Population Structure, Mating Type, and Mefenoxam Sensitivity of *Phytophthora nicotianae* in Virginia Tobacco Fields

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ABSTRACT

Parkunan, V., Johnson, C. S., Bowman, B. C., and Hong, C. X. 2010. Population structure, mating type, and mefenoxam sensitivity of *Phytophthora nicotianae* in Virginia tobacco fields. Plant Dis. 94:1361-1365.

Black shank is an important disease of tobacco (*Nicotiana tabacum*) caused by the fungus-like organism, *Phytophthora nicotianae*. Three physiological races (0, 1, and 3) have been documented in the United States. Shifts in the pathogen population structure have become a concern due to the widespread use of cultivars possessing resistance to race 0 arising from a single gene (Ph_p or Ph_l). A comprehensive statewide survey conducted throughout major tobacco-growing areas during summer 2006 and supplemented by additional isolates in 2007 and 2008 yielded 217 isolates from flue-cured, burley, and dark fire-cured tobacco fields. After determining species identity using a single-strand conformational polymorphism fingerprinting technique, the race identity of isolates was assessed via greenhouse tests using three differential cultivars (Hicks, L8, and NC1071). Approximately 76% of the isolates belonged to race 1, 21% to race 0, and the remaining 3% were race 3. This race structure was comparable with those in the other tobacco-producing states in the United States. Approximately 94% of isolates belonged to A² mating type and merely 6% were A¹. These data suggest that it is unlikely that sexual recombination serves as a major mechanism enhancing the genetic diversity of the pathogen in Virginia. All isolates were also evaluated against mefenoxam at 5 µg/ml. None were insensitive; 98% of isolates were either highly sensitive or sensitive and the remaining 2% were intermediately sensitive. These results indicate that mefenoxam remains effective for control of black shank in Virginia. The results of this study can assist breeders to develop cultivars possessing the most appropriate set of disease resistance traits, as well as extension specialists, county agents, and tobacco growers in their decision-making process to manage tobacco black shank in Virginia.

Black shank is a root and stem rot disease of tobacco (*Nicotiana tabacum* L.) caused by a fungus-like soilborne pathogen, *Phytophthora nicotianae* Breda de Haan (= *P. nicotianae* var. *nicotianae*). It is one of the most widespread and damaging diseases of cultivated tobacco in the southeastern United States and other tobacco-producing regions worldwide (4,10,12,28). Three physiological races (0, 1, and 3) have been reported in the United States (16–18,34). Aggressiveness of *P. nicotianae* has been shown to differ widely from one population to another (2,7,33,35). All phenological stages of the plants may be affected, resulting in symptoms such as wilting of leaves, chlorosis, stunting, necrosis of the lower stem and root, and disk-ing of the pith (9). Infection occurring during early stages of plant growth causes severe stunting, collapsing of leaves, and death, which worsen under hot weather

and drought conditions (9). Severe yield losses occur in all tobacco types, sometimes reaching 100% when disease management practices are not implemented (31). Disease severity and occurrence have been shown to be influenced by the tobacco cultivars grown (30). Resistant cultivars have played a major role in reducing crop losses since their release in 1943 (5,36). Resistance has been incorporated inter- and intraspecifically into cultivated tobaccos from three sources: *N. tabacum* 'Florida 301', *N. longiflora*, and *N. plumbaginifolia* (27,32). Resistance from the latter two sources involves single, dominant genes (*viz.*, Ph_l and Ph_p , respectively). Both genes are highly effective against race 0 but do not provide any protection against race 1 of *P. nicotianae* (22–24). Resistance derived from Florida 301 is effective against both races of black shank and, for this reason, has been the preferred source of resistance for approximately four decades (3,37). However, resistance derived from Florida 301 provides only partial protection from disease, requiring supplemental soil fungicide applications and crop rotation for growers to meet their production goals. Release of a series of hybrid tobacco cultivars (NC71, NC72, NC291, NC297, RG H51, Speight H20,

