

To the Graduate Council:

I am submitting herewith a thesis written by Pedro Ricardo Jones entitled "Identification and Occurrence of Races of Phytophthora parasitica var. nicotianae on Dark Tobacco." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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Thesis

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IDENTIFICATION AND OCCURRENCE OF RACES OF PHYTOPHTHORA
PARASITICA VAR. NICOTIANAE ON DARK TOBACCO

A Thesis

Presented for the

Master of Science

Degree

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ABSTRACT

In 1978 isolations of Phytophthora parasitica var. nicotianae were made from black shank-infected plants from Tennessee counties where dark fire- and air-cured tobacco are grown. The race of eight representative isolates were identified with the following differential tobacco cultivars: Madole, DF-300, L8 and A23. Seedlings were grown in the greenhouse in artificial media in 12-unit aluminum muffin pans, each supported by an aluminum cake pan. After 14 days the seedlings were inoculated with mycelium, removed and root system indexed for black shank severity. Reaction of known races 0, 1 and 3 were similar to reactions reported previously. Five of the eight unknown isolates were identified as race 0 and three identified as race 1.

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CHAPTER I

INTRODUCTION

Tobacco, with a value of \$192,000,000, is one of the major crops grown in Tennessee, second in value only to soybean. In 1977 dark tobacco production in Tennessee was 13,500,000 kg with a value of \$40,100,000. Black shank, caused by Phytophthora parasitica var. nicotianae Van Breda de Haan is an important disease of tobacco in the Southeastern part of the United States. Losses due to black shank on dark tobacco in Tennessee in 1977 were estimated at \$1,329,000 (2). The use of resistant varieties is necessary for tobacco production in Tennessee due to the lack of satisfactory black shank control with chemicals and rotation.

Four races of P. parasitica var. nicotianae are known to occur. The occurrence and distribution of race 0 and 1 have been reported on burley, flue-cured and Connecticut shade tobacco types. Race 3 is known to occur in Connecticut while race 2 occurs in South Africa. No information is available on the occurrence or distribution of the pathogen in the areas where dark tobacco is produced in Tennessee. Such knowledge about the pathogen population is important to the existing dark-fire tobacco breeding program. The objective of this study was to identify and determine the occurrence of races of P. parasitica var. nicotianae on dark tobacco in Tennessee.

CHAPTER II

LITERATURE REVIEW

I. THE DISEASE

Black shank of tobacco (Nicotiana tabacum L.) was first described by Van Breda de Haan in 1896 in Java (27). Black shank first appeared in the U.S. in the southern part of Decatur Co., Georgia, in 1915 (24) and in Tennessee in Robertson Co., in 1935 (12).

II. THE PATHOGEN

P. parasitica var. nicotianae, incitant of black shank of tobacco, is a semi-aquatic fungus (19). The fungus is a member of the class Oomycetes belonging to the order Peronosporales (1). The aerial hyphae, 3-11 μ in diameter, are straight, colorless and are usually non-septate. Inter- and intracellular hyphae in pith tissue may be straight or irregular, sometimes swollen and knotty in appearance. Young hyphae are hyaline and turn light yellow with age. The sporangia are oval to lemon shaped with an average size of 25 x 35 μ . The sporangia possess apical papillae, through which zoospores are discharged. Each sporangium produces 5 to 30 ovoid zoospores 7 to 11 μ in length and bluntly pointed at the anterior end. Each zoospore has two flagella of unequal length. Chlamydospores develop frequently in diseased tissue. The chlamydospores

are ovoid, non-papillate and hyaline to brown in color. The sexual stage is difficult to produce in culture and apparently rare in nature. The oogonia are spherical and hyaline (19).

III. SYMPTOMS

Black shank is a disease affecting primarily roots and basal parts of stems (8, 19). However, all parts of a tobacco plant in any stage of growth may be infected (28). The disease is usually first noticed in low, wet spots in a field or plant bed. The first indication of the disease is a sudden wilting of the leaves, followed by chlorosis. Symptoms of the disease vary with age and weather conditions. All or part of the root system may be infected and turn black with the stem lesions extending several cm up the stalk (8, 19, 24). Often, when the stem of a diseased plant is split lengthwise through the lesions, the pith is dry, discolored and is separated into plate-like disks. During periods of rainy weather, necrotic lesions may develop on the leaves (19).

IV. VARIABILITY OF PATHOGEN

Several workers have reported variation among P. parasitica var. nicotianae isolates. Apple and others (3, 4, 22) found isolates which were weakly pathogenic on a susceptible variety and highly pathogenic on a moderately resistant variety. Apple reported strains of P. parasitica var. nicotianae to differ culturally and physiologically (4). Hendrix and Apple (13) observed variability in race 0 isolates used to stem inoculate L8.

