Relationships Between the Development of Root Systems of Tobacco and Infection by *Phytophthora parasitica* var. *nicotianae*

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ABSTRACT

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Early infection of tobacco root systems by *Phytophthora parasitica* var. *nicotianae* was evaluated after plant growth in autoclaved and nonautoclaved field soils infested with a defined inoculum density of the pathogen. Incidence of plant infection and average numbers of infections per infected plant were similar for resistant and susceptible tobacco cultivars in either soil. Low efficiencies of inoculum for infection implied infrequent successful contact between susceptible root tissues and pathogen propagules. Root growth of both tobacco cultivars was not altered consistently by infection. Numbers and total lengths of elements of various root orders and branching ratios of healthy and infected plants differed significantly in autoclaved soil of one experiment only. The pattern of root system growth of Speight G-28 tobacco was more consistent than that of Hicks tobacco.

Incidence of black shank of tobacco (*Nicotiana tabacum* L.) has been shown to be related to initial density and aggregation of inoculum of the soilborne pathogen, *Phytophthora parasitica* Dast. var. *nicotianae* (Breda De Haan) Tucker (6,9,15). These relationships have been demonstrated in experiments conducted in plant growth rooms and under field conditions. Such relationships have been used to provide interpretations of the types of interactions that occur between populations of tobacco plants and this pathogen in the course of epidemic development. More detailed evaluations of patterns of initial infections and progressive colonization of individual root systems by the pathogen would provide greater insight into specific events associated with disease development in populations of plants. This would provide information on which more effective strategies of disease control could be based.

Estimates of levels of infection of individual plant root systems at defined inoculum densities have been made for pinto bean, infected by *Fusarium solani* f. sp. *phaseoli*; alfalfa, infected by *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp.; and peanut, infected by *Cylindrocladium crotalariae* (7,13,23). These estimates have been reported in terms of infections per unit length of root. Unfortunately, the types of roots infected were not always defined clearly, and the significance of particular patterns of infection could not be assessed in terms of tissue susceptibility.

Generally root system development has been evaluated at single points in time by using a descriptive scheme based on chronological order of appearance of roots (3,21). Difficulties in interpretation of infection patterns may be related to such visualizations of root system structure.

In this study, infections of individual root systems of tobacco associated with a defined inoculum density of *P. p. nicotianae* were assessed in short-term experiments in a plant growth room. Patterns of infection were assessed in relation to various root tissues as defined in a quantitative root analysis system (10).

MATERIALS AND METHODS

Patterns of early root infections of susceptible and resistant tobacco plants by *P. p. nicotianae* were evaluated in field soil (Blichton sand) in the presence of indigenous microorganisms or in soil being recolonized by microorganisms after autoclaving. Soil was passed through a 1-mm-mesh sieve before use. Autoclaved soil was treated for 1 hr on each of two successive days and was kept covered until use.

Isolate P-230 of the pathogen was used in all infection experiments. Chlamydospores of this pathogen were produced axenically in liquid culture by the method of Tsao (24). Chlamydospore inoculum, free of viable mycelium, was prepared according to the method of Ramirez and Mitchell (20). Concentrations of chlamydospores in the resulting suspensions were determined from counts of propagules in 20 hemacytometer fields.

Suspensions of chlamydospores were added to both autoclaved and nonautoclaved field soils to establish inoculum densities of 50 chlamydospores per gram of soil. It had been determined previously that more than 90% of the tobacco plants became infected after 14 days of growth in either soil infested at this inoculum level. Infested soil was added to 100-ml polypropylene beakers according to the infested soil layer method of Kannwischer and Mitchell (15). Sixty-five grams of autoclaved or nonautoclaved soil infested with the pathogen was layered over approximately 15 g of autoclaved builder's sand. A final layer consisting of 35 g of either autoclaved or nonautoclaved, noninfested field soil was placed over the infested soil layer. This procedure was used to allow undamaged root growth of tobacco plants from the upper, noninfested layer of soil into the infested layer of soil below. A 2-wk-old seedling of the susceptible cultivar, Hicks, or the resistant cultivar, Speight G-28, grown previously in autoclaved vermiculite, was transplanted into the noninfested layer of soil. Fifteen seedlings of each tobacco cultivar were transplanted in this manner into beakers of both autoclaved and nonautoclaved soils. Control treatments consisted of six 2-wk-old seedlings transplanted singly into polypropylene beakers containing autoclaved or nonautoclaved soil not infested with the pathogen. Transplanted seedlings were placed in watering trays, covered with clear plastic, and grown in a plant growth room at 25 $\pm$ 2 C and under 16 hr of light (700 μE/m^2/sec) per day. Plants were watered from below on alternate days.

