

# Effect of Certain Nematodes on the Growth of *Pinus edulis* and *Juniperus monosperma* seedlings<sup>1</sup>

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**Abstract:** *Pinus edulis* and *Juniperus monosperma* seedlings were inoculated separately with each of seven nematode species, and grown for 9 months at 20 C soil temperature. *Hoplolaimus galeatus*, *Rotylenchus pumilis*, *Tylenchus exiguus*, and *Xiphinema americanum* parasitized *P. edulis* seedlings, but did not significantly reduce seedling growth. *Pinus edulis* was not a host for *Tylenchorhynchus cylindricus*, *Aphelenchoides cibolensis*, or *Criconeoides humilis*. *Xiphinema americanum* and *R. pumilis* parasitized *J. monosperma* seedlings, and reduced their root weights and root collar diameters. *Juniperus monosperma* was not a host for *A. cibolensis* and *T. exiguus*, and parasitism of this tree species by *T. cylindricus* and *C. humilis* remains uncertain. **Key words:** forest trees, tree seedlings, pine, juniper.

Many plant-parasitic nematode species have been found in the rhizospheres of *Pinus edulis* Engelm. and *Juniperus monosperma* (Ergelm.) Sarg. in central New Mexico (4). No information is available, however, on the effects of nematodes on the growth of these southwestern tree species. The objectives of my study were to determine the parasitism and pathogenicity of seven nematode species on *P. edulis* and *J. monosperma* seedlings.

## MATERIALS AND METHODS

The potting mixture used was composed of six parts forest soil, one part silica sand, and one part peat moss. The forest soil was a loam of granular structure derived from limestone parent material. The potting mixture was fumigated with ethylene dibromide (1,2-dibromoethane) at the rate of 56.1 liters/hectare (6 gal/acre) for 7 days in sealed containers, and aerated for 4 days. The fumigant killed all nematodes in the mixture.

Seeds of either *P. edulis* or *J. monosperma*, previously washed in flowing tap water for 48 hr and soaked in 30% hydrogen peroxide for 30 min, were planted in 1300 g of the potting mixture contained in each of 96 one-liter plastic pots—48 pots/tree species. Eight pots of each tree species were randomly placed in each of six temperature tanks. After 1 month, the seedlings were thinned to one/pot.

The seven nematodes used in the experiment were *Xiphinema americanum* Cobb, *Tylenchus exiguus* deMan, *Aphelenchoides cibolensis* Riffle, *Hoplolaimus galeatus* (Cobb) Thorne, *Rotylenchus pumilis* (Perry) Sher, *Tylenchorhynchus cylindricus* Cobb, and *Criconeoides humilis* Raski and Riffle. *Hoplolaimus galeatus* specimens were obtained from a population originally recovered from a forest stand in 1968 and maintained on *Pinus ponderosa* Laws. seedlings in a greenhouse. Inoculum of *A. cibolensis* was obtained from a population isolated from a *Juniperus deppeana* Steud. rootlet in 1965 and maintained on cultures of *Suillus granulatus* (Fries) Kuntze. This ectomycorrhizal fungus was isolated in 1967 from tissue plantings of a sporocarp collected at Bandelier National Monument near Los Alamos, New Mexico. The remaining five species were recovered from soil collected from a *P. ponderosa* stand in central New Mexico.