and others) has dramatically enhanced the use of host resistance among growers and reduced soil fungicide use significantly (6,23). These hybrids possess a single dominant gene (Ph_p), originally derived from *N. plumbaginifolia*, but also hold varying levels of Florida 301 resistance (6,23). Until recently, the Ph_p gene was mainly incorporated into flue-cured tobacco, while the Ph_l gene was incorporated more commonly into burley tobacco (16,23,34). Since the widespread deployment of hybrid flue-cured tobacco cultivars possessing Ph_p in the 1990s, the frequency of race 1 populations has increased dramatically in Georgia and North Carolina (3,8,9,29). Occurrence of race 1 populations has been documented since the 1960s (3,9,14,20,27). Race 1 populations can emerge within one to two growing seasons after deployment of complete resistance to race 0 populations via the Ph_p gene-mediated resistance (34). Apparent race shifts have resulted in complete failures of tobacco crops under commercial field conditions over relatively short periods of time. Race shifts within the black shank pathogen populations in these states prompted a statewide survey in Virginia to determine whether or not a similar shift was occurring. Our main objectives were to describe the population structure and distribution of *P. nicotianae* in commercial tobacco fields in Virginia. Additional goals were to assess the mefenoxam sensitivity and mating type structure of these populations.

MATERIALS AND METHODS

Isolate collection. A statewide survey was conducted during the summer of 2006 to collect isolates of *Phytophthora* from tobacco fields in Virginia's flue-cured, burley, and dark fire-cured tobacco-producing regions. Additional isolates were obtained in 2007 and 2008 from additional fields identified through Virginia Cooperative Extension diagnostic activities. Tobacco plants exhibiting black-shank-like symptoms, representing cultivars with or without the Ph_p or Ph_l gene, were collected from 12 counties (Fig. 1). Basal stem portions exhibiting a blackened lesion were transported to the laboratory at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA for isolation of *Phytophthora* spp.

Isolations, purification, and storage of isolates. Portions of pith along the leading

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Accepted for publication 29 July 2010.

edge of the lesion within each stem were aseptically removed and cut into 0.5- to 1.0-cm² pieces. Three to four pieces from each sample were subjected to a commercial immunodetection assay kit (Alert-LF; Neogen Europe Ltd., Auchincruive, Scotland, UK) to confirm the presence of *Phytophthora* spp. Samples testing positive for *Phytophthora* spp. were further processed for isolation as follows. In all, 5 to 10 remaining pieces of pith were surface sterilized with 0.5% sodium hypochlorite for 30 s and, subsequently, with 70% ethanol for 60 s, then rinsed twice in sterile deionized water, before plating on PARPH-V8 agar, selective for *Phytophthora* spp. (13). Emerging colonies were subcultured onto PARP-V8 agar and purified cultures were maintained on clarified V8 agar. Isolates were maintained in sterile deionized water with or without sterile hemp seed in 2-ml screw-cap tubes at 15°C (12).

Species identity. A colony polymerase chain reaction (PCR) was performed using internal transcribed spacer (ITS)6 (5'-GAA GGT GAA GTC GTA ACA AGG-3') and ITS7 (5'-AGC GTT CTT CAT CGA TGT GC-3') primers. DNA fingerprints were obtained from a single-strand conformational polymorphism (SSCP) analysis of PCR products (25). The PCR conditions included initial denaturation of DNA for 2 min at 96°C; followed by 30 cycles of 30 s of denaturation at 94°C, 45 s of annealing at 55°C, and 60 s of extension at 72°C; and, finally, 10 min of incubation at 72°C. *Phytophthora* isolates were identified to the species level by comparisons with the standard PCR-SSCP fingerprints (15).

Inoculum preparation. Purified isolates were grown in 10% clarified V8 broth in 10-cm-diameter petri dishes for 1 week under ambient laboratory temperature (22°C) in the dark (1). Five 5-mm-diameter plugs of actively growing mycelium of each isolate were transferred into approximately 10 ml of 10% clarified V8 broth. Three plates were maintained for each isolate. After 1 week of incubation in the dark, the clarified V8 medium was carefully removed from the culture plate without disturbing the mycelial colonies, using a separate Pasteur pipette for each

isolate. After adding 1% cold, sterile soil-water extract, plates were incubated for 3 to 4 h under fluorescent light. Soil-water extract was prepared by mixing rhizosphere soil into deionized water and filtering through Whatman no. 4 and 1 papers. Plates were then checked under the microscope (×100) to ensure that zoospores were released from the intact sporangia. The suspensions from all three plates (approximately equaling 10 ml from each plate) of each isolate were combined and transferred into a 50-ml tube. After mixing the contents of each 50-ml tube thoroughly, a 1-ml aliquot was transferred into a 2-ml Eppendorf tube. The Eppendorf tube was then vortexed for 60 s to encyst the zoospores, and zoospores were counted in two 10-μl samples of the aliquot suspension using a hemacytometer (1). The mean values were used to estimate the approximate number of zoospores in 1 ml of suspension (1). Final volume was adjusted by diluting the 30-ml suspension based on number of zoospores in 1 ml of suspension to obtain a final inoculum density of 15,000 zoospores/ml.

