Population Density of Phytophthora parasitica var. nicotianae in Relation to Pathogenesis and Season

R. A. Flowers and James W. Hendrix

Department of Plant Pathology, University of Kentucky, Lexington 40506. Senior author now Assistant Plant Pathologist, University of Georgia, College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, Georgia 31794.

Kentucky Agricultural Experiment Station Journal Series Paper No. 71-11-129.

Appreciation is expressed to J. W. Gulley for assistance.

Accepted for publication 22 November 1971.

ABSTRACT

Numbers and location of soil-borne propagules of *Phytophthora parasitica* var. *nicotianae* are determined largely by pathogenesis. Population densities were almost undetectable at the time tobacco plants were transplanted into the field, but increased rapidly in the rhizospheres of plants of susceptible cultivars, and reached maxima at the time plants were killed. Plants of a moderately resistant cultivar were not killed by the pathogen, and populations of the fungus rose slowly and reached maxima near the end of the growing season. Populations failed to rise to a detectable level in the rhizosphere of plants of the cultivar M S Burley 21 X L8 in a field infested with race 0, to which this cultivar is highly resistant; but populations rose sharply in the rhizospheres of plants of this cultivar in a field infested with race 1, to which this cultivar is susceptible. Propagule populations were high directly beneath infected plants, but low 6 and 18 inches away. They were high in the upper 3 inches of soil, low in the next 3 inches, and not detected 6 to 12 inches deep. The pathogen readily overwintered in the upper 3 inches of soil. Population levels of total *Pythium* species were not affected by these factors.

Phytopathology 62:474-477.

The black shank disease has long been the major threat to flue-cured and burley tobacco production in the United States. The disease is controlled primarily by use of cultivars having moderate resistance derived

from the cultivar Florida 301. Following the development of resistance from *Nicotiana longiflora* and *N. plumbaginifolia*, a new race (race 1) of the causal fungus which is highly pathogenic to tobacco with this resistance was discovered (1, 12). The rapid development of race 1 in fields heavily infested with race 0 (3, 7) has limited the usefulness of cultivars with this resistance (3, 11).

The pathogen, *Phytophthora parasitica* Dastur var. *nicotianae* (B. de Haan) Tucker, becomes well established in some fields soon after initial infestation, and may persist for several years in the absence of tobacco (2, 4, 5, 8, 9, 10); however, interpretation of these studies is prevented by lack of information on the relationships of the fungus to soil beyond rough estimates of disease potential of soils. This report on distribution of populations of *Phytophthora parasitica* var. *nicotianae* in relation to pathogenesis and seasons provides information relevant to this problem.

MATERIALS AND METHODS.–Two fields in continuous tobacco cultivation were selected. The field in Scott County, Kentucky, was naturally infested with race 0, determined as before (6, 7). The field in Smith County, Tennessee, was naturally infested with both race 0 and race 1.

At each location, soil samples were collected from the rhizospheres of plants of the following cultivars of tobacco: Burley 21, susceptible to both races; Burley 37, moderately resistant to both races; and M S Burley 21 X L8, highly resistant to race 0, but susceptible to race 1. Five subsamples were taken from the top 3 inches of soil and were combined to make a composite sample of at least 10 g for each plant. Three plants of each cultivar were sampled. Samples were taken immediately after transplanting (early June), at 2-week intervals during the growing season, and at 4-week intervals during the following winter and spring months.

Additional soil samples were collected during February, March, and April of 1969 in the Smith County field for studying vertical distribution of the fungus during and following winter. Plants were selected at random each month in areas where the soil had not been disturbed by previous sampling or by cultural procedures following the 1968 crop.

Quantitative estimates of propagule population densities of the black shank fungus and *Pythium* spp. were made by our plating procedure (6). The selective medium was prepared 1 day prior to collecting soil samples for these studies. Six plates/sample were seeded with 1 ml of 1:25 soil suspension (5 g soil/125 ml 0.5% agar) and incubated 36 hr at 24 C in the dark. Standard errors were computed for each mean for three plants.

