Relative Susceptibilities of Five Pine Species to Three Populations of the Pinewood Nematode

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ABSTRACT

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Seedlings of eastern white, loblolly, pond, slash, and Virginia pines were inoculated in a greenhouse at the rate of 4,000 nematodes per tree with three populations of the pinewood nematode (*Bursaphelenchus xylophilus*) from declining Virginia pines. Twelve weeks after inoculation, pine mortality and susceptibility rank were highest for slash pine, moderate for eastern white, loblolly, and pond pines, and lowest for Virginia pine, which was highly resistant. Mortality was also significantly related to differences in virulence of nematode populations. There was a highly significant correlation between percent mortality and mean number of nematodes recovered from recently killed seedlings. The greatest number was recovered from slash pine, and successively fewer were recovered from eastern white, loblolly, pond, and Virginia pines.

Additional key words: *Pinus elliottii* var. *elliottii, P. serotina, P. strobus, P. taeda, P. virginiana*

Although the impact of the pinewood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle (= *B. lignicolus* Mamiya & Kiyohara), on pine forests in Japan has been well documented (11), the role of the nematode in mortality of pines in the United States is still being defined (14). As Malek and Appleby (10) recently noted, two very different systems involving *B. xylophilus* may be operating in the United States. Where native pines are grown under minimal environmental stress, the nematode may be a secondary invader transmitted during oviposition by cerambycid beetles; however, where exotic pines are planted and exposed to environmental stress, the nematode may be a primary pathogen.

In the southeastern United States, the pinewood nematode was not considered a problem until it was discovered in dying slash (*Pinus elliottii* Engelm. var. *elliottii*), sand (*P. clausa* (Chapm.) Vasey), and Virginia (*P. virginiana* Mill.) pines in several seed orchards (G. M. Blakeslee, R. Esser, and T. Miller, *personal communications*; 4). Since most of the seedlings produced in the South for reforestation are from genetically improved pines on 3,970 ha of seed orchards, diseases that disturb this production system are of particular concern to forest resource managers. Furthermore, disease occurrences in seed orchards may signal future problems in pine plantations and natural forest stands as they become more intensively managed.

This study was undertaken to determine the relative susceptibilities of seedlings of five pine species to the pinewood nematode, the pathogenic variation of three nematode populations, and the population dynamics of the nematode in inoculated pine seedlings. A preliminary report has been presented (3).

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MATERIALS AND METHODS

Three-year-old seedlings of eastern white (*P. strobus* L.), loblolly (*P. taeda* L.), pond (*P. serotina* Michx.), slash, and Virginia pines were inoculated in a greenhouse with three populations of the pinewood nematode isolated from declining Virginia pines in two seed orchards in Alabama (AL) and South Carolina (SC) and a progeny test in Georgia (GA). Seedlings were grown in plastic flats (33 × 13 × 11 cm) containing a mixture of soil, pine bark, and sand (2:1:1, v/v). Each flat contained seven or eight seedlings that had been transplanted when 1 yr old.

Nematode populations used as inocula were reared on *Botrytis cinerea* Pers. growing on potato-dextrose agar at 25 C. Nematodes were extracted by a pie-tin technique (1) from 10-day-old cultures, then collected on a 325-mesh screen. Inoculum density of each population was adjusted to 10,000 nematodes per milliliter.

To add a stress factor, seedlings in the flats were not watered for 1 wk before inoculation. To inoculate each seedling, an area of bark on the second-year wood of the main stem was removed, then a piece of sterile, absorbent cotton containing 4,000 nematodes was attached with Parafilm over the wound. About 21 seedlings (seven in each of three flats) were inoculated with each nematode population. Equal numbers of wounded but uninoculated seedlings served as controls. The study began in mid-December 1983 and concluded 12 wk after inoculation.

Seedlings were examined weekly, and recently killed seedlings were sampled for nematodes by extracting from a 25-mm section of the stem directly below the inoculation point and another at the base of the seedling. Sections were incubated in 5 ml of water in separate test tubes for 24 hr. Extracted nematodes in a 1-ml aliquot were counted and expressed as the number per gram of fresh wood weight. At the conclusion of the study, remaining seedlings were also sampled for nematodes.

RESULTS

The pinewood nematode caused significant mortality of seedlings in four of the five pine species tested. Data for the three nematode populations were pooled to calculate the percent mortality vs. time curves for the five species (Fig. 1). Mortality was first recorded 3 wk after inoculation for pond, loblolly, slash, and Virginia pines. Eastern white pines began to die at 5 wk. Pond pines died rapidly, and there was no further mortality after 5 wk. Although loblolly pines continued to die until 9 wk, most of the mortality had occurred by 5 wk. Slash and eastern white pines continued to die until the conclusion of the study. Only one of the Virginia pines and none of the control seedlings died.

