PATHOGENICITY AND SURVIVAL OF TYLENCHORHYNCHUS BREVIDENS AS RELATED TO ENVIRONMENT AND HOST DEVELOPMENT

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CHAPTER I

INTRODUCTION

There have been numerous reports (2,7,14,21) dealing with the effects of temperature on host-parasite relationships of various plants and several species of nematodes. As far as can be determined, there has been no report of the effects of host development as it relates to the expression of nematode pathogenicity.

Tylenchorhynchus brevidens Allen has been demonstrated to be pathogenic to small grains (12). It was suspected from this study that the ability of the nematode to induce disease symptoms, at least on wheat, was closely related to host development and temperature.

Knowledge concerning the pathogenic nature of T. brevidens with a susceptible host, is considered basic to an understanding of the host-parasite relationships involved with this nematode.

The objectives of the present investigation were:
(1) to determine the stage or stages in wheat development most vulnerable to the pathogenic activities of T. brevidens and to relate these activities to the environment, particularly temperature; (2) to determine the effect of host development and other seasonal factors on population trends of T. brevidens in the field; and (3) to try to increase this nematode in tissue culture for the purpose of obtaining a continuous source of aseptic inoculum.

CHAPTER II

REVIEW OF LITERATURE

The genus Tylenchorhynchus, described by Cobb (4) in 1913, has a worldwide distribution, although the known distribution of many individual species is very restricted (1). Representatives of the genus occur in most types of soil and are often very numerous (25). Some species have been shown to be important plant pests (10). The species, Tylenchorhynchus brevidens, with which this investigation is concerned, was described by Allen in 1955. However, relatively little has been published on the host-parasite relationships of this species.

Langdon et al. (12), in Oklahoma, reported T. brevidens as causing stunt symptoms of small grains. Norton (15), in Texas, found T. brevidens, usually in large numbers, associated with roots of small grains and grasses, but he was unable to obtain evidence that it was pathogenic. Oostenbrink et al. (18) reported T. brevidens as common root parasite on small grains in Dutch soils, but its significance was not demonstrated. Krusberg and Hirschmann (11) recovered large numbers of T. brevidens from soil samples taken in the middle of the dry season in mountains of Peru having an elevation of about 6562-16406 feet. The soil was reported as extremely hard and powdery dry. This nematode has been observed frequently in heavy clay soils (15).

Numerous studies have been reported on the effects of temperature

on the reactions of various crops to several species of nematodes. Dropkin (7) showed that temperature affected the ability of soybeans (Glycine max (L.) Merr.), and tobacco (Nicotiana tabacum L.) to support growth of Meloidogyne spp. When Meloidogyne incognita acrita Chitwood was inoculated on soybean, variety Adams, the namatodes developed to maturity at temperatures between 21.5° and 25°C in greater numbers than at temperatures between 31.5° and 35°C, but there were more larvae reaching maturity at the higher temperatures in the variety Chief. Flue-cured tobacco, var. 402, was found to be favorable a host at higher temperatures than tobacco from breeding line GH-90-193, and breeding line GH-60-1004. Dropkin concluded that temperature offers a convenient tool for control of host reaction in the studies of the nature of resistance. Presumably, metabolic differences in the soybean variety Chief, grown at different temperatures, accounts for its change from a poor host at low root temperature to a better host at the higher temperatures.

Lownsbery and Maggenti (14) reported an effect of soil temperature and soil moisture on population levels of Xiphinema americanum Cobb. They reported that X. americanum maintained itself at high population levels on Alpine strawberry (Fragaria vesca L,) and Mazzard cherry (Prunus avium L.) for 5 months at 21°C. Populations declined at 16°, 27°, and 32°C, and where temperature fluctuated with a mean of 27°C. When a 21°C temperature was coupled with low amplitude moisture fluctuation, populations of X. americanum had increased after 5 months on strawberry.

Response to the Lahontan variety of alfalfa (Medicago sativa L.) inoculated with Ditylenchus dipsaci (Kuhn) Filipjev was reported by Grundbacher and Stanford (8) to vary with temperature. This variety was more

resistant at 11°C than at 16° or 21°C; an alfalfa introduction from Iran and the variety Talent were resistant at all 3 temperatures.

In countries with a temperate climate, nematode populations in general often increase in the spring (22). Dieter, as cited by Wallace (22) considered that climatic conditions in the spring were not of primary importance. He suggested that the degree of development of the host plant determined the infection level of Heterodera avenae (Mortensen et al.) Filipjev in the host plant at a time when environmental conditions were optimal for larval hatch. Lownsbery (13) reported that the greatest injury to peach trees (vars. S-37 and Lovell) in California by species of Criconemoides occurred in winter and spring when the nematode population levels were highest. During summer and autumn the higher temperatures and drier conditions appeared to inhibit population increases.

