Distribution of *Phytophthora parasitica* var. *nicotianae* Races and Their Sensitivity to Metalaxyl in Georgia

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ABSTRACT

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.

Tobacco (*Nicotiana tabacum* L.) samples displaying typical black shank symptoms were collected in 1990–1992 throughout the tobacco-producing area of Georgia. Cultures of *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker (Ppn) isolated from samples were stored in culture until the fall of each of the years. The tobacco cultivars K 326 (low Ppn resistance), Speight G 70 (moderate Ppn resistance), Coker 371 Gold (high Ppn resistance), and the line 1071 (immune to race 0, susceptible to race 1) were grown in a greenhouse and used to determine virulence and races. Isolates were cultured on toothpicks impregnated with V8 juice agar, and the toothpicks were pushed into the stems of test plants to inoculate them. The cultivars K 326 and Speight G 70 were susceptible to all isolates, with K 326 being the most susceptible. Only 12 isolates of the 75 collected caused disease on line 1071. Coker 371 Gold demonstrated very high stem resistance, even though isolates were collected from decaying roots and stems of the cultivar. Race 1 was isolated from the entire tobacco-growing area, suggesting widespread distribution. Isolates were variable in sensitivity to metalaxyl, with a range of ED_{50} of 0.96 to <0.01 μg/ml. Typical field rates of metalaxyl may not be sufficient to control isolates that have a low level of sensitivity.

Additional keywords: *Nicotiana plumbaginifolia,* Ridomil

Black shank of tobacco incited by *Phytophthora parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker continues to be a serious pathogen of tobacco (*Nicotiana tabacum* L.) in Georgia. Stunting, wilting, leaf yellowing, and root and lower stem necrosis are symptoms associated with tobacco black shank. In most cases, plants wilt and collapse before leaves mature and can be harvested. Often, plant collapse occurs very suddenly, especially under stress of drought or hot weather. Although control techniques have been recommended for management of the disease (10), levels of the disease appear to be on the increase in Georgia. The use of long-term rotations is widely recommended, but they are uneconomical to growers. Resistance to the disease organism is commercially available, but cultivars with high resistance have less desirable agronomic qualities. The use of metalaxyl is widespread in the state, but the cost of the chemical is high, and many growers tend to use less than optimum rates for control. Under Georgia conditions in high-disease areas, metalaxyl is recommended at 2.24–3.36 kg a.i./ha preplant incorporated. Split applications of metalaxyl at lower rates are recommended, with at least 1-yr rotation and the use of a resistant cultivar (10).

Accepted for publication 14 January 1994.

Even when these management methods are incorporated, control is often poor. The incidences of poor control have led us to investigate the loss of effectiveness of metalaxyl and the development of new virulent strains and races. Experience in experimental plots in North Carolina has led Apple (1,2) to believe that race 1 of the pathogen may develop if highly resistant cultivars are repeatedly grown in fields infested at very high populations of the fungus. Similar observations have been made in Georgia. Apple (1,2) noted that race 0 was defined as the common race, which is not pathogenic (or rarely so) on *Nicotiana plumbaginifolia* Viv., and race 1 as those biotypes pathogenic on plants of *N. plumbaginifolia.*

This study was initiated to determine the distribution of races 0 and 1 of *P. p. nicotianae* in the tobacco-growing belt of Georgia. Also, isolates collected from these disease areas were evaluated for sensitivity to metalaxyl, the only fungicide recommended for black shank control in Georgia.

MATERIALS AND METHODS

Tobacco samples exhibiting symptoms of black shank were collected from across the tobacco-growing belt of Georgia in 1990–1992. Samples were transported in coolers from the field to the laboratory in most cases, but some were received in the mail by the Cooperative Extension Service.

P. p. nicotianae was isolated from infested tissue by placing bits of infected pith in autoclaved tap water for 24–72 hr to encourage sporangia formation. Tissues were then placed on water agar amended with 0.5 g/L Ampicillin (D[−]-α-Aminobenzylpenicillin) sodium salt and 0.4 cm^3/L, (Pimaricin 2.5% aqueous suspension [Sigma]). In instances where contaminants occurred, the sporulating tissue was placed under the amended water agar and allowed to grow to the surface of the agar. Isolates were maintained on V8 juice agar under ambient laboratory conditions until the fall of each year; then race determinations were made in the greenhouse.

