Effects of Soil Matric Potential on Infection of Tobacco by *Phytophthora parasitica* var. *nicotianae*

H. D. Shew

Assistant professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650.
Journal Series Paper 8593 of the North Carolina Agricultural Research Service, Raleigh.
The author gratefully acknowledges the technical assistance of D. T. Glover in this study.
This research was supported in part by a grant from the North Carolina Tobacco Foundation, Inc.
Accepted for publication 16 March 1983.

ABSTRACT

Shew, H. D. 1983. Effects of soil matric potential on infection of tobacco by *Phytophthora parasitica* var. *nicotianae*. Phytopathology 73:1160-1163.

Soil naturally infested with *Phytophthora parasitica* var. *nicotianae* was placed on Büchner funnel tension plates to control soil matric potential. A single 1-mo-old cultivar Hicks tobacco seedling was transplanted into each funnel and incubated for 21 days. With an initial inoculum density of 5,000 propagules per kilogram of soil, 60, 27, and 7% of the plants were infected at constant matric potentials of −10, −20, and −50 millibars (mb), respectively. When plants were exposed to a single 24-hr saturation period after 1 wk of incubation at −30 or −50 mb, infection was significantly increased in comparison to plants not exposed to the saturation period. Initial inoculum densities of 40, 80, 170, 300, 700, 1,300, 2,700, and 5,300 propagules per kilogram of soil resulted in 73, 47, 80, 73, 80, 80, 100, and 93% infection, respectively, when plants were exposed to a 24-hr saturation period. Saturation periods as short as 0.5 hr overcame the inhibitory effect on infection of soil matric potentials of −30 to −50 mb. The mechanism of infection enhancement by soil saturation was apparently stimulation of zoospore release and dispersal.

The soilborne fungus *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker is an important root disease pathogen of tobacco (*Nicotiana tabacum* L.) in the southeastern United States (8). Inoculum level and soil moisture greatly influence the development of diseases caused by *Phytophthora*. For example, Kannwischer and Mitchell (5) recently demonstrated a direct effect of initial inoculum density (chlamydospores and zoospores) of *P. parasitica* var. *nicotianae* on infection of the highly susceptible tobacco cultivar Hicks. In addition, the effect of initial inoculum density of some other *Phytophthora* spp. on subsequent disease incidence and severity of their respective hosts was described by Mitchell (11,12). The importance of soil water potential on various aspects of the life cycle and in development of diseases caused by soilborne *Phytophthora* spp. was clearly demonstrated by Duniway (3,4,9). For instance, the specific requirements of *Phytophthora* for high soil water matric potentials to form sporangia (3,4) and to release zoospores (9) has been described. Also, the importance of high soil matric potentials for the diffusion of exogenous nutrients to the surface of germinating propagules such as the chlamydospore was described by Sterne et al (13). Soil flooding has been shown to enhance disease development through predisposing the host to infection by *Phytophthora* spp. (1,7).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

The effect of soil water potential on inoculum density-infection relationships with *Phytophthora* spp., however, is not clear. The objective of this study was to determine the effects of constant and varying soil matric potentials on infection of plants of a susceptible tobacco cultivar in soil infested with several levels of propagules of *P. parasitica* var. *nicotianae*.

MATERIALS AND METHODS

The source of inoculum used in all tests was a sandy loam field soil naturally infested with *P. parasitica* var. *nicotianae*. The soil was collected from the top 15 cm in a field with a history of tobacco black shank disease. To ensure a constant and reliable source of infested soil, inoculum was subsequently produced in the greenhouse by mixing two volumes of the infested soil with one volume of coarse sand (0.5–2.0 mm in diameter). The mixture was placed in flats (50 × 35 × 6 cm) into which 1-mo-old Hicks tobacco seedlings were transplanted and watered. Following mortality of the seedlings, either additional seedlings were transplanted into the flats or the soil was collected for use in experiments. Prior to use, the soil from flats was allowed to dry to approximately 5% moisture by weight, sieved through a 2-mm sieve to remove larger root fragments, and then assayed to determine inoculum density of *P. parasitica* var. *nicotianae*. Inoculum density was determined by suspending each of five 5-g soil samples in 30 ml of deionized water and then dispensing each sample onto five plates of the Kannwischer and Mitchell selective medium (5), modified by substituting V-8 juice agar (75 ml of V-8 juice and 20 g of agar per

liter) as the basal medium. Variation in inoculum density was obtained by diluting the infested soil with noninfested soil and coarse sand to maintain a soil:sand ratio of 2:1 (v/v). All soil dilutions were assayed with the selective medium to determine the inoculum density of the pathogen propagules in each treatment. The noninfested soil (collected from a noninfested portion of the same field as the infested soil) was steamed for 30 min at 80 C and air-dried prior to use. Assays with the selective medium showed that steamed noninfested soil contained no viable propagules of the pathogen.

