Invasive Plant Science and Management 2016 9:96–111

Regional and Local Genetic Variation in Japanese Stiltgrass (*Microstegium vimineum*)

Theresa M. Culley, Cynthia D. Huebner, and Ari Novy*

Nonnative *M. vimineum* has been expanding rapidly in the eastern United States, where it can negatively affect plant communities. Locally, the species is assumed to spread from roadsides into nearby forests, where it can form dense populations after disturbances, especially in light gaps. Using microsatellite markers, we quantified patterns of genetic variation and structure among populations at nine sites in West Virginia. We then examined patterns of local dispersal within each population, focusing on subpopulations along the roadside, those coalescing nearby along the forest edge, and subpopulations in the interior forest. We found that levels of genetic variation of *M. vimineum* were relatively low overall across populations but with genetic structure present among populations ($F_{st} = 0.60$). Within populations, subpopulations along the roadside were genetically variable, containing 4 to 22 unique, multilocus genotypes. Many of these genotypes were also identified in the adjacent forest, consistent with local, diffusive spread from the roadway. However, several genotypes in the interior forest were unique to the population, indicating that dispersal from other sites may also occur. Overall, it appears that genetic diversity and structure in *M. vimineum* reflects a variety of processes, including localized dispersal and long-distance migration.

Nomenclature: Japanese stiltgrass, *Microstegium vimineum* (Trin.) A. Camus.

Key words: Genetic structure, invasive, local dispersal, microsatellites.

Understanding the beginning stages of the spread of a newly introduced plant species is critical for biologists studying invasion dynamics and for land managers concerned with eradication or control of invasive weeds in their natural areas. Both the rate and pattern of spread can be instrumental in determining whether an introduced species has the potential to become invasive and ultimately exert ecological and economic effects on the environment. For example, weed risk assessments often incorporate questions dealing with propagule dispersal or aggressiveness of spread (e.g., Fox et al. 2008; NatureServe 2004; Pheloung et al. 1999). Newly invading species are generally considered to spread across suitable habitats in the landscape in either a locally dispersed, stepwise fashion (*diffusion*) or through long-distance dispersal events (*saltation*) at the front of an advancing wave (Davis and Thompson 2000; Sakai et al. 2001; Smith et al. 1999), with infill progressing from the colonization points. The type of spread depends in part on reproductive output, method of propagule dispersal, and establishment ability. Understanding how the spread of an invasive species occurs at a local scale is critical to creating effective management strategies for newly invading species. For example, if initial spread in a locality is observed to be rapid and expansive, aggressive eradication and control efforts can be started in other nearby areas to prevent further invasion.

Microstegium vimineum is a rapidly spreading invasive plant affecting at least 24 states primarily east of the Mississippi River in the United States (USDA 2016). This shade-tolerant, C_4 annual grass is found in disturbed (Eschtruth et al. 2006; Marshall and Buckley 2008b; Orwig and Foster 1998), managed (Marshall and Buckley 2008a; Oswalt et al. 2007), and relatively undisturbed forests (Barden 1987; Horton and Newfeld 1998; Huebner, 2007; Winter et al. 1982), with specific niche requirements (see Warren et al. 2011 for a review). Although herbicide and manual control can be effective (Flory 2010; Flory and Lewis 2009; Ward and Mervosh 2012), *M. vimineum* cover in untreated plots can reach

DOI: 10.1614/IPSM-D-15-00055.1

* Professor, Department of Biological Sciences, University of Cincinnati, 614 Rieveschl Hall, Cincinnati, OH 45221; Research Scientist, Northern Research Station, U.S. Forest Service, U.S. Department of Agriculture, 180 Canfield Street, Morgantown, WV 26505; Executive Director, U.S. Botanic Garden, 245 First Street SW, Washington, DC 20024 and Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC. Corresponding author's E-mail: theresa.culley@uc.edu

Management Implications

Our research highlights the importance of evaluating local and regional patterns of genetic diversity when defining management strategies of an invasive plant. *Microstegium vimineum* has low genetic diversity, which could potentially make it susceptible to disease or unable to adequately respond to other stochastic events. Despite this paucity of diversity, *M. vimineum* has evolved within its invasive range, and the presence of at least one effective pathogen (*Bipolaris* spp.) has not forestalled its rapid spread.

Our results show that roadsides, despite having a relative abundance of chasmogamous seeds, are not a significant source of genetic variation. Instead, any new genotypes in an area are likely the product of long-distance dispersal. Second, our results indicate that forest interior subpopulations are more often not the product of spread from the immediately adjacent roadside but, instead, may come from a long-distant source. Finally, our study shows there is genetic differentiation among regional populations, which could serve as a source of new or highly fit (i.e., able to withstand a range of environments) genetic information.

The key to stopping this influx of new genotypes into a site appears to be stopping or slowing the long-distance dispersal of the seed. Effective preventative measures could include strategically located cleaning stations (e.g., highway intersections, rest areas, gas stations) for vehicles and equipment and the use of certified clean gravel and other road construction/maintenance materials. Based on our results, priority locations would be intersections between the regionally different populations.

Although our research highlights the complexity of the success of *M. vimineum* as an invader, it also supports a focus on preventative management to reduce long-distance spread.

$>$ 90% with 590 to 1,160 seedlings m^{-2} (55 to 108 seedlings ft^{-2}) (Ward and Mervosh 2012). These extensive mats can reduce native species richness in invaded areas (Adams and Englehardt 2009; Flory and Clay 2010a; Oswalt et al. 2007), change soil nutrients and pH (Ehrenfeld et al. 2001; Kourtev et al. 1998, 1999; McGrath and Binkley 2009), and reduce native plant and arthropod diversity (McGrath and Binkley 2009; Oswalt et al. 2007; Simao et al. 2010). Native tree and herbaceous species regeneration has been slowed in the presence of *M. vimineum* and its extensive thatch (Aronson and Handel 2011; Flory and Clay 2010a,b; Webster et al. 2008). *M. vimineum* may potentially be allelopathic (Pisula and Meiners 2010) and gain a competitive advantage over other plant species in the presence of white-tailed deer (Eschtruth and Battles 2008). Its seed production can be prolific but is often quite variable—a single tiller can reportedly produce between 3 (Gibson et al. 2002) and 70 seeds (Cheplick 2005), depending on environmental conditions and climate. A single plant has been documented as having anywhere between 5 and 13 tillers, depending on the site (Gibson et al. 2002). A persistent seed bank is common, with seeds remaining dormant in the soil for up to 3 yr (Barden 1987; Gibson et al. 2002).

At a given site, a population of *M. vimineum* is hypothesized to form as seed or propagule dispersal occurs from roadsides to the forest edge and eventually into the forest itself, creating a series of subpopulations potentially in a source–sink relationship (Warren et al. 2011). For example, *M. vimineum* often first becomes established in disturbed, sunny, and often wet corridors, such as along roads or skidtrails of harvested forests, and then, appears in adjacent undisturbed sites, such as shady forests. Most of the seed from a given plant is not dispersed by wind but, instead, falls within 1 m (3.3 ft) of the parent plant (Huebner 2010a; Rauschert et al. 2010), although long-distance dispersal may also occur (e.g., Anderson et al. 2013; Christen and Matlack 2009).

The coalescing subpopulations of *M. vimineum* plants in the forest edge, which develop perpendicularly from the larger roadside population, suggest that each subpopulation is composed of individuals that originated from one or more roadside individuals. The lack of coalescence occurring farther into the forest interior, where individual plants occur, suggests that simple spread by diffusion is slow. It is also possible that individual plants in the forest interior originate from long-distance dispersal events, especially when dispersed via vectors that physically and inadvertently move the seed or soil containing the seed long distances (e.g., floods, people, equipment, or animals; Warren et al. 2011). This type of two-phase dispersal, consisting of long-distance dispersal to a new site followed by short-distance, localized dispersal, may be an important mechanism behind the rapid expansion of this plant (Anderson et al. 2013; Barden 1987). Long-distance dispersal of *M. vimineum* may predominantly occur along corridors, such as roads and rivers (Mortensen et al. 2009), and may also be episodic in time (Miller and Matlack 2010). Higher genetic diversity may be associated with long-distance dispersal, compared with short-distance dispersal, assuming that not all new populations originate from the same source or that selection is occurring at the different sites, which presumably differ environmentally. If spread into forests is dependent on diffusion from adjacent, invaded roadside corridors (i.e., a local or short-distance process) and mutation rates are low, one would expect equal or less genetic diversity in the forest-interior plants compared with the adjacent roadside subpopulation, the original source.

