Relationship of Isolate Origin to Pathogenicity of Race 0 and 1 of *Phytophthora parasitica* var. *nicotianae* on Tobacco Cultivars

A. S. Csinos, Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton

ABSTRACT

Csinos, A. S. 2005. Relationship of isolate origin to pathogenicity of race 0 and 1 of *Phytophthora parasitica* var. *nicotianae* on tobacco cultivars. Plant Dis. 89:332-337.

Flue-cured tobacco cultivars were evaluated for their reaction to race 0 and race 1 of *Phytophthora parasitica* var. *nicotianae*, the incitant of the disease tobacco black shank. Seventeen commercial tobacco cultivars having resistance derived from Fla 301 or a combination of Fla 301 and Fla 105 were subjected to root or stem inoculation by 22 different isolates of *P. parasitica* var. *nicotianae* collected from across the Georgia tobacco-growing belt. An adapted stem inoculation technique using field-grown tobacco indicator cvs. K-326, NC-71, Coker 371 Gold, and the breeding line NC-1071 was used to determine races of *P. parasitica* var. *nicotianae*. Typically, in greenhouse evaluations, cultivars stem inoculated with race 1 of *P. parasitica* var. *nicotianae* were killed. Cultivars that had resistance from both Fla 301 and Fla 105 (*Ph* gene) were not killed, with few exceptions, when inoculated with race 0. Fifty-seven tobacco specimens having black shank symptoms from the Georgia and Florida tobacco belt were evaluated for *P. parasitica* var. *nicotianae* race using the adapted stem inoculation technique. Of the samples evaluated from commercial tobacco fields, 83% yielded race 1 compared with a similar evaluation made in 1994, where only 16% of the samples yielded race 1. The increase in race 1 incidence may be related to the increase in use of cultivars which have the *Ph* gene for resistance. The use of rotations and metalaxyl or mefenoxam may be required where race 1 is found.

Phytophthora parasitica Dast. var. *nicotianae* (Breda de Haan) Tucker causes a serious root and stem disease of tobacco (*Nicotiana tabacum* L.) called black shank and is distributed throughout the United States and in many tobacco-growing regions of the world (11). Although management programs utilizing cultural practices, genetic resistance, and chemical treatments exist, losses in some years can be severe (6,12,13). Both race 0 and race 1 of the pathogen are present in the tobacco-growing areas of the United States, and race 1 was first reported in Georgia in 1994 (6). Commercial tobacco cultivars have resistance to *P. parasitica* var. *nicotianae* derived from Florida (Fla) 301, which is inherited in a complex fashion (1,2,4). This resistance is inherited quantitatively and confers partial resistance to both races; however, because of the high disease pressures encountered in Florida and Georgia, it may not be useful to growers (13). The resistance from Fla 105, a selection from

Charlie Deans shade tobacco nursery in Florida (7), demonstrated a high level of resistance to race 0 of *P. parasitica* var. *nicotianae*.

Apple (1) demonstrated that NC-1071 was resistant to race 0 of *P. parasitica* var. *nicotianae* but was susceptible to race 1 of the pathogen. In 1994, Csinos and Bertrand (6) reported that Coker 371 Gold (371 G) had resistance that was similar to NC-1071, a breeding line with monogenic race 0 black shank resistance derived from *N. plumbaginifolia*. In 1999, Csinos (5) demonstrated that cultivars that were developed for resistance to black shank utilizing Fla 105 were resistant to infection of the stem by race 0 of the pathogen, but not race 1. In addition, cultivars that were developed using Fla 301 resistance did not demonstrate resistance in the stems of the plants but demonstrated varying levels of root resistance, and severity of root decay was isolate dependent. These findings were important because, in Georgia, lower stem infections can occur in the absence of root infection (5).

Cv. 371 G, first released in 1987, has black shank resistance derived from Fla 105. This cultivar demonstrated good resistance to race 0 of the pathogen but, because of poor root growth and a tendency to lodge, was not popular with growers. Carlson et al. (3), using double haploids derived from 371 G, determined that the cultivar had a single dominant gene that conferred complete resistance to

race 0, but not race 1 of *P. parasitica* var. *nicotianae*. They demonstrated that black shank resistance in 371 G is derived from a single dominant gene, designated *Ph*, pyramided upon the Fla 301 quantitative genetic resistance. In 2002, Johnson et al. (9) reported that the origin of the *Ph* gene in 371 G was *N. plumbaginifolia* Viv.

Resistance of tobacco to *P. parasitica* var. *nicotianae* has been evaluated in several different systems using leaves (15,16), stems (5,6,8,17), and roots (5,6,9,18) as the inoculation target. For screening of potential tobacco cultivars for resistance to *P. parasitica* var. *nicotianae*, the seedling techniques described by Litton et al. (10) and Van Jaarsveld et al. (18) may be the most rapid and reliable. The technique best suited for determination of races of *P. parasitica* var. *nicotianae* may depend on individual situations and either the relative difficulty in producing test cultivars for evaluation or the expertise or facilities required for fungal culture and storage.