RESULTS.–*Seasonal changes in population densities.*–In the field infested with race 0 only (Scott County), populations of *Phytophthora parasitica* var. *nicotianae* were low the first 6 weeks after transplanting. The populations increased sharply during August in the rhizospheres of Burley 21 plants and about 1 month later in the rhizospheres of Burley 37 plants (Fig. 1-A). Very few propagules were detected at 6 and 18 inches from the plants. The fungus was not detected in samples taken from the rhizospheres of M S Burley 21 X L8 plants. Populations were maximum when Burley 21 plants developed severe black shank symptoms in mid-August. Neither Burley 37 nor M S Burley 21 X L8 plants wilted or developed stem lesions during the growing season.

In the field infested with both races (Smith County), populations were also extremely low at transplanting time, but increased immediately in the rhizospheres of Burley 21 plants, which were killed within 3 or 4 weeks (Fig. 1-B). The fungus was not detected 6 or 18 inches from stems. No aboveground symptoms of black shank occurred on the Burley 37 plants, but populations increased steadily during the growing season (Fig. 1-B). Low populations were detected at 6 and 18 inches from stems. M S Burley 21 X L8 plants had severe black shank symptoms, and died 6 to 8 weeks after transplanting. Populations at a distance of 6 inches from the M S Burley 21 X L8 plants also increased during the growing season to a maximum of about 50 propagules/g dry soil, which was about 5% of the population density directly beneath stems. Populations 18 inches from the stems were very low.

In both fields, populations remained high well into and throughout the winter months.

Vertical distribution and overwintering. – Populations of *P. parasitica* var. *nicotianae* were highest in the top 3 inches of soil beneath plants of M S Burley 21 X L8 which appeared to have been killed by black shank the previous season (Table 1). Few propagules were present below 3 inches, and none was detected below 6 inches. While the fungus was isolated from soil from the rhizosphere of all plants selected, population densities were extremely variable.

In contrast, populations of *Pythium* species were relatively consistent at all depths tested, and were not restricted to the top 6 inches of soil (Table 1).

DISCUSSION.–This study suggests that propagule population levels of *Phytophthora parasitica* var. *nicotianae* are determined by the amount and rate of pathogenesis. If pathogenesis was not evident (M S Burley 21 X L8 in the field infested only with race 0), increases in propagules did not occur. When pathogenesis occurred, population densities increased until plants were killed. These maxima were reached early in the season when plants were killed by the pathogen, or late when plants were killed by frost.

The rate of pathogenesis apparently influenced the total numbers of propagules produced. Propagules were produced rapidly and in large numbers directly beneath the rapidly killed Burley 21 plants, but propagules were seldom detected 6 or 18 inches away. However, Burley 37 and M S Burley 21 X L8 were not killed so rapidly, and new roots were probably produced after the initial infections occurred. These roots provided new substrates for the fungus, and the total number of propagules produced was probably much higher on resistant plants than on highly susceptible ones when pathogenesis was