Genetic variation in *M. vimineum* is also likely influenced by its breeding system. The species exhibits mixed mating, involving chasmogamy (florets are located in open inflorescences that are facultatively outcrossed) and cleistogamy (florets located in the closed sheaths of the internodes and are self-pollinated). Individual *M. vimineum* plants growing along roadsides are much taller (about 1 m) and produce larger chasmogamous (CH) inflorescences as well as more cleistogamous (CL) seeds

Figure 1. Map of regions sampled in West Virginia for populations of *Microstegium vimineum*. Three populations were sampled in each of the three regions (see Table 1 for locality information). The shaded area represents the Monongahela National Forest, which encompasses the Cheat–Potomac Ranger District (split into two ranger districts, CRD and PRD). The Fernow populations are located in district CRD. The Cooper populations are located within Coopers Rock State Forest and the West Virginia University Forest (WVU). The area west of the dark line represents the Allegheny Plateau region of the state and the area east of the line represents the Ridge and Valley Province (RVP), which contains the PRD populations. (Color for this figure is available in the online version of this article.)

because they have more and longer internodes with closed inflorescences than the shorter plants (often $<$ 7 cm [2.8 in] tall) inhabiting the forest interior (Cheplick 2008; Huebner 2010a). The forest-interior plants, consequently, have comparatively fewer CH and CL seeds than the roadside plants (Huebner 2011). However, the ratio of CH to CL seeds is typically greater for the forest interior (shade-grown) *M. vimineum* plants (Cheplick 2006). CL seeds are also significantly less viable than CH seeds are (Huebner 2011). Thus, the larger number of CH seeds associated with roadsides may indicate that roadside populations should be genetically more diverse than individuals from the forest interior. Nonetheless, the large number of available CL seeds, despite being less viable than CH seeds are, may result in more of the roadside individuals originating from selfed or potentially inbred seed compared with the forest-interior plants. If an increase in patch size at a given site is due to diffusive spread, a coalescing subpopulation along the forest edge should contain more genetically similar individuals than the roadside subpopulation has and possibly than the forest interior subpopulation has.

To examine patterns of regional and local spread of *M. vimineum* for its potential for further expansion, we used microsatellite markers to analyze nine populations located at three different regions in West Virginia (Figure 1). Within each population, samples were collected to represent three different stages of spread: roadside subpopulations (*roadside*), coalescing subpopulations within the nearby forest edge (*edge*), and individual plants present in the adjacent forest interior (*interior*). We quantified the level of genetic diversity and degree of inbreeding within the populations and across the three regions and then compared multilocus genotypes of the three subpopulations (roadside, edge, and interior) within each population and region to determine the number and distribution of multilocus genotypes.

Using these genetic data, we addressed the following two questions: (1) Does *M. vimineum* spread within sites through local diffusion or long-distance dispersal or both? (2) Are forest edge and interior subpopulations more prone to inbreeding than are the adjacent roadside subpopulations? Based in this information, we discuss genetic variability and structure within the species as related to its ability to adapt and potentially spread into different environments. Answers to these questions will help inform management efforts to more effectively slow the spread of this species.

Materials and Methods

Study Area. Nine sites were selected, which consisted of a contiguous roadside *M. vimineum* population adjacent to a $\geq$ 70-yr-old, closed-canopy, deciduous forest, with no evidence of anthropogenic disturbance and with *M. vimineum* appearing in the forest. The forests were located within three regions in West Virginia: (1) three in the Fernow Experimental Forest (38.883N, 79.283W), Cheat-Potomac Ranger District of the Monongahela National Forest (MNF) in the Allegheny Plateau (Fernow); (2) three in Cooper's Rock State Forest and the West Virginia University Forest (39.650N, 79.783W) and in the Allegheny Plateau (Cooper); and (3) three in the Seneca Rocks area (39.033N, 79.70W) of the Cheat–Potomac Ranger District of the MNF in the Ridge and Valley Province (RVP; Figure 1). Populations at these sites were a subset of populations used in the Huebner (2010b) study.

The Fernow and Cooper sites are mixed-mesophytic forests, and the RVP sites are oak (*Quercus* spp.)-dominated forests. Although the Fernow and Cooper sites are similar in topography and plant species composition, the Cooper sites have less species-rich understories than the Fernow sites have (C.D.H., perssonal observation). The

Table 1. Descriptive information for nine sampled populations of *Microstegium vimineum* in West Virginia, USA with the number of plants (N) indicated that were collected within each stage of potential spread (Roadside, forest Edge, and forest Interior). Universal Transverse Mercator (UTM) coordinates are listed in decimal degrees (Lat, Long). Also provided is the type of road (gravel or paved) nearest to the sampled population.

Population	Region	Global positioning system coordinates	Roadside	Edge	Interior	Road type
			No.			
Cooper12	Cooper, Northern Allegheny Plateau	39.636116N, 79.799950W	50	29	30	Gravel
Cooper3	Cooper, Northern Allegheny Plateau	39.649877N, 79.784572W	50	50	52	Paved
WVU13	Cooper, Northern Allegheny Plateau	39.672221N, 79.769128W	50	30	30	Paved
CRD14	Fernow, Southern Allegheny Plateau	39.033142N, 79.702804W	50	0	50	Gravel
CRD23	Fernow, Southern Allegheny Plateau	39.039913N, 79.697493W	51	0	50	Gravel
CRDhemlock	Fernow, Southern Allegheny Plateau	39.028438N, 79.689039W	50	50	50	Gravel
PRD14	Ridge and Valley Province	38.855809N, 79.312975W	51	51	52	Gravel
PRD23	Ridge and Valley Province	38.911847N, 79.262657W	50	50	50	Gravel
PRD5	Ridge and Valley Province	38.979200N, 79.260251W	50	25	25	Paved

Fernow, Cooper, and RVP sites range in elevations between 585 and 707 m, 664 and 720 m, and 592 and 902 m, respectively. Annual precipitation for the RVP sites is about 79 cm, whereas it averages about 160 cm for the Fernow and Cooper sites (Clarkson 1964). The highest average temperature for the RVP sites is 30 C (86 F), but temperature averages 23 C to 25 C for the Fernow and Cooper sites (NCDC 2015). All three of the Fernow sites were located on gravel roads; in both the Cooper and RVP regions, one site was on a paved road, and the remaining two sites were located next to gravel roads (Table 1). The Cooper 12 and Cooper 3 sites are separated from the West Virginia University (WVU) 13 site by a road.

At each of the nine sites, the *M. vimineum* populations were divided into three subpopulations (roadside, edge, and interior). The roadside (termed R) plants were located between the road and the forest canopy. Ditches, culverts, and other potentially different roadside structures were excluded. This contiguous roadside subpopulation was < 10 m and ≥ 1 m wide, parallel to the road. The edge subpopulation (E) was located as part of the forest edge, which occurred between the forest canopy edge (the defined edge of the roadside subpopulations) and ≤ 10 m into the forest interior. This subpopulation was defined by its own structure (evident coalescence or grouping of stems), not by the forest structure. Although the two are likely related, our interest in differentiating this subpopulation was to capture potential genetic similarities that one would expect with diffusive spread vs. the separate, single stems found scattered farther into the forest interior. Forest light levels (measured with a quantum light meter [Spectrum Technologies USA Inc] and a densiometer [Robert E. Lemmon, Forest Densiometers]) also dropped significantly beyond 10 m. Edge effects (sunfleck frequency, degree of soil compaction, litter depth, among others) could extend deeper into the forest, but these edge effects did not appear to affect the degree of coalescence in the subpopulations and were not the focus of this study. The interior subpopulation (I) was composed of individual plants located in the forest interior, extending from the 10-m roadside area 50 m or less into the forest, and were not yet coalescing into patches.

Tissue Sampling. Between 25 and 51 individuals were sampled from each of the three subpopulations at each of the nine populations, taking one leaf from each individual plant. Every effort was made to select single plants that were not visibly connected to any other plant. Leaves associated with each population and subpopulation were stored in their own glassine envelope and sealed in a large plastic bag filled with silica gel for ≤ 12 h. Coalescing populations could not be differentiated at two population sites within the Fernow region (CRD14 and CRD23) and, thus, had no collections made for this subpopulation type (a lack of coalescing subpopulations may indicate a young invasion).

DNA Extraction and Polymerase Chain Reaction. DNA was extracted from field-collected samples, initially using a standard cetyltrimethylammonium bromide (CTAB) extraction technique (modified from Doyle and Doyle 1987) for single individuals. This method was eventually replaced by a high-throughput, 96-well plate method, using a modified CTAB approach developed in the Culley laboratory (T.M.C. and R. Stokes, unpublished data) to increase the speed of sample extraction. DNA was then stored at –40 C until further analysis.

Microsatellite regions were amplified using 16 primers created de novo or previously developed in *M. vimineum* or

Table 2. List of the microsatellite primers used in *Microstegium vimineum* that were developed for the species (Novy et al. 2012), *Miscanthus sinensis* (Hung et al. 2009), and *Zea mays* (Cai et al. 2005; K. Aitken, personal communication). Three of these markers (contigs 1366, 1262, 760) captured two separate and independent loci each and were, thus, scored separately, resulting in 19 microsatellite loci used in the study. Shown are the primer names, forward (F) and reverse (R) primer sequences, repeat motif, size of the fragments, number of alleles, dye used for multiplexing, and the taxon in which it was first developed. The last column contains the reference for previously published markers or in the case of newly developed microsatellite markers, the Genbank accession number.

