Effect of Soil Type and Soil Matric Potential on Infection of Tobacco by *Phytophthora parasitica* var. *nicotianae*

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ABSTRACT

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Black shank development was observed in greenhouse-grown tobacco seedlings growing in soil material collected from 12 soil series. When infested with similar levels of *Phytophthora parasitica* var. *nicotianae*, the soils varied in conduciveness to disease development; seedling infection ranged from 8 to 92%. Disease incidence was correlated with soil texture, drainage class of the parent soil, fumigation, and nutrient status. In four of the soil materials maintained at four controlled matric potential regimes, infection of tobacco seedlings was reduced in each soil material at −40 millibars (mb) compared to higher potentials. Disease suppression at −40 mb was attributed to restricted zoospore release and dispersal. Infection was greatest in each soil material when plants at −40 mb were exposed to a single 24-hr saturation period (−40 mb +S) 1 wk into a 3-wk test. Infection was lower in two disease-suppressive soil materials at −10, −20, and −40 mb + S than in two disease-conducive soil materials. Propagule survival over a 6-mo period in the different soil materials was not correlated with disease conduciveness even though survival was lower in some untreated compared to fumigated soils. The nature of disease suppression appeared to be a combination of microbial, physical, and chemical factors.

Black shank of tobacco (*Nicotiana tabacum*) caused by the soilborne fungus *Phytophthora parasitica* (Dast) var. *nicotianae* (Breda de Haan) Tucker is a destructive and economically important disease in the southeastern United States (11). Host resistance, fungicide use, cultural practices and soil properties affect black shank development (11).

The importance of soil properties to black shank severity was demonstrated when Dukes and Apple (4) evaluated soil materials from 99 locations representing 23 soil series in North Carolina for their conduciveness to black shank. Soil series, parent material, drainage class, texture, pH, and available Ca++ and Mg++ were correlated with disease incidence. More disease also was observed in some soils following fumigation with methyl bromide, suggesting the presence of soil organisms antagonistic to the pathogen. Unfortunately, initial inoculum density was not quantified and the watering regime was not controlled in this study. The importance of water potential in development of Phytophthora root rots has since been studied and recently reviewed (6). The incidence and severity of other Phytophthora root rots have been correlated with soil series, position on the landscape, and sites with poor internal drainage (1,2,8,14,15).

Soil matric potential is important in the life cycle of *Phytophthora* spp. because of its effects on sporangium and chlamydospore production, zoospore release and dissemination, and disease development (6). A direct effect of matric potential on black shank development in tobacco seedlings has been reported (17). Seedling infection decreased from 60 to 7% as matric potential decreased from −10 to −50 millibars (mb); however, when soil maintained at −50 mb was saturated for 24 hr, 100% seedling infection occurred. Disease enhancement was attributed to increased zoospore production and dispersal (17). However, it is not known if the effects of matric potential on disease development are similar in soils of different textures or in soils that differ in conduciveness to black shank development. Soil texture determines pore size distribution and may influence disease development through an effect on zoospore dissemination. For example, zoospores of *P. cryptogea* moved 25–35 mm through a coarse-textured U.C.-type soil mix at matric potentials less than −1 mb, but only 5 mm in a silt loam or fine sandy loam (5). Also, behind a horizontal wetting front in a packed soil column, zoospores of *P. megasperma* moved 35 mm in a sand, 44 mm in a sandy clay loam, and 48 mm in a loam, but failed to move in a silt loam (19).

The purposes of this study were to screen soil materials in the greenhouse for relative conduciveness to black shank development in susceptible and moderately resistant tobacco seedlings, to observe disease development in plants growing in selected soils maintained at different matric potential regimes, and to determine the effects and possible interactions of matric potential and soil type on propagule survival and disease development.

MATERIALS AND METHODS

Greenhouse experiment. Soil materials representing 12 soil series (Table 1) were tested for conduciveness to black shank development. Soils were collected from the upper 15 cm of the AP horizon in fallow fields in March, air-dried, sieved through a 2-mm screen, and stored in the greenhouse in polyurethane bags. *P. p.* var. *nicotianae* was not present initially in any of the soils as determined by soil assays (17). Half of each soil material was moistened and fumigated with methyl bromide at 0.5 kg/70 kg of soil. Methyl bromide was allowed to dissipate for several days before the soil was used in the experiments.