Infection of root systems was assessed after 2 wk of tobacco growth. Fifteen asymptomatic plants of each cultivar were removed gently from both autoclaved and nonautoclaved, infested soils. Each beaker containing a seedling was inverted and submerged in a container of water. With slight agitation of the container, each seedling floated to the surface. Tops of seedlings were removed and root systems were surface-disinfested in 70%

ethanol and rinsed in deionized water.

Each root system was dissected completely according to the classification scheme established in the morphometric root analysis system described by Fitter (10). In this scheme any root that terminates in an apical meristem is defined as a first-order root (Fig. 1). Where two first-order roots merge, there begins a second-order root. Where two second-order roots merge, there begins a third-order root and so forth. The union of a particular root element with that of a higher order does not alter the classification of the element of the latter root order.

Dissected roots were plated individually onto a selective medium (17). After incubation for 48 hr in the dark at 25 C, roots were examined for emergence of colonies of *P. p. nicotianae*. Experiments established to evaluate root infections were performed twice. Root system infection was evaluated in relation to cultivar and soil by planned F tests (16).

Growth of root systems of both tobacco cultivars also was evaluated after 2 wk of plant growth in infested soils. An additional six infected seedlings were removed from both autoclaved and nonautoclaved, infested soils. Six healthy seedlings were removed from each noninfested soil. Each root system was spread carefully on acetate film, and the numbers and lengths of root elements in selected root classes, as defined in the morphometric scheme, were recorded using the Micro-comp data acquisition system (Southern Micro Instruments, Inc., Atlanta, GA).

Evaluations of changes in total root system structure associated with infection also were made by estimating branching ratios as described by Fitter (10). This parameter describes the number of roots in each order relative to the number of roots in the next higher order. Branching ratio is a function of how root elements within a root system are joined together; it is derived from the linear relationship between the log of numbers of elements and root order number. The antilog of the absolute value of the slope of this line is defined as the branching ratio. Influences of treatments on tobacco root system development were evaluated by planned F tests.

The consistency of root system development of tobacco over repeated experiments was evaluated by comparing root systems of each cultivar in these experiments to predicted root system development as estimated from results of earlier experiments evaluating tobacco growth during a 15-day period (8). Within the earlier study, exponential curves describing time-related increases in numbers and total lengths of first- and second-order roots per plant were transformed using the natural log transformation. First-order linear equations derived by regression analyses described relationships between transformed values and time very well. By interpolation, estimates were made of the expected mean numbers and total lengths of first- and second-order roots of Hicks and Speight G-28 tobacco plants after 14 days of growth in autoclaved or nonautoclaved, noninfested soil. The 95% confidence intervals also were estimated for these expected values.

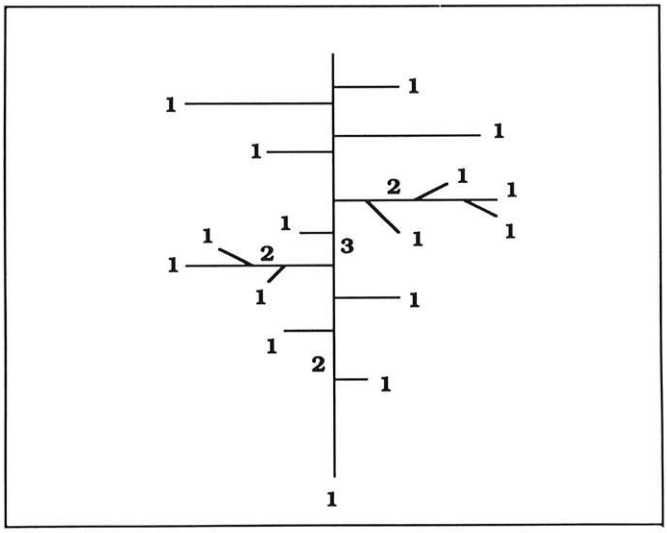

Fig. 1. Schematic representation of a tobacco root system as described by the classification scheme of the morphometric root analysis system; 1, 2, 3 = root orders.