Primer name	Primer sequences	Repeat motif	Size range	No. of alleles	Dye	Developed in	Reference or GenBank accession
MV01[a]	F: CCAGTGAATGTCATTTGTCC R: GCGTGAATTGAAATG ATTG	$(AG)_{10}$	208–228	9	6-FAM	*M. vimineum*	Novy et al. 2012
MV03[b]	F: GTCTGACCACCAACATTCTG R: TCAGGAAAGCTACCCTATG	$(AAG)_{16}$	322–367	12	NED	*M. vimineum*	Novy et al. 2012
MV05[b]	F: CATGCCAACCCTATTCTATC R: GAGAAACAAGGTGCAAAGAG	$(AAC)_7$	329–410	16	NED	*M. vimineum*	Novy et al. 2012
MV07[a]	F: CCTCCTTCAGACAGTCATTG R: TACAACAGATGCCGACTACC	$(AAC)_8$	343–362	4	6-FAM	*M. vimineum*	Novy et al. 2012
MV09[a]	F: TCATCC ATCTCCATAACTCC R: TTGCCATCTTCCCTACTAAC	$(ACAT)_{11}$	107–123	4	PET	*M. vimineum*	Novy et al. 2012
MV10[b]	F: TGAAGACAATGAGGCAAGTC R: TCGTCCTTGTGAGTCATGAC	$(AAAC)_6$	249–265	11	NED	*M. vimineum*	Novy et al. 2012
MV1042[a]	F: GGCTGAAATGAAGTGAACAC R: ACTTCTGCTATGCCAATGAC	$(ATC)_7$	234–259	15	VIC	*M. vimineum*	KP793028
MV1262[b]	F: TATGTGCTTCCATTCCACTC R: CAATATTGGGCCTTTAAGTC	$(ATC)_{10}$	117–147	9	NED	*M. vimineum*	KP793029
MV1366[a]	F: GATGTAACGAAATGGCTCAG R: AGCGTTAAATAGGGTGACAC	$(AG)_{10}$	146–162	7	NED	*M. vimineum*	KP793030
MV1675[b]	F: ATCCGAATATGCATGAGAAG R: CATGCGCTAACACTACTTTC	$(GTT)_{29}$	296–368	22	VIC	*M. vimineum*	KP793031
MV2358[a]	F: GCGCTAATTAAACCTTTGTC R: GACGTTCGTTTCAATACCAC	$(ACT)_9$	142–172	13	PET	*M. vimineum*	KP793032
MV2712[a]	F: ACCACGTGCCTTATTATCTC R: AGGATAGACGCATGTTCTTG	$(AGT)_{11}$	98–102	3	6-FAM	*M. vimineum*	KP793033
MV10tc[a]	F: CGTCGCAGAGATAAGAGCAC R: GGAGTCCTGGTGTAAGTGTT	$(CA)_4$	326–337	6	PET	*M. vimineum*	KP793034
M&M15[a]	F: ACACCGAAGCACGCCTGAT R: ATAATGTGCAATGCAGTCTAG	$(CA)_3(TA)_3(TG)_2$	69–84	7	6-FAM	*Miscanthus sinensis*	Hung et al. 2009
SSCIR26[b]	F: AAAATCAGACAAACAGCAT R: AGAAGAAGCAGATACAGGT	$(GA)_{17}$	128–148	8	VIC	*Zea mays*	Cai et al. 2005
SSCIR33[b]	F: GCTCATATATCTTCCTGGTC R: AGTGGTCTGGTGCTTTGG	$(GT)_{14}(GA)_9 \ldots (GA)_9$	97–107	5	6-FAM	*Zea mays*	Cai et al. 2005

[a] Primer mix 1.
[b] Primer mix 2.

other grass species (Table 2). These primers included *M&M15* (developed for eulaliagrass [*Miscanthus sinensis* Anderss.]; Hung et al. 2009); *SSCIR26* and *SSCIR33* (corn [*Zea mays* L.]; Cai et al. 2005, primer sequences obtained directly from K. Aitken, personal communication); and *MV01*, *MV03*, *MV05*, *MV07*, *MV09*, and *MV10* (developed for *M. vimineum* by Novy et al. 2012). Primer set *MV-10tc* was developed by the Culley Laboratory, using the Glenn and Schabel (2005) nonradioactive method. Six other primer pairs (contigs *1042*, *1262*, *1366*, *1675*, *2358*, and *2712*) were developed using next-generation sequencing, as described in Novy et al. (2012), and were subsequently tested by T.M.C. Because variation for three markers (contigs *1366* and *1262* and *MV05*) was commonly detected as different fixed alleles across populations, each marker was assumed to be capturing

two separate and independent loci and was, thus, scored separately (e.g., contig *1366* was scored as contigs *1366A* and *1366B*). A subsequent analysis for linkage disequilibrium indicated that these sister loci were not linked, and it was appropriate to analyze them independently (T.M.C., data available upon request). This resulted in a total of 19 loci analyzed in this study.

DNA was amplified in multiplexed reactions via polymerase chain reaction (PCR) using the Qiagen (Valencia, CA) Multiplexing Kit in 10 µl reaction volumes as follows: 5 µl Multiplex PCR Master Mix, 1 µl Primer Mix (consisting of 2 µM reverse primer and 2 µM forward-labeled primer for each marker), 3.8 µl H_2O, and 0.2 µl DNA. Samples were then amplified on an Eppendorf (Hamburg, Germany) thermocycler with the following conditions: initial denaturation of 95 C for 15 min, followed by 35 cycles each at 94 C for 30 s, 57 C for 90 s, and 72 C for 60 s, followed by a final extension of 60 C for 30 min. Each DNA sample was run twice, using two different primer mixes (Table 2). PCR products were then sent to Cornell University's Life Sciences Core Laboratory Center (New York, NY) for fragment analysis on a 3730 xl sequencer (Applied Biosystems, Fortune City, CA) using a LIZ 500 internal size standard. The resulting fragment-analysis data were analyzed using GeneMarker software (version 1.85, SoftGenetics, State College, PA) to identify alleles.

Genetic Analysis. Measures of genetic variation were calculated in GenAlEx software (version 6.5; Peakall and Smouse 2012) for each population as follows: number of alleles per locus (A) and per polymorphic loci (A_p), the percentage of polymorphic loci at the 95% level (P_p), observed heterozygosity (H_o) and the expected proportion of heterozygous loci per individual (H_e), and the fixation index (F; the same as F_{is}). Descriptive statistics were also calculated for each subpopulation (roadside, edge, and interior) within each population. Selfing rates (s) were estimated for each subpopulation from the outcrossing rate (t) as $1 - t$, where $t = (1 - F)/(1 + F)$, in which F is the fixation index (Brown 1979; Jain 1979).

Genetic differentiation was quantified among all populations and subpopulations using an analysis of molecular variance to calculate F_{st} in GenAlEx. To visually examine the clustering relationships of genotypes across populations, a principal coordinates analysis (PCoA) was used. Relationships of subpopulations within each site were also examined using an unweighted pair group method with arithmetic mean (UPGMA) phenogram based on pair-wise, coancestry identity values for all subpopulations within populations.

Because *M. vimineum* tends to spread and root at the nodes, potentially interdigitating within a population and thus creating overlapping genotypes within a specific patch, we also analyzed the microsatellite data from a multilocus perspective and in some cases, using a haplotype analysis in GenAlEx. We then investigated whether the same multilocus genotype could be found in the different subpopulations at each population and also across populations, using the software package Poppr (Kamvar et al. 2014) in R (R Core Team 2014). For this analysis, the data set of 974 individuals was first refined to eliminate genotypes with more than $>$ 20% missing alleles (69 genotypes were removed) and loci with more than $>$ 20% missing data (*M&M15* and contigs *2712* and *1304* were removed, leaving 16 loci). This resulted in a final data set of 905 individuals.

Finally, to determine whether individuals within the same subpopulation, population, or both expressed similar genetic composition, the data were first analyzed in STRUCTURE, a software program (Pritchard et al. 2000) that uses a Bayesian approach of model-based clustering to assign each individual to membership in K groups, which may or may not correspond to the number of sampled populations. Based on this analysis, the percentage of membership in each K group was calculated for each sample, and those values were graphed to allow the researcher to visually determine whether the K groups matched with expectations based on field collection. An admixture ancestry model was run with correlated allele frequencies and a burn-in period of 10,000 and a run of 100,000 iterations for each K from 2 to 10, with five replicates at each K value. The Pritchard et al. (2000) method was used to identify the best K, in which the lowest K is selected for which the estimated log likelihood of $\Pr(X|K)$ first reaches a plateau. In this analysis, seven groups were detected as indicated by the model that generated the highest probability for the data [$\Pr(X|K) = -6132.2$].

Because some assumptions of the STRUCTURE analysis (i.e., nonrandom mating and Hardy-Weinberg equilibrium) were violated in the partially CL and facultatively outbreeding *M. vimineum*, the program InStruct (Gao et al. 2007) was then used to assign the samples into K clusters based, in part, on estimated population-level selfing rates. Five independent Markov Monte Carlo chains were run in a population-based model to infer population structure and population selfing rates for $K = 7$ to 9 (based on the previous STRUCTURE analysis); each chain consisted of 500,000 burn-in iterations for a total of 1,000,000 iterations, with a thinning interval of 10 interactions between retained draws. The Gelman-Rudin statistic was used to check convergence, and the optimal K was inferred. The InStruct analysis was also repeated separately for each population using each subpopulation (roadside, edge, or interior) as

separate populations to determine whether subpopulations in the same population clustered together or separately.

Results and Discussion

Local Patterns of Spread. Although *M. vimineum* may rapidly invade disturbed sites where it can detrimentally affect the ecosystem (e.g., Adams and Englehardt 2009; McGrath and Binkley 2009; Webster et al. 2008), its pattern of spread on a local scale has received relatively little attention (however, see Miller and Matlack 2010). Consequently, we wanted to determine whether genetic patterns were consistent with local diffusion of the species from the roadside into the forest or whether long-distance dispersal from populations at other sites had a role. Within the West Virginia populations sampled here, both mechanisms appeared to be at work. For example, if invasive spread of *M. vimineum* was due solely to local diffusion from the roadside into the forest edge and then into the forest interior, we should find the greatest genetic diversity along the road and subsequently lower levels diversity with distance from the road (because of colonization events and genetic bottlenecks). Although this pattern was detected in three of the nine populations (Cooper12, PRD23, and PRD5; Table 3), it was not found in the other six populations, in which most genetic variation was detected in the forest interior or forest edge subpopulations. However, when analyzed across all populations, estimates of genetic variation did not differ significantly between the roadside and forest interior locations, the two extremes (paired t test; $P > 0.141$).