The determination of the race of *P. parasitica* var. *nicotianae* is critical to formulating a recommendation to growers (12,13). If an isolate is race 0, then the options for management are a great deal broader than they are if a grower determines that he has race 1 on his farm. Typically, our laboratory would receive samples in the summer months and the pathogen would be isolated, cultured, stored, and then tested in the fall of the year in a greenhouse. This long delay between receiving samples and the testing procedure was time consuming and often resulted in lost or contaminated cultures and loss of virulence. A simpler, more timely evaluation method was needed.

The objective of this work was to evaluate commercial tobacco cultivars grown in Georgia for their response to race 0 and 1 of *P. parasitica* var. *nicotianae* to both root and stem inoculation. A second objective was to evaluate a stem inoculation method adapted from work of Hendrix and Apple (8) on field-grown indicator plants to distinguish between race 0 and race 1 of the pathogen. This work also attempts to elucidate the relationship of origin (cultivar) of the isolate to the race and pathogenicity of that isolate to commercial tobacco cultivars grown in Georgia.

MATERIALS AND METHODS

Greenhouse. *P. parasitica* var. *nicotianae* used in this study was isolated from tobacco in Tifton County, GA, from K-326

Corresponding author: A. S. Csinos
E-mail: csinos@tifton.uga.edu

Financial assistance was provided by Philip Morris Tobacco Company and the Georgia Commodity Commission for Tobacco.

Accepted for publication 1 November 2004.

DOI: 10.1094/PD-89-0332
© 2005 The American Phytopathological Society

in 1996 (race 0, isolate 364) and from NC-1071 in 1997 (race 1, isolate 381) for studies conducted in 1998. Isolates used in 1999 were isolated from NC-1071 (isolate 381 and 393), NC-71 (isolate 395), NC-72 (isolate 396), and K-326 (isolate 397). The origin of isolates used for inoculations in 2002 are listed in Table 1. Cultures were stored at room temperature on V8 juice agar as previously described (6) and transferred monthly to fresh media.

Inocula were prepared by sterilizing and infesting toothpicks with the test organism (5,6). Toothpicks were autoclaved in V8 juice for 15 min at 121°C, allowed to cool, placed on potato dextrose agar-filled petri plates, and inoculated with a 5-mm plug from actively growing cultures of *P. parasitica* var. *nicotianae*. Plates were incubated at 27°C in the dark for 10 to 14 days, allowing the fungus to grow across the plates and into the media-impregnated toothpicks. Plants were inoculated by aseptically pushing the infested toothpicks into stems 2 to 3 cm above the soil line or into root systems near the base of the plant. Noninfested toothpicks served as controls.

Cultivars and their resistance to race 0 and 1 of *P. parasitica* var. *nicotianae* that were evaluated in 1998, 1999, and 2002 are listed in Tables 1 to 3.

Tobacco cultivars were seeded and grown 6 to 8 weeks to transplant size in styrofoam float tray systems as recommended by the Georgia Cooperative Extension Service (13). Seedlings were 15 to 20 cm tall, with a stem diameter of 5 mm, and were transferred to separate trays just prior to inoculation. Greenhouse temperature was managed with heaters and fan-assisted cooling pads, which maintained temperatures in the range of 15 to 25°C during the tests. Each treatment consisted of six single-plant replications. The cultivar-inoculation combinations were kept in separate trays and placed in separate float baths to prevent cross contamination during the test period.

Race determination was based on the work of Apple (1) and Hendrix and Apple

(8). NC-1071 (*N. plumbaginifolia*-derived line) and K-326 were used as the plant indicators in these tests. If both tobacco indicators were killed, the isolate was race 1. If only K-326 was killed, the isolate was race 0. A subjective severity rating was used by choosing a rating number between 1 and 10, where 1 was a healthy plant and 10 was stem collapsed and plant dead (5,6). Ratings were recorded on stems 3 to 14 days post inoculation depending on the test. Roots were evaluated by washing them and then rating them on a scale of 0 to 100% of root necrosis. Stem disease progress was evaluated in all tests, but root necrosis was evaluated only in root inoculation tests.