McIntyre and Taylor found race 3 to differ significantly from race 0 and 1 physiologically and in certain biochemical traits (21).

Variation has been found among isolates of the pathogen from different geographic locations (4, 5). In general, isolates from locations where the disease was severe were the most highly pathogenic (3). Isolates from infested fields planted to resistant varieties yielded a higher percentage of virulent biotypes than those fields having no history of being planted to resistant varieties (4, 7). It was postulated that continuous planting of resistant varieties exerted a selection pressure favoring highly virulent biotypes (4, 5).

V. OCCURRENCE AND DISTRIBUTION OF RACES

To date four races of P. parasitica var. nicotianae have been identified. Race 0 and race 1 can be separated using a differential reaction between cultivars with Florida 301 type resistance and those with resistance developed from either Nicotiana plumbaginifolia Viv. or Nicotiana longiflora Cav. The latter are highly resistant to race 0 and are completely susceptible to race 1 of the fungus (10, 11, 17, 23). In South Africa isolates of the fungus were found which appeared to be different from the two races found in America. The race 2 isolates were unable to infect the cultivar Delcrest 202 which is susceptible to races 0 and 1 of the fungus (15, 16). In 1976 McIntyre and Taylor (20) presented evidence for occurrence of a third race in Connecticut. Their isolates designated

C was pathogenically unlike race 0 and 1. Tobacco cultivars Nicotiana nesophila Johnst. and N. tabacum 1071 were both resistant to C. Tobacco cultivar L8 was moderately susceptible to C with stem necrosis extending only several cm above and below inoculation site. This C isolate was later designated as race 3 (21).

Race 0 is the most frequently encountered form of the pathogen (10, 13). In a study of races of P. parasitica var. nicotianae in Middle Tennessee, Hendrix and Flowers (14) isolated race 0 from plants of susceptible varieties or plants having Florida 301 resistance. Gupton (11) reported the presence of race 1 in East Tennessee and the wide distribution of race 1 in Middle Tennessee. Race 1 was first isolated from infected greenhouse plants with N. longiflora type resistance (26). In North Carolina race 1 was isolated from infected cultivars with N. tabacum x N. plumbaginifolia type background (6). In Tennessee and Kentucky race 1 has been isolated from infected plants in fields where L8 type hybrids have been grown (18). Race 1 appeared to occur in fields infested with race 0 in response to selection pressure of planting N. plumbaginifolia and certain lines derived from N. plumbaginifolia x N. tabacum hybrid (10).

CHAPTER III

MATERIALS AND METHODS

I. COLLECTION OF ISOLATES AND ISOLATION OF PATHOGEN

Twenty-four isolates of the fungus were collected in August of 1978 from plants suspected of having black shank. The distribution of the collection sites is shown in Figure 1. The pathogen was isolated by surface sterilizing infected tissue with 0.1% sodium hypochlorite, rinsing with tap and sterile water and blotting dry. A section of tissue was removed from the lesion margin and placed on a medium containing corn meal, Pimaracin, Polymyxin B Sulfate, and Penicillin G (Corn meal PPP) (8). The isolates were maintained by transferring every four weeks to freshly prepared potato dextrose agar (PDA).

II. INDEXING METHOD

The indexing method used to determine the race of the isolates was similar to that described by Troutman, Henderson and LaPrade (25). Seeds of tobacco cultivars Madole, DF-300, L8 and A23 were sown in sterile four-inch plastic pots in "Jiffy Mix" (1:1:1, peat:perlite:vermiculite). Seedlings were transplanted after 35 days into sterile muffin pans containing the same medium. One and one-half gram Osmocote (N:P:K, as 14:14:14) in 2.91 tap water were added to the cake pan. Each