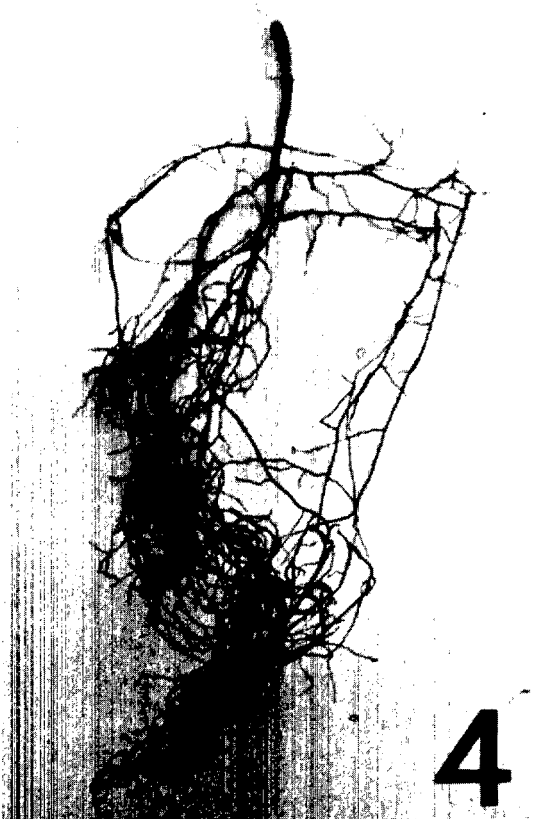
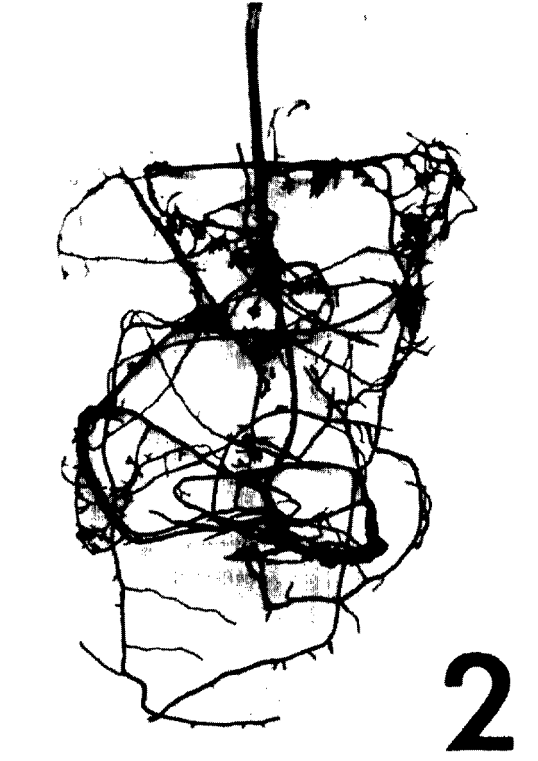
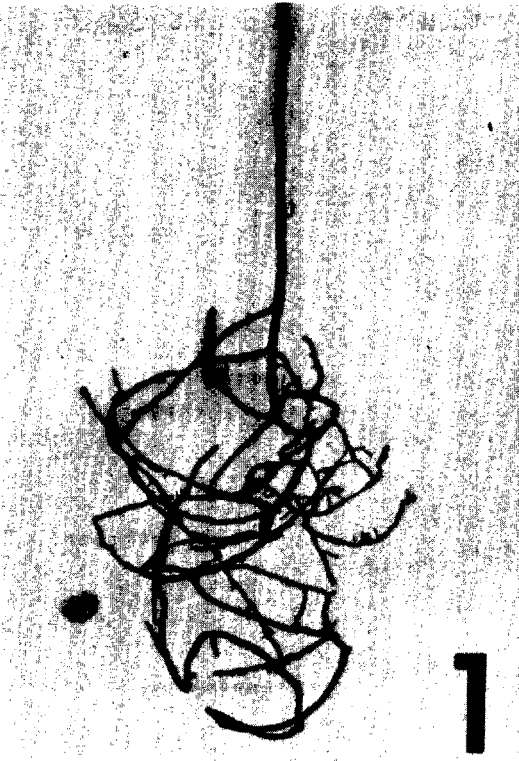
Nematodes were added to the seedlings 1 to 4 weeks after thinning by making two holes (5 mm X 150 mm) in the mixture near the root collar of each seedling, and adding a water suspension of 600 nematodes of one species. Each nematode species was replicated six times on each tree species. Water decanted from beakers containing each nematode species was added to six pots of each tree species to serve as controls.

The experiment was continued for 9 months. Seedlings were grown under artificial light (2000 ft-c) at a day-length of 16 hr. Ambient temperatures averaged about 23 C at foliage level, but ranged from 6 to 36 C during the experiment. The wide temperature range occurred as a result of radiant heat from daily cycling of the lights. Soil temperatures were maintained at 20 ± 1 C. The average moisture content at -0.1 bar matric potential (an

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approximation of soil moisture content at field capacity) of 12 randomly selected soil samples was determined with a pressure plate apparatus (3), and the moisture content thus determined (23%) was re-established in all pots every 2-3 days by weighing.

Final nematode populations were determined by processing the potting mixture of each pot, including root rinse water, by the Cobb sifting and gravity method. Residue on the screen was collected in 100 ml of water and processed by the centrifugal flotation technique (1). The roots of all seedlings were misted under an intermittent mist (2) for a minimum of 7 days to recover nematodes.

The effects of the nematodes on seedling growth were determined by analyses of: (i) total stem length; (ii) oven-dry weight of stems; (iii) root-collar diameter; and (iv) oven-dry weight of the root systems. All weights were determined after oven-drying for 48 hr at 65 C.

## RESULTS AND DISCUSSION

*Rotylenchus pumilis*, *T. exiguus*, *X. americanum*, and *H. galeatus* parasitized *P. edulis* seedlings, but did not significantly reduce seedling growth (Table 1). The final mean populations of *X. americanum* and *H. galeatus* were less than the initial number added to each pot. However, reproduction occurred because gravid females and larvae of both species were recovered from the soil from all pots to which

they were added, and many *X. americanum* eggs were recovered from the soil from three of the six *X. americanum* pots. Population increases of this nematode species apparently were limited by reduced rootlet density, because seedlings in three of the pots containing these nematodes had few or no fine feeder rootlets (Fig. 1).

Bifurcate, double bifurcate, and coralloid forms of ectomycorrhizae (Fig. 2) were found on *P. edulis* seedlings in all treatments. Mycorrhizal formation was confirmed by examination of sections of several ectomycorrhizae under a microscope. Since the seedlings were grown in open pots, the fungal symbiont of the ectomycorrhizae remains unknown. Ectomycorrhizae on seedlings in pots containing the parasites were dark brown to black, similar to pine ectomycorrhizae formed by *Cenococcum graniforme* (Sow.) Ferd. and Winge, while those on control seedlings were predominantly white to light tan. The feeding of the nematode parasites might have been responsible for this discoloration.