Local diffusion or long-distance dispersal of the species should also be evident in the distribution of multilocus genotypes (MLGs), which ranged from 3 to 28 within each population (Table 3; Figure 2). If invasive spread of *M. vimineum* progressed from the roadside into the forest edge before continuing on into the forest interior, most genotypes should be found along the road, with a smaller subset of those genotypes in the forest (because of founder events). Alternatively, a cluster of different genotypes in the forest edge or interior (especially genotypes not seen along the roadside) would be consistent with long-distance dispersal and immigration. The former pattern of local dispersal from the roadside was found in some populations, such as Cooper12, with 22 MLGs identified along the roadside and with many of these genotypes present in the adjacent forest (Table 3). However, a pattern of local dispersal was not observed in other populations, such as PRD14, where most MLGs were found along the forest edge ($n = 28$). In addition, several genotypes in the interior were unique within many of the populations, consistent with occasional, long-distance dispersal from other locations. Novel genotypes in the interior forest were unlikely to represent a historical seed bank because *M. vimineum* seeds may only be viable for about 3 yr and are not considered long lived (Bardon 1987; Huebner 2011).

In some cases, the number of genotypes within a subpopulation was exceedingly low, even given a large sample size. For example, interior or roadside subpopulations at CRD23 only contained four genotypes, despite 37 to 42 samples being analyzed (edge samples were not available because of the age of the site). Although it is possible that our estimated number of roadside genotypes was biased because of dense vegetative growth of plants in these areas, we tried to minimize the possibility of inadvertently collecting leaves from multiple stems of the same individual. Other subpopulations exhibited many more genotypes. For example, 10 or more genotypes were detected within the roadside, forest edge, or forest interior samples in the CRDhemlock population, which occupied a relatively shady location. There were also relatively shorter plants at that site in all subpopulations (including the roadside), which could ultimately lead to more outcrossed progeny (Cheplick 2006), although selfing rates at this site indicated that selfing predominated (see below).

There was also substantial genetic differentiation among all subpopulations ($F_{st} = 0.603$, $P < 0.001$), which is consistent with local dispersal and relative genetic isolation on a regional scale. In general, subpopulations clustered together within each site, as visualized by the UPGMA analysis (Figure 3). The exception was the PRD populations, in which samples were generally intermixed with one another but were still separate from the Cooper, WVU, and CRD samples. In the InStruct analysis, conducted separately for subpopulations within each site, the optimal K ranged from 2 to 4, but individuals from subpopulations did not always cluster with one another within each site (Table 4). The Gelman-Rubin statistic for the convergence of log likelihood in the separate analyses ranged from 0.862 to 1.670 (with an exception of 4.181 for Cooper3), indicating good convergence in both log likelihood and selfing rates across different chains (Gao et al. 2007).

Previously, the few studies of local dispersal in *M. vimineum* have focused on the role of roadways in facilitating spread (e.g., Christen and Matlack 2006, 2009; Miller and Matlack 2010) because roadways can act as a movement corridor composed of linear, continuous habitat that enables nonnative species to gain a foothold into a natural area. Although observations of invasive species along roadsides may be an artifact of observer accessibility (Christen and Matlack 2006), *M. vimineum* is recognized to spread along roadways, especially those that are paved (e.g., Christen and Matlack 2009). Propagules may be carried in car tires, dormant seeds can be moved in gravel piles during road maintenance (Christen and Matlack 2009), or seeds may spread via wind turbulence

Table 3. Genetic variation within each of nine populations of *Microstegium vimineum* in which samples were located along the roadside (R), present in forest edge subpopulations perpendicular to the road (E), or scattered individuals in the forest interior (I). Shown are the mean population sample size averaged across loci, mean number of alleles (*A*), percentage of polymorphic loci (% of *P*), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index or inbreeding coefficient (*F*), selfing rate (% *s*), the number of multilocus genotypes, and the total number of samples analyzed.

Population	Subpopulation	Mean size	*A*	% of *P*	H_o	H_e	*F*	% *s*	No. of MLGs	Total no.
Cooper–Northern Allegheny Plateau										
Cooper12	I	22.05	2.47	68.42	0.082	0.173	0.706	82.77	11	22
	E	17.58	1.95	57.89	0.095	0.136	0.457	62.73	10	18
	R	36.26	3.21	89.47	0.096	0.216	0.620	76.55	22	35
Cooper3	I	27.89	1.90	63.16	0.061	0.104	0.607	75.55	6	23
	E	40.58	1.47	52.63	0.064	0.072	0.589	74.14	10	43
	R	43.10	1.21	26.32	0.056	0.047	0.380	55.07	6	48
WVU13	I	25.37	1.26	26.32	0.101	0.068	0.280	43.75	7	27
	E	20.10	1.26	26.32	0.105	0.072	0.271	42.64	5	20
	R	37.21	2.53	73.68	0.093	0.295	0.755	86.10	16	41
Mean		30.01	1.92	53.80	0.084	0.131	0.518	66.59	10.3	30.8
Fernow–Southern Allegheny Plateau										
CRD14	I	42.79	2.05	63.16	0.065	0.287	0.748	85.58	13	46
	E	—	—	—	—	—	—	—	—	—
	R	42.26	2.26	63.16	0.062	0.293	0.756	86.15	12	43
CRD23	I	38.32	1.21	21.05	0.053	0.036	0.500	66.67	4	42
	E	—	—	—	—	—	—	—	—	—
	R	35.16	1.10	21.05	0.047	0.034	0.545	70.55	4	37
CRDhemlock	I	39.58	2.16	73.68	0.136	0.303	0.627	77.08	18	39
	E	35.74	2.68	89.47	0.115	0.268	0.705	82.70	14	35
	R	44.00	2.16	78.95	0.069	0.296	0.817	89.93	16	47
Mean		39.69	1.95	58.65	0.078	0.217	0.671	79.81	11.6	41.3
Ridge and Valley Province										
PRD14	I	42.84	1.53	42.11	0.051	0.068	0.613	76.01	9	44
	E	43.00	2.21	63.16	0.105	0.177	0.613	76.01	28	45
	R	44.21	2.37	73.68	0.074	0.155	0.746	85.45	16	45
PRD23	I	46.32	1.53	47.37	0.041	0.077	0.710	83.04	7	48
	E	33.63	1.32	26.32	0.030	0.060	0.554	71.30	9	32
	R	31.37	1.90	63.16	0.071	0.109	0.778	87.51	12	29
PRD5	I	20.84	1.21	21.05	0.105	0.066	−0.220	36.07	3	22
	E	22.21	1.16	15.79	0.057	0.041	0.051	9.71	6	24
	R	46.74	1.42	36.84	0.058	0.072	0.458	62.83	10	50
Mean		36.80	1.63	43.28	0.066	0.092	0.478	65.33	11.1	37.7
Grand Mean		35.14	1.83	51.73	0.076	0.143	0.550	70.08	11.0	36.2

caused by rapidly moving vehicles (Bäumer et al. 2005). The species can also spread in other ways, such as stream flooding (Christen and Matlack 2009) and along deer trails (Miller and Matlack 2010). Once introduced into a site, expansion of the species may depend on several environmental variables, including the amount of bare soil (Huebner, 2010b; Warren et al. 2011), the soil moisture, the light availability (Christen and Matlack 2009) or the canopy openness (Huebner 2010b), ammonium-N, and pH (Nord et al. 2010), in addition to propagule pressure.

Populations of *M. vimineum* may exhibit source–sink dynamics as a metapopulation (Warren et al. 2011). If so, sink populations in shady, forested habitats should not be self-supporting because of difficulty with recruitment and reproduction, such that their continued persistence requires constant seed input from nearby roadside (source) populations. In this case, interior forest subpopulations should contain a subset of genotypes detected along the roadside. This was seen in some populations, such as PRD14, PRD5, and WVU13, in which fewer genotypes

