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

GREGORY R. NOEL and B. F. LOWNSBERY

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 X 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 fuchsins was poured into the dish to kill the nematodes in situ.

RESULTS

At all temperatures T. clarus suppressed...
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.

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

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Nematode inoculation</th>
<th>Fresh weight (gm)*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tops</td>
<td>Roots</td>
<td>Total plant</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>47.3 ± 2.5</td>
<td>30.1 ± 2.0</td>
<td>87.7 ± 3.8</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>35.9 ± 3.2</td>
<td>21.8 ± 3.5</td>
<td>56.4 ± 7.4</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>53.2 ± 3.0</td>
<td>29.2 ± 3.0</td>
<td>82.4 ± 5.1</td>
</tr>
<tr>
<td>24</td>
<td>+</td>
<td>46.9 ± 2.8</td>
<td>21.7 ± 2.0</td>
<td>68.6 ± 5.1</td>
</tr>
<tr>
<td>27</td>
<td>-</td>
<td>63.6 ± 2.7</td>
<td>32.1 ± 3.3</td>
<td>95.7 ± 5.3</td>
</tr>
<tr>
<td>27</td>
<td>+</td>
<td>50.4 ± 4.0</td>
<td>22.3 ± 2.9</td>
<td>72.7 ± 7.6</td>
</tr>
</tbody>
</table>

Analysis of variance:

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Tops</th>
<th>Roots</th>
<th>Total plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>5</td>
<td>563.71**</td>
<td>138.02*</td>
<td>1269.69**</td>
</tr>
<tr>
<td>A = T. clarus</td>
<td>1</td>
<td>1082.41**</td>
<td>662.24**</td>
<td>1747.65**</td>
</tr>
<tr>
<td>B = Temperature</td>
<td>2</td>
<td>808.75**</td>
<td>9.72</td>
<td>1059.14*</td>
</tr>
<tr>
<td>Interaction A X B</td>
<td>2</td>
<td>59.32</td>
<td>4.22</td>
<td>125.34</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>55.72</td>
<td>48.70</td>
<td>206.50</td>
</tr>
</tbody>
</table>

*Mean and standard error of 6 replications.

*Total of three cuttings.

*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, Penicilli um spp., Fusarium solani (Mart.) Appel & Wr. emend Snyder & Hans., Fusarium oxysporum Schl. emend Snyder & 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

Modification of a Computer Simulation Model for a Plant-Nematode System

H. FERRIS

Abstract: New data on egg development and death rates, and refinements of logic concerning interaction of the nematode and host, were incorporated into a simulation model of a Meloidogyne arenaria and grapevine system. Simulations of field data improved but other areas of weakness in the model were discovered. Two peaks in the egg population curve suggested that the nematode was able to complete two life cycles before host dormancy and declining temperatures limited physiological activity. Key Words: Meloidogyne arenaria, population dynamics, nematode-host interaction.

The development of a computer simulator (MELSIM) of a nematode-plant system (2, 3) has assisted in directing research efforts (4, 6). Experimentation with the simulator has exposed errors which required refinements in the logic of the model. The result is that the simulator must be updated periodically. It is hoped that the simulators predictive ability will improve as knowledge of the system is gained. This paper reports the effects of incorporating data on egg development and death rates (4) into the model, and of some logic refinements in the relationship of numbers of nematodes to plant damage and the influence of the physiological status of the host. Trial simulations are compared with field data (5).

Egg development and death rates: Experiments on Meloidogyne arenaria egg development relative to temperature (4) indicated the need for two regression models, one to describe the development and hatch rate of 74% of the egg population and the other to describe the slower hatch of the remainder. The simulator was modified to partition each cohort of newly deposited eggs into two developmental/hatch groups. Eggs developed according to a temperature-dependent rate (proportion of development completed per h):

\[ D = (25.5 T - 235)(10^{-6}) \]

Seventy-four percent of the eggs hatched at maturity while the remainder hatched at a rate (egg hatch/egg/h):

\[ H = (8T - 30.5)(10^{-9}) \]

Studies of the effects of temperature on egg death rate (4) were used to formulate models based on temperature and length of exposure. Death rates (deaths/egg/h) during the first week of exposure to sub-optimal temperature were described by:

\[ R = (459.5 - 38.5T + 0.9T^2)(10^{-6}) \]

and during subsequent exposures by:

\[ R = (10855.9 - 606.2T + 14.7T^2 + 20.85L^2 - 1.9TL^2 + 0.04T^2L^2)(10^{-7}) \]

where \( T \) = temperature and \( L \) = length of exposure in days.

Since temperature experiences of each daily cohort of eggs will vary, the deaths of each age group of eggs are determined individually. The data base for the models was developed at constant temperatures (4), and the simulations involve fluctuating temperature. It could be argued, therefore, that the temperature-death rate models are somewhat misused in the simulator since the age of an egg is not a measure of time.

Received for publication 17 October 1977.

1 Assistant Nematologist, Department of Nematology, University of California, Riverside, California 92521. Supported in part by USDA-CSRS Grant 316-15-63.