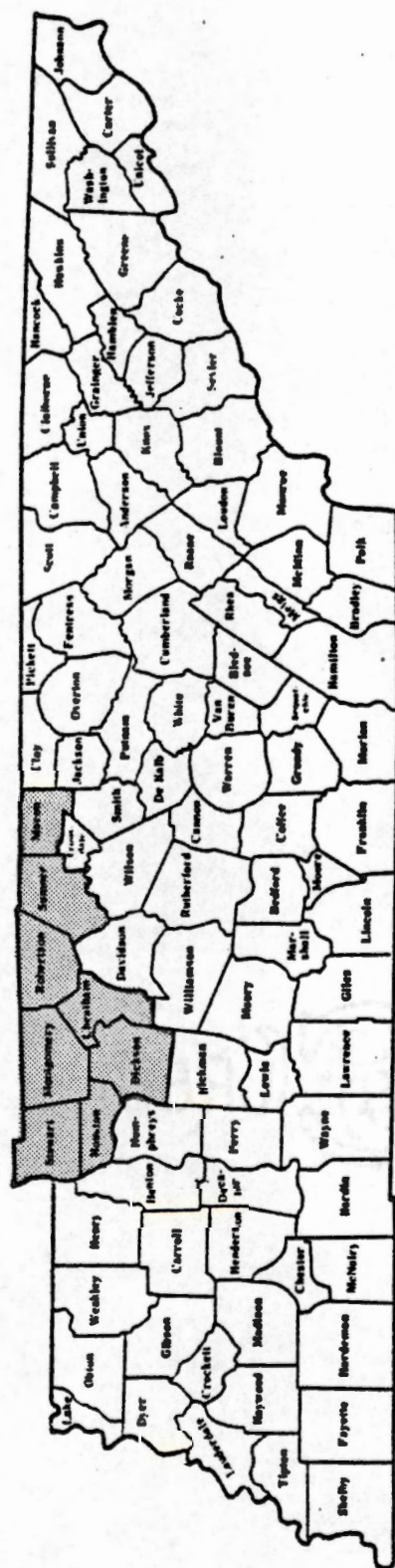


Figure 1. The collection sites of isolates identified as races of *P. parasitica* var. *nicotianae* from the areas of dark tobacco in Tennessee

One isolate from each shaded county was used in the study

muffin pan contained twelve plants, three each of the four cultivars. The plants were placed on greenhouse benches after transplanting and were watered as needed. Materials used in the indexing method is shown in Figure 2.

The cultivar Madole, which is susceptible to race 0 and 1 of P. parasitica var. nicotianae, was used to verify the presence of the pathogen. DF-300, which is moderately resistant to race 0 and 1, was used to indicate differences in virulence among the isolates. L8, which is susceptible to race 1, was used to indicate the presence of race 1. A23, which is susceptible to races 0, 1 and 3, was used to indicate the presence of race 2. Eight of the 24 unknown isolates and one isolate each of known races 0, 1 and 3 were used in the study. Race 0, race 1 and race 3 were obtained from Dr. C. C. Litton and Dr. J. L. McIntyre, respectively.

III. INOCULUM PREPARATION

Inoculum was prepared by transferring the fungus from stock cultures to Corn meal PPP medium. Agar plugs from these cultures were used to seed 200-ml prescription bottles containing 50 ml of potato dextrose broth (PDB). The cultures were kept at room temperature for three weeks. The broth containing the mycelial mats from two bottles was homogenized for three seconds in a Waring blender. The homogenate was diluted with tap water to 1600 ml. One hundred ml of this mycelial suspension was added to water in the cake pans.



Figure 2. Arrangement of plant in muffin tin supported by cake pan used to characterize the isolates as to race of P. parasitica var. nicotianae

IV. RATING SYSTEM

Two weeks after inoculation each plant was removed from the pans and the root system was rated for disease severity. The following rating scale was used:

- 0 no symptom
- 1 0- 25% of roots infected with crown symptom
- 2 25- 50% of roots infected with crown symptom
- 3 50- 75% of roots infected with crown symptom
- 4 75-100% of roots infected with crown symptom
- 5 plant dead

The seedlings were 7 and 8 weeks old prior to inoculation in trial 1 and 2, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

I. SYMPTOM DEVELOPMENT

Wilting of plants was noted four to seven days after inoculation. Stem necrosis, collapsing and death occurred within 7-14 days. Blackening of the stem of the susceptible cultivars sometimes extended into the bud of the plant, especially of small plants. The plants that appeared to be healthy continued to grow, but later developed leaf chlorosis. Control plants also developed this chlorosis. The percentage of dead, diseased and healthy plants at 14 days after inoculation are presented in Table 1.

