# **BIOLOGICAL AND MECHANICAL CONTROL OF JAPANESE**

# STILTGRASS (MICROSTEGIUM VIMINEUM)

by

Samantha Nestory

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Entomology

Summer 2016

© 2016 Samantha Nestory All Rights Reserved ProQuest Number: 10190879

All rights reserved

INFORMATION TO ALL USERS The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



ProQuest 10190879

Published by ProQuest LLC (2016). Copyright of the Dissertation is held by the Author.

All rights reserved. This work is protected against unauthorized copying under Title 17, United States Code Microform Edition © ProQuest LLC.

> ProQuest LLC. 789 East Eisenhower Parkway P.O. Box 1346 Ann Arbor, MI 48106 - 1346

# BIOLOGICAL AND MECHANICAL CONTROL OF JAPANESE STILTGRASS (*MICROSTEGIUM VIMINEUM*)

by

Samantha Nestory

Approved:

Judith Hough-Goldstein, Ph.D. Professor in charge of thesis on behalf of the Advisory Committee

Approved:

Jacob Bowman, Ph.D. Chair of the Department of Entomology and Wildlife Ecology

Approved:

Mark Rieger, Ph.D. Dean of the College of Agriculture and Natural Resources

Approved:

Ann L. Ardis, Ph.D. Senior Vice Provost for Graduate and Professional Education

### ACKNOWLEDGMENTS

I would like to thank everyone who helped me through this process. Ben, who was there through all of the ups and downs; Emily, Julian, Sean, and Kaitlin, for being the most supportive, hilarious, and loving labmates I could ask for and setting the bar very high for co-workers in the future; and Judy, who was unendingly kind and helpful and who gave me so many wonderful opportunities and experiences in my four and a half years working with her.

# **TABLE OF CONTENTS**

LIST	OF TABLES	vi
LIST	OF FIGURES	. vii
ABST	FRACT	X
01		
Chapt	ter	
1	TEST PLANT LIST FOR MICROSTEGIUM VIMINEUM	1
	Introduction	1
	Nature of the Problem	1
	Proposed Action	1
	Target Weed Information	2
	Taxonomy	2
	Description	3
	Distribution of the Target Weed	3
	Taxonomically Related Plants	5
	Distribution of Taxonomically Related Plants	8
	Life History	9
	Impacts	. 10
	Alternative Management Options.	. 13
	Known Host Range of Candidate Biological Control Agent	. 14
	Test Plant List	. 15
	Category 1: Genetic types of the target weed species found in North	
	America and the native range	15
	Category 2: Species in the same genus as the target weed	15
	Category 3: Species in other genera in the same family as the target	
	weed	. 16
	Category 4: Threatened and endangered species in the same family as	
	the target weed	20
	Category 5: Species in other families in the same order that have some	
	phylogenetic, morphological, or biochemical similarities to the target	
	weed	23

	Category 6: Species in other orders that have some morphological or biochemical similarities to the target weed Category 7: Any plants on which the proposed biological control agent or its close relatives have been previously found or recorded to feed	. 26
	and/or reproduce	. 27
	Perspective of Risk	. 31
2	EFFECT OF MOWING HEIGHT, MOWING TIMING, AND LEAF MULCH ADDITION ON JAPANESE STILTGRASS	. 33
	Introduction Materials and Methods	. 33 . 35
	Study Area Experimental Design Data Collection Data Analysis	. 35 . 35 . 36 . 37
	Results and Discussion	. 37
	Treatment Effects on Japanese Stiltgrass Treatment Effects on Plant Community	. 37 . 43
3	EFFECT OF CUTTING AT DIFFERENT INTERNODAL REGIONS ON JAPANESE STILTGRASS GROWTH IN THE FIELD AND GREENHOUSE	. 52
	Introduction Materials and Methods	. 52 . 53
	Study Area Experimental Design Data Collection Data Analysis	. 53 . 54 . 55 . 56
	Results	. 56
	Field Experiment Greenhouse Experiment	. 56 . 62
	Discussion	. 67
REFE	RENCES	. 72

# LIST OF TABLES

Table 1	Taxonomic relationship of plants proposed for host-plant testing for <i>Microstegium viminium</i> (Stevens 2001, Soreng et al. 2015). Order Poales except where noted.	5
Table 2	Threatened and endangered grass species (Poaceae) listed by the U.S. Fish and Wildlife Service (USFWS, ECOS)	.21
Table 3	List of plant species recommended for testing to determine potential host range of candidate biological control agent of <i>Microstegium</i> <i>vimineum</i> (Trin.) A. Camus (Poales, Poaceae). Footnotes indicate important literature references: <sup>1</sup> Simao et al. 2010, <sup>2</sup> Redman 1995, <sup>3</sup> Emery et al. 2011, <sup>4</sup> Flory et al. 2007, <sup>5</sup> Beauchamp et al. 2013, <sup>6</sup> Schafale and Weakley 1990, <sup>7</sup> Cipollini et al. 2013, <sup>8</sup> Lee et al. 2012, <sup>9</sup> Boyd and Moffett 2003, <sup>10</sup> Abrams and Johnson 2012, <sup>11</sup> Oswalt et al. 2007.	. 27
Table 4	Average cover (±SE) per plot of the most abundant species in the plant communities before treatment (28 August) and six weeks after treatment (9 October), 2014.	. 44
Table 5	Average cover (±SE) per plot of the most abundant species in the plant communities before treatment (24 April, 30 July, or 11 September) and in October following treatment, 2015	. 47
Table 6	Results of ANOVA evaluating Japanese stiltgrass maximum final height, biomass, and number of seed spikelets across treatments at two sites.	. 58
Table 7	Survival, growth, and seed types produced in the greenhouse by Japanese stiltgrass stem cuttings cut at varying intermodal regions	. 63

# **LIST OF FIGURES**

Figure 1	Distribution of <i>Microstegium vimineum</i> in Asia. Black dots indicate presence within the country or province (CABI 2014)
Figure 2	Distribution of <i>Microstegium vimineum</i> in the United States (EDDMapS 2016)
Figure 3	Phylogenetic relationships among families in the Order Poales (Stevens 2001)
Figure 4	Average ( $\pm$ SE) Japanese stiltgrass (A) percent cover and (B) biomass per plot at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, <i>p</i> < 0.05)
Figure 5	Average ( $\pm$ SE) Japanese stiltgrass seed spikelets per plot at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ )
Figure 6	Average ( $\pm$ SE) Japanese stiltgrass (A) percent cover and (B) biomass per plot at the end of the season in 2015 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, $p <$ 0.05)
Figure 7	Average (±SE) Japanese stiltgrass seed spikelets per plot at the end of the season in 2014 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ )

Figure 8	Average ( $\pm$ SE) non-Japanese stiltgrass resident plant community (A) percent cover and (B) biomass at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ ).	45
Figure 9	Average (±SE) resident non-Japanese stiltgrass plant community (A) percent cover and (B) biomass at the end of the season in 2015 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ ).	49
Figure 10	Weekly average maximum height of Japanese stiltgrass per field sample from Site 1 after cutting at varying internodal regions (Control, no cutting; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 9) are not significantly different (Tukey's HSD, $p < 0.05$ ).	59
Figure 11	Weekly average maximum height of Japanese stiltgrass per field sample from Site 2 after cutting at varying internodal regions (Control, no cutting; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 9) are not significantly different (Tukey's HSD, $p < 0.05$ ).	60
Figure 12	Average aboveground biomass (±SE) of Japanese stiltgrass per field sample nine weeks after cutting at varying internodal regions (C, control [no cutting]; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ )	61
Figure 13	Average (±SE) number of Japanese stiltgrass seed spikelets produced per field sample nine weeks after cutting at varying internodal regions (C, control [no cutting]; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node). Different letters indicate significant differences among treatments (Tukey's HSD, $p < 0.05$ )	62

Figure 14	Weekly average maximum height of Japanese stiltgrass per	
	greenhouse sample from Site 1 after transplant from mist room.	
	Treatments were B5, below 5th node; B4, below 4th node, B3, below	
	3rd node; B2, below 2nd node; B1, below 1st node. Means with the	
	sam letters (lowercase for week 1 and uppercase for week 6) are not	
	significantly different (Tukey's HSD, $p < 0.05$ ).	. 65

#### ABSTRACT

Japanese stiltgrass (*Microstegium vimineum*) is an invasive grass that poses a major threat to native biodiversity and restoration efforts in invaded areas. Preliminary research suggests that there may be potential biological control agents that can be used in a classical biological control program in the United States. We compiled a test plant list for submission to Technical Advisory Group for Biological Control Agents of Weeds. Using a phylogenetic approach, we chose 59 plants species that will help to determine the host specificity of any potential biological control agents for Japanese stiltgrass. We will submit this test plant list to TAG-BCAW for review and comment.

We also investigated non-chemical control methods for Japanese stiltgrass. Several studies suggest that mowing can be an effective control method, but none have explicitly explored the effects of mowing height. Additionally, no studies have assessed the effectiveness of leaf mulch as a management strategy. We explored the effects of mowing timing, mowing height, and leaf mulch addition on Japanese stiltgrass in White Clay Creek State Park in Newark, DE. In 2014, we established small plots at two sites: secondary forest understory and early successional field. Plots were mowed at one of three heights (10 cm, 5 cm, 0 cm) in late August before seed set. In 2015, we established small plots in secondary forest understory. Treatments included leaf mulch addition in April (3 cm or 8 cm), mowing in July (0 cm or 10 cm), and mowing in September (0 cm or 10 cm). Treatments were compared to untreated plots. Vegetation surveys were conducted prior to treatment and at the end of the season. Aboveground biomass was harvested and sorted into Japanese stiltgrass vegetation and all other vegetation before drying and weighing. Seed spikelets were counted, dried, and weighed. All treatments except the 3-cm leaf mulch addition reduced cover and biomass of Japanese stiltgrass. Mowing at ground level in July or September were the most effective treatments, reducing Japanese stiltgrass cover, biomass, and seed production by as much as 99%. The 8-cm leaf mulch addition also reduced Japanese stiltgrass cover, biomass, and seed production, but not as effectively. However, mowing at ground level also significantly reduced the cover and biomass of the resident plant community, which implies that land managers should consider the composition and desirability of the resident plant community when choosing their management strategy.

Lastly, we explored the effect of cutting at different internodal regions on Japanese stiltgrass growth in the field and stem cutting growth in the greenhouse. No studies have investigated the effectiveness or viability of Japanese stiltgrass stem cuttings, which could be useful for large-scale production for a biological control program or future experiments. In July 2014, we established samples at two sites in the White Clay Creek State Park System: a secondary forest understory and an early successional field. Each sample had ten stems of Japanese stiltgrass that were all cut at the same internodal region: below the first node, below the second node, below the third node, below the fourth node, or below the fifth node. Treatments were compared to uncut samples. Maximum height and seed spikelet presence data were collected weekly. The stem remnants from the Japanese stiltgrass that was cut were planted in the greenhouse and monitored for stem survival, maximum height, and date of first seed emergence. At the end of the season, aboveground biomass from field and greenhouse plants was harvested and seed spikelets were sorted into chasmogamous

xi

(CH) and cleistogamous (CL) seed spikelets before drying and weighing. In the field experiment, all cutting treatments reduced Japanese stiltgrass biomass and seed production. However, stems cut at higher nodes recovered better than stems cut at lower nodes and had greater height, biomass, and seed production at the end of the season. Additionally, Japanese stiltgrass height and biomass were greater at the sunnier site. These results suggest that mowing height is important and effects may vary by light conditions. In the greenhouse experiment, >99% of stem cuttings survived and produced seed. Biomass and seed production did not differ among treatments, but stem cuttings from higher nodes produced seed more quickly. These results suggest that stem cuttings are an efficient and viable method for propagating Japanese stiltgrass.

#### Chapter 1

## TEST PLANT LIST FOR *MICROSTEGIUM VIMINEUM*

## Introduction

### Nature of the Problem

*Microstegium vimineum*, commonly known as Japanese stiltgrass, is a nonnative invasive annual grass that was accidentally introduced to the United States in the early 1900s, possibly through packing material for porcelain. *Microstegium vimineum* was first identified in 1919 in Knoxville, Tennessee, and since its introduction it has spread to 28 states in the eastern U.S. It is a significant threat to natural areas, especially forest ecosystems, and can be a nuisance in residential areas. As the only representative of the genus *Microstegium* in North America, this species is a promising target for biological control.

#### Proposed Action

This host plant list is to notify TAG-BCAW of our intent to begin a biological control program for the control of *Microstegium vimineum*. This species has been declared a noxious weed in Alabama and is considered a target for biological control. Your comments on the accuracy, appropriateness, and thoroughness of this list are appreciated.

#### **Target Weed Information**

Taxonomy

Order: Poales

Family: Poaceae Subfamily: Panicoideae Tribe: Andropogoneae Subtribe: Saccharinae

*Microstegium vimineum* is most commonly known as Japanese stiltgrass, but may also be called Chinese packing grass, Nepalese browntop, Nepal grass, Japanese grass, annual jewgrass, Mary's grass, or bamboo grass. Synonyms include: *Andropogon vimineus* Trin.; *Microstegium willdenowianum*, Nees ex Lindl.; *Eulalia viminea* (Trin.) Kuntze; *Eulalia viminea* (Trin.) Kuntze var. *variabilis* Kuntze; *Pollinia viminea* (Trin.) Merr.; and *Microstegium vimineum* (Trin.) A. Camus var. imberbe (Nees) Honda.

K. B. von Trinius first described this species as *Andropogon vimineus* in 1832 with a specimen collected by Nathaniel Wallich in Nepal. In 1836, C. G. Nees von Esenbeck placed it in a new genus *Microstegium* as the type species, renaming it to *Microstegium willdenowianum*. In 1891, O. Kuntze placed it into the genus *Eulalia* as *Eulalia viminea* and then E. D. Merrill placed it into the genus *Pollinia* as *Pollinia viminea* in 1932. Finally, in 1922, A. Camus replaced the species into *Microstegium* and named it *Microstegium vimineum*, which is the currently accepted binomial. There has been much debate regarding the significance of the presence of an awn, which gave rise to awned and awnless taxonomic varieties for many of the above

classifications. However, it is now generally accepted as a single taxon with no subspecific varieties (Fairbrothers and Gray 1972).

#### Description

*Microstegium vimineum* is a small, weak-stemmed annual grass. It has a sprawling growth habit with ascending growing tips and can grow 1.2-1.5 m high in good growing conditions. The thin stems are smooth with periodic nodes from which roots may sprout, especially from lower nodes. The leaves are regularly spaced and alternately arranged along the stem. Leaves are lanceolate and slightly asymmetrical, averaging 5-20 mm wide and 3-8 cm long. The leaves have smooth edges and a shiny pale midrib on the upper leaf surface. The root system is shallow, fibrous, and weak and the plant is easily pulled. *M. vimineum* produces two types of flower spikelets. The outcrossing, chasmogamous flowers occur along terminal racemes that may occur in groups of 1-3 racemes. The self-pollinating, cleistogamous flowers occur along axillary racemes that are enclosed in leaf-sheaths. Seeds are small and oblong with an awn. In the fall, the plants may turn brown or reddish-purple. The stems remain as bright tan colored thatch throughout the winter.

#### Distribution of the Target Weed

*Microstegium vimineum* is native to eastern Asia, including China, Japan, North Korea, South Korea, eastern Russia, northeast India, Iran, Myanmar, Bhutan, Nepal, Taiwan, Thailand, Vietnam, Malaysia, and the Philippines (Fig. 1). It has been introduced to Turkey and the Caucuses, as well as the Democratic Republic of the Congo, Costa Rica, and the United States, including Puerto Rico.



Figure 1 Distribution of *Microstegium vimineum* in Asia. Black dots indicate presence within the country or province (CABI 2014).

