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Variation in Pathogenicity and Host Range of *Bipolaris* sp. Causing Leaf Blight Disease on the Invasive Grass *Microstegium vimineum*

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Microstegium vimineum is a widespread invasive grass that poses significant threats to forests and disturbed areas throughout the United States. Often, the large-scale, rapid spread of *Microstegium* prohibits management by traditional methods. Control of *Microstegium* may be possible through the use of a pathogen (referred to here as *Bipolaris* Mv) that causes leaf blight on *Microstegium*. Members of the fungal genus *Bipolaris* are known pathogens of many plants, including important agronomic crops. However, little is known about the biology and host range of *Bipolaris* Mv. We used a series of growth chamber and light bank experiments to determine the variation in *Bipolaris* Mv from different geographic origins and its ability to cause foliar lesions and chlorosis on *Microstegium*. We used petri plate and soil infestation assays to determine the effects of *Bipolaris* Mv on *Microstegium* emergence from seed, biomass, and root necrosis. Finally, we tested the host range of these fungi on economically and ecologically important plant species. All isolates increased disease on *Microstegium* foliage relative to controls, although the effects varied among isolates. Isolates increased root necrosis by 97% in petri plate assays and by 4% in soil infestation trials compared to controls. Infestation of soils with *Bipolaris* Mv reduced emergence of *Microstegium* from seed by 31% compared to controls, but did not affect root or stand biomass. *Bipolaris* Mv produced lesions on a range of grasses, including corn, sorghum, rye, and wheat, although lesion size varied with isolate. These results indicate that *Bipolaris* Mv may be an effective pathogen on *Microstegium*, but its use as a bioherbicide may be impractical because of its effects on a wide range of grasses.

Nomenclature: Microstegium (Mary's grass, stiltgrass), *Microstegium vimineum* (Trin.) A. Camus; rimini rye, *Secale cereale* L.; sorghum, *Sorghum bicolor* (L.) Moench ssp. bicolor; wheat, *Triticum aestivum* L.; corn, *Zea mays* L.

Key words: Biocontrol, fungi, invasive grass, pathogen, reservoir hosts.

Microstegium vimineum (stiltgrass, hereafter referred to as *Microstegium*) is a C_4 shade-tolerant annual grass that is a widespread invader of forests and disturbed habitats in the eastern United States as far west as Missouri and Arkansas (U.S. Department of Agriculture–Natural Resources Conservation Service 2005). Once established, *Microstegium* can produce dense stands that alter native plant composition (Bauer and Flory 2011; Flory and Clay 2010a), tree regeneration and succession (Flory and Clay 2010b), decomposition (Ehrenfeld et al. 2001), and soil nutrients (DeMeester and Richter 2010) and carbon cycling (Strickland et al. 2010). Invasions also affect the composition of arthropod assemblages (Civitello et al. 2008; Simao et al. 2010) and feeding preferences of vertebrate herbivores (Knight et al. 2009). Methods such as PRE or POST herbicides, mowing, and hand removal may be effective at controlling small-scale invasions (Flory 2010). However, the large-scale and rapid spread of *Microstegium* invasions has prevented its effective management in most cases. One potential solution is the use of pathogens as bioherbicides (Hallett 2005; Upadhyay et al. 2011). Pathogenic fungi have been used as biocontrol organisms for other invasive plants with some success (Den Breeyen and Charudattan 2009). However, currently there are no biocontrol organisms available for management of *Microstegium*.

Leaf blight disease is a newly described fungal disease on invasive *Microstegium* caused by a *Bipolaris* species that is similar, but not identical, to *Bipolaris zeicola* (Kleczewski and Flory 2010). We refer to the pathogen here as *Bipolaris* Mv. Leaf blight disease is characterized by the presence of expanding foliar and stem lesions; blighting and melting out of entire patches of *Microstegium* has been observed in West Virginia field sites (Russ Richardson, personal communication). In the greenhouse, relatively low levels of foliar infection by *Bipolaris* Mv resulted in a nearly 40% reduction in seed head production (Kleczewski and Flory 2010). Experimental studies have also shown that *Bipolaris* Mv can reduce *Microstegium* performance in forest habitats. When chlorothalonil fungicide was applied repeatedly to diseased *Microstegium* populations over a growing season, lesion area was reduced by up to 68% and *Microstegium* biomass and seed head production was up to 50 and 200% greater, respectively, than in untreated control plots (Flory et al. 2011). In addition to causing foliar symptoms, some members of the *Bipolaris* genus can cause root and crown necrosis on susceptible hosts (Liljeroth et al. 1993, 1996; Mirabolfathy and Ershad 2006) and overwinter in soil or thatch (Duczek et al. 1996; Hodges and Watschke 1975), resulting in persistent disease outbreaks in established areas (Al-Sadi and Deadman 2010; Smiley et al. 2009) and diminished host productivity (Martinez et al. 2010). Newly emerged plants may be particularly susceptible to root infection and blight, as is the case in wheat and rye planted in fields infested with *Bipolaris sorokiniana* (Al-Sadi and Deadman 2010) and tall fescue soils infested with *Cochliobolus sativus* (teleomorph of *B. sorokiniana*; Trevathan 1992, 1996). Seedling emergence also can be reduced in infested soils (Trevathan 1992, 1996). Previous studies have focused on infection levels of *Microstegium* foliage (Kleczewski and Flory 2010; Flory et al. 2011), but the effects of infested soils on emergence of *Microstegium* from seed and seedling productivity are unknown.