Inoculation procedure and race identity. A greenhouse assay was conducted using three tobacco entries: Hicks, L8, and NC1071 (18). Hicks is susceptible to races 0, 1, and 3 of *P. nicotianae*; L8 and NC1071 are resistant to race 0 but susceptible to race 1; and L8 is also susceptible to race 3. A 15,000 zoospore/ml suspension (2 ml) was applied to the foliage of each 5-week-old plant (with four true leaves); runoff from the leaves drained into the soilless media (Metro Mix 360; Sun Gro Horticulture, Bellevue, WA) around the roots of each plant in a 5-by-5-cm round cell. Five plants of each cultivar were inoculated. Inoculated plants were maintained under greenhouse conditions with night and day temperatures ranging from 18 to 32°C in separate 3-by-5-cell multi-well trays for each isolate. Inoculated plants were maintained for 1 to 2 weeks in most cases. Isolates that didn't produce symptoms during the initial 2-week period were maintained for an additional 2 weeks before evaluation for symptoms. Races of *P. nicotianae* were identified based on differential response of

cultivars. Isolates that caused unclear symptoms or no symptoms at all were retested. If no symptoms were observed at the end of the second test, tissue samples were collected separately for each cultivar and plated on PARPH-V8 agar medium after surface sterilization. Races were identified based on the recovery of isolates from the three differential cultivars. Because some isolates produced no symptoms on host differential cultivars, the only reliable way to identify whether or not they colonized the plant was by plating leaf, shoot, and root tissue onto selective medium after surface sterilization. When isolates were recovered from all three cultivars, they were identified as race 1; if recovered from only Hicks and not L8 and NC1071, isolates were identified as race 0; and if isolates were recovered from Hicks and L8 but not from NC1071, they were identified as race 3. This new method of race identification was used only for isolates that did not produce any symptoms. The same rationale was used for this method compared with race identification via symptom production by three host differential cultivars.

Mating type. The mating types of 217 isolates were determined by pairing known A¹ and A² testers of *P. meadii* or *P. nicotianae* on 10% clarified V8 juice agar medium. The unknown isolate was placed on one side of the 60-mm-diameter petri dish and the known A¹ or A² tester was placed on the other side. The petri dishes were incubated in the dark at 25°C for 4 to 8 weeks or until oospores were formed. Mating type was identified for unknown isolates based on the presence of oospores. If pairing with the known A¹ tester produced oospores, then the unknown isolate was determined to be A², and vice versa. If isolates did not produce oospores after 8 weeks, a second attempt was made using previously identified *P. nicotianae* testers.

Mefenoxam sensitivity. Isolates were assessed for their sensitivity to mefenoxam, the active ingredient in Ridomil Gold SL ((R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylaminio]-propionic acid methyl ester; Syngenta Crop Protection, Greensboro, NC). In total, 217 isolates were screened at the field application concentration of mefenoxam at 5 μg/ml in 10% clarified V8 agar medium. Plates used were 60 mm in diameter and replicated three times, along with control plates without the amendment of mefenoxam. Plugs (5 mm) were placed in the center of the plate and incubated at 25°C for 7 days. At the end of 7 days, the mycelial diameter of radial growth was measured in two directions; mean values were used to calculate growth compared with control plates. The experiment was repeated once under the same conditions. Based on the mean relative growth of isolates, mefenoxam sensitivity was classified into four categories: highly sensitive, 0 to 10% growth;

Fig. 1. Race distribution of *Phytophthora nicotianae* in flue-cured, burley, and dark tobacco fields in Virginia during 2006 to 2008.

sensitive, 11 to 25% growth; intermediately sensitive, 26 to 50% growth; and insensitive, 51 to 100% growth.

RESULTS

Species and race identity. As identified by morphology and the SSCP fingerprinting technique, 217 isolates of the 219 samples tested belonged to *P. nicotianae*. The remaining two isolates were identified as *P. inundata* based on morphological characteristics and ITS sequencing.

