Resistance to Black Shank in the Field Predicted by a Test of Tobacco Seedlings

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ABSTRACT

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Roots of 10 seedlings of 17 lines of tobacco in petri dishes were inoculated with 1,000–5,000 zoospores of *Phytophthora parasitica* var. *nicotianae*. Although the relation between the number of seedlings killed and the number of plants of the same lines that became diseased in the field was strongly nonlinear, the relation between the calculated numbers of infections of seedlings and infections of plants in the field was more nearly linear ($r = 0.86$). If lines estimated by the multiple infections correction to have more than 20 infections per 10 seedlings had been discarded, all resistant lines—and only resistant lines—would have been kept.

Development of disease-resistant cultivars is facilitated when resistance can be detected in young plants grown in the laboratory or greenhouse. Several seedling tests have been proposed for black shank of tobacco caused by *Phytophthora parasitica* Dast var. *nicotianae* (B. de Haan) Tucker. Inoculating detached leaves did not always reflect whole plant resistance in the field (8). Inoculating the lower stem of seedlings with eight or more leaves gave a better correlation with field resistance (9).

Troutman et al (7) grew seedlings in modified aluminum muffin pans so that roots grew through a wire mesh into a pan of water below. Inoculum was introduced through water. Correlation with field results was $r = 0.8$. Stokes and Litton (5) modified this method by growing seedlings in vermiculite in plastic tubes with a hole in the bottom. Roots were inoculated by dipping most of the tube into a suspension of motile zoospores. Suspending the tubes over tanks of evenly heated water improved uniformity of results (3). Correlation with known field performance was good. All these tests require considerable time and special or modified equipment.

McIntyre and Taylor (4) used whole seedlings at the five-leaf stage with roots washed free of growing medium and arranged in plastic petri dishes. A standardized concentration of zoospores was sprayed over all the seedlings in open dishes, which then were closed and held for 1 wk at 27 C in controlled-temperature chambers with 12 hr of light daily. Taylor et al (6) improved uniformity by dripping 1 ml of a 5×10^3 zoospore suspension onto the roots of plants arranged with roots to the center of the dishes. With this method, field susceptibility and resistance of test lines at the extremes, but not of all those at intermediate ranges, could be predicted. The work reported here is a further evaluation of this technique.

MATERIALS AND METHODS

Seedlings. Seedlings of test lots were grown, prepared for inoculation, and inoculated as described previously (6). Ten seedlings with four to five leaves were arranged in a 100-mm petri dish with the roots resting on a central disk of moist paper, and two 0.5-ml volumes of a suspension of zoospores of *P. parasitica* var. *nicotianae* race 3 were dropped onto the roots. After many lines were tested, 14 with a range of responses were chosen. Each of five tests from 24 March to 14 July 1978 included four replicates of 10 plants of each of the 14 lines and of three standards: WS 117, highly susceptible to black shank; L, moderately resistant (polygenic); and the breeding line FI, highly resistant (polygenic). Concentration of zoospores was 5,000 per milliliter except in test 3, in which it was about 1,000 per milliliter. Plants were held at approximately 27 C with 14 hr of light daily. The number of dead plants was recorded 6 days after inoculation and also usually after 4 and 7 days; in test 3, observations were continued for 14 days. In each test we selected the time when every plate had at least one diseased plant and about one dish in five contained only dead plants. These incubation periods were 6, 5, 14, 6, and 6 days in the five tests, respectively.

Field. Plants were grown in the greenhouse to transplant size in 96-hole trays now widely used by Connecticut growers (1). They were transplanted to a field infested naturally with *P. parasitica* var. *nicotianae*. Test lines were planted in 10-m rows spaced 98 cm apart. The first and then every sixth plant were the highly susceptible WS 117; these were designated witness plants. Plants were spaced about 35 cm apart for a total of 25–30 per row. There were five replicates. Plots were rototilled between rows and hoed to control weeds. The number of plants with stems blackened at or above the soil line was recorded several times during the season, with the final recording on 25 September.

Multiple infection transformation. Among N plants, the probability of a plant remaining healthy is $(N-1)/N$ if one infection occurs and $[(N-1)/N]^x$ if x infections occur. The probability of a plant being diseased is then $1-[(N-1)/N]^x$.

