Effect of Host Resistance on Spread of *Phytophthora parasitica* var. *nicotianae* and Subsequent Development of Tobacco Black Shank Under Field Conditions

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ABSTRACT

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Four flue-cured tobacco cultivars representing different levels of quantitative resistance to the black shank disease were planted in small plots in a field with no history of tobacco production. After the last cultivation, a single plant in each row was inoculated by inserting 3 ml of an aqueous suspension of *Phytophthora parasitica* var. *nicotianae* into the stem at the soil line. The field was not reentered until the end of the growing season, when disease incidence was assessed. In the average-rainfall growing season of 1982, spread of the pathogen was detected in rows of all cultivars. Spread was detected in 78, 56, 17, and 17% of the rows with inoculated plants and percentage of total plants expressing symptoms was 38, 15, 10, and 8% for the susceptible cultivar Hicks, low-resistance cultivar Coker 319, moderate-resistance cultivar Coker 411, and high-resistance cultivar NC 82, respectively. Symptomatic plants were not continuous down a row from the point of inoculation, but *P. p.* var. *nicotianae* was recovered from soil and root assays from all intervening asymptomatic plants, indicating that pathogen spread was continuous. Movement of the pathogen from the inoculated plant was detected in 75% of the rows that contained no symptomatic plants except the inoculated plant. In the dry summer of 1983, spread was detected only in plots of the cultivar Hicks, with spread occurring in 20% of rows that contained an inoculated plant.

Black shank is a root and crown rot disease of tobacco (*Nicotiana tabacum* L.) induced by the soilborne fungus *Phytophthora parasitica* (Dast.) var. *nicotianae* (Breda de Haan) Tucker (12). Black shank is controlled by the use of fungicides, cultural practices, and host resistance (12). The source of black shank resistance in most flue-cured tobacco cultivars is the resistant parent, Florida 301 (12). The resistance is thought to be controlled by a partially dominant gene that is expressed to varying degrees because of the presence of modifier genes in the susceptible parents (14). Currently grown cultivars have low to high levels of black shank resistance. The level of black shank resistance used to describe a cultivar is a subjective rating assigned by extension specialists and is based on disease development under field conditions over several years.

Campbell et al (1,2) analyzed epidemics of black shank that occurred over several years on tobacco cultivars ranging in resistance to *P. p.* var. *nicotianae* from susceptible to highly resistant. More than half of the 50 disease progress curves examined were best described by the logistic model. Because the pattern of diseased plants was generally random, they proposed that spread of the pathogen within rows was not the reason for the logistic increase in disease. They concluded that a combination of other factors may have accounted for the sigmoid-shaped curve, including the pattern of root growth and the effects of environmental stresses on the physiology of the host plant.

Disease progress best described by a logistic model does not necessarily mean that secondary inoculum is involved in disease development (1,18). In fact, empirical evidence for the importance of secondary inoculum in disease spread within a growing season is limited to a few host-pathogen systems (3,8,17,19). Mycelial growth from plant to plant is the primary mechanism of dispersal in these examples and possibly of the movement of zoospores in Aphanomyces root rot (19). Kannwischer and Mitchell (11) assumed epidemics of tobacco black shank were polycyclic but stated that evidence of spread due to production and dispersal of secondary inoculum needed to be obtained. Soilborne *Phytophthora spp.*, including *P. p.* var. *nicotianae* (7,11), reproduce profusely on and in infected roots, and pathogen dispersal and subsequent disease development have been documented for several host-pathogen combinations (23–25). Spread in these systems is generally along drainage patterns over a period of years (24,25). Quantitative information is lacking on the spread of *Phytophthora* spp. within a growing season from a point source of inoculum in a row crop.

The objectives of this study were to determine if *P. p.* var. *nicotianae* is dispersed from a point source of inoculum within a growing season under field conditions and to determine the effect of host resistance on the extent of pathogen dispersal and subsequent disease development.

MATERIALS AND METHODS

Plots were established in 1982 and 1983 at the Upper Coastal Plain Research Station, Rocky Mount, NC, in a field with no history of tobacco production. Extensive sampling of the field for *P. p.* var. *nicotianae* before transplanting indicated no background level of the pathogen. The 1983 test was conducted in a portion of the field fallowed in 1982 and separated from the area infested in 1982 by meadow strips. Tobacco seedlings were planted in three-row plots in 1982 and four-row plots in 1983. There were 11–13 plants per row, with a plant spacing of 0.56 m and a row spacing of 1.1 m. Four cultivars, Hicks, Coker 319, Coker 411, and NC 82, were chosen to represent a susceptible cultivar and low, moderate, and high levels of resistance to black shank, respectively. Each treatment was replicated six times in 1982 and five times in 1983 in a randomized complete block design. Recommended practices for tobacco production were followed, except the plants were not topped (flower heads removed) and no chemicals were applied to prevent growth from axillary buds at leaf nodes. Plots were not irrigated in either year of the study. Rainfall and number of rain days for the 1982 and 1983 growing seasons are given in Table 1.