Field. Field stem inoculations of tobacco test cultivars were conducted in

2002 and 2003. The test site was located at the University of Georgia, College of Agriculture and Environmental Sciences, Coastal Plain Experiment Station in Tifton. Soil type was fuquay loamy sand (loamy, siliceous thermic Arenic Plinthic Paleudults, 88% sand, 9% silt, 3% clay, and 0.74% organic matter, pH 6.0). A site was selected that had not been used for tobacco culture for several years and had no history of black shank. In 2002 and 2003, the following cultivars were transplanted into two rows, 80 m long each, on 2 and 23 April, respectively: K-236, NC-1071, NC-71, and 371 G. K-326 has a low level of root resistance to both race 0 and race 1 derived from Fla 301, whereas the other three cultivars have the monogenic resistance *Ph* gene

Table 2. Response of tobacco cultivars root and stem inoculated with race 0 and race 1 of *Phytophthora parasitica* var. *nicotianae*, 1998^a

Cultivars	Root inoculation				Stem inoculation ^y	
	Stem infection ^w		Root infection (%)		Stem infection	
	I-364	I-381	I-364	I-381	I-364	I-381
371 G ^x	1.0 d	4.3 b	4 d	47 b-d	1.2 d	9.9 a
NC-1071 ^x	1.0 d	8.4 a	1 d	97 a	1.1 d	9.9 a
SP G 28 ^y	5.4 ab	2.2 bc	52 b	23 c-g	10.0 a	10.0 a
SP G 70 ^y	4.4 bc	1.6 c	56 b	7 g	9.9 a	10.0 a
Hicks ^z	7.7 a	7.0 a	87 a	68 b	9.9 a	10.0 a
K-326 ^y	5.7 ab	2.2 bc	33 bc	16 e-g	9.9 a	10.0 a
NC-71 ^x	4.3 bc	7.5 a	17 cd	51 bc	1.4 d	9.9 a
NC-72 ^x	1.5 d	2.0 bc	26 cd	13 fg	2.2 c	9.9 a
OX-207 ^y	5.6 ab	2.2 bc	49 b	39 c-e	10.0 a	10.0 a
SP 168 ^x	1.1 d	3.4 bc	2 d	33 c-f	1.4 d	8.7 bc
SP 172 ^y	6.9 a	2.1 bc	55 b	11 fg	6.5 b	10.0 a
SP 200 ^y	6.0 ab	3.2 bc	25 cd	3 g	9.8 a	8.6 c
SP NF3 ^y	2.9 cd	2.2 cd	21 cd	26 d-g	10.0 a	9.8 ab

^a Isolate 364 is race 0 isolated from K-326 and isolate 381 is race 1 isolated from NC-1071. Noninfested toothpick controls were rated from 1 to 1.2 on the stem infection scale (not shown). Means within a column followed by the same letter are not significantly different according to Fisher's least significant difference mean separation test, $P = 0.05$.

^y Mean of two tests. Each test evaluated six plants per cultivar and per race.

^w Stem infection ratings were based on a scale of 1-10, where 1 is healthy and 10 is stem collapsed and plant dead for stem both inoculated and root inoculated plants. Infections were subjectively rated between the two extremes based on severity. Root infection ratings were based on a scale of 0-100%.

^x Cultivar with *Ph* gene for resistance to *P. parasitica* var. *nicotianae* pyramided on to Fla 301 resistance.

^y Cultivar with Fla 301 resistance to *P. parasitica* var. *nicotianae*.

^z Cultivar without Fla 301 or *Ph* gene for *P. parasitica* var. *nicotianae* resistance.

Table 1. Greenhouse evaluation of *Phytophthora parasitica* var. *nicotianae* isolates on tobacco cultivars, fall 2002

Cultivars	Isolate (sample origin) disease rating ^x															Control
	417 (NC71)	418 (SP168)	419 (NC71)	421 (K326)	423 (NC71)	424 (NC95)	425 (NC2326)	426 (NC1071)	427 (VP129)	428 (SP168)	430 (NC297)	431 (371G)	434 (SP168)	435 (K297)	436 (NC71)	
K-326 ^y	5.0 ab	6.9 ab	5.4	7.2 a	6.6 a	8.6 ab	7.3 a	1.8 ab	6.1	5.0	7.7 ab	6.9 ab	9.4 ab	6.4	6.8	1.0
NC-1071 ^z	6.3 a	7.9 a	3.2	1.0 b	3.8 ab	8.9 a	1.8 b	1.6 ab	7.4	7.1	9.3 a	9.8 a	9.9 a	8.7	6.5	1.0
NC-71 ^z	4.0 ab	4.1 c	1.9	1.0 b	3.8 ab	5.6 bc	1.6 b	1.4 b	4.5	5.7	5.2 b	5.1 b	7.2 bc	7.0	5.5	1.0
NC-72 ^z	3.5 ab	5.0 bc	1.8	1.0 b	3.9 ab	5.3 c	1.3 b	1.3 b	4.6	5.9	6.5 ab	6.8 ab	7.7 abc	8.3	5.0	1.0
SP 168 ^z	2.9 b	4.9 bc	1.4	1.0 b	3.9 ab	4.0 c	1.3 b	2.8 a	5.3	5.2	6.0 ab	5.5 b	5.7 c	5.4	2.3	1.0
371 G ^z	2.3 b	3.9 c	1.2	1.0 b	2.9 b	6.6 abc	1.3 b	2.5 ab	5.3	5.8	4.6 b	5.9 b	7.5 bc	6.4	4.3	1.0