Tobacco seedlings for tests were produced in the greenhouse. Seeds were sown into vermiculite in 22.5-cm-diameter round aluminum pans with holes made in the bottoms. These were placed into larger pans which contained water and allowed watering from beneath. When seedlings were approximately 4 wk old (four- to six-leaf stage) they were transferred to speedling floating trays containing Pro-Mix BX. Water was supplied through the bottoms of the floating trays. Additional fertilization was supplied by adding 0.2 g/L of 20-20-20 (N-P-K) in the float water. Acephate (0.1 g/L) was added to the water to control insects. Seedlings were allowed to grow until they reached transplant size of 15–20 cm tall with a stem diameter of 4–6 mm. Cultivars used to determine virulence and races were K 326 (low resistance to black shank), Speight G 70 (moderate resistance), Coker 371 Gold (high resistance), and tobacco line 1071 (2), which is immune to race 0 of *P. p. nicotianae* but susceptible to race 1 (6).

Inoculum for the studies was prepared by cutting round toothpicks in half, autoclaving them for 20 min at 121 C, and reautoclaving in V8 juice agar 1 day later. They were placed on petri plates filled with V8 juice agar, and the center of each plate was inoculated with 3-mm cork borer plugs of mycelium from the growing edge of the test isolates and incubated for 1–2 wk at 27 C in the dark to allow the fungus to infest the toothpicks.

Seedlings were inoculated by pushing infested toothpicks into their stems, a method adapted from Kanlong and Hendrix (8). Each isolate was inoculated into six plants of each cultivar, and the test repeated. In 1991, a third test was

conducted with three plants each for some of the cultivars. Observations on reactions were made daily for 7 days after inoculation, at which time plants were rated on a 1–5 scale, where 1 = no reaction; 2 = darkening around inoculation point; 3 = lesion ≤5 mm found on stem; 4 = lesion >5 mm on stem, plant wilting; and 5 = stem collapsed, plant dead.

Isolates were evaluated for their tolerance to metalaxyl in the laboratory. Cooled (45 C) potato-dextrose agar (PDA) was amended with metalaxyl at 0.01, 0.1, 1.0, and 10.0 μg/ml, poured

Table 1. Source and disease rating of isolates of *Phytophthora parasitica* var. *nicotianae* collected in 1990

Isolate no.	County	Host cultivar	Disease rating[x]				
			K 326	Speight G 70	Coker 371 Gold	Line 1071	ED 50 (μg/ml)
218	Brantley	K 326	4.3 a[y]	3.9 a	1.0 b	1.0 b[z]	0.06
219	Brooks	Speight G 70	3.3 a	3.0 a	1.0 b	1.0 b	0.33
220	Tift	K 326	3.7 a	3.2 a	1.0 b	1.0 b	0.34
221	Lanier	K 326	3.6 a	3.2 a	1.0 b	1.0 b	0.48
222	Irwin	Speight G 70	3.1 a	3.3 a	1.0 b	1.0 b	0.07
223	Bacon	K 326	4.0 a	3.2 a	1.0 b	1.0 b	0.07
224	Wayne	K 326	4.0 a	2.9 b	1.0 c	1.0 c	0.56
225	Wayne	K 326	4.1 a	3.4 a	1.0 b	1.0 b	0.08
226	Lanier	K 326	3.5 a	2.4 b	1.0 c	1.0 c	0.08
227	Worth	Speight G 70	3.3 a	2.8 a	1.0 b	1.0 b	0.92
228	Worth	K 326	4.1 a	2.5 b	1.0 c	1.0 c	0.88
229	Worth	K 326	3.4 a	3.0 a	1.0 b	1.0 b	0.85
230	Coffee	Speight G 70	3.4 a	3.1 a	1.0 b	1.0 b	0.96

[x]Disease rating scale was 1–5; 1 = no reaction; 2 = darkening around inoculation point; 3 = lesion ≤5 mm on stem; 4 = lesion >5 mm on stem, plant wilting; and 5 = stem collapsed, plant dead.
[y]Numbers in rows followed by the same letter are not different ($P = 0.05$) according to the Waller-Duncan k-ratio t test. Numbers in columns are not different ($P = 0.05$).
[z]Rating of ≥1.5 in line 1071 are considered pathogenic, therefore race 1.