RESULTS

After 14 days of growth in autoclaved or nonautoclaved soil infested with *P. p. nicotianae*, a minimum of 67% of tobacco plants of either cultivar were infected by the pathogen; in most infested soils more than 87% of the plant population was infected. At the end of the growth period, however, only one or two plants in any treatment combination had died from black shank. All dead plants exhibited symptoms typical of black shank, but they were not evaluated further in this study. Previously, however, direct microscopic observation of stained root systems of such plants had revealed that first-order roots within the vicinity of the root crown, as well as the lower stem tissues themselves, always had been colonized by the pathogen (English, *unpublished*). Plants that did not exhibit aboveground symptoms did not exhibit root necrosis.

For each cultivar, the number of infected roots per infected plant in autoclaved or nonautoclaved soil did not differ significantly ($P > 0.05$). No significant differences were observed between numbers of infected roots per infected Hicks or Speight G-28 tobacco plant in either autoclaved or nonautoclaved soil (Table 1). The average numbers of infected roots observed per infected plant ranged from 10.1 to 16.1. Typically, between 1 and 31 infected roots were observed within any single infected root system. More than 80% of all infections per infected plant of either tobacco cultivar occurred on first-order roots.

The efficiency of inoculum of a pathogen describes the proportion of propagules that infect roots when available (noninfected) root surface area is not limiting. Within the present study, the number of chlamydospores added to a defined amount of soil was controlled. The efficiency of inoculum for observed infections therefore was defined as the ratio of the total number of infected roots observed per tobacco root system to the total number of chlamydospores added to the volume of soil in which each plant was grown (approximately 3,250 chlamydospores). Average efficiencies of chlamydospores of this pathogen for observed infections of both tobacco cultivars in autoclaved and nonautoclaved soils were very low and varied between 0.002 and 0.005 (Table 1). Inoculum efficiency did not vary significantly between cultivars within either autoclaved or nonautoclaved soil. Similarly, efficiency for infection of either Hicks or Speight G-28 tobacco did not differ significantly between plants grown in autoclaved or nonautoclaved, infested soil. Similar inoculum

TABLE 1. Observed infections on roots of tobacco by *Phytophthora parasitica* var. *nicotianae* and inoculum efficiency after 14 days of plant growth in autoclaved or nonautoclaved field soil infested with 50 chlamydospores of the pathogen per gram of soil

		Infected roots/infected plant[a]					
		Root order				Total	Inoculum
Cultivar	Soil	1[b]	2	3	4	plant	efficiency[c]
Hicks	Autoclaved	8.1	1.6	0.4	0.0	10.1[d]	0.003[d]
	Nonautoclaved	10.1	2.2	0.5	0.0	12.8	0.004
Speight	Autoclaved	13.3	2.5	0.2	0.1	16.1	0.005
G-28	Nonautoclaved	8.5	2.1	0.3	0.0	10.9	0.003

[a] Numbers of infected roots per root order or individual plant were determined as the average of up to 15 asymptomatic, infected root systems that had been dissected completely by root order and plated onto selective medium (17).

[b] Root orders are as defined in the morphometric root analysis system (10).

[c] Efficiencies were determined from the average of the ratios of numbers of infected roots per plant to total number of chlamydospores within the volume of soil containing each plant (approximately 3,250 chlamydospores).

[d] No significant differences ($P > 0.05$) in total infected roots or inoculum efficiency were observed in relation to cultivar or soil.

efficiencies and patterns of root infection were observed when the experiment was repeated.

In these experiments, most of the root growth of tobacco occurred in the infested soil layer. During 14 days of growth in infested soils, the development of tobacco root systems was not altered consistently by infection with *P. p. nicotianae*. Estimates of root growth parameters were made only for first- and second-order roots as roots of higher orders formed very late in the course of experiments. Numbers of first- and second-order roots and total lengths of first-order roots of healthy plants were significantly greater ($P < 0.05$) than those of infected plants of both cultivars only in autoclaved soil (Figs. 2–5). However, when the experiment was repeated, these differences were not significant. In autoclaved or nonautoclaved soil, numbers and total lengths of roots of healthy and infected Hicks tobacco did not differ significantly from those of healthy and infected plants of Speight G-28 tobacco. Within either autoclaved or nonautoclaved soil, average lengths of root elements per first- or second-order roots of healthy plants did not differ significantly from those of infected plants of either cultivar (Table 2).