*M. vimineum* was introduced to the U.S. in the early 1900s, possibly through packing material for porcelain products from Asia. It was first identified in 1919 in Knoxville, Tennessee (Fairbrothers and Gray 1972) and is now present in 28 states in the eastern United States, from New Hampshire south to Florida and west to Texas (Fig. 2). *M. vimineum* invades a wide variety of habitats, including floodplains, riverbanks, fields, mesic slopes, woodland edges, roadsides, wetlands, meadows, forest understories, and lawns. Its spread is largely human-mediated through shoes, clothing, or contaminated equipment and spread rates increase drastically in areas adjacent to roadsides (Mortensen et al. 2009). There is evidence that *M. vimineum* may be rapidly evolving in the United States, allowing the species to expand northward in its invasive range (Novy et al. 2013).



Figure 2 Distribution of *Microstegium vimineum* in the United States (EDDMapS 2016).

# Taxonomically Related Plants

Table 1 shows the taxonomic relationship of plants discussed in this document.

Poacae phylogeny is based on a recent review (Soreng et al. 2015). The Catalogue of

New World Grasses (Poaceae) is also available online

(<u>http://www.tropicos.org/Project/CNWG</u>). The relationship of other families is based on Stevens (2001).

Table 1Taxonomic relationship of plants proposed for host-plant testing for<br/>*Microstegium viminium* (Stevens 2001, Soreng et al. 2015). Order Poales<br/>except where noted.

Family	Subfamily	Tribe	Subtribe	Genus & species
Poaceae	Panicoideae	Andropogoneae	Saccharinae	Microstegium vimineum Miscanthus sinensis Saccharum officinarum Saccharum ravennae

Family	Subfamily	Tribe	Subtribe	Genus & species
			Andropogoninae	Andropogon arctatus
				Andropogon gerardii
				Schizachyrium littoral
				Schizachyrium niveum
				Schizachyrium
				scoparium
			Anthistiriinae	Bothriochloa
				ischaemum
				Heteropogon
				melanocarpus
			Sorghinae	Sorghastrum nutans
				Sorghum bicolor
				Sorghum halepense
			Rottboelliinae	Coelorachis rugosa
				Eremochloa
				ophiuroides
			Tripsacinae	Tripsacum floridanum
			1	Zea mays
			Arthraxoninae	Arthraxon hispidus
			Unknown	Imperata cvlindrica
		Paniceae		Paspalum laeve
				Digitaria sanguinalis
				Dichanthelium hirstii
				Oplismenus hirtellus
				ssp. undulatifolius
				Panicum virgatum
				Stenotaphrum
				secundatum
		Chasmanthieae		Chasmanthium
				latifolium
	Aristidoideae	Aristideae		Aristida oligantha
	Arundinoideae	Arundineae		Arundo donax
		Molinieae		Hakonechloa macra
	Danthonoideae	Danthonieae		Danthonia spicata
	Chloridoideae	Cynodonteae		Eleusine indica
	Chiofidoladad	eynouonicue		Cynodon dactylon
				Muhlenbergia
				schreberi
				Orcuttia inaeaualis
				Tridens flavus
	Orvzoideae	Orvzeae		Leersia viroinica
	Oryzolacae	51 9 2000		Orvza sativa
				Zizania texana
				America - i - mater
	Bambusoideae	Arundinarieae		Αγμημημητή σισμητομ
	Bambusoideae Pooideae	Arundinarieae Meliceae		<i>Arunainaria gigantea</i> <i>Glyceria striata</i>
	Bambusoideae Pooideae	Arundinarieae Meliceae Poeae		Artinainaria gigantea Glyceria striata Avena sativa
	Bambusoideae Pooideae	Arundinarieae Meliceae Poeae		Arunainaria giganiea Glyceria striata Avena sativa Calamagrostis
	Bambusoideae Pooideae	Arundinarieae Meliceae Poeae		Arunainaria gigantea Glyceria striata Avena sativa Calamagrostis canadensis
	Bambusoideae Pooideae	Arundinarieae Meliceae Poeae		Arunainaria gigantea Glyceria striata Avena sativa Calamagrostis canadensis Poa alsodos

Family	Subfamily	Tribe	Subtribe	Genus & species
				Festuca rubra
				Lolium perenne ssp.
				perenne
				Schedonorus
				arundinaceus
		Triticeae		Hordeum vulgare
				Secale cereale
				Triticum aestivum
Xyridaceae				Xyris tennesseensis
Eriocaulaceae				Eriocaulon
				decangulare
Juncaeae				Juncus tenuis
Cyperaceae				Carex pensylvanica
				Cyperus croceus
Bromeliaceae				Hechtia glomerata
Typhaceae				Sparganium
				eurycarpum
ORDER COM	IMELINALES/ F	amily Commelin	naceae	Commelina communis
ORDER ZINC	GIBERALES/ Fai	mily Cannaceae		Canna indica

There are many important plant species within the tribe Andropogoneae. In the subtribe Saccharinae, Chinese silvergrass (*Miscanthus sinensis*) and Ravenna grass (*Saccharum ravennae*) are widely planted as ornamentals. Sugarcane (*Saccharum officinarum*) is an important crop that is produced in Florida, Louisiana, Hawaii, and Texas. In the closely related subtribe Andropogoninae, *Andropogon arctatus* (pinewoods bluestem) is threatened in Florida, *Schizachyrium littorale* (shore little bluestem) is endangered in Ohio and rare in Pennsylvania, and *Schizachyrium niveum* (pinescrub bluestem) is endangered in Florida. In another closely related subtribe, Sorghinae, sorghum (*Sorghum bicolor*) is an important crop species that is produced in the Great Plains states and Texas. In the subtribe Rottboelliinae, *Coelorachis rugosa* (wrinkled jointtail grass) is endangered in Maryland and New Jersey and centipede grass (*Eremochloa ophiuroides*) is a turfgrass species. In subtribe Tripsacinae,

*Tripsacum floridanum* (Florida gamagrass) is threatened in Florida and corn (Zea mays) is the most important crop in the United States.

Within the subfamily Panicoideae and in the closely related tribe Panicae, *Stenatophrum secundatum* (St. Augustine grass) is an important turf grass species. In the tribe Chasmanthieae, *Chasmanthium latifolium* (Indian woodoats) is a popular native ornamental grass. In the related subfamily Arundinoideae, *Hakonechloa macra* is a popular shade-tolerant ornamental. In the subfamily Chloridoideae, Bermudagrass (*Cynodon dactylon*) is an important turf grass species and *Orcuttia inaequalis* (San Joaquin Valley Orcutt grass) is endangered in CA.

There are many important species in the more distantly related grass subfamilies Oryzoideae and Pooideae. In Oryzoideae, rice (*Oryza sativa*) is an important crop grown in the southeast and California, and Texas wildrice (*Zizania texana*) is endangered in Texas. In Pooideae, there are multiple important turfgrass species: Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and tall fescue (*Schedonorus arundinaceus*). There are also many important crop species: barley (*Hordeum vulgare*), cereal rye (*Secale cereale*), wheat (*Triticum aestivum*), oats (*Avena sativa*), and perennial ryegrass (*Lolium perenne* ssp. *perenne*), which is an important forage crop for livestock.

### Distribution of Taxonomically Related Plants

The ornamental grasses *Miscanthus sinensis* and *Saccharum ravennae* are widely planted throughout the United States, and *M. sinensis* has naturalized populations in disturbed, high light habitats in many eastern states (Dougherty et al. 2014). Sugarcane (*Saccharum officinarum*) is grown and produced in Florida, Louisiana, Texas, and Hawaii. *Andropogon arctatus* occurs in wet pinewoods in North

Carolina, Alabama, and Florida. Schizachyrium littorale occurs mostly in dry secondary dunes in states with coastlines along the Great Lakes, the Gulf of Mexico, and the Atlantic Ocean. Schizachyrium niveum is endemic to Florida and occurs in sandy rosemary, pine, or oak scrub. Sorghum (Sorghum bicolor) is produced in the Great Plains states and Texas. Coelorachis rugosa is distributed throughout the mid-Atlantic and southern states. Tripsacum floridanum occurs only in Florida in pine rockland and other dry sites. Corn (Zea mays) is grown in many states throughout the country but production is concentrated in the Midwestern states. Orcuttia inaequalis occurs in vernal pools only in California. The turf grasses (Stenatophrum secundatum, Cynodon dactylon, Poa pratensis, Festuca rubra, Schedonorus arundinaceus, and *Eremochloa ophiuroides*) are planted in lawns and turfs throughout the United States. Hakonechloa macra is planted in many shady landscapes and gardens throughout the more temperate regions of the U.S. *Chasmanthium latifolium* is also planted in landscapes throughout the U.S. but it can occur naturally in eastern and southern states. Rice (Orvza sativa) is produced in the southeast and California. Texas wildrice (Zizania texana) occurs only in Texas in healthy flowing streams. Barley is produced mostly in Idaho, Minnesota, Montana, North Dakota, Oregon and Washington. Rye, wheat, oats, and perennial ryegrass can occur throughout the U.S. in agricultural fields.

#### Life History

*Microstegium vimineum* is an annual  $C_4$  grass. Plants that use  $C_4$  photosynthetic pathways fix carbon in a way that reduces wasteful fixation and limits losses by photorespiration. This pathway requires more energy from ATP, and  $C_4$  plants are usually most successful in high sunlight, high temperature conditions.

However, *M. vimineum* is a shade-adapted  $C_4$  plant that takes advantage of highintensity sunflecks in understory habitats (Horton and Neufeld 1998).

In the mid-Atlantic region, *M. vimineum* typically germinates in mid to late spring and grows slowly throughout the summer, reaching peak productivity in August (Barden 1987). In late August and September, *M. vimineum* produces chasmogamous and cleistogamous seed spikelets. Chasmogamous seed spikelets are produced on terminal racemes and reproduce sexually. Cleistogamous seed spikelets are produced on sheathed axillary racemes that self-fertilize. The seed bank is persistent and can remain viable for multiple years (Barden 1987, Webster et al. 2008). While chasmogamous seeds are longer-lived and more viable than cleistogamous seeds, cleistogamous seeds can ensure the persistence of *M. vimineum* populations when conditions are unfavorable for chasmogamy (Huebner 2011).

*M. vimineum* seed production can be very high, especially when light is not limiting, with each plant capable of producing hundreds of seeds (Gibson et al. 2002). However, seeds can still mature in low sunlight conditions, which is unusual for a  $C_4$  plant (Cheplick 2005, Warren et al. 2012). It is also unusually intolerant of drought for a  $C_4$  plant (Webster et al. 2008). Plants typically senesce and die in October and November, although the stems remain as thatch throughout the winter and can build up significantly over many seasons (Flory and Clay 2010a).

#### Impacts

*Microstegium vimineum* was used as packing material for porcelain in the early 1900s and the culms may currently be used for basket weaving. However, there are no reports of purposeful planting of *M. vimineum* for ornamental, erosion, or forage purposes (Tu 2000).

*Microstegium vimineum* is considered one of the worst invasive species in the eastern United States. It is currently classified as a Class C noxious weed in Alabama, an invasive and banned weed in Connecticut, and a prohibited weed in Massachusetts. It invades a wide variety of habitats, including floodplains, lawns, riverbanks, woodland edges, roadsides, wetlands, meadows, and forest understories (Fairbrothers and Gray 1972, Hunt and Zaremba 1992, Redman 1995). *M. vimineum* is unusually shade-tolerant for a C<sub>4</sub> grass and can invade and spread quickly in areas with as little as 5% full sunlight (Winter et al. 1982). *M. vimineum* is an aggressive competitor that outcompetes native and invasive species, such as *Lonicera japonica*, an established and aggressive invader (Leicht et al. 2005, Belote and Weltzin 2006).

*M. vimineum* poses a particular threat to forest ecosystems. *M. vimineum* can invade very quickly from roadsides into adjacent forest understories if there is significant ground, litter, or canopy disturbance (Cole and Weltzin 2004, Oswalt et al. 2007, Oswalt and Oswalt 2007, Marshall and Buckley 2008). These invasions can reduce native plant richness, biomass, and cover (Adams and Engelhardt 2009, Tekiela and Barney 2015), and these negative effects may worsen with time and increasing *M. vimineum* density (Flory and Clay 2010a). *M. vimineum* also significantly impedes forest regeneration by reducing the seasonal recruitment of small-seeded tree species. These effects on recruitment can compound to reduce the natural regeneration of important woody species in forest understories by 400% over 3 seasons (Flory and Clay 2010b).

The negative impacts of *M. vimineum* are exacerbated by the overabundance of white-tailed deer throughout the eastern U.S. Deer preferentially feed on native herbs, which reduces their competitive ability. Deer feeding habits also create disturbances

that facilitate *M. vimineum* invasion and spread (Eschtruth and Battles 2009, Knight et al. 2009). Herbivory may also interact with other factors, such as invasive earthworm presence or drought, to further impede native plant communities and exacerbate *M. vimineum* impacts (Webster et al. 2008, Dávalos et al. 2015).

*M. vimineum* can have significant effects on nutrient cycling. *M. vimineum* allocates more nitrogen to its shoots compared to native plants, which allocate most nitrogen to their roots. With large-scale invasions, this can change the nitrogen partitioning in forest understories from belowground to aboveground (Fraterrigo et al. 2011). Furthermore, *M. vimineum* invasion can increase potential nitrification rates and cause soil nitrate to become the dominant form of inorganic nitrogen. This is the preferred form of inorganic nitrogen for *M. vimineum* and at high densities, positive feedback responses to increased nitrate can further promote *M. vimineum* growth and persistence (Lee et al. 2012). *M. vimineum* invasions can also accelerate carbon cycling in the presence of increased nitrogen, leading to net losses in soil carbon in eastern U.S. forests (Strickland et al. 2011, Craig et al. 2015).

The impacts of *M. vimineum* invasion on different animal groups have been largely unexplored. Most of the published studies have focused on arthropod communities, but the results are inconsistent across studies. Some observational studies suggest that *M. vimineum* invasion corresponds to an increase in arthropod abundance and species richness, especially with herbivores (Tang et al. 2012, Metcalf and Emery 2015). This may be due to the large influx of carbon from *M. vimineum* invasion, which may provide a plentiful resource for herbivorous arthropods (Bradford et al. 2010). However, the only experimental study evaluating these impacts suggests that *M. vimineum* invasion leads to reduced arthropod abundance and species richness

and a significant shift in arthropod composition (Simao et al. 2010). A study exploring higher trophic level impacts demonstrated that American toads are adversely affected by *M. vimineum* invasion due to increased spider presence and predation in invaded areas (DeVore and Maerz 2014).

Controlling *M. vimineum* invasions can be very costly, especially in natural areas where access and herbicide use may be limited or restricted. While no reports concerning specific costs of control for *M. vimineum* have been published, one 7-hectare invasion in Florida cost approximately \$490/hectare to achieve significant control using postemergent herbicide (EPPO 2014).

#### Alternative Management Options

*Microstegium vimineum* can be controlled through a variety of chemical and non-chemical methods. Hand-weeding may be effective for small-scale invasions if performed late in the season. However, it is labor-intensive and may disturb the soil, which can promote further *M. vimineum* seed germination (Flory and Clay 2009). Mowing can be an effective treatment if it is done once a season, just before *M. vimineum* sets seed in late summer (Flory and Lewis 2009). Mowing regularly throughout the season is ineffective and results in shorter grass that can still set seed from lower axillary stems (Tu 2000). It is important that hand-weeding and mowing treatments are applied every year in order to deplete the seed bank, which may take several years depending on the age of the invasion (Judge et al. 2008, Flory and Lewis 2009). Prescribed burns in spring or fall do not control *M. vimineum* and can actually increase recruitment and performance in following years (Emery et al. 2013, Wagner and Fraterrigo 2015).

A variety of preemergent and postemergent herbicides can be effective in controlling *M. vimineum*. Pendimethalin is a preemergent herbicide that can provide adequate control, but only if applied at the correct time at a high rate (Swearingen and Adams 2008). While it does not affect already established vegetation, it prevents germination of native seeds (Flory and Clay 2009). Glyphosate and glufosinate are two non-selective postemergent herbicides that can eliminate seed production and provide excellent control of *M. vimineum*, but they severely impact all other treated vegetation, reducing native plant cover and richness post-treatment (Pomp et al. 2010, Ward and Mervosh 2012). Imazapic, also a non-selective herbicide, can be applied as a preemergent before germination or as a postemergent herbicide any time before seed set. Imazapic can significantly reduce *M. vimineum* cover, biomass, and seed production, but it also impacts the native plant community, though not as severely as glyphosate (Judge et al. 2005a, Ward and Mervosh 2012). Fluazifop-p-butyl is a selective postemergent herbicide that can adequately control *M. vimineum* with minimal effect on the non-graminoid native plant community (Judge et al. 2005b). Fenoxaprop-*p*-ethyl, a selective postemergent herbicide, provides excellent control of M. vimineum and can maintain or even increase cover and richness of native species post-treatment (Judge et al. 2005a, b, Judge et al. 2008, Pomp et al. 2010, Ward and Mervosh 2012).

