# Response of Pinus ponderosa Seedlings to Stylet-Bearing Nematodes

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*Abstract:* Of 12 stylet-bearing nematodes used for inoculations, *Pratylenchus penetrans, P. brachyurus, P. vulnus, Ditylenchus destructor, Meloidogyne incognita, M. javanica,* and *M. hapla* reproduced on *Pinus ponderosa,* while *Xiphinema index, Aphelenchus avenae, Paratylenchus neoamblycephalus, Tylenchulus semipenetrans,* and *Macroposthonia xenoplax* did not. *P. vulnus, P. brachyurus, P. penetrans, A. avenae, D. destructor, T. semipenetrans,* and *P. neoamblycephalus* significantly suppressed both the shoot and root wet weights of ponderosa pine seedlings obtained from stands in five different locations. *X. index* significantly suppressed root wet weights, *M. xenoplax* significantly suppressed shoot wet weight, and *M. incognita, M. javanica,* and *M. hapla* suppressed neither at the inoculation levels used. Injurious nematodes tended to suppress root growth more than shoot growth. Seedlings from two locations produced greater shoot growth wet weight than did seedlings from the other three locations. The more injurious nematodes tended to cause an increase in the water content of shoots. Frequency analyses of seedling population shoot-root ratios indicated that ponderosa pine seedlings could be selected for better shoot-root ratios as well as for resistance to several pathogenic nematodes.

The forest industry is responding to increasing demands for wood and wood products by improving processing, utilization and production so that timber yields will be substantially increased per unit area and per unit time. In anticipation of severe limitations of nonrenewable resources in the foreseeable future, interest has focused on the feasibility of conservation by developing alternative renewable resources. To satisfy these needs, silviculture and forest management must become intensive enough to produce forests that grow at much nearer their biological capacity (1). Among a series of proposals in a recent scheme to attain biological potential (18), two are based on nonengineering procedures—genetic selection, and pest and disease protection.

The inhibition of forest seedling growth by plant-parasitic nematodes has been established (6, 7, 10, 11, 12, 13, 15, 21, 24, 25, 26). Despite repeated demonstrations that nematodes are important in the forest biome (16), their role has been largely ignored by both industrial and public forest management. Forest nematology in the western coastal states of the USA has received little attention until recently (10, 24, 25). This report is concerned with the nature of the response of the progeny of five stands of ponderosa pine (*Pinus ponderosa* Laws. var. ponderosa) from the northern and central Sierra Nevada to twelve species of stylet-bearing nematodes.

## MATERIALS AND METHODS

Each seed lot obtained from the Institute of Forest Genetics (Pacific Southwest Forest and Range Experiment Station), Placerville, California (Table 1), was a composite collection of seeds (provenance) from a number of trees in a stand. Seeds were stratified at 4 C for 90 days and then placed on filter paper moistened with a suspension of benomyl (50 mg/kg active ingredient) to germinate at 25 C under fluorescent light (16-h photoperiod). Germinating seeds with radicles about 10 mm long were transferred to benomyl-moistened filter paper in another petri dish and returned to 4 C to reduce further growth. At the end of germination (about 2 weeks) the germinants were each planted in a styrofoam cup containing 150 ml of sand (equal parts fine white sand and coarse washed sand) premixed with benomyl (50 mg/kg active ingredient) and ethazole (30 mg/kg active ingredient, 5-ethoxy-3-trichloromethyl-1,2,4-thiodiazole). The cups, pierced for drainage, were placed on a greenhouse bench under natural light for 2 months (late April to late June 1975) and watered as needed with 50% Hoagland's solution (8). At 3, 6, and 9 weeks after planting, each cup was

TABLE 1. Population samples of *Pinus ponderosa* obtained from the Institute of Forest Genetics.

| Provenance designation | Source | Latitude | Longitude | Approximate elevation (meters) | Crop year |
|---|---|---|---|---|---|
| Plu 3 | Spring Garden Plumas County, CA | 39° 53′ | 120° 47′ | 1500 | 1967 |
| Sie 2 | Mountain House Sierra County, CA | 39° 30′ | 120° 54′ | 1400 | 1967 |
| Eld 15 | Silver Fork El Dorado County, CA | 38° 46′ | 120° 19′ | 1200 | 1970 |
| Eld 113 | Salmon Falls El Dorado County, CA | 38° 45′ | 121° 03′ | 150 | 1970 |
| Eld 12 | Georgetown Junction El Dorado County, CA | 38° 47′ | 120° 12′ | 1650 | 1971 |

drenched with an aqueous suspension of benomyl (50 mg/kg active ingredient) and ethazole (30 mg/kg active ingredient) for the continued suppression of pathogenic fungi.

The seedlings from each lot were divided into 15 groups of equal size, with the number of replicates per group varying from 27 to 34 depending on the viability of the seed lot. By late June, the shoot growth of all seedlings was 10–15 mm as measured from the base of the lowermost needles. Thirteen of these groups were infested with nemas as indicated in Table 2. The inoculum number selected for each species was the amount that experience indicated should produce an effect on a moderately susceptible host plant.

The treatments were divided into two trials to facilitate harvest handling and counting. The plants were allowed to grow for 4 months after inoculation in trial 1, and for 5 months in trial 2. Roots were washed, and the shoots were excised just below the lowermost needles. The shoots and roots were blotted with a towel and their wet weights were determined. The shoots were dried overnight at 100 C and weighed after cooling. Individual root systems inoculated with endoparasitic nematodes were chopped and incubated for a week in a mist chamber (9) for collection of nematodes and counting.