Tobacco root system branching was not altered by infection. No significant differences were observed in branching ratios of healthy and infected plants of either tobacco cultivar in either soil (Table 3). Similarly, within each soil no significant differences were observed between branching ratios of Hicks and Speight G-28 plants.

The average numbers and total lengths of first- and second-order roots per tobacco plant in the present root infection experiments generally fell within the range of expected values for various treatment combinations. Virtually all observed values of the root growth parameters for healthy or infected Speight G-28 tobacco plants (Figs. 2–5) in autoclaved or nonautoclaved soil fell within the ranges of expected values (Tables 4 and 5). Root system development of healthy or infected Hicks tobacco plants in autoclaved and nonautoclaved soils was less inconsistent. Very often, observed numbers and total lengths of first- and second-order roots per plant of this cultivar in both soils fell below the minimum values expected. Patterns of deviation of these values from expected ranges were not obvious, but they were not related to infection of plants by *P. p. nicotianae*.

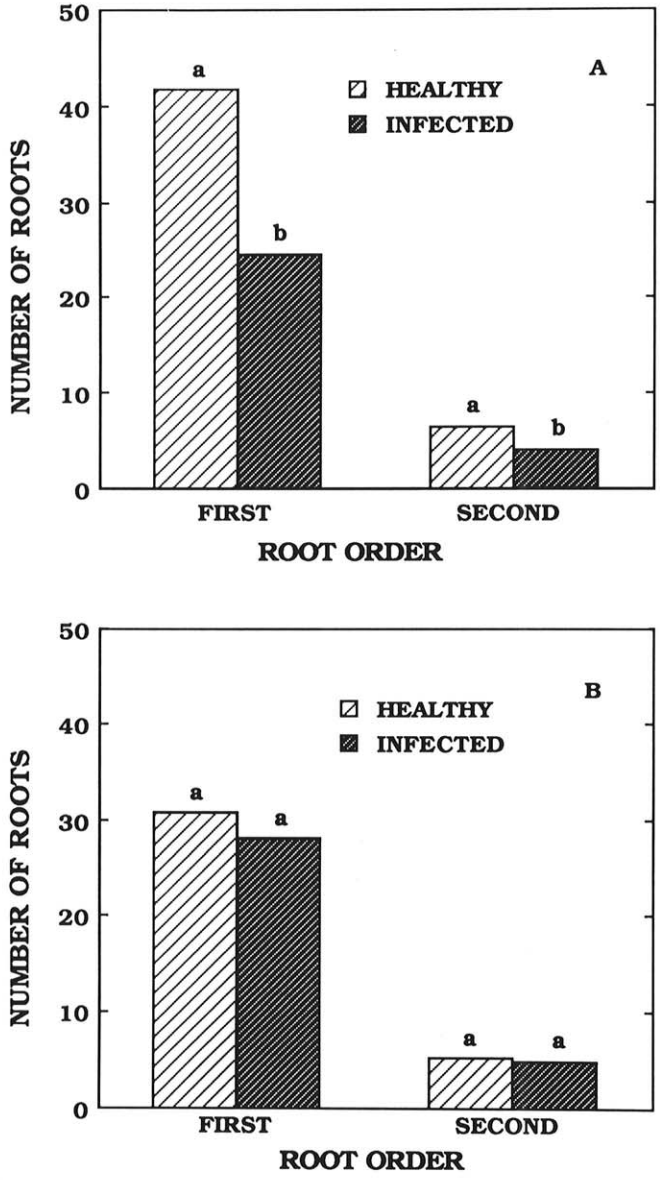

Fig. 2. Number of first- and second-order roots per healthy or infected Hicks tobacco plant after 14 days of growth in **A**, autoclaved or, **B**, nonautoclaved soil artificially infested with *Phytophthora parasitica* var. *nicotianae*. Within root orders, columns with different letters differ significantly ($P < 0.05$).