Species of *Bipolaris* have been considered as bioherbicides for invasive plants such as johnsongrass [*Sorghum halepense* (L.) Pers.] (Winder and Vandyke 1990), cogongrass [*Imperata cylindrical* (L.) Beauv.] (Yandoc and Charudattan 2004), wild

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poinsettia (*Euphorbia heterophylla* L.) (de Lima Nechet et al. 2006) and painted spurge (*Euphorbia geniculata* L.) (Lakshmanan et al. 1990). However, the host ranges for members of this fungal genus are often broad, and their use could pose threats to other plant species (Stack 1992). For example, the USDA lists 40 known host species for *B. zeicola*, including corn, rice, sorghum, and many native grass species (Farr and Rossman 2011). *Bipolaris* are highly diverse at the species and isolate levels (Arabi and Jawhar 2007; Berbee et al. 1999; Jawhar and Arabi 2009) and have great potential for interspecific hybridization and developing pathogenicity on multiple hosts (Dodd 1993; Nelson and Kline 1964). As a result, new species or races can evolve rapidly and isolates can vary in terms of virulence and host range (Dodd 1993; Nelson 1960).

We currently do not know if *Bipolaris* Mv represents a new species or race of *Bipolaris* unique to invading populations of *Microstegium* or if this pathogen is an undescribed species introduced from native *Microstegium* populations in Asia. Regardless, little is known about the host range of *Bipolaris* Mv. In a previous study, we demonstrated that this pathogen can infect a range of grasses native to the United States (Flory et al. 2011). Managed plants are likely to encounter invasive plants at the crop–noncrop interface, particularly as the range of the invasive species increases (Tscharntke et al. 2007). An understanding of the infection potential of this pathogen on economically important plants is required before we pursue *Bipolaris* Mv as a bioherbicide for use on *Microstegium*.

The objective of this research was to assess the pathogenicity and host range for multiple isolates of *Bipolaris* Mv collected from diseased *Microstegium* populations. The pathogenicity of the fungi was assessed on *Microstegium* foliage of differing ages, on roots of newly germinated plants, and on emergence and growth of *Microstegium* from seed. In addition, we conducted a host range assay to determine if these pathogens pose a potential threat to economically or ecologically important grasses or crops. These results provide a critical first step in determining the potential of *Bipolaris* Mv to control *Microstegium.*

Materials and Methods

Sources of *Bipolaris* Mv and *Microstegium*. We sampled diseased *Microstegium* at Big Oaks National Wildlife Refuge (BONWR), Indiana (38°58′41″N, 85°25′40″W; BP1), and separate populations in Crummies Creek Demonstration Forest (CCDF), West Virginia (38°46′42″N, 81°04′23″W; BP2, BP3, and BP4). Sample vouchers were sent to the U.S. Department of Agriculture National Fungus Collection (Beltsville, MD). The procedures used to isolate and identify these fungi as pathogenic on *Microstegium* have been detailed previously (Kleczewski and Flory 2010). The capacity of BP4 to incite foliar lesions has been described (see Kleczewski and Flory 2010) and was included here as a positive control for disease experiments. All *Microstegium* used in these experiments were germinated from seed collected from disease-free populations of *Microstegium* at BONWR in 2009.

Induction of *Bipolaris* Mv Sporulation. Isolates of pathogenic *Bipolaris* Mv were obtained from vouchers stored on corn meal malt agar slants. Mycelia was transferred to petri plates containing modified V8 agar (V8Mv) and incubated at room temperature under a light bank equipped with four 40-watt cool white fluorescent bulbs set on a 12-h light cycle (Kleczewski and Flory 2010). V8Mv was prepared by boiling 40 g fresh weight *Microstegium* in 1 L dH_20 for 20 min. The liquid extract was strained through four layers of sterile cheesecloth, added to 0.70 g KH_2PO_4, 1.50 g $MgSO_4$, 1.5 g $CaCO_3$, 20 g agar, and 90 ml V8 juice, and autoclaved for 20 min (Winder and Vandyke 1990). After 10 days, petri plates containing pure fungal isolates were cut into 1-cm blocks, placed agar-side down onto petri plates containing V8Mv, and incubated under the light bank previously described. Spores were typically produced on the upper side of the agar blocks within 5 days, after which the agar blocks were transferred into 50-ml Falcon tubes (BD-Mississauga, ON, Canada) containing 25 ml sterile dH_20 and vortexed for 2 min. Spores were separated from mycelia and agar blocks by filtering the test tube contents through four layers of cheesecloth. For all experiments, sporulation and harvesting of spores was timed such that their application to plants of the appropriate age could be carried out within 8 h.