Known Host Range of Candidate Biological Control Agent No candidate agents are proposed at this time.

## **Test Plant List**

Category 1: Genetic types of the target weed species found in North America and the native range

There are no known genetic types of *Microstegium vimineum*. However, there is some evidence that this species has evolved rapidly following introduction to North America about 100 years ago. Flory et al. (2011) found significant variation in biomass among populations collected from different sites and planted in multiple common gardens, suggesting that they were genetically variable and different from populations in the native range. In addition, in growth chambers there was a clear cline in flowering time and biomass from populations collected along a latitudinal range from the northern to the southern extreme of the *M. vimineum* distribution in the U.S., with northern populations flowering earlier and producing less biomass than southern populations regardless of photoperiod regime (Novy et al. 2013). This suggests that these characters are under genetic control and have responded to adaptive selection. Populations may also have evolved in response to different biotic environmental conditions (Ziska et al. 2014). In addition, separate genetic lineages have been identified in amplified genomic DNA from geographically isolated populations (Baker and Dyer 2011). However, none of these genetic differences among U.S. populations are likely to affect herbivory. Host plant testing should use plant material from M. *vimineum* populations near where release is intended.

Category 2: Species in the same genus as the target weed

There are no species in the same genus as *Microstegium vimineum* in North America.

Category 3: Species in other genera in the same family as the target weed

The family Poaceae is very large and diverse. Recent molecular and morphological studies have divided it into 12 subfamilies, 51 tribes, and 80 subtribes (Soreng et al. 2015). As noted, *Microstegium vimineum* is a member of the tribe Saccharinae within the tribe Andropogoneae. Although the relationships between subtribes in Andropogoneae are still somewhat unclear, recent evidence suggests that Saccharinae is likely most related to the subtribes Sorghinae, Ischaeminae, and Dimeriinae (Soreng et al. 2015). Other subtribes within the tribe include Arthraxoninae, Tripsacinae, Chionachninae, Coicinae, Rottboelliinae, Germainiinae, Andropogoninae, and Anthistiriinae.

The tribe Andropogoneae is part of the supertribe Andropogonodae, which also includes the tribe Arundinelleae. Supertribe Andropogonodae is related to supertribe Panicodae, which includes tribes Paniceae and Paspaleae (Soreng et al. 2015). Other tribes within Panicoideae that are basal to both supertribes are Thysanolaeneae, Cyperochcloeae, Centotheceae, Chasmanthieae, Zeugiteae, Tristachyideae, and Gynerieae. Subfamily Panicoideae is part of the PACMAD clade in which the most basal subfamily is Aristoideae, which is sister to the rest of the clade. Panicoideae is the next most basal group, followed by sister groups Arundinoideae and Micrairoideae and then sister groups Danthonioideae and Chloridoideae. The PACMAD clade is ancestral to the BOP clade, which includes subfamilies Oryzoideae, Bambusoideae, and Pooideae. Subfamilies Anomochlooideae, Pharoideae, and Puelioideae are ancestral to these two clades (Soreng et al. 2015).

According to the USDA plants database, the subfamilies Anomochlooideae, Pharoideae, and Puelioideae are not represented in North America (USDA, NRCS 2016). Micrairoideae of the PACMAD clade also has no representatives in North

America. All other subfamilies have species that occur in the same range or habitat as Japanese stiltgrass in its invasive range. Other members of the subfamily Panicoideae are often found co-occurring with M. vimineum or are important ecosystem components, including species within Andropogon, Schizachyrium, Dichanthelium, Panicum, Paspalum, Chasmanthium, and Digitaria (Schafale and Weakley 1990, Flory and Clay 2009, Cheplick 2010, Simao et al. 2010, Emery et al. 2011). There are thousands of species in Panicoideae so we included multiple species from this group, representing at least 6 subtribes and 3 tribes, in order to understand the risk of attack within the target weed's subfamily. Species within the subfamily Chloridoideae also co-occur with *M. vimineum*, including members of *Tridens*, *Muhlenbergia*, and Eragrostis (Redman 1995, Cipollini et al. 2013, Stricker et al. 2016). We chose five species representing different genera and categories of importance for this subfamily. Species in Pooideae can also co-occur with *M. vimineum*, such as members of *Poa*, Calamagrostis, Schedonorus, and Glyceria (Flory et al. 2007, Simao et al. 2010, Emery et al. 2011, Lee et al. 2012). We chose habitat associates to represent the environmentally important species in this group. We also included at least one representative of all other subfamilies present in the U.S., focusing on habitat associates and species with similar morphological characteristics.

There are many economically important species within Poaceae, particularly related to agriculture. Sugarcane (*Saccharum officinarum*), a member of the target weed's subtribe, accounts for approximately for 45% of U.S. sugar production (USDA-ERS). The closely related subtribe Sorghinae includes sorghum (*Sorghum bicolor*), which is harvested for grain and silage and had an estimated economic value of \$2 billion in 2015 (USDA-NASS). Corn (*Zea mays*) is a member of the target

weed's tribe. The United States is the number one producer of corn worldwide, with over 90 million acres of corn planted per year, which provides 95% of total feed grain used in the U.S. (USDA-ERS). Corn production was valued at over \$49 billion in 2015 (USDA-NASS). Corn is also processed into starch, sweeteners, corn oil, beverage and industrial alcohols, and ethanol for fuel. Rice (Oryza sativa) is a member of the family Oryzoideae and is produced primarily in the southern United States. In 2015, rice produced in the U.S. had an economic value of over \$2.5 billion dollars. Major uses for rice include beer, pet food, and human consumption, and domestic demand continues to increase yearly (USDA-ERS). Oats (Avena sativa) are mostly used for livestock feed, with a small percentage used for human consumption, particularly breakfast foods. For 2015, oat production was valued at \$220 million. Perennial ryegrass (Lolium perenne ssp. perenne) is a highly nutritious forage species that is planted by farmers and ranchers in the Midwest and northern U.S. It is also used for turf and erosion control. Barley (Hordeum vulgare) is an important grain in animal feed, but it is also used for malt production, seed, and human consumption. In 2015, barley production was valued at over \$1.1 billion (USDA-NASS). Cereal rye (Secale cereale) is used in flour and production value was estimated at \$75 million in 2015 (USDA-NASS). Wheat (*Triticum aestivum*) is the third most important crop by acreage and gross production value in the U.S. Wheat is the principal food grain in the U.S. and is sold mostly as flour. In 2015, wheat production was valued at over \$10 billion dollars (USDA-NASS).

There are also Poaceae species that are economically important in horticulture and landscape. Bermudagrass (*Cynodon dactylon*), Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), tall fescue (*Schedonorus arundinaceus*), St.

Augustine grass (*Stenatophrum secundatum*), zoysiagrass (*Zoysia japonica*), bahiagrass (*Paspalum notatum*), and centipede grass (*Eremochloa ophiuroides*) are all important turfgrass species that are grown for sod and seed throughout the U.S. Chinese silvergrass (*Miscanthus sinensis*) and ravennagrass (*Saccharum ravennae*) are members of the subtribe Saccharinae and are planted in landscapes across the country as ornamental species. *Miscanthus sinensis* is also being considered for cultivation as a source of biofuel (Dougherty et al. 2014). Other common ornamental grasses include fountain grass (*Pennisetum setaceum*), ribbon grass (*Phalaris arundinacea*), purple muhly grass (*Muhlenbergia capillaris*), and Hakone grass (*Hakonechloa macra*). Native grasses are also used in landscapes, such as big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), prairie dropseed (*Sporobolus heterolepis*), and Indian woodoats (*Chasmanthium latifolium*).

We included all important crop species in our test plant list because they are all members of different genera and each yields product that is valued at millions or billions of dollars each year. We included three turfgrass species well-suited to southern lawns (Bermudagrass, centipede grass, and St. Augustine grass) and three species well-suited to northern lawns (Kentucky bluegrass, red fescue, and tall fescue). These turfgrass species are widespread and provide valuable services directly (e.g. aesthetics) and indirectly (e.g. employment) to people throughout the U.S. However, they also represent genera like *Festuca* and *Poa* that have many other important species, and augment representation in groups closely related to Japanese stiltgrass. We included Chinese silvergrass and ravennagrass not only because they are popular ornamental grasses, but more importantly because they are members of the same subtribe as the target weed. We included Hakone grass as a representative of

subfamily Arundinoideae, because it has economic and aesthetic value, unlike many other species in Arundinoideae. We also included Indian woodoats because it is valuable in landscapes and natural ecosystems, has been reported as a habitat associate of Japanese stiltgrass, and represents the only genus in subtribe Chasmanthieae that is present in the U.S.

All untested species in Poaceae are represented at least at the subfamily level. In certain subfamilies, like Pooideae, untested species may be represented at the tribe level as well, which allows for better inferences about the potential for attack. Inferences about untested species in subfamilies with one or two representatives on our list will be more limited. Considering the representation within tribe Andropogoneae, we will likely be able to draw robust conclusions about risk of attack in the target weed's tribe.

Category 4: Threatened and endangered species in the same family as the target weed

Of the grass species that are identified as threatened, endangered, or candidate species according to the U.S. Fish and Wildlife Service (Table 2), only *Dichanthelium hirstii* occurs in the same range and habitat as *Microstegium vimineum*. However, considering the number of species within genus *Orcuttia* that are endangered or threatened at the federal level, we included *Orcuttia inaequalis* as a representative of this group. We also included *Zizania texana* because although *M. vimineum* has not been reported in any counties where *Z. texana* is present, *M. vimineum* is present in Texas and the likelihood of spread is high, as with many invasive species.

Considering this relatively low representation of federally listed threatened and endangered species, we also included all species within the tribe Andropogoneae that were threatened or endangered at the state level based on listings through the USDA

plants database (USDA, NRCS 2016). Within subtribe Andropogoninae, *Andropogon arctatus* is threatened in Florida, *Schizachyrium littorale* is endangered in Ohio and rare in Pennsylvania, and *Schizachyrium niveum* is endangered in Florida. These species not only represent populations that may be particularly vulnerable, but act as representatives for a subtribe that is closely related to Saccharinae. We also included *Coelorachis rugosa* of subtribe Rottboelliinae, which is endangered in Maryland and New Jersey, although it is present in a number of eastern states. Finally, we included *Tripsacum floridanum*, which is threatened in Florida. This species is a member of subtribe Tripsacinae, the same subtribe to which corn belongs.

The species included on this list thoroughly represent state-listed species within the target weed's tribe and federally threatened or endangered species within the target weed's range. There are many other Poaceae species that are listed as threatened or endangered at the state level, but we chose to represent those that are most phylogenetically related based on the prediction that they are at greater risk of attack than more distantly-related species.

Subfamily	Tribe	Scientific Name	Common Name	Status	Range
Panicoideae	Andropogoneae	Ischaemum byrone	Hilo ischaemum	Endangered	HI
Panicoideae	Paniceae	Digitaria pauciflora	twospike crabgrass	Candidate	FL
Panicoideae	Paniceae	Cenchrus agrimonioides	Kamanomano	Endangered	HI
Panicoideae	Paniceae	Dichanthelium hirstii	Hirst's panicgrass	Candidate	NJ, DE, NC, GA
Panicoideae	Paniceae	Panicum fauriei	Carter's	Endangered	HI

Table 2Threatened and endangered grass species (Poaceae) listed by the U.S.Fish and Wildlife Service (USFWS, ECOS).

Subfamily	Tribo	Scientific	Common	Status	Danga	
Sublamily	1 ribe	Name	Name	Status	Kange	
		var. carteri	panicgrass			
Panicoideae	Paniceae	Panicum	Lau`ehu	Endangered	HI	
		niihauense				
Aristidoideae	Aristideae	Aristida	Chase's	Endangered	PR	
		chaseae	threeawn			
Aristidoideae	Aristideae	Aristida	Pelos del	Endangered	PR	
		portoricensis	diablo			
Chloridoideae	Eragrostideae	Eragrostis	Fosberg's love	Endangered	HI	
		fosbergii	grass			
Chloridoideae	Cynodonteae	Neostapfia	Colusa grass	Threatened	CA	
		colusana				
Chloridoideae	Cynodonteae	Orcuttia	California	Endangered	CA	
		californica	Orcutt grass			
Chloridoideae	Cynodonteae	Orcuttia	San Joaquin	Threatened	CA	
		inaequalis	Orcutt grass			
Chloridoideae	Cynodonteae	Orcuttia pilosa	Hairy Orcutt	Endangered	CA	
			grass			
Chloridoideae	Cynodonteae	Orcuttia tenuis	Slender Orcutt	Threatened	CA	
			grass			
Chloridoideae	Cynodonteae	Orcuttia viscida	Sacramento	Endangered	CA	
			Orcutt grass			
Chloridoideae	Cynodonteae	Tuctoria	Greene's	Endangered	CA	
		greenei	tuctoria			
Chloridoideae	Cynodonteae	Tuctoria	Solano grass	Endangered	CA	
		mucronata	-			
Chloridoideae	Cynodonteae	Swallenia	Eureka Dune	Endangered	CA	
		alexandrae	grass			
Oryzoideae	Oryzeae	Zizania texana	Texas wild-	Endangered	TX	
-			rice			
Pooideae	Poeae	Calamagrostis	Hillegrand's	Endangered	HI	
		hillebrandii	reedgrass			
Pooideae	Poeae	Festuca	Moloka'I	Endangered	Hi	
		molokaiensis	fescue			
Pooideae	Poeae	Festuca ligulata	Guadalupe	Candidate	TX	
		_	fescue			
Pooideae	Poeae	Alopecurus	Sonoma	Endangered	CA	
		aequalis var.	shortawn			
		sonomensis	foxtail			
Pooideae	Poeae	Poa	San	Endangered	CA	
		atropurpurea	Bernardino			
			bluegrass			
Pooideae	Poeae	Poa mannii	Mann's	Endangered	HI	
			bluegrass			
Pooideae	Poeae	Poa napensis	Napa	Endangered	CA	
		-	bluegrass			
Pooideae	Poeae	Poa	Hawaiian	Endangered	HI	
		sandvicensis	bluegrass	L Č		
Pooideae	Poeae	Poa	Kauai	Endangered	HI	

Subfamily	Tribe	Scientific Name	Common Name	Status	Range
		siphonoglossa	bluegrass		

### Category 5: Species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to the target weed

According to the Angiosperm Phylogeny Classification System, Poaceae is a member of the terminal clade of the order Poales, known as the graminids, in which Poaceae is the terminal family and is most closely related to sister groups Joinvilleaceae and Ecdeiocoleaceae and less related to Flagellariaceae (Stevens 2001; Fig. 3). Poaceae is also most morphologically similar to these families. However, Poaceae is the only graminid family with species in North America.

The graminid clade is most closely related to the restiid clade, which consists of Anarthriaceae and sister groups Centrolepidaceae and Restionaceae. All three of these families lack representatives in North America. Poaceae is next most related to the cyperids, which is a clade that includes sister groups Xyridaceae and Eriocaulaceae at the base of the clade, followed by Mayacaceae, Thurniaceae, Juncaceae, and Cyperaceae (Fig. 3). Thurniaceae has no species in North America and although Mayaceae has a single representative in North America, it is a submerged aquatic species and so was not included in our list. Poaceae is less related to Rapataceae, which is a single branch between the cyperids and the bromeliads. Rapataceae has no species in North America. Poaceae is least related to the most ancestral groups in Poales, the bromeliads, which consists of Typhaceae and Bromeliaceae. These phylogenetic relationships are largely supported by other recent phylogenetic studies on Poales (Bouchenak-Khelladi et al. 2014).