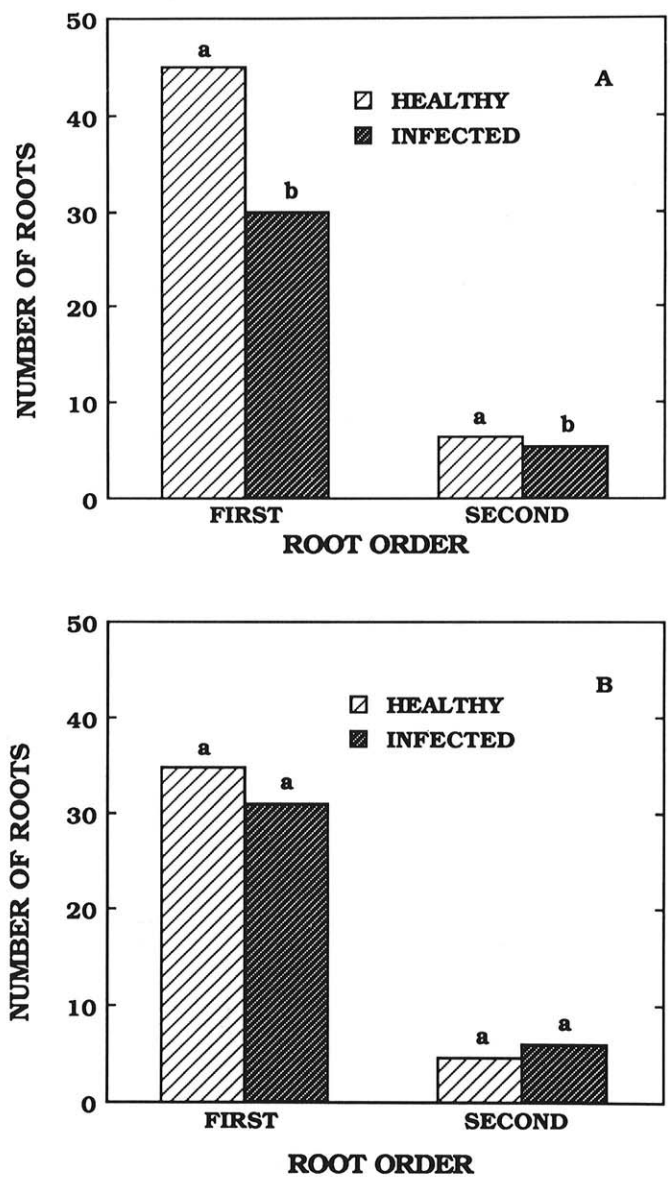

Fig. 3. Number of first- and second-order roots per healthy or infected Speight G-28 tobacco plant after 14 days of growth in **A**, autoclaved or, **B**, nonautoclaved soil artificially infested with *Phytophthora parasitica* var. *nicotianae*. Within root orders, columns with different letters differ significantly ($P < 0.05$).

DISCUSSION

Processes involved in the development of epidemics associated with soilborne pathogens generally have been evaluated through quantification of disease in terms of incidence on a whole plant basis. Disease incidence, as a measure of disease progression, represents the end result of numerous cycles of interactions between populations of a host plant and pathogen. Typically such incidence values have been transformed on the basis of mathematical models to provide biological interpretations of the processes of disease development (1,2,14,22,25). Unfortunately, models have been based on contentious assumptions and interpretations of processes involved in disease development have come under challenge. Investigations of early root infection of tobacco by *P. p. nicotianae* were established to measure more directly the relationship of inoculum and susceptibilities of root tissues to infection.

An imposed short period of growth of tobacco in soil infested with this pathogen allowed for quantification of early interactions between roots of these plants and *P. p. nicotianae*. Efforts to provide such detailed descriptions for infection of tobacco in field situations have been hampered by extremely low initial densities of