After the last cultivation, and 45–50 days after transplant, one plant in each row in 1982 and one plant in each of the two center rows of the four-row plots in 1983 was selected at random and stem inoculated 2 cm below the soil line by wounding the stem and inserting 3 ml of a suspension of *P. p.* var. *nicotianae* made by blending 1-mo-old cultures grown on oatmeal agar (one 9-cm-diameter petri plate culture per 30 ml of distilled water). The suspension contained viable hyphal fragments, sporangia, and chlamydospores of *P. p.* var. *nicotianae*. A mixture of five isolates

of *P. p.* var. *nicotianae* originally isolated from flue-cured tobacco from five counties in North Carolina was used to inoculate plants. After inoculation, the field was not reentered until the end of the growing season, when plants were rated individually for disease symptoms on the stem. Plant death was attributed to black shank if the characteristic blackened stem lesion was present at or above the soil line.

Movement of the pathogen was determined in some rows by collecting and assaying roots of symptomatic and asymptomatic plants and by assaying soil collected 15, 30, and 60 cm from the inoculated plant. Roots were removed from the soil, washed in running tap water, and surface-sterilized in 0.5% NaOCl for 30 sec before plating on a selective agar medium (11). Soil samples consisted of three soil cores (2 cm diameter by 15 cm deep) collected at each specified distance along the side of the bedded row. The three cores from each distance were bulked and assayed on a modified PARP selective agar medium (11,21). Randomness of infected plants in each row was determined by runs analysis (1,13).

RESULTS

Pathogen spread was observed in both years of the study. The extent of spread within rows, as determined by aboveground symptom expression, was dependent on the level of host resistance and on the environmental conditions (Table 2). During the average-rainfall growing season of 1982, disease spread was observed in 78% of the rows of the susceptible cultivar and in 17% of the rows of the moderately and highly resistant cultivars. The maximum spread possible within a row was nine plants (5 m); however, because of the location of the inoculated plant, maximum spread possible in most rows was five or six plants. Maximum spread observed in 1982 was nine plants in the susceptible cultivar and two plants (1.1 m) in the highly resistant cultivar. Final disease incidence decreased with increasing level of resistance (Table 2). In contrast, in the dry growing season of 1983, spread was limited to only 20% of the rows of the susceptible cultivar. Maximum spread was one plant. No spread was observed in the resistant cultivars.

Based on results from runs analysis, occurrence of symptomatic plants within a row was random in 93% of the rows where spread was observed in 1982. Roots taken from asymptomatic plants located between symptomatic plants were frequently infected with *P. p.* var. *nicotianae* (Table 3). When both the infected but asymptomatic and symptomatic plants were included in the runs analyses, occurrence of diseased plants within a row was nonrandom and spread of the pathogen was continuous from the source plant. Most spread occurred along a gentle slope of 1–2% in the field (Tables 3 and 4).

Movement of the pathogen from the point of inoculation was detected in many rows where no disease symptoms developed except on the inoculated plant. The pathogen was recovered from 68, 50, and 40% of the soil samples collected 15, 30, and 60 cm from the inoculated plant in 1982. The pathogen was recovered on assay

TABLE 1. Average annual rainfall and the total rainfall and rain days recorded for 1982 and 1983 at the Upper Coastal Plain Research Station, Rocky Mount, NC

	1982[a]		1983[a]		
Month	Total rainfall	No. rain days[b]	Total rainfall	No. rain days	30-yr av.
May	2.75	–	3.60	–	3.19
June	6.98	2	2.53	–	4.58
July	4.77	8	1.94	4	5.70
Aug	5.94	9	3.46	4	5.33

[a] Plants were inoculated on 22 June 1982 and 2 July 1983. Final disease rating on 30 August 1982 and 29 August 1983.
[b] Number of days rainfall exceeded 25 mm after the date of inoculation.