A two-way analysis of variance of the percent mortality data at 12 wk revealed that pine species × nematode population was highly significant (Table 1). This interaction was due largely to the uniform response of pond pine to the three nematode populations and the low level of mortality in Virginia pine. The main effects, which were also highly significant, were considered more valuable in interpreting the results. The SC nematode population was significantly less virulent than the GA and AL populations. Susceptibility ranks of pines were: slash, highly susceptible; eastern white, loblolly, pond, all moderately susceptible; and Virginia, highly resistant.

Within pine species, the number of nematodes recovered did not vary significantly among nematode populations. Therefore, population data were pooled within pine species and subjected to one-way analysis of variance. There was a marked seedling-to-seedling variation in the number of nematodes recovered; however, the number per gram of fresh weight in base samples and the mean number varied significantly among pine species (Table 2). The greatest numbers occurred in slash pine. The least were extracted from the single Virginia pine that had died. The correlation between the number of nematodes immediately below the inoculation site and in the base of the seedling was not significant for eastern white, loblolly, and pond pines but was highly significant for slash pine. In slash, eastern white, and loblolly pines, the greatest concentration of nematodes was in the base of the seedlings. At the conclusion of the experiment, nematodes were not recovered from the remaining inoculated slash, loblolly, pond, or Virginia pines or from the controls; however, nematodes were extracted from 18% of the apparently healthy, inoculated eastern white pines. There was a highly significant correlation (r = 0.98) of mean percent mortality (Table 1) with mean number of nematodes recovered (Table 2).

DISCUSSION

Pine species vary in susceptibility to infection by *B. xylophilus* (2,5,7). The results of this study and those of previous reports, however, can be compared only with caution because of differences in methods, inoculum density, host age and genotype, number of trees inoculated, length of experiments, and environmental conditions. Eastern white pine, for example, was reported resistant by Kondo et al (7), Futai and Furuno (5), and Dropkin et al (2) when the inoculum density was 1,000–2,000 nematodes per tree. At an inoculum density of 20,000 nematodes per tree, Kondo et al (7) reported that all eastern white pine seedlings died. Meyers (12) reported that inoculated eastern white pine seedlings died rapidly but appeared to be a poor host since only a few nematodes were recovered. Futai and Furuno (5) concluded that loblolly and slash pines were highly resistant to nematode infection. The data of Dropkin et al (2) indicate that slash and Virginia pines are moderately susceptible. Luzzi and Tarjan (9) reported that all 18-mo-old seedlings of slash, sand, and loblolly pines inoculated with 500 or 1,000 nematodes per tree died within 40 days.

Variation in virulence among cultures of *B. xylophilus* derived from different populations has been reported in both Japan and the United States (11,13). Kondo et al (7) reported that a population from Saga, Japan, was more pathogenic to Scots (*P. sylvestris* L.) pine than a population from Ashland, MO, but that there was no difference in the two populations on jack (*P. banksiana* Lamb.), Austrian (*P. nigra* Arnold), and shortleaf (*P. enchinata* Mill.) pines. Wingfield et al (13) found that *B. xylophilus* extracted from balsam fir (*Abies balsamea* (L.) Mill.) killed balsam fir seedlings but not red (*P. resinosa* Ait.) or Scots pine seedlings, whereas *B. xylophilus* from pine killed only pine seedlings.

Kiyohara and Suzuki (6) indicated that symptom development was related to growth of nematode populations. In our study, there was a highly significant correlation of percent mortality with mean number of nematodes recovered per gram of fresh stem weight. This correlation, however, should not be interpreted as a direct relationship