Ectoparasitic nematodes were recovered by sieving (4) and placing the sieve contents on a Baermann funnel for 48 h for addi-

TABLE 2. Sources and inoculum levels of nematodes used to infest *P. ponderosa* seedlings.

| Nematode | Source | Inoculum/pot |
|---|---|---|
| 1. *Xiphinema index* (ectoparasitic) | Fig roots | 100 |
| 2. *Pratylenchus brachyurus* (endoparasitic) | Carrot slices | 600 |
| 3. *Meloidogyne hapla* (endoparasitic) | Tomato roots | 1200 |
| 4. *P. vulnus* (endoparasitic) | Carrot slices | 600 |
| 5. *P. penetrans* (endoparasitic) | Carrot slices | 600 |
| 6. *M. incognita* (endoparasitic) | Tomato roots | 1200 |
| 7. *M. javanica* (endoparasitic) | Tomato roots | 1200 |
| 8. *Tylenchulus semipenetrans* (endoparasitic) | Persimmon roots | 1200 |
| 9. *Ditylenchus destructor* (endoparasitic) | *Alternaria tenuis* culture | 2000 |
| 10. *Aphelenchus avenae* (nonparasitic) | *Fusarium solani* culture | 2000 |
| 11. Combined *D. destructor* + *A. avenae* | *A. tenuis* culture | 1000 |
| | *F. solani* culture | 1000 |
| 12. *Macroposthonia xenoplax* (ectoparasitic) | Plum roots | 3000 |
| 13. *Pratylenchus neoamblycephalus* (ectoparasitic) | Plum roots | 2000 |
| 14. Control, Trial 1 | Not inoculated | 0 |
| 15. Control, Trial 2 | Not inoculated | 0 |

tional purification and concentration. In addition, sand from endoparasitic *D. destructor* and *T. semipenetrans* inoculations was similarly processed for estimates of the population. *P. brachyurus* was not so treated; it was learned from subsequent counsel that this nematode differs from the other two *Pratylenchus* species in that the sand often contains a greater proportion of the population than does the root system.

In terms of several plant-yield parameters the provenance means, combining all treatments, and the treatment means, combining all provenances were ranked, after analysis of variance, by Duncan's multiple-range test.

## RESULTS AND DISCUSSION

As an inoculum check 1 week after inoculation, a seedling from each treatment employing an endoparasitic nematode was washed free of sand; the root system was stained with lactophenol-acid fuchsin and examined for nematodes *in situ*. Many nemas were found in the roots of plants infested with *Meloidogyne* spp., *Pratylenchus* spp., *Tylenchulus semipenetrans*, and *Ditylenchus destructor*. Also examined were wet sievings of sand from a cup from each of the other nematode treatments, and live nematodes were found in all cases but controls.

Roots of all seedlings were examined macroscopically and microscopically before being chopped to determine mycorrhizal formation, but no Hartig nets, mantles, or dichotomously branched short roots were found. The fungicidal treatment apparently inhibited mycorrhizal formation. In preliminary tests without fungicidal treatment, seedlings of comparable age invariably developed mycorrhizae.

Certain nematodes (*P. vulnus*, *P. penetrans*, *D. destructor*) reproduced well and were recovered in large numbers (Tables 3, 4). Some (*Meloidogyne* spp. and *P. brachyurus*) were recovered in very low numbers, while others (all ectoparasites) were not recovered.

Considering the treatment means at the bottom of Table 4, which include all provenances, the shoot weights for *A. avenae* plus *D. destructor* do not differ from those of the untreated control (P = 0.01), but

these two treatments differ from all others, including *D. destructor* or *A. avenae* separately.

When treatments were grouped together to test the general influence of provenance, two provenances, Eld 113 and Eld 15, had larger mean shoot wet weights. However, because of variability, the interaction of treatment × provenances was not statistically significant.

When provenances were grouped together to test the general influence of nematode treatments, shoot dry weights discriminated more differences than shoot wet weights. Variable water content can apparently obscure the effect of treatment on the seedlings.

The ratio shoot-wet-weight/shoot-dry-weight was analyzed to evaluate the effect of nematode treatment on water content of the plant shoots. The more injurious treatments tend to increase the proportion of water in the shoots. Within the genera *Meloidogyne* and *Pratylenchus* there were no significant differences between species for wet- or dry-weight of shoots, although significant differences occurred in wet/dry-weight ratios of shoots between *M. incognita* and *M. hapla* as well as between *P. vulnus* and the other two *Pratylenchus* spp. The relative ranking of provenances according to mean shoot wet weights and mean shoot dry weights was greatly different from that obtained using the ratios of shoot wet weight to shoot dry weight. Using the ratios also reduced variability, revealing differences in this parameter between the provenance-nematode combinations. The provenances appeared randomly distributed, whereas the nematode treatments were more grouped by nematode over the range of ratios. The kind of nematode attacking the plant roots had a substantial impact upon the moisture content of the seedling shoots, even though the shoots of some uninfested provenances inherently had a somewhat higher water content.

