Effects of Temperature on the Pathogenicity of Tylenchorhynchus clarus to Alfalfa and Observations on Feeding[1]

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Abstract: The involvement of *Tylenchorhynchus clarus* in plant disease is reported. Addition of a suspension of surface-axenized nematodes reduced top and root growth of alfalfa. Reproduction of *T. clarus* was greater at 24 and 27 than at 21 C. The interaction of nematodes with temperature did not produce significant effects on alfalfa growth in the 4.5-mo experimental period. *T. clarus* fed endo- and ectoparasitically. *Key Word:* stunt nematode.

Tylenchorhynchus clarus Allen is widely distributed in California, where it is associated with many crops (11). Although *T. clarus* has received scant attention it appears to be worldwide in occurrence (3, 6, 11). Radewald et al. (9) observed an increase in head weight of lettuce (*Lactuca sativa* L.) in *T. clarus*-infested fields following fumigation with 1,3-dichloropropene but not with 1,2-dibromoethane even though the population densities of *T. clarus* were reduced by both fumigants. *T. clarus* did not reduce the growth of lettuce in the greenhouse (9). This species is one of the nematodes most frequently recovered from alfalfa fields in California (11). This study was initiated to determine the effects of temperature on the pathogenicity of *T. clarus* to alfalfa (*Medicago sativa* L.).

MATERIALS AND METHODS

A two-factor factorial experiment was designed to test the effect of two levels of *T. clarus* at three temperatures on the growth of 'Moapa 69' alfalfa. Seeds were planted in 1.2-liter pots containing a heat-treated sandy loam consisting of 78% sand, 14% silt, and 8% clay. Seedlings were thinned to four/pot soon after emergence. When they were 1 mo old the soil was infested with 1,800 *T. clarus*/pot. An equal number of uninfested pots served as controls. Centrifugal flotation was used to extract nematode inoculum from cultures on alfalfa growing in the greenhouse (5). Before being inoculated the nematodes were axenized for 12 hr in a solution containing Aretan at 130 μg/ml (3% w/w mercury; Plant Protection Ltd. Yalding, Kent, England) and dihydrostreptomycin sulfate at 6×10^4 μg/ml. Pots were placed in temperature tanks at 21, 24, and 27 C. There were six replicates of both the nematode-infested and uninfested pots at each temperature. Supplemental incandescent lighting of 2,700 lux (measured at the soil surface) with a 13-hr photoperiod was supplied during the period of short day lengths. The plants were fertilized regularly with a 7% N, 6% P_2O_5, 19% K_2O fertilizer containing no urea and little ammonia, which have been shown to injure nematodes in greenhouse cultures (8).

During the 4.5-mo experimental period, three cuttings were harvested. Plants were cut, 5 cm above the crown, when the first bloom appeared on any plant in the experiment. Harvest of tops at first bloom allowed for maximum growth and storage of root reserves and enabled the plants to be cut at a uniform physiological age rather than a chronological age (12). Final nematode populations were extracted from the soil and root washings by centrifugal flotation. Roots were cultured for fungi. Root sections were placed in 0.5% NaOCl for 20 sec, rinsed in sterile water, blotted dry, and cultured on 2% water agar, potato-dextrose agar (PDA), PDA acidified with lactic acid, and a medium selective for *Pythium* and *Phytophthora* (7).

To determine the feeding sites of *T. clarus*, 2-day-old alfalfa seedlings growing in sand in a petri dish were inoculated and placed at 27 C. After 72 hr, hot lactophenol containing acid fuchsin was poured into the dish to kill the nematodes *in situ*.

RESULTS

At all temperatures *T. clarus* suppressed

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the top, root, and total weights of 'Moapa 69' alfalfa below those of uninoculated controls (Table 1). Top weights and total plant weights increased with temperature, but root weight was not affected by the range of temperatures studied. The nematode X temperature interaction was not statistically significant since the reduction in plant growth caused by *T. clarus* was similar at all temperatures (Table 1).

Nematode feeding resulted in fewer feeder roots (Fig. 1). No lesions or other abnormalities were observed. Nematode populations had increased to 5,580 ± 1556 at 21 C. This was smaller ($P < 0.05$; using independent comparisons) than the populations of 15,133 ± 5220 and 17,433 ± 5053 respectively recovered at 24 and 27 C. There was no difference between the final populations at 24 and 27 C.