^x Isolate 417 originated from Suwanee County, FL; 418 from Cook County; 419 from Bacon County; 421 (race 0) from Appling County; 423, 435, and 434 from Toombs County; and 436 from Pierce County, GA. Isolates 424, 425, 426, 427, 428, 430, and 431 are from the CPES Disease Nursery, Tift County. Cultivars in brackets are the original cultivars from which *P. parasitica* var. *nicotianae* was isolated. Stem infection ratings were based on a scale of 1 to 10, where 1 is a healthy plant and 10 is stem collapsed and plant dead. Infections were subjectively rated between the two extremes based on severity. Data are means of three tests each with six replications performed on 29 October, 11 November, and 18 December 2002. Means in the same column followed by the same letter are not different ($P = 0.05$) according to Fisher's least significant difference test. Columns of data containing no letters indicate no significance.

^y Cultivar with Fla 301 resistance.

^z Cultivars with *Ph* gene for *P. parasitica* var. *nicotianae* resistance pyramided onto Fla 301 resistance.

pyramided onto the Fla 301 resistance (1–3,9).

Tobacco production practices followed the Georgia Cooperative Extension recommendations (13). County agents and tobacco specialists were requested to send to us diseased tobacco samples displaying typical black shank symptoms (10). Samples from commercial fields were sent via next-day mail and processed immediately on receipt. In addition, samples were obtained from the tobacco Regional Small Plot Variety Test located at the Coastal Plain Experiment Station, Tifton, GA.

Isolates were identified as to location and cultivars from which they were isolated (Table 4). Samples were washed using a stiff bristle brush under running water to remove soil, debris, and stalks split at or near the stem lesion. Splinters of tobacco stalk and pith were removed from the area transcending the diseased–healthy interface and placed in autoclaved tap water in petri plates for transport to the field or incubated at ambient room temperature. Samples placed in water were observed for the typical lemon-shaped papillate sporangia produced by *P. parasitica* var. *nicotianae* over the next two days (11). In most cases, the same samples that were used for observation of sporulation also were used for plant inoculation. Stems of test tobacco cultivars were split aseptically using a scalpel and the sliver of tissue inserted into the split stem. The incision was sealed using parafilm and wrapped with tape to prevent dehydration. Each sample was inoculated on three plants of each indicator cultivar for a total of 12 inoculations per sample and tagged

for identification. Individual inoculation points were scored 3 to 5 days later for positive or negative infection based on typical stem necrosis. Samples were removed from the field plants and placed on V8 ampicillin-pimaricin-amended media (5,6) for culturing and identification. Isolates were maintained on V8 juice agar under ambient laboratory conditions for testing purposes.

The inoculation points were made on the upper half of the plant where plants tended to be more succulent. Previous data has indicated that susceptibility of tobacco to *P. parasitica* var. *nicotianae* is higher the farther the inoculation point moves from the soil line (5). Data was analyzed by analysis of variance or general linear model procedures of SAS (SAS Institute, Cary, NC). Significant difference among means was determined by Fisher's least significant difference mean separation at $P = 0.05$ (14).

RESULTS

Greenhouse. Inoculated stems of susceptible cultivars demonstrated a black necrosis of the stem followed by a rapid tissue collapse. These reactions were observed within 3 to 10 days, depending on the succulence of the tissue being inoculated and the temperature of the greenhouse at inoculation. Often, a noncompatible reaction would cause some discoloration of the stem but would not result in tissue deterioration. These scores typically were less than 2.0 on the stem disease rating scale. Stem disease reactions of 2.0 or less were considered resistant reactions, whereas those above 2.0 are considered susceptible reactions.

Tobacco cultivars evaluated in the greenhouse either by stem or root inoculation showed a consistent reaction to race 0 or race 1 of *P. parasitica* var. *nicotianae* depending whether the *Ph* gene was part of its pedigree (Tables 1 to 3). With few exceptions, all cultivars having the *Ph* gene were highly resistant to race 0 of *P. parasitica* var. *nicotianae* but were susceptible to race 1 of the pathogen.

In 1998, NC-71 scored a mean reaction of 4.3 in two trials for isolate 364 (race 0) for stem reaction to a root inoculation (Table 2). However, in the same series of tests, the stem inoculation of isolate 364 scored only 1.4 on NC-71, which is considered a resistant reaction. In another study in 1999 (Table 3), the cultivar NC-71 scored a reaction of 4.1 when stem inoculated with isolate 395 originally isolated from NC-71. Inoculation of NC-1071 and K-326 indicated that isolate 395 is race 0. In 2002, NC-71 inoculated with isolate 419 suggested a resistant reaction; whereas, with NC-1071 a susceptible reaction was detected (Table 1). In 2002–03 in the field evaluations, samples originating from NC-71, 371 G, and NC-72 proved to be race 0 (Table 4) even though those cultivars have the *Ph* gene for resistance.