Wallace (22) commented that as nematode population growth was determined by the rate of reproduction, which in turn, was influenced by the environment, it was not surprising that nematode populations in the field show wide fluctuations the year around and that the changes in population density vary from one year to another. Oostenbrink (17) stated that although there was a linear relationship between the logarithm of initial populations of Pratylenchus penetrans (Cobb) Filipjev and Stekhoven and growth reduction in susceptible crops, this relationship varies greatly with crop, soil, year and other factors affecting plant growth. Sasser and Nusbaum (20) reported that nematode populations fluctuate widely even with the same crop in the same season. This was shown for root-knot nematodes (M. incognita (Kofoid and White) Chitwood and M. arenaria (Neal) Chitwood) in 2-year tobacco rotation experiments. Such population changes

were often caused by weather conditions and may have affected the number of plant parasitic nematodes in the soil as well as in the host tissues.

Marked changes in nematode populations have often been associated with periods of rainfall. Seinhorst, as cited by Wallace (22), reported an increased activity of D. dipsaci as associated with high rainfall and moisture. Parris (19) considered that increased root-knot nematode (H. marioni (Cornu) Goody) activity and increased galling on potato (Solanum tuberosum L.) roots were related to increased soil moisture. In his studies he was able to obtain higher yields in fumigated as well as in infested soils, but the higher the soil moisture, the greater the injury to tubers in an infested soil through galling caused by H. marioni. In the presence of the nematode, in dry soil, yields were lower. Norton (15) reported populations of T. brevidens were at low levels during dry periods and increased in density only after rainfall.

Seasonal fluctuations in populations of an unidentified species of Tylenchorhynchus and T. martini Fielding in rice (Oryza sativa L.) were closely correlated with rainfall (9). Heavy rainfall or flooding was associated with low population levels and dry periods with high populations.

Wehunt (23) studied population trends of Tylenchorhynchus sp. on white clover (Trifolium repens L.) and found that the maximum nematode population coincided with maximum crop growth. Di Edwardo (5) studied soil populations of Pratylenchus penetrans in strawberry beds and found that numbers of nematodes reached a maximum in the soil in June and in the roots in July. At the end of July, the concentration of nematodes per unit volume of root decreased with increased root growth but increased in September with the invasion of new roots. The influence of environment on nematode populations was evident in the experiment of Dolliver (6) who

showed that the ability of P. penetrans to reproduce in peas (Pisum sativum L.), Wando variety, was related to the physiological status of the plant attacked. Treatments such as less favorable light and temperature, excision of plant parts and limitation of nutrient supply that reduced plant dry weight, increased nematode numbers. On the other hand, with treatments that reduced dry weight considerably, there were reduced numbers of nematodes. Dolliver concluded that population increased in various treatments indicated the intimate interaction between the parasite, host and environment.

Winslow (25), in summarizing host-parasite relationships as they pertain to nematodes, stated that the expression of host-parasite relationships was affected by such factors as the genetic characteristics of the host, the inherent parasitic abilities of the parasite, the inoculum potential, the number of parasites involved and environmental conditions.

CHAPTER III

MATERIALS AND METHODS

A wheat field of silt-clay loom soil about 3 miles west of the Oklahoma State University Campus, Stillwater, Oklahoma, was chosen as the site for field studies. All subsequent soil samples, unless otherwise specified, were taken from this field. Severe stunting of wheat and barley, restricted to localized spots, had been observed in this field for at least the preceding 2 growing seasons. Soil samples taken from around the roots of wheat where stunting was observed showed high populations of T. brevidens compared to samples taken from adjacent areas where apparently healthy wheat plants were growing. Extraction of nematodes from the soil was done by employing Seinhorst's inverted flask technique as modified by Chapman (3). However, a sieve with 30 μ openings was used instead of a sieve with 43 μ openings during the latter part of the investigation, after it had been found that an increased recovery of nematodes resulted by using 30 μ opening sieve

Fluctuations in nematode populations were followed in the field. Sampling for nematode counts was done approximately at monthly intervals. The soil samples were gathered at various points at random in the infested area, mixed thoroughly and the nematodes were extracted. Rainfall data were obtained from the Agronomy Farm, Oklahoma State University, about a mile and a half away from the experimental field.

To determine the longevity of T. brevidens in soil, in the absence of the host, samples of the infested field soil were brought to the laboratory. Without disturbing the lumps, the soil was divided into 2 equal lots and each lot was again subdivided into 3 parts, each of which was placed in a brown paper sack. One lot, consisting of 3 parts, was placed in the laboratory at a highly fluctuating temperature ranging from 70-90°F, while the other lot was placed in a room at a temperature ranging from 40°-45°F. No water was added to the soil throughout the duration of the experiment. The initial nematode population and the moisture content (dry weight basis) of the soil samples were determined just before the start of the experiment. Subsequent samplings for nematode counts and soil moisture determinations were done at approximately 2-month intervals.