TABLE 2. Effect of host resistance on spread *Phytophthora parasitica* var. *nicotianae* from one inoculated plant per row and subsequent development of tobacco black shank

	Rows in which spread observed (%)[b]		Disease (%)[c]	
Cultivar[a]	1982	1983	1982	1983
Hicks	78	20	38	6.1
Coker 319	56	0	15	4.6
Coker 411	17	0	10	4.6
NC-82	17	0	8	4.6

[a] Cultivars representing four levels of resistance to tobacco black shank: Hicks, susceptible; Coker 319, low resistance; Coker 411, moderate resistance; and NC-82, high resistance.
[b] Spread occurred if any plants other than the inoculated plant within a row were symptomatic at the final disease rating on 30 August 1982 and 29 August 1983.
[c] Percentage of plants showing symptoms of tobacco black shank at the final disease rating.

TABLE 3. Pattern of spread of *Phytophthora parasitica* var. *nicotianae* from an inoculated plant and subsequent development of tobacco black shank in four rows of cultivar Hicks tobacco in 1982 at the Upper Coastal Plains Research Station, Rocky Mount, NC

	Row			
Plant no.[a]	A	B	C	D
1	–[b,c]	–	–	–
2	–	–	–	–
3	–	–	–	S
4	–	–	–	S
5	I	–	+	+
6	+	I	I	I
7	S	S	+	+
8	+	S	+	+
9	+	+	S	S
10	S	S	+	S
11	–	–	+	–

[a] A slope of 1–2% ran from plant 1 toward plant 11.
[b] I = plant inoculated by inserting an aqueous suspension of *P. p.* var. *nicotianae* into the stem after the last cultivation, S = blackened stem lesion characteristic of tobacco black shank present at or above the soil line, + = root isolations for *P. p.* var. *nicotianae* positive on a selective agar medium but no aboveground symptoms of black shank observed; – = no aboveground symptoms of black shank and root isolations for *P. p.* var. *nicotianae* negative.
[c] Distribution of symptomatic plants in each row determined to be random based on runs analysis. Distribution of diseased plants, based on root infections, was nonrandom based on results of runs analysis.

TABLE 4. Spread of tobacco black shank from a point source of inoculation in four flue-cured tobacco cultivars as determined by presence of aboveground symptoms

Slope direction[a]	Plant no.[b]	Percent symptomatic[c]			
		Hicks	Coker 319	Coker 411	NC-82
Uphill	1	28	11	6	6
	2	22	11	0	0
	3	28	11	0	0
	4	28	6	0	0
	5[d]	17	0	0	0
Downhill	1	39	11	0	6
	2	39	0	6	6
	3	39	6	6	0
	4	22	6	0	0
	5	22	6	0	0
	6	33	0	0	0
	7	11	6	0	0
	8	6	0	0	0
	9	6	0	0	0

[a] A 1–2% slope was present in the field.
[b] Number refers to plants either uphill or downhill starting from the inoculated plant.
[c] Black shank resistance in the four cultivars: Hicks, susceptible; Coker 319, low; Coker 411, moderate; and NC-82, high. Value is percentage of total plants in 18 rows of tobacco for each cultivar.
[d] No plants beyond the fifth plant became symptomatic for any cultivar.

plates as chlamydospores free in soil and as colonized root fragments.

DISCUSSION

Tobacco black shank was a polycyclic disease (22) under the conditions in this study. In the absence of cultivation, which would serve to disperse inoculum, movement of the pathogen from a point source of inoculum was detected and subsequent disease development was observed in both years. In both years of this study, no disease was observed in the fields before the inoculation treatment, and in 1983, no disease developed except on inoculated plants with the exception of the two rows of Hicks where the plant adjacent to the inoculated plant became symptomatic. These observations, along with the general distribution of disease within rows (Table 4) and the failure to recover the pathogen in soil samples taken before transplanting, indicate that there was no background level of *P. p.* var. *nicotianae* inoculum in the field. Spread of *P. p.* var. *nicotianae* and the subsequent development of black shank was dependent on the level of host resistance and on environmental conditions. As the level of host resistance increased, the number of rows in which spread occurred, and the extent of spread in those rows, decreased (Table 4). The slower rate of disease development observed in the resistant cultivars could possibly be related to a reduction or delay in secondary inoculum production and dispersal, a reduction in inoculum efficiency on the resistant cultivars, or a reduction in the rate of root colonization after infection. Kannwischer and Mitchell (11) reported that, under field conditions, secondary inoculum production by *P. p.* var. *nicotianae* was delayed on the resistant tobacco cultivar Speight G-28 compared with that on the susceptible cultivar Hicks. Similar results were obtained with root-zone populations of *P. p.* var. *nicotianae* in the four cultivars used in this study (H. D. Shew, *unpublished*). A delay in the production of secondary inoculum would result in fewer cycles of secondary inoculum production, which would slow epidemic development, and lower final disease incidence. The effect of host resistance on the latent period and infectious period (period of secondary inoculum production) of *P. p.* var. *nicotianae* on tobacco has not been quantified.