Figure 3 Phylogenetic relationships among families in the Order Poales (Stevens 2001).

Poaceae shares morphological similarities with Juncaeae and Cyperaceae and biochemical similarities with Cyperaceae, namely the presence of C<sub>4</sub> photosynthetic

pathways. In fact, Cyperaceae is the second most C<sub>4</sub> species-rich family after Poaceae (Besnard et al. 2009). Species in Cyperaceae and Juncaceae are highly likely to be found in the same habitat as *Microstegium vimineum*. Many studies have noted co-occurrence of *M. vimineum* and species such as *Carex radiata, Carex pensylvanica, Cyperus croceus, Juncus tenuis*, and *Scirpus atrovirens* (Flory et al. 2007, Oswalt et al. 2007, Abrams and Johnson 2012). There are also reports of some Xyridaceae species, like the endangered *Xyris tennesseensis*, co-occurring with *M. vimineum* (Boyd and Moffett 2003). In order to represent these co-occurring families, we chose to include *Carex pensylvanica* and *Cyperus croceus* as representatives of Cyperaceae, *Juncus tenuis* as a representative of Juncaceae, and *Xyris tennesseensis* as a represent to represent Bromeliaceae, and *Sparganium eurycarpum* to represent Typhaceae. Even though we found no record of these three species co-occurring with *M. vimineum*, their inclusion will help further clarify host specificity within Poales.

The species proposed for testing represent all families within Poales that have species present in North America. We included two species with different genera to represent Cyperaceae, considering it is a frequently reported habitat associate of *M. vimineum*. While there are limitations to the conclusions we can draw considering the small number of species we included, the six families that Poaceae is most related to are not present in the North America, which implies significant genetic divergence between the target family and the next most susceptible family in the considered range.

# Category 6: Species in other orders that have some morphological or biochemical similarities to the target weed

Poales is ancestral to Commelinales and Zingiberales and all three orders are part of the commelinids, making them somewhat related. Other orders have families that share biochemical properties with Poaceae through the convergent evolution of C<sub>4</sub> photosynthetic pathways (Sage 2001). Some of these families include Amaranthaceae, Euphorbiaceae, Asteraceae, Polygonaceae, and Caryophyllaceae (Sage 2001). For the test plant list, we included *Commelina communis* to represent Commelinales because it co-occurs with *M. vimineum*, and *Canna indica* to represent Zingiberales because it is present in a number of southeastern and southern states in the U.S. where there is a possibility of co-occurrence.

Aside from the C<sub>4</sub> photosynthetic pathway, little is known about the biochemistry of *M. vimineum*. Poaceae in general are thought to lack the variety of secondary compounds found in dicotyledons, with herbivore defense due largely to their high silicate content (Tscharntke and Greiler 1995). However, Vicari and Bazely (1993) noted a large array of secondary compounds present in many grasses, most of which have been studied in cereal crop cultivars. Kaneta and Sugiyama (1973) identified various flavonoid compounds in grass species, including tricin (5,7,40-trihydroxy-30,50-dimethoxyflavone) in *M. vimineum*. Subsequent work, primarily in crop species, showed that tricin is a dominant flavone in cereal crops, including wheat, rice, barley, sorghum, oats, and maize (Zhou and Ibrahim 2010). Thus the crop species already on the test plant list will cover those plants known to have biochemical similarities to the target.

Category 7: Any plants on which the proposed biological control agent or its close relatives have been previously found or recorded to feed and/or reproduce

There is no proposed biological control agent at this time.

Table 3List of plant species recommended for testing to determine potential host<br/>range of candidate biological control agent of *Microstegium vimineum*<br/>(Trin.) A. Camus (Poales, Poaceae). Footnotes indicate important<br/>literature references: <sup>1</sup>Simao et al. 2010, <sup>2</sup>Redman 1995, <sup>3</sup>Emery et al.<br/>2011, <sup>4</sup>Flory et al. 2007, <sup>5</sup>Beauchamp et al. 2013, <sup>6</sup>Schafale and Weakley<br/>1990, <sup>7</sup>Cipollini et al. 2013, <sup>8</sup>Lee et al. 2012, <sup>9</sup>Boyd and Moffett 2003,<br/><sup>10</sup>Abrams and Johnson 2012, <sup>11</sup>Oswalt et al. 2007.

SPECIES NAME	COMMON NAME	ORIGIN	SIM. HAB.	CONSERV. STATUS	ECON. IMP.	REASON FOR INCLUSION			
ORDER Poales/ Family Poaceae									
Su	Subfamily Panicoideae/ Tribe Andropogoneae/ Subtribe Saccharinae								
Microstegium vimineum	Japanese stiltgrass	non- native				Target weed			
Miscanthus sinensis	Chinese silvergrass	non- native			Ornamental	Same subtribe			
Saccharum officinarum	sugarcane	non- native			Agriculture	Same subtribe, economically important			
Saccharum ravennae	ravennagrass	non- native			Ornamental	Same subtribe			
	Panicoideae/	Andropog	oneae/ Su	btribe Androp	ogoninae				
Andropogon arctatus	pinewoods bluestem	native		Threat. in FL		Same tribe, threatened in the same family			
Andropogon gerardii	big bluestem	native	yes <sup>1</sup>			Same tribe, associated habitat			
Schizachyrium littorale	shore little bluestem	native		Endang. in OH; Rare in PA		Same tribe, threatened in the same family			
Schizachyrium niveum Schizachyrium	pinescrub bluestem	native		Endang. in FL		Same tribe, threatened in the same family			
scoparium	little bluestem	native				Same tribe			

	Panicoideae	e/ Andropo	goneae/ S	ubtribe Anthis	tiriinae			
Bothriochloa	yellow	non-						
ischaemum	bluestem	native				Same tribe		
Heteropogon	sweet							
melanocarpus	tanglehead	native				Same tribe		
C 1	Panicoide	ae/ Androj	pogoneae/	' Subtribe Sorg	hinae	1		
Sorghastrum	Indianaraas	notivo				Sama triba		
nutans	Indialigrass	native				Same tribe		
Sorohum		non-				econom		
bicolor	sorghum	native			Agriculture	important		
Sorghum		non-						
halepense	Johnsongrass	native				Same tribe		
	Panicoideae	e/ Andropo	goneae/ S	ubtribe Rottbo	elliinae			
						Same tribe,		
~						threatened in		
Coelorachis	wrinkled			Endang. in		the same		
rugosa	Jointtail grass	native		MD, NJ		family		
eremocnioa	grass	non- native			Turf	Same tribe		
ophiarotaes								
	r anicoluea	le/ Anurop	ogoneae/			Same tribe		
						threatened in		
Tripsacum	Florida			Threat. in		the same		
floridanum	gamagrass	native		FL		family		
						Same tribe,		
_		non-				econom.		
Zea mays	corn	native			Agriculture	important		
	Panicoideae	/ Andropo	goneae/ S	ubtribe Arthra	xoninae			
						Same tribe,		
Arthraxon	small	non-	2			associated		
nispiaus		native	yes			naoitat		
T	Panicoide	eae/ Andro	pogoneae.	/ Subtribe unk	nown			
Imperata	cogongrass	non- native				Same tribe		
Cytinarica	cogoligiass	Daniaaida	 	Daniagaa	1	Same tribe		
		Panicolue	ae/ Tribe			Same		
						subfamily		
						associated		
Paspalum laeve	field paspalum	native	yes <sup>3</sup>			habitat		
Digitaria		non-				Same		
sanguinalis	crabgrass	native				subfamily		
						Same		
						sublamily,		
Dichanthelium	Hirst's			Candidate		the same		
hirstii	panicgrass	native		species		family		
Oplismenus	wavyleaf	non-	ves <sup>5</sup>			Same		

hirtellus ssp. undulatifolius	basketgrass	native				subfamily, associated habitat			
						Same			
Daniaum						subfamily,			
r unicum virgatum	switchgrass	native	ves <sup>1</sup>			habitat			
Stenotaphrum	St Augustine	native	yes			Same			
secundatum	grass	native			Turf	subfamily			
Panicoideae/ Tribe Chasmanthieae									
Chasmanthium	Indian					Same			
latifolium	woodoats	native	yes <sup>6</sup>		Ornamental	subfamily			
	Subfa	mily Aristi	doideae/	Tribe Aristidea	e				
Aristida	prarie three-								
oligantha	awn	native				Same family			
	Subfam	ily Arundi	noideae/	Tribe Arundin	eae				
Arundo donax	giant reed	non- native				Same family			
	A	rundinoid	eae/ Trib	e Molinieae	<u> </u>	· · · · ·			
Hakonechloa		non-							
macra	Hakone grass	native			Ornamental	Same family			
	Subfamily Danthonoideae/ Tribe Danthonieae								
						Same family,			
Danthonia	poverty		7			associated			
spicata   oatgrass   native   yes'   habitat									
Subfamily Chloridoideae/ Tribe Cynodonteae									
Flowsing indiag	Indian	non-				Same family			
Cunodon	goosegrass	non				Same family			
dactvlon	Bermudagrass	native			Turf	Same family			
						Same family.			
Muhlenbergia						associated			
schreberi	nimblewill	native	yes <sup>2</sup>			habitat			
						Same family,			
	San Joaquin			T . 1		threatened in			
orcuttia	valley Orcutt	nativa		federally		family			
indequalis	grass	native		tilleaterieu		Same family			
	nurnleton					associated			
Tridens flavus	tridens	native	ves <sup>7</sup>			habitat			
y	Sub	family Orv	zoideae/ '	Tribe Orvzeae		1			
						Same family,			
Leersia						associated			
virginica	whitegrass	native	yes <sup>2</sup>			habitat			
						Same family,			
	riaa	non-			A ani a14	economically			
Oryza sativa	псе	native			Agriculture	important			
Zizania texana	Texas wildrice	native		Federally		Same family,			

				endangered		threatened in the same				
Subfamily Rambusoideae/ Tribe Arundinariese										
	Sublamity Dambusolucae/ 111be Al unumaricae									
Arundinaria	giont cono	notivo				associated				
giganiea	<u>sigumeu</u>   giant cane   native   yes       nabitat									
	Su					Same family				
Gheoria striata	fowl	nativa	ves <sup>8</sup>			associated				
Giyceria siriaia	mannagrass	Pooide	yes ae/ Tribe	Poeae		Ildollat				
						Same family.				
Avena sativa	common oat	non- native			Agriculture	economically important				
					0	Same family,				
Calamagrostis						associated				
canadensis	bluejoint	native	yes <sup>1</sup>			habitat				
						Same family,				
Dog glandog	grove	nativo	4			associated				
Fou disoues	Kentucky	native	yes			naunai				
Poa pratensis	bluegrass	native			Turf	Same family				
Fosting willing	rad facaua	non-			Turf	Sama family				
Testucaruora	Teu Tescue	native			1 411	Same family				
Lolium perenne	perennial	non-			Agriculture	economically				
ssp. perenne	ryegrass	native			/Turf	important				
Schedonorus		non-				Same family, associated				
arundinaceus	tall fescue	native	yes <sup>3</sup>		Turf	habitat				
	•	Pooidea	e/ Tribe T	riticeae	•	•				
						Same family,				
Hordeum	common	non-				economically				
vulgare	barley	native			Agriculture	important				
						Same family,				
Secale cereale	cereal rve	native			Agriculture	important				
Secure cer cure	cerearrye	native			righteutture	Same family				
Triticum	common	non-				economically				
aestivum	wheat	native			Agriculture	important				
	OI	RDER Poal	les/ Famil	y Xyridaceae						
						Same order,				
						associated				
	Tennessee					nabitat,				
Xvris	vellow-eved			Federally		the same				
tennesseensis	grass	native	yes <sup>9</sup>	endangered		order				

		Poales/ Fa	mily Erio	caulaceae		
Eriocaulon decangulare	ten-angle pipewort	native		Endangered in TN		Same order, threatened in the same order
		Poales/	- Family Ji	Incaeae		
Juncus tenuis	poverty rush	native	ves <sup>4</sup>			Same order, associated habitat
		Poales/ F	amily Cv	peraceae		
Carex pensylvanica	Pennsylvania sedge	native	yes <sup>10</sup>			Same order, associated habitat
Cyperus croceus	Baldwin's flatsedge	native	yes <sup>11</sup>			Same order, associated habitat
	]	POALES/ ]	Family Br	omeliaceae		
Hechtia glomerata	guapilla	native				Same order
Poales/ Family 7	Гурһасеае					
Sparganium eurycarpum	broadfruit bur- reed	native				Same order
	ORDER	Commelin	ales/ Fam	ily Commelina	iceae	
Commelina communis	Asiatic dayflower	non- native	yes <sup>11</sup>			Closely related, associated habitat
	ORD	ER Zingib	erales/ Fa	mily Cannacea	e	
Canna indica	Indian shot	non- native				Closely related

## **Perspective of Risk**

Poaceae is a large, diverse, and economically important family, which necessitates extensive testing within this group. Our list thoroughly explores species within the subtribe Saccharinae, containing at least one representative from all genera present in the continental U.S. and Canada. Within the tribe Andropogoneae, we included at least one representative from most genera present in the continental U.S. and Canada. We also included at least one representative of each subfamily with species in the continental U.S. and Canada. There is considerable representation from subfamily Pooideae due to the significant number of agriculture, turf, and important native species. Although Poales contains 17 families, relatively few have species present in the continental U.S. and Canada and we included at least one representative species for all of those that do. We believe that our list represents Poaceae very well and untested species are likely to be represented at the subtribe or tribe level, which allows for better inferences to be made about risk of attack.

The largest risk associated with a biological control agent of *Microstegium vimineum* is the potential damage to economically important grass species. Considering the close phylogenetic relatedness of *M. vimineum* and sugarcane, allowable attack within the phylogenetic hierarchy is limited. Genomic mapping suggests that sorghum and sugarcane are genetically similar, especially in comparison with other grass crop species, such as corn (Dufour et al. 1997). Thus, based on the hypothesis that phylogenetically related species are more likely to be susceptible to attack than distantly related species, sorghum and sugarcane are at the greatest risk and if significant damage is observed on either of these, the biological control agent cannot be considered. Thus, a successful agent would likely be genus or species-specific to *M. vimineum*.

#### Chapter 2

## EFFECT OF MOWING HEIGHT, MOWING TIMING, AND LEAF MULCH ADDITION ON JAPANESE STILTGRASS

#### Introduction

Previous studies have explored multiple chemical and nonchemical control methods for Japanese stiltgrass. Hand-pulling is a time-intensive method that does not significantly reduce cover or biomass in large invasions (Flory and Lewis 2009; Ward and Mervosh 2012). While some herbicides (e.g. glufosinate, glyphosate, and fenoxaprop-*p*-ethyl) have proved effective in controlling Japanese stiltgrass, these methods are controversial, limited by permits or bans, or harmful to resident native plant populations, even at low doses (Judge et al. 2005a; Judge et al. 2005b; Ward and Mervosh 2012). Prescribed fires applied in the fall can reduce Japanese stiltgrass cover by 79% and biomass by 90%, but these fires also kill small shrubs and trees and therefore detrimentally affect the resident native community (Flory and Lewis 2009; Ward and Mervosh 2012). Mowing is a potential method for successful control as it can reduce Japanese stiltgrass cover and biomass (Flory and Lewis 2009). While the recommended time to mow is between flowering and seed set, mowing any time after midsummer can reduce Japanese stiltgrass cover and biomass similarly (Shelton 2012; Ward and Mervosh 2012). However, the effect of mow height on Japanese stiltgrass regrowth and reproductive output and on the resident plant community has not been evaluated.

Leaf litter disturbance plays a significant role in the spread and success of Japanese stiltgrass. Japanese stiltgrass presence is positively and significantly associated with shallower litter depths (Barden 1987; Huebner 2010), and in a study where leaf litter was removed, the linear spread of Japanese stiltgrass was 4.5 times greater than in areas where leaf litter was undisturbed (Oswalt and Oswalt 2007). In areas that were sown with Japanese stiltgrass seeds and covered with a double-thick layer of litter, ultimate seed establishment and survival were not significantly reduced, although when seeds were sown on top of a thick layer of litter, germination rates decreased significantly (Schramm and Ehrenfeld 2010).

