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Population Response to Temperature in the Subfamily Tylenchorhynchinae[1]

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Abstract: The effects of temperature on population development of 11 species of stunt nematodes in the subfamily Tylenchorhynchinae were compared on red clover or Kentucky bluegrass in constant-temperature tanks at 5-degree intervals from 10 to 35 C. The optimum temperature for population increase on red clover in 90 days was 30 C for *Tylenchorhynchus agri, T. nudus, T. martini,* and *T. clarus,* 25 C for *T. sylvaticus* and *T. dubius,* and 20 C for *T. canalis, Merlinius brevidens,* and *Quinisulcius capitatus.* The optimum was 30 C for *T. robustoides* and 25 C for *T. maximus* on Kentucky bluegrass. The temperature range for population increase was 20–35 C for *T. agri, T. nudus, T. martini,* and *T. clarus,* 20–30 C for *T. sylvaticus* and *T. robustoides,* 15–25 C for *T. maximus,* 10–25 C for *T. dubius,* and 10–20 C for *M. brevidens* and *Q. capitatus. T. canalis* increased only at 20 C. All species were recovered in numbers near their inoculum level at 10 C. There was no survival of *T. sylvaticus, T. dubius, T. canalis, T. robustoides, T. maximus, M. brevidens,* and *Q. capitatus* at 35 C, or of the last three of these species at 30 C. Temperature had no effect on sex ratio in final populations. Population increase was greatest in *T. martini* and least in *T. canalis. Key Words:* stunt nematodes, *Tylenchorhynchus, Merlinius, Quinisulcius,* red clover, Kentucky bluegrass, population development.

Numerous species of stunt nematodes in the subfamily Tylenchorhynchinae occur in soils of the North Central region of the United States (14, 21). Little attention has been given to the host-parasite relationships and ecological requirements of these nematodes, although certain species are frequently encountered in abundance and in polyspecific communities in the rhizosphere of herbaceous plants.

While most of the native species of stunt nematodes are easily cultured on several mutual hosts, marked population fluctuations that vary with the species and season of the year have been noted by the author during attempts at increasing greenhouse populations for biological studies. Moreover, in Illinois certain species that are common associates of herbaceous perennials, such as turfgrasses and clovers, are seldom encountered on the major annual row crops corn and soybeans, which are suitable hosts under greenhouse conditions. Other species are frequent associates of both crop types. The reasons for those interspecific distributional differences are unknown, although differences in soil temperature regimes between crop types may be partially responsible.

Temperature requirements among stunt nematodes are known only for certain populations of *Tylenchorhynchus clarus* (13), *T. claytoni* (11), *T. dubius* (3, 15) and *T. nudus* (18, 20). This paper presents results of studies of the temperature preferences of 11 species of stunt nematodes apparently indigenous to all or part of the North Central United States.

MATERIALS AND METHODS

Species of stunt nematodes studied, and their original sources, were: *Tylenchorhynchus agri* Ferris, soybean (*Glycines max* [L.] Merr.), Marion County, Illinois; *T. canalis* Thorne and Malek, western wheatgrass (*Agropyron smithii* Rydb.), Pennington Co., South Dakota; *T. clarus* Allen, creeping bentgrass (*Agrostis palustris* Huds.), Hamilton Co., Ohio; *T. dubius* Bütschli, Kentucky bluegrass (*Poa pratensis* L.), Champaign Co., Ill.; *T. martini* Fielding, oats (*Avena sativa* L.), Champaign Co., Ill.; *T. maximus* Allen, Kentucky bluegrass, Champaign Co., Ill.; *T. nudus* Allen, soybean, Brookings Co., S. Dak.; *T. robus-*

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toides Thorne and Malek, western wheatgrass, Pennington Co., S. Dak.; *T. sylvaticus* Ferris, sugar maple (*Acer saccharum* Marsh), Champaign Co., Ill.; *Merlinius brevidens* (Allen) Sidiqi, corn (*Zea mays* L.), Pope Co., Ill.; and *Quinisulcius capitatus* (Allen) Siddiqi, Kentucky bluegrass, Champaign Co., Ill. All but two species were cultured on 'Kenland' red clover (*Trifolium pratense* L.) in a greenhouse and tested on that host. *T. maximus* and *T. robustoides* were studied on 'Newport' Kentucky bluegrass, because they failed to increase on red clover in repeated tests.

