RESISTANCE OF TOBACCO CULTIVARS AND CANDIDATE CULTIVARS TO *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANAE*

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Twenty-eight official tobacco (*Nicotiana tabacum* L.) cultivars, 35 candidate cultivars and one breeding line were evaluated for resistance to *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucker at field locations in Georgia, South Carolina, and North Carolina and, in greenhouse tests in North Carolina and Virginia. Cultivars which were considered resistant when they were released ranged from 49 to 100% killed in field locations and 59-100% killed in a greenhouse test. New candidate cultivars ranged from 100% killed to almost completely resistant to black shank. Correlation coefficients of % disease for field data vs. greenhouse data on candidate cultivars ranged from 0.57 to 0.76 ($P = 0.0001$). Data for % disease between field locations had correlation coefficients from 0.81 to 0.86 ($P = 0.0001$). The Scott-Knot procedure of cluster analysis was used to separate means into distinct groups without overlapping. This method of statistical analysis facilitates researchers and extension specialists to give verbal ratings for cultivars according to their resistance to black shank.

INTRODUCTION

Black shank of tobacco (*Nicotiana tabacum* L.) incited by *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucker is widely spread over the flue cured belt in the eastern U.S.A. (8). The disease causes loss estimates of 0.7-1.7% of the $1.5 billion crop annually (3). The use of crop rotation and metalaxyl (3,4,11,13) aid in reducing the losses from this persistent soil-borne fungus, but these methods are costly and do not eradicate the disease.

The most practical and economical method for controlling black shank is the use of resistant cultivars. However, commercially available cultivars are not adequately resistant to completely control the disease in areas with moderate to heavy infestations of the pathogen. Apple (1) showed that *P. parasitica* var. *nicotianae* isolates obtained from tobacco cultivars resistant to black shank were more likely to be highly pathogenic than isolates of the pathogen obtained from susceptible cultivars. Apple also predicted that available resistance to the pathogen would diminish with successive crops of the resistant cultivar and emphsized that evaluation of resistance to the pathogen should be conducted in areas where the highly pathogenic biotypes have evolved.

The work reported here evaluates official tobacco cultivars and candidate tobacco cultivars for resistance to *P. parasitica* var. *nicotianae*. The test locations have a long history of tobacco black shank and represent areas that have highly pathogenic biotypes of the fungus.

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MATERIALS AND METHODS

Greenhouse

Race 0 of the black shank pathogen *Phytophthora parasitica* var. *nicotianae* was used in the greenhouse studies. One month old tobacco plants grown from seed were transplanted to pie pans or muffin pans containing vermiculite (14) with 9-12 seedlings per pan. Each entry was replicated three times (3 pans each). Plants were grown in pans for 1-3 weeks to overcome transplant shock and develop new roots.

At location one (Blackstone, Va.) the inoculum was grown at ambient temperature (Ca. 21 C) for 12 days on potato-dextrose-broth in medicine bottles. Inoculum was diluted 1:3 with water and plants were inoculated by introducing 50 ml into bread pans (used for subirrigation) supporting the muffin pans. The muffin pans had holes in the bottoms for irrigation and passage of the pathogen (14). Beginning four days after inoculation numbers of plants infected were counted each day for four days. Percent disease was calculated for the entries at the end of the test. Disease index (DI) was calculated using the following formula:

$$DI = \left(\frac{\sum_{i=1}^{N} Xi}{\frac{K}{\sum_{i=1} ni}} \right) X\ 100$$

where N = maximum number of dead plants per replication per rating, Xi = number of dead plants per replication per rating, K = number of ratings, ni = initial number of plants per replication, 100 = disease index expressed as percent.

At location two (Raleigh, N.C.) inoculum consisted of three-week-old oatmeal agar cultures grown at ambient temperature and blended in 300 ml of sterile deionized water/10 cm plate. Inoculum was added as 50 ml of suspension per pan between rows of plants in trenches cut with a knife. Disease development was recorded every 5-6 days for a total of four readings. Percent disease was calculated at the end of the test for each entry. Disease index was calculated as follows:

$$DI = \frac{\sum_{i=i}^{n} Xi\ [100 - (i-1)\ 10^{n}]}{I}$$

where i = ordinal evaluation number, n = number of evaluations (excluding initial stand count), X = number of dead plants since last count, and I = initial number of plants in plot (3).