In trial 1, the root wet-weight means of the plants infested with the root-knot nematode (*Meloidogyne* spp.) were clumped around the mean of the uninfested control (Table 3, bottom). That is not surprising, since it is well known that deleterious effects of these root parasites on agricultural crop hosts in light infestations may be compen-

TABLE 3. Growth measurements[a] of ponderosa pine progeny grown 4 months in soil infested with different stylet-bearing nematodes, or uninfested; and estimates of nematode populations[b] attained in this time, Trial 1.

| Provenance | Growth measurements and nematodes recovered | Nematode treatments | | | | | | | | Provenance x̄ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Uninfested Control | M. incognita [c] | M. javanica [c] | M. hapla | X. index | P. vulnus [c] | P. brachyurus [c] | P. penetrans [c] | |
| Eld 113 | Shoot wet wt (g) | 6.71 | 5.92 | 5.59 | 6.24 | 5.55 | 5.29 | 5.44 | 4.30 | 5.63 a |
| | Shoot dry wt (g) | 1.84 | 1.58 | 1.53 | 1.76 | 1.42 | 1.44 | 1.38 | 1.08 | 1.51 A |
| | Root wet wt (g) | 11.50 MN | 12.13 M | 10.54 MNOPQRS | 11.26 MNO | 9.26 PQRSTUVW | 9.08 QRSTUVWX | 8.56 STUVWXYZ | 7.36 WXYZ | 9.96 α |
| | Shoot wet wt/shoot dry wt | 3.69 pqrstuvwx | 3.73 opqrstuvw | 3.70 pqrstuvwx | 3.55 vwxyz | 3.94 lmn | 3.67 qrstuvwx | 3.96 lmn | 4.02 l | 3.79 χ |
| | Shoot wet wt/root wet wt | 0.59 | 0.49 | 0.54 | 0.56 | 0.60 | 0.58 | 0.64 | 0.59 | 0.57 ψ |
| | Nematodes recovered | 0 | + | + | 0 | 0 | 477 | 33 | 5970 | |
| Eld 15 | Shoot wet wt (g) | 6.32 | 5.17 | 5.73 | 5.81 | 5.78 | 5.62 | 4.75 | 5.16 | 5.54 a |
| | Shoot dry wt (g) | 1.85 | 1.41 | 1.64 | 1.69 | 1.48 | 1.54 | 1.26 | 1.36 | 1.53 A |
| | Root wet wt (g) | 9.90 NOPQRST | 9.53 NOPQRSTU | 10.79 MNOPQR | 10.88 MNOPQR | 8.59 STUVWXYZ | 8.09 TUVWXYZ | 7.40 WXYZ | 7.13 XYZ | 9.04 β |
| | Shoot wet wt/shoot dry wt | 3.42 z | 3.65 rstuvwxy | 3.53 wxyz | 3.46 yz | 3.91 lmno | 3.64 rstuvwxy | 3.77 mnopqrstuv | 3.82 lmnopqrs | 3.65 ω |
| | Shoot wet wt/root wet wt | 0.63 | 0.58 | 0.53 | 0.54 | 0.68 | 0.69 | 0.65 | 0.73 | 0.63 X |
| | Nematodes recovered | 0 | + | + | 0 | 0 | 890 | 52 | 8640 | |
| Sie 2 | Shoot wet wt (g) | 5.63 | 5.88 | 5.69 | 5.21 | 5.54 | 4.70 | 4.81 | 4.37 | 5.23 a |
| | Shoot dry wt (g) | 1.59 | 1.58 | 1.59 | 1.46 | 1.44 | 1.30 | 1.22 | 1.13 | 1.41 A |
| | Root wet wt (g) | 9.57 NOPQRSTU | 11.50 MN | 10.37 MNOPQRS | 10.42 MNOPQRS | 8.75 STUVWXYZ | 8.65 STUVWXYZ | 7.80 UVWXYZ | 7.15 XYZ | 9.27 β |
| | Shoot wet wt/shoot dry wt | 3.57 vwxyz | 3.72 opqrstuvw | 3.59 tuvwxyz | 3.62 stuvwxyz | 3.88 lmnopq | 3.63 rstyvwxy | 3.98 lm | 3.88 lmnop | 3.73 χψ |
| | Shoot wet wt/root wet wt | 0.59 | 0.52 | 0.55 | 0.50 | 0.64 | 0.54 | 0.62 | 0.61 | 0.57 ψ |
| | Nematodes recovered | 0 | + | + | 0 | 0 | 1110 | 287 | 5610 | |
| Eld 12 | Shoot wet wt (g) | 5.62 | 4.68 | 4.76 | 4.49 | 4.25 | 3.96 | 4.16 | 4.43 | 4.54 b |
| | Shoot dry wt (g) | 1.59 | 1.27 | 1.27 | 1.23 | 1.16 | 1.09 | 1.08 | 1.16 | 1.23 B |
| | Root wet wt (g) | 11.03 MNOPQ | 9.18 QRSTUVW | 9.77 NOPQRSTU | 8.96 RSTUVWXYZ | 6.99 Z | 7.03 Z | 7.10 XYZ | 7.06 YZ | 8.39 α |
| | Shoot wet wt/shoot dry wt | 3.53 wxyz | 3.67 qrstuvwx | 3.76 nopqrstuv | 3.61 tuvwxyz | 3.65 rstuvwxy | 3.61 tuvwxyz | 3.88 lmnopq | 3.83 lmnopqr | 3.69 ψω |
| | Shoot wet wt/root wet wt | 0.51 | 0.51 | 0.48 | 0.50 | 0.60 | 0.55 | 0.58 | 0.63 | 0.54 ψ |
| | Nematodes recovered | 0 | + | + | + | 0 | 1100 | 66 | 5860 | |
| Plu 3 | Shoot wet wt (g) | 4.16 | 5.21 | 4.94 | 4.09 | 4.62 | 4.07 | 4.36 | 4.06 | 4.44 b |
| | Shoot dry wt (g) | 1.17 | 1.41 | 1.41 | 1.16 | 1.22 | 1.12 | 1.14 | 1.08 | 1.21 B |
| | Root wet wt (g) | 9.42 OPQRSTUV | 10.88 MNOPQR | 11.16 MNOP | 9.04 RSTUVWXY | 7.84 UVWXYZ | 7.92 TUVWXYZ | 7.80 UVWXYZ | 7.46 VWXYZ | 8.94 βα |
| | Shoot wet wt/shoot dry wt | 3.58 uvwxyz | 3.64 rstuvwxy | 3.49 xyz | 3.54 wxyz | 3.78 mnopqrstu | 3.64 rstuvwxy | 3.84 lmnopqr | 3.80 mnopqrst | 3.67 ω |
| | Shoot wet wt/root wet wt | 0.44 | 0.47 | 0.45 | 0.46 | 0.58 | 0.51 | 0.57 | 0.53 | 0.50 Ω |
| | Nematodes recovered | 0 | 0 | + | + | 0 | 2100 | 67 | 6110 | |
| Treatment x̄ | Shoot wet wt (g) | 5.69 a' | 5.37 a' | 5.34 a'b' | 5.17 a'b'c' | 5.15 a'b'c' | 4.73 b'c'd' | 4.70 c'd' | 4.46 d' | |
| | Shoot dry wt (g) | 1.61 A' | 1.45 A'B'C; | 1.49 A'B' | 1.46 A'B'C' | 1.34 B'C'D' | 1.30 C'D'E; | 1.22 D'E' | 1.16 E' | |
| | Root wet wt (g) | 10.28 α' | 10.64 α' | 10.53 α' | 10.11 α' | 8.29 β' | 8.16 β' | 7.73 β'α' | 7.23 α' | |
| | Shoot wet wt/shoot dry wt | 3.56 ω' | 3.68 ψ' | 3.62 ω' | 3.56 ω' | 3.83 χ' | 3.64 ψ'ω' | 3.89 χ' | 3.87 χ' | |
| | Shoot wet wt/root wet wt | 0.55 Ψ'Ω' | 0.51 Ω' | 0.51 Ω' | 0.51 Ω' | 0.62 X' | 0.58 X'Ψ' | 0.61 X' | 0.62 X' | |