Soil matric potential was controlled by using Büchner funnels as tension plates (3,4). The reference points for matric potential were the tops of both the glass plate and water reservoir. After the water columns were attached to the funnels, a 1.5-cm layer of infested soil was placed in each funnel and leveled. A 1.5-cm layer of the steamed noninfested soil was then placed over the infested soil and leveled. The soil was packed to give a bulk density of approximately 1.3 g/cm^3 of soil. The test soils were brought to the initial matric potentials either by wetting from the bottom or by slowly adding water to the soil surface. Funnels were supported on plywood racks (104 × 41 cm). Transpiration and evaporation were reduced by covering each rack with a plexiglass cover. Humidity was kept high by moistening paper towels placed on the racks.

A single 1-mo-old Hicks tobacco seedling was transplanted into the upper 0.5 cm of the noninfested soil, which allowed uninjured root tips to grow into the infested soil (5,10). Unless otherwise stated, plants were maintained in the funnels for 19–21 days removed, washed thoroughly in running tap water, dipped in 70% alcohol, blotted dry, and placed on the selective medium. Plates were incubated in the dark at room temperature and observed for growth of *P. parasitica* var. *nicotianae* from the root system. Final disease incidence was determined by including all plants that became infected. Disease severity was rated on a 1 to 5 scale with 1 = healthy, 2 = <5% root rot, 3 = 6–50% root rot, 4 = >50% root rot and/or crown rot, and 5 = dead plant.

All experiments were conducted in controlled environment chambers (2) with 14-hr photoperiods and day:night temperatures of 28:24 C. Light intensity was either 13,000 or 23,000 lux. Plants were fertilized once a week with 8 ml/funnel of a complete nutrient solution (2) or by mixing approximately 0.5 g of 14-14-14 slow-release fertilizer into the soil before use. All experiments were done in a randomized complete block design and replicated over time. Treatments consisted of three to six observations and were replicated three to five times.

Since infection was expressed on a percent basis, data were arc sine transformed to $\sqrt{(\text{percent})}$. Transformed data were analyzed by analysis of variance. Some treatment effects were tested for significance by single-degree-of-freedom contrasts and regression analysis was used to test for linear effects.

Variations in matric potential over time. After 1 wk of growth, which allowed time for roots of the seedlings to enter the infested soil layer, matric potential was adjusted in some treatments to either higher or lower potentials for the final 2 wk of the experiments. In addition, in some treatments initially at −30 or −50 mb, the matric potential was adjusted to 0 mb for various periods of time and then returned to −50 mb for the final 2 wk of the experiments.

To determine the effect of the various saturation periods on zoospore production, soil matric potential was adjusted to −30 mb with or without tobacco seedlings present. After 1 wk of incubation, the soil in the funnels was flooded (water level brought to approximately 3 mm above the soil surface) and sampled after 0.5–25 hr for the presence of zoospores by removing 2-ml aliquot samples of the surface water and placing them on the selective medium. The number of colonies of *P. parasitica* var. *nicotianae* on the plates was counted after 48 hr and, whenever possible, each colony was traced back to its origin to determine whether it was a zoospore.

The length of time soil remained wetter than −25 mb after termination of the saturation period was determined by periodically collecting soil samples and determining their water content. Water content was then converted to matric potential by using the drying curve of a soil moisture characteristic (Fig. 1).

RESULTS

Infection of cultivar Hicks tobacco seedlings by *P. parasticia* var. *nicotianae* decreased as matric potential decreased (Table 1). At constant matric potentials of −10, −20, and −50 mb, percentage of plants infected were 60, 27, and 7, respectively. Regression analysis of these data indicated a highly significant linear effect of constant matric potentials on infection.

When seedlings were exposed to a 24-hr saturation period after 1 wk at −50 mb and then returned to −50 mb for 2 wk, 100% infection occurred. A single-degree-of-freedom contrast was used to compare the saturation treatment to the other water potential treatments. The saturation treatment increased infection ($P = 0.01$) compared to all other treatments.

