguous ha. The forest is comprised predominantly of yellow-poplar (*Liriodendron tuipifera* L.), American beech (*Fagus grandifolia* Ehrh.), red maple (*Acer rubrum* L.), and sweetgum (*Liquidambar styraciflua* L.). The midstory is largely absent, but where present, mostly comprised of *F. grandifolia*. The understory is predominantly *M. vimineum* and sparse Christmas fern (*Polystichum acrostichoides* Michx.). The site was chosen primarily because of the high-density coverage of *M. vimineum*.

Experimental Design

Within the study site, three study areas (~ 30-m^2) were designated as treatment blocks. Within each study area, four treatments (see below) were implemented as well as a control (*n* = 5 treatment plots per block) among individual treatment plots of 2 x 2 m (4-m^2) with a 1-m buffer in between. Basal area of overstory trees in each treatment block was determined within a 11.3-m fixed radius plot, and average canopy height of dominant and codominant trees measured using a clinometer (Ashton and Kelty 2018). Percent *M. vimineum* in each plot was estimated using an ocular estimation of cover in 10 percent interval categories (e.g., 0,10, 20, 30, …, 100 percent) (Ward and Mervosh 2012). All understory vegetation within the 4-m^2 treatment plot was identified. Understory species richness was determined from the number of unique species in each plot. Understory species diversity was determined from the Shannon-Wiener Diversity Index. For species richness and diversity, *M. vimineum* was not included in calculations. Study areas had similar *M. vimineum* understory coverage density, based on ocular observation.

Treatment Applications

To assess the effectiveness of different *M. vimineum* control methods, four treatments were identified that were easily accessible to landowners: (1) herbicide application (glyphosate), (2) mechanical removal (mechanical trimmer), (3) manual removal (hand pulling), and (4) herbicide alternative (vinegar). Each removal treatment was then compared to a no treatment (control). The herbicide application consisted of a 2 percent solution of glyphosate, a broad-spectrum active ingredient, readily available to consumers using the pre-mixed solution Roundup® Ready-to-Use and its built-in spraying wand [2 percent glyphosate (isopropylanite salt), 2 percent pelargonic acid and related fatty acids, 96 percent other ingredients] Monsanto Company, Marysville, OH. The herbicide alternative application consisted of common white vinegar (acetic acid content of 5 percent) applied using a spray bottle. Both spray solutions were applied in all seasons during dry conditions with no precipitation during the 24- to 48-hour post-application window. The mechanical removal application removed aboveground *M. vimineum* stems but left roots intact using a STIHL Pro Series FS70 R string trimmer. The manual removal application removed whole stems and root systems of *M. vimineum* biomass via hand-pulling. Any cut *M. vimineum* biomass remaining after manual treatment was removed from the plot and discarded outside of the study area.

Post-Treatment Data Collection and Analysis

All treatments were applied in early March 2020, early July 2020, and late September 2020, coinciding with phenological stages of *M. vimineum* development in the Piedmont of North Carolina. These stages were immediately after germination, after full stem expansion and moderate growth into the middle of growing season, and prior to seed-set for *M. vimineum*. Six weeks post-treatment, percent coverage of *M. vimineum*, understory vegetation species richness, and understory species diversity were measured for each treatment using the same methods for pre-treatment assessments. Post-treatment measurements were compared to pre-treatment measurements to determine treatment impacts (e.g., treatment type and timing) on *M. vimineum* coverage.

RESULTS

Removal of *M. vimineum* was most effective in the herbicide treatment with glyphosate (98 ± 0 percent average removal), followed by the herbicide alternative treatment with vinegar (79 ± 21 percent removal), and mechanical removal treatment (48 ±15 percent removal) (fig. 1). Hand pulling was the least effective treatment for the removal of *M. vimineum* at 28 ± 14 percent (fig. 1). Glyphosate had consistent results for removal across seasons. Control of *M. vimineum* via vinegar and mechanical removal were more effective in the summer and fall treatment applications than spring (fig. 1). Hand pulling was slightly more effective at *M. vimineum* removal in the summer compared to spring or fall treatment applications, but never resulted in more that 50 percent removal in any seasonal application (fig. 1).

Average species diversity, as measured by the Shannon-Weiner Index, was low across all plots during the pre-treatment period due *M. vimineum* dominance (table 1). Six weeks following treatment applications, species diversity increased across all treatments and seasons. Following spring treatments, increases in plant diversity were modest, while greater increases in diversity were observed following summer and fall treatments. Manual removal had resulted in the largest overall plant diversity in spring (0.99), herbicide alternative treatments with vinegar resulted in the largest overall plant diversity in summer (1.32), and mechanical removal resulted in the largest overall plant diversity in the fall (1.03). The highest species richness in any treatment plot was observed after the summer application (1.32) with