[a] Using analysis of variance and Duncan's multiple-range test, average growth measurements followed horizontally by the same designations do not differ $(P = 0.01)$; also, treatments and provenance averages followed by the same designation do not differ $(P = 0.01)$.

[b] + sign indicates some root galls, larvae, and adult females with eggs were found.

[c] Nematodes extracted from roots only.

TABLE 4. Growth measurements[a] of ponderosa pine progeny grown 5 months in soil infested with different stylet-bearing nematodes, or uninfested; and estimates of nematode populations attained in this time (Trial 2).

| Provenance | Growth measurements and nematodes recovered | Nematode treatment | | | | | | | Provenance x̄ |
|---|---|---|---|---|---|---|---|---|---|
| | | Uninfested Control | A. avenae / D. destructor | P. neoamblycephalus | T. semipenetrans | D. destructor | M. xenoplax | A. avenae | |
| Eld 15 | Shoot wet wt (g) | 8.60 | 7.15 | 6.99 | 6.36 | 6.64 | 6.91 | 6.37 | 7.00 a |
| | Shoot dry wt (g) | 2.52 | 2.21 | 2.03 | 1.65 | 1.82 | 1.98 | 1.68 | 1.97 A |
| | Root wet wt (g) | 13.67 PQR | 12.26 QRST | 12.62 PQRST | 9.27 WXYZ | 8.84 WXYZ | 12.35 PQRST | 9.86 UVWXY | 11.27 β |
| | Shoot wet wt/shoot dry wt | 3.41 yz | 3.36 yz | 3.43 yz | 3.91 pqr | 3.65 vwx | 3.50 xyz | 3.77 rstuvw | 3.58 ω |
| | Shoot wet wt/root wet wt | 0.63 | 0.58 | 0.55 | 0.68 | 0.75 | 0.55 | 0.64 | 0.63 X |
| | Nematodes recovered | 0 | 970 | 0 | 0 | 1540 | 0 | 0 | |
| ·Eld 113 | Shoot wet wt (g) | 8.15 | 7.64 | 6.42 | 6.49 | 6.45 | 6.30 | 5.98 | 6.78 a |
| | Shoot dry wt (g) | 2.46 | 2.24 | 1.83 | 1.75 | 1.68 | 1.73 | 1.56 | 1.89 A |
| | Root wet wt (g) | 16.29 0 | 14.32 PQ | 12.29 QRST | 10.80 TUVW | 9.59 WXYZ | 12.44 PQRST | 9.96 UVWX | 12.23 α |
| | Shoot wet wt/shoot dry wt | 3.33 z | 3.42 yz | 3.51 xy | 3.72 stuvw | 3.85 qrst | 3.67 uvw | 3.84 qrst | 3.62 ω |
| | Shoot wet wt/root wet wt | 0.51 | 0.53 | 0.52 | 0.60 | 0.67 | 0.51 | 0.59 | 0.56 Ψ |
| | Nematodes recovered | 0 | 1340 | 0 | ·0 | 1510 | 0 | 0 | |
| Sie 2 | Shoot wet wt (g) | 5.97 | 6.54 | 5.76 | 5.99 | 5.60 | 5.60 | 5.20 | 5.81 b |
| | Shoot dry wt (g) | 1.75 | 1.90 | 1.67 | 1.58 | 1.43 | 1.52 | 1.30 | 1.59 B |
| | Root wet wt (g) | 12.98 PQRS | 12.77 PQRST | 13.39 PQRS | 9.85 UVWXYZ | 7.86 YZ | 11.48 STUV | 9.29 WXYZ | 11.11 βα |
| | Shoot wet wt/shoot dry wt | 3.41 yz | 3.46 yz | 3.45 yz | 3.80 qrstuv | 3.96 pq | 3.69 tuvw | 4.30 p | 3.69 ψ |
| | Shoot wet wt/root wet wt | 0.46 | 0.52 | 0.43 | 0.61 | 0.70 | 0.48 | 0.56 | 0.54 Ψ |
| | Nematodes recovered | 0 | 1150 | 0 | 0 | 694 | 0 | 0 | |
| Eld 12 | Shoot wet wt (g) | 6.76 | 6.47 | 5.52 | 5.28 | 4.97 | 4.86 | 5.68 | 5.65 b |
| | Shoot dry wt (g) | 2.03 | 1.94 | 1.59 | 1.37 | 1.36 | 1.34 | 1.48 | 1.59 B |
| | Root wet wt (g) | 13.81 PQ | 12.41 PQRST | 11.74 RSTU | 8.82 WXYZ | 8.02 XYZ | 9.69 VWXYZ | 9.48 WXYZ | 10.57 α |
| | Shoot wet wt/shoot dry wt | 3.34 yz | 3.38 yz | 3.49 xyz | 3.87 pqrs | 3.65 vwx | 3.63 wx | 3.83 qrstu | 3.60 ω |
| | Shoot wet wt/root wet wt | 0.50 | 0.52 | 0.47 | 0.59 | 0.62 | 0.48 | 0.59 | 0.54 Ψ |
| | Nematodes recovered | 0 | 416 | 0 | 0 | 1070 | 0 | 0 | |
| Plu 3 | Shoot wet wt (g) | 6.52 | 5.88 | 5.25 | 5.05 | 5.41 | 5.29 | 3.94 | 5.33 b |