Host resistance may determine the importance of environment in development of Phytophthora root rots (5). This is especially true if the mechanism of resistance is to slow the rate of internal root colonization by the pathogen (5). The rate of root colonization after initial infection will determine the number of primary and secondary infections required to cause sufficient root necrosis to induce symptoms. Nusbaum (15) reported that black shank resistance derived from the cultivar Florida 301 reduced the rate of root colonization compared with the rate of colonization of susceptible cultivars. Because the number of environmental periods favorable for the production and dispersal zoospores of *Phytophthora* spp. (soil saturation) (5) is limited within a growing season, disease development and spread thus should have been much less in the resistant cultivars.

Specific environmental effects were probably determining factors in the spread of *P. p.* var. *nicotianae* from a point source of inoculum. Most spread in 1982 was in the direction of a slope of 1–2% in the field. Because no cultivation took place after inoculation, this unidirectional spread probably indicates movement of pathogen propagules with moving water. Movement of *Phytophthora* propagules, primarily zoospores, along drainage patterns is well documented (5,24). The extent of disease spread (up to 5 m in Hicks) indicates that pathogen movement probably included zoospore dispersal in surface water. In 1982, there were 24 days of measurable rainfall between inoculation and final disease rating, with rainfall exceeding 25 mm on 19 days. In 1983, there were only 9 days of measurable rainfall and eight of these rainfalls exceeded 25 mm. Spread of *P. p.* var. *nicotianae* was detected in 1983, but symptom development was very limited. In addition, the field site used in 1983 was better drained than the 1982 site and surface water was not observed during the growing season. Zoospore movement thus was probably limited primarily to movement through the soil matrix. Dispersal of zoospores in the soil matrix is dependent on the soil type and soil water potential and is very limited compared with dispersal in surface water (4,5). Root contact also may have accounted for the limited spread of *P. p.* var. *nicotianae* to plants adjacent to the inoculated plant in the susceptible cultivar Hicks in 1983. Roots of adjacent tobacco plants normally grow together by midseason (16), making root contact between plants common. The growth rate of *P. p.* var. *nicotianae* through host tissue is not known, but growth of *P. cinnamomi* in susceptible host tissue is reported to be 18 mm/day (20). A similar growth rate for *P. p.* var. *nicotianae* could result in spread to adjacent plants within the time frame of the experiment.

The random occurrence of symptomatic plants within rows was unexpected. Campbell et al (1) reported a random occurrence of diseased plants within rows, but they worked in fields with a history of black shank occurrence and thus did not start with a single source of inoculum within a row. The pattern of diseased plants in their study may have been a reflection of inoculum pattern or environment at particular sites within a row, or both. In the present study, soil and root assays showed that pathogen dispersal was continuous down a row, resulting in a nonrandom pattern of dispersal for *P. p.* var. *nicotianae*. Ferrin and Mitchell (6) reported a nonrandom pattern of inoculum for *P. p.* var. *nicotianae* in soil. The location and/or number of root infections on root systems of plants within a row must have varied, resulting in the random development of symptoms. The proximity of infections to the crown of the plant or the total number of infection sites per plant may have affected the time required for onset of symptoms. Results from this study suggest that the sole use of aboveground symptoms as an indicator of pathogen spread may not provide a true biological interpretation of the nature of the disease cycle for tobacco black shank.

This study demonstrated that tobacco black shank can be a polycyclic disease and that secondary inoculum production is important in final disease incidence. Because disease was only rated at the end of each growing season, a logistic increase could not be demonstrated for the disease in these tests. However, a logistic increase for black shank epidemics on the cultivars used in this study has been observed in previous (1) and subsequent years (H. D. Shew, *unpublished*). Factors other than secondary inoculum production and dispersal also may result in a logistic type increase in root diseases (1,9,18). These factors include the pattern of root growth (9) and environmental effects on plant stress and resistance expression (10). Additional research is required to determine the role of these factors on development of black shank epidemics. Knowledge of how these factors are related should improve our ability to predict the effectiveness of new disease management practices through their effect on both the host and the pathogen.

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