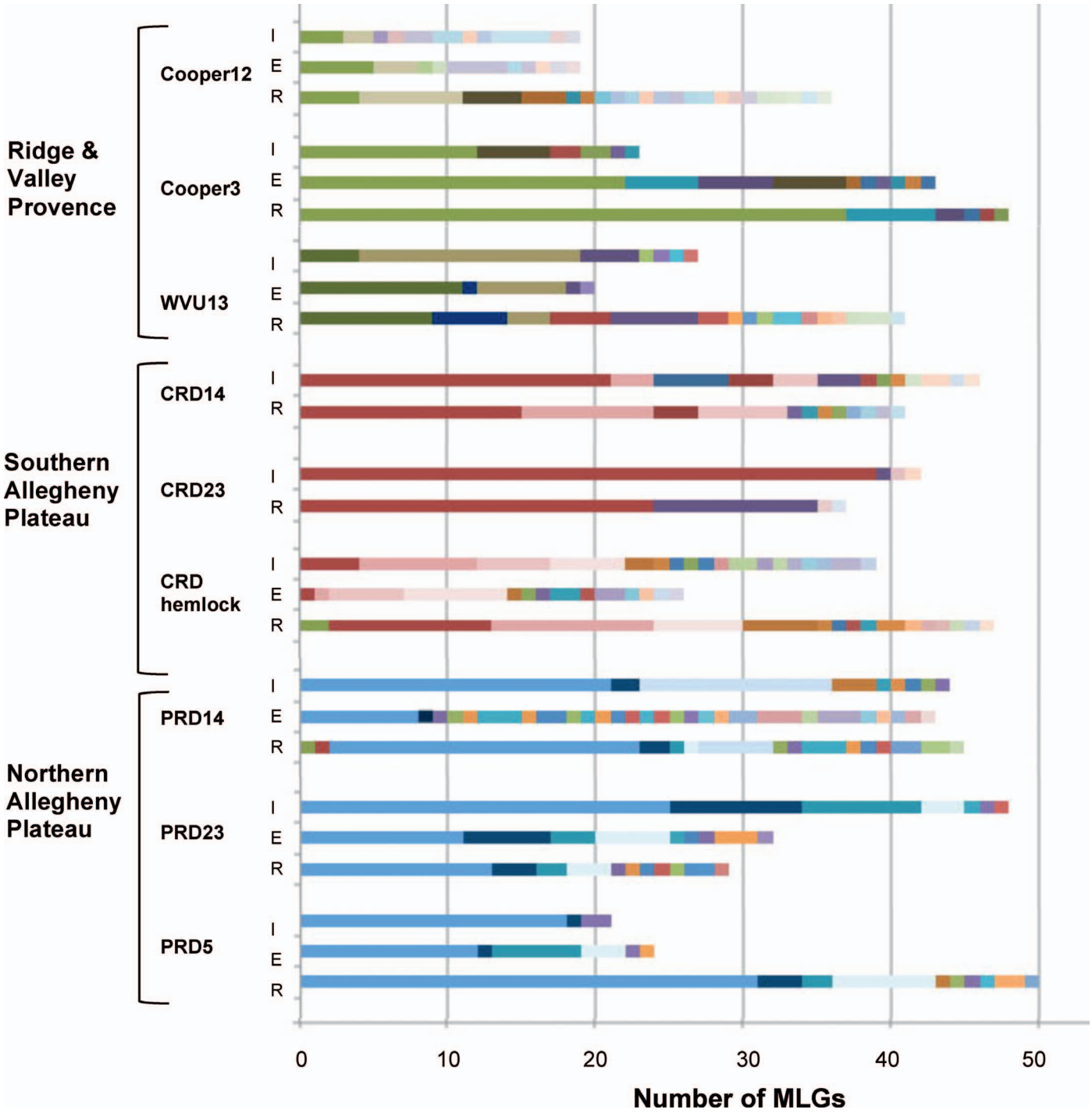

Figure 2. Distribution of multilocus genotypes (MLGs) within each population that were detected along the roadside (R), coalescing in the forest edge (E), and individuals within the forest interior (I). Numbers along the x-axis indicate the numbers of individuals within each genotype. Each MLG is represented by a different color. (Color for this figure is available in the online version of this article.)

were detected in the forest interior as compared along the roadside; furthermore, these forest genotypes were usually a subset of those detected along the roadside, except for one or two rare samples (see Figure 4). Within other populations, however, there were more genotypes detected in the forest interior than identified along the roadside; furthermore, several of these genotypes were different (Figure 4). This could reflect outcrossing events or seed

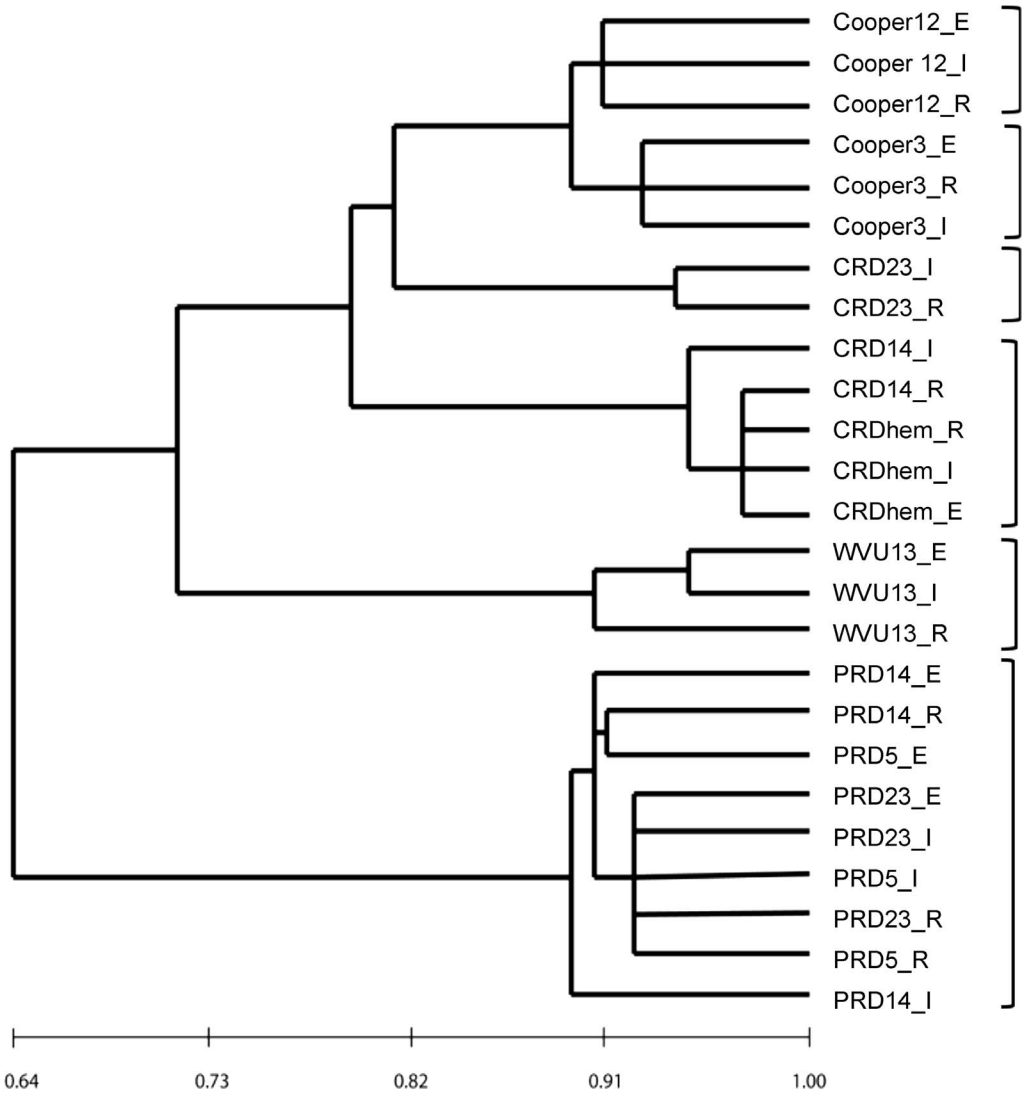

Figure 3. Unweighted pair group method with arithmetic mean phenogram showing clustering relationships among the roadside (R), forest edge (E), and forest interior (I) subpopulations of *Microstegium vimineum* across nine sites in West Virginia.

dispersal from other more-distant areas, which would be incompatible with hypothesized localized source–sink dynamics in *M. vimineum*. Alternatively, genotype differences among subpopulations at a site could result from inconsistent sampling; if our sampling was not comprehensive enough along the roadside, we may have missed "novel" forest interior genotypes, which may, in fact, already exist along the roadside. However, the discovery of only a few MLGs in some roadside subpopulations, despite large sampling (e.g., four MLGs in CRD23 found in 37 samples), indicates that the sampling was sufficient. Interior subpopulations may also not be true sinks because individuals in low-light environments can still produce flowers and seeds that can disperse away, albeit at lower relative numbers than in the high-light environments along the roadside (e.g., Huebner 2010). Seeds produced in the forest interior also exhibited moderate germination rates (Huebner 2011), and seedlings were able to establish and spread within those sites (Christen and Matlack 2009). Taken together, these results indicate that interior populations have the capacity to become self-sustaining, rather than acting solely as a sink.

Inbreeding in the Roadside vs. the Forest Edge and Forest Interior. The CH/CL breeding system of *M. vimineum* may be differentially expressed in roadside vs. forested sites and, thus, may also affect local genetic patterns. Although CL flowers in *M. vimineum* are automatically self-pollinated, CH flowers can be either outcrossed or selfed (if donor pollen is unavailable), as shown in a genetic analysis of plants grown from CH (mean $F_{is} = 0.443$) and CL seed ($F_{is} = 0.924$) obtained

Table 4. Results of the InStruct software analysis conducted separately for each population, focusing on subpopulations (R, roadside; E, forest edge; I, forest interior) and tested for $K = 1$ to 4 (except for CRD14 and CRD23 where $K = 1$ to 3).