Nematodes were killed *in situ* on and in roots of the alfalfa seedlings. They were most abundant in the zone of differentiation, but were observed also in the zone of elongation and the meristematic region. Nematodes that penetrated the roots did so primarily in the zone of differentiation, with one-third to one-half of their bodies inside the root, whereas others were entirely within the roots (Fig. 2). Fewer than 10% of the nematodes observed had completely penetrated the root.

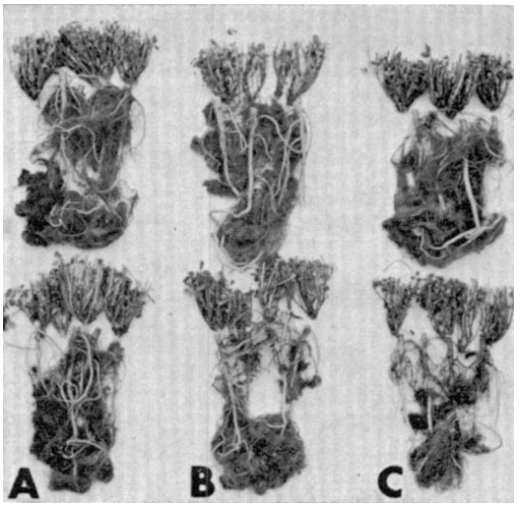

FIG. 1-(A-C). Roots and stubble of 'Moapa 69' alfalfa 4.5 months after inoculation with *Tylenchorhynchus clarus* at soil temperatures of 27, 24, or 21 C. *A)* 27 C. *B)* 24 C. *C)* 21 C. Uninoculated controls are the top members of each pair, and nematode-inoculated plants are the bottom members.

TABLE 1. Effects of soil temperature and *Tylenchorhynchus clarus* on growth of 'Moapa 60' alfalfa.

Temperature (C)	Nematode inoculation	Fresh weight (gm)[a]		
		Tops[b]	Roots	Total plant[c]
21	−	47.3 ± 2.5	30.1 ± 2.0	87.7 ± 3.8
21	+	35.9 ± 3.2	21.8 ± 3.5	66.4 ± 7.4
24	−	53.2 ± 3.0	29.2 ± 3.0	92.4 ± 5.1
24	+	46.9 ± 2.8	21.7 ± 2.0	78.0 ± 5.1
27	−	65.6 ± 2.7	32.1 ± 3.3	109.5 ± 5.3
27	+	50.4 ± 4.0	22.3 ± 2.9	82.1 ± 7.6

Analysis of variance:

Source of variation	df	Mean squares		
		Tops	Roots	Total plant
Treatments	5	563.71**	138.02*	1269.69**
A = *T. clarus*	1	1082.41**	662.24**	3971.51**
B = Temperature	2	808.75**	9.72	1059.14*
Interaction A X B	2	59.32	4.22	125.34
Error	30	55.72	48.70	206.50

[a]Mean and standard error of 6 replications.
[b]Total of three cuttings.
[c]Total of three cuttings, roots, and stubble.
*$P < 0.05$.
**$P < 0.01$.

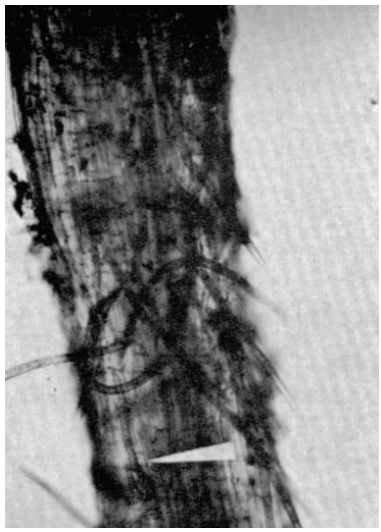

FIG. 2. Root segment of a 'Moapa 69' alfalfa seedling infected with *Tylenchorhynchus clarus*. One nematode is completely within the root (arrow).

Fungi isolated from roots of infected and uninfected plants were: *Trichoderma viridae* Pers., *Alternaria alternata* (Fr.) Keissler, *Penicillium* spp., *Fusarium solani* (Mart.) Appel & Wr. emend Snyd. & Hans., *Fusarium oxysporum* Schl. emend Snyd. & Hans., *Epicoccum nigrum* Link, *Aspergillus* sp., *Paecilomyces* sp., and *Chaetomium globosum* Kunze ex Fr. All of the fungi isolated are recognized as common soil saprophytes. The incidence of the fungi was the same for nematode-infected and uninfected plants except for *E. nigrum*, which was recovered from a nematode-infected plant grown at 21 C.