*Pinus edulis* apparently was not a host for *C. humilis*, *T. cylindricus*, and *A. cibolensis* (Table 1) because low populations were recovered from all pots, no gravid females or larvae were found, and many of the *T. cylindricus* specimens recovered were starved. Since *A. cibolensis* has been reported to feed on 50 of 53 species of ectomycorrhizal fungi (5) and has

TABLE 1. Mean nematode populations and their effect on growth of *Pinus edulis* seedlings.

Nematode species	Final nematode population per pot <sup>a</sup>		Seedling growth <sup>b</sup>			
	Soil	Roots	Root collar diameter (mm)	Root weight (mg)	Stem weight (mg)	Stem height (mm)
<i>Rotylenchus pumilis</i>	1410	18	2.4	499	319	37
<i>Tylenchus exiguus</i>	778	—	2.5	511	358	37
<i>Xiphinema americanum</i>	535	—	2.3	477	301	39
<i>Hoplolaimus galeatus</i>	187	35	2.3	468	312	39
<i>Tylenchorhynchus cylindricus</i>	43	—	2.4	519	400	43
<i>Criconemoides humilis</i>	14	—	2.4	525	335	38
<i>Aphelenchoides cibolensis</i>	<1	—	2.6	591	355	43
Control	—	—	2.7	715	429	47

<sup>a</sup>Population after 9 months; initial nematode population was 600. Numbers are means of six replications.

<sup>b</sup>Means in columns 4 through 7 are not significantly different from each other at 5% level according to Duncan's multiple range test.



FIG. 1-4. Effect of *Xiphinema americanum* on *Pinus edulis* and *Juniperus monosperma* root systems after exposure for 9 months to an initial population of 600 nematodes: 1. *P. edulis* inoculated, no ectomycorrhizae and few feeder roots; 2. *P. edulis* uninoculated, many ectomycorrhizae; 3. *J. monosperma* inoculated, few fine roots; 4. *J. monosperma* uninoculated.

TABLE 2. Mean nematode populations and their effect on growth of *Juniperus monosperma* seedlings.

Nematode species	Final nematode population per pot <sup>a</sup>		Seedling growth			
	Soil	Roots	Root collar diameter	Root weight	Stem weight	Stem height
			(mm)	(mg)	(mg)	(mm)
<i>Rotylenchus pumilis</i>	1584	49	1.5b <sup>b</sup>	223bc	214ab	55a
<i>Tylenchus exiguus</i>	50	—	1.8a	253ab	293a	55a
<i>Xiphinema americanum</i>	621	—	1.3b	152c	117b	48a
<i>Hoplolaimus galeatus</i>	7	<1	1.5b	171bc	164b	47a
<i>Tylenchorhynchus cylindricus</i>	27	—	1.5b	206bc	184ab	47a
<i>Criconemoides humilis</i>	18	—	1.5b	180bc	196ab	46a
<i>Aphelenchoides cibolensis</i>	<1	—	1.8a	256ab	297a	53a
Control	—	—	1.9a	329a	292a	54a

<sup>a</sup>Population after 9 months; initial nematode population was 600. Numbers are means of six replications.

<sup>b</sup>Means followed by same letter are not significantly different from each other at 5% level according to Duncan's multiple range test.

the potential to feed on established ectomycorrhizae, it would appear that a population of this nematode should have been maintained on the *P. edulis* ectomycorrhizae. Perhaps these ectomycorrhizae produced metabolites that killed the nematode or inhibited its feeding or reproduction. It has been suggested that metabolic products might have been responsible for the development of limited populations of *A. cibolensis* on many root-inhabiting fungi (5).

Two nematode species entered the roots of *P. edulis* and *J. monosperma*. Low numbers of *R. pumilis* were recovered from all root systems of both tree species. In addition, low populations of *H. galeatus*, a species known to feed endoparasitically on pine roots (6), were recovered from all *P. edulis* root systems and from four of six *J. monosperma* root systems (Tables 1, 2).

*Xiphinema americanum* and *R. pumilis* fed and reproduced on *J. monosperma* and reduced root weights and root collar diameters (Table 2, Fig. 3, 4). Gravid females and larvae of both nematodes and eggs of *X. americanum* were recovered from the soil of all pots containing these nematodes. The feeding of *X. americanum* destroyed nearly all the fine rootlets on these seedlings (Fig. 3).

Seedlings of *Juniperus monosperma* did not support populations of *Aphelenchoides cibolensis* and *Tylenchus exiguus*; and they are not considered a host for these nematodes (Table 2).

*Juniperus monosperma* root weights and root collar diameters were reduced by *T.*

*cylindricus*, *C. humilis*, and *H. galeatus* even though their populations declined during the experiment (Table 2). A decline in nematode density may result when a host is intolerant and is severely damaged by a few nematodes. It is also possible that the populations declined as a result of low reproduction rates and heavy mortality after inoculation because of handling during the extraction period. Additional studies with a wide range of initial nematode densities must be made to clarify the effects of these nematodes on the growth of this tree species.

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