Experiment 1—*Bipolaris* Mv Pathogenicity on *Microstegium* of Different Ages. Ontogenetic resistance may significantly alter susceptibility of plants to fungal pathogens (Heil 2010), and plant age can impact disease expression (Bournival et al. 1994; Chang and Hwang 2003; Nyvall and Hu 1997). Therefore, we conducted experiments on *Microstegium* of different ages (30 days and 55 days POST) to determine how plant age may influence leaf blight disease of *Microstegium*. The experiment was a completely randomized design with three replicates per fungal isolate per experiment, plus controls ($n = 15$ for each experiment). *Microstegium* seeds were germinated three to a pot and grown in the greenhouse in 12-cm-diam plastic pots containing MetroMix 360 (Sun Gro, British Columbia, Canada). We prepared solutions of 5-day-old fungal spores for each isolate, and adjusted solutions to a concentration of 10^4 spores ml^{-1} with sterile distilled water. Three replicate pots of *Microstegium* were sprayed with spores to wetness (Kleczewski and Flory 2010; Winder and Vandyke 1990). Three additional pots of *Microstegium* were sprayed with dH_20 as controls. We applied BP1 at a concentration of 10^3 spores ml^{-1} for the first experiment, because of difficulties in obtaining sufficient quantities of spores. Following inoculation, plants were covered with clear plastic bags to increase humidity and improve pathogen infection, randomized within a growth chamber, and incubated at 26 C for four days (Kleczewski and Flory 2010). Plastic bags were then removed and the plants were grown in the growth chamber for an additional 11 days at 30 C. We visually assessed the percentage of chlorotic and necrotic foliage immediately after removal from the growth chamber. Then, for each pot a random subsample of 15 to 20 leaves was electronically scanned and percent lesion area was evaluated with APS Assess v2.0, Image Analysis Software for Plant Disease Quantification (APS Press, St. Paul, MN). Each of the age experiments was replicated twice in separate growth chambers. The 30-day and 55-day experiments were analyzed independently due to confounding effects of age and block.

Experiment 2—*Bipolaris* Mv Pathogenicity on *Microstegium* Roots. We conducted a petri plate assay to determine the capacity for *Bipolaris* pathogens to cause root necrosis on

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Table 1. Responses of 14 potential host species to *Bipolaris* Mv isolates applied at 1×10^4 spores ml^{-1} and incubated in the greenhouse for 10 days. $n = 6$ for all plant × isolate combinations.[a]

Plant identity and seed source			*Bipolaris* Mv isolate			
Scientific name	Common name	Cultivar/variety	BP1	BP2	BP3	BP4
Microstegium vimineum (Trin.) A. Camus	Stiltgrass[b]		LL	ML	SL	SL
Brassica napus L.	Canola[c]		I	I	I	I
Citrullus lanatus (Thumb.) Matsum. & Nakai	Watermelon[d]	Charleston Grey	I	I	I	I
Cucumis sativus L.	Cucumber[d]	Straight Eight	I	I	I	I
Daucus carota L.	Carrot[d]	Short 'n Sweet	I	I	I	I
	Tall sescue[e]	AR542	ML	SL	SL	SL
Lolium arundinaceum (Schreb.) S. J. Darbyshire	Tall fescue[e]	Jesup	SL	SL	SL	SL
Phaseolus vulgaris L	Green bean[d]	Stringless Green Pod	I	I	I	I
Poa pratensis L.	Kentucky bluegrass[e]	KY 31	ML	SL	SL	SL
Secale cereale L.	Rimini rye[f]		SL	ML	SL[k]	SL
Solanum lycopersicum L.	Tomato[g]	Red Cherry	I	I	I	I
Sorghum bicolor (L.) Moench ssp. bicolor	Sorghum[b]		LL	SL	SL	SL
	Sorghum × sudangrass[h]	Super Su 22	ML	SL	ML	SL
Triticum aestivum L.	Wheat[i]		SL	SL	SL	SL
Zea mays L.	Seed corn[i]		LL	ML	ML	ML
Zea mays L.	Sweet corn[i]	Ambrosia	LL	SL	SL	SL
Zea mays L.	Sweet corn[i]	Incredible	ML	ML	ML	SL
Zea mays L.	Sweet corn[j]	Powwow F1	LL	ML	ML	SL
Zea mays L.	Sweet corn[j]	Fastlane F1	LL	ML	ML	ML

[a] Abbreviations: I, immune; SL, lesions < 1-mm length; ML, lesions 1–5-mm length; LL, lesions > 5-mm length.
[b] Locally acquired.
[c] Johnson Seed Co.
[d] W. Atlee Burpee & Co.
[e] Agresearch, Inc.
[f] Turner Seed Co.
[g] Ferry-Morse Seed Co.
[h] Resource Seeds, Inc.
[i] Pioneer Hi-Bred International, Inc.
[j] Harris-Moran Seed Co.
[k] Indicates visible symptoms of stem necrosis.