Inoculum for infesting soil was produced in a portion of the fumigated half of each soil material by infecting and killing successive plantings of tobacco seedlings (17). Mycelial mats of five isolates of *P. p.* var. *nicotianae*, grown for 1 wk on oatmeal agar, were stripped from the agar and blended in 1 L of deionized water for 1 min. Five hundred milliliters of the mycelial suspension were mixed into each soil material and the soil was placed in flats. One-month-old cultivar Hicks tobacco seedlings were transplanted into the soil, and these were killed by the pathogen within 3 days. Two successive crops of seedlings were planted in the same soil which established inoculum densities ranging from 150–300 propagules per gram (p/g) of dry soil. Prior to determining inoculum density of *P. p.* var. *nicotianae* in the soil by using a selective agar medium (9), soil was allowed to air dry to

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approximately 5% moisture by weight. The infested soil then was diluted with either fumigated or untreated soil to obtain an average inoculum density as determined by subsequent assay of 6 p/g (range 3–14 p/g) for use in tests.

A 6-cm layer of infested soil at the final inoculum density was placed in 10-cm-diameter clay pots. A 3-cm layer of noninfested soil of the same soil type and treatment (fumigated or untreated) was then placed over the infested soil and either a 1-mo-old cultivar Hicks (susceptible) or cultivar Speight G-28 (moderately resistant) tobacco seedling was transplanted into the noninfested soil layer. Disease development was evaluated over a 30-day period by using three methods of evaluation: a disease severity rating of 0–30 which was scored inversely according to the time when aboveground wilting and stem lesion symptoms first appeared (symptoms observed on day 1 = 30, on day 10 = 20, and on day 29 = 1, etc.); a root rot severity rating of roots excised after 30 days (0 = healthy root system, 1 = < 20% of the root system with lesions, 2 = ≥ 20% of the root system with lesions, 3 = crown rot, and 4 = dead plant; and the percentage of seedlings infected as determined by plating the entire root system from each seedling (after evaluation for root rot) on the selective medium (9) and observing for growth of *P. p.* var. *nicotianae*. Root systems were surfaced sterilized in 10% Clorox for 15 sec prior to being placed on the selective medium. The experiment was a completely randomized block, 13 × 2 × 2 factorial (13 soils, two tobacco cultivars, and fumigated or untreated soil), with three replications and four plants per replication.

Growth chamber experiment. Disease development was observed in four soil materials (Norfolk-2, Grantham, Duplin, and Coxville) at four matric potential regimes. Regimes included constant matric potentials of −10, −20, and −40 mb (1 mb = 0.1 kPa), and one regime maintained at −40 mb with a single 24-hr saturation period 1 wk into each 3-wk test (−40 mb + S). Inoculum was established in the soils by using a mixture of isolates as described above. Infested soil was stored in polyurethane bags at room temperature until used. Inoculum densities averaging 10.5 p/g soil (range 2–20 p/g in different replications over time determined at planting) were established for each replication of the experiment. Inoculum densities did not differ between treatments for a given soil material within a replication. Experiments were replicated over time in a controlled-environment growth chamber.

Soil matric potential was controlled by using Büchner funnel tension plates (7). The reference points for matric potential were the top of the glass plate and the water reservoir. A 15-mm layer of infested soil covered by a 15-mm layer of noninfested soil was placed in each funnel, and a single 1-mo-old cultivar Speight G-28 tobacco seedling was transplanted into the noninfested layer. Soils were initially brought to the desired matric potentials by adding water to the soil surface. Plastic collars covering the surface of the soil were placed around each seedling to reduce evaporation from the soil, and relative humidity was maintained as described previously (17). The experiment was conducted in controlled-environment chambers with a 14-hr photoperiod, light intensity of 23,000 lux, and day:night temperatures of 28:24 C. Root rot rating and percentage of seedlings infected were determined after 3 wk as described for the greenhouse experiment. The experiment was a completely randomized split-plot design with matric potential as the whole plot and soil type as the subplot. The experiment was replicated over time with four replications and six observations (funnels) per replication.

Propagule survival. Five fumigated and 13 untreated soil materials were infested to obtain an inoculum density of approximately 40 p/g. Infested soil was wetted to approximately 11% water content by weight, placed in glass vials with plastic caps that allowed air exchange, and stored at 24 C. Inoculum density was determined from each vial every month for 6 mo. There were three replicate vials for each soil material.