II. REACTION OF DIFFERENTIALS TO ISOLATES

The reaction of the four differentials cultivars to eight isolates is presented in Table 2. Isolate A appeared to be the most virulent isolate tested. Madole and A23 were highly susceptible to this isolate, while DF-300 was moderately susceptible. L8 was highly resistant to isolate A. In response to isolate B, Madole was moderately susceptible and DF-300, L8 and A23 were highly resistant. Cultivars Madole and DF-300 were highly resistant to isolate C, and L8 and A23 were moderately susceptible. Reaction of DF-300, L8 and A23 indicated high resistance to isolate D, whereas Madole was moderately susceptible. Madole and L8 were highly susceptible to isolate

TABLE 1. The percentage of differential cultivars dead, diseased and healthy 14 days after inoculation with isolates of *P. parasitica* var. *nicotianae*

Isolates	Differentials or Cultivars									
	Madole		DP-300		L8		A23			
	Dead ¹	Diseased ²	Healthy ³	Dead	Diseased	Healthy	Dead	Diseased	Healthy	Dead
A	100	0	0	0	100	0	0	0	100	44
B	22	45	33	0	33	67	0	0	100	56
C	0	33	67	0	78	22	11	45	44	0
D	22	56	22	0	33	67	0	0	100	0
E	44	56	0	0	67	33	33	56	11	89
F	0	56	44	0	44	56	0	0	100	11
G	33	45	22	0	67	33	0	0	100	11
H	89	11	0	0	67	33	11	89	0	0

¹The percentage of dead plants include plants with a disease rating of 5.

²The percentage of diseased plants include all plants with disease rating of 1 to 4.

³The percentage of healthy plants correlates with 0 of the rating scale.

TABLE 2. Reaction of four cultivars used as differentials to isolates of P. parasitica var. nicotianae

Tobacco cultivars or differentials	ISOLATES							
	A	B	C	D	E	F	G	H
Madole	S ¹	S	R ²	S	S	S	S	S
DF-300	S	R	R	R	R	R	R	R
L8	R	R	S	R	S	R	R	S
A23	S	R	S	R	S	S	S	S

¹Designations of "susceptibility" based on disease indices of 1.4 or more.

²Designations of "resistant" based on disease indices of 1.3 or less.

E. DF-300 was highly resistant to isolate E, while A23 was moderately susceptible. Madole was slightly susceptible to isolate F. DF-300 and L8 were highly resistant and A23 was moderately susceptible to isolate F. The response of Madole and A23 indicated moderate susceptibility to isolate G, in comparison to the highly resistant response of DF-300 and L8 to isolate G. Madole was highly susceptible, L8 and A23 were moderately susceptible and DF-300 was highly resistant to isolate H.

The ratings were tested by analysis of variance. The Mean square table for the analysis is given in Table 3. Where differences occurred, the mean was separated using Duncan's Multiple Range Test. These results are presented in Tables 4, 5, and 6.

III. CHARACTERIZATION OF ISOLATES

The race classification of the isolates is presented in Table 6. Based on differential reaction on cultivars Madole, DF-300, L8 and A23, isolates A, B, D, F and G were classified as race 0 and isolates C, E and H were classified as race 1. Results for race 3 are presented in Table 7. Race 3 was highly pathogenic on Madole and slightly pathogenic on cultivars DF-300 and L8. Race 3 was moderately pathogenic on cultivar A23. This is the first report of race 3 having been tested on cultivars Madole and DF-300. The results with the known races

TABLE 3. Mean Square of Analysis of Variance on data from Trial 2

Source	Degree of Freedom	Cultivars			
		Madole	DF-300	L8	A23
Isolates	10	25.2061 ²	3.912 ³	17.9616 ²	14.1061 ³
Replication	2	3.2780	2.1313	.9394	4.8586
(Isolates & ¹ Replication)	20	2.8061	1.0313	1.3616	3.5364
Plants (Iso-					
Plants (Iso-					
lates, repli-					
cation)	66	1.789	.3030	.5152	.8687

¹ Isolates x Replication was used as error term to test race.

² Significant at .01.

³ Significant at .05.

TABLE 4. Mean disease severity indices of Trial 1 on tobacco cultivars 14 days after inoculation with isolates characterized as races of P. parasitica var. nicotianae

Tobacco Cultivars	Isolates							
	A	B	C	D	E	F	G	H
	Trial ¹ 1							
Madole	4.22c	4.22c	4.75a	4.67a	4.67a	4.33a,c	4.11c	4.67a
DF-300	3.56b	3.33b,c	1.63e	1.89d	2.67d	3.33b,c	1.89e	4.22a
L8	1.67c	0d	3.88a	0d	3.11b	2.22d	.111d	4.11a
A23	2.33b	3.00a,b	4.13a	3.44a,b	3.00a,b	3.67a,b	2.89a,b	4.00a

¹Seedlings in Trial 1 were 7 weeks old at the time of inoculation.

Means in each row followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test.

Means are averages of nine plants.