newly germinated *Microstegium* plants. The experiment was a completely randomized design with five replicates of each fungal isolate, plus controls ($n = 25$). Isolates of *Bipolaris* Mv were grown on V8Mv under the light bank for 10 days. For each fungus, five 1-cm-diam agar plugs were transferred to the center of individual petri plates containing 1.5% water agar. Isolate 0315 of the watermelon-specific pathogen *Fusarium oxysporum* f.sp. *niveum* was used as a negative control (Egel et al. 2005). We aseptically germinated *Microstegium* in sealed petri plates containing moistened, autoclaved filter paper and then placed two seedlings into each plate such that the root was in contact with the plug margin. Plates were sealed with parafilm, randomized, and incubated under the same light bank previously described. We quantified the average amount of root necrosis per plate after 11 days with the use of a 12-point Horsfall–Barrett scale (Horsfall and Barratt 1945). We chose an 11-day assessment period because pilot studies indicated that *Microstegium* plants begin to show symptoms of nutrient deficiency on water agar approximately 13 days POST (N.M.K., unpublished data). The experiment was replicated a second time 10 days after completion of the first experiment.

Experiment 3—*Bipolaris* Pathogenicity in Soil. We conducted a second experiment to determine the impacts of soil infestation with *Bipolaris* Mv on *Microstegium* emergence from seed and seedling growth and survival. All methods were based on those presented in Trevathan (1992). The experiment was a completely randomized design, with five replicates of each treatment plus controls ($n = 30$). Inoculum was produced by growing *Bipolaris* Mv on 10 plates of V8Mv for 15 days. For each isolate, agar and mycelia were removed from petri plates and homogenized in 300 ml of sterile tap water. Next, we mixed 100 ml of the fungal slurry in 12-cm-diam pots containing autoclaved field soil, diluted 1:1 (vol:vol) with autoclaved sand to lighten the texture and promote root recovery at harvest. Before dilution with sand, the soil had a pH of 7.0 and contained 1.4% organic matter; 28 ppm available P; 74 ppm K; 110 ppm Mg; 800 ppm Ca; and a cation exchange capacity of 5.1 (A & L Great Lakes Laboratories, Inc., Fort Wayne, IN). Controls consisted of pots containing untreated soil in addition to pots containing soil mixed with 100 ml of V8Mv homogenate. Twenty seeds of *Microstegium* were placed onto the soil surface, and the pots were transferred to the light chamber described previously and randomized. Air and soil temperatures were maintained at 26 ± 2 C. Emergence data were recorded over a 25-day period. After 25 days plants were removed from pots, and roots were scored for necrosis with the use of a 12-point Horsfall–Barrett scale. Roots and shoots were dried to constant mass at 60 C and weighed. This experiment was replicated a second time 3 days after completion of the first experiment.

Experiment 4—*Bipolaris* Mv Host Range on Agronomic Plants. The host ranges of *Bipolaris* Mv isolates were tested against a set of 14 plant species representing seven common C_4 and C_3 agricultural grasses (plus *Microstegium*) and six nongrass agricultural crops (Table 1). Two lines of tall fescue [*Lolium arundinaceum* (Schreb.) S.J. Darbyshire] and five lines of corn were included in the analysis, resulting in a total of 19 distinct plant genotypes. Plants were sown into 12-cm-diam pots containing MetroMix360 and then grown for

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Table 2. MANOVA table for the plant age trials (experiment 1) indicating the overall treatment effects and interactions due to fungal isolate and replicated experiments on foliar lesion area, foliar chlorosis, and foliar necrosis of *Microstegium* inoculated with *Bipolaris* Mv or water controls. Decreasing values of the Wilks lambda statistic indicate effects that contribute more to the model.

Source	Wilks lambda	*F*	*P*
30-day-old *Microstegium*			
Fungal isolate	0.034	9.8	< 0.001
Experimental replication	0.773	1.7	0.212
F × E	0.499	1.2	0.344
55-day-old *Microstegium*			
Fungal isolate	0.065	6.4	< 0.001
Experimental replication	0.771	1.6	0.232
F × E	0.485	1.1	0.372

30 days in a greenhouse maintained at 25 C. Supplemental lighting was provided by metal halide lamps that delivered 300 µmol m^2 sec^{-1} PPFD at the bench surface for 12 h day^{-1}. Spores were prepared as previously described and applied to three replicate plants to wetness at a concentration of 10^4 spores ml^{-1}. Sterile water applied to three replicate plants per species served as negative controls, and 30-day-old *Microstegium* treated with the different *Bipolaris* Mv isolates served as positive controls. Plants were then randomized in the greenhouse, covered with plastic bags, and incubated for 72 h. Bags were removed and plants were grown in the greenhouse for an additional 7 days. At the conclusion of the experiment (10 days postinoculation) plants were assessed for lesions on the following scale: I = no lesions; SL = small lesions < 1 mm diameter; ML = medium lesions 1 to 3 mm in diameter; LL = large lesions > 3 mm in diameter (based on Winder and Vandyke 1990). Numerous distinct lesions were required to categorize a plant as susceptible. To confirm pathogenicity, we harvested and surface disinfested a subsample of three symptomatic leaves per treatment and allowed the leaves to incubate on water agar as described previously. The experiment was replicated a second time on a separate bench in the greenhouse.