In this study, we explored the effects of mow height, mow time, and presence of added leaf mulch on the regrowth and reproductive output of Japanese stiltgrass, as well as the effect on the resident plant community. Although previous studies have examined the effects of litter depth on the survival and germination of sown Japanese stiltgrass seeds, we studied the effects of different leaf litter depths on the already present Japanese stiltgrass population. Additionally, mowing height has not been explored in depth. If mowing at a higher height can provide similar results as mowing at ground level, land managers may better control large-scale invasions for which string trimmers are an uneconomical option. Lastly, land managers may find it difficult to monitor intensely enough to act within the window between flowering and seed set, so we explored the interaction between mowing height and mowing time to determine whether these factors may act synergistically to substantially reduce Japanese stiltgrass populations. Overall, we aimed to gather information that may improve management plans for Japanese stiltgrass.

#### **Materials and Methods**

#### Study Area

We conducted this study in the White Clay Creek State Park, a 1,375-hectare park located near Newark in New Castle County, Delaware. In 2014, we established plots at a site on the east side of the park, located at 39.712207 N, 75.757684 W. In 2015, we established plots at a different site nearby, located at 39.709762 N, 75.7562403 W. Our sites were in modified successional forest dominated by tuliptree (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), wild black cherry (*Prunus serotina* Ehrh.), sycamore (*Platanus occidentalis* L.), and American beech (*Fagus grandifolia* Ehrh.). The understory and shrub layers were dominated by spicebush (*Lindera benzoin* (L.) Blume), sassafras (*Sassafras albidum* (Nutt.) Nees), multiflora rose (*Rosa multiflora* Thunb.), and autumn olive (*Elaeagnus umbellata* Thunb.). The average annual precipitation in Newark, Delaware is 117.4 cm and the average temperature during the summer months is 24.1°C (75.3°F; NOAA 2015).

#### Experimental Design

In 2014, we established 20 0.5-m by 0.5-m plots at least 1 m apart in late August, arranged in a randomized complete block design with five replicates and four treatments: 1) mow at ground level, 2) mow at 5 cm, 3) mow at 10 cm, or 4) control (no mowing). Due to the size of the plots, the grass was cut using shears to simulate a mowing treatment. All treatments were applied in late August between flowering and seed set of Japanese stiltgrass.

In 2015, we established 70 0.5-m by 0.5-m plots at least 1 m apart in late April, arranged in a randomized complete block design with 10 replicates of seven treatments: 1) leaf mulch applied 3 cm deep (M3), 2) leaf mulch applied 8 cm deep

(M8), 3) early (July) mow at 0 cm (E0), 4) early (July) mow at 10 cm (E10), 5) late (September) mow at 0 cm (L0), 6) late (September) mow at 10 cm (L10), or 7) control (C; no mulch or mow). The leaf mulch consisted of leaves that were collected from private properties by a local landscaping company in fall 2014 and left in an unturned pile throughout the winter and early spring. Subsample analysis indicated the leaf mulch was dominated by oak species. Species identified include: *Quercus alba* L., *Q. falcata* Michx., *Q. ilicifolia* Wangenh., *Q. palustris* Münchh., *Q. phellos* L., *Q. rubra* L., *Q. velutina* Lam., *Acer rubrum, Fagus grandifolia, Prunus americana* Marshall, and *Magnolia* sp. Leaf mulch was applied in late April before germination of Japanese stiltgrass vegetative growth, and the late mows occurred in mid-September, between flowering and seed set of Japanese stiltgrass in our plots. Due to the size of the plots, the grass was again cut using hedge shears to simulate a mowing treatment.

#### Data Collection

Prior to the application of treatments, all plots were surveyed for species composition and percent cover for all species. In 2014, surveys were conducted on 28 August, and in 2015 surveys were conducted on 24 April, 30 July, and 11 September. The same surveys were conducted in October both years, and plots were then destructively harvested. In the laboratory, the harvested vegetation was sorted into Japanese stiltgrass and non-Japanese stiltgrass vegetation ("other"). Japanese stiltgrass seed spikelets, if present, were removed, counted, and classified as chasmogamous (CH) or cleistogamous (CL). The seeds, Japanese stiltgrass vegetation, and "other" vegetation were dried separately in a large agronomy oven at 37 °C to constant mass and then weighed.

#### Data Analysis

We used two-way ANOVAs by block and treatment to analyze the effects of treatment on Japanese stiltgrass cover and biomass, Japanese stiltgrass seed production and seed biomass, and "other" vegetation biomass. Data were log-transformed to improve normality (Shapiro-Wilks test) and equality of variances (Levene's test) when necessary; non-transformed means and standard errors are shown in graphs. Post-hoc Tukey HSD tests were used to determine differences between treatments.

## **Results and Discussion**

Treatment Effects on Japanese Stiltgrass

In 2014, all mowing treatments provided some level of Japanese stiltgrass control. Mowing at 10 cm was least effective, reducing cover by 57% and biomass by 59% (Fig. 4). Mowing at 5 cm was moderately effective, reducing cover by 90% and biomass by 79%. Mowing at ground level reduced stiltgrass cover by 98% and biomass by >99%, making it the most effective treatment. All treatments reduced seed production by at least 85% (Fig. 5). Mowing at ground level reduced seed production by >99%. These results suggest that when mowing between flowering and seed set, mowing lower is more effective at reducing Japanese stiltgrass cover, biomass, and seed production.



Figure 4 Average ( $\pm$ SE) Japanese stiltgrass (A) percent cover and (B) biomass per plot at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).



Figure 5 Average ( $\pm$ SE) Japanese stiltgrass seed spikelets per plot at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

In 2015, all treatments except the 3-cm leaf mulch (M3) addition significantly reduced cover and biomass of Japanese stiltgrass compared to control plots (Fig. 6). Mowing at 10 cm had a similar impact regardless of timing. Mowing at 10 cm in July (E10) reduced cover by 56% and biomass by 57%, while mowing at 10 cm in September (L10) reduced cover by 63% and biomass by 68% (Fig. 6). Seed production was reduced by 63% and 76% with these two treatments, respectively (Fig. 7). Applying an 8-cm layer of leaf mulch and mowing at ground level in July controlled stiltgrass more effectively, with the 8-cm layer of mulch (M8) reducing

cover by 91% and biomass by 94%, and mowing at ground level in July (E0) reducing cover by 92% and biomass by 97%. Seed production was reduced by 88% and 92% with these two treatments, respectively. The most effective treatment was mowing at ground level in September (L0), which reduced cover, biomass, and seed production by 98%.



Figure 6 Average ( $\pm$ SE) Japanese stiltgrass (A) percent cover and (B) biomass per plot at the end of the season in 2015 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).



Figure 7 Average ( $\pm$ SE) Japanese stiltgrass seed spikelets per plot at the end of the season in 2014 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

Prior to this study, mowing height had not been explicitly investigated and our results suggest that it can significantly impact the success of mowing on reducing Japanese stiltgrass cover and seed production. Previous studies have found that mowing near the ground with a string trimmer just before seed set can reduce cover by 70-99% (Flory and Lewis 2009; Pomp et al. 2010; Ward and Mervosh 2012). Fewer studies have examined the effects of mowing on Japanese stiltgrass biomass, but Flory and Lewis (2009) found that mowing with a string trimmer can reduce biomass by 95%. We observed similar reductions in cover and biomass with our late season,

ground-level mowing treatment. Concerning seed production, Ward and Mervosh (2012) found that mowing did significantly reduce seed production but not as effectively as herbicides, such as fenoxaprop-*p*-ethyl and glyphosate, which achieved >99% reductions. However, our results demonstrated that mowing just before seed production at ground level can reduce seed production as effectively as herbicide treatments.

Although land managers generally recommend mowing Japanese stiltgrass just before seed set, previous studies suggest that the timing of mowing does not affect its efficacy as a control treatment (Shelton 2012; Ward and Mervosh 2012). However, our results suggest that timing can be an important factor. Interestingly, we observed no significant differences in cover or biomass between our early and late mows at 10 cm, but our late mow at 0 cm had significantly lower cover and seed production compared the early mow at 0 cm. This suggests that Japanese stiltgrass that is mowed low just before seed set does not have the time or the physical structure left to sufficiently regrow before frost kills the plants. Although we did not conduct a multiyear study, other studies demonstrate that even if mowing is not effective the first year, multiple years of treatment can reduce Japanese stiltgrass cover by 82% and decrease the seed bank by 93% after 3 years (Judge et al. 2008). Although the number of years of treatment necessary may vary depending on invasion history, these results emphasize the need for yearly treatment to effectively deplete the seed bank.

Leaf mulch additions varied in their effectiveness depending on thickness. A previous study by Schramm and Ehrenfeld (2000) found that Japanese stiltgrass germination, establishment, and growth were not affected by a litter layer artificially thickened to twice the natural litter layer, ranging from 3.4 to 5.0 cm thick when

doubled. The results from our 3-cm leaf mulch addition confirm these findings as we did not see significant reductions in Japanese stiltgrass cover, biomass, or seed production at that depth. However, when we applied 8 cm of leaf mulch, reductions in cover, biomass, and seed production rivaled those of mowing at ground level. This suggests that there may be a minimum barrier that must be achieved to impact germination or seedling establishment. A significant leaf mulch barrier may control Japanese stiltgrass by limiting sunlight to seeds, reducing water infiltration to the soil layer, or providing a physical barrier for seeds or seedlings (Xiong and Nilsson 1999). These effects may be enhanced due to the very small size of Japanese stiltgrass seeds (Kostel-Hughes et al. 2005). Halvorson et al. (2016) demonstrated that herbaceous seeds that were washed with extracts from overstory hardwood tree species (i.e. maple, oak, and poplar) had reduced germination rates. High litter extract concentrations from these tree species lead to increased concentrations of positively charged ions, such as magnesium, calcium, and potassium, which may increase osmolality and salinity. These factors are known to reduce and delay successful germination (Almansouri et al. 2001; Li et al. 2015). Considering our leaf mulch was dominated by oak and maple species, the combination of specific ion toxicities and osmotic stresses from leaf chemicals may have reduced Japanese stiltgrass germination, but only at sufficient thickness.

### Treatment Effects on Plant Community

In 2014, before application of the mowing treatments in August, plant communities in the plots were dominated by Japanese stiltgrass, but also included Japanese honeysuckle, Virginia creeper, and poison ivy (Table 4). In October 2014, six weeks after treatment, plant communities in plots mowed at 10 cm were still

dominated by Japanese stiltgrass, but included Japanese honeysuckle and greenbrier at higher percent cover than prior to treatment. Plant communities in plots mowed at 5 cm and 0 cm plots became dominated by Japanese honeysuckle with relatively low cover of Japanese stiltgrass (Table 4). Remaining cover was composed of less abundant species or ground cover (i.e. bare ground, lichens, litter), which we did not measure.

Table 4Average cover (±SE) per plot of the most abundant species in the plant<br/>communities before treatment (28 August) and six weeks after treatment<br/>(9 October), 2014.

	Cover (±SE) Before Treatment	Cover (±SE) After Treatment
Control		
Microstegium vimineum (Japanese stiltgrass)	$72.0 \pm 3.39$	$77.0 \pm 4.64$
Lonicera japonica (Japanese honeysuckle)	$7.0 \pm 1.22$	$8.0 \pm 1.14$
Rosa multiflora (multiflora rose)	$4.0 \pm 1.0$	$2.0 \pm 1.0$
Parthenocissus quinquefolia (Virginia creeper)	$3.5 \pm 1.5$	$5.0 \pm 0.0$
Mow at 10 cm		
Microstegium vimineum (Japanese stiltgrass)	$79.0 \pm 3.85$	$32.8 \pm 6.71$
Lonicera japonica (Japanese honeysuckle)	$5.8 \pm 1.63$	$16.6 \pm 2.20$
Parthenocissus quinquefolia (Virginia creeper)	$3.0 \pm 0.0$	$1.0 \pm 0.0$
Smilax spp. (greenbrier)	$1.0 \pm 0.0$	$6.5 \pm 2.47$
Mow at 5 cm		
Microstegium vimineum (Japanese stiltgrass)	$69.0 \pm 3.85$	$7.8 \pm 2.89$
Lonicera japonica (Japanese honeysuckle)	$7.2 \pm 2.16$	$11.0 \pm 1.74$
<i>Toxicodendron radicans</i> (poison ivy)	$4.0 \pm 0.71$	$0.0 \pm 0.0$
Mow at 0 cm		
Microstegium vimineum (Japanese stiltgrass)	$77.0 \pm 4.82$	$1.4 \pm 0.61$
Lonicera japonica (Japanese honeysuckle)	$10.0 \pm 2.83$	$6.2 \pm 1.21$

Overall, only mowing at 10 cm affected the total cover of non-Japanese stiltgrass vegetation, which increased by 127% compared to control plots (Fig. 8A).

Japanese honeysuckle and cat greenbrier cover increased most noticeably. Biomass of non-Japanese stiltgrass vegetation was only significantly impacted when mowed at ground level, which reduced biomass by 81% (Fig. 8B). These results suggest that mowing does not control other plant species as well as it controls Japanese stiltgrass in this environment. In fact, mowing at 10 cm and 5 cm seems to stimulate growth of the invasive vine Japanese honeysuckle. However, considering that increases in cover did not correlate with increases in biomass, we suggest that mowing may have an effect on the health of individual plants, leading to disproportionately low biomass despite increases in cover. Overall, the only treatment that significantly repressed the resident plant community in 2014 was mowing at ground level.



Figure 8 Average ( $\pm$ SE) non-Japanese stiltgrass resident plant community (A) percent cover and (B) biomass at the end of the season in 2014 for each treatment (control, mow at 10 cm, mow at 5 cm, and mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

In 2015, before application of the mulch treatments in April, Japanese stiltgrass had not yet germinated, so plant communities in the plots were dominated by violets and Japanese honeysuckle. In October 2015, nearly 6 months following treatment, plant communities were still dominated by Japanese stiltgrass, but also included spicebush, oriental bittersweet, Japanese honeysuckle, and ash seedlings (Table 5). Before application of the mowing treatments in July and September, plant communities in mowing plots were dominated by Japanese stiltgrass, with relatively low cover of Japanese honeysuckle, oriental bittersweet, spicebush, and ash seedlings. In October, 2.5 months following the early mowing treatments, plant communities in the plots mowed at 10 cm (E10) were still dominated by Japanese stiltgrass while plant communities in the plots mowed at 0 cm (E0) had similar cover of Japanese stiltgrass and oriental bittersweet. One month following the late mowing treatments, plant communities in the plots mowed at 10 cm (L10) were still dominated by Japanese stiltgrass and plots mowed at ground level (L0) had low cover of oriental bittersweet, Japanese stiltgrass, and Japanese honeysuckle (Table 5). Remaining cover was composed of less abundant species or ground cover (i.e. bare ground, lichens, litter), which we did not measure.

Table 5Average cover (±SE) per plot of the most abundant species in the plant<br/>communities before treatment (24 April, 30 July, or 11 September) and<br/>in October following treatment, 2015.