| | Shoot dry wt (g) | 1.92 | 1.76 | 1.57 | 1.33 | 1.41 | 1.54 | 1.01 | 1.51 B |
| | Root wet wt (g) | 14.40 P | 14.31 PQ | 13.09 PQRS | 9.91 UVWXY | 9.45 WXYZ | 13.31 PQRS | 7.72 Z | 11.74 αβ |
| | Shoot wet wt/shoot dry wt | 3.40 yz | 3.34 yz | 3.35 yz | 3.82 qrstu | 3.84 qrstu | 3.43 yz | 3.88 pqrs | 3.58 ω |
| | Shoot wet wt/root wet wt | 0.46 | 0.41 | 0.40 | 0.51 | 0.57 | 0.40 | 0.50 | 0.46 Ω |
| | Nematodes recovered | 0 | 536 | 0 | 0 | 1280 | 0 | 0 | |
| Treatment x̄ | Shoot wet wt (g) | 7.20 a' | 6.74 a' | 5.99 b' | 5.83 b' | 5.82 b' | 5.79 b' | 5.43 b' | |
| | Shoot dry wt (g) | 2.13 A' | 1.99 A' | 1.74 B' | 1.54 C'D' | 1.54 C'D' | 1.62 B'C: | 1.41 D' | |
| | Root wet wt (g) | 14.21 α' | 13.22 β' | 12.63 β' α' | 9.73 ζ' | 8.75 ε' | 11.88 α' | 9.26 ζ' ξ' | |
| | Shoot wet wt/shoot dry wt | 3.38 ω' | 3.39 ω' | 3.45 ω' | 3.82 φ' χ' | 3.79 χ' | 3.58 ψ' | 3.87 φ' | |
| | Shoot wet wt/root wet wt | 0.51 Ω' | 0.51 Ω' | 0.47 Ω' | 0.60 Ψ' | 0.66 X' | 0.49 Ω' | 0.58 Ψ' | |

[a]Using analysis of variance and Duncan's multiple-range test, average growth measurements followed horizontally by the same designations do not differ ($P = 0.01$); also, treatment and provenance averages followed by the same designation do not differ ($P = 0.01$).

sated for by root proliferation and gall formation. Among the lesion nematodes that suppress root growth, *Pratylenchus penetrans* was significantly more injurious than *P. vulnus*. The nematodes in trial 2 produced an even greater spread in mean root wet weights (Table 4) than those of trial 1. The mutually antagonistic effect of *A. avenae* and *D. destructor* appeared again in root wet weights.

The effect of provenance appeared to be greater on root growth than on shoot growth. The provenance ranking was essentially the same in both trials.

The significant interactions in this combination treatment × provenance were shown by the components of variance. Interactions based upon mean root wet weights fell into 14 different subgroups in trial 1, and into 12 in trial 2. The provenances were broadly distributed over the range in both trials, whereas the treatments tended to be grouped more according to nematode infestation. In the nematode-plant systems studied, the kind of nematode was more influential than provenance in determining root weight.

The larger proportion of a typical ponderosa seedling wet weight was in the root system (Tables 3, 4). Although shoot wet weights and root wet weights were reduced by injurious nematodes, analyses of the mean ratios of shoot-wet-weights/root-wet-weights (S/R) showed a proportionally greater loss in root wet weights than in shoot wet weights. The loss averaged 3% (range–7% to 13%) over controls of trial 1, and 8% (range–8% to 29%) over controls of trial 2.

The effect of provenance sampled, on grouped treatments indicated a 25% difference in the range of S/R ratios over the low in trial 1, and a 37% difference in trial 2. Eld 15 and Eld 113 produced essentially the same shoot growth when attacked by nematodes, but Eld 15 produced this shoot growth with significantly less root growth. Conversely, Plu 3 required a proportionally greater root growth to support less shoot growth.