Statistical Analysis. All statistical analyses were conducted with the use of SPSS v.18 (Chicago, IL). Variables were examined to assure that they met all assumptions of ANOVA using the explore function of SPSS. All proportion data were arcsin–square root transformed prior to analysis. For experiments 1 and 3 we used multivariate ANOVA (MANOVA) to determine if the mean differences among multiple measured variables were due to chance. Further univariate ANOVAs were conducted on individual measured variables if initial MANOVA analysis indicated a significant overall treatment effect. We combined the results of the repeated experiments if no significant experiment by treatment interactions were present. Means for treatments were compared with the use of Fisher's protected least significant difference at P = 0.05. Untransformed data are presented in text and figures as least-squares means ± 1 SE.

Results and Discussion

Experiment 1—*Bipolaris* Pathogenicity on *Microstegium* of Different Ages. For both 30- and 55-day-old *Microstegium,* treatment with *Bipolaris* Mv had an overall effect on the mean differences of measured dependent variables; however, these effects did not interact with experimental replication (Table 2). Foliar lesion areas were affected by treatment with *Bipolaris* Mv for both 30- and 55-day-old trials (Table 3). The four isolates of *Bipolaris* Mv were variable in their capacity to produce lesions on *Microstegium* foliage on both 30- and 55-day-old plants. This supports other studies of this genus that indicate a high degree of isolate-level variability in virulence (Ghazvini and Tekauz 2007, 2008; Traut and Warren 1993). On 30-day-old plants, lesion areas were the largest for isolate BP1 (3.92 ± 0.24%), followed by BP3 (1.87 ± 0.25%), whereas BP2 and BP4 produced similar lesion areas (1.20 ± 0.28%; 1.12 ± 0.24%; Figure 1a). Surprisingly, BP1 produced lesion areas approximately 2.5 times greater than the other isolates on average, even though it was inoculated at tenfold lower concentrations, indicating much greater virulence of this isolate. On 55-day-old *Microstegium*, BP1 again produced the greatest lesion areas (11.91 ± 0.94%), followed by BP2 (7.50 ± 0.94%), BP4 (4.70 ± 1.05%), and BP3 (2.45 ± 0.94%; Figure 1a). Overall, lesion areas were 4.62% greater on 55-day-old plants when compared to 30-day-old plants.

On 30-day-old *Microstegium, Bipolaris* Mv isolates increased foliar chlorosis 194% (BP2) to 291% (BP4) compared to untreated controls (Table 3; Figure 1b). Similarly, there was an overall increase in foliar chlorosis on 55-day-old *Microstegium*. However, the range of chlorosis compared to controls ranged from 187 (BP4) to 274% (BP2; Figure 1b). Although we were not able to test it statistically, the overall levels of chlorosis were lower for 55-day-old plants (average for 30-day-old plants: 15.66 ± 5.55%; average for 55-day-old plants: 8.66 ± 2.77%). *Bipolaris* Mv did not affect foliar necrosis levels for *Microstegium* of either age group (Table 3).

The results of both the 30- and 55-day-old trials indicate that *Bipolaris* Mv not only is a pathogen capable of damaging *Microstegium* of different ages by producing necrotic lesions, but it also can increase the level of chlorosis or plant

Table 3. Overall univariate ANOVA table for the plant age trials (experiment 1), showing effects and interactions due to fungal isolate and replicated experiments on foliar lesion area, foliar chlorosis, and foliar necrosis of *Microstegium* inoculated with *Bipolaris* Mv or water controls.

	Foliar lesion area			Foliar chlorosis			Foliar necrosis		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
30-day-old *Microstegium*									
Fungal isolate	4	35.9	< 0.001	4	7.0	< 0.001	4	0.7	0.613
Experimental replication	1	00.0	< 0.960	1	4.8	< 0.039	1	0.6	0.635
Error	23								
55-day-old *Microstegium*									
Fungal isolate	4	15.9	< 0.001	4	9.3	< 0.001	4	0.6	0.673
Experimental replication	1	03.7	< 0.069	1	1.0	< 0.324	1	0.7	0.675
Error	22								

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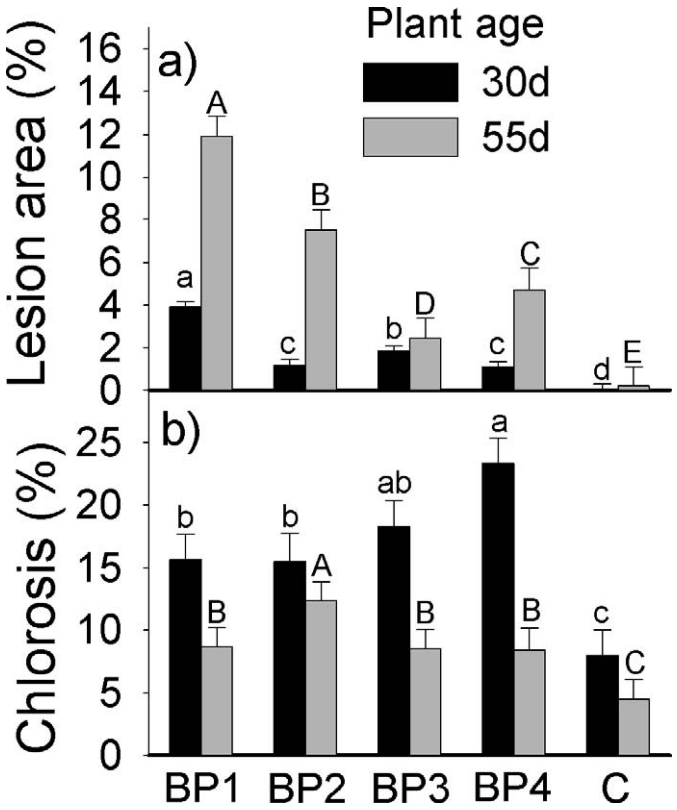