Data analysis. Data for all experiments were analyzed by analysis of variance and the Waller-Duncan *k*-ratio *t*-test. Some treatment effects were tested for significance by single-degree-of-freedom contrasts. Stepwise regression with greatest increase in R^2 as the decision criterion (16) was used to determine significant linear variables in the greenhouse experiment. Variables tested included: pH; drainage class of the parent soil material (well = 1, moderately well = 2, somewhat poorly = 3, and poorly = 4); percent sand; cation exchange capacity; percent base saturation; percent acid saturation; humic matter content; and concentrations of NO_3^-, NH_4^+, K^+, Ca^{++}, Mg^{++}, Mn^{++}, Na^+, Zn^{++}, Cu^{++}, and S^-. All variables except drainage class were determined by the North Carolina

TABLE 1. Soil series, taxonomic classification, drainage class, pH, textural class, and percent sand, silt, and clay for soil materials used in tests for conduciveness to black shank development

Soil series	Classification[x]	Drainage class[y]	pH	Textural class[z]	Sand (%)	Silt (%)	Clay (%)
Norfolk-1	FL Typic Paleudult	W	5.9	SL	75	6	19
Norfolk-2	FL Typic Paleudult	W	5.4	SL	65	30	5
Goldsboro	FL Aquic Paleudult	MW	6.6	SL	55	33	13
Lynchburg	FL Aeric Paleaqult	SWP	5.7	SL	61	28	11
Rains	FL Typic Paleaqult	P	5.7	L	38	46	16
Granville	FL Typic Hapludult	W	5.0	SL	78	16	6
Aycock	FS Typic Hapludult	W	6.0	L	49	37	15
Nahunta	FS Aeric Paleaqult	SWP	5.1	SIL	28	57	15
Grantham	FS Typic Paleaqult	P	5.5	SIL	15	67	17
Marlboro	C Typic Paleudult	W	5.0	SL	69	22	9
Duplin	C Aquic Paleudult	MW	4.7	SL	55	34	11
Coxville	C Typic Paleaqult	P	4.6	SL	51	33	16
Cecil	C Typic Hapludult	W	5.7	SCL	61	14	25

[x]Taxonomic classifications: FL = fine loamy, siliceous, thermic; FS = fine silty, siliceous, thermic; and C = clayey, kaolinitic, thermic.
[y]Drainage classes: W = well, MW = moderately well, SWP = somewhat poorly, and P = poorly.
[z]Textural classes: SL = sandy loam; SIL = silt loam, L = loam; and SCL = sandy clay loam.

Department of Agriculture Soil Testing Laboratory. Percent sand, silt, and clay was determined by using the hydrometer method (3).

RESULTS

Greenhouse experiment. Tobacco cultivar, soil material, and fumigation influenced the percentage of seedlings infected, root rot rating, and disease severity. Disease incidence and severity on cultivar Hicks seedlings were equal or greater than that on cultivar Speight G-28 seedlings in all soils. Differences ($P = 0.05$) in disease development among the soils were observed only with cultivar Speight G-28 seedlings in untreated soil (Table 2). In fumigated soil and with cultivar Hicks seedlings, differences among the soils were not significant, although the ranking of the relative conduciveness of soils to disease was similar to the ranking with cultivar Speight G-28 seedlings in untreated soil.

TABLE 2. Percent infection, root rot rating, and severity rating of black shank disease development of cultivar Speight G-28 tobacco seedlings grown in 13 unfumigated soil materials

Soil material	Infection[w] (%)	Root rot rating[x]	Severity rating[y]
Grantham	92 a[z]	3.6 a	22 a
Duplin	75 ab	1.0 cd	2 cde
Goldsboro	67 abc	1.8 bc	0 e
Aycock	58 abc	2.3 abc	4 cde
Cecil	58 abc	2.3 abc	6 b
Granville	50 bc	1.3 cd	0 e
Norfolk-1	42 bcd	1.1 cd	0 e
Marlboro	42 bcd	1.3 cd	3 cde
Rains	33 cd	1.2 cd	2 cde
Nahunta	33 cd	2.8 ab	5 cd
Norfolk-2	33 cd	1.5 bcd	1 de
Lynchburg	8 d	2.0 bc	4 cde
Coxville	8 d	0.2 d	0 e

[w] Infection determined by presence or absence of *Phytophthora parasitica* var. *nicotianae* in root systems plated on selective medium.
[x] Root rot rating: 0 = healthy root system, 1 = <20% root rot, 2 = ≥20% root rot, 3 = crown rot, and 4 = dead plant.
[y] Severity rating scale 0-30 (wherein disease observed on day 1 = 30, day 10 = 20, day 29 = 1, etc.) based on aboveground symptoms of wilting and stem lesions.
[z] Values in the same column followed by same letter do not differ, $P = 0.05$, according to the Waller-Duncan k-ration t-test.