*Pathogenic relations:* It was evident that the bulk of the residual photosynthate had gone into production of the root portion. S/R was the ratio selected for analysis of individual plant responses to a nematode

attack. The individual observations of each nematode-provenance combination were grouped into six classes according to the formula of Yule (27):

$$\text{Number of classes} = 2.5\sqrt[4]{\text{number in sample}}$$

The summation curves presenting the class frequencies of the combined population samples of *P. ponderosa* appeared to fall into three groups. The first group comprised uninfested controls and *M. hapla*-infested plants (illustrated by uninfested controls, Fig. 1). This group had a greater proportion of its higher class-frequencies at the lower S/R ratios; four of the six curves showed maxima in the frequency class of 0.45 to 0.55. The second group consisted of root lesion nematode infestations, *Pratylenchus* spp. (illustrated by *P. penetrans*, Fig. 2), in which all six curves had maxima in the same S/R frequency class. However, the frequencies in the classes with S/R ratios
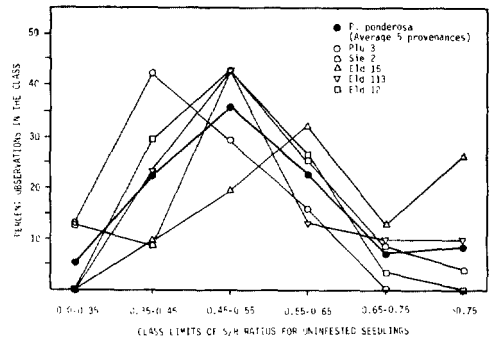


FIG. 1. The proportion of the observations of S/R ratios falling within designated limits of the 6 selected classes for different stands of uninfested seedlings of *P. ponderosa.*
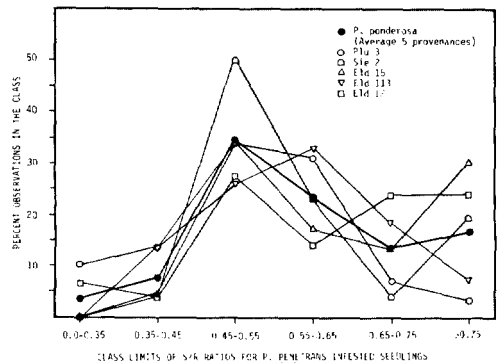


FIG. 2. The proportion of the observations of S/R ratios falling within designated limits of the 6 selected classes for different stands of seedlings of *P. ponderosa* infested with *P. penetrans.*

below that of the maximum were lower, and the frequencies in the classes with S/R ratios above that of the maximum were greater than for Group 1. The third group consisted of *X. index* and *D. destructor* infestations (illustrated by *X. index*, Fig. 3), in which the maximum frequency was in the S/R ratio class of 0.55 and 0.65, reflecting the greater portion of frequencies with higher S/R values. This frequency difference between low and high S/R ratios could be interpreted as a measure of pathogenicity since the differences in S/R were largely functions of differences in root wet weight. There was substantial variability of the plant population samples in the frequency at which each was found in an S/R class for nematode infestations. There appeared to be no consistency among plant population samples in their responses to the seven nematode treatments analyzed, indicating that population class-frequency curves may have been a reflection of specific nematode infestations.

*Nematode population-density distribution within seedling samples of P. ponderosa:* Replicate observations of populations within any provenance-nematode combination varied widely; therefore, a frequency distribution analysis was made. Frequency classes were selected by the method described above. With *P. penetrans*, which was very precocious on ponderosa pine, the frequencies were greatest in the midrange of class limits (Fig. 4). The higher frequencies of *P. vulnus* occurred toward the lower end of the range (Fig. 5). Those of the *D. destructor* (Fig. 6) occurred even lower than those of *P. vulnus*. It appeared that Sierra Nevada populations of *P. ponderosa* could vary greatly in response according to the attacking nematode. Furthermore, there may have been substantial differences between different ponderosa pine populations as to suitability as a host for a particular nematode. Neither did there appear to be a consistency in the susceptibility of any provenance to all the nematodes.

## CONCLUSIONS

The stylet-bearing nematodes used in this experiment were of agricultural origin. That is not to say, however, that these species cannot occur in the forest biome.

The nematode fauna populating the forest soils of California remain largely unexplored. A number of the nematodes used in these experiments have been shown to be injurious to ponderosa pine seedlings.
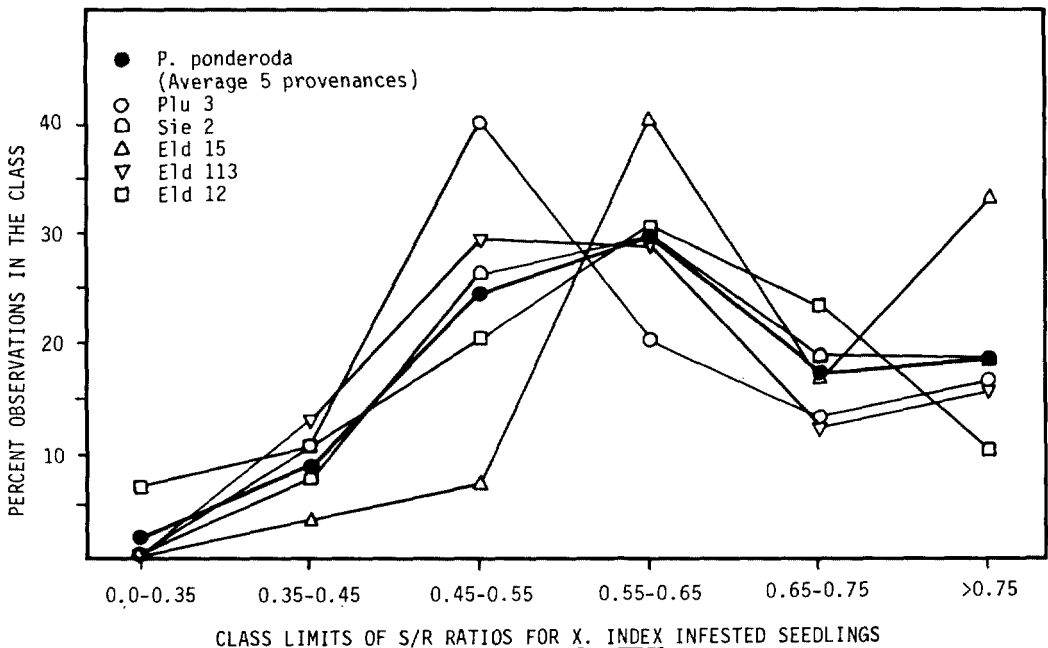