Plants were grown in 10-cm-diam plastic pots containing 500 cm^3 of Sparta loamy fine sand. A 15-ml centrifuge tube was pressed 5 cm into the soil in the center of each pot at planting. Clover seeds were planted in soil infested with commercial *Rhizobium* inoculant, and the seedlings were later thinned to four/pot. Pots for bluegrass were sown with 100 seeds apiece.

Nematodes for inoculation were extracted by a modification of the method of Christie and Perry (6). Two weeks after seeding, a 25-ml suspension of 200 nematodes of the appropriate species in all developmental stages was poured into the hole resulting from removal of the tube. The hole was filled with moist soil, and the pot was watered gently. The pots were arranged randomly on a greenhouse bench where ambient temperatures averaged 25 C.

Three days after inoculation, each pot was placed in an impervious plastic container. The rim of the pot was 2 cm below that of the container and flush with its side. Four replications for each nematode species were placed randomly in each of six Cornell-type constant-temperature tanks (10) so that the soil line was 2 cm below the water level. The tanks were maintained at 10, 15, 20, 25, 30, or 35 $\pm$ 1 C. The pots received 50 ml of a 23% N–19% P_2O_5–17% K_2O fertilizer solution at days 0 and 45 and 100 ml of distilled water when the soil surface became dry. Natural lighting was supplemented with fluorescent to provide a 14-hr day length.

Tests of individual species were initiated consecutively so that they could be terminated at intervals of 3–4 days. Nematodes were extracted 90 days after inoculation by the same method used to obtain inoculum. Numbers/pot were estimated from counts of replicated one-ml aliquots of a 200-ml extract suspension or counted *in toto* when numbers were low. Plant shoots and root systems were over-dried at 80 C for 3 days and weighed for comparison of growth among temperatures.

RESULTS

There were distinct differences among the species in response to temperature and ability to utilize red clover as a host (Fig. 1). Temperature-related differences in population development included optimum for reproduction, range for reproduction, and survival at upper extremes.

Maximum population increases of *T. agri, T. nudus, T. martini, T. clarus,* and *T. robustoides* occurred at 30 C. There was little difference in final populations of *T. robustoides* between 25 and 30 C, indicating that its optimum may lie between those temperatures. Increases were greatest at 25 C in *T. sylvaticus, T. dubius,* and *T. maximus,* and at 20 C in *T. canalis, M. brevidens,,* and *Q. capitatus.*

T. agri, T. nudus, T. martini, and *T. clarus* increased in population over a range of 20–35 C, whereas *T. sylvaticus* and *T. robustoides* increased only between 20 and 30 C. Final populations were relatively low at 20 C in these species. In contrast, *M. brevidens* and *Q. capitatus* reproduced within the relatively low temperature range of 10–20 C. *T. dubius* and *T. maximus* required an intermediate range of 15–25 C, though the former increased in numbers slightly at 10 C. *T. canalis,* for which clover was a poor host, increased only at 20 C.

All species survived temperatures as low as 10 C, but populations increased at that temperature only in *T. dubius, M. brevidens,* and *Q. capitatus.* Populations of *T. agri, T. nudus, T. martini,* and *T. clarus* increased substantially at the relatively high temperature of 35 C. The last three of these species increased as well at that temperature as at 25 C. None of the other species survived at 35 C, where examination of sieve residues following extraction revealed only a few dead nematodes. At 30 C, there was little survival of *T. canalis* and *T. dubius,* and none of *T. maximus, M. brevidens,* and *Q. capitatus.*