Of the 217 *P. nicotianae* isolates tested, 165 isolates (76%) were race 1, 46 isolates (21%) were race 0, and six isolates (3%) were race 3 (Table 1). Approximately 97% of the isolates collected from *Ph_p* or *Ph_i* gene-containing cultivars were race 1 and the remaining 3% were race 0. Likewise, 14% of the isolates collected from cultivars without the *Ph_p* or *Ph_i* gene were race 1, 76% of the isolates were race 0, and the remaining 10% were race 3 (Table 2). All race 3 isolates were collected from cultivars without the *Ph_p* or *Ph_i* gene (Table 2).

Mating type. Among the 217 *P. nicotianae* isolates tested, 94% were A² and the remaining 6% were A¹ (Table 1). The proportion of A² to A¹ in *Ph* gene-containing cultivars and non-*Ph* gene-containing cultivars was very similar, 93:7 and 94:6, respectively (Table 2). All isolates recovered from burley cultivars were A² mating type, regardless of the presence or absence of the *Ph* gene. Isolates from flue-cured and dark fire-cured tobaccos represented both mating types (Table 2). Only a single mating type was recovered from 88% of the fields sampled, with the A² mating type accounting for 84% of the fields; both mating types were recovered from 12% of the fields (*data not shown*).

Mefenoxam sensitivity. Of the 217 *P. nicotianae* isolates tested, 68% were highly sensitive and 30% were sensitive, whereas only 2% were intermediately sensitive and none were insensitive (Table 1). All isolates recovered from the *Ph*-gene-containing cultivars were either highly sensitive or sensitive and none were

intermediately sensitive. Isolates recovered from cultivars not possessing the *Ph* genes represented all three categories of mefenoxam sensitivity.

DISCUSSION

This study is the first state-wide survey conducted in Virginia tobacco fields to assess the population structure of *P. nicotianae* in terms of host race, mating type, and mefenoxam sensitivity. From our race distribution results, it is clear that race 1 now dominates Virginia tobacco fields, followed by races 0 and 3. Host resistance mediated via the *Ph_p* or *Ph_i* gene is the most economical and widely used strategy for black shank management. Because complete resistance to race 1 of *P. nicotianae* is not available in commercial tobacco cultivars, growers must rely on mefenoxam fungicides or cultivars possessing greater levels of Florida 301 resistance to manage the black shank pathogen. Studies to locate complete resistance to race 1 populations among wild species of tobacco have been

Table 1. Race distribution, mating type structure, and mefenoxam sensitivity of tobacco black shank pathogen *Phytophthora nicotianae* populations recovered from flue-cured, burley, and dark fire-cured tobacco fields in Virginia during 2006 to 2008^a

<i>Ph</i> gene	Tobacco type	Cultivar	Race distribution			Mating type		Mefenoxam sensitivity		
			Race 0	Race 1	Race 3	A ¹	A ²	HS	S	IS
–	Flue-cured	K326	21 (88)	0 (0)	3 (13)	1 (4)	23 (96)	7 (29)	16 (67)	1 (4)
–	Flue-cured	K346	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
–	Flue-cured	VA116	1 (50)	0 (0)	1 (50)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)
–	Flue-cured	Oxford 414	0 (0)	4 (100)	0 (0)	1 (25)	3 (75)	1 (25)	2 (50)	1 (25)
–	Burley	KT204	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
–	Burley	TN90	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	1 (50)	1 (50)
–	Dark	VA359	15 (100)	0 (0)	0 (0)	1 (7)	14 (93)	3 (20)	10 (67)	2 (13)
<i>Ph_p</i>	Flue-cured	CC27	0 (0)	17 (100)	0 (0)	0 (0)	17 (100)	17 (100)	0 (0)	0 (0)
<i>Ph_p</i>	Flue-cured	GL350	1 (11)	8 (89)	0 (0)	0 (0)	9 (100)	8 (89)	1 (11)	0 (0)
<i>Ph_p</i>	Flue-cured	NC291	1 (5)	18 (95)	0 (0)	2 (11)	17 (89)	19 (100)	0 (0)	0 (0)
<i>Ph_p</i>	Flue-cured	NC471	0 (0)	8 (100)	0 (0)	0 (0)	8 (100)	6 (75)	2 (25)	0 (0)
<i>Ph_p</i>	Flue-cured	NC71	2 (7)	28 (93)	0 (0)	0 (0)	30 (100)	25 (83)	5 (17)	0 (0)
<i>Ph_p</i>	Flue-cured	NC72	0 (0)	13 (100)	0 (0)	1 (8)	12 (92)	9 (69)	4 (31)	0 (0)
<i>Ph_p</i>	Flue-cured	SP168	0 (0)	5 (100)	0 (0)	2 (40)	3 (60)	4 (80)	1 (20)	0 (0)
<i>Ph_p</i>	Flue-cured	SP225	0 (0)	4 (100)	0 (0)	0 (0)	4 (100)	4 (100)	0 (0)	0 (0)
<i>Ph_p</i>	Flue-cured	SP227	0 (0)	5 (100)	0 (0)	1 (20)	4 (80)	5 (100)	0 (0)	0 (0)
<i>Ph_p</i>	Flue-cured	RGH51	0 (0)	14 (100)	0 (0)	3 (21)	11 (79)	12 (86)	2 (14)	0 (0)
<i>Ph_i</i>	Burley	KT206	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
<i>Ph_i</i>	Burley	14xL8	0 (0)	2 (100)	0 (0)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)
N/A	N/A	N/A	5 (12)	35 (85)	1 (2)	1 (2)	40 (98)	22 (54)	19 (46)	0 (0)
Overall	...	...	46 (21)	165 (76)	6 (3)	13 (6)	204 (94)	147 (68)	65 (30)	5 (2)