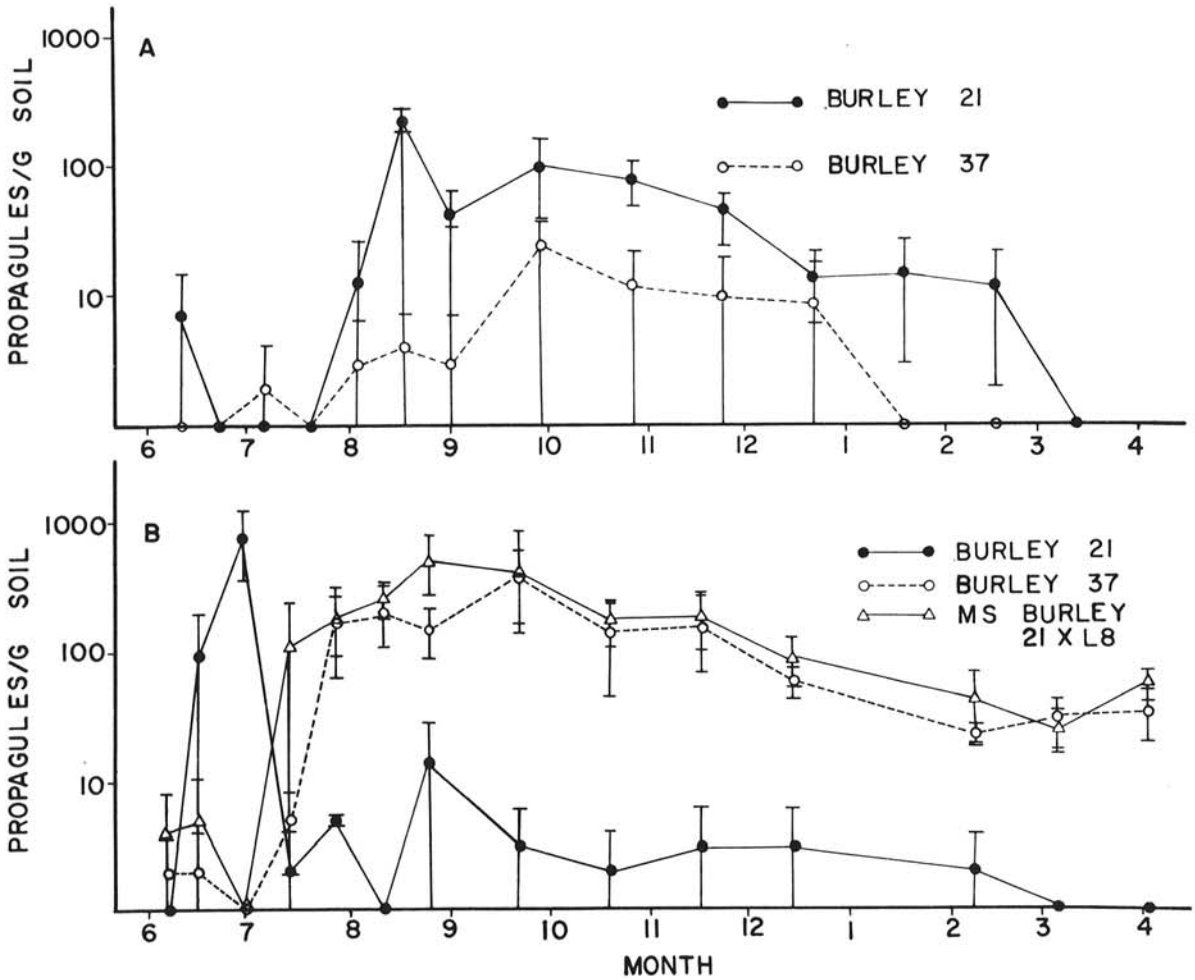

Fig. 1. Populations of *Phytophthora parasitica* var. *nicotianae* in the rhizospheres of tobacco plants grown in infested fields. **A)** Field infested with race 0 only (Scott County, Kentucky). **B)** Field infested with both race 0 and race 1 (Smith County, Tenn.). Brackets enclose standard errors of the means for three plants.

TABLE 1. Depth of occurrence and propagule numbers of *Phytophthora parasitica* var. *nicotianae* and *Pythium* spp. beneath black shank-affected M S Burley 21 X L8 plants (Smith County field)

Sampling depth (inches)	Collection time					
	February		March		April	
	Phytophthora	*Pythium*	*Phytophthora*	*Pythium*	*Phytophthora*	*Pythium*
	Propagules/g dry soil [a]					
0-3	94±89	402±52	55±26	502±22	923±399	521±117
3-6	23±13	451±58	0	452±29	38±36	505±70
6-9	0	409±66	0	452±109	0	476±31
9-12	0	355±42	0	372±17	0	396±71

[a]Means for three plants, with standard errors.

extensive, as it was in the Smith County field (Fig. 1-B). Burley 21 plants were not killed so rapidly in the Scott County field, and higher populations were reached with these plants than with plants of Burley 37 (Fig. 1-A).

The distribution of propagules in soils also suggests that propagule density and total number are determined by the amount and location of roots parasitized. Populations were high in the upper 3 inches of soil, where roots were abundant; and they

were progressively lower with depth, where roots were less abundant. Populations were high directly beneath diseased plants, but were lower 6 or 18 inches away. Apparently the fungus is relatively immobile in soil, and is distributed throughout soil by plowing. High populations were found beneath diseased plants as late as April, but the fungus was seldom isolated after the land was plowed for a new crop.

