Reproduction of Mixed Populations of *Tylenchorhynchus clarus* and *Pratylenchus* spp. on 10 Host Plants

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ABSTRACT

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Tylenchorhynchus clarus increased more than 10-fold in 3 mo on wild ryegrass, sweet corn, and parsley and more than eightfold on tomato cv. V.F. 145 and Yellow Dock in unsterilized soil. Multiplication was greatest on alfalfa cv. Lahonton, at 20 C; this 15-fold multiplication was twice that at 25 or 30 C and was not accompanied by injury to alfalfa roots. *T. clarus* reproduced rapidly on alfalfa seedlings growing on agar medium under high light intensity but reproduced slowly under low light intensity, probably because seedling growth was reduced. *Pratylenchus* spp. (97% *P. thornei*) in the soil increased 57-, 35-, 34-, 25-, and 22-fold on wild ryegrass, tomato cv. Cherry, barley cv. Briggs, tomato cv. V.F. 145, and parsley, respectively.

Additional key words: host range, laboratory culture, *Pratylenchus thornei*, temperature effects

Although *Tylenchorhynchus clarus* Allen (1) has been associated with 120 plant species in California (10), little is known about the actual host range or pathogenicity of this plant-parasitic nematode. In two field trials in the Palo Verde Valley of California, growth of lettuce improved after soil fumigation to control *T. clarus* (9). Two of the most important crops with which *T. clarus* is associated are alfalfa and barley (10). This study was done to obtain more information about reproduction of *T. clarus* and its effects on these crops.

MATERIALS AND METHODS

Host range. The population of *T. clarus* used in our experiments was obtained from an alfalfa field north of Davis, California. The clay loam field soil (3) was well mixed and placed into clay pots 15 cm in diameter (1,200 cm^3 volume). The initial population of *T. clarus* in two 250-cc samples of naturally infested soil was determined using Cobb's sieving and gravity method (4).

The pots were sunk in pine shavings to maintain favorable temperatures (18–24 C). Alfalfa (*Medicago sativa* L. cv. Lahonton and Moapa), tomato (*Lycopersicon esculentum* Mill., cv. Cherry, V.F. 145, and V.F.), barley (*Hordeum vulgare* L. cv. Briggs), sweet corn (*Zea mays* L. cv. Saccharata), parsley (*Petroselinum crispum* cv. Hamburg), Yellow Dock (*Rumex* spp.), and wild ryegrass (*Lolium* sp.) were planted five seeds per pot. Plants were thinned to one to three per pot depending on the species. A fallow treatment was included to evaluate survival of nematodes in the absence of host plants. There were seven replicates of the 11 treatments.

In this and other greenhouse experiments, the plants were weeded frequently, irrigated with distilled water, and fertilized with Ra-Pid-Gro soluble plant food (Ra-Pid-Gro Corp., Danville, NY). White flies and mites were controlled by regular sprays with Malathion 50. The initial population of *T. clarus* was 500 nematodes per pot.

After 3.5 mo of growth, plant roots were removed from the soil, which was then mixed well for uniformity, and nematodes were recovered from a 250-cc sample using Cobb's method (4). Counts for 250 cm^3 of soil were multiplied by the appropriate factor to obtain the total nematode population per pot. Student's *t* test was used to compare the mean populations for plants and the fallow treatment. Two *Pratylenchus* spp. (approximately 97% *P. thornei* and 3% *P. minyus*) were in this field soil. *Pratylenchus* in the soil around plants in this test were counted at the end of the experiment.

Effects of soil temperature. Nine 15-cm diameter clay pots, filled with field soil naturally infested with *T. clarus* and seeded with alfalfa cv. Lahonton, were placed in crocks immersed in water maintained at 20, 25, and 30 C in three constant temperature tanks (5). After 3.5 mo, fresh weights of roots and nematode populations were determined.

Laboratory culture. Alfalfa cv. Moapa seedlings were grown on the medium used by Lownsbery et al (6) except that vitamin stock and hormones were deleted. Alfalfa seeds were sterilized with 95% ethanol for 5 min, then rinsed twice with sterile distilled water. Seeds were then treated with 0.001 gm/ml of mercuric chloride for 7 min and washed twice with sterile distilled water. Approximately 20 surface-sterilized seeds were added to 150-ml capacity autoclaved Duraglas jars, each containing 40 ml of medium.