Stock cultures of T. brevidens were started from handpicked individuals obtained from field soil and were maintained on wheat (variety Concho) grown in steamed soil in 6-in. clay pots in a room maintained at 70°F. Adequate precautions were taken to prevent contamination between pots. Further increase in nematode populations was obtained by using small amounts of infested soil or suspending the nematodes from the stock culture in water then adding these to steam sterilized soil with wheat. A 12-hr daylight photoperiod was maintained in the room by incandescent and flourescent lights, producing an intensity of about 2400 foot candles at the center of the bench. Incandescent lights were turned on a few minutes ahead of the flourescent lights by an automatic switch.

Since modifications in procedures in pathological studies were made from time to time to meet existing needs, these procedures, as well as

those of pathogenicity and tissue culture studies are presented in the section on Results.

CHAPTER IV

RESULTS

Field Observations

Stunting and yellowing of wheat and barley plants due to pathogenic activities of T. brevidens, have been observed in distinct patches in the field. These areas have been most noticeable when the plants were at the boot stage of development. Examination of the root systems of affected plants revealed a depletion in total root volume; the roots being shorter, having stubby tips, and fewer branch rootlets. The tissues were dark in color, often with the cortex sloughed off, and the roots appeared to be in a state of decay, presumably due to invasion by secondary organisms.

Fluctuations in the population of T. brevidens in the field over a 1-year period are shown in Fig. I. The population trends of the nematode followed closely the development of the host. The availability of adequate moisture in the later part of August allowed volunteer plants to grow. From a low nematode population after harvest, sampling showed an increase in nematode population when there were volunteer plants. When the field was plowed again and the volunteer plants removed, there was a decline in population. Increase in nematode numbers was again noted a few weeks after planting and germination. The trends of population increase over a period of one year were not affected by rainfall or by intermittent cold periods during