In contrast, population levels of total *Pythium* species were not obviously affected by seasons, vertical or horizontal distance from the bases of plants, or cultivars.

It is clear that *Phytophthora parasitica* var. *nicotianae* readily overwinters in high populations in the upper portions of soils. It is not certain from these studies whether or not populations decline during the growing season or fall and winter. Since we sampled soil from the same plants (Fig. 1), the apparent decline seen in this study could be due to depletion of rhizosphere soil by sampling, especially in the case of susceptible cultivars where killing of plants limited the amount of rhizosphere soil.

This study provides new information, but not reasons, for the frequent observation that black shank becomes more severe in some fields than in others. Disease development was much slower in the Scott County field than in the Smith County field, and population densities were considerably lower in the former (Fig. 1-A) than in the latter (Fig. 1-B). Differences in the rates of disease development could be due to differences in population densities at the beginning of the season, but we could not test this hypothesis because plowing apparently dilutes the propagules below the level of reliable measurement by our method. However, our data (Fig. 1) suggest that populations at the beginning of the season should be considerably lower in the Scott County field than in the Smith County field.

It is also not apparent why M S Burley 21 X L8 plants, considered highly susceptible to race 1 (11, 12), were not killed as rapidly as were Burley 21 plants in the Smith County field. The reason may be that the initial race 0 population was much higher than the race 1 population, or that pathogenesis by both races results in more rapid death of Burley 21 plants than M S Burley 21 X L8, which is affected only by race 1. It is also possible that the race 0 strains in the Smith County field are more virulent than either the race 1 strains in that field or the race 0 strains in the Scott County field.

LITERATURE CITED

1. APPLE, J. L. 1962. Physiological specialization within Phytophthora parasitica var. nicotianae. Phytopathology 52:351-354.
2. APPLE, J. L. 1963. Persistance of Phytophthora parasitica var. nicotianae in soil. Plant Dis. Reptr. 47:632-634.
3. APPLE, J. L. 1967. Occurrence of race 1 of Phytophthora parasitica var. nicotianae in North Carolina and its implications in breeding for disease resistance. Tobacco Sci. 11:79-83.
4. CLAYTON, E. E., J. G. GAINES, T. E. SMITH, K. J. SHAW, & T. W. GRAHAM. 1944. Control of flue-cured tobacco root diseases by crop rotation. U.S. Dep. Agr. Farmers' Bull. 1952. 12 p.
5. DUKES, P. D., & J. L. APPLE. 1968. Inoculum potential of Phytophthora parasitica var. nicotianae as related to factors of the soil. Tobacco Sci. 12:200-207.
6. FLOWERS, R. A., & J. W. HENDRIX. 1969. Gallic acid in a procedure for isolation of Phytophthora parasitica var. nicotianae and Pythium spp. from soil. Phytopathology 59:725-731.
7. HENDRIX, J. W. & R. A. FLOWERS. 1968. Development of races of Phytophthora parasitica var. nicotianae in mid-Tennessee in 1966. Tobacco Sci. 12:134-135.
8. JOHNSON, E. M., & W. D. VALLEAU. 1954. Disappearance of black shank from Kentucky soils. Kentucky Agr. Exp. Sta. Bull. 615. 11 p.
9. LUCAS, G. B. 1965. Diseases of tobacco [2nd ed.]. The Scarecrow Press, Inc. p. 190-191.
10. MATTHEWS, E. M., W. KROONTJE, & R. G. HENDERSON. 1960. Effect of length of rotation on losses from black shank in flue-cured tobacco varieties. Tobacco Sci. 4:156-158.
11. STOKES, G. W., & C. C. LITTON. 1966. Source of black shank resistance in tobacco and host reaction to race 0 and race 1 of Phytophthora parasitica var. nicotianae. Phytopathology 56:678-680.
12. VALLEAU, W. D., G. W. STOKES, & E. M. JOHNSON. 1960. Nine years experience with Nicotiana longiflora factor for resistance to Phytophthora parasitica var. nicotianae in the control of black shank. Tobacco Sci. 4:92-94.