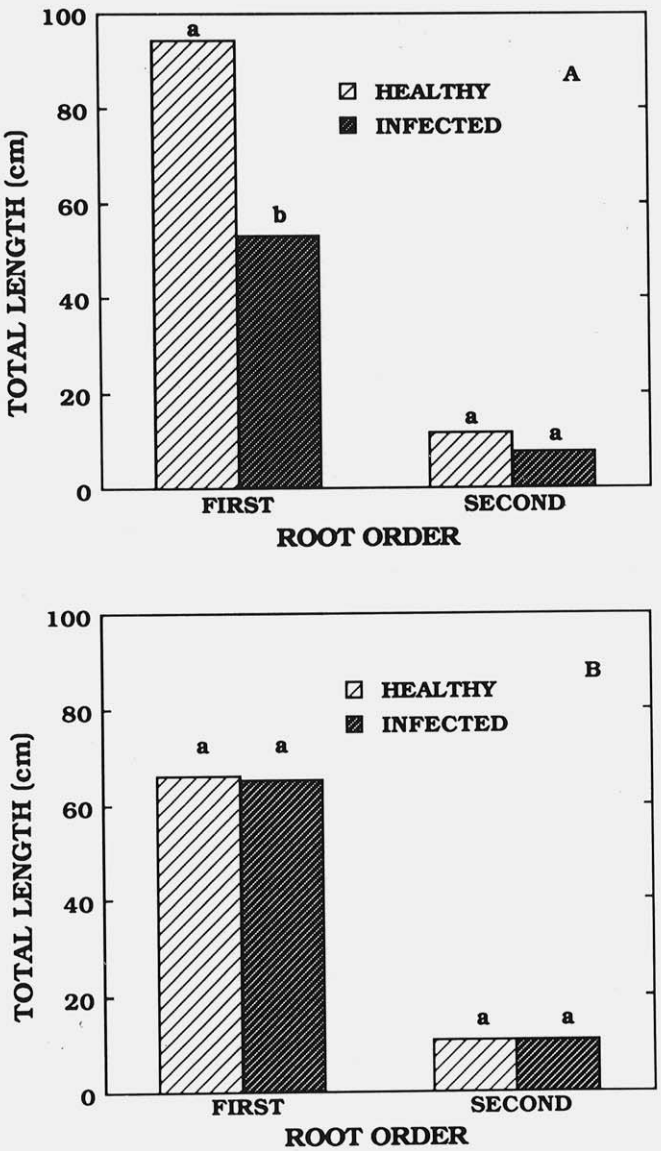

Fig. 4. Total lengths of first- and second-order roots per healthy or infected Hicks tobacco plant after 14 days of growth in **A,** autoclaved or, **B,** nonautoclaved soil artificially infested with *Phytophthora parasitica* var. *nicotianae*. Within root orders, columns with different letters differ significantly ($P < 0.05$).

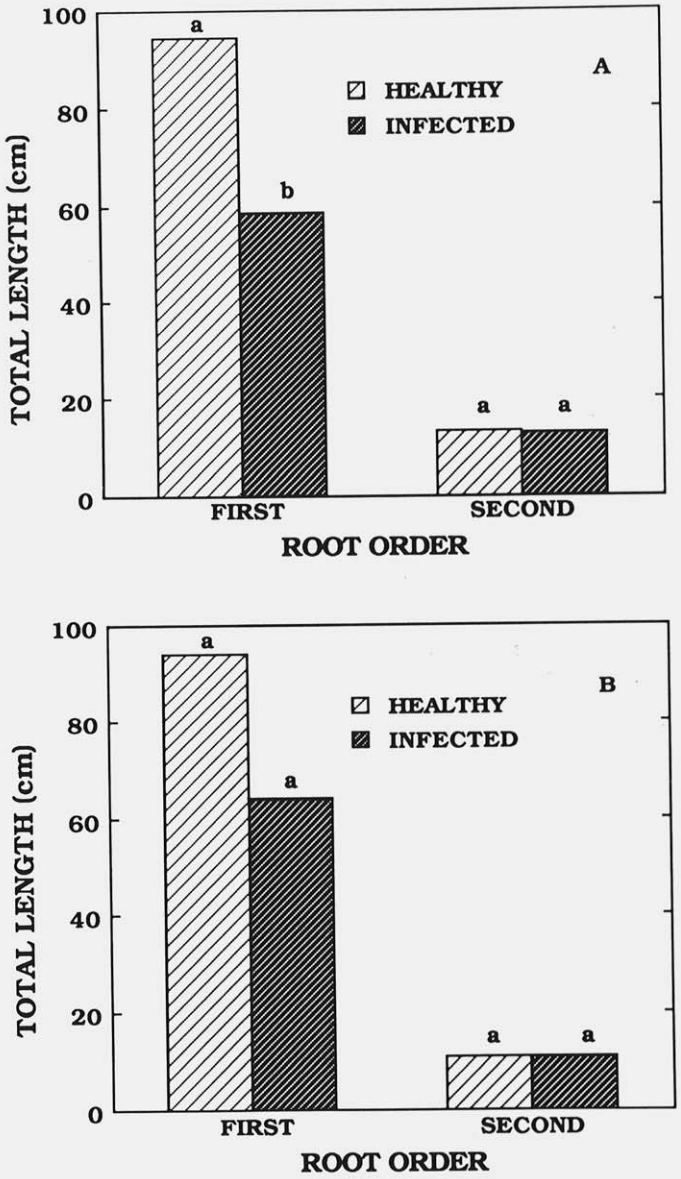

Fig. 5. Total lengths of first- and second-order roots per healthy or infected Speight G-28 tobacco plant after 14 days of growth in **A,** autoclaved or, **B,** nonautoclaved soil artificially infested with *Phytophthora parasitica* var. *nicotianae*. Within root orders, columns with different letters differ significantly ($P < 0.05$).