Seedlings were allowed to grow for 2 wk before inoculation with handpicked axenic females of *T. clarus*. Separatory funnels linked vertically (8) were used for axenization. The nematode suspension passed through the top funnel, which contained 133 ppm Aretan, a 3% organic mercury formulation (Plant Protection Ltd., Yalding, Kent, England), and was held for 12 hr in the second funnel, which contained penicillin G (625 units/ml). The suspension was removed from the receiver tube at the bottom of the apparatus and shaken for homogeneity. A sterile 5-ml syringe was used to transfer 1 ml of the nematode suspension

Table 1. *Tylenchorhynchus clarus* and *Pratylenchus* in naturally infested soil after 3-mo

Plant	Number per pot[a]	
	T. clarus	*Pratylenchus* spp.
Wild ryegrass	8,472 ± 1,251 x	2,870 ± 860
Sweet corn cv. Golden Blend	7,654 ± 481 x	571 ± 307
Parsley cv. Hamburg	7,139 ± 978 x	1,079 ± 393
Tomato cv. V.F. 145	5,124 ± 855 y	1,236 ± 322
Yellow Dock	5,090 ± 739 y	594 ± 148
Barley cv. Briggs	3,743 ± 254 z	1,700 ± 391
Tomato cv. Cherry	2,514 ± 640 z	1,747 ± 1,630
Alfalfa cv. Moapa	2,487 ± 330 z	700 ± 208
Tomato cv. V.F.	1,836 ± 206 z	722 ± 373
Alfalfa cv. Lahonton	1,545 ± 251 z	64 ± 39
Fallow	295 ± 60 z	0 ±

[a] Average of seven replicates and standard error. In each column averages followed by the same letter do not differ significantly ($P = 0.05$). Initially, each pot contained approximately 500 *T. clarus* and 50 *Pratylenchus*. Nematodes were extracted only from soil, not from roots.

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Table 2. Numbers of *Tylenchorhynchus clarus* per pot and fresh weights of roots of alfalfa cv. Lahonton after 3.5 mo of growth

Temperature (C)	*T. clarus* per pot[a]	Root weight (g)
20	9,189 ± 2,059 y	9.3 y
25	4,088 ± 2,569 z	10.4 y
30	4,689 ± 2,050 z	9.1 y

[a] Average of nine replicates and standard error. In each column averages followed by the same letter do not differ significantly ($P = 0.05$). Initially, each pot contained 500 *T. clarus*.

(approximately 50 *T. clarus*) to each jar. By this procedure some Aretan and penicillin G was transferred to the alfalfa cultures and may have inhibited growth of undesired microorganisms in the cultures. During inoculation, two 1-ml samples were taken from the suspension for nematode counts.

All jars were placed on a laboratory bench at approximately 23 C, with light from a nearby window ranging from about 0 to 12 lux. After 6 wk, half the jars were placed under a fluorescent light bank where the temperature was approximately 29 C and the incident light intensity (night vs. day) during storage ranged from 12 to 23 lux. Lights were on 24 hr a day. After 4 mo, *T. clarus* were extracted from each jar under a heated intermittent mist (7) and counted.

RESULTS AND DISCUSSION

Host range. Wild ryegrass, sweet corn, and parsley were the best hosts (Table 1). On these plants, *T. clarus* increased more than 10-fold in 3 mo. The next best hosts were tomato, cv. V.F. 145, and Yellow Dock, on which *T. clarus* increased more than eightfold. When Duncan's multiple range test was used to separate means, the poorer hosts were the same group as the fallow treatment (Table 1). The mean for any plant, however, was greater ($P < 0.01$) than for the fallow.

The *Pratylenchus* spp. (largely *P. thornei*) present, initially in small numbers, reached a large population level in the soil around some plants (Table 1) and could have had a competitive influence on *T. clarus*. However, the largest soil populations of *Pratylenchus* were around wild ryegrass, one of the best hosts for *T. clarus*.

Effect of soil temperature. Greatest multiplication (15-fold) of *T. clarus* on alfalfa cv. Lahonton occurred at 20 C, the lowest temperature tested (Table 2), and perhaps the optimum temperature for this nematode is lower than 20 C. Although the *T. clarus* population attained in 3.5 mo at 20 C was twice that at 25 or 30 C, the final weights of alfalfa roots at the three temperatures did not differ and injury by *T. clarus* was not detected. Because uninfected controls at these same temperatures were not included, conclusions regarding pathogenicity cannot be definite.

Tylenchorhynchus spp. are generally believed to have a simple, browsing, ectoparasitic relation with their host, resulting in few diagnostic symptoms and often less pathogenicity than species in other genera (2). Nevertheless, some are pathogens (2), and *T. clarus* might have a detrimental effect on alfalfa over a longer period than we studied.

Laboratory culture. *T. clarus* increased on alfalfa seedlings growing on the agar medium. More nematodes developed under higher light intensity (4,119 nematodes per jar). This was probably due in part to better seedling growth at the higher light intensity; better growth was noted, although seedlings were not weighed. Seedling growth at the higher light intensity was no more than twice that at the lower intensity, but the *T. clarus* increase at the higher light intensity was approximately nine times that at the lower intensity. At lower light intensity, the nematode reproduced slower (466 nematodes per jar), probably because plants were smaller.

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