	Cover (±SE)	Cover (±SE)
	Before Treatment	After Treatment
24 April		
Control (C)		
Microstegium vimineum (Japanese stiltgrass)	$0.0 \pm 0.0$	$73.5 \pm 5.88$
<i>Viola</i> sp.	$13.7 \pm 2.21$	$0.0 \pm 0.0$
Lonicera japonica (Japanese honeysuckle)	$6.4 \pm 1.50$	$4.1 \pm 1.07$
<i>Celastrus orbiculatus</i> (oriental bittersweet)	$0.0 \pm 0.0$	$7.6 \pm 1.52$
3 cm of leaf mulch (M3)		
Microstegium vimineum (Japanese stiltgrass)	$0.0 \pm 0.0$	$57.5 \pm 6.41$
<i>Viola</i> sp.	$15.4 \pm 3.46$	$0.0 \pm 0.0$
Lindera benzoin (spicebush)	$3.0 \pm 0.71$	$9.5 \pm 4.60$
Lonicera japonica (Japanese honeysuckle)	$4.0 \pm 0.62$	$4.3 \pm 0.70$
Celastrus orbiculatus (oriental bittersweet)	$0.0 \pm 0.0$	$7.4 \pm 1.04$
8 cm of leaf mulch (M8)		
Microstegium vimineum (Japanese stiltgrass)	$0.0 \pm 0.0$	$6.2 \pm 1.50$
Viola sp.	$12.1 \pm 1.45$	$0.0 \pm 0.0$
Lonicera japonica (Japanese honeysuckle)	$8.0 \pm 2.87$	$3.8 \pm 0.52$
Fraxinus sp.	$0.0 \pm 0.0$	$4.3 \pm 0.98$
<i>Celastrus orbiculatus</i> (oriental bittersweet)	$0.0 \pm 0.0$	$3.0 \pm 0.40$
30 July		
Control (C)		
Microstegium vimineum (Japanese stiltgrass)	$60.0 \pm 6.79$	$73.5 \pm 5.88$
Celastrus orbiculatus (oriental bittersweet)	$8.4 \pm 1.56$	$7.6 \pm 1.52$
Lonicera japonica (Japanese honeysuckle)	$3.3 \pm 0.89$	$4.1 \pm 1.07$
Smilax spp.	$4.0 \pm 1.0$	$4.0 \pm 0.71$
Early mow at 10 cm (E10)		
Microstegium vimineum (Japanese stiltgrass)	$54.0 \pm 7.84$	$32.5 \pm 5.16$
Celastrus orbiculatus (oriental bittersweet)	$5.9 \pm 1.18$	$8.5 \pm 1.31$
Lonicera japonica (Japanese honeysuckle)	$5.7 \pm 1.98$	$3.5 \pm 0.66$
Fraxinus sp. (ash seedlings)	$3.0 \pm 0.94$	$0.0 \pm 0.0$
Early mow at 0 cm (E0)		
Microstegium vimineum (Japanese stiltgrass)	595+665	$63 \pm 0.69$
<i>Celastrus orbiculatus</i> (oriental bittersweet)	$8.3 \pm 1.29$	$6.6 \pm 1.13$

Lonicera japonica (Japanese honeysuckle) Fraxinus sp. (ash seedlings)	$5.8 \pm 1.51$ $3.8 \pm 1.24$	$1.8 \pm 0.18$ $0.0 \pm 0.0$
11 September		
Control (C)		
Microstegium vimineum (Japanese stiltgrass)	$68.5 \pm 5.0$	$73.5 \pm 5.88$
Celastrus orbiculatus (oriental bittersweet)	$5.4 \pm 0.58$	$7.6 \pm 1.52$
Lonicera japonica (Japanese honeysuckle)	$4.4 \pm 0.90$	$4.1 \pm 1.07$
Smilax spp.	$3.0 \pm 0.0$	$4.0 \pm 0.71$
Late mow at 10 cm (L10)		
Microstegium vimineum (Japanese stiltgrass)	$59.5 \pm 6.26$	$27.3 \pm 5.25$
Celastrus orbiculatus (oriental bittersweet)	$6.0 \pm 0.67$	$9.3 \pm 1.38$
Lindera benzoin (spicebush)	$4.0 \pm 0.71$	$0.0 \pm 0.0$
Lonicera japonica (Japanese honeysuckle)	$3.7 \pm 1.32$	$1.7 \pm 0.26$
Late mow at 0 cm (L0)		
Microstegium vimineum (Japanese stiltgrass)	$70.5 \pm 6.30$	$1.4 \pm 0.15$
Celastrus orbiculatus (oriental bittersweet)	$4.9 \pm 0.73$	$3.0 \pm 0.42$
Lonicera japonica (Japanese honeysuckle)	$4.0 \pm 1.54$	$1.0 \pm 0.0$

Overall, many of the treatments did not significantly reduce the total cover and biomass of the non-Japanese stiltgrass plant community in 2015. Mowing at ground level in July (E0) did not significantly reduce cover but it did reduce biomass by 75% (Fig. 9). Mulching at 8 cm had a moderate effect on both, reducing cover by 52% and biomass by 58%. Mowing at ground level in September had the most significant effect on other vegetation, reducing cover by 80% and biomass by 89%.



Figure 9 Average ( $\pm$ SE) resident non-Japanese stiltgrass plant community (A) percent cover and (B) biomass at the end of the season in 2015 for each treatment. Treatments were C (control), M3 (3 cm of leaf mulch), E10 (July mow at 10 cm), L10 (September mow at 10 cm), M8 (8 cm of leaf mulch), E0 (July mow at 0 cm), L0 (September mow at 0 cm). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

Other mowing experiments have demonstrated reductions in cover and biomass of the resident plant community similar to those we observed in 2015 (Judge et al. 2008). Others have demonstrated no change (Flory and Lewis 2009) and some demonstrated increases in species richness, cover, and biomass, especially of native species (Pomp et al. 2010; Ward and Mervosh 2012). There seems to be little evidence of a consistent effect of mowing on non-target plants, even in studies conducted over multiple years, which may be due in part to site-specific characteristics. Our site was highly invaded by woody invasive plant species. Some studies indicate that woody species are especially impacted by mowing, which may partly explain the extreme reductions in non-Japanese stiltgrass cover and biomass from ground level mowing (Judge et al. 2008). However, other studies suggest that Japanese honeysuckle growth is stimulated by mowing (Stransky 1984; Nuzzo 1997). Despite Japanese honeysuckle being present in all of our plots, we only observed this effect after mowing at 10 cm in 2014 (Table 4). The inconsistent response of Japanese honeysuckle and other invasive plant species to mowing may be a product of mowing height, the age of the invasion, or other environmental characteristics. Considering the low presence and cover of desirable native species at our study site, we cannot draw conclusions about the impact of mowing on the native plant community in this environment.

Mulching had a less pronounced impact on the biomass and cover of the resident non-Japanese stiltgrass plant community. Evidence suggesting that thick layers of leaf litter are more detrimental to small-seeded species than large-seeded species may explain why Japanese stiltgrass, which has very small seeds, was controlled under a thick layer of leaf mulch, while other species that have larger seeds, such as spicebush (*Lindera benzoin*) and ash (*Fraxinus* sp.), were able to emerge through the leaf mulch. Additionally, litter has a stronger effect on germination than establishment (Xiong and Nilsson 1999). Thus, our leaf mulch may have controlled early season growth and annual plants, such as Japanese stiltgrass, more effectively than perennial plants.

Overall, most treatments provided some level of Japanese stiltgrass control, which suggests that there is potential for effective Japanese stiltgrass control using non-chemical methods. If land managers are less concerned with preserving the

resident plant community (i.e. if the other plants are mostly invasive species), mowing just before seed production in the fall at ground level can provide excellent control even over one season. However, management will need to continue yearly for multiple seasons to ensure sufficient reductions in seed production and eventual depletion of the seed bank. If land managers are concerned about preserving the resident plant community, leaf mulch application at sufficient depths (8 cm or more in mid-Atlantic forests) may provide adequate control of Japanese stiltgrass while still allowing for native shrubs and perennials to emerge and survive. It is important to note that leaf mulch should consist of hardwood leaves, such as maple and oak, because seasonal decomposition rates are low (Halvorson et al. 2016). Application of leaf mulch can occur once per season any time before Japanese stiltgrass germination, which provides more temporal flexibility for land managers. Leaf mulch may even be added as a barrier to invasion of Japanese stiltgrass if areas of great concern are threatened. Furthermore, the efficacy of these Japanese stiltgrass control methods may be improved with the integration of restoration plantings of competitive native species. A study on invasive mile-a-minute weed (Persicaria perfoliata (L.) H. Gross) found an additive effect of native species seeding with direct weed control in suppressing milea-minute weed (Cutting and Hough-Goldstein 2013). This integrated approach may be especially important at sites that are invaded by multiple different invasive species, such as our study site, to prevent reinvasion by secondary invasive plants and promote recovery of the native plant community (Lake et al. 2013). This strategy of pro-active leaf mulch treatment is promising for limiting the spread of Japanese stiltgrass and together with native plant seeding and other non-chemical or chemical management strategies, may lead to a reduction in Japanese stiltgrass populations and their damage.

#### Chapter 3

## EFFECT OF CUTTING AT DIFFERENT INTERNODAL REGIONS ON JAPANESE STILTGRASS GROWTH IN THE FIELD AND GREENHOUSE

#### Introduction

Although most control efforts for Japanese stiltgrass focus on chemical and mechanical control methods, a few researchers are currently exploring the possibility of classical biological control. Classical biological control, in the context of controlling an invasive plant species, is a management strategy that involves identifying a pest species' native range and surveying that range for host-specific herbivores, most often insects. If an insect demonstrates a high degree of host specificity in the native country, this insect can be exported to guarantine facilities in the invaded range, where host range testing must be conducted to determine host specificity. In China, field surveys have been conducted by Jialiang Zhang and Jianging Ding of the Chinese Academy of Sciences to identify insects that feed on Japanese stiltgrass in its native range that may act as potential biological controls agents in the United States (J. Ding, personal communication). After preliminary host range testing was conducted at facilities in China, an unidentified mealybug (Sternorrhyncha: Pseudococcidae) showed promise. If this insect is imported to quarantine facilities in the United States, the facility will require many Japanese stiltgrass plants not only for host range testing purposes, but also to feed and sustain the insect colony.

Most studies in which Japanese stiltgrass has been propagated for experimentation used plants grown from seed (Judge et al. 2005; Craig et al. 2011; Huebner 2011; Novy et al. 2013) or seedlings transplanted from the field (Cole and Weltzin 2005; Touchette and Romanello 2010). As an alternative method, we wanted to determine if field cuttings of Japanese stiltgrass are effective and reproductively viable when planted in a greenhouse setting. We believe this may be possible considering the growth habit of Japanese stiltgrass. Seeds germinate in late spring and seedlings grow upright to about 25 cm, after which tillers begin to bend over. Aerial roots then sprout from nodes along these tillers and establish in the soil, which can lead to a sprawling growth habit (Lee et al. 2014). If these stem cuttings are viable, we also wanted to determine whether the number of nodes below the point of cutting affect the growth and reproductive abilities of the cutting in the greenhouse. With this, we aimed to gain insight into the efficiency of growing a large quantity of plants from cuttings in the hope that it may be faster than growing from seed, which could be helpful in future experiments. We also wanted to determine if the number of nodes below the point of cutting affects the growth and reproductive abilities of the Japanese stiltgrass left in the field. We hoped to gain insight into optimal cutting heights for the most successful limitation of Japanese stiltgrass regrowth and reproduction.

#### **Materials and Methods**

#### Study Area

We conducted this study in White Clay Creek State Park, a 1,375-hectare park located near Newark in New Castle County, Delaware. We established plots at two sites that were dominated by Japanese stiltgrass but varied in environmental conditions and successional stage. Site 1 (39.7027 N, 75.7057 W) was located in an early successional, partly open field. Other vegetation included goldenrod (*Solidago* spp.), Japanese honeysuckle (*Lonicera japonica*), wineberry (*Rubus phoenicolasius* Maxim.), and Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.). Site 2 (39.7129 N, 75.7573 W) was located in modified successional forest. The canopy layer was dominated by tuliptree (*Liriodendron tulipifera* L.), red maple (*Acer rubrum* L.), wild black cherry (*Prunus serotina* Ehrh.), sycamore (*Platanus occidentalis* L.), and American beech (*Fagus grandifolia* Ehrh.). The understory and shrub layers included spicebush (*Lindera benzoin* (L.) Blume), sassafras (*Sassafras albidum* (Nutt.) Nees), multiflora rose (*Rosa multiflora* Thunb.), and autumn olive (*Elaeagnus umbellata* Thunb.). The average annual precipitation in Newark, Delaware is 117.4 cm and the average temperature during the summer months is 24.1°C (75.3°F, NOAA 2015).

#### Experimental Design

For the field experiment, we established 10 replicates of six treatments at each site in a randomized block design in late July 2014. For each sample, ten Japanese stiltgrass stems were gathered and each stem was cut at an internodal region determined by one of six treatments: (B1) below the first node (i.e. near ground level), (B2) between the first and second nodes, (B3) between the second and third nodes, (B4) between the third and fourth nodes, (B5) between the fourth and fifth nodes, and (C) control treatment (no cutting). The remaining parts of the cut stems were loosely tied together using colored flagging tape that corresponded to the appropriate treatment. For the greenhouse experiment, the cut stems from all B1, B2, B3, B4, and B5 treatment samples were collected and planted in the greenhouse. Each sample was planted in a small pot using vermiculite with a thin layer of peat moss at the bottom to prevent loss of vermiculite through the drainage holes. The potted samples were placed in a greenhouse mist room for approximately two weeks. Upon removal from the mist room, each sample was transplanted into a 12.7-cm diameter pot using ProMix potting soil and placed in a greenhouse with temperatures ranging from 22-24°C. Plants were watered daily and fertilized weekly with Peters Excel Multi-Purpose 21-5-20, mixed at 200 ppm N.

#### Data Collection

In the field experiment, samples were monitored weekly after cutting for nine weeks. Each week, we recorded maximum height, presence of seed spikelets (yes/no), and general condition for each sample. Aboveground biomass was harvested in late September 2014. For all samples, seed spikelets were removed, counted, and classified as chasmogamous (CH) or cleistogamous (CL). In the greenhouse experiment, stem survival and maximum height data were collected for each sample after transplant from the mist room. Weekly maximum height data and dates of first seed emergence (checked daily) were collected over six weeks for samples from Site 1 and seven weeks for samples from Site 2. In early October 2014, final maximum height data were collected and aboveground biomass was harvested. Seed spikelets were removed, counted, and classified as chasmogamous (CH) or cleistogamous (CL) for two randomly selected replicates from each site. These data were used to estimate reproductive success for the rest of the replicates. The seeds and vegetation from both

the field and greenhouse experiments were dried in a large agronomy oven at 27°C for five weeks and weighed for reproductive and aboveground biomass data.

#### Data Analysis

We used two-way ANOVAs by block and treatment to analyze the effects of treatment on Japanese stiltgrass height, biomass, time to seed set, seed production, and seed biomass. Biomass data were square-root-transformed and height and seed data were log-transformed to improve normality (Shapiro-Wilks test) and equality of variances (Levene's test) when necessary; non-transformed means and standard errors are shown in graphs. Post-hoc Tukey HSD tests were used to determine differences between treatments. T-tests were used to compare average values per plot at the two sites for data where significant block effects were observed.

#### Results

## Field Experiment

For the Japanese stiltgrass that recovered after cutting, we observed resprouting from the node immediately below the cut, leading to loss of apical dominance and a multi-stemmed plant. Japanese stiltgrass that was cut below the first node rarely recovered, but if it did, new growth emerged from roots or nodes hidden under soil. The maximum height of the stems nine weeks after cutting differed significantly by treatment (Table 6). There was also a significant block effect (Table 6), probably due to differential growth at the two sites. On average, Japanese stiltgrass stems at Site 1, the sunnier site, were taller than those at Site 2 immediately after cutting (t(12) = 6.98, p < 0.0001) and at the end of the season (t(13) = 7.07, p <0.0001; Figs. 10, 11). At Site 1, weekly maximum height trends differed among treatments (Fig. 10). Average maximum height of control stems increased from 52.5 cm immediately after cutting to 96.7 cm at the end of the season. Stems cut at B4 and B5 also demonstrated increasing maximum heights throughout the season, with B5 increasing from 38.1 cm to 76.7 cm at the end of the season. Stems cut at B1 and B2 demonstrated decreasing maximum heights, with B1 decreasing from 6.5 cm to 3.7 cm and B2 decreasing from 12.8 cm to 11.8 cm (Fig. 10). At Site 2, weekly maximum height trends differed less distinctly (Fig. 11). Maximum height of control stems increased from 30.1 cm immediately after cutting to 39.0 cm at the end of the season. Stems cut at B1 decreased from 2.7 cm to 2.4 cm (Fig. 11).