Field

Tobacco cultivars and candidate cultivars were evaluated in the same test. Official varieties tested were those listed in **Table 1,** except 1071 which is a breeding line and is used as a *P. parasitica* var. *nicotianae* race indicator. Candidate cultivars tested are listed in **Table 2,** except Va. Gold, NC 95 and NC 2326 which are official cultivars used as standards. Tobacco plants were produced in methyl bromide treated seed beds using recommended practices (6,7,9,10). Cultivars were planted into plots 10-12 m long, with an inter-row spacing of 122 cm and inter-plant spacing of 45-56 cm. Entries were replicated three times at each location. Areas in three locations, Tifton, Georgia, Florence, South Carolina, and Rocky Mount, North Carolina were selected which have histories of severe black shank. Seedlings were transplanted in 1983 on 16 April in Georgia, 19 May in South Carolina and 6 May in North Carolina. Recommended fertilization, insecticides, and cultural practices were followed for each location (6,7,9,10). Plots were irrigated as required. Percent disease was calculated at the end of the test for each entry. Numbers of living plants were counted every two weeks starting two weeks after transplanting. A plant was considered dead when it was permanently wilted with visible symptoms of black lesions extending above ground. Disease indices were calculated as described under greenhouse tests (location two).

Data were analyzed using ANOVA, Duncan's multiple range test, a cluster analysis method for mean separation developed by Scott and Knott (5,12) and regression analysis.

Table 1. Evaluation of official tobacco cultivars for disease caused by *Phytophthora parasitica* var. *nicotianae* under field and greenhouse conditions.

	Field [1]		Greenhouse [2]	
Tobacco Cultivar	% Disease	Disease Index	% Disease	Disease Index
Va Gold [3]	100 a	81 a	100 a	100 a
Hicks Broadleaf [3,4]	100 a	85 a	100 a	83 a
Reams 266 (1601) [4,5]	98 a	42 c	nd	nd
Coker 319 [3]	96 a	58 b	100 a	95 a
Coker 316 [4,5]	89 a	36 c	100 a	91 a
NK 326	89 a	42 c	nd	nd
NC 567 [3]	85 a	38 c	100 a	81 a
Coker 176	82 a	37 c	82 a	64 a
NC 628 [3]	82 a	34 c	100 a	89 a
NC 22 NF [3]	80 a	32 c	59 b	43 b
Speight G-58 [3]	80 a	36 c	89 a	55 b
PD 4 [3]	80 a	36 c	89 a	80 a
Coker 347 [3]	79 a	41 c	96 a	78 a
Coker 411 [3,5]	79 a	35 c	nd	nd
McNair 373	77 a	33 c	84 a	70 a
Speight G-28	75 a	32 c	67 b	48 b
Speight G-70	65 b	27 d	89 a	68 a
NC 50 [3]	62 b	29 d	70 b	54 b
Va 182 [3]	62 b	27 d	89 a	82 a
Coker 209	61 b	22 d	93 a	72 a
Coker 48	60 b	27 d	85 a	67 a
McNair 944 [3]	57 b	24 d	93 a	81 a
Coker 298 [3]	57 b	33 c	96 a	74 a
NK 399 [3]	57 b	22 d	93 a	80 a
NC 82 [3]	56 b	23 d	89 a	74 a
Coker 51 [3,5]	49 b	19 d	nd	nd
1071 [4]	32 b	14 d	18 c	11 c

[1] Means in columns followed by the same letter are not significantly different according to Scott-Knot procedure (P = 0.05).
[2] The greenhouse disease data for the official varieties was determined only at North Carolina; nd is not determined.
[3] Not included in Georgia field trials.
[4] Tobacco breeding line not included in South Carolina field trials.
[5] Not included in North Carolina field trials.

RESULTS AND DISCUSSION

In field tests 49 to 100% of plants in the official cultivars were killed **(Table 1).** Similar, but higher percent disease values were recorded in the greenhouse for those cultivars. The resistance currently available in commercial cultivars would be unacceptable in fields moderately to highly infested with *P. parasitica* var. *nicotianae*. Some cultivars that had similar values for % disease differed in their disease indices. The lower the disease index, the later in the season the cultivar began to wilt. Cultivars with low disease indices would be advantageous in some instances, allowing growers to harvest a portion of the crop prior to plant death.

Candidate tobacco cultivars ranged from being almost completely killed to highly resistant to *P. parasitica* var.

Table 2. Evaluation of candidate tobacco cultivars for disease caused by *Phytophthora parasitica* var. *nicotianae* under field and greenhouse conditions.