TABLE 3. Survival of *Phytophthora parasitica* var. *nicotianae* in fumigated and untreated soil materials[x]

Soil material	Propagule survival[y]	
	Unsterile soil	Sterile soil
Nahunta	95 a[y,z]	...
Rains	76 ab	62 a
Grantham	75 abc	...
Goldsboro	74 abc	...
Marlboro	69 abc	...
Cecil	69 abc	...
Norfolk-2	52 bcd	64 a
Granville	47 cd	...
Norfolk-1	41 de	...
Aycock	40 def	...
Coxville	32 def	88 a
Duplin	18 ef	74 a
Lynchburg	14 f	99 a

[x] Soil fumigated with methyl bromide at 0.5 kg/70 kg of soil.
[y] Percent of original inoculum recovered after incubation at 24 C for 6 mo.
[z] Average of three replications. Values in a column followed by same letter do not differ, $P = 0.05$, according to the Waller-Duncan k-ratio t-test.

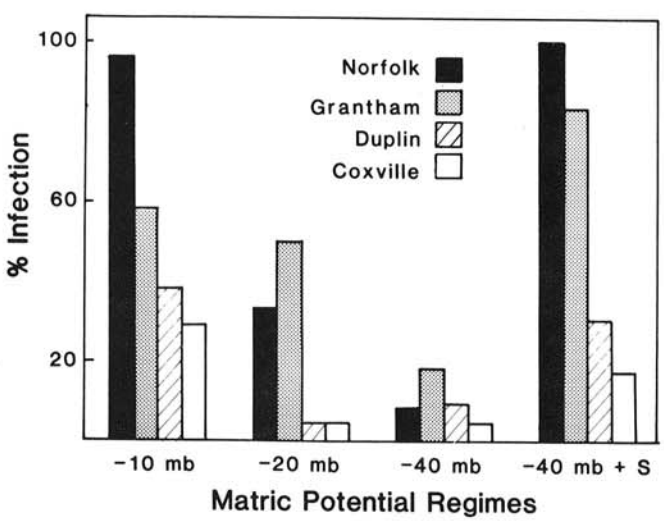

Fig. 1. Percent seedling infection of 1-mo-old cultivar Speight G-28 tobacco seedlings by *Phytophthora parasitica* var. *nicotianae* in four soil materials maintained at four matric potential regimes.

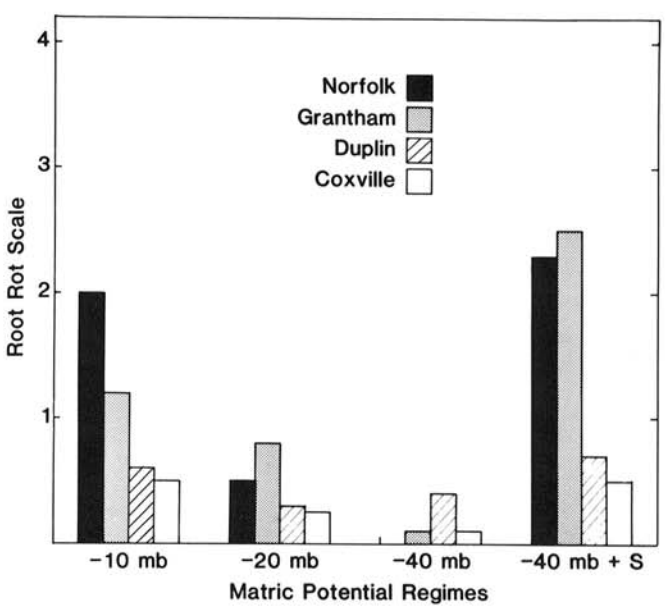

Fig. 2. Root rot rating of 1-mo-old cultivar Speight G-28 tobacco seedlings in four soil materials infested with *Phytophthora parasitica* var. *nicotianae* maintained at four matric potential regimes. Zero = healthy root system, 1 = <20% of root system with lesions, 2 = ≥20% of root system with lesions, 3 = crown rot, and 4 = dead plant.

Stepwise regression was used to determine significant variables affecting infection and root rot ratings on cultivar Speight G-28 seedlings in untreated soil. In a linear model ($R^2 = 0.790$), Mg^{++} and Zn^{++} content were positively correlated and P^-, drainage class, and percent sand were negatively correlated with disease incidence.