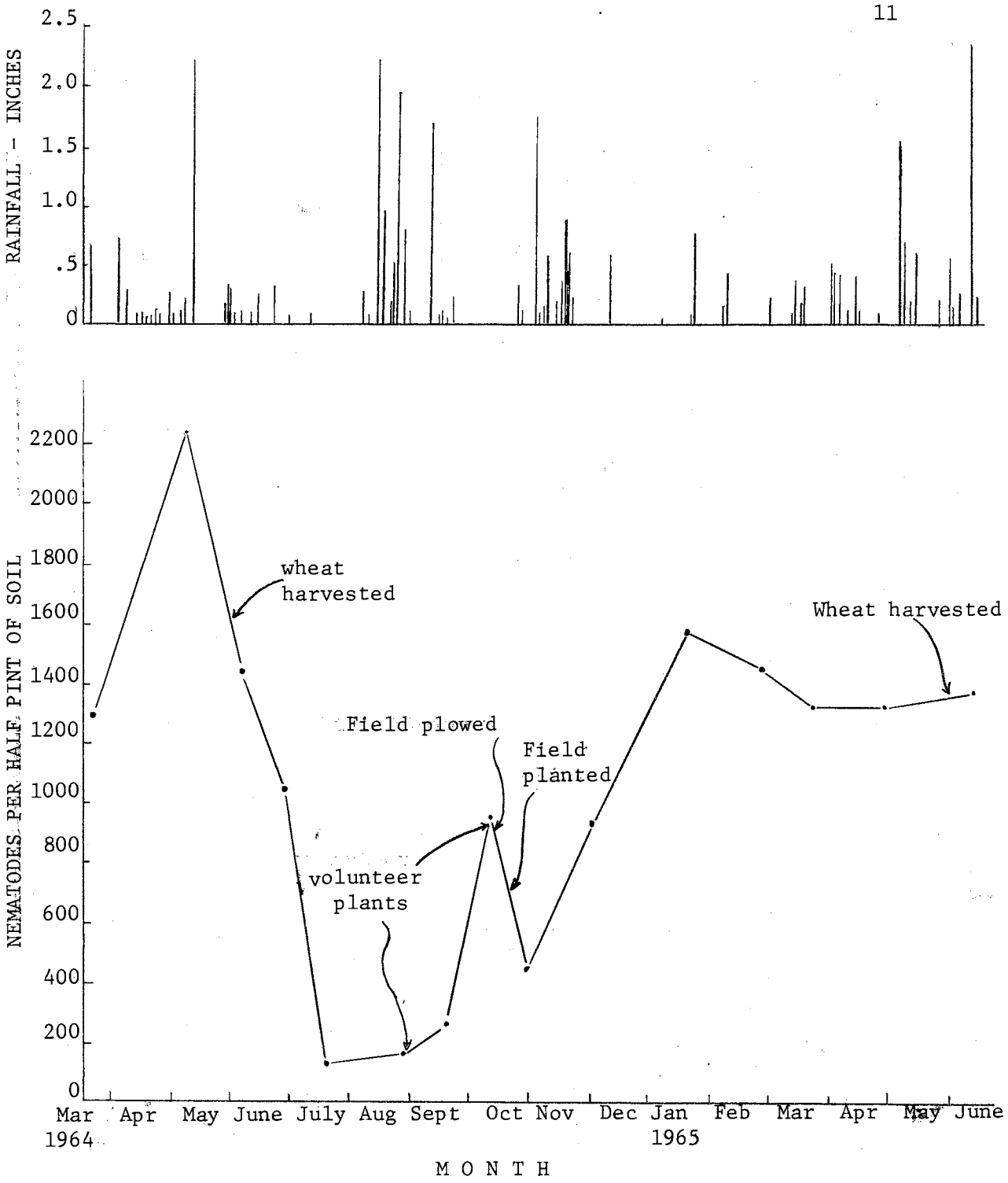


Fig. 1. Effect of soil moisture, seasonal changes, and the presence or absence of a host on population trends of Tylenchorhynchus brevidens in the field.

winter. A decline in populations was noted, only after the wheat plants were harvested.

Effects of temperature on T. brevidens in the laboratory

Infested field soil without a host was stored in the laboratory at 70-90°F for a period of 16 months. At the beginning of the storage period there was an average of 858 T. brevidens per half pint of soil. At that time the moisture content of the soil was 18.1% (dry weight basis). At the end of the storage period, the nematode population had dropped to an average of 58 nematodes per half pint of soil, and the soil moisture content was down to 0.41%.

In a separate set of observations, it was found that greater numbers of T. brevidens survived in the soil, in the absence of host, at the lower temperatures of 40-45°F than at 70-90°F during the 11 months period (Fig. 2). The moisture content of these samples after 11 months was 0.63% at 40-45°F and 0.42% at 70-90°F. There was no determination made for nematodes after the 11th month due to insufficient soil samples, as it had been anticipated that the nematodes would not survive in the soil, in the absence of host, for more than 4 months. From the data in Fig. 2, it was evident that this nematode was capable of surviving under adverse conditions for a longer period than had been expected.

Host development as related to pathogenicity

Experiment I. - In an attempt to study the effects of T. brevidens at different stages of plant development, the following experiments were conducted. About 200 nematodes, suspended in water, were added to each of the test pots at the following stages of plant development: (1) planting, (2) start of vernalization, (3) half through vernalization, and (4) end of vernalization. An uninoculated control, also vernalized,