Tobacco Entry	Field [1] % Disease	Field [1] Disease Index	Greenhouse % Disease	Greenhouse Disease Index
Va Gold [2,3]	100 a	81 a	100 a	83 a
Va 101	95 a	49 b	50 b	38 b
Va 103	95 a	52 b	58 b	48 b
Speight G-99M	92 a	49 b	64 b	51 b
Va 102 [3]	90 a	46 b	60 b	48 b
NC 95 [3]	90 a	40 c	64 b	50 b
Speight G-84	89 a	38 c	60 b	40 b
NC TG-24	86 a	43 b	46 b	37 b
Reams 119	85 a	42 b	58 b	53 b
NC TG-25 [3]	85 a	45 b	39 c	36 b
NC 2326 [3]	84 a	43 b	61 b	43 b
NC 1418	83 a	31 c	73 b	55 b
Speight G-92M	82 a	34 c	58 b	46 b
PD 48	82 a	36 c	68 b	51 b
PD 88	80 a	38 c	50 b	35 b
Reams 216	80 a	35 c	39 c	27 c
Speight G-96	79 a	34 c	63 b	45 b
PD 9	75 b	35 c	52 b	42 b
NC 745 USDA	74 b	25 d	49 b	37 b
Speight G-80	72 b	30 c	62 b	47 b
Va 104	71 b	31 c	57 b	41 b
Rogers 82-7-346	70 b	28 d	42 c	29 c
NC 70 USDA	69 b	30 c	54 b	40 b
PD 279	66 b	27 d	43 c	36 b
Rogers 82-31-3411	64 b	26 d	62 b	51 b
NK 94	59 b	24 d	51 b	38 b
Rogers 82-49-910	58 b	22 d	30 c	20 c
NK 2142	56 b	21 d	39 c	34 b
NC 1523	56 b	26 d	28 c	43 b
NK 279	55 b	23 d	30 c	24 c
NC 48 USDA	45 c	19 d	47 b	35 b
Coker 206Y	44 c	20 d	31 c	24 c
NK 2120	23 d	6 e	39 c	36 b
Coker 82-226Y2	18 d	9 e	24 c	18 c
Coker 82-234Y	13 d	3 e	7 d	7 c
NK 2117	9 d	3 e	30 c	24 c
Coker 82-211Y	6 d	4 e	0 d	0 c
Coker 82-721Y	5 d	3 e	17 d	15 c

[1] Means in columns followed by the same letter are not significantly different according to the Scott-Knot procedure ($P = 0.05$).
[2] Not included in Georgia field trials.
[3] Tobacco cultivars included as standard cultivars for disease evaluation.

nicotianae indicating that acceptable levels of resistance can be attained **(Table 2)**. Similar responses were recorded in the greenhouse tests.

Separation of cultivars or breeding lines according to their resistance was accomplished by the use of the Scott-Knott procedure. The Duncan's multiple range test gave up to 10 overlapping classes, which made separation based on disease reaction difficult (5). Official tobacco cultivars tested in the field were separated into only two groups for percent disease, and into four groups for disease index **(Table 1)**. Similar separation occurred for the cultivars tested in the greenhouse. Candidate cultivars were separated into four groups according to % disease and five and three groups for disease index in field and greenhouse tests, respectively **(Table 2)**.

Correlation coefficients of candidate tobacco varieties **(Table 2)** for % disease of field data at three locations vs. greenhouse data at two locations ranged from 0.57 to 0.76 ($P. = 0.0002$) and ranged from 0.50 to 0.71 ($P. = 0.002$) for disease indices. Correlation coefficients for % disease and disease index between field locations ranged from 0.86 to 0.81 ($P. = 0.0001$) and 0.87 to 0.84 ($P. = 0.0001$), respectively. These data indicate that there was good correlation between field and greenhouse data, and between field locations, although considerable variability occurred among locations for some cultivars.

The breeding line 1071 is used as an indicator for evaluation of tobacco black shank **(Table 1)**. The breeding line has resistance to race 0 of *P. parasitica* var. *nicotianae* but none to race 1 (2). Eighteen percent of the 1071 plants died when inoculated with race 0, but 100% died when inoculated with race 1 (unpublished data). Thirty-two percent of 1071 died in the field tests, suggesting that a mixed population of race 0 and 1 existed in the field locations. However, the severity of disease recorded for cultivars, once considered resistant, indicates that the test areas have populations of the fungus that have become highly pathogenic over time (1). Testing in greenhouses using specific races of the fungus could yield important data on candidate cultivars as to specificity of resistance. However, conclusions drawn strickly from greenhouse data where only a single biotype of the pathogen is used may yield results that are not in agreement with data generated in black shank nurseries. Testing in areas where the pathogen has become very pathogenic under continuous tobacco culture assures that candidate cultivars that are selected under these conditions will have high resistance in commercial fields for several years.

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