DISCUSSION

Although generally considered to be weakly pathogenic, some species of *Tylenchorhynchus* are harmful to grasses (1, 15) and others are associated with diseases of dicotyledonous plants (4, 10). The present study demonstrated the involvement of *T. clarus* in disease of alfalfa.

Species of *Tylenchorhynchus* and related genera generally vary in their feeding behavior from cursory browsing to semi-endoparasitism with their heads embedded in the root (2). They have seldom been observed feeding endoparasitically. Steiner (14) reported *Tylenchorhynchus claytoni* Steiner to be an endoparasite of tobacco (*Nicotiana tabacum* L.), and Wasilewska (15) recovered *Merlinius brevidens* (Allen) Siddiqi from roots of alfalfa. In the present study *T. clarus* was found feeding endoparasitically as well as ectoparasitically. This finding was made after final populations were determined by extraction from soil and root washings without incubation of the roots for extraction of endoparasites. Therefore, final populations were undoubtedly larger than indicated.

The higher numbers of *T. clarus* obtained at 24 and 27 C are a result of the direct effect of temperature on the nematode rather than an effect of the availability of food, since roots weights were not affected by temperature.

LITERATURE CITED

1. BIRCHFIELD, W., and W. J. MARTIN. 1956. Pathogenicity on sugarcane and host plant studies of a species of Tylenchorhynchus. Phytopathology 46:277-280.
2. BRIDGE, J., and N. G. M. HAGUE. 1974. The feeding behaviour of Tylenchorhynchus and Merlinius species and their effect on growth of perennial ryegrass. Nematologica 20:119-130.
3. ELMILIGY, I. A. 1969. Redescription of Tylenchorhynchus clarus Allen, 1955. Nematologica 15:288-290.
4. GOWEN, S. R. 1971. Tylenchus emarginatus and Tylenchorhynchus dubius associated with Sitka spruce (Picea sitchensis) seedlings. Pl. Path. 20:69-72.
5. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48:692.
6. KNOBLOCH, N. A. 1975. Quinisulcius tarjani sp. n. (Nematoda: Tylenchorhynchinae) with key to Quinisulcius species and notes on other plant-parasitic nematodes from Mexico. Proc. Helminthol. Soc. Wash. 42:52-56.
7. MIRCETICH, S. M., and H. W. FOGLE. 1969. Role of Pythium in damping-off of peach. Phytopathology 59:356-358.
8. MOJTAHEDI, H., and B. F. LOWNSBERY. 1976. The effects of ammonia-generating fertilizer on Criconemoides xenoplax in pot cultures. J. Nematol. 8:306-309.
9. RADEWALD, J. D., P. G. MOWBRAY, A. O. PAULUS, F. SHIBUYA, and J. M. RIBLE. 1969. Preplant soil fumigation for California head lettuce. Plant Dis. Rep. 53:385-389.
10. ROSS, J. P., C. J. NUSBAUM, and H. HIRSCHMANN. 1967. Soybean yield reduction by lesion, stunt, and spiral nematodes. Phytopathology 57:463-464 (Abstr.).
11. SIDDIQUI, I. A., S. A. SHER, and A. M. FRENCH. 1973. Distribution of plant parasitic nematodes in California. State of Calif., Dept. of Food and Agric., Div. of Plant Ind. 324 p.

12. SMITH, E. 1972. Cutting schedules and maintaining pure stands. Pages 481-496 *in* C. H. Hanson, ed. Alfalfa science and technology. Am. Soc. Agron., Madison, Wisc.
13. SMOLIK, J. D., and R. B. MALEK. 1973. Effect of Tylenchorhynchus nudus on growth of Kentucky bluegrass. J. Nematol. 5:272-274.
14. STEINER, G. 1937. Opuscula miscellanea nematologica, V. (1) Tylenchorhynchus claytoni, n. sp., an apparently rare nemic parasite of the tobacco plant. Proc. Helminthol. Soc. Wash. 4:33-34.
15. WASILEWSKA, L. 1967. Analysis of the occurrence of nematodes in alfalfa crops. I. Species composition of nematodes in two alfalfa crops of different age and penetration of species from soil to plants. Ekol. Pol. (Series A) 15:31-74.