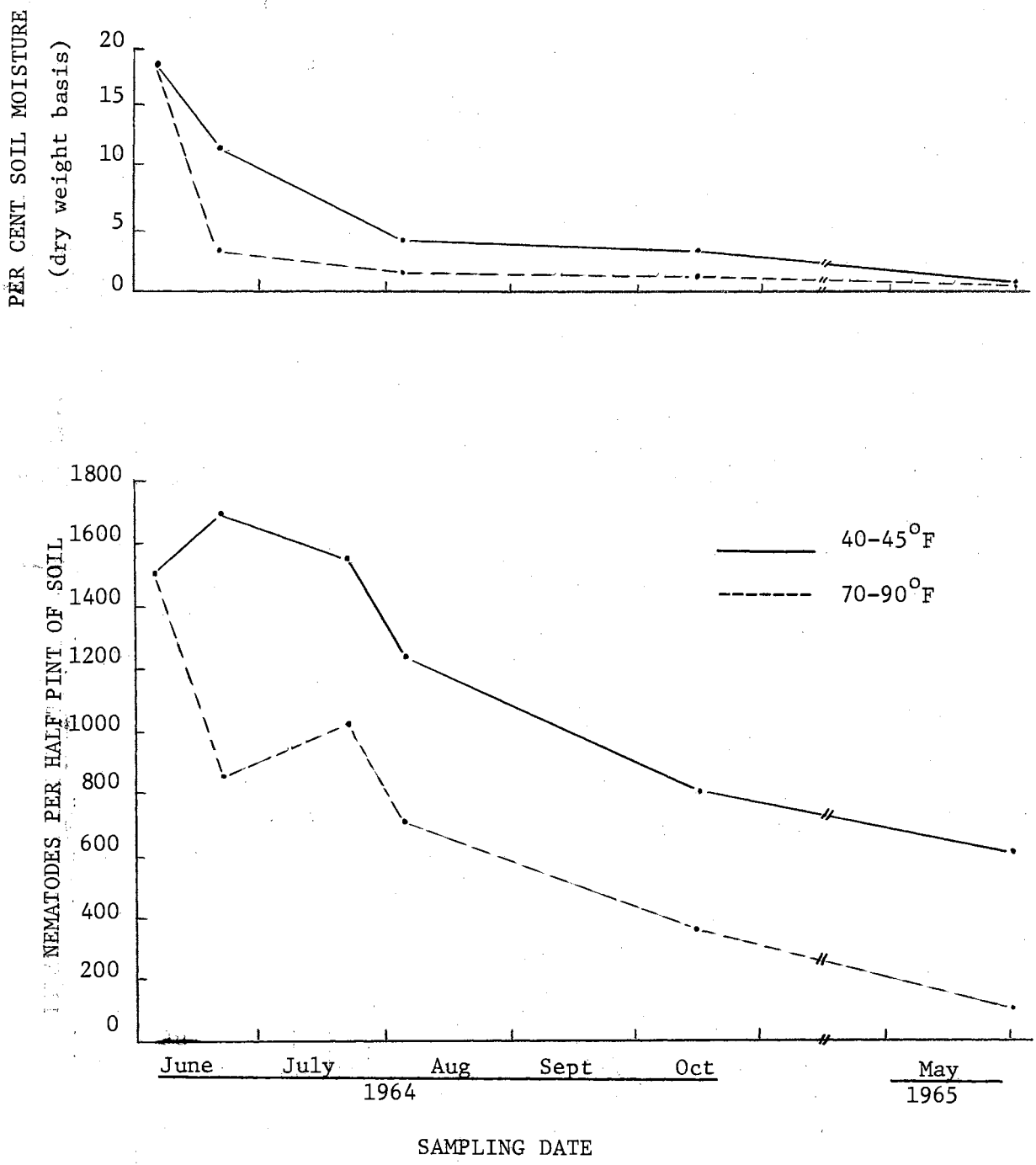


Fig. 2. Survival of Tylenchorhynchus brevidens in field soil, in the absence of host, stored at 40-45° and 70-90°F for 11 months.

served as the fifth treatment. Each of the above treatments consisted of 3 replicates. There were 10 plants per pot. A 12-hr daylight photoperiod was maintained with light intensity of about 2400 foot candles. Approximately 4 weeks after growing at 70°F, they were transferred to a chamber at a constant temperature of 40°F for vernalization where they were given the same photoperiod and light intensity. Since the plants appeared to be adversely affected by the cold temperature, vernalization was carried on for less than 3 weeks. After vernalization, the plants were brought back to the room at 70°F. At this temperature, the plants recovered and produced new leaves of light green color. Some of the plants had a tendency to lodge because the leaves were long and weak. After vernalization, the plants were kept in this room for about 7 weeks. Since this length of time was thought to be sufficient for the stimulation of flowering. However, none of the plants showed signs of floral differentiation at the end of this time. Another treatment of 3 replicates was planted with the same number of plants per pot and inoculated with T. brevidens at planting. These latter plants were not vernalized and were grown at 70°F throughout the entire experiment.

Even though flowering was not induced, significant differences in gross fresh weights of roots of wheat plants were found with treatments 1 and 2 compared to treatments 3, 4 or 5 (Table I). The reduction in top growth of wheat plants from treatments 1, 2 and 3 compared to treatments 4 or 5 was evident from visual observation.

Wheat plants grown at a constant temperature of 70°F, and not vernalized, inoculated with 200 T. brevidens, did not show any measurable damage to roots or any apparent stunting of the tops. This result confirmed the observations made by Langdon et al. (12) that there were no

stunt symptoms induced by T. brevidens on wheat grown at a constant temperature of 70°F.

Experiment II. - The same procedures were followed here as in Experiment I except that in Experiment II the following modifications in procedure were made. A 14-hr daylength was provided in both the 70° and the 40°F chambers. The plants were vernalized for about 4 weeks instead of less than 3 weeks. The number of nematodes added per pot was increased from 200 in Experiment I to 600 with treatments 1 and 3 and 500 with treatments 2 and 4, respectively. Liquid fertilizer (20-20-20) was applied at 2-week intervals to plants in all treatments throughout the entire experiment. In Experiment II, as in Experiment I, the nematodes, suspended in water, were added at: (1) planting, (2) start of vernalization, (3) half through vernalization, and (4) end of vernalization. An uninoculated control, also vernalized, served as the fifth treatment.

Since it had been shown in Experiment I that damage to roots of wheat due to nematodes was likely occurring before or during vernalization, it was decided that additional inoculum added at later times, might also produce a response. To test this point, as a part of Experiment II, an additional 3 replicates, each inoculated with 900 nematodes, were included with treatment 3, and 3 replicates each with 1200 nematodes, were added with treatment 4.

Seven weeks after vernalization, wheat plants were examined for symptoms of response to T. brevidens. No statistically significant difference was found in gross fresh weights of plant tops with any of the treatments. There was, however, a significant reduction in root weights with treatment 1 or 2 compared to treatments 3, 4, or 5 (Table I). In general, the plants in this experiment grew more vigorously than did those

in Experiment I. This was probably due to several applications of liquid fertilizer; although the plants may have had too much fertilizer. This latter might account for the failure to get differences in top growth with any of the nematode treatments. Most of the plants, with all treatments, produced inflorescences.

It was found that the addition of 900 and 1200 T. brevidens, to plants during or after vernalization produced no additional measurable effect over that caused by 500 and 600 nematodes, respectively.