Root infection for isolate 364 was typical for what would be expected from race 0 of *P. parasitica* var. *nicotianae*. Cultivars that had Fla 301 resistance plus the *Ph* gene typically showed little root infection (Table 2). Hicks, which has neither resistance, had the highest level of root disease. Race 1 tended to cause more root necrosis on plant cultivars with the *Ph* gene than on cultivars that had only the Fla 301 resistance (Table 2).

Stem necrosis following stem inoculation was more definitive than root necrosis as an indicator of a susceptible and resistant reaction. Cultivars which contained the *Ph* gene demonstrated little or no necrosis when stem inoculated by race 0. However, they were killed by stem inoculation of race 1.

In a trial in 1999, cultivars having the *Ph* gene were resistant when stem inoculated with race 0 of *P. parasitica* var. *nicotianae*, but were highly susceptible to race 1 of *P. parasitica* var. *nicotianae*. However, the reaction of NC-71 to isolate 395 was atypical for cultivars with the *Ph* gene (Table 3). In a similar study in 2002 using a wider array of isolates, isolates 421 and 425 from K-326 and NC-2326, respectively, demonstrated a distinctive race 0 reaction. All other isolates tested were race 1 (Table 1). Isolate 419 demonstrated erratic results, not producing the expected results with respect to the presence of the *Ph* gene. Isolate 426 demonstrated a weak response on all tobacco cultivars.

Field. Fifty-seven tobacco samples suspected of being infected with *P. parasitica* var. *nicotianae* based on symptomatology and presence of sporangia were subjected

Table 3. Greenhouse evaluation of *Phytophthora parasitica* var. *nicotianae* isolates stem inoculated to tobacco cultivars, 1999^w

Cultivars	Isolate (specimen origin) disease rating ^x					Control
	381 (NC-1071)	393 (NC-1071)	395 (NC-71)	396 (NC-72)	397 (K-326)	
C 371 Gold ^y	10.0 a	5.6	1.3	10.0 a	1.3 c	1.0
NC- 1071 ^y	10.0 a	5.5	1.0	10.0 a	1.0 c	1.0
NC-71 ^y	10.0 a	5.1	4.1	10.0 a	1.2 c	1.0
NC-72 ^y	10.0 a	6.1	1.2	10.0 a	1.3 c	1.0
SP-168 ^y	10.0 a	6.4	1.2	10.0 a	1.0 c	1.0
K-346 ^z	10.0 a	10.0	5.6	10.0 a	8.2 a	1.0
SP G-70 ^z	10.0 a	10.0	5.6	10.0 a	9.8 a	1.0
NC-95 ^z	10.0 a	9.5	5.0	9.4 ab	9.3 ab	1.0
NC-236 ^z	10.0 a	9.9	6.3	10.0 a	8.3 a	1.0
K-326 ^z	10.0 a	10.0	6.5	10.0 a	8.9 a	1.0
SP 200 ^z	9.6 b	8.9	3.4	9.9 ab	9.4 a	1.0
SP 200A ^z	10.0 a	6.5	5.0	8.7 b	4.6 b	1.0

^w Stem infection ratings were based on scale of 1 to 10, where 1 is a healthy plant and 10 is stem collapsed and plant dead. Infections were subjectively rated between the two extremes based on severity. Data are means of two tests with six replications each performed on 3 and 19 March 1999. Means in the same column followed by the same letter are not different ($P = 0.05$) according to Fisher's least significant difference test. Columns of data containing no letters indicate no significant difference among means.

^x Isolates of *P. parasitica* var. *nicotianae* with specimen origin in parentheses. Isolate number 381 originated at Blackshank Farm, and isolates 393, 395, 396, and 397 were cultured from Blackshank Nursery Area, Tifton, GA. Cultivars listed in parentheses are the original cultivar from which *P. parasitica* var. *nicotianae* was inoculated.

^y Cultivar with *Ph* gene for resistance to *P. parasitica* var. *nicotianae* pyramided onto Fla 301 resistance.