Figure 1. Least-squares means and standard errors for (a) percent lesion area and (b) percent chlorosis resulting from treatment of 30- and 55-day-old *Microstegium* seedlings with spores of different isolates of *Bipolaris* Mv (BP1-BP4) and water controls (c). Measurements were taken 15 days after spores were applied to foliage. Different letters indicate significant mean separations with Fisher's LSD. $n = 6$ for each treatment within an age group.

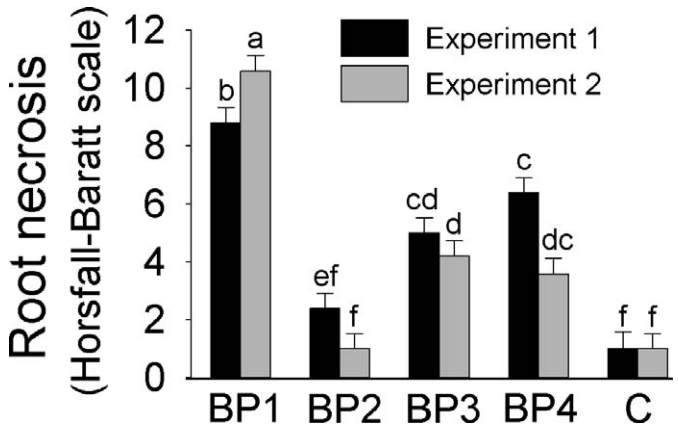

Figure 2. Least-squares means and standard errors for root necrosis ratings of *Microstegium* seedlings treated with plugs of different isolates of *Bipolaris* Mv (BP1-BP4) and a nonpathogenic fungal control (c). Different letters indicate significant mean separations using Fisher's LSD. $n = 5$ for each treatment.

senescence under certain conditions. This outcome supports findings for *B. sorokiniana*, which can increase chlorosis and senescence of barley and wheat (Dehne and Oerke 1985; Kaur et al. 2004). Increased levels of chlorosis or senescence may have important consequences on the growth and competitive interactions between *Microstegium* and co-occurring native plants in the invasive range (Flory et al. 2011). The increase in lesion areas on 55-day-old plants suggests that plant age plays a role in the expression of disease symptoms on *Microstegium*. Consequently, timing of foliar *Bipolaris* Mv application relative to plant age should be further explored to determine the age at which optimal symptom expression occurs, thereby improving the biocontrol potential of *Bipolaris* Mv. We did not detect any effects of *Bipolaris* Mv on the percent of necrotic (dead) foliage. This result may have differed if the length of the experiment was extended because chlorotic foliage would have senesced and increased necrosis ratings of treated plants relative to controls. Therefore, although under these conditions foliar necrosis was unaffected, this may not be true in longer-term studies.

Experiment 2—*Bipolaris* Pathogenicity on *Microstegium* Roots. Our results demonstrate that *Bipolaris* Mv can cause root necrosis on *Microstegium* under the appropriate conditions; however, the degree of necrosis between isolates and experiments was highly variable (Table 4; Figure 2). BP1 caused the greatest levels of root necrosis for both experimental runs of the petri plate assay, averaging ratings of 8.8 and 10.6 for experiments 1 and 2, respectively. These values correspond to approximately 75 to 87% and 94 to 97% of necrotic root length per plant (Horsfall and Barratt 1945). In the first experimental run, BP4 caused the second greatest amount of root necrosis (equivalent to approximately 50% total root length) followed by BP3 (equivalent to approximately 25% total root length). Low levels of root necrosis were detected for BP2, and controls remained free of necrosis. In the second experimental run, BP3 caused the second greatest level of root necrosis (equivalent to approximately 12% total root length) followed by BP4 (equivalent to approximately 10% total root length). No necrosis was observed for controls or BP2 in the second experimental run. The variation in virulence of *Bipolaris* Mv isolates between experiments indicates that infection of roots differs substantially with subtle changes in the host, pathogen, or environment, as is true for most bioherbicidal organisms (Hallett 2005). Regardless, our results demonstrate that *Bipolaris* Mv can cause necrosis of host roots, which is consistent with other members of this genus (Liljeroth et al. 1993, 1996; Mirabolfathy and Ershad 2006). Consequently, *Bipolaris* Mv has the potential to influence *Microstegium*

Table 4. Overall univariate ANOVA table for the root necrosis trials (experiment 2), showing effects and interactions due to fungal isolate and replicated experiments on the degree of root necrosis on *Microstegium* inoculated with *Bipolaris* Mv.