Experiment III. - The third experiment was a refinement of the first two experiments. Procedures as in Experiment II were followed, except for the following modifications: the length of time from planting to vernalization was shortened from 4 weeks in Experiment II to 3 weeks, and liquid fertilizer was applied only at the beginning of and after vernalization.

To obtain further information on the effect of adding increased amounts of inoculum during and after vernalization, 3 additional replicates were used at each of these treatment times. Numbers of nematodes with these treatments were increased to 900, added during vernalization (Treatment 3), and to 750 after vernalization (Treatment 4). More nematodes were not used in this latter treatment because they were not available.

Seven weeks after vernalization, plants in treatments 1 and 2 exhibited a striking difference in both top and root growth compared to plants in treatments 3, 4 or 5. Since practically all plants produced inflorescences, stunt symptoms with plant tops in treatments 1 and 2 were very evident. Gross fresh weights of tops and roots with treatments 1 and 2 were statistically highly significantly different than

those with treatments 3, 4 or 5 (Table I). Flowering stalks and heads with treatments 1 and 2 were shorter than with treatments 3, 4 or 5. Comparison of root systems of wheat inoculated at planting with T. brevidens with root systems from the uninoculated control is shown in Fig. 3. As in Experiment II, no measurable difference was noted in root systems of plants inoculated with increased numbers of nematodes during and after vernalization.

Wheat plant development as related to time required to identify initial root damage by T. brevidens. - On the basis of results from the preceding 3 experiments, there was good evidence that damage to roots of wheat plants by T. brevidens had occurred during the early stages of plant development. To determine further the approximate time required for such injury to occur, another experiment was set up.

Wheat was planted in twenty 4-in. clay pots containing sterile soil, and grown at 70°F. Each of 15 pots was inoculated with 700-800 nematodes before planting. The 5 remaining pots served as uninoculated controls. Plant roots from 3 inoculated pots and one control pot were examined at weekly intervals to determine the amount of injury present. At the end of the first week after planting, there were no recognizable symptoms of injury to roots. At the end of the second week after planting, examination of the roots revealed characteristic root-stunt symptoms in all 3 replicates and injury to roots appeared to be in an advanced stage. Some of the root tissues were dark and appeared in a state of decay. Of the 700-800 T. brevidens inoculated per pot, an average of 639 nematodes was recovered after 2 weeks.

To pin down more exactly the number of days required for root injury to occur by T. brevidens, the following experiment was conducted. Three

TABLE I

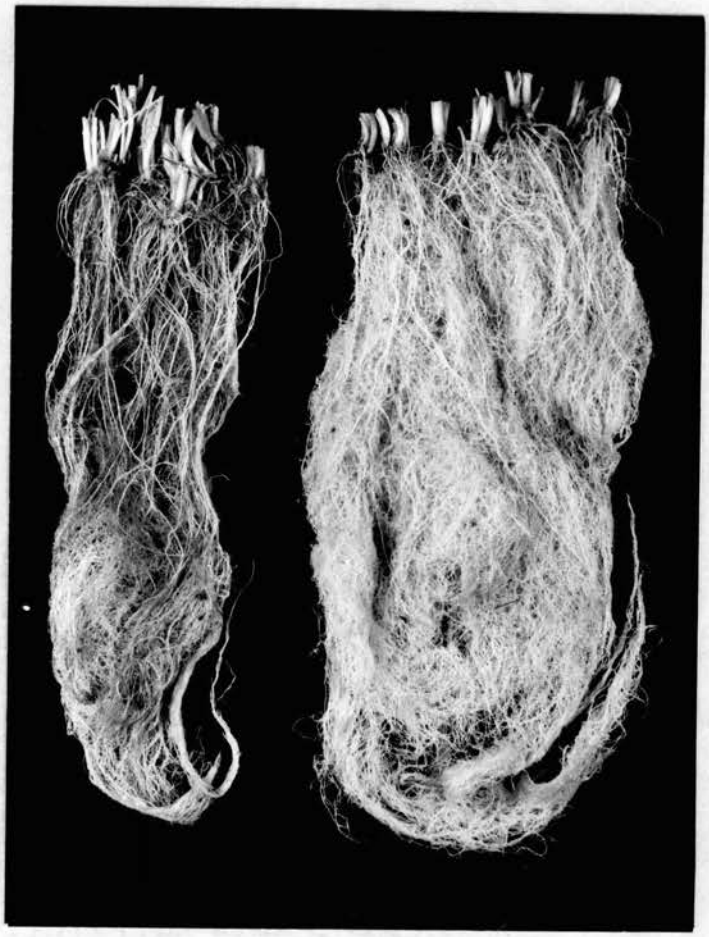
EFFECT OF TYLENCHORHYNCHUS BREVIDENS ON WHEAT INOCULATED AT VARIOUS STAGES OF PLANT DEVELOPMENT[a]