The 24-hr saturation period increased ($P = 0.01$) infection over the unsaturated treatment at initial inoculum densities between 40

TABLE 1. The effect of constant and varying soil matric potential on black shank incidence and severity on cultivar Hicks tobacco[u]

Matric potential (−mb)	Infection (%)[v]	Severity[w]
10[x]	60	3.3
20	27	1.9
50	7	1.4
10-50[y]	33	2.2
20-50	27	1.9
50-10	75	3.1
50-S-50[z]	100	5.0

[u] Average inoculum density of 4,000 propagules per kilogram of soil.
[v] Average of three replications, six observations per replication.
[w] Severity scale of 1–5 with 1 = healthy and 5 = dead plant.
[x] Matric potential for entire 3-wk period of the experiment.
[y] First number represents matric potential for first week and second number represents matric potential for final 2 wk of the experiment.
[z] Plants grown at −50 mb for the duration of the experiment except for a 24-hr saturation period (S) after 1 wk.

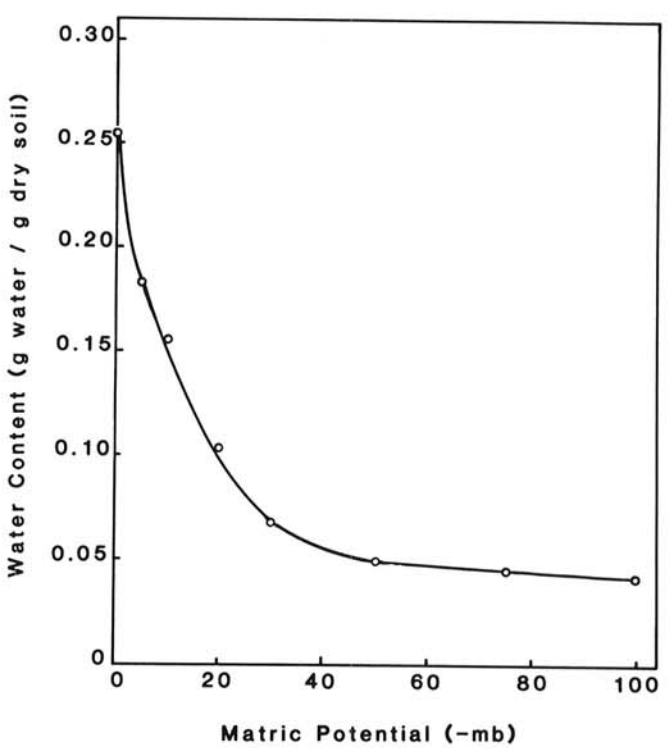

Fig. 1. Drying curve of the soil moisture characteristic for the sandy loam soil.

and 5,300 propagules per kilogram of soil (Table 2). Percent infection was 47–100 in the saturated treatment and 0–40 in the unsaturated treatment. No interaction occurred (as determined by analysis of variance) between initial inoculum density and the saturation treatment, which indicated a similar response to soil saturation, regardless of initial inoculum level.

All soil saturation periods tested (0.5–24 hr) increased percent infection of cultivar Hicks tobacco seedlings over the unsaturated treatment (Table 3). In fact, no infection was detected in the seedlings maintained at −30 mb for the 3-wk duration of the tests. In most cases, plants in the saturation treatments died prior to termination of the tests, resulting in the high disease severity ratings. Zoospores were detected in the water above the infested soil as soon as 0.5 hr following flooding of the soil (Table 4).

TABLE 2. The influence of a single 24-hr soil saturation period on infection of cultivar Hicks tobacco plants at different initial inoculum densities of *P. parasitica* var. *nicotianae*

Inoculum density[x] (propagules per kg of soil)	Infection (%)[y,z]	
	Not saturated	Saturated
5,300	20	93
2,700	40	100
1,300	7	80
700	13	80
300	20	73
170	13	80
80	7	47
40	0	73

[x] Average inoculum density in five replications.
[y] Average of five replications with three seedlings per replication.
[z] The saturation period increased percent infection significantly at all initial inoculum densities ($P = 0.01$).

TABLE 3. The effect of length of period of soil saturation on black shank incidence and severity on cultivar Hicks tobacco[w]

Saturation time (hr)	Infection (%)[x]	Severity[y]
0.0[z]	0	1.0
0.5	100	4.6
1.0	92	4.4
2.0	92	4.2
4.0	100	4.8
8.0	100	4.8
16.0	100	4.8
24.0	100	4.3

[w] Average inoculum density of 5,200 propagules per kilogram of soil.
[x] Average of four replications, four observations per replication.
[y] Scale of 1–5 with 1 = healthy and 5 = dead.
[z] Plants maintained at −30 mb for the 3 wk duration of the test, except during single saturation period of length indicated.