Population	Optimal *K*	Gelman-Rudin statistic	Selfing rate ($K = 1$)	Proportion membership in each *K* cluster					
				Subpopulation	Cluster1	Cluster2	Cluster3	Cluster4	No.
Cooper–Northern Allegheny Plateau									
Cooper12	4	0.896	0.365	I:	0.423	0.403	0.1	0.074	25
				E:	0.408	0.512	0.051	0.029	20
				R:	0.329	0.491	0.113	0.061	43
Cooper3	3	3.181	0.165	I:	0.42	0.418	0.162		34
				E:	0.413	0.412	0.176		46
				R:	0.417	0.416	0.167		48
WVU13	4	1.146	0.475	I:	0.855	0.134	0.005	0.006	27
				E:	0.788	0.201	0.005	0.006	22
				R:	0.318	0.241	0.386	0.054	41
Fernow–Southern Allegheny Plateau									
CRD14	3	1.563	0.716	I:	0.247	0.642	0.111		48
				R:	0.485	0.42	0.096		48
CRD23	2	1.67	0.166	I:	0.93	0.071			43
				R:	0.852	0.148			41
CRDhemlock	3	0.862	0.641	I:	0.594	0.328	0.079		43
				E:	0.69	0.19	0.12		39
				R:	0.56	0.389	0.051		49
Ridge and Valley Province									
PRD14	4	1.13	0.403	I:	0.803	0.032	0.162	0.004	45
				E:	0.299	0.301	0.345	0.054	46
				R:	0.636	0.018	0.254	0.091	47
PRD23	3	1.325	0.249	I:	0.427	0.304	0.269		50
				E:	0.431	0.305	0.263		38
				R:	0.416	0.308	0.276		36
PRD5	3	1.038	0.05	I:	0.347	0.332	0.321		22
				E:	0.332	0.332	0.336		24
				R:	0.337	0.332	0.33		50

from roadside plants (T.M.C. and C.D.H., unpublished data). Because of abundance alone, more roadside plants were likely derived from CH seeds than from CL seeds, depending on the overall CL seed viability. Therefore, we expected to find increased outcrossing (i.e., decreased inbreeding) in the roadsides, compared with the forest edge and forest interior.

The results of our genetic study suggest that a mixed-mating system is present in the West Virginia subpopulations of *M. vimineum* but with no clear pattern across the

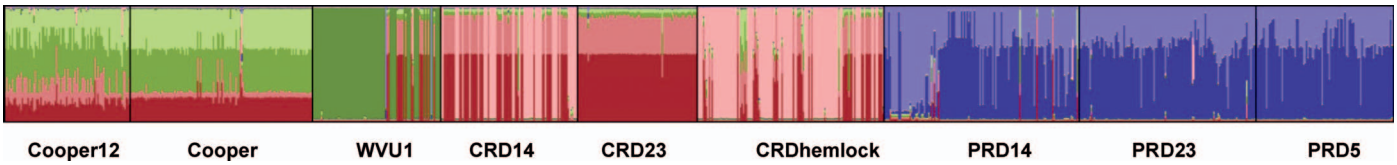

Figure 4. Bar plot from InStruct software showing the grouping of all samples into $K = 8$ groups. Each group is represented by a different color, and each column is a different individual. The proportion of color within each column represents the percentage of membership of the plant sample in that particular group. Data were obtained by running five independent Markov Monte Carlo chains in a population-based model to infer population structure and population selfing rates; each chain consisted of 500,000 burn-in iterations within 1,000,000 total iterations, and a thinning interval of 10 interactions between retained draws. (Color for this figure is available in the online version of this article.)

subpopulations. Overall, selfing rates were generally moderate to high in all 25 subpopulations, ranging from 42.6 to 89.9% (Table 3), with the exception of the PRD5 edge (9.7%) and interior (36.1%) subpopulations. Similarly, the fixation index was moderate to substantial (ranging from 0.27 to 0.82) within each subpopulation (R, E, I) at every site, with the exception of the PRD5 edge ($F = 0.05$) and interior ($F = -0.22$) samples (Table 3). Consequently, our expectation of consistently high inbreeding in the forest relative to the roadside was not supported. For example, in many populations (WVU13, CRDhemlock, PRD14, PRD23, and PRD5), interior and edge subpopulations had relatively lower fixation indices and selfing rates than did roadside subpopulations. Although most interior subpopulations exhibited substantial selfing rates (s; range = 44 to 86%; Table 3), these values were significantly greater than roadside subpopulations in only two of the nine populations (Cooper12, Cooper3). In the case of subpopulations along the forest edge, inbreeding and selfing were prevalent, but values still tended to be less than along the roadside for all populations, except for Cooper3. The discovery of substantial inbreeding in the roadside subpopulations indicates that many plants may be derived from CL seeds or selfed CH seeds, or they may be siblings, despite previous assumptions that outcrossing should predominate in this type of location.

Although midrange to high selfing rates are consistent with a CH/CL system, inbreeding dynamics in the subpopulations could also be complicated by the presence of multiple, identical genotypes in *M. vimineum*, especially along the roadsides. Because F_{is} (the same as F in our study) is the probability that two homologous alleles within an individual are identical by descent relative to the rest of the population (Hedrick 2005), it measures the reduction in heterozygosity from nonrandom mating. Although reduced heterozygosity is most often associated with inbreeding or population substructure (i.e., the Wahlund effect), the same result may also be achieved when multiple copies of different multilocus genotypes increase the probability of alleles being identical within a population (i.e., low genetic diversity captured by the marker system). This is similar in overall effect to the formation of a series of genetic lines within a highly selfing population. In *M. vimineum*, higher values of F (Table 3) were usually found in subpopulations with many unique multilocus genotypes. In addition, the high fixation index for each subpopulation was also consistent with the existence of identical genotypes. The appearance of multiple, identical multilocus genotypes was not completely unexpected, given the low overall genetic diversity in this species; alternatively, these genotypes may suggest the need for more loci in the analysis to maximize the detection of different genotypes.

Genetic Variation and Structure in the Species. Although it can be difficult to know exactly how much genetic variation, if any, is necessary to foster invasive spread in different environments, we conclude that *M. vimineum* does not contain substantial amounts of genetic variation, as measured by these neutral microsatellite markers, despite its aggressive ability to spread. Levels of genetic variation were relatively low to moderate overall in the species (Table 5), with strong a genetic structure among populations ($F_{st} = 0.60$) and generally among sampling regions (Figure 5). The number of alleles per locus ranged from 1.21 to 4.47, with an average of 2.58. The percentage of polymorphic loci averaged 71.9% for the species, but the range of values was highly variable (21 to 100%). Levels of heterozygosity were very low, as measured by H_e (range: 0.03 to 0.30; mean = 0.161) and H_o (range: 0.05 to 0.10; mean = 0.07). The latter value was much lower than the H_o mean of 0.51 reported for other species with mixed mating (Nybom 2004). Most of the loci were out of Hardy-Weinberg equilibrium in most populations, except PRD5; however, this was not unexpected, given the mixed-mating system of the species.

There were also regional patterns of genetic variation detected across populations. Although the CRD23 population contained the lowest amount of genetic variation, populations within the Ridge and Valley Province typically exhibited low variation in general (Table 5). This is consistent with the fact that, compared with the Northern and Southern Allegheny Plateau, RVP sites were drier, with more sun, resulting in plants that were taller, with a greater proportion of CL reproduction; this may explain the higher selfing rate detected in the Ridge and Valley Province (s = 85.6), compared with the Southern (s = 80.2) and Northern (s = 66.4) Allegheny Plateau (Table 5). Overall, the low levels of genetic variation observed in these West Virginian populations were generally consistent with other investigations of the same species (Baker and Dyer 2011), including those that estimated across the United States (H_o = 0.00; A. Novy, unpublished data) and within the native Chinese range (H_o = 0.03; A. Novy, unpublished data).

Significant genetic structure was detected among populations of *M. vimineum* ($F_{st} = 0.60$, $P < 0.001$). This was only slightly greater than an investigation of the species in the neighboring state of Virginia (F_{st} = 0.55 with amplified fragment length polymorphism [AFLP] markers; Baker and Dyer 2011) but was much greater than multiple populations across the United States (F_{st} = 0.39; A. Novy, unpublished data). In addition, 185 MLGs were detected across the 905 individuals; of the 43 MLGs found in more than a single individual, 24 were restricted to a single population, 15 were found across populations within a single region, and 4 MLGs occurred across two or more regions (Figure 6). For example, the most common MLG

Table 5. Descriptive genetic statistics for populations of *Microstegium vimineum* sampled at nine sites in West Virginia (based on 974 total individuals). For each population, samples collected from the roadside, forest edge, and forest interior areas are grouped together. Shown are the sample size averaged across loci, the mean number of alleles (*A*), the percentage of polymorphic loci (% *P*), observed heterozygosity (H_o), expected heterozygosity (H_e), the fixation index or inbreeding coefficient (*F*), the estimated selfing rate (% *s*), and the number of multilocus genotypes.

Population	Sample size	*A*	% *P*	H_o	H_e	*F*	% *s*	No. of MLGs
Cooper12	75.90	4.47	94.74	0.093	0.215	0.694	81.94	35
Cooper3	111.58	2.37	73.68	0.060	0.072	0.601	75.08	16
WVU13	82.68	2.79	84.21	0.100	0.208	0.770	42.02	22
Mean	90.05	3.21	84.21	0.084	0.165	0.688	66.35	24.3
CRD14	85.05	2.37	73.68	0.063	0.302	0.718	83.58	21
CRD23	73.47	1.21	21.05	0.050	0.035	0.523	68.68	6
CRDhemlock	119.32	3.21	100.00	0.104	0.303	0.789	88.20	35
Mean	92.61	2.26	64.91	0.072	0.213	0.677	80.15	20.7
PRD14	130.05	2.90	84.21%	0.077	0.154	0.693	81.87	46
PRD23	111.37	2.42	78.95%	0.049	0.091	0.786	88.01	20
PRD5	89.79	1.47	36.84%	0.070	0.068	0.378	87.00	13
Mean	110.40	3.26	66.67	0.065	0.104	0.619	85.63	26.3
Grand mean	97.68	2.58	71.93%	0.074	0.161	0.661	77.37	23.7

(MLG 45) was detected in 162 individuals located in the PRD14, PRD23, and PRD5 populations. Furthermore, genetic structure was also evident in the PCoA (Figure 5), in which some of the populations were genetically distinct from each other but with a large amount of overlap among individuals from different populations (e.g., PRD populations tended to cluster toward the left, whereas CRD populations grouped toward the right). The first two axes explained 79.30% of the variation, indicating the robustness of this analysis. The InStruct analysis also showed that several populations were differentiated from one another, with those from the same region exhibiting membership in similar clusters (Figure 4). The optimal *K* was 8, and the Gelman-Rubin statistic for the convergence of the log likelihood was 0.9501, indicating strong convergence in the log likelihood and selfing rates across chains (Gao et al.