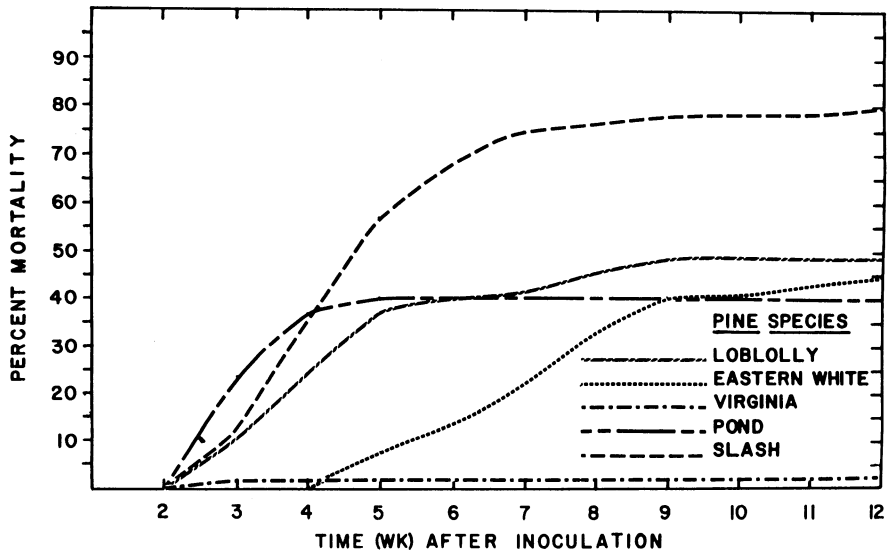

Fig. 1. Mortality (%) of five pine species inoculated with the pinewood nematode (*Bursaphelenchus xylophilus*) over time.

Table 1. Percent mortality of 3-yr-old seedlings of five pine species 12 wk after inoculation with three populations of the pinewood nematode

	Nematode population[y]			
Pine species	Georgia	Alabama	South Carolina	Mean[z]
Slash	89	95	54	79 a
Loblolly	48	68	26	47 b
Eastern white	46	57	30	44 b
Pond	41	38	41	40 b
Virginia	5	0	0	2 c
Mean[z]	46 a	52 a	30 b	

[y] Mortality based on three replicate flats, each containing seven or eight seedlings, per host-population combination.
[z] Species or population means not followed by the same letter are significantly different (P = 0.01) according to Duncan's multiple range test.

Table 2. Average number of pinewood nematodes recovered from stem segments of dead, previously inoculated 3-yr-old seedlings of five pine species

		No. of nematodes per gram fresh weight[y]			
Pine species	No. of seedlings	Inoculation site	Stem base	Mean of two segments	r[z]
Slash	48	894	1,310 a	1,102 a	0.54**
Eastern white	32	404	821 b	612 b	0.16
Loblolly	30	203	829 b	516 b	0.12
Pond	25	419	428 b	423 b	0.30
Virginia	1	30	60 b	45 b	···
Weighted mean		532	918	725	

[y] For each column, means not followed by the same letter are significantly different (P = 0.05) according to Kramer's (8) extension of multiple range tests to group means with unequal numbers of replications. For below the inoculation site, means are not separated because the analysis of variance was not significant.
[z] Correlation coefficient for number of nematodes recovered from stem base and immediately below inoculation site. ** = Significance at P = 0.01.

between tree mortality and population dynamics because variation in the number of nematodes extracted from the recently killed seedlings was extremely high. In slash pine, for example, the mean number of nematodes per gram of fresh stem weight ranged from 23 to 9,250, and the coefficient of variation was 135%. The correlation, therefore, indicates only that relative susceptibility was related to the adequacy of the pine species to serve as a host for the parasite.

Kiyohara and Suzuki (6) in Japan reported that there was no significant difference in the distribution of the nematode in seedlings according to direction and height. In our study as well as a previous pathogenicity test (4), there was a trend toward a greater concentration of the nematodes at the base of the seedlings. The lack of correlation, however, between the number of nematodes recovered from below the inoculation point and from the seedling base for eastern white, loblolly, and pond pines tends to confirm the observation (6) that the nematodes are randomly distributed throughout the stem. There was, however, a significant correlation for slash pine, which indicates that there was a gradient from the inoculation point to the base of the seedling in this pine species.

Although field studies are needed to further define the relative susceptibilities of southeastern pine species to *B. xylophilus*, this greenhouse study provides some insight into the host relations of the pathogen in the South. The nematode populations used were from declining Virginia pine, a species that has proven highly resistant to the pinewood nematode in this and a previous test (4). The source trees were planted outside their natural range, and in Alabama, were stressed by pitch canker infections (caused by *Fusarium moniliforme* Sheld. var. *subglutinans* Wollenw. & Reink.) and severe root deterioration (4). Therefore, the pinewood nematode may have been a secondary invader. In slash pine, a highly susceptible species according to this study, however, *B. xylophilus* may function as a primary pathogen resulting in tree mortality. Mortality associated with pinewood nematode has been observed in previously healthy, unstressed, vigorous, and rapidly growing slash pines in seed orchards in Florida (G. M. Blakeslee, R. Esser, and T. Miller, *personal communications*).

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