TABLE 2. The relationship between infection of tobacco by *Phytophthora parasitica* var. *nicotianae* and the average lengths of roots after 14 days of plant growth in autoclaved or nonautoclaved field soil artificially infested with propagules of the pathogen

Cultivar	Soil	Chlamydospores/ g soil	Mean root length (cm)/ root order 1[a]	2
Hicks	Autoclaved	0	2.3(0.1)[b,c]	1.7(0.1)[c]
		50	2.1(0.2)	1.9(0.2)
	Nonautoclaved	0	2.2(0.1)	2.7(0.5)
		50	2.4(0.2)	2.3(0.2)
Speight G-28	Autoclaved	0	2.1(0.1)	2.1(0.3)
		50	2.0(0.1)	2.3(0.2)
	Nonautoclaved	0	2.7(0.1)	2.4(0.4)
		50	2.1(0.2)	1.8(0.2)

[a] Root orders are as defined in the morphometric root analysis system (10).
[b] Values are the averages of mean lengths of elements per root order of six plants; within infested soil, averages are for infected root systems only. Values in parentheses are standard errors.
[c] Within autoclaved or nonautoclaved soil, no significant differences ($P > 0.05$) in mean root lengths were observed in relation to plant infection.

inoculum of this pathogen in soil. Development of extensive root systems is likely to occur before susceptible tissues contact propagules of the pathogen; evaluations of patterns of root infection under these conditions would be virtually impossible with present technology. An inoculum density much greater than that typically found as initial inoculum in the field was used in the present experiments; however, this density did not appear to overwhelm the system in the 2 wk of testing. The large proportions of asymptomatic plants and the low average numbers of observed infected roots per infected plant supports this contention. The variation in numbers of infections observed per infected plant supports as well the probabilistic nature of the infection process in situ. Such variations in numbers of infections would be expected at early stages of any epidemic.

The majority of observed infections occurred on recently formed first-order roots. A question arises as to how infections that were observed on roots of higher order developed. It may be that at early stages of epidemic development infections at these points occurred when tissues were younger and belonged to the first-order class. Root extension and branching subsequent to infection would have led to infected tissues now being within second and higher order root classes. This scenario is supported by results of previous experiments in which first-order roots were highly susceptible to infection with small numbers of zoospores; second-order roots were rarely infected (8). Growth of first-order roots generally continued after inoculation. In the present study, root necrosis was observed rarely. As an alternative scenario, *P. p. nicotianae* may have infected first-order root tissues and subsequently grown into second- and higher order root tissues. Further investigations are required to evaluate these possibilities.

TABLE 3. The relationship between infection of tobacco by *Phytophthora parasitica* var. *nicotianae* and branching ratios of root systems after 14 days of plant growth in autoclaved or nonautoclaved field soil artificially infested with propagules of the pathogen

Cultivar	Soil	Chlamydospores/ g soil	Branching ratio
Hicks	Autoclaved	0	5.32(0.55)[a,b]
		50	4.90(0.27)
	Nonautoclaved	0	5.53(0.24)
		50	4.37(0.47)
Speight G-28	Autoclaved	0	5.52(0.65)
		50	4.95(0.33)
	Nonautoclaved	0	4.37(0.47)
		50	5.53(0.26)

[a] Values are the averages of branching ratios of six plants; within infested soil, averages are for infected root systems only. Values in parentheses are standard errors.

[b] Within autoclaved or nonautoclaved soil, no significant differences ($P > 0.05$) in branching ratios were observed in relation to plant infection.

TABLE 4. Expected ranges of values of numbers of elements within root orders of tobacco after 14 days of growth in autoclaved or nonautoclaved field soil[a]

Cultivar	Soil	Root order[b]	Number of roots		
			min.[c]	mean	max.
Hicks	Autoclaved	1	34.1	43.3	55.1
		2	6.7	8.3	10.2
	Nonautoclaved	1	38.5	46.1	55.1
		2	5.3	6.3	7.5
Speight G-28	Autoclaved	1	27.9	40.0	87.4
		2	4.4	6.2	8.7
	Nonautoclaved	1	18.5	32.5	56.8
		2	2.4	4.9	9.7

[a] Expected ranges calculated from root growth data in English (8).