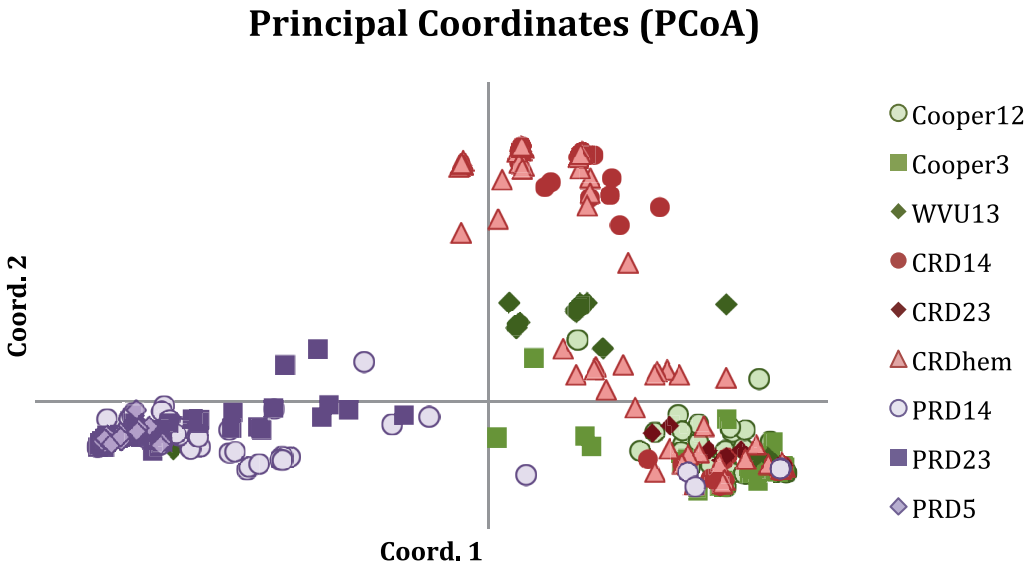

Figure 5. Principal coordinates analysis (PCoA) showing relationships of all individuals of *M. vimineum* in ordination space, based on the 19 microsatellite loci. Samples are color-coded to match the populations. Coordinate 1 and 2 axes contain 47.42 and 20.06% of the variation, respectively. (Color for this figure is available in the online version of this article.)

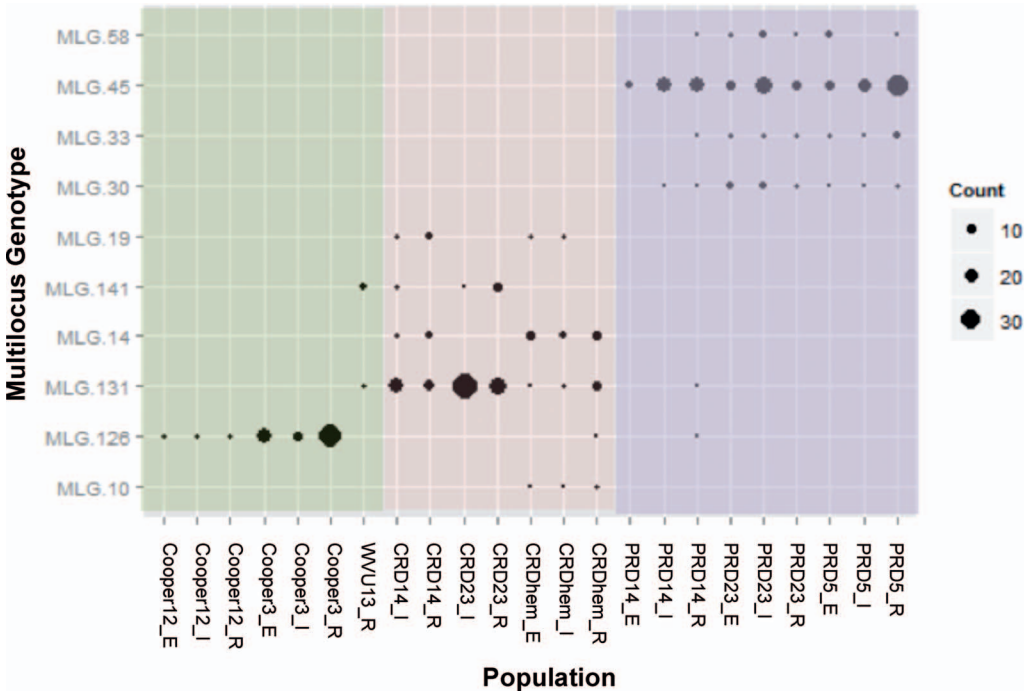

Figure 6. The 10 most-frequent multilocus genotypes (MLGs) detected across regions, populations (see Table 1), and subpopulations (R, roadside; E, forest edge; I, forest interior) of *Microstegium vimineum* in West Virginia. Each dot represents the presence of that particular MLG within the sampling location indicated on the x-axis; the size of each dot represents the number of individuals that possess that MLG. The three sampling regions (see Figure 1) are indicated by the shading color. The edge and interior subpopulations for the WVU population are not shown because they did not contain any of the MLGs depicted here. (Color for this figure is available in the online version of this article.)

2007). Further examination of the five replicate runs for $K = 8$ revealed that the same clusters persisted, but their relative positions shifted slightly across the predefined populations in two of the five replicates. Populations within each region were typically well differentiated from one another.

The discovery that regions differed significantly from one another with detectable genetic clusters is consistent with either separate introductions of this species, selection within each region, or both. In contrast to nonnative species that have been purposely introduced into the United States multiple times through horticulture or other means, *M. vimineum* was accidentally introduced, possibly as a contaminant in packing for ceramics. Consequently, it is difficult to know whether low genetic diversity is due to few initial founder events or it reflects repeated bottlenecks since arrival. Regardless, the potential for adaptation of this species to new environments appears not to be hampered by its low genetic diversity because spread of *M. vimineum* continues unabated in many areas of the United States. Furthermore, despite low genetic diversity, as measured by neutral markers and a lack of a traditional source–sink relationship, the species has demonstrated rapid evolution of phenology during invasive range expansion, suggesting sufficient genetic diversity to drive adaptively significant evolution (Novy et al. 2013). In other monocots, invasive spread can still occur in a relatively genetically depauperate species if one or more genotypes are particularly vigorous and able to tolerate a range of different habitat types. Indeed the seed of Ridge and Valley Province plants were significantly smaller in size and mass than the seeds of plants from the Allegheny Plateau, suggesting potential fitness differences across the regions (Huebner 2011). Consequently, it would be particularly helpful to examine the growth and reproduction of different genotypes of *M. vimineum* in different environments that are characteristic of the introduced range to determine whether these genotypes exhibit fitness differences. In so doing, we might be better able to understand reasons behind the rapid spread of this species and consequently develop more-effective means of management and control.

Acknowledgments

The authors thank L. Chaney for field collection of samples, as well as S. Stancheva, S. Yadav, and S. Albers for assistance with the genetic analysis of the PRD and WVU populations. We thank several anonymous reviewers for their valuable comments. This research was funded by the

Northern Research Station, U.S. Department of Agriculture, Forest Service, Morgantown, WV, with Joint Venture Agreement 10-JV11242303-047.

Literature Cited

Adams SN, Englehardt KAM (2009) Diversity declines in *Microstegium vimineum* (Japanese stiltgrass) patches. Biol Conserv 142:1003–1010

Anderson DP, Turner MG, Pearson SM, Albright TP, Peet RK, Wieben A (2013) Predicting *Microstegium vimineum* invasion in natural plant communities of the southern Blue Ridge Mounts, USA. Biol Invasions 15:1217–1230

Aronson MFJ, Handel SN (2011) Deer and invasive plant species suppress forest herbaceous communities and canopy tree regeneration. Nat Areas J 31:400–407

Baker SA, Dyer RJ (2011) Invasion genetics of *Microstegium vimineum* (Poaceae) within the James River Basin of Virginia, USA. Conserv Genet 12:793–803. DOI: 10.1007/s10592-011-0186-0

Barden LS (1987) Invasion of *Microstegium vimineum* (Poaceae), an exotic, annual, shade-tolerant, C_4 grass, into a North Carolina floodplain. Am Mid Nat 118:40–45

Bäumer D, Vogel B, Fiedler F (2005) A new parameterization of motorway-induced turbulence and its application in a numerical model. Atmos Environ 39:5750–5759

Brown AHD (1979) Enzyme polymorphism in plant populations. Theor Pop Biol 15:1–42