TABLE 5. Mean disease severity indices of Trial 2 on tobacco cultivars 14 days after inoculation with isolates characterized as races of P. parasitica var. nicotianae

Tobacco Cultivars	Isolates							
	A	B	C	D	E	F	G	H
	Trial ¹ 2							
Madole	5.00a	1.78b	.778c	2.44b	4.33a	1.4b,c	2.22b	4.89a
DF-300	2.67a	.333c	.778b,c	.333c	.778b,c	.778b,c	.778b,c	.778b,c
I8	0 ^c	0 ^c	2.00b	0c	3.56a	0c	0c	3.33a
A23	4.33a	.778d	1.78b	.444d	3.33b	2.44b,c	1.78c	2.89b

¹Seedlings in Trial 2 were 8 weeks old at the time of inoculation.

Means in each row followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test.

Means are averages of nine plants.

0 and 1 isolates were similar to results reported previously by other researchers (17, 18, 21).

Variations in disease development between replications was noted. The amount of variation in disease development between trial 1 and trial 2 could be contributed to plant size, and/or greenhouse conditions. Plants in trial 1 were seven weeks at the time of inoculation while plants in trial 2 were eight weeks old. In both trials plants which were severely affected were 2.54 to 5.08 cm in height whereas the average height of the plants was about 13.97 cm. The results of both trial 1 and trial 2 are given in this study, however, the author believes that the results of the second trial are more representative due to differences in plant age at inoculation. It appeared that some increases in susceptibility were associated with plant age in trial 1. The additional 7 days growth prior to inoculation in trial 2 appeared to prevent this problem. The cause of chlorosis to the leaves of the healthy plants and control was probably due to nutritional effects.

TABLE 6. Sources of isolates of P. parasitica var. nicotianae and race identification

Isolates Designation	Source	Race Determination
(A)	Robertson County	Race 0
(B)	Dickson County	Race 0
(C)	Sumner County	Race 1
(D)	Houston County	Race 0
(E)	Macon County	Race 1
(F)	Cheatham County	Race 0
(G)	Montgomery County	Race 0
(H)	Stewart County	Race 1

TABLE 7. Mean disease severity indices and reactions of four tobacco cultivars to race 3 of P. parasitica var. nicotianae.

Tobacco Cultivar	Reaction ¹	Mean of Disease Severity ²	
		TRIALS	
		1	2
Madole	S ³	4.44	4.78
DF-300	R	3.44	1.11
L8	R	1.22	.333
A23	S	3.89	2.78

¹Reactions are from Trial 2.

²Means are the averages of nine plants.

Seedlings in Trial 1 were 7 weeks old at the time of inoculation.

Seedlings in Trial 2 were 8 weeks old at the time of inoculation.

³Designations of "susceptibility" based on disease indices of 1.4 or more.

Designations of "resistance" based on disease indices of 1.3 or less.

control was probably due to nutritional effects.

The differences between isolate A, isolates B,D,F,G, isolate C and isolates E and H indicate only the variation in pathogenicity of the isolates on the differentials tested. Isolate A appeared to be a virulent strain of race 0 while C was a race-1 strain of low virulence. The reaction of race 3 on the differentials tested supplies additional information about this race. Other studies of race 3 on DF-300 should be conducted to test the advisability of the continued use of Florida 301 type resistance in dark tobacco.

Wilting, as indicated by Troutman (25), was not a good measure of susceptibility. Collapsing of plants, blackening of stems and root deterioration were good indications of susceptibility and useful in indexing disease severity. Collapsed plants and those completely girdled with stem necrosis were recorded as dead. As reported by Troutman, it is important to include susceptible, moderate and highly resistant varieties as standards for comparison. The indexing method used in this experiment was sufficient for determining the races of particular isolates. However, in a breeding program or other studies that require more quantitative results, more controlled conditions would have to be adopted.

CHAPTER V

SUMMARY AND CONCLUSION

The identification and occurrence of races of P. parasitica var. nicotianae on dark tobacco in Tennessee was determined by race characterization of eight isolates as to race using a small plant indexing method. All isolates tested on cultivars Madole and A23 responded with varying degrees of pathogenicity. DF-300 exhibited high resistance to all isolates except isolate A. L8 typically demonstrated high levels of resistance to those isolates classified as race 0 and moderate to high levels of susceptibility to those races classified as race 1. L8 also demonstrated a high level of resistance to race 3. None of the isolates tested were characterized as race 3.

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VITA

Pedro Ricardo Jones was born in Charleston, South Carolina on August 3, 1955. He attended elementary school in Charleston and was graduated from Charles A. Brown High School in May, 1973. The following September, he entered Knoxville College where he received a Bachelor of Science Degree in June, 1977. He became a graduate student in the Department of Agricultural Biology with a graduate research assistantship in September of 1977.

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