Table 2. Source and disease rating of isolates of *Phytophthora parasitica* var. *nicotianae* collected in 1991

Isolate no.	County	Host cultivar	Disease rating[x]				
			K 326	Speight G 70	Coker 371 Gold	Line 1071	ED 50 (μg/ml)
275	Mitchell	K 326	5.0 a A[y]	5.0 a A	1.9 cd B	1.0 e C[z]	0.07
276	Mitchell	K 326	5.0 a A	4.8 a A	1.0 f B	1.0 e B	0.09
277	Mitchell	K 326	4.0 c–f A	3.4 e A	1.3 ef B	1.0 e B	0.02
278	Toombs	K 326	5.0 a A	4.8 a A	1.0 f B	1.0 e B	<0.01
279	Toombs	K 326	5.0 a A	4.7 a–c A	1.3 ef B	1.0 e B	0.08
280	Toombs	K 326	4.8 a–c A	4.8 ab A	1.0 f B	1.0 e B	<0.01
281	Toombs	K 326	4.7 a–d A	4.7 a–c A	1.4 d–f B	1.0 e B	0.02
282	Lanier	K 326	4.5 a–e A	5.0 a A	1.4 d–f B	1.0 e B	0.07
283	Lanier	K 326	4.8 ab A	4.7 ab A	1.0 f B	1.0 e B	<0.01
284	Lanier	K 326	5.0 a A	4.7 a–c B	1.0 f C	1.0 e C	<0.01
285	Coffee	K 326	4.0 c–f A	4.5 a–d A	2.6 b B	1.0 e C	0.03
286	Coffee	K 326	3.6 fg A	3.4 e A	1.0 f B	1.0 e B	<0.01
287	Lanier	C 371 G	5.0 a A	5.0 a A	5.0 a A	5.0 a A	0.01
288	Colquitt	K 326	3.2 g B	4.7 ab A	5.0 a A	5.0 a A	<0.01
289	Berrien	K 326	4.5 a–e A	4.5 a–c A	5.0 a A	4.2 bc A	<0.01
290	Lanier	K 326	4.7 a–d A	4.7 a–c A	4.9 a A	4.1 c B	<0.01
291	Irwin	K 326	5.0 a A	4.1 b–e B	4.9 a AB	4.6 ab AB	0.60
292	Pierce	C 371 G	4.7 a–d A	4.6 a–c A	4.6 a A	4.0 c B	0.05
293	Appling	K 326	4.7 a–d A	4.3 a–d A	1.6 c–e B	1.3 e B	0.01
294	Atkinson	K 326	4.1 b–f A	4.6 a–c A	1.0 f C	1.9 d B	0.43
295	Appling	K 326	4.4 a–e A	3.8 de A	1.1 f C	2.3 d B	0.01
296	Appling	K 326	5.0 a A	4.0 c–e B	1.0 f C	1.0 e C	0.08
297	Wayne	K 326	4.7 a–d A	4.9 a A	1.0 f B	1.0 e B	0.07
298	Wayne	K 326	3.9 d–g A	3.6 e A	1.3 ef B	1.0 e B	0.08
299	Jeff Davis	Speight G 70	5.0 a A	4.5 a–d B	1.0 f C	1.0 e C	0.40
300	Berrien	K 326	3.8 e–g A	3.6 e A	1.0 f B	1.0 e B	0.07
301	Coffee	K 326	5.0 a A	4.5 a–c B	1.0 f C	1.0 e C	0.01
302	Coffee	Speight G 70	5.0 a A	5.0 a A	1.0 f B	1.0 e B	0.07
303	Coffee	K 326	5.0 a A	4.9 a A	1.3 ef B	1.3 e B	0.30
304	Coffee	Speight G 70	5.0 a A	4.7 a–c B	1.0 f C	1.0 e C	0.01
305	Jeff Davis	Speight G 70	5.0 a A	5.0 a A	1.0 f B	1.0 e B	0.60
306	Jeff Davis	K 326	5.0 a A	5.0 a A	1.0 f B	1.0 e B	0.07
307	Jeff Davis	K 326	5.0 a A	5.0 a A	2.0 c B	1.0 e C	0.90
308	Grady	K 326	4.8 a A	4.7 a–d A	1.0 f B	1.0 e B	0.70
Control			1.0 h A	1.0 f A	1.0 f A	1.0 e A	ND