[b] Root orders are as defined in the morphometric root analysis system (10).

[c] Minimum and maximum values are the limits of the 95% confidence intervals about means; the mean values were derived from interpolations of the linear regressions of root length parameters on time. Root systems were measured every 3 days for 15 days.

The low values of inoculum efficiency in this study suggested a low probability of compatible interactions between susceptible root tissues and pathogen propagules in soil during only 2 wk of tobacco growth. Efficiency would be expected to increase with increasing time of plant growth in infested soil. The low values also may have been an artifact associated with estimations derived from the ratios of numbers of infected roots to numbers of pathogen propagules. It was not possible to determine if more than one infection had occurred per infected root. If such an occurrence was common, then true values of inoculum efficiency would have been greater than those observed.

Estimations of efficiency of inoculum of soilborne pathogens have been provided in only one other pathosystem. Tomimatsu and Griffin (23) reported the efficiency of microsclerotia of *Cylindrocladium crotalariae* for infection of peanuts at 103%. This value, however, was estimated on the basis of numbers of infections per germinated sclerotium placed within the region of the root surfaces of peanut plants. Only 0.27–0.28% of these observed infections resulted in necrosis of roots.

Differences in observed numbers of infected roots per infected tobacco plant of cultivars susceptible and resistant to *P. p. nicotianae* were not significant. The equivalence of early infection patterns may be unique to the conditions imposed in the present experiments. Comparisons of the behaviors of these cultivars under other environmental conditions would be appropriate. Alternatively, lack of differences in infections between the cultivars suggests that resistance may be expressed at stages of disease development beyond initial infection. Several authors have reported such a lack of differential response of susceptible and resistant plant cultivars to initial infections by *Phytophthora* spp. (4,5,11,12,18,19). Resistance instead was reported to be expressed through reductions in rates and extensiveness of progressive root tissue colonization by pathogens. In most of those studies, however, susceptibilities of cultivars to infection were compared by immersing root tips or root systems in concentrated suspensions of zoospores. Mechanisms of resistance to initial infection may have been overwhelmed by the high numbers of zoospores that encysted and infected within a limited region behind root tips. Within soil such large numbers of zoospores are not likely to be available for infection.

The relationship of tobacco root growth to infection by *P. p. nicotianae* was inconsistent. Lack of consistent, detectable differences in branching ratios of healthy and infected plants suggests that insufficient time had elapsed for plants to respond to early infection events.

To evaluate the contribution of host plant growth to the development of an epidemic, control of that component of a

TABLE 5. Expected ranges of values of total lengths of elements within root orders of tobacco after 14 days of growth in autoclaved and nonautoclaved field soil[a]

Cultivar	Soil	Root order[b]	Total length (cm)		
			min.[c]	mean	max.
Hicks	Autoclaved	1	79.8	103.5	134.5
		2	8.4	13.3	21.1
		All orders[d]	89.1	112.2	141.2
	Nonautoclaved	1	72.2	96.5	129.0
		2	8.1	14.0	24.3
		All orders	75.9	108.9	156.3
Speight G-28	Autoclaved	1	48.9	74.4	113.3
		2	4.2	13.1	40.4
		All orders	57.4	83.1	120.3
	Nonautoclaved	1	39.6	77.5	151.4
		2	7.5	14.3	24.5
		All orders	47.5	91.8	177.7

[a] Expected ranges calculated from root growth data in English (8).

[b] Root orders are as defined in the morphometric root analysis system (10).

[c] Minimum and maximum values are the limits of the 95% confidence intervals about means; the mean values were derived from interpolations of the linear regressions of root growth parameters on time. Root systems were measured every 3 days for 15 days.

[d] Includes all root orders per plant.

pathosystem must be achieved consistently. Growth of Speight G-28 tobacco was controlled well enough that numbers and lengths of roots fell within expected ranges during the 14 days of plant growth in infested soils. Root growth of Hicks tobacco was controlled less effectively. The degree of control attained, however, was encouraging when considering the variations that could be expected from using transplants. Variations in patterns of root growth might be less in trials in which plants were begun from seed that had been screened for uniformity. Increased control of plant growth is certainly desirable in evaluations of the contributions of the host root component to pathosystem behavior.

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