Source	df	*F*	*P*
Fungal isolate	4	82.5	< 0.001
Experimental replication	1	3.5	< 0.066
F × E	4	5.2	< 0.01
Error	39		

Table 5. MANOVA table for the soil infestation trial (experiment 3), overall treatment effects, and interactions due to fungal isolate and replicated experiments on percent emergence from seed, stand biomass, root biomass, and root necrosis of *Microstegium* germinated in soil infested with *Bipolaris* Mv. Decreasing values of the Wilks lambda statistic indicate effects that contribute more to the model.

Source	Wilks lambda	*F*	*P*
Fungal isolate	0.024	4.0	< 0.001
Experimental replication	0.743	4.3	< 0.050
F × E	0.542	1.5	< 0.081

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Table 6. Overall univariate ANOVA table for the soil infestation trial, showing effects and interactions due to fungal isolate and replicated experiments on percent emergence, stand biomass, root biomass, and root necrosis of *Microstegium* germinated in soil infested with *Bipolaris* Mv or controls.

	Percent emergence			Stand biomass			Root biomass			Root necrosis		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Fungal isolate	5	2.4	< 0.050	5	1.8	0.137	5	1.5	< 0.213	5	12.9	< 0.001
Experimental replication	1	3.5	< 0.068	1	0.5	0.485	1	4.7	< 0.050	1	1.0	< 0.316
Error	53			53			53			53		

through direct effects on the roots of emerging seedlings. More research is needed to determine how soils infested with this pathogen affect the roots and productivity of mature *Microstegium*.

Experiment 3—*Bipolaris* Pathogenicity in Soil. Soil treatments and experimental replication had main effects on dependent variables, but did not interact with one another (Table 5). With the exception of BP1, isolates of *Bipolaris* Mv reduced seedling emergence by approximately 31% relative to V8 agar controls (Table 6, Figure 3). This is in accordance with other studies of this genus (Kumar et al. 2002; Trevathan 1992). This difference in emergence did not translate into differences in stand biomass at the time of harvest, which was likely due to plastic growth responses under reduced plant densities. Although *Bipolaris* Mv increased visible symptoms of root necrosis relative to controls (Table 5; Figure 4), no differences were detected in root biomass. This may have been a result of the relatively low levels of necrosis observed on roots, which on average scored a Horsfall–Barrett rating of 1.9 (equivalent to 3 to 4% total root length with necrosis). These results suggest that if *Bipolaris* Mv already is present in the soil, levels of *Microstegium* establishment may be reduced compared to uninfested areas. However, these initial effects may not be sufficient to curtail growth of this grass if levels of root infection are low, as was observed in this study. Other aspects of the soil, such as temperature, pH, organic matter content and quality, and soil microbial composition, may influence the outcome of *Bipolaris* Mv infection of *Microstegium* roots (Fradkin and Patrick 1985; Scardaci 1981) and therefore plant growth. The form of inoculum (i.e., spores vs. mycelium) also can influence infection of roots by *Bipolaris* (Duzcek et al. 1996; Trevathan 1992). Further work on this pathogen should explore the effects of various soil properties and inoculum levels and types on *Microstegium* growth and germination.

Experiment 4—*Bipolaris* Host Range on Agronomic Plants. Our results show that *Bipolaris* Mv can cause disease on grasses of agronomic and ecological importance. All eight of the grasses tested were susceptible to each isolate of *Bipolaris* Mv to some degree, although there was variation in lesion development among fungal isolates and plant species/lines (Table 1). BP1 produced the largest lesions on tested grasses, producing large lesions on *Microstegium*, sorghum, seed corn, and sweet corn (Ambrosia, PowWow F1, and Fastland F1; Table 1). BP1 produced medium lesions on tall fescue (AR542), Kentucky bluegrass, sorghum × sudangrass, and sweet corn (Incredible); whereas this isolate only produced small lesions on tall fescue (Jesup), rimini rye, and wheat (Table 1). BP2 produced medium lesions on *Microstegium*, rimini rye, and corn, and small lesions on tall fescue (AR542 and Jesup), Kentucky bluegrass, sorghum, sorghum × sudangrass, wheat, and sweet corn (ambrosia; Table 1). BP3 produced medium lesions on sorghum × sudangrass, seed corn, and sweet corn (Incredible, PowWow F1, Fastlane F1), while producing small lesions on *Microstegium*, tall fescue (Ar542 and Jesup), Kentucky bluegrass, rimini rye, sorghum, wheat, and sweet corn (Ambrosia; Table 1). BP4 produced medium lesions on seed corn and sweet corn (Fastlane F1), while producing small lesions on *Microstegium*, tall fescue (AR542 and Jesup), Kentucky bluegrass, rimini rye, sorghum, sorghum × sudangrass, wheat, and sweet corn (Ambrosia, Incredible, and PowWow F1; Table 1). Of the 14 tested plant species, none of the six nongrasses, including canola, carrot, cucumber, green bean, tomato, and watermelon, developed lesions in response to