[x]Disease rating scale was 1–5; 1 = no reaction; 2 = darkening around inoculation point; 3 = lesion ≤5 mm on stem; 4 = lesion >5 mm on stem, plant wilting; and 5 = stem collapsed, plant dead.
[y]Means in columns followed by the same lowercase letter (a–f) and rows followed by the same uppercase letter (A–C) are not different ($P = 0.05$) according to the Waller-Duncan k-ratio t test. Means for K 326 were calculated from two tests, while means of the other two cultivars and one breeding line were calculated from three tests.
[z]Rating of ≥1.5 in line 1071 is considered pathogenic, therefore race 1.

into 85-mm-diameter plastic petri plates, and allowed to gel. The plates were inoculated with a 3-mm cork borer plug of test isolate at the edge of each plate and incubated at 27 C in the dark. The growth rate was recorded. Mean separation was by the Waller-Duncan *k*-ratio *t* test at $P = 0.05$ (12).

RESULTS

P. p. nicotianae was often difficult to isolate in pure culture. Contamination from bacteria, the most difficult problem, frequently was eliminated by allowing the fungus to grow upward through water agar. In 1990, 59 samples were collected, but because of desiccation and the poor condition of many of the samples, only 13 viable pure cultures were recovered. In 1991, all 35 samples collected yielded pure cultures of *P. p. nicotianae,* but one culture was lost during storage. In 1992, 28 pure culture isolates were recovered from 37 diseased tobacco plants.

All isolates produced abundant sporangia when plugs of culture were placed in sterile tap water for 24–48 hr, and no obvious differences in growth characteristics were evident between cultures of race 0 and race 1, or among cultures within a race. The fungi readily grew on and into the autoclaved medium-soaked toothpicks.

In 1990, the 13 isolates cultured from diseased tobacco samples exhibiting typical black shank symptoms originated from cultivars that have low to moderate resistance to black shank (Table 1). All isolates caused severe disease reactions on K 326 and moderate disease reactions on Speight G 70. Only in one instance (isolate 222) was the average reaction of the isolate on Speight G 70 greater than on K 326. None of the 13 isolates collected in 1990 caused a disease reaction on Coker 371 Gold or the line 1071. These differential varietal reactions indicate that all 13 isolates were race 0.

In 1991, all 34 isolates caused severe disease reactions on K 326 and Speight G 70 (Table 2). Reaction ratings ranged from 3.2 to 5.0 on these cultivars. Only six of the isolates caused a less severe reaction on Speight G 70 than on K 326, whereas only one isolate caused a more severe reaction on Speight G 70 than on K 326. All isolates except those numbered 287–292 were less pathogenic to Coker 371 Gold and/or line 1071 than to K 326 and Speight G 70. All isolates except those which caused severe disease on line 1071 were nonpathogenic or weakly pathogenic on Coker 371 Gold. Isolates 287–292 were highly pathogenic to line 1071, and isolate 295 was moderately pathogenic to line 1071. These isolates are considered to be race 1. Isolates 293, 294, and 303, although highly pathogenic to K 326 and Speight G 70, were weakly pathogenic or nonpathogenic to Coker 371 Gold and line 1071. Reaction ratings of ≤1.5 typically represented a single plant that showed a reaction in one of the three tests used to calculate the mean, and was not repeatable. These isolates are considered race 0.

All 28 isolates collected from commercial tobacco fields in 1992 were highly pathogenic on both K 326 and Speight G 70 (Table 3). Pathogenicity ratings ranged from 4.2 to 5.0 on those cultivars. Seven of the isolates were less pathogenic on Speight G 70 than on K 326, but none of the isolates were more pathogenic on Speight G 70 than on K 326. Isolates 358, 365, 366, and 367 were low to moderately pathogenic (ratings ranged from 2.0 to 2.9) on Coker 371 Gold and line 1071, and were considered race 1. Isolate 361 caused a weakly pathogenic reaction (mean rating 1.1) in one plant in one of the tests and was considered race 0.