Japanese stiltgrass biomass also differed significantly by treatment and by block (Table 6), again probably due to site differences. Overall, biomass at Site 1 was greater than biomass at Site 2 (t(18) = 5.65, p < 0.0001). Including replicates from both sites, cutting below the 4th node (B4) reduced the final aboveground biomass by 78% and cutting below the 1st node (B1) reduced the aboveground biomass by 98% (Fig. 12). The number of weeks it took to produce seed differed significantly by block but not by treatment (Table 6). Japanese stiltgrass produced seed more quickly at Site 1, taking an average of 6.5 weeks compared to 7.5 weeks at Site 2 (t(10) = -9.89, p < 0.0001). Total seed production differed significantly by treatment and by block (Table 6). Japanese stiltgrass at Site 1 produced more than three times as many seed spikelets as Japanese stiltgrass at Site 2 (Site 1:  $21.2 \pm 0.87$ ; Site 2:  $6.75 \pm 0.36$ ; t(10) = 3.52, p = 0.005). Across both sites, cutting below the 5th node (B4) reduced the number of seed spikelets by 46% and cutting below the 1st node (B1) reduced the number of seed spikelets by 76%. Cutting below the 1st node (B1) reduced the number of seed

spikelets to 0 (Fig. 13). There was also a significant block effect on seed type (F(19,45) = 3.33, p = 0.0005). At Site 1, 52.6% of seeds produced were chasmogamous and at Site 2, 71.5% of seeds were chasmogamous (t(18) = -3.98, p = 0.0008). Seed type did not differ significantly by treatment (F(4,45) = 1.51, p = 0.22).

Table 6Results of ANOVA evaluating Japanese stiltgrass maximum final height,<br/>biomass, and number of seed spikelets across treatments at two sites.

		Max Final	timum Height	Biomass		Weeks to Seed Set		Seed Spikelets	
Source	df	F	р	F	р	F	р	F	р
Treatment	5	34.58	<0.0001	35.70	<0.0001	1.63	0.16	81.52	<0.0001
Block	19	4.55	<0.0001	4.86	<0.0001	4.28	<0.0001	3.29	<0.0001



Figure 10 Weekly average maximum height of Japanese stiltgrass per field sample from Site 1 after cutting at varying internodal regions (Control, no cutting; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 9) are not significantly different (Tukey's HSD, p < 0.05).


Figure 11 Weekly average maximum height of Japanese stiltgrass per field sample from Site 2 after cutting at varying internodal regions (Control, no cutting; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 9) are not significantly different (Tukey's HSD, p < 0.05).



Figure 12 Average aboveground biomass ( $\pm$ SE) of Japanese stiltgrass per field sample nine weeks after cutting at varying internodal regions (C, control [no cutting]; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).



Figure 13 Average ( $\pm$ SE) number of Japanese stiltgrass seed spikelets produced per field sample nine weeks after cutting at varying internodal regions (C, control [no cutting]; B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

## Greenhouse Experiment

Despite some visual differences in health and growth among samples, 100% of the samples had at least partial stem survival and 99% of the samples produced seed. Stem survival averaged 8-9 of the original 10 stems, with no significant differences by treatment (Table 7). After samples were removed from the mist room and placed in the greenhouse, we observed large increases in aerial root growth from the lower nodes of the stem cuttings, which established in the soil and created thick masses of roots. There were significant block effects on average maximum height of stem cuttings after removal from the mist room (F(19,76) = 14.05, p < 0.0001). Stem cuttings from Site 1 were taller than stem cuttings from Site 2 (t(15) = 5.57, p < 0.0001; Figs. 14, 15). Cuttings from both sites gradually increased in height for the first 5-6 weeks with a growth spurt in the last week, at which point the treatments were very similar in height (Figs. 14, 15).

Although B1 stem cuttings were taller, B4 and B5 stem cuttings produced seeds more quickly (F(4,75) = 4.90, p = 0.001; Fig. 16). Overall, it took B1 stem cuttings an average of 27.6 days to produce seed, while B5 stem cuttings took an average of 23.3 days. There were also differences in days to first seed production by block (F(19,75) = 14.55, p < 0.0001). On average, stem cuttings from Site 1 took 19.5 days to seed and stem cuttings from Site 2 took 30.9 days to seed (t(14) = -8.49, p < 0.0001). There were no significant differences among treatments for the rest of the measured variables (Table 7). The average aboveground biomass per sample nine weeks after planting was 7.11 g and the average total number of seed spikelets produced was 286.7, of which 84.5% were chasmogamous on average.

Table 7Survival, growth, and seed types produced in the greenhouse by Japanese<br/>stiltgrass stem cuttings cut at varying intermodal regions.

	Stems Alive After Transplant (#/10)	Aboveground Biomass (g)	Chasmogamous Seed Spikelets	Cleistogamous Seed Spikelets
Treatment			r	<b>r</b>
Below 1 <sup>st</sup> node	$8.9 \pm 0.23$	$7.06 \pm 0.407$	$137.5 \pm 15.86$	$162.5 \pm 14.38$
Below 2 <sup>nd</sup> node	$8.3 \pm 0.42$	$7.54 \pm 0.450$	$119.3 \pm 26.73$	$164.3 \pm 42.70$
Below 3 <sup>rd</sup> node	$9.0 \pm 0.21$	$7.17 \pm 0.405$	$136.3 \pm 17.21$	$155.0 \pm 6.22$
Below 4 <sup>th</sup> node	$9.1 \pm 0.25$	$6.87 \pm 0.364$	$138.0 \pm 19.52$	$175.8 \pm 26.24$

Below 5 <sup>th</sup> node	$9.0 \pm 0.29$	$6.89 \pm 0.404$	$112.5 \pm 36.15$	$132.3 \pm 38.44$
F <sub>(4)</sub>	1.49	0.91	0.44	0.47
Р	0.21	0.46	0.78	0.75



Figure 14 Weekly average maximum height of Japanese stiltgrass per greenhouse sample from Site 1 after transplant from mist room. Treatments were B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 6) are not significantly different (Tukey's HSD, p < 0.05).



Figure 15 Weekly average maximum height of Japanese stiltgrass per greenhouse sample from Site 2 after transplant from mist room. Treatments were B5, below 5th node; B4, below 4th node, B3, below 3rd node; B2, below 2nd node; B1, below 1st node. Means with the same letters (lowercase for week 1 and uppercase for week 7) are not significantly different (Tukey's HSD, p < 0.05).



Figure 16 Average ( $\pm$ SE) days after stem cuttings were planted until Japanese stiltgrass seed production for greenhouse samples across both sites (B1, below the first node; B2, below the second node; B3, below the third node; B4, below the fourth node; B5, below the fifth node). Different letters indicate significant differences among treatments (Tukey's HSD, p < 0.05).

## Discussion

The results from the field experiment suggest that the number of nodes that remain after cutting is important to the regrowth and reproductive ability of Japanese stiltgrass. This may have implications for mowing height considering that samples cut at lower nodes had significantly lower heights after cutting and those samples recovered less effectively or not at all over the rest of the season. The reductions we observed in aboveground biomass in our 10-stem samples are similar to those observed in larger mowing studies. Previous studies have found that mowing Japanese stiltgrass near to ground level can reduce biomass by 95-98% and seed production by 98% (Flory and Lewis 2009; Chapter 2, this thesis). However, our results also suggest that site-specific characteristics and environmental conditions may affect how well Japanese stiltgrass can recover. At the sunnier site, Japanese stiltgrass had increased height, greater biomass, shorter time to seed emergence, and greater seed production throughout the season, which are trends supported by previous studies. Cheplick and Fox (2011) found that Japanese stiltgrass produced fewer chasmogamous and cleistogamous seed spikelets in the shade and Cheplick (2005) reported that chasmogamous spikelets comprised a larger percentage of reproductive output in shady populations than in sunny populations, which we also observed in our study. These consistent differences in growth between the shady and sunny sites are likely a result of reduced photosynthetic activity in low light conditions (Horton and Neufeld 1998). Soil moisture may have also factored into these differences. Japanese stiltgrass performs better with higher soil moisture, leading to increased germination, biomass, seed production, and seed size (Huebner 2011; Warren et al. 2011; Warren et al. 2013). However, since we did not measure soil moisture, we cannot confirm these results.

The results from the greenhouse experiment demonstrate that cuttings can be an efficient and viable method of producing Japanese stiltgrass plants. To our

68

knowledge, only one previous study has used stem cuttings as a method of growing Japanese stiltgrass plants. Winter et al. (1982) established stem cuttings in the greenhouse, transferred them outdoors for a light-limitation experiment, and harvested them for dry weights 45 days after transfer. No further information about methodology or stage of plant growth (i.e. if the plants produced seed) was provided. However, stem cutting is a method used to propagate other grass species, especially in agriculture, horticulture, and biofuel production. Sugarcane and *Miscanthus* species, which are closely related to Japanese stiltgrass, have been successfully propagated through stem cuttings (Atkinson 2009). Other Poaceae species, including various bamboos, may also be propagated through stem or culm cuttings (Ramanayake 2006). Studies regarding propagation of these and other species suggest that node developmental position and age can significantly affect rooting and growth of stem cuttings (Atkinson 2009). Nodes that are lower on the stem are more "mature" than those higher on the stem, and these "mature" nodes have increased rooting potential. Although we did not directly measure rooting, we observed lower final height for stem cuttings from below the fifth node compared to stems that had been cut lower on the plants, but only with cuttings from the shadier site (Fig. 15). However, considering the overall success of all other stem cuttings, we suggest that node age is not a significant factor for Japanese stiltgrass, which could be attributed to Japanese stiltgrass' annual life cycle or its high growth rate.

Other studies suggest that the success of stem cuttings varies with season and temperature. Stem cuttings of giant reed (*Arundo donax* L.) performed significantly better when collected and grown during the summer months (Wijte et al. 2005). Although we did not investigate temperature, a previous study by Warren et al. (2011)

69

demonstrated that Japanese stiltgrass growth and reproduction is strongly positively correlated with increasing temperature, so it is likely that the consistently high greenhouse temperatures played a role in the success of our cuttings. Considering that Japanese stiltgrass is an annual plant, seasonality is likely not a factor. Interestingly, we observed significant differences in timing of first seed emergence in the greenhouse cuttings by both treatment and site. Shorter cuttings produced seed more quickly, which may be related to rooting potential. Previous studies have reported higher rooting percentage in shorter cuttings of woody plants, such as loblolly pine (Pinus taeda L.) and African teak (Milicia excelsa (Welw.) C.C. Berg) (Ofori et al. 1997; Foster et al. 2000). If Japanese stiltgrass exhibits similar trends, better rooting in shorter cuttings would increase nutrient uptake, which could lead to healthier plants and faster seed production. Cheplick (2005) reported that Japanese stiltgrass seeds collected from sunny populations and grown in the greenhouse partitioned significantly more biomass to seed spikelets compared to seeds from shady populations, but overall seed production did not vary between populations. This difference in initial biomass partitioning may partially explain why stem cuttings from the sunny site produced seed more quickly.

Overall, the success of our stem cuttings was extraordinary, especially considering the lack of chemical pretreatment or root induction medium. These results provide a potential protocol for propagation of Japanese stiltgrass stem cuttings that can produce reproductively viable Japanese stiltgrass plants in 25 days. The success and speed of this method of growing Japanese stiltgrass plants has many implications. First, since height of the cutting and nodal age did not generally affect ultimate growth, cuttings may be taken throughout the growing season. This offers greater

70

temporal flexibility than growing from seed collected in the field, which is restricted to late summer or early fall when Japanese stiltgrass produces seeds. Second, although the cuttings are clones of the parental plants, greater temporal flexibility provides more time to collect cuttings from various populations to increase genetic and phenotypic diversity. In addition, cuttings from sites with different growing conditions did not vary in their ultimate success, which allows for introduction of further genetic and phenotypic diversity. Finally, growing from cuttings eliminates the germination process associated with growing from seeds, which can save time and supplies. The ability to produce many Japanese stiltgrass plants in a short amount of time will be essential to efficient host-range testing for potential biological control agents by providing experimental plants, as well as diet for rearing sufficient populations.

## REFERENCES

- Abrams, M. D., & Johnson, S. E. (2012). Long-term impacts of deer exclosures on mixed-oak forest composition at the Valley Forge National Historical Park, Pennsylvania, USA. The Journal of Torrey Botanical Society, 139(2).
- Adams, S. N., & Engelhardt, K. A. M. (2009). Diversity declines in *Microstegium vimineum* (Japanese stiltgrass) patches. Biological Conservation, 142(5), 1003–1010.
- Almansouri, M., Kinet, J., & Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant and Soil, 231, 243–254.
- Atkinson, C. J. (2009). Establishing perennial grass energy crops in the UK: A review of current propagation options for *Miscanthus*. Biomass and Bioenergy, 33(5), 752–759.
- Baker, S. A., & Dyer, R. J. (2011). Invasion genetics of *Microstegium vimineum* (Poaceae) within the James River Basin of Virginia, USA. Conservation Genetics, 12, 793-803.
- Barden, L. (1987). Invasion of *Microstegium vimineum* (Poaceae), an exotic, annual, shade-tolerant, C4 grass, into a North Carolina floodplain. American Midland Naturalist, 118(1), 40–45.
- Beauchamp, V., Koontz, S., Suss, C., Hawkins, C., Kyde, K. L., & Schnase, J. L. (2013). An introduction to *Oplismenus undulatifolius* (Ard.) Roem. & Schult. (wavyleaf basketgrass), a recent invader in Mid-Atlantic forest understories. Journal of the Torrey Botanical Society, 140(4), 391–413.
- Belote, R. T., & Weltzin, J. F. (2006). Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. Biological Invasions, 8(8), 1629–1641.
- Besnard, G., Muasya, A. M., Russier, F., Roalson, E. H., Salamin, N., & Christin, P. A. (2009). Phylogenomics of C4 photosynthesis in sedges (Cyperaceae): multiple appearances and genetic convergence. Molecular Biology and Evolution, 26(8), 1909-1919.

- Bouchenak-Khelladi, Y., Muasya, A. M., & Linder, H. P. (2014). A revised evolutionary history of Poales: Origins and diversification. Botanical Journal of the Linnean Society, 175(1), 4–16.
- Boyd, R. S., & Moffett, J. M. (2003). Management of *Xyris tennesseensis* (Tennesee yellow-eyed grass), a federally-endangered plant species. Georgia Department of Transportation, Forest Park.
- Bradford, M. A., DeVore, J. L., Maerz, J. C., McHugh, J. V., Smith, C. L., & Strickland, M. S. (2010). Native, insect herbivore communities derive a significant proportion of their carbon from a widespread invader of forest understories. Biological Invasions, 12(4), 721–724.
- CABI (2014). Invasive Species Compendium. *Microstegium vimineum*. http://www.cabi.org/isc/datasheet/115603; last accessed April 11, 2016.
- Cheplick, G. P. (2005). Biomass partitioning and reproductive allocation in the invasive, cleistogamous grass *Microstegium vimineum*: Influence of the light environment. Journal of the Torrey Botanical Society, 136(4), 500–519.
- Cheplick, G. P. (2010). Limits to local spatial spread in a highly invasive annual grass (*Microstegium vimineum*). Biological Invasions, 12(6), 1759–1771.
- Cheplick, G. P., & Fox, J. (2011). Density-dependent growth and reproduction of *Microstegium vimineum* in contrasting light environments. Journal of the Torrey Botanical Society, 138(1), 62–72.
- Cipollini, M. L., Strahl, M., Gorden, N. S., Tomlinson, P., & Ware, R. (2013). Vegetative survey of Martha's Meadow, an open limestone habitat in northwestern Georgia. Southeastern Naturalist, 12(2), 317–338.
- Cole, P. G., & Weltzin, J. F. (2004). Environmental correlates of the distribution and abundance of *Microstegium vimineum* in east Tennessee. Southeastern Naturalist, 3(3), 545–562.
- Cole, P. G., & Weltzin, J. F. (2005). Light limitation creates patchy distribution of an invasive grass in eastern deciduous forests. Biological Invasions, 7(3), 477– 488.
- Craig, M. E., Pearson, S. M., & Fraterrigo, J. M. (2015). Grass invasion effects on forest soil carbon depend on landscape-level land use patterns. Ecology, 96(8), 2265–2279.