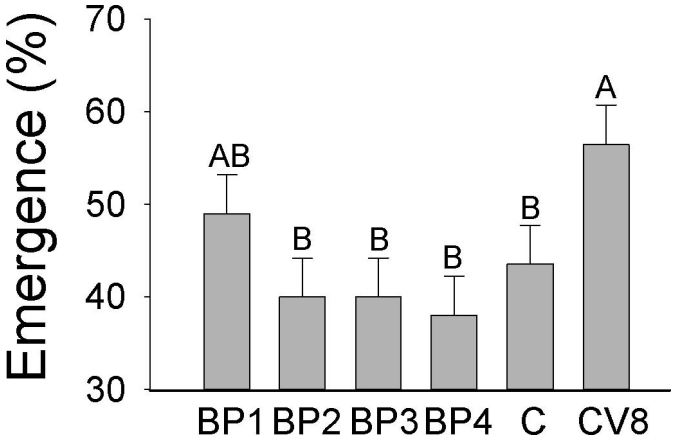

Figure 3. Least-squares means and standard errors for the percent of *Microstegium* emerging 25 days after treatment of soil with different isolates of *Bipolaris* Mv plus V8Mv agar (BP1-BP4), soil treated with only V8Mv agar (CV8), and untreated soil (c). Different letters indicate significant mean separations using Fisher's LSD. $n = 10$ for each treatment.

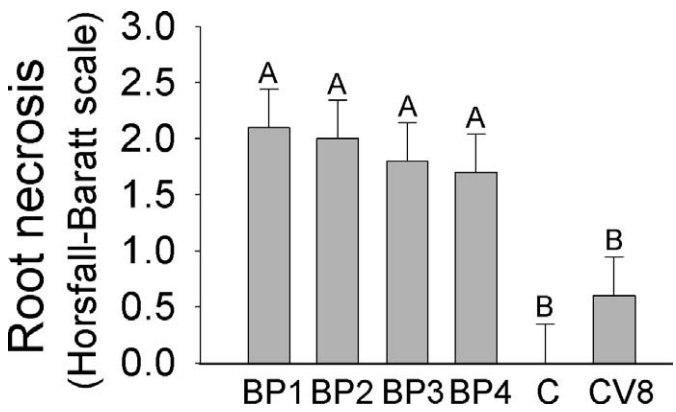

Figure 4. Least-squares means and standard errors for root necrosis ratings of *Microstegium* emerging from soil treated with slurries of different isolates of *Bipolaris* Mv and V8Mv agar (BP1 to BP4), soil treated with only V8Mv agar (CV8), and untreated soil (c). Different letters indicate significant mean separations using Fisher's LSD. $n = 10$ for each treatment.

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inoculation with *Bipolaris* Mv (Table 1). Water controls remained free of lesions, and in all cases only *Bipolaris* Mv was observed sporulating on lesions, confirming pathogenicity for the isolate on compatible hosts.

The susceptible grasses tested in this experiment are commonly found in yards, agronomic fields, and other managed settings (e.g., golf courses, athletic fields) throughout the United States. The goal for this experiment was to provide a basic range of hosts for *Bipolaris* Mv, not to measure the effects of this pathogen on susceptible plants. Lesion size has been associated with susceptibility to *B. zeicola* in corn (Traut and Warren 1993), *B. sorokiniana* in barley (Bashyal et al. 2011) and wheat (Domiciano et al. 2010), and *B. oryzae* in rice (Carvalho et al. 2010). The variation in lesion size observed here may provide an estimate of potential effects of infection by *Bipolaris* Mv on these grasses (Kaur et al. 2004). The impact of *Bipolaris* Mv infection on these plants may not be severe in managed settings, as fungal diseases are often heavily managed through fungicide applications. Support for the effectiveness of a fungicide for controlling *Bipolaris* Mv has been reported in a previous study (Flory et al. 2011), and this fungus is likely to be sensitive to other fungicides utilized to control related fungi in other systems. Regardless, the direct effects of *Bipolaris* Mv on the productivity and health of these grasses warrants further study.

Earlier studies have demonstrated the potential negative impacts of *Bipolaris* Mv on native plants co-occurring with *Microstegium* (Flory et al. 2011). Managed grasses potentially can serve as corridors to spread the disease to adjacent populations of *Microstegium* and associated native grasses, as has been demonstrated in other systems (Tscharntke et al. 2007). In a study of the effects of *B. sorokiniana* on wheat, Kaur et al. (2004) found that sporulation was greater in isolates producing smaller lesions relative to those producing larger lesions. Therefore, even if infection by *Bipolaris* Mv does not cause significant amounts of disease on managed grasses, these plants may still serve as reservoirs, allowing pathogen spillover onto native grass populations. Indeed, this pathogen may pose equal threats on managed or natural grass populations. It is apparent that there are numerous unresolved questions that need to be addressed before *Bipolaris* Mv can be used as a bioherbicide of *Microstegium*.

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