The expected number y of plants diseased when x infections occur at random is calculated from the binomial distribution:

$$y = N\,(1-[(N-1)/N]^x) \qquad (1)$$

Using the Poisson approximation, Gregory (2) provided a table of x and y. For the 5–25 plants we examined, however, we used equation 1 to estimate infections x from observations y. Sometimes when N was 5–10 and disease was severe, all plants were diseased and equation 1 estimated an infinite y, which is nonsense. Accordingly, when y equaled N, we substituted N–0.3 for y in equation 1, which produced an orderly progression of x for $y = 1, 2, 3,\ldots,N$.

RESULTS

Seedling test. The mean number of dead seedlings per dish of each line and the calculated number of infections are shown in Table 1. The standard errors of the means were 0.35 for dead seedlings and 1.48 for infections, with a highly significant variation among the 17 lines. The 20 replicates, ie, four replicates in each of five tests, did not cause significant variation. The variance ratio F for lines was slightly greater for infections than for number of dead seedlings.

Disease in the field. Since the number N of plants of the lines in each replicate was 20–25 with a mean of 22.7, the number of diseased plants per plot was adjusted by dividing by N and

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multiplying by 23. The mean of this number of diseased plants of each line per plot is shown in Table 1. The variation of the means was highly significant.

The means of calculated numbers x of infections of 23 plants are also shown in Table 1. They varied highly significantly, although the variance ratio F was less than when y was analyzed.

The number y of diseased plants in a plot was correlated (r = 0.36) with the number of diseased witness plants in the plot (Fig. 1). Also, the calculated number x of infections in a plot was correlated (r = 0.50) with the calculated number of infections of witness plants. Adjustment for these correlations did not, however, change the outcome greatly.

Prediction of disease in the field by the seedling test. A curved relation was observed between the number of dead seedlings in the laboratory and the number of diseased plants in the field. Disease in the field was slight for lines with 0–6 dead seedlings per dish but considerable for lines with 7–10 dead seedlings. Thus, the linear correlation coefficient r was only 0.75. The relation between the calculated numbers of infections of seedlings and of plants in the field, however, was more nearly linear and r was 0.86 (Fig. 2). Adjustment for witnesses did not increase the closeness of relations between means of field disease and means of seedling disease.

DISCUSSION

An incubation of exactly 6 days was proposed earlier for the seedling test (6). Experience has shown, however, that progress of disease will vary even with a constant number of spores and constant temperature. Here we used incubation to a given amount of mortality rather than a fixed time and thereby eliminated significant variation among repetitions and replicates and demonstrated highly significant variation among lines.

Although witness plants revealed differences in inoculum and other factors causing variation in extent of disease from place to place in the field, they provided only a moderate increase in the variance ratio for differences among lines and did not increase the correlation between disease in seedlings and disease in the field. The use of witness plants failed to increase the correlation because an average relation between disease in the witness plants and disease in the test lines was necessarily used for both susceptible and resistant lines. The consequent poor fit is evident in Fig. 1, where resistant lines have few infections and susceptible lines have many in plots where witness plants have more than 10 infections.

The relation between calculated infections x and number y of diseased plants is curved, especially as y approaches the number N of plants available. The proportion of diseased plants often was high in the seedling tests but never exceeded 0.81 in the field. Hence, the relation between disease in field and disease in seedlings is sharply curved. It follows that transformation of the variables to infections straightens the relation (Fig. 2).

The question of whether resistance in the field can be predicted by this inexpensive and rapid test of seedlings is resolved by Fig. 2.

TABLE 1. Comparison of seedling tests and field tests of 17 tobacco lines for resistance to black shank

	Seedlings[a]		Field[b]	
Line	Dead	Infections[c]	Diseased	Infections[c]
FI	2.6	3.0	0.2	0.2
17	3.8	5.0	0.2	0.2
25	5.0	7.8	0.2	0.2
44	5.1	7.5	0.4	0.4
24	6.0	11.3	0	0
30	6.2	10.2	0.4	0.4
L	6.6	11.3	0.2	0.2
41	6.8	11.8	0.6	0.6
27	7.0	12.5	2.2	2.5
22	7.2	13.4	1.4	1.5
19	7.7	15.4	0.4	0.4
21	8.0	17.5	1.3	1.3
8	9.3	27.0	4.2	5.4
28	9.4	29.4	7.1	8.8
33	9.5	29.0	3.4	3.7
WS 117	9.7	30.3	4.6	5.4
1	9.8	31.6	7.4	11.6

[a] Mean numbers among 10 seedlings in each of four replicates in five tests.
[b] Mean numbers adjusted for an average of 23 plants per field plot in each of five replicates.
[c] Number of infections calculated with the multiple infection correction.