TABLE 4. Recovery of zoospores of *P. parasitica* var. *nicotianae* following flooding of a naturally infested soil for different periods of time

Flooding time (hr)	Number of zoospores[z]	
	With plants	Without plants
0.5	11	21
1.0	7	46
1.5	14	8
2.0	36	71
3.0	9	15
4.0	29	14
5.0	16	19
6.0	34	53
13.5	6	7
25.0	3	3

[z] Number of zoospores recovered from eight funnels in each treatment by placing a 2-ml aliquot sample from each funnel on a plate of the selective medium at each time period.

Numbers of zoospores recovered were generally, but not significantly, greater in the treatment without plants than in those with plants. Saturation periods were terminated by lowering the reservoirs below tension plates. The soil drained to −15 mb in 15 min and to −20 mb within 1 hr when the reservoirs were lowered to give a final matric potential of −30 mb (Fig. 2).

DISCUSSION

Soil matric potential had a highly significant effect on infection of seedlings of the susceptible tobacco cultivar Hicks in soil naturally infested with *P. parasticia* var. *nicotianae* (Table 1). Matric potentials conducive to infection of tobacco were generally higher than −30 mb and are much higher (less negative) than the matric potentials found by Sterne et al (13) to limit infection of avocado (*Persea indica* L.) seedlings by *P. cinnamomi*. Sterne et al (13) reported 50–100% infection at −100 mb, but found very little infection at −250 mb. They attributed this reduction in infection at −250 mb to a lack of nutrient availability to directly germinating propagules of *P. cinnamomi* and not to a direct effect of matric potential on the pathogen. The failure of *P. parasitica* var. *nicotianae* to cause infection at matric potentials lower than those that favor zoospore production (9) indicates that under the conditions of the present study direct germination and infection of tobacco by propagules of *P. parasitica* var. *nicotianae* other than the zoospore was not an important mode of infection. The primary infective propagule of *P. parasticia* var. *nicotianae* in nature is not known, but the disease is most severe in wet areas (8), which are conducive to zoospore production and dispersal. The importance of matric potential effects on direct germination and infection was further supported by the fact that a single soil saturation period that induced zoospore release (Table 4) also induced a great increase in infection over a wide range of initial inoculum densities (Tables 2 and 3). In fact, after saturation, high levels of infection were observed at all initial inoculum densities ranging from approximately 40 to 5,300 propagules per kilogram of soil.

Kannwischer and Mitchell (5) found a significant linear effect of increasing initial inoculum density on infection of tobacco by *P. parasitica* var. *nicotianae*. They reported ID_{50} values of 132 chlamydospores per kilogram of soil (eight to nine chlamydospores per plant) and 42 zoospores per plant. Plants were exposed to similar initial inoculum densities in the present study; however, the

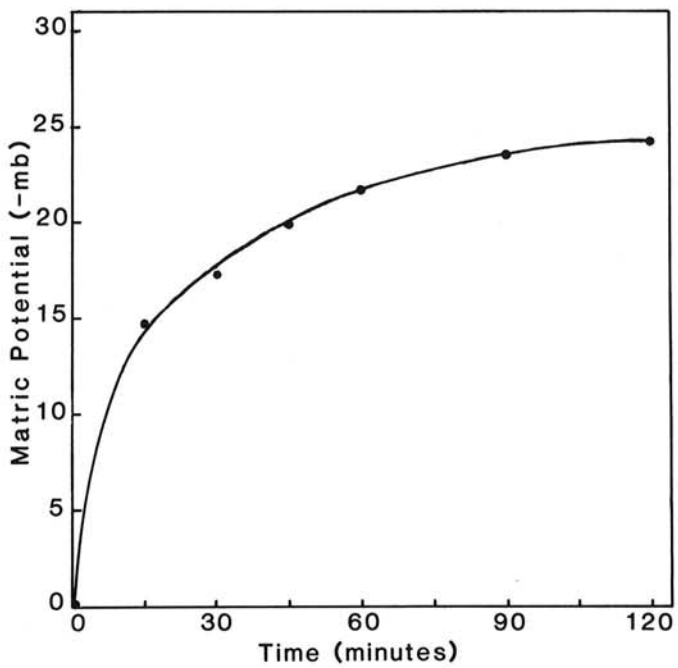

Fig. 2. Relationship between matric potential of the soil and time after returning a saturated soil to −30 mb water potential.

final percentages of seedling infection were similar regardless of initial inoculum density whenever the soil was saturated (Table 2). At the lowest inoculum density tested in this study, an average of three to four propagules of *P. parasitica* var. *nicotianae* were present in each funnel. If each of these propagules produced one or more sporangia and released zoospores during the saturation period, the zoospore ID_{50} value would quickly be reached for cultivar Hicks tobacco. In only one of the initial inoculum densities was final infection below 50% (Table 2).