Locations of race 1 isolates in the Georgia tobacco-growing belt were randomly distributed (Fig. 1). Although an attempt was made to obtain a random sample of diseased tobacco plants from across the growing area, the enthusiasm of county agents for the project was

Table 3. Source and disease rating of isolates of *Phytophthora parasitica* var. *nicotianae* collected in 1992

Isolate no.	County	Host cultivar	Disease rating[x]				
			K 326	Speight G 70	Coker 371 Gold	Line 1071	ED 50 (μg/ml)
340	Tattnall	K 326	5.0 a A[y]	4.6 a–d B	1.0 c C	1.0 c C[z]	0.01
341	Colquitt	Coker 371 Gold	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.04
342	Lanier	K 326	5.0 a A	4.9 ab A	1.0 c B	1.0 c B	0.01
343	Appling	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.20
344	Appling	K 326	5.0 a A	4.8 a–c A	1.0 c B	1.0 c B	0.06
345	Appling	K 326	5.0 a A	4.3 cd B	1.0 c C	1.0 c C	0.09
346	Appling	McNair 944	5.0 a A	4.6 a–d B	1.0 c C	1.0 c C	0.01
347	Jeff Davis	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.05
348	Echols	K 346	4.6 b A	4.2 d A	1.0 c B	1.0 c B	0.06
349	Lanier	Speight G 108	5.0 a A	4.4 a–d B	1.0 c C	1.0 c C	0.01
350	Cook	Speight G 70	5.0 a A	4.3 b–d B	1.0 c C	1.0 c C	0.07
351	Worth	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	<0.01
352	Brooks	Speight G 70	5.0 a A	4.8 a–c A	1.0 c B	1.0 c B	0.09
353	Colquitt	K 326	5.0 a A	4.6 a–d B	1.0 c C	1.0 c C	0.06
354	Colquitt	Coker 371 Gold	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.90
355	Colquitt	Speight G 70	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.06
356	Colquitt	K 394	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.01
357	Colquitt	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.01
358	Irwin	K 326	5.0 a A	5.0 a A	2.9 a B	2.8 a B	0.60
359	Pierce	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.01
360	Pierce	Speight G 70	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.60
361	Colquitt	Speight G 70	5.0 a A	4.5 a–d B	1.0 c C	1.1 c C	0.06
362	Colquitt	Coker 371 Gold	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.40
363	Coffee	K 326	5.0 a A	5.0 a A	1.0 c B	1.0 c B	0.30
364	Mitchell	K 326	5.0 a A	4.8 a–c A	1.0 c B	1.0 c B	0.30
365	Bulloch	K 326	5.0 a A	5.0 a A	2.3 b B	2.0 b B	0.20
366	Toombs	Coker 371 Gold	5.0 a A	4.8 a–c A	2.8 a B	2.6 a B	0.50
367	Tift, BSF	Coker 371 Gold	5.0 a A	5.0 a A	2.1 b B	2.8 a B	0.50
Control			1.0 c A	1.0 e A	1.0 c A	1.0 c A	ND

[x]Disease rating scale was 1–5; 1 = no reaction; 2 = darkening around inoculation point; 3 = lesion ≤5 mm on stem; 4 = lesion >5 mm on stem, plant wilting; and 5 = stem collapsed, plant dead.

[y]Means in columns followed by the same lowercase letter (a–f) and rows followed by the same uppercase letter (A–C) are not different ($P = 0.05$) according to the Waller-Duncan *k*-ratio *t* test. Means are of two tests.

[z]Rating of ≥1.5 in line 1071 is considered pathogenic, therefore race 1.