Cai Q, Aitken KS, Fan YH, Piperidis G, Jackson P, McIntyre CL (2005) A preliminary assessment of the genetic relationship between *Erianthus rockii* and the "*Saccharum* complex" using microsatellite (SSR) and AFLP markers. Plant Sci 169:976–984

Cheplick GP (2005) Biomass partitioning and reproductive allocation in the invasive, cleistogamous grass *Microstegium vimineum*: Influence of the light environment. J Torrey Bot Soc 132:214–224

Cheplick GP (2006) A modular approach to biomass allocation in an invasive annual (*Microstegium vimineum*; Poaceae). Am J Bot 93:539–545

Cheplick GP (2008) Growth trajectories and size-dependent reproduction in the highly invasive grass *Microstegium vimineum*. Biol Invasions 10:761–770

Christen D, Matlack G (2006) The role of roadsides in plant invasions: a demographic approach. Conserv Biol 20:385–391

Christen D, Matlack G (2009) The habitat and conduit functions of roads in the spread of three invasive species. Biol Invasions 11:453–465

Davis MA, Thompson K (2000) Eight ways to be a colonizer; two ways to be an invader: A proposed nomenclature scheme for invasion biology. Bull Ecol Soc Am 81:226–230

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Ehrenfeld JG, Kourtev P, Huang W (2001) Changes in soil functions following invasions of exotic understory plants in deciduous forests. Ecol Appl 11:1287–1300

Eschtruth AK, Battles JJ (2008) Acceleration of exotic plant invasion in a forested ecosystem by a generalist herbivore. Conserv Biol 23:388–399

Eschtruth AK, Cleavitt NL, Battles JJ, Evans RA, Fahey TJ (2006) Vegetation dynamics in declining eastern hemlock stands: 9 years of forest response to hemlock woolly adelgid infestation. Can J For Res 36:1435–1450

Flory SL (2010) Management of *Microstegium vimineum* invasions and recovery of resident plant communities. Restor Ecol 18:103–112

Flory SL, Clay K (2010a) Non-native grass invasion alters native plant composition in experimental communities. Biol Invasions 12:1285–1294

Flory SL, Clay K (2010b) Non-native grass invasion suppresses forest succession. Oecologia 164:1029–1038

Flory SL, Lewis J (2009) Nonchemical methods for managing Japanese stiltgrass (*Microstegium vimineum*) Invasive Plant Sci Manag 2:301–308

Fox AM, Gordon DR, Dusky JA, Tyson L, Stocker RK (2008) IFAS Assessment of Non-Native Plants in Florida's Natural Areas—Status Assessment. http://plants.ifas.ufl.edu/assessment/. Accessed November 4, 2014

Gao H, Williamson S, Bustamante CD (2007) A Markov chain Monte Carol approach for joint inference of population structure and inbreeding rates from multilocus genotype data. Genetics 176:1635–1651

Gibson DJ (2002) Life history of *Microstegium vimineum* (Poaceae), an invasive grass in southern Illinois. J Torrey Bot Soc 129:207–219

Glenn TC, Schable M (2005) Isolating microsatellite DNA loci. Methods Enzymol 395:202–222

Hedrick PW (2005) Genetics of Populations. Boston: Jones and Bartlett

Horton JL, Neufeld HS (1998) Photosynthetic responses of *Microstegium vimineum* (Trin.) A. Camus, a shade-tolerant, C_4 grass, to variable light environments. Oecologia 114:11–19

Huebner CD (2007) Strategic management of five deciduous forest invaders using *Microstegium vimineum* as a model species. Pages 19–28 *in* Cavender N, ed. Proceedings of the Ohio Invasive Plants Research Conference: Continuing Partnerships for Invasive Plant Management. Columbus, OH: Ohio Biological Survey

Huebner CD (2010a) Spread of an invasive grass in closed-canopy deciduous forests across local and regional environmental gradients. Biol Invasions 12:2081–2089

Huebner CD (2010b) Establishment of an invasive grass in closed-canopy deciduous forests across local and regional environmental gradients. Biol Invasions 12:2069–2080

Huebner CD (2011) Seed mass, viability, and germination of Japanese stiltgrass (*Microstegium vimineum*) under variable light and moisture conditions. Invasive Plant Sci Manag 4:274–283

Hung KH, Chiang TY, Chiu CT, Hsu TW, Ho CW (2009) Isolation and characterization of microsatellite loci from a potential biofuel plant *Miscanthus sinensis* (Poaceae). Conserv Genet 10:1377–1380

Jain SK (1979) Estimation of outcrossing rates: some alternative procedures. Crop Sci 19:23–26

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

Kleczewski NM, Flory SL (2010) Leaf blight disease on the invasive grass *Microstegium vimineum* caused by a *Bipolaris* sp. Plant Dis 94:807–811

Kourtev PS, Ehrenfeld JG, Huang WZ (1998) Effects of exotic species on soil properties in hardwood forests of New Jersey. Water Air Soil Pollut 105:493–501

Kourtev P, Ehrenfeld JG, Huang W (1999) Differences in earthworm densities and nitrogen dynamics under exotic and native plant species. Biol Invasions 1:237–245

Marshall JM, Buckley DS (2008a) Effects of microsites created by selective harvesting on growth of *Microstegium vimineum* in a Central Hardwood forest. For Sci 54:534–542

Marshall JM, Buckley DS (2008b) Influence of litter removal an mineral soil disturbance on the spread of an invasive grass in a Central Hardwood forest. Biol Invasions 10:531–538

McGrath DA, Binkley MA (2009) *Microstegium vimineum* invasion changes soil chemistry and microarthropod communities in Cumberland Plateau forests. Southeast Nat 8:141–156

Miller NP, Matlack GR (2010) Population expansion in an invasive grass, *Microstegium vimineum*: a test of the channeled diffusion model. Divers Distrib 16:816–826

Mortensen DA, Rauschart ESJ, Nord AN, Jones BP (2009) Forest roads facilitate the spread of invasive plants. Invasive Plant Sci Manag 2:191–199

NatureServe (2004) An Invasive Species Assessment Protocol: Evaluating Non-Native Plants for Their Impact on Biodiversity. http://www.natureserve.org/library/invasiveSpeciesAssessmentProtocol.pdf. Accessed November 4, 2014

[NCDC] National Climatic Data Center. NOAA National Centers for Environmental Information. http://www.ncdc.noaa.gov/. Accessed June 30, 2015.

Nord AN, Mortensen DA, Rauschert ESJ (2010) Environmental factors influence early population growth of Japanese stiltgrass (*Microstegium vimineum*). Invasive Plant Sci Manag 3:17–25

Novy A, Flory SL, Honig JA, Bonos S, Hartman JM (2012) Characterization of polymorphic microsatellites for the invasive grass *Microstegium vimineum* (Poaceae). Am J Bot 99:e56–e58

Novy, A, Flory SL, Hartman JM (2013) Evidence for rapid evolution of phenology in an invasive grass. J Evol Biol 26:443–450

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155

Orwig DA, Foster DR (1998) Forest response to the introduced hemlock woolly adelgid in southern New England, USA. J Torrey Bot Soc 125:60–73

Oswalt CM, Oswalt SN, Clatterbuck WK (2007) Effects of *Microstegium vimineum* (Trin.) A. Camus on native woody species density and diversity in a productive mixed-hardwood forest in Tennessee. Forest Ecol Manag 242:727–732

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel: population genetic software for teaching and research-an update. Bioinformatics (Oxf) 28:2537–2539

Pheloung PC, Williams PA, Halloy SR (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. J Environ Manag 57:239–251

Pisula NL, Meiners SJ (2010) Relative allelopathic potential of invasive plant species in a young disturbed woodland. J Torrey Bot Soc 137:81–87

Pritchard JK, Stephens M, Donnelley P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–995

R Core Team (2014) R: A Language and Environment for Statistical Computing. Version 3.1.0. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org. Accessed July 1, 2014

Rauschert ESJ, Mortensen DA, Bjornstad ON, Nord AN, Peskin N (2010) Slow spread of the aggressive invader, *Microstegium vimineum* (Japanese stiltgrass). Biol Invasions 12:563–579

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN, Weller SG (2001) The population biology of invasive species. Ann Rev Ecol Syst 32:305–332

Simao MCM, Flory SL, Rudgers JA (2010) Experimental plant invasion reduces arthropod abundance and richness across multiple trophic levels. Oikos 119:1553–1562

Smith HA, Johnson WS, Shonkwiler JS, Swanson SR (1999) The implications of variable or constant expansion rates in invasive weed infestations. Weed Sci 47:62–66

[USDA NRCS] US Department of Agriculture, National Resources Conservation Service (2016) The PLANTS Database. http://plants.usda.gov. Accessed April 21, 2016

Ward JS, Mervosh TL (2012) Nonchemical and herbicide treatments for management of Japanese stiltgrass (*Microstegium vimineum*). Invasive Plant Sci Manag 5:9–19

Warren RJ, Wright JP, Bradford MA (2011) The putative niche requirements and landscape dynamics of *Microstegium vimineum*: an invasive Asian grass. Biol Invasions 13:471–483

Webster CR, Rock JH, Froese RE, Jenkins MA (2008) Drought-herbivory interaction disrupts competitive displacement of native plants by *Microstegium vimineum*, 10-year results. Oecol 157:497–508

Winter K, Schmitt MR, Edwards GE (1982) *Microstegium vimineum*, a shade adapted C_4 grass. Plant Sci Lett 24:311–318

Received December 3, 2015, and approved April 4, 2016.

Associate Editor for this paper: John F. Gaskin, USDA-ARS