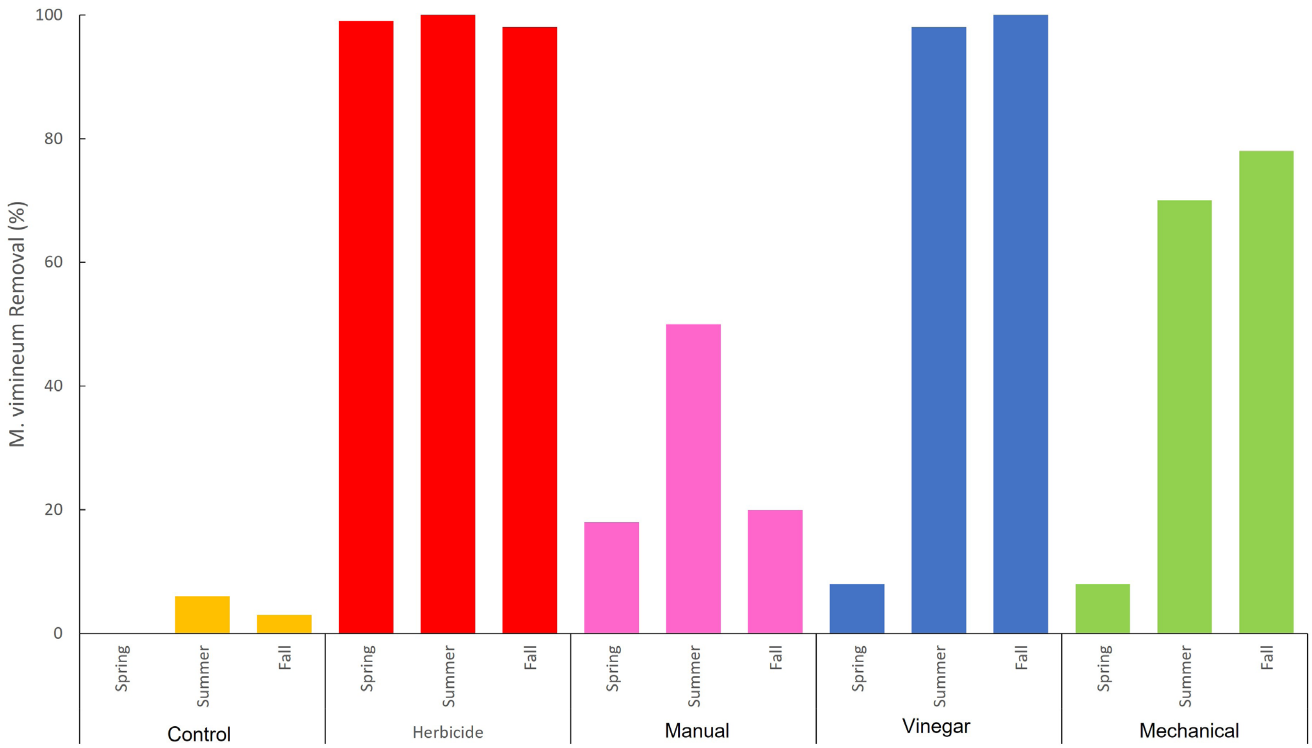

Figure 1—Percent *Microstegium vimenium* removed for each treatment type and time of application.

five species present among 23 individual stems. Glyphosate, despite being one of the more effective treatments for biomass removal, had adverse effects on species richness and had the lowest species richness and diversity over all the plots and application times (table 1).

The greatest variety of species returning 6 weeks post-treatment occurred in plots treated with vinegar in the summer with five different species emerging post-treatment, followed by the summer mechanical removal plot which had four different species emerge (table 1). The control plot and manual removal plots had similar numbers, but different species emerged post-treatment in each plot (two to three different species); however, the herbicide plots had the lowest number of species emerge post-treatment (one to two different species per plot) (table 1).

DISCUSSION

Herbicide application with glyphosate was the most effective treatment at reducing *M. vimineum* biomass. Post-emergent applications of fluazifop-P-butyl, acetic acid, pelargonic acid, imazapic, fenoxaprop-p-ethyl, glufosinate, glyphosate, clethodim, quinclorac, sethoxydim, and Monosodium Methanearsonate have all been considered effective treatments (Flory 2010, Frey and Schmitt 2015, Hall and others 2014, Judge and others 2005, Ward and Mervosh 2012). Flory (2010) found that the application of a post-emergent herbicide was more effective than hand pulling in treating an area for *M. vimineum*. Despite being the most effective treatment at removing *M. vimineum*, herbicide had a negative correlation with species richness and diversity.

Glyphosate is a broad-spectrum, non-selective herbicide often killing plants within 2 weeks (Franz and others 1997). Six weeks after treatment, few species could have recovered or established due to the wide-ranging impact of glyphosate. In their review of glyphosate impacts on natural communities, Sullivan and Sullivan (2003) found vascular plant species richness was unaffected or increased in most (83 percent) studies after treatment. Miller and Miller (2004), along with others in a special issue of *The Wildlife Society Bulletin*, emphasized the short-term negative impacts (3-5 years) and selective impacts of forest herbicide applications on plant biodiversity, including broad spectrum glyphosate. However, Miller and Miller (2004) also showcased the unique advantages of selective herbicides for targeting unwanted plant pests. Future studies should explore selective herbicide impacts on M. *vimineum* and associated species richness and diversity. Such approaches could also help identify co-existing heterospecifics that may be released by treatments and tolerant of *M. vimineum* allelopathy (Pisula and Mieners 2010) such as other exotic species. Monitoring species richness and diversity recovery over longer periods (>6 weeks post-treatment) could also identify optimal control methods for concomitant *M. vimineum* control and native plant species conservation.