^a Data shown here are number of isolates from 217 total isolates, with percentage of isolates in parentheses. N/A = presence of *Ph* gene, tobacco type, and cultivar data not available for these isolates. HS: highly sensitive; S: sensitive; IS: intermediately sensitive; and I: insensitive (none of the isolates were insensitive).

Table 2. Summarized race distribution, mating type, and mefenoxam sensitivity data by *Ph* gene and tobacco type of tobacco black shank pathogen *Phytophthora nicotianae* from Virginia tobacco fields during 2006 to 2008^a

<i>Ph</i> gene	Tobacco type	Race distribution			Mating type		Mefenoxam sensitivity		
		Race 0	Race 1	Race 3	A ¹	A ²	HS	S	IS
–	Flue-cured	22 (71)	5 (16)	4 (13)	2 (6)	29 (94)	9 (29)	20 (65)	2 (6)
–	Burley	0 (0)	2 (67)	1 (33)	0 (0)	3 (100)	1 (33)	1 (33)	1 (33)
–	Dark	15 (100)	0 (0)	0 (0)	1 (7)	14 (93)	3 (20)	10 (67)	2 (13)
–	Flue, burley, and dark	37 (76)	7 (14)	5 (10)	3 (6)	46 (94)	13 (27)	31 (63)	5 (10)
<i>Ph_p</i>	Flue-cured	4 (3)	120 (97)	0 (0)	9 (7)	115 (93)	109 (88)	15 (12)	0 (0)
<i>Ph_i</i>	Burley	0 (0)	3 (100)	0 (0)	0 (0)	3 (100)	3 (100)	0 (0)	0 (0)
<i>Ph_p</i> or <i>Ph_i</i>	Flue, burley, and dark	4 (3)	123 (97)	0 (0)	9 (7)	118 (93)	112 (88)	15 (12)	0 (0)

^a Data shown here are the subtotal number of isolates for each tobacco type with the presence or absence of *Ph* gene from 217 total isolates, with percentage of isolates in parentheses. These data do not include isolates with unknown background information. HS: highly sensitive; S: sensitive; IS: intermediately sensitive; and I: insensitive (none of the isolates were insensitive).

unsuccessful. Li et al. (26) found that none of the 97 accessions from 37 species of *Nicotiana* delivered complete resistance against race 1 of *P. nicotianae*. In total, nine nondomesticated *Nicotiana* spp. have been shown to partially suppress race 1 populations of *P. nicotianae* (26). However, the mode of inheritance for this resistance has not been elucidated. Therefore, it is critical that farmers continue applying fungicide to partially resistant commercial cultivars, in addition to crop rotation, in order to simultaneously manage populations of multiple races of the black shank pathogen.

The similarity in race distribution results from Virginia compared with other tobacco-growing states indicates that, since 1997, *P. nicotianae* races have adapted to the widespread planting of *Ph_p* gene-containing hybrid cultivars over much of the flue-cured tobacco-growing region of the United States (3,8,9,14,20,27). Earlier work showed that race 1 populations can predominate over race 0 within one to two generations of growing *Ph_p*-gene-containing flue-cured tobacco cultivars (3,34). The basis for the shift from race 0 to race 1 and vice versa has been shown to involve selection pressure imposed by resistant or susceptible cultivars (34). However, specific differences in mechanisms involved in pathogenicity of race 0 versus race 1 populations have not been elucidated, although some reports have compared and quantified the aggressiveness of race 0 versus 1 isolates (16,33,34). Based on these reports, race 0 isolates seem to be more aggressive and fit than race 1 isolates against cultivars with moderate to high levels of partial resistance. These reports suggest that isolate aggressiveness varies with the type and level of resistance present in the host tissue from which the isolate was obtained (18,33).