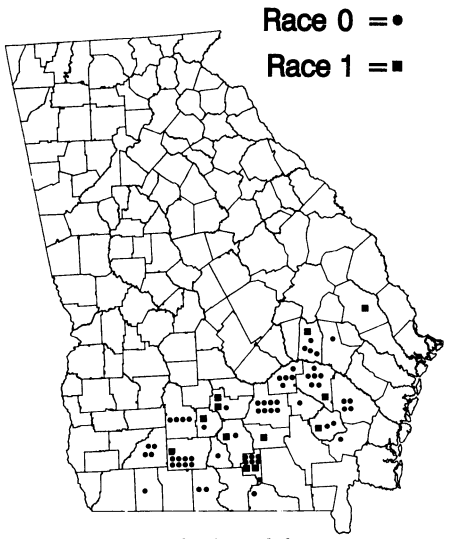

Fig. 1. Isolates of *Phytophthora parasitica* var. *nicotianae* determined to be race 0 = • or race 1 = ■ in the tobacco-growing belt of Georgia sampled in 1990–1992.

variable and was reflected in the response.

Isolates recovered from commercial tobacco fields were evaluated for their sensitivity to metalaxyl. Isolates ranged from very sensitive to metalaxyl (ED_{50} < 0.01 μg/ml) to mildly tolerant (ED_{50} > 0.96 μg/ml) (Tables 1–3). Growth of most isolates was inhibited completely at 1.0 μg/ml.

DISCUSSION

Over the past few years we have observed that tobacco black shank severity and incidence is increasing in Georgia. The evaluation of isolates from the tobacco growing belt in Georgia would suggest that there are two possible mechanisms at work. This is the first survey of *P. p. nicotianae* race distribution and evaluation of metalaxyl sensitivity in Georgia. Gaines (5) and Thompson et al (11) have discussed the occurrence and distribution of black shank but did not discuss race development or distribution.

Over the 3-yr period (1990–1992), 75 isolates of *P. p. nicotianae* were recovered from diseased tobacco plants exhibiting typical black shank symptoms. Sixteen percent of those isolates were determined to be race 1. None of the commercially available tobacco cultivars have high resistance to race 1 of the black shank pathogen. Development of race 1 has been reported in other states (2,4,7,9) and may develop from the use of cultivars with resistance to race 0 (2). The black shank resistant cultivar, Coker 371 Gold, in these stem inoculation studies demonstrated remarkable resistance, almost equivalent to that of line 1071. The derivation of resistance for Coker 371 Gold is not clear, but the disease reaction in stems more closely resembled that of line 1071, *N. plumbaginifolia*-derived resistance, than it resembled the Fl 301 resistance found in Speight G 70 and K 326. The use of Coker 371 Gold in black shank areas has increased in Georgia since the cultivar's release in 1986. Although only four of 12 of the isolates found to be race 1 were isolated from Coker 371 Gold, this does not preclude the possibility of Coker 371 Gold being grown in that field before the collection was made. However, we have no direct evidence that race 1 developed in Georgia with the continuous culture of Coker 371 Gold in heavily infested fields.

The sensitivity to metalaxyl of *P. p. nicotianae* isolates varies by almost 100 times (ED_{50} ranged from 0.96 to <0.01 μg/ml). We speculate that field applications of metalaxyl at 3.36 kg/ha would translate to approximately 1–2 μg/g, and many of the isolates evaluated in this study may not be sufficiently inhibited at that level to control the disease in the field. The authors are fully cognizant that in vitro studies may not relate well to in vivo activity, but plainly the variability does exist, and thus we suggest that reduced sensitivity may play a role in the occasional lack of control of the disease at common use rates. We do not know whether this variation in sensitivity is natural variation or represents selection of decreased sensitivity by repeated use of metalaxyl.

We interpret from the data that race 0 can infect and kill Coker 371 Gold, since some of the Coker 371 Gold samples were infected with a typical race 0 reaction isolate. We also suggest that the stem resistance of Coker 371 Gold may be greater than that found in the roots, since stems showed no reaction from some isolates recovered from Coker 371 Gold. Several researchers have discussed the differential resistance reactions among plant parts (2,3,6,13). Although the precise relationship of resistance among plant parts is not known, the method described by Hendrix and Apple (6) and Kanlong and Hendrix (8) and used here provides distinct reactions that can be used to differentiate races.

ACKNOWLEDGMENTS

We thank the Georgia Cooperative Extension Service county agents for assistance in locating samples, Lewis Mullis and Wanda Tillery for technical assistance, and Philip Morris Tobacco Co. for funding.

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