Growth chamber experiment. Soil material and matric potential were related to percent infection and root rot rating (Figs. 1 and 2). Seedlings planted in the Norfolk and Grantham soils had a higher percent infection (incidence) and root rot rating (severity) than those in Duplin and Coxville for all matric potential regimes except −40 mb at which disease incidence was uniformly low. In the conducive soils, disease incidence was greater in the Norfolk-2 than in the Grantham soil at −10 mb, while at −20 mb, disease incidence was greater in the Grantham soil as determined by single-degree-of-freedom contrast statements (Fig. 1). Disease incidence was similar in Norfolk-2 and Grantham at −40 mb and −40 mb + S, and in Duplin and Coxville at all matric potential regimes.

Propagule survival. Inoculum densities decreased in all soils over the 6-mo assay period, but inoculum was still present after 6 mo in all soils tested. The rate of propagule survival varied among untreated soils but not among fumigated soils. Fumigation significantly increased survival of propagules of *P. p.* var. *nicotianae* in the Lynchburg, Duplin, and Coxville soils (Table 3).

DISCUSSION

Soil material affected the incidence (percent seedling infection) and severity (root rot rating and disease severity rating) of black shank in greenhouse and in growth chamber experiments. Texture (percent sand), drainage class of the parent soil, and available Ca^{++}, Mg^{++}, Zn^{++}, and P^- were correlated with disease development in the greenhouse test. These findings agree with the results of Dukes and Apple (4) and other workers (10,20) and may reflect actual naturally occurring differences in disease development among soils. For instance, drainage class of the parent soil would affect percent organic matter, soil microorganims, and texture of the soil material due to position on the landscape and height of the seasonally high water table. However, differences among soils in disease incidence and severity observed in the greenhouse also may reflect the lack of control over soil moisture in pots. Finer-textured soils were often water-logged which reduced root development of tobacco seedlings and may have influenced the severity of root infections. Coarser-textured soils remained drier, and this may have reduced infection by limiting production and dispersal of zoospores. For example, percent seedling infection in Norfolk-2 was only 33% in the greenhouse but was greater than 90% when soil infested to a similar inoculum density was maintained at matric potentials of −10 mb and −40 mb + S in the growth chamber. These results further emphasize the need for defining soil water conditions when experimenting with disease development of soilborne *Phytophthora* spp., and they should be considered when interpreting results from previous studies (e.g., 4).

At controlled matric potentials, disease incidence in all soil materials followed the general trends described by Shew (17). As matric potential decreased from −10 to −40 mb, disease incidence decreased, while a period of saturation overcame the suppressive effect of low matric potentials. Since high matric potentials give greater disease incidence and are required for zoospore release and dissemination (12,13), it appears likely that zoospores were the major infective propagules under our test conditions. Although general trends were similar, a difference in disease incidence in the two conducive soils was observed at −10 and −20 mb. Percent seedling infection was lower at −20 mb than at −10 mb in the Norfolk-2 sandy loam but not in the Grantham silt loam. Textural differences in the soils may account for these observations. Texture affects pore size distribution and, therefore, the percentage of pores that are water filled at a given matric potential. A significant reduction in water-filled pore spaces occurred between −10 to −20 mb in the Norfolk-2 sandy loam, but not in the somewhat finer-textured Grantham silt loam (18). This may have caused a reduction in disease incidence in the Norfolk-2 soil due to a greater restriction on zoospore dispersal. Under field conditions, texture and soil aggregation (structure) may similiarly influence infection under high soil moisture and may determine if a soil and site are conducive to black shank development, especially where tobacco is irrigated.

The nature of disease suppression in the Duplin and Coxville soils is unclear. Their pH was lower than in other soils, but pH did not appear to be an important factor in the regression of factors contributing to disease incidence. Adjustment of the pH also failed to alter the suppressive nature of these soils. The Duplin soil material had a high disease incidence (75%) in the greenhouse, but plants exhibited a low root rot and severity rating. At optimum matric potentials, disease incidence of seedlings in Duplin was low (42%) relative to conducive soil materials (Fig. 1). Propagule survival in unfumigated Duplin and Coxville soil material over a 6-mo period was greatly reduced compared to survival in the fumigated soil fraction, suggesting the presence of microbial antagonists in these soils. Sporangium production also was reduced in the Duplin and Coxville soil materials compared to sporangium production in the Grantham soil (18). This suppression was not altered by either autoclaving soils for 1 hr on 3 successive days, steam pasturizing soil for 1 hr at 60 C, raising pH with KOH, or wetting the soils with a complete nutrient solution instead of deionized water. Further research is required to determine the mechanism of sporangium suppression and reduced propagule survival in the suppressive soil materials.

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