Temperature had no significant effect on

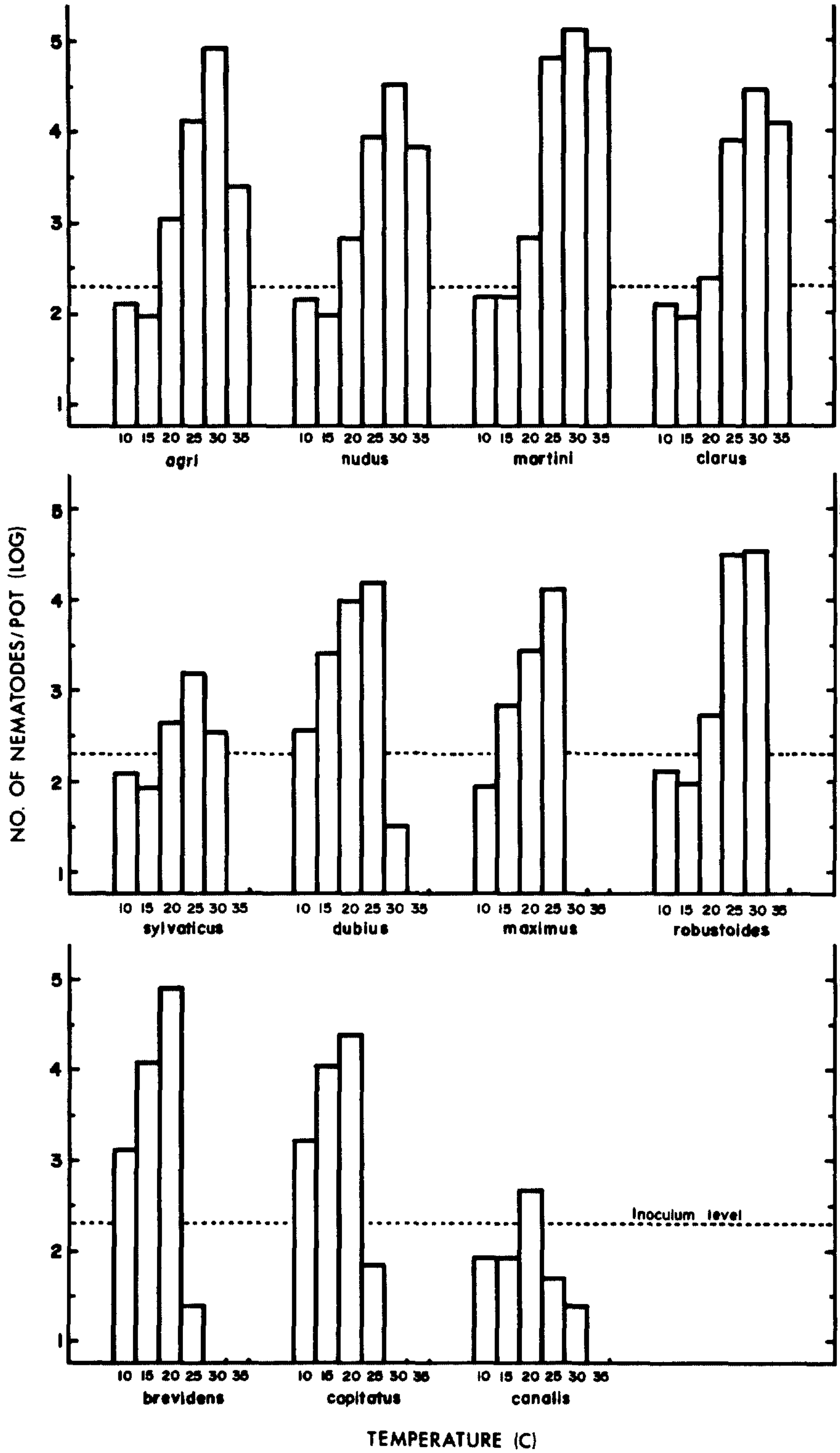

FIG. 1. Effect of temperature on population development of *Tylenchorhynchus agri, T. canalis, T. clarus, T. dubius, T. martini, T. nudus, T. sylvaticus, Merlinius brevidens,* and *Quinisulcius capitatus* on red clover, and of *T. maximus* and *T. robustoides* on Kentucky bluegrass.

the final adult population sex ratio, which was similar to that in the inoculum. The female:male ratio at the optimum temperature for each bisexual species was: *T. agri,* 2.0; *T. nudus,* 2.6; *T. sylvaticus,* 0.9; *T. dubius,* 1.0; *T. robustoides,* 1.1; *T. canalis,* 5.8; and *Q. capitatus,* 1.1. One male of *T. maximus* was noted at 20 C and two of *M. brevidens* at 10 C, but none were found at other temperatures or anywhere in the remaining monosexual species.

Populations of most species increased rapidly at and near their optimum temperatures. *T. martini* reached the highest level (125,000) of any species and *T. canalis* the lowest (475). Increases of the different species at their optima on red clover were: *T. martini,* 625×; *T. agri,* 430×; *M. brevidens,* 418×; *T. nudus,* 157×; *T. clarus,* 136×; *Q. capitatus,* 121×; *T. dubius,* 92×; *T. sylvaticus,* 9×; and *T. canalis,* 2×. *T. robustoides* and *T. maximus* populations respectively increased 171 and 65× on Kentucky bluegrass.