Table 1—Species richness as measured of unique individuals with number of stems in parentheses, and Species diversity as measured by the Shannon Wiener Index (SWI) in study plots prior to and 6 weeks post-treatment applications across all three seasons[a]

Treatment	Pre-treatment		Post-treatment	
	Species richness	SWI	Species present	SWI
Spring				
Herbicide	*Parthenocissus quinquefolia* (2)	0.00	*Polystichum archo-stichoides* (1) *Vitus rotundifolia* (2)	0.64
Mechanical removal	*Allium canadense* (2) *Ligustrum sinense* (1)	0.64	*Allium canadense* (4) *Polystichum archostichoides* (3)	0.68
Manual removal	*Ligustrum sinense* (1) *Polystichum achrostichoides* (1)	0.69	*Lespedeza sp.* (5) *Ligustrum sinense* (2) *Polystichum archostichoides* (2)	0.99
Vinegar	*Acer rubrum* (1) *Ligustrum sinense* (2)	0.64	*Acer rubrum* (3) *Allium canadense* (1) *Ligustrum sinense* (10)	0.76
Control	No other species present[b]	0.00	*Allium canadense* (1) *Polystichum achrostichoides* (2)	0.64
Summer				
Herbicide	*Ligustrum sinense* (2)	0.00	*Ligustrum sinense* (1) *Polystichum achrostichoides* (3)	0.56
Mechanical removal	*Ligustrum sinense* (3)	0.00	*Allium canadense* (1) *Ligustrum sinense* (5) *Polystichum archostichoides* (3) *Vitus rotundifolia* (1)	1.17
Manual removal	*Ligustrum sinense* (2)	0.00	*Lespedeza sp.* (5) *Ligustrum sinense* (2) *Vitus rotundifolia* (1)	0.90
Vinegar	*Ligustrum sinense* (3)	0.00	*Allium canadense* (4) *Lespedeza sp.* (2) *Ligustrum sinense* (15) *Polystichum achrostichoides* (4) *Rubus fruticosis* (3)	1.32
Control	*Ligustrum sinense* (2)	0.00	*Ligustrum sinense* (3) *Panicum virgatum* (1) *Lespedeza* (1)	0.95
Autumn				
Herbicide	*Parthenocissus quin-quefolia* (3)	0.00	*Allium canadense* (5)	0.00
Mechanical removal	*Allium canadense* (2) *Ligustrum sinense* (2)	0.69	*Allium canadense* (10) *Ligustrum sinense* (9) *Vitus rotundifolia* (4)	1.03
Manual removal	*Ligustrum sinense* (1) *Polystichum archo-stichoides* (2)	0.64	*Ligustrum sinense* (3) *Polystichum archostichoides* (4)	0.68
Vinegar	N/A	0.00	*Carya tomentosa* (1) *Ligustrum sinense* (4) *Polystichum archostichoides* (3)	0.97
Control	*Ligustrum sinense* (1)	0.00	*Carya tomentosa* (1) *Ligustrum sinense* (5)	0.45

[a]For species richness and diversity, *Microstegium vimineum* was not included in calculations.
[b]Only *Microstegium vimineum* present.

After herbicide application, vinegar (an herbicide alternative) and mechanical removal were the next most effective controls of *M. vimineum*. These two treatments also had the greatest species richness and diversity measures, even greater than the untreated control plot. The removal of *M. vimineum* by vinegar and mechanical removal likely stimulated the seedbank and provided access resources to other native species. The ability of vinegar and mechanical removal to reduce *M. vimineum* cover and promote species richness and diversity would make them attractive options for private landowners with management goals that favor a more diverse herbaceous understory compared to herbicide application. These two treatments had the greatest success in the summer and fall applications. That is likely because *M. vimineum* was unable to recover from having expended resources earlier in the growing season. Understanding the seasonal dynamics of *M. vimineum* control options is critical to ensure treatments are effective both ecologically and economically to the landowner. Additionally, understanding how repeated annual applications of these treatments affects *M. vimineum* is an important next step in developing guidance for landowners to control *M. vimineum*.

Hand pulling was the least effective treatment application for *M. vimineum* removal. Hand pulling was the most labor intensive of all the treatment applications and required every stem and root system to be extracted from the soil to be effective (Flory 2010). Given the physical labor required to achieve <50 percent *M. vimineum* removal rate through hand pulling, this treatment application is less likely to be adopted by landowners and land managers when other less intensive and more successful treatment options exist.

CONCLUSIONS

Preliminary results suggest that chemical applications of commercial and alternative herbicides (e.g., glyphosate and vinegar) were most successful at reducing *M. vimineum* coverage and alternative herbicides likewise resulted in higher species diversity in treatment plots. Due to the success of glyphosate and vinegar in the first portion of the project, these two treatments will be replicated across three new studies sites using the same methods for the second portion of this study.

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