Treatments	Experiment I				Experiment II				Experiment III			
	Nematodes		Gross Fresh wt[b]		Nematodes		Gross Fresh wt		Nematodes		Gross Fresh wt	
	Inoculated	Recovered[c]	Tops	Roots	Inoculated	Recovered	Tops	Roots	Inoculated	Recovered	Tops	Roots
	no.	no.	g	g	no.	no.	g	g	no.	no.	g	g
Nematodes inoculated at:												
1. Planting	200	410	21.4	41.5	500	415	34.1	45.4	400	683	27.4	48.2
2. Start of vernalization	200	461	25.6	40.9	600	658	40.2	47.2	400	1156	30.1	57.4
3. Half through vernalization	200	203	30.4	68.2	500	447	46.5	68.3	450	450	43.5	72.5
					900	421	48.9	69.8	900	878	48.7	80.4
4. End of vernalization	200	185	35.9	74.5	600	168	56.5	85.8	450	755	46.2	79.7
					1200	507	57.0	79.6	750	483	43.9	84.7
5. Uninoculated control, vernalized	0	0	34.2	78.6	0	0	49.7	88.9	0	0	41.7	93.5

[a]Statistical analysis was done by employing Duncan's New Multiple Range Test. Treatment means not enclosed in the same bracket are significantly different from one another at the 5% level.

[b]Means of 3 replicates

[c]Nematodes per half pint of soil



(A) (B)

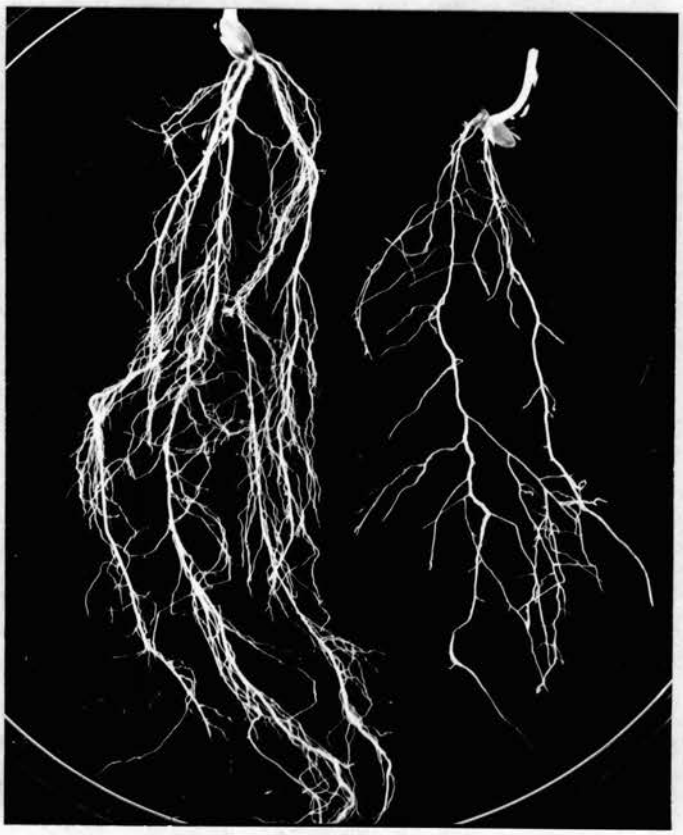
Fig. 3. Roots of wheat plants 105 days after planting. (A) inoculated with Tylenchorhynchus brevidens at planting, and (B) uninoculated control. Both lots of plants had been vernalized. Note drastic reduction of root systems in (A).

replicates each in paper cups containing sterile soil, were inoculated with 100, 200, 300, and 400 T. brevidens immediately after the cups were planted to wheat. An uninoculated control was provided for each time the roots were to be examined. The plants were grown at 70°F. The first examination was made 3 days after planting and plants were examined every day thereafter until root injury was observed.

There was no observed injury to roots of wheat from the third through the tenth day after planting. At the end of 11 days, plants in one cup, inoculated with 400 nematodes showed root symptoms (Fig. 4). Two hundred sixty six nematodes were recovered from this cup, while the other 2 cups at this inoculum level had 116 and 83 nematodes, respectively. At the end of 12 days after planting, plants in all 3 replicates inoculated with 400 T. brevidens per cup showed root symptoms. A root system from a cup with 400 nematodes, allowed to develop for about 1 month is shown in Fig. 5 along with an uninoculated control. The other treatments inoculated with 100, 200, or 300 T. brevidens did not show root symptoms at the end of 12 days after planting. There were insufficient replicates for observations beyond this time.

(A) (B)

Fig. 4. Wheat root systems from plants 11 days after planting showing (A) uninoculated control, and (B) inoculated with 400 Tylenchorhynchus brevidens at planting.



(A) (B)

Fig. 5. Wheat root systems from plants one month after planting showing (A) uninoculated control, and (B) inoculated with 400 Tylenchorhynchus brevidens at planting.