Shoot and root weights of red clover were highest at 20 C in all nematode treatments. Weights of Kentucky bluegrass were greatest at 25 C for shoots and at 20 C for roots. The optimum temperature for population development of *T. agri, T. nudus, T. martini,* and *T. clarus* was well above the optimum for plant growth. The optimum for other species was the same or only slightly above that for the host.

DISCUSSION

There was a relatively wide range of temperature preferences among the species of stunt nematodes, as well as some correlation between morphology and response to temperature. The group containing *T. agri, T. nudus, T. martini,* and *T. clarus,* which have smooth female tail tips, required relatively high temperatures for maximum reproduction. The optima for those species may be higher than 30 C, and the upper limit for reproduction appears to be above 35 C. Poor plant growth above 30 C, however, probably restricted feeding sites and thus the degree of population development. A second group, consisting of *T. dubius, T. maximus,* and *T. canalis,* with annulated female tail tips, preferred an intermediate temperature and could not survive high temperatures. *T. robustoides* and *T. sylvaticus* were exceptions to the morphological-physiological correlation. Although they are most similar to species in the first group, their temperature responses resembled those of the second group. *M. brevidens* and *Q. capitatus,* which differ from the *Tylenchorhynchus* spp. in having more than four lateral lines, required relatively low temperatures for reproduction and could not survive at or above 30 C. Those species appear to have a threshold for reproduction slightly below the 10 C limit of testing. This distinct physiological difference from the other species biologically supports the division of the genus *Tylenchorhynchus sensu lato* into the genera proposed by Siddiqi (16, 17).

The temperature requirements for population development of *T. nudus* and *T. dubius* were similar to those reported by other workers. Smolik (18) and Smolik and Malek (20) found that the optimum for the same population of *T. nudus* used in this study is also 30 C on sorghum (*Sorghum bicolor* Pers.) and spring wheat (*Triticum aestivum* L.). Sharma (15) reported that 25 C was the preferred temperature for a Netherlands population of *T. dubius* on ryegrass (*Lolium perenne* L.). Brzeski (3) indicated that 20 C was the optimum for a Polish population on cabbage (*Brassica oleracea* L.), a response that was probably related to the cool temperature requirements of the host. The reaction of the Ohio isolate of *T. clarus* to temperatures below 30 C was similar to that recorded for a California population of the species on alfalfa (*Medicago sativa* L.) by Noel and Lownsbery (13), whose upper limit of study was 27 C.

Results of this study appear to explain the difficulties encountered in increasing and maintaining cultures of many of these species in the greenhouse, where seasonal temperature fluctuations are often extreme. Several years of culturing at this laboratory have shown that species in the *T. agri* group increase very little during the winter, when soil temperatures often cannot be maintained above 20 C. In summer, these species can be increased rapidly to high population levels. The converse is true of *M. brevidens* and *Q. capitatus.* Large numbers can be obtained in winter, whereas populations drop to low levels during the summer when daily soil temperatures frequently rise above 30 C. Little difficulty has been encountered with the other species, although *T. canalis* and *T. sylvaticus* have been slow to increase on all of the several hosts tested earlier.

The differences among the species in response to temperature emphasize the need for adequate temperature control in biological studies of these nematodes, particularly in obtaining a true picture of their reproductive potentials and pathogenic capabilities. Suboptimum temperatures, at which population increase was slow and/or plants were tolerant of parasitism, may have been major factors in the inability of past research to demonstrate pathogenicity of species in the *T. agri* group on red clover (1, 2, 4). Relatively low temperatures may also have been a factor in some cases of nematode-induced stimulation of plant growth noted by several workers (1, 2, 5, 7, 12).

The results indicate also that differences in soil temperature regimes among crop types is a factor controlling intraregional distribution patterns of some species of stunt nematodes, particularly in dark-colored soils. The infrequent row-crop association of the low-temperature-requiring *M. brevidens* and *Q. capitatus* and other species that cannot withstand high temperatures may be due to the lack of insulation of the soil surface and upper levels of the profile during early stages of crop growth. The effect of insolation on much of the profile is enhanced by planting preparation and cultivation of fields before canopy formation in row crops. Members of the *T. agri* group apparently can withstand, and in fact seem in prefer, the high temperatures of this environment. Routine analysis of turf samples in Illinois have shown that these species seldom reach the population levels encountered in row crops in this area by the author and Ferris and Bernard (8, 9). Soil temperatures beneath turf in Illinois rarely rise above 25 C, which is suboptimum for reproduction of these species. Stunt nematode populations beneath this and other perennial cover crops are frequently dominated by *T. dubius, T. maximus,* and *Q. capitatus,* which prefer cooler soils. In eastern South Dakota, however, where temperatures beneath turf are somewhat higher in summer, the thermophilic *T. nudus* is an abundant associate of turfgrasses (19).