^z Cultivar with Fla 301 resistance to *P. parasitica* var. *nicotianae*.

to a differential cultivar screen for determination of race (Table 4). The differential indicator tobacco cultivars used were K-326 and the breeding line NC-1071. NC-71 and 371 G were additional test cultivars, both having the *Ph* gene for resistance. Of the samples evaluated employing

the field race determination screen, 49 were positive for *P. parasitica* var. *nicotianae*. Four samples did not have a viable pathogen and four samples were *Rhizoctonia solani*. In all, 88% of the samples identified as *P. parasitica* var. *nicotianae* were determined to be race 1. Five race 0 iso-

lates originated from cultivars with the *Ph* gene, and two race 0 isolates originated from cultivars not having the *Ph* gene. Of the isolates which originated from cultivars that did not have the *Ph* gene for resistance, 66% were race 1. All of the isolates originating from cultivars having the

Table 4. Race determination of *Phytophthora parasitica* var. *nicotianae* using stem inoculation of field-grown indicator tobacco cultivars and lines, 2002 and 2003

Sample no.	Source (county) ^x	Sample origin (cultivar)	<i>Ph</i> gene ^y	Race determination ^z	Pathogenicity ^w	
					NC-71	C 371 G
01-02	Atkinson	NC-297	+	1	+	+
02-02	Suwannee, FL	NC-71	+	1	+	+
03-02	Cook	SP 168	+	1	+	+
04-02	Bacon	NC-71	+	0	nd	nd
05-02	Bacon	NC-297	+	0	+	+
06-02	Appling	K-326	–	1	+	+
07-02	Toombs	SP 168	+	1	+	+
08-02	Toombs	NC-71	+	1	+	+
09-02	Coffee	NC-71	+	1	+	+
10-02	Atkinson	NC-72	+	1	+	+
11-02	Pierce	NC-71	+	N	nd	nd
12-02	Toombs	SP 168	+	N	nd	nd
13-02	Toombs	NC-297	+	1	+	–
14-02	Toombs	SP 168	+	1	+	–
15-02	Toombs	NC-297	+	1	+	–
01-03	Tifton Nursery	NC-1071	+	1	+	+
02-03	Tifton Nursery	K-326	–	0	–	–
03-03	Tifton Nursery	NC-71	+	1	+	+
04-03	Tifton Nursery	371 G	+	0	+	–
05-03	Treutlen	NC-297	+	1	+	–
06-03	Tifton Nursery	K-326	–	0	+	+
07-03	Tifton Nursery	NC-71	+	1	+	+
08-03	Tifton Nursery	NC-1071	+	1	–	+
09-03	Tifton Nursery	371 G	+	1	+	–
10-03	Tifton Nursery	NC-72	+	0	+	–
11-03	Pierce	SP G 70	–	0	–	–
12-03	Pierce	NC-71	+	1	+	–
13-03	Cook	NC-71	+	R	–	–
14-03	Cook	NC-72	+	R	–	–
15-03	Pierce	NC-71	+	R	–	–
16-03	Pierce	NC-72	+	R	+	–
17-03	Tifton Nursery	K-326	–	1	+	+
18-03	Tifton Nursery	NC-71	+	1	+	+
19-03	Tifton Nursery	NC-1071	+	1	+	+
20-03	Tifton Nursery	371 G	+	1	+	+
21-03	Tifton Nursery	NC-1071	+	N	N	N
22-03	Tifton Nursery	K-326	–	1	+	+
23-03	Tifton Nursery	NC-71	+	1	+	+
24-03	Tifton Nursery	371 G	+	1	+	+
25-03	Tifton Nursery	SP 225	–	1	+	+
26-03	Tifton	NC-72	+	1	+	+
27-03	Tifton	NC-72	+	1	+	+
28-03	Tifton	NC-72	+	1	+	+
29-03	Tifton	NC-72	+	1	+	+
30-03	Tifton	NC-72	+	1	+	+
31-03	Pierce	NC-297	+	1	+	+
32-03	Tifton Nursery	NC-1071	+	1	+	+
33-03	Tifton Nursery	K-326	–	1	+	+
34-03	Tifton Nursery	NC-71	+	1	+	+
35-03	Tifton Nursery	371 G	+	1	+	+
36-03	Tifton Nursery	NC-1071	+	1	–	+
37-03	Tifton Nursery	K-326	–	1	+	+
38-03	Tifton Nursery	NC-71	+	1	+	+
39-03	Tifton Nursery	371 G	+	1	+	+
40-03	Suwannee, FL	NC-297	+	0	–	–
41-03	Suwannee, FL	NC-297	+	0	–	–
42-03	Suwannee, FL	NC-297	+	N	–	–

^w Symbols: + indicates that at least one of the three inoculations produced stem necrosis; nd = not determined.

^x Counties in Georgia unless otherwise indicated; Tifton Nursery = samples obtained from Regional Small Plot Variety Test Disease Nursery, Tifton, GA.

^y The *Ph* gene is derived from *Nicotiana glauca* conferring black shank resistance to tobacco.