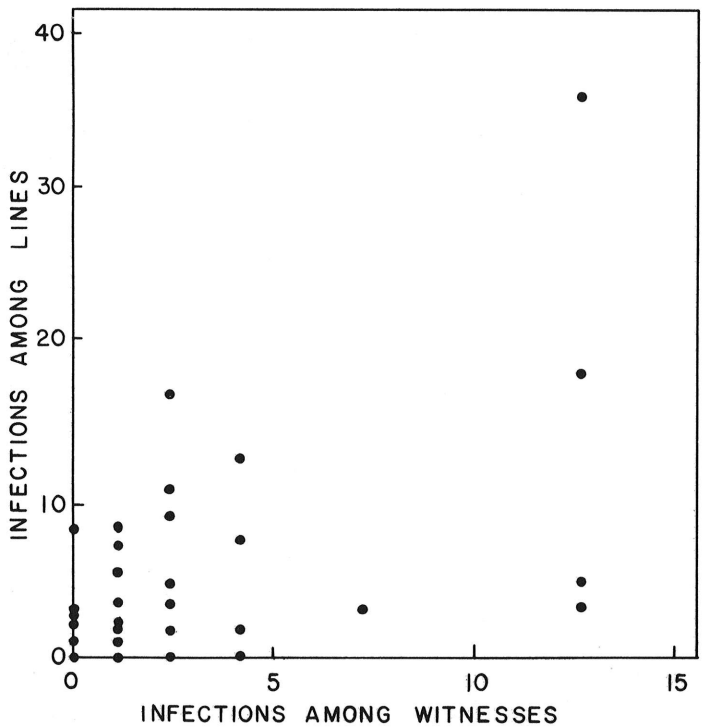

Fig. 1. Relation between black shank infections among 23 plants of a tobacco line and five witness plants (susceptible line) in the same field plot. Numbers of infections were calculated from numbers of diseased plants by means of the multiple infection correction.

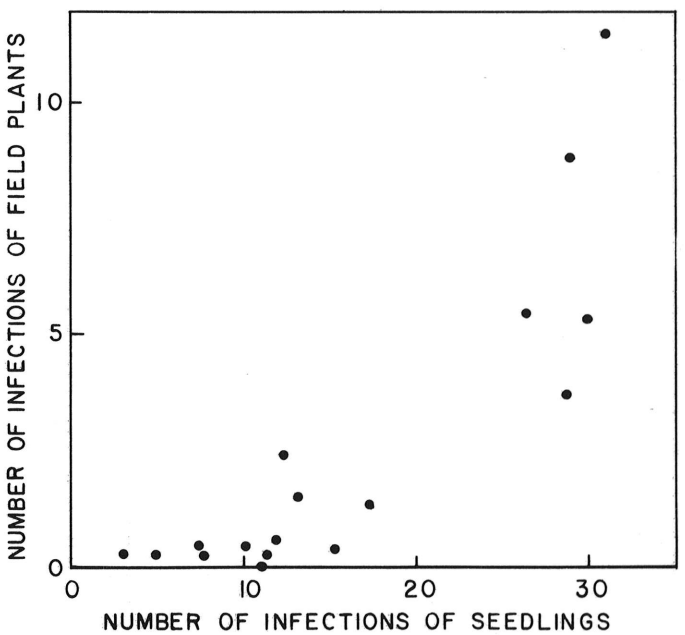

Fig. 2. Relation between black shank infections of 23 plants of a tobacco line in the field and 10 seedlings of the same line inoculated in a petri dish. Numbers of infections were calculated from numbers of diseased plants by means of the multiple infection correction.

Points in the lower left region represent plants resistant in both laboratory and field, and points in the upper right region represent plants susceptible in both places; there are no points for plants resistant in one place and susceptible in the other. In other words, if lines with more than 20 infections per 10 seedlings had been discarded, all the resistant lines—and only the resistant lines—would have been kept. Thus, the test is not only reliable but uses smaller plants and less time than inoculating stems (9) and simpler equipment than that needed for inoculating roots in a growing medium held at controlled temperatures (3,5).

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