Differences in incubation period among isolates in our greenhouse assay could be due to the innate aggressiveness of isolates resulting from the varying types and levels of black shank resistance possessed by the cultivars from which they were originally isolated (8).

In fact, 27 of the 165 race 1 isolates initially showed unclear or no symptoms on the differential cultivars; 15 of these 27 isolates did not cause clear symptoms during the first trial. During the second trial, the same 15 isolates caused disease symptoms on all three cultivars but the rate of symptom expression was delayed 4 to 5 days compared with the 138 race 1 isolates initially identified. Twelve isolates did not cause symptoms even 3 to 4 weeks after inoculation in the second trial. Symptomless infection has been reported in other *Phytophthora* spp., such as *P. ramorum* and *P. kernoviae* on rhododendron and holm oak (11). These 12 isolates, all originally collected from *Ph*-gene-containing

cultivars, failed to produce symptoms in either trial of the differential assay. However, *P. nicotianae* was consistently recovered from surface-sterilized, asymptomatic plant tissue of all three cultivars when inoculated with these 12 isolates. These 12 isolates were characterized as race 1 because the pathogen was reisolated from asymptomatic parts of all three differential cultivars. Symptomless infections of tobacco by *P. nicotianae* has not been reported earlier and deserves further investigation because this could significantly affect host physiology and resistance.

Of the 127 isolates originally recovered from *Ph_p*-gene-containing flue-cured cultivars, 4 were typed as race 0 in our host differential assay: 2 isolates originally collected from NC71 and 1 isolate each from GL350 and NC291. The cause of these discrepancies is not known. Possible explanations might include occasional misidentification of the cultivars in the sampled fields or, perhaps, some compromise of the *Ph* gene resistance mechanisms by a disease complex or dual infection or other unknown agents that might enable a race 0 isolate to penetrate and colonize normally resistant tissue.

Information on distribution of A¹ and A² mating types is critical to determine the genetic variability within the field pathogen population. Only one-tenth of the Virginia tobacco fields had both mating types, and all those samples originated from flue-cured tobacco fields, indicating a low genetic diversity. The relative ratios of A¹ and A² mating types could suggest the level of sexual recombination occurring in the natural field conditions, as well as the potential for oospore production. Predominance of a single mating type in the majority of Virginia tobacco fields may indicate genetically less diverse populations, and this may play a role in maintaining isolate sensitivity to mefenoxam throughout the state.

Although high levels of mefenoxam resistance have been reported in *P. nicotianae* isolates collected from ornamental nurseries and *P. cactorum* in strawberries (19,21), none of the tobacco isolates evaluated in this study showed resistance to mefenoxam. Our results were similar to earlier reports from other tobacco-growing states (9,16), indicating that mefenoxam remains an effective tool for controlling tobacco black shank in Virginia for some time to come.

In summary, this study demonstrated that race 1 populations of *P. nicotianae* now dominate tobacco fields in Virginia. Due to the lack of complete resistance to race 1, growers from race-1-dominated counties should select a tobacco cultivar with at least partial resistance to all races of the pathogen. Because none of the tested isolates displayed resistance to mefenoxam, growers should be able to continue protecting their crop by using this

fungicide in addition to host resistance, crop rotation, and other integrated management practices. Improved monitoring of fluctuations in pathogen race structure under field conditions will be an important factor in identifying more effective management strategies for multiple races of the black shank pathogen. Quick and reliable diagnostic methods for black shank pathogen races using DNA fingerprinting techniques would be tremendously helpful in assisting growers and plant breeders to select cultivars more appropriately and to address concerns about shifts in pathogen population race structure in a preventative versus remedial approach.

ACKNOWLEDGMENTS

This project was supported in part by grants from Altria Client Services and Philip Morris International. We thank C. Gallup (David Shew laboratory), North Carolina State University; D. Reed and R. Wells at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center (AREC), Blackstone; and other members of the Hong lab at the Hampton Roads AREC, Virginia Beach for their assistance.

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