^z The tobacco cultivar K-326 and the breeding line NC-1071 were used as indicator plants. Race was determined by inoculation of K-326 and NC 1071. If only K-326 became infected, the isolate was race 0. If both cultivars were infected the isolate was race 1. Three inoculations per cultivar for each isolate were made. N = nonviable culture and R indicates that *Rhizoctonia solani* was cause of disease on submitted sample.

Ph gene for resistance yielded race 1 of the pathogen, with the exception of one each from 371 G, NC-71, and NC-72 (Table 4).

DISCUSSION

There is a good relationship among isolates of race 0 or race 1 on their ability to infect cultivars depending on whether the *Ph* gene is present. Very few exceptions were found to this observation. The exception was associated primarily with NC-71 in three trials and 371 G and NC-72 in one trial. These results are confusing and suggest, perhaps, that the pathogen may have several levels of pathogenicity, virulence is inadequately evaluated by our systems, dual cultures of race 0 and 1 may have been involved, or race 0 resistance in some cultivars is not complete. Other pathogens, such as *R. solani*, have been problematic on NC-71, and *Pythium* spp. have been isolated frequently from NC-71. This is curious because the *Ph* gene is pyramided onto Fla 301 resistance (2,3) and would not be expected to alter its resistance to other pathogens.

In 1994 (6), this laboratory reported that 16% of the isolates collected from the Georgia Tobacco belt and evaluated for race between 1990 and 1992 were race 1. We speculated that a shift from race 0 to race 1 of *P. parasitica* var. *nicotianae* may be occurring with the use of 371 G, a cultivar that has the *Ph* gene for resistance (1,3,9). In the study reported here, 83% of the *P. parasitica* var. *nicotianae* isolates collected from commercial tobacco fields in the Georgia tobacco-growing belt and Suwannee County, FL, during 2002 and 2003 were race 1.

The *Ph* gene derived from *N. plumbaginifolia* imparts resistance to cultivars for race 0 but not race 1. All new flue-cured cultivars being developed for *P. parasitica* var. *nicotianae* resistance derive their resistance from Fla 301 and Fla 105 (via 371 G). NC-71 was released in 1995 and was the first of the black shank-resistant cultivars with the *Ph* gene that had good agronomic characteristics and high yields. By 2002, the *Ph* gene was in 64% of all tobacco produced in Georgia (13). As the popularity of cultivars having the *Ph* gene has grown in Georgia, so has the incidence of race 1.

In the past, our laboratory has isolated and cultured *P. parasitica* var. *nicotianae* from samples, held them until fall, and then conducted race evaluations in the greenhouse. The use of field-grown indicator cultivars as a screen for race determination of *P. parasitica* var. *nicotianae* has eliminated much work and time and improved the recovery and efficiency of the procedure. The reactions that we have observed in the field tended to be decisive (Table 4) and very rapid. In 1994, we reported that, of the 131 diseased tobacco samples being evaluated for race, only 75

or 57% of the samples yielded a culture or survived the testing procedure. In this current study employing the field screening technique, only 4 of the 57 (5%) samples evaluated did not yield a pathogen and four were *R. solani* (Table 4). Our observations indicate that *R. solani* inoculated into stems yielded a slow, nonaggressive disease reaction and a brown lesion typical of sore shin (11). This reaction with the direct observation in water culture made distinguishing between the two pathogens relatively simple.

Carlson et al (3) indicated that, in their studies in North Carolina, race 1 of *P. parasitica* var. *nicotianae* is less aggressive than race 0. In our studies, we found a very wide range of virulence in race 1, and many isolates were more aggressive than our race 0 isolates. The development of new races with the introduction of monogenic resistance has been documented before (1,2,6,11) and is not surprising. Carlson et al. (3) stated that the combination of Fla 301 and the *Ph* gene should provide resistance to both races of *P. parasitica* var. *nicotianae*. However, in just a few years after the introduction of the *Ph* gene in Georgia, the relative incidence of race 1 has increased dramatically. The increased incidence of race 1 of *P. parasitica* var. *nicotianae* in Georgia is of great concern. New cultivars being developed having resistance to *P. parasitica* var. *nicotianae* use a combination of Fla 301 and Fla 105 (*Ph* gene) resistance. However, under the heavy pathogen pressures encountered in the Florida and Georgia tobacco belt and the predominance of race 1, this resistance alone is inadequate to manage *P. parasitica* var. *nicotianae*. It is interesting to note that cultivars that do not have the *Ph* gene in their pedigree still yielded race 1 of *P. parasitica* var. *nicotianae*. This would suggest that race 1 of *P. parasitica* var. *nicotianae* is well adapted and may establish itself and survive very well even in the absence of cultivars that have the *Ph* gene. We have maintained race 1 in culture for years and have some cultures that remain very virulent on tobacco cultivars that have the *Ph* gene. We suspect that any field that has a history of black shank and has been planted to a cultivar that has the *Ph* gene will be a likely candidate to develop race 1.