FIG. 3. The proportion of the observations of S/R ratios falling within designated limits of the 6 selected classes for different stands of seedlings of *P. ponderosa* infested with *X. index*.
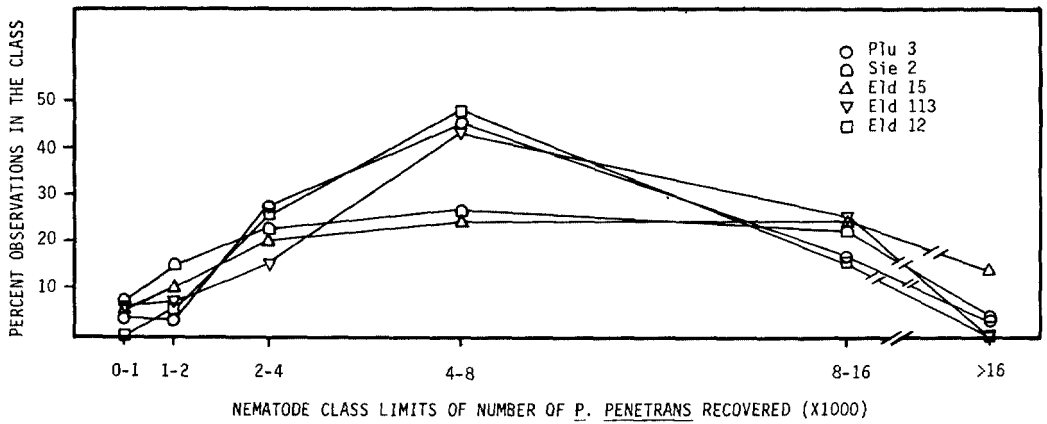
FIG. 4. The proportion, of the observations of *P. penetrans* numbers recovered at harvest, falling within designated limits of 6 selected classes for different population samples of *P. ponderosa*.

*Pratylenchus* spp. and *D. destructor* reproduced well and were recovered at harvest time. *Meloidogyne* spp. also reproduced on ponderosa. Certain pine populations, however, appeared to resist some of these species. Age may have been a factor in the susceptibility of this pine to rootknot; however, it remains to be established which *Meloidogyne* spp. are injurious to ponderosa pine seedling growth but were not recovered at the end of the experiment. Perhaps ponderosa pine was resistant, or susceptibility was related to host age; more likely, it was a consequence of poor environmental control during growth. The pine seedlings were grown from the spring through fall, and the greenhouse facilities

were unable to cope with the occasional extraordinary hot spells of summer in the Sacramento Valley. The plants normally recover from such transient periods with no visible aftereffects; however, it is not uncommon for ectoparasitic nematode populations maintained in small containers to be lost under such conditions.

Of particular interest was the role of *A. avenae*, generally regarded as a fungus feeder (22), when infecting the rhizosphere of *P. ponderosa* not exhibiting the typical mycorrhizal absorption system. Since *A. avenae* and *D. destructor* as single-species inocula inhibit pine growth, it was surprising that in a mixed inoculum there appeared to be mutual antagonism, so that
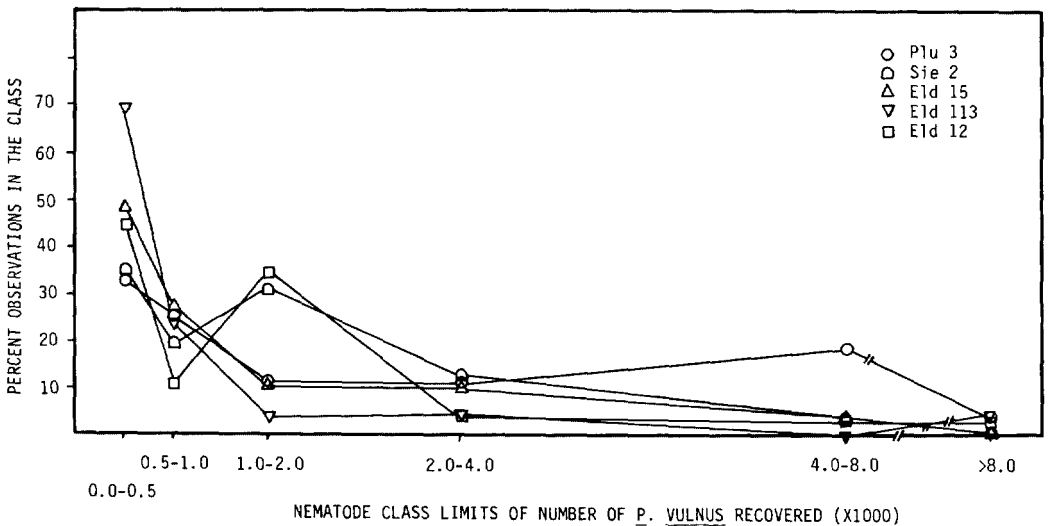