- Craig, S., Kannadan, S., Flory, S. L., Seifert, E. K., Whitney, K. D., & Rudgers, J. A. (2011). Potential for endophyte symbiosis to increase resistance of the native grass *Poa alsodes* to invasion by the non-native grass *Microstegium vimineum*. Symbiosis, 53(1), 17–28.
- Cutting, K. J., & Hough-Goldstein, J. (2013). Integration of biological control and native seeding to restore invaded plant communities. Restoration Ecology, 21(5), 648–655.
- Dávalos, A., Nuzzo, V., & Blossey, B. (2015). Interactive effects of deer, earthworms and non-native plants on rare forest plant recruitment. Biological Conservation, 187, 173–181.
- DeVore, J. L., & Maerz, J. C. (2014). Grass invasion increases top-down pressure on an amphibian via structurally mediated effects on an intraguild predator. Ecology, 95(7), 1724–1730.
- Dougherty, R. F., Quinn, L. D., Endres, A. B., Voigt, T. B., & Barney, J. N. (2014). Natural history survey of the ornamental grass *Miscanthus sinensis* in the introduced range. Invasive Plant Science and Management, 7(1), 113–120.
- Dufour, P., Deu, M., Grivet, L., D'Hont, A., Paulet, F., Bouet, A., Lanaud, C., Glaszmann, J. C., & Hamon, P. (1997). Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. Theoretical and Applied Genetics, 94(3-4), 409–418.
- EDDMapS. 2016. Early Detection & Distribution Mapping System. The University of Georgia Center for Invasive Species and Ecosystem Health. Available at http://www.eddmaps.org/
- Emery, S. M., Luke Flory, S., Clay, K., Robb, J. R., & Winters, B. (2013). Demographic responses of the invasive annual grass *Microstegium vimineum* to prescribed fires and herbicide. Forest Ecology and Management, 308, 207– 213.
- Emery, S. M., Uwimbabazi, J., & Flory, S. L. (2011). Fire intensity effects on seed germination of native and invasive Eastern deciduous forest understory plants. Forest Ecology and Management, 261(8), 1401–1408.
- EPPO (2014). Pest risk analysis for *Microstegium vimineum*. EPPO, Paris. Available at http://www.eppo.int/QUARANTINE/Pest\_Risk\_Analysis/PRA\_intro.htm

- Eschtruth, A. K., & Battles, J. J. (2009). Acceleration of exotic plant invasion in a forested ecosystem by a generalist herbivore. Conservation Biology, 23(2), 388–399.
- Fairbrothers, D. E., & Gray, J. R. (1972). *Microstegium vimineum* (Trin.) A. Camus (Gramineae) in the United States. Journal of the Torrey Botanical Society, 99(2), 97–100.
- Flory, S. L., & Clay, K. (2009). Invasive plant removal method determines native plant community responses. Journal of Applied Ecology, 46(2), 434–442.
- Flory, S. L., Long, F., & Clay, K. (2011). Invasive Microstegium populations consistently outperform native range populations across diverse environments. Ecology, 92(12), 2248–2257.
- Flory, S. L., & Clay, K. (2010a). Non-native grass invasion alters native plant composition in experimental communities. Biological Invasions, 12(5), 1285– 1294.
- Flory, S. L., & Clay, K. (2010b). Non-native grass invasion suppresses forest succession. Oecologia, 164(4), 1029–1038.
- Flory, S. L., & Lewis, J. (2009). Nonchemical methods for managing Japanese stiltgrass (*Microstegium vimineum*). Invasive Plant Science and Management, 2(4), 301–308.
- Flory, S. L., Rudgers, J. A., & Clay, K. (2007). Experimental light treatments affect invasion success and the impact of *Microstegium vimineum* on the resident community. Natural Areas Journal, 27(2), 124–132.
- Foster, G. S., Stelzer, H. E., & McRae, J. B. (2000). Loblolly pine cutting morphological traits: Effects on rooting and field performance. New Forests, 19(3), 291–306.
- Fraterrigo, J. M., Strickland, M. S., Keiser, A. D., & Bradford, M. A. (2011). Nitrogen uptake and preference in a forest understory following invasion by an exotic grass. Oecologia, 167(3), 781–791.
- Gibson, D. J. D., Spyreas, G., & Benedict, J. (2002). Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. Journal of the Torrey Botanical Society, 129(3), 207–219.

- Halvorson, J. J., Belesky, D. P., & West, M. S. (2016). Inhibition of forage seed germination by leaf litter extracts of overstory hardwoods used in silvopastoral systems. Agroforestry Systems.
- Horton, J. L., & Neufeld, H. S. (1998). Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C4 grass, to variable light environments. Oecologia, 114(1), 11–19.
- Huebner, C. D. (2011). Seed mass, viability, and germination of Japanese stiltgrass (*Microstegium vimineum*) under variable light and moisture conditions. Invasive Plant Science and Management, 4(3), 274–283.
- Hunt, D. M., & Zaremba, R. E. (1992). The northeastward spread of *Microstegium vimineum* (Poaceae) into New York and adjacent states. Rhodora, 94(878), 167–170.
- Judge, C. A., Neal, J. C., & Derr, J. E. (2005a). Preemergence and postemergence control of Japanese stiltgrass (*Microstegium vimineum*). Weed Technology, 19(1), 183–189.
- Judge, C. A., Neal, J. C., & Derr, J. F. (2005b). Response of Japanese stiltgrass (*Microstegium vimineum*) to application timing, rate, and frequency of postemergence herbicides. Weed Technology, 19(4), 912–917.
- Judge, C. A., Neal, J. C., & Shear, T. H. (2008). Japanese Stiltgrass (*Microstegium vimineum*) management for restoration of native plant communities. Invasive Plant Science and Management, 1(2), 111–119.
- Kanata, M., & Sugiyama, N. (1973). Identification of flavone compounds in eighteen Gramineae species. Agricultural and Biological Chemistry, 37(11), 2663-2665.
- Knight, T. M., Dunn, J. L., Smith, L. A., Davis, J., & Kalisz, S. (2009). Deer facilitate invasive plant success in a Pennsylvania forest understory. Natural Areas Journal, 29(2), 110–116.
- Kostel-Hughes, F., Young, T. P., & Wehr, J. D. (2005). Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. Journal of the Torrey Botanical Society, 132(1), 50–61.
- Lake, E. C., Hough-Goldstein, J., & D'Amico, V. (2013). Integrating management techniques to restore sites invaded by mile-a-minute weed, *Persicaria perfoliata*. Restoration Ecology, 22, 127-133.

- Lee, M. R., Flory, S. L., & Phillips, R. P. (2012). Positive feedbacks to growth of an invasive grass through alteration of nitrogen cycling. Oecologia, 170(2), 457– 465.
- Leicht, S. A., Silander, J. A., & Greenwood, K. (2005). Assessing the competitive ability of Japanese stilt grass, *Microstegium vimineum* (Trin.) A. Camus. Journal of the Torrey Botanical Society, 136(4), 500–519.
- Li, Q., Tan, J., Li, W., Yuan, G., Du, L., Ma, S., & Wang, J. (2015). Effects of environmental factors on seed germination and emergence of Japanese brome (*Bromus japonicus*). Weed Science, 63, 641–646.
- Marshall, J. M., & Buckley, D. S. (2008). Influence of litter removal and mineral soil disturbance on the spread of an invasive grass in a Central Hardwood forest. Biological Invasions, 10(4), 531–538.
- Metcalf, J. L., & Emery, S. M. (2015). Non-native grass invasion associated with increases in insect diversity in temperate forest understory. Acta Oecologica, 69, 105–112.
- Mortensen, D. A., Rauschert, E. S. J., Nord, A. N., & Jones, B. P. (2009). Forest roads facilitate the spread of invasive plants. Invasive Plant Science and Management, 2(3), 191–199.
- National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information (2015). Data Tools: 1981-2010 Normals. (https://www.ncdc.noaa.gov/cdo-web/datatools/normals). Accessed: April 20, 2016.
- Novy, A., Flory, S. L., & Hartman, J. M. (2013). Evidence for rapid evolution of phenology in an invasive grass. Journal of Evolutionary Biology, 26(2), 443–450.
- Nuzzo, V. (1997). Element stewardship abstract for *Lonicera japonica*. The Nature Conservancy, Arlington, VA. http://www.invasive.org/weedcd/pdfs/tncweeds/lonijap.pdf
- Ofori, D., Newton, A. C., Leakey, R. R. B., & Grace, J. (1997). Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: Effects of maturation, coppicing, cutting length and position on rooting ability. Journal of Tropical Forest Science, 10(1), 115–129.

- Oswalt, C. M., & Oswalt, S. N. (2007). Winter litter disturbance facilitates the spread of the nonnative invasive grass *Microstegium vimineum* (Trin.) A. Camus. Forest Ecology and Management, 249(3), 199–203.
- Oswalt, C. M., Oswalt, S. N., & Clatterbuck, W. K. (2007). Effects of *Microstegium vimineum* (Trin.) A. Camus on native woody species density and diversity in a productive mixed-hardwood forest in Tennessee. Forest Ecology and Management, 242(2-3), 727–732.
- Pomp, J., McGill, D., Grafton, W., Chandran, R., & Richardson, R. (2010). Effects of mechanical and chemical control on *Microstegium vimineum* and its associates in central West Virginia. Proceedings of the 14th Biennial Southern Silvicultural Research Conference. Gen. Tech. Rep. SRS-121. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 614 pp.
- Ramanayake, S. M. S. D., Meemaduma, V. N., & Weerawardene, T. E. (2006). In vitro shoot proliferation and enhancement of rooting for the large-scale propagation of yellow bamboo (*Bambusa vulgaris* "Striata"). Scientia Horticulturae, 110(1), 109–113.
- Redman, D. E. (1995). Distribution and habitat types for Nepal Microstegium [*Microstegium vimineum* (Trin.) Camus] in Maryland and the District of Columbia. Castanea, 60(3), 270–275.
- Sage, R. (2001). Environmental and evolutionary preconditions for the origin and diversification of the C4 photosynthetic syndrome. Plant Biology, 3(C), 202– 213.
- Schafale, M. P., & Weakley, A. S. (1990). Classification of the natural communities of North Carolina: Third approximation. North Carolina Natural Heritage Program, N.C.
- Schramm, J. W., & Ehrenfeld, J. G. (2010). Leaf litter and understory canopy shade limit the establishment, growth and reproduction of *Microstegium vimineum*. Biological Invasions, 12(9), 3195–3204.
- Shelton, A. L. (2012). Mowing any time after midsummer can manage Japanese stiltgrass. Invasive Plant Science and Management, 5(2), 209–216.
- Simao, M. C. M., Flory, S. L., & Rudgers, J. A. (2010). Experimental plant invasion reduces arthropod abundance and richness across multiple trophic levels. Oikos, 119(10), 1553–1562.

- Soreng, R. J., Peterson, P. M., Romaschenko, K., Davidse, G., Zuloaga, F. O., Judziewicz, E. J., Filgueiras, T. S., Davis, J. I., & Morrone, O. (2015). A worldwide phylogenetic classification of the Poaceae (Gramineae). Journal of Systematics and Evolution, 53(2), 117–137.
- Stevens P.F. (2001 onwards). Angiosperm Phylogeny Website. Version 12, July 2012 [with continuous updates since]. URL http://www.mobot.org/MOBOT/research/APweb/
- Stransky, J. J. (1984). Forage yield of Japanese honeysuckle after repeated burning or mowing. Journal of Range Management, 37(3), 237–238.
- Stricker, K. B., Harmon, P. F., Goss, E. M., Clay, K., & Luke Flory, S. (2016). Emergence and accumulation of novel pathogens suppress an invasive species. Ecology Letters, 19, 469-477.
- Strickland, M. S., DeVore, J. L., Maerz, J. C., & Bradford, M. A. (2011). Loss of faster-cycling soil carbon pools following grass invasion across multiple forest sites. Soil Biology and Biochemistry, 43(2), 452–454.
- Swearingen, J. M., & Adams, S. (2008). WeedUS: database of plants invading natural areas in the United States of America. Plant Conservation Alliance, Alien Plant Working Group Weeds Gone Wild. http://www.nps.gov/plants/alien/list/a.htm
- Tang, Y., Warren, R. J., Kramer, T. D., & Bradford, M. A. (2012). Plant invasion impacts on arthropod abundance, diversity and feeding consistent across environmental and geographic gradients. Biological Invasions, 14(12), 2625– 2637.
- Tekiela, D. R., & Barney, J. N. (2015). System-level changes following invasion caused by disruption of functional relationships among plant and soil properties. Ecosphere, 6, 1–16.
- Touchette, B. W., & Romanello, G. A. (2010). Growth and water relations in a central North Carolina population of Microstegium vimineum (Trin.) A. Camus. Biological Invasions, 12(4), 893–903.
- Tscharntke, T., & Greiler, H. J. (1995). Insect communities, grasses, and grasslands. Annual Review of Entomology, 40, 535-538.
- Tu, M. (2000). Elemental Stewardship Abstract for *Microstegium vimineum*. Arlington, VA: The Nature Conservancy. http://www.invasive.org/weedcd/pdfs/tncweeds/micrvim.pdf. Accessed: April 9, 2016.

- USDA-ERS (2015). Crops. Available at http://www.ers.usda.gov/topics/crops/sugarsweeteners/background.aspx
- USDA-NASS (2016). "Quick Stats". Available at http://quickstats.nass.usda.gov/
- USDA, NRCS (2016). National Plant Data Center. The PLANTS Database. http://plants.usda.gov
- USFWS, ECOS (2016). Environmental Conservation Online System. Threatened and Endangered Species. http://ecos.fws.gov/
- Vicari, M. & Bazely, D. (1993). Do grasses fight back? The case for antiherbivore defenses. TREE, 8 (4), 137-141.
- Wagner, S. A., & Fraterrigo, J. M. (2015). Positive feedbacks between fire and nonnative grass invasion in temperate deciduous forests. Forest Ecology and Management, 354, 170–176.
- Ward, J. S., & Mervosh, T. L. (2012). Nonchemical and herbicide treatments for management of Japanese stiltgrass (*Microstegium vimineum*). Invasive Plant Science and Management, 5(1), 9-19.
- Warren, R. J., Bahn, V., & Bradford, M. A. (2012). The interaction between propagule pressure, habitat suitability and density-dependent reproduction in species invasion. Oikos, 121(6), 874–881.
- Warren, R. J., Bahn, V., & Bradford, M. A. (2013). Decoupling litter barrier and soil moisture influences on the establishment of an invasive grass. Plant and Soil, 367(1-2), 339–346.
- Warren, R. J., Bahn, V., Kramer, T. D., Tang, Y., & Bradford, M. A. (2011). Performance and reproduction of an exotic invader across temperate forest gradients. Ecosphere, 2(2), 1–19.
- Webster, C. R., Rock, J. H., Froese, R. E., & Jenkins, M. A. (2008). Droughtherbivory interaction disrupts competitive displacement of native plants by *Microstegium vimineum*, 10-year results. Oecologia, 157(3), 497–508.
- Wijte, A. H. B. M., Mizutani, T., Motamed, E. R., Margaret, L., Miller, D. E., Alexander, D. E., & Journal, I. (2005). Temperature and endogenous factors cause seasonal patterns in rooting by stem fragments of the invasive giant reed, *Arundo donax* (Poaceae). International Journal of Plant Science, 166(3), 507– 517.

- Winter, K., Schmitt, M. R., & Edwards, G. E. (1982). *Microstegium vimineum*, a shade adapted C4 grass. Plant Science Letters, 24, 311–318.
- Xiong, S., & Nilsson, C. (1999). The effects of plant litter on vegetation: A metaanalysis. Journal of Ecology, 87(6), 984–994.
- Zhou, J.-M., & Ibrahim, R. K. (2010). Tricin a potential multifunctional nutraceutical. Phytochemical Review, 9, 413-424.
- Ziska, L. H., Tomecek, M. B., Valerio, M., & Thompson, J. P. (2014). Evidence for recent evolution in an invasive species, *Microstegium vimineum*, Japanese stiltgrass. Weed Research, 55, 260-267.