Management of *P. parasitica* var. *nicotianae* without the use of resistant cultivars will become more and more difficult. The use of resistance in cultivars is the least expensive and the easiest method for management of the disease. Agronomic techniques and chemicals can be used, but are less convenient, more costly, or both. Rotation away from tobacco generally is recommended for black shank management. However, proximity of fields to

curing barns, water sources, and general accessibility all play major roles in decisions as to where fields will be located. Metalaxyl and mefenoxam are the only recommended fungicides for use on *P. parasitica* var. *nicotianae* (13). They are expensive and require timely post-plant applications for best results. Cultivars with polygenic resistance to all races of *P. parasitica* var. *nicotianae* will be required to provide durable resistance to tobacco black shank.

ACKNOWLEDGMENTS

I thank J. Laska, L. Mullis, and D. Hankinson for technical assistance; C. LaHue for typing; and Philip Morris Tobacco Company and the Georgia Commodity Commission for Tobacco for financial assistance.

LITERATURE CITED

1. Apple, J. L. 1962. Physiological specialization within *Phytophthora parasitica* var. *nicotianae*. *Phytopathology* 52:351-354.
2. Apple, J. L. 1967. Occurrence of race 1 of *Phytophthora parasitica* var. *nicotianae* in North Carolina and its implications in breeding for disease resistance. *Tob. Sci.* 11:79-83.
3. Carlson, S. R., Wolff, M. F., Shew, H. D., and Wernsman, E. A. 1997. Inheritance of resistance to race 0 of *Phytophthora parasitica* var. *nicotianae* from the flue-cured tobacco cultivar Coker 371-Gold. *Plant Dis.* 81:1269-1274.
4. Chaplin, J. F. 1966. Comparison of tobacco black shank resistance from four sources. *Tob. Sci.* 10:55-58.
5. Csinos, A. S. 1999. Stem and root resistance to tobacco black shank. *Plant Dis.* 83:777-780.
6. Csinos, A. S., and Bertrand, P. F. 1994. Distribution of *Phytophthora parasitica* var. *nicotianae* races and their sensitivity to metalaxyl in Georgia. *Plant Dis.* 78:471-474.
7. Csinos, A. S., and Whitty, E. B. 1995. Evaluation of Coker 371-Gold parentage for resistance to *Phytophthora parasitica* var. *nicotianae*. (Abstr.) Tobacco Workers Conference, Tampa, FL.
8. Hendrix, J. W., and Apple, J. L. 1967. Stem resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco derived from *Nicotiana longiflora* and *N. plumbaginifolia*. *Tob. Sci.* 11:148-150.
9. Johnson, E. S., Wolff, M. F., Wernsman, E. A., Atchley, W. R., and Shew, H. D. 2002. Origin of the black shank resistance gene, *Ph*, in tobacco cultivar Coker 371-Gold. *Plant Dis.* 86:1080-1084.
10. Litton, C., Legg, P. D., and Collins, G. B. 1970. A greenhouse technique for screening tobacco seedlings for black shank resistance. *Tob. Sci.* 14:124-125.
11. Lucas, G. B. 1975. *Diseases of Tobacco*, 3rd ed. Biological Consulting Associates, Raleigh, NC.
12. Melton, T. A. 1998. Disease management. Pages 92-112 in: *Flue-Cured Tobacco Information*, North Carolina Cooperative Extension Service, North Carolina State University, College of Agriculture and Life Science, Raleigh, NC.
13. Moore, J. M., Bertrand, P., Jones, D., Givan, W. D., Sumner, P., Harris, G. P., Kingtlingee, K. D., and Raines, G. C. 2003. Page 195 in: *Georgia Tobacco Growers Guide*. Univ. Ga. Coop. Ext. Serv. Coll. Agric. Environ., Tifton, GA.
14. Steel, R. G. D., and Torrie, J. H. 1980. *Principles and Procedures for Statistics*, 2nd ed. McGraw-Hill, New York.

15. Tedford, E. C., Miller, T. L., and Nielsen, M. T. 1990: A detached-leaf technique for detecting resistance to *Phytophthora parasitica* var. *nicotianae* in tobacco. Plant Dis. 74, 313-316.
16. Willis, W. H. 1971: A comparison of black shank resistance of tobacco measured in inoculated whole plants and excised leaf tissue. Tob. Sci. 15:47-50.
17. Willis, W. H., and Moore, L. D. 1971: Response of some cultivars and lines of tobacco to stem inoculation with *Phytophthora parasitica* var. *nicotianae*. Tob. Sci. 15:51-53.
18. Van Jaarsveld, E., Wingfield, M. J., and Drenth, A. 2003. A rapid seedling screening technique to assay tobacco for resistance to *Phytophthora nicotianae*. J. Phytopathol. 151:389-394.