LITERATURE CITED

1. AMOSU, J. O., and D. P. TAYLOR. 1974. Interaction of Meloidogyne hapla, Pratylenchus penetrans and Tylenchorhynchus agri on Kenland red clover, Trifolium pratense. Indian J. Nematol. 4:124-131.

2. AMOSU, J. O., and D. P. TAYLOR. 1974. Stimulation of growth of red clover by Tylenchorhynchus agri. Indian J. Nematol. 4:132-137.

3. BRZESKI, M. W. 1970. The effect of temperature on development of Heterodera schachtii Schm. and Tylenchorhynchus dubius Büt. (Nematoda, Tylenchida). Roczn. Nauk Roln., Ser. E, 1(1):205-211.

4. CHAPMAN, R. A. 1959. Development of Pratylenchus penetrans and Tylenchorhynchus martini on red clover and alfalfa. Phytopathology 49:357-359.

5. CHAPMAN, R. A. 1963. Population development of Meloidogyne arenaria in red clover. Proc. Helminthol. Soc. Wash. 30:233-236.

6. CHRISTIE, J. R., and V. G. PERRY. 1951. Removing nematodes from soil. Proc. Helminthol. Soc. Wash. 18:106-108.

7. COURSEN, B. W. 1957. Biology and host-parasite relationships of the nematode Paratylenchus projectus. M.S. Thesis, Univ. Md., 41 p.

8. FERRIS, V. R., and R. L. BERNARD. 1961. Seasonal variations of nematode populations in soybean field soil. Plant Dis. Rep. 45:789-793.

9. FERRIS, V. R., and R. L. BERNARD. 1971. Crop rotation effects on population densities of ectoparasitic nematodes. J. Nematol. 3:119-122.

10. FERRIS, J. M., B. LEAR, A. W. DIMOCK, and W. F. MAI. 1955. A description of Cornell temperature tanks. Plant Dis. Rep. 39:875-878.

11. KRUSBERG, L. R. 1959. Investigations on the life cycle, reproduction, feeding habits and host range of Tylenchorhynchus claytoni Steiner. Nematologica 4:187-197.

12. MALEK, R. B., and W. R. JENKINS. 1964. Aspects of the host-parasite relationships of nematodes and hairy vetch. N. J. Agr. Exp. Sta. Bull. 813, 31 p.

13. NOEL, G. R., and B. F. LOWNSBERY. 1978. Effects of temperature on the pathogenicity of Tylenchorhynchus clarus to alfalfa and observations on feeding. J. Nematol. 10:195-198.

14. NORTON, D. C., O. J. DICKERSON, and J. M. FERRIS. 1968. Nematology in the North Central region, 1956-1966. N. C. Reg. Res. Public. 187, 20 p.

15. SHARMA, R. D. 1971. Studies on the plant parasitic nematode Tylenchorhynchus dubius. Meded. Landbouwhog. Wageningen 71(1):54-58.

16. SIDDIQI, M. R. 1970. On the plant-parasitic nematode genera Merlinius gen. n. and Tylenchorhynchus Cobb and the classification of the families Dolichodoridae and Belonolaimidae n. rank. Proc. Helminthol. Soc. Wash. 37:68-77.

17. SIDDIQI, M. R. 1971. Structure of the oesophagus in the classification of the superfamily

Tylenchoidea (Nematoda). Indian J. Nematol. 1: 25-43.

18. SMOLIK, J. D. 1977. Effects of Trichodorus allius and Tylenchorhynchus nudus on growth of sorghum. Plant Dis. Rep. 61:855-858.

19. SMOLIK, J. D., and R. B. MALEK. 1972. Tylenchorhynchus nudus and other nematodes associated with Kentucky bluegrass turf in South Dakota. Plant Dis. Rep. 56:898-900.

20. SMOLIK, J. D., and R. B. MALEK. 1972. Temperature and host suitability studies on Tylenchorhynchus nudus. Proc. S. Dak. Acad. Sci. 51:142-145.

21. THORNE, G., and R. B. MALEK. 1968. Nematodes of the northern Great Plains. I. Tylenchida (Nemata: Secernentea). S. Dak. Agr. Exp. Sta. Tech. Bull. 31, 111 p.