FIG. 5. The proportion, of the observations of *P. vulnus* numbers recovered at harvest, falling within designated limits of 6 selected classes for different population samples of *P. ponderosa*.
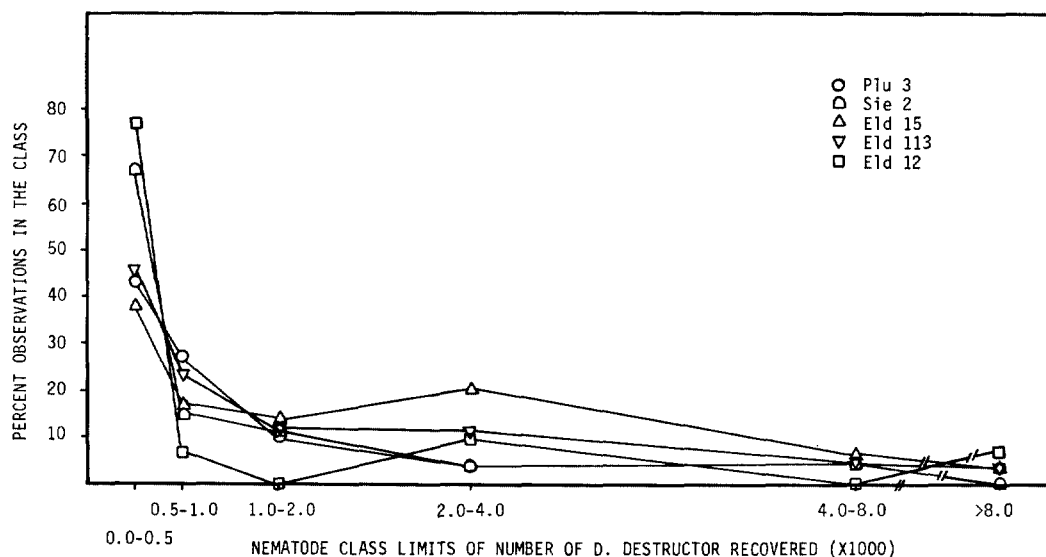
FIG. 6. The proportion, of the observations of *D. destructor* numbers recovered at harvest, falling within designated limits of 6 selected classes for different population samples of *P. ponderosa.*

plant growth was comparable to that by uninfected controls.

The shoot-wet-weight/root-wet-weight ratios of *P. ponderosa,* which for the combined plant population samples maximizes at about 0.5, indicated that about two-thirds of the photosynthate produced by the plant for biomass was used for root formation. The effect of injurious nematodes was to shift the ratio to greater values, apparently by reducing root growth more than shoot growth. The findings in this report are in substantial agreement with those of Stein (20) with 2-year-old *P. ponderosa* juveniles in field plots. Stein's final determination of T/R at the end of the second year in field plots was 0.71 with the best soil, and between 1.5 and 2.0 with poor soil. It was his observation that the poor soil inhibited root growth preferentially so that T/R ratios were greater, similar to the greater ratios attributed to injurious nematodes in this report. According to Stein, seedlings in his plots probably competed somewhat with each other by the end of the second season, but, even so, the growth capability demonstrated in the good soil was far above that usually observed in the forest. Furthermore, he concluded that the trees he used had a reservoir of juvenile growth capability far in excess of actual growth normally found in nature. The present findings are consistent with that conclusion. If, in this case,

early heritable growth differences forecast later growth differences (2; and F. C. Franklin, private communication), the frequency curves of Fig. 1 based on seedling responses of *P. ponderosa* indicate that the five stands of ponderosa pine selected from the northern and central Sierra Nevada had a potential for a proportional increase in the amount of photosynthate redirected toward top growth. If Stanley's conclusion (19) can be accepted that genetic differences exist between pines from different sources in the capacity of roots for nutritional uptake or in different metabolic efficiencies at the enzyme level, then a genetic potential also exists for selection in *P. ponderosa* for redirection of photosynthate. The feasibility of such an approach does not seem unfavorable, especially when it is considered that the S/R ratios are 1.5–2.0 for seedlings of annuals (e.g., cotton and tomato), and in excess of 3 for perennials such as four- and eight-year-old apple trees and four-year-old peach trees (3, 5).

The frequency curves (Figs. 4–6) suggested there were differences in susceptibility not only between populations of *P. ponderosa* but also within populations of four nematode species. If the lower numbers of nematodes recovered were assumed to reflect a lack of susceptibility, then the class frequencies at the lower end of the range would provide a rudimentary measure

of the reservoir of resistance available within each stand to the various nematodes. The class frequencies were high at the extreme low end of the range for *P. penetrans*. Correspondingly, selection for resistance to *P. vulnus* and *D. destructor* should be favorable, less so for *P. brachyurus*, and perhaps difficult for *P. penetrans* (although substantially less difficult than attempting to transfer resistant germplasm from another species into ponderosa pine). Since *P. ponderosa* appeared to have resistance to four parasitic nematodes, some resistance to other nematodes is probable, although a tree that is resistant to one kind of nematode is not necessarily resistant to other kinds. Nematodes are not mixed uniformly in nature; different species normally predominate in different areas. Nevertheless, the principle of selection for resistance of ponderosa pine to nematodes has been upheld; the outlook is extremely promising and consistent with the observation of Roth (14) with regard to the usefulness in breeding *P. ponderosa* for resistance to natural diseases. These experiments constituted a very elementary step; the bulk of the developmental work remains to be done. The argument of Tigerstedt (23) for application of the principle of ecological genetics merits serious consideration in any forest-tree breeding scheme; however, at least with nematodes, the simple fact remains that in any mature or overmature planting that is in near balance or decline, plant removal followed by replanting with the same or similar cultivars without nematode countermeasures of chemical or biological design, leads to an avoidable loss of growth.

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