Flooding of soil has been shown to enhance diseases caused by soilborne *Phytophthora* spp. on several hosts (1,6,7). The known mechanisms of disease enhancement by flooding include predisposition of the host (1,7) and the direct effect of high matric potentials on the pathogen through increasing zoospore production and dispersal (4,6,9). Data obtained in this study show the importance of an increase in the inoculum density through stimulation of zoospore production in saturated or flooded soils (Table 2). As little as 0.5 hr of saturation was sufficient to enhance infection (Table 3) and induce the release of detectable numbers of zoospores (Table 4) at an initially high inoculum density (4,000 to 5,000 propagules per kilogram of soil). Additional experiments are needed to determine the nature of interactions between initial inoculum density and the duration of the saturation periods required for host infection.

Percent infection ranged from 0 to 40% in seedlings maintained at −30 mb for 3 wk (Tables 2 and 3). One possible explanation for the difference observed between experiments was that 8 ml of a nutrient solution was added to each funnel once a week in the tests where infection was detected in treatments without a saturation period (Table 2) but was not added in the experiment where no infection was detected at −30 mb (Table 3). The addition of the nutrient solution may have been adequate to increase the matric potential in some of the soil long enough to enhance infection. This problem was avoided in subsequent tests (Tables 3 and 4) by mixing a slow-release fertilizer directly into the soil.

Naturally infested soil was used in this study and inoculum densities of *P. parasitica* var. *nicotianae* present in the soil were determined by assaying the soil on a selective medium. Therefore, only approximate inoculum densities were known. It is not known what percentage of the total propagules of *P. parasitica* var. *nicotianae* present in the soil were detected by the isolation procedure. However, in the soil dilution series (Table 2), expected inoculum densities for the various dilutions (based on inoculum density determined in nondiluted soil) were usually very close to recovered inoculum densities, suggesting that the assay technique was equally sensitive over a wide range of inoculum densities. Also, pathogen propagules in the soil were not all of one form, since both chlamydospores and small root fragments yielded colonies of *P. parasitica* var. *nicotianae* on the selective medium. For more direct comparison to previous inoculum density studies, the effects of controlled water potentials should be tested using soil infested with a known number of a given propagule of *P. parasitica* var. *nicotianae*. Results obtained in this study demonstrate the very dramatic effect that soil water potential has on the inoculum density-infection relationship of *P. parasitica* var. *nicotianae* on seedlings of a susceptible tobacco cultivar and should also illustrate the importance of defining soil water conditions when reporting inoculum density effects on diseases caused by other *Phytophthora* spp.

LITERATURE CITED

1. Blaker, N. S., and MacDonald, J. D. 1981. Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to Phytophthora root and crown rot. Phytopathology 71:831-834.
2. Downs, R. J., and Bonaminio, V. P. 1976. Phytotron procedural manual. Tech. Bull. 224. N. C. State Univ., Raleigh. 38 pp.
3. Duniway, J. M. 1975. Formation of sporangia by *Phytophthora drechsleri* in soil at high matric potentials. Can. J. Bot. 53:1270-1275.
4. Duniway, J. M. 1976. Movement of zoospores of *Phytophthora cryptogea* in soils of various textures and matric potentials. Phytopathology 66:877-882.
5. Kannwischer, M. E., and Mitchell, D. J. 1981. Relationship of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. Phytopathology 71:69-73.
6. Kenerley, C. M., Papke, K., Grand, L. F., and Bruck, R. I. 1982. Effect of flooding on Phytophthora root rot of Fraser fir. (Abstr.) Phytopathology 72:359.
7. Kuan, T.-L., and Erwin, D. C. 1979. Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. Phytopathology 70:981-986.
8. Lucas, G. B. 1975. Diseases of Tobacco. 3rd ed. Harold E. Parker and Sons, Fuquay-Varina, NC. 621 pp.
9. MacDonald, J. D., and Duniway, J. M. 1978. Influence of the matric and osmotic components of water potential on zoospore discharge in *Phytophthora*. Phytopathology 68:751-757.
10. Mitchell, D. J. 1975. Density of *Pythium myriotylum* oospores in soil in relation to infection of rye. Phytopathology 65:570-575.
11. Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to infection of several hosts. Phytopathology 68:1754-1759.
12. Shew, H. D., and Benson, D. M. 1983. Influence of soil temperature and inoculum density of *Phytophthora cinnamomi* on root rot of Fraser fir. Plant Dis. 67:522-524.
13. Sterne, R. E., Zentmyer, G. A., and Kaufmann, M. R. 1977. The influence of matric potential, soil texture, and soil amendment on root disease caused by *Phytophthora cinnamomi*. Phytopathology 67:1495-1500.