Attempts at getting T. brevidens in tissue culture

Root tissue of many different plants was used in tissue culture in an attempt to grow and maintain aseptic cultures of T. brevidens for use as inoculum. White's (24) nutrient agar was used, modified into a 0.7% agar medium and at a pH of 5.4. Water agar, 1.5%, was also tried with less success because the roots seemed to grow actively only for a short period of time.

Excised roots of wheat (Triticum aestivum L. var. Concho), barley (Hordeum vulgare L. var. Will 930), oats (Avena sativa L. var. Arkwin), cotton (Gossypium hirsutum L., undetermined variety), pepper (Capsicum frutescens L. var. California Wonder), cucumber (Cucumis sativa L. var. Marketer), squash (Cucurbita maxima L. var. Golden Summer), peanut (Arachis hypogaea L. var. Argentine 1163-1172), sorghum (Sorghum vulgare Pers. var. OK 612), sweet corn (Zea mays var. saccharata (Sturtev.) Bailey, undetermined variety), Pop corn (Zea mays var. everta (Sturtev.) Bailey var. Trucker's Favorite), and tomato (Lycopersicon esculentum Mill. var. Rutgers) were tried. The seeds were surface sterilized in 10% commercial Chlorox (5.25% sodium hypochlorite) for about 20 minutes and then were rinsed once in sterile water. These sterilized seeds were placed aseptically and spread uniformly on moist sterile filter paper in sterile Petri dishes for germination. When roots were about 2-3 cm long, they were severed aseptically and transferred to the agar in the plates where they were allowed to grow and develop for about 3-4 days. After this time, the plates were screened for contaminations and poor root growth.

Alfalfa plants were also tried. A whole alfalfa plant was used per plate because the plants grew slowly and could be maintained in the Petri

dish for several months without crowding. The alfalfa seeds were surface sterilized in concentrated HCl for about 10 minutes, then rinsed twice in sterile water. Seeds were allowed to germinate on moist sterile filter paper in sterile Petri dishes for 2-3 days, then the whole plant was transferred to White's medium or to water agar in plates.

The nematodes, mature females and larvae, were disinfected for 20 minutes in 3700 ppm streptomycin sulfate solution, then rinsed twice in sterile water before being transferred aseptically, by a sterile micropipette, to the roots in the Petri dishes. On the average, 20-30 T. brevidens were used per plate.

The nematode-inoculated plates were inverted and placed in plastic bags kept at 70°F in the dark. Examinations of the plates for nematode reproduction and feeding were made periodically. Due to exposure of the plates during examination, contamination was a major problem.

No reproduction of T. brevidens was observed with roots of any plant tested, although nematodes remained alive for about 4 months near the roots of alfalfa plants growing on agar. No feeding by the nematode was observed, although they were repeatedly seen aggregated around the roots in the area from the region of cell elongation to the root tip. It was observed that the nematodes followed the developing roots and dead nematodes were left behind near old roots.

Of the plants tried, alfalfa roots supported live nematodes the longest. T. brevidens survived with roots of barley, oats and wheat only about 2-6 weeks. No nematodes survived after 1 week with any of the other root cultures.

CHAPTER V

DISCUSSION

One of the objectives of this investigation has been to determine the influence of host development and the environment as they relate to pathogenic activities of T. brevidens. Evidence has been presented from several different experiments to show that measurable effects of nematode activity could not be demonstrated unless the nematode was present during the earlier stages of wheat plant development, i.e., germination or at the start of vernalization. Wheat inoculated with T. brevidens half through or after vernalization did not respond with reduced root systems or stunted tops. Increasing the amount of nematode inoculum by 2 to 3 times during or after vernalization did not produce disease symptoms.

It was observed that as a consequence of nematode activity during the early stages of wheat development, tillering was considerably reduced. Since winter wheat must undergo a thermoperiod change to complete its normal reproductive cycle, it is important that plants have adequate root systems and tillers to subsequently mature and produce normal yields (16).

Evidence was presented that T. brevidens was as pathogenic at 40° as it was at 70°F. From what is known regarding temperature relations

of other plant-parasitic nematodes, this would be considered a very unusual situation. So far as can be determined, no other nematode has been demonstrated to be pathogenic at the lower temperature, 40°F.

CHAPTER VI

SUMMARY

It was shown that measurable damage to wheat, var. Concho, was produced by Tylenchorhynchus brevidens only when this nematode was present during germination or the early phases of vernalization. Adding T. brevidens, even in increased numbers, to soil in which wheat was growing at later stages of development did not result in a measurable plant response. At 70°F, the earliest observable injury to roots of wheat plants, inoculated with 400 nematodes, occurred about 11 days after planting.

As measured by response of wheat plants, T. brevidens was equally pathogenic at 40° or 70°F.

Fluctuations of population of T. brevidens in the field were apparently more related to host developments than to temperature or moisture.

At 70-90°F, T. brevidens survived, in the laboratory, in the absence of a host, for 1 year and 4 months. In a separate test with field soil in the laboratory for a period of 11 months, more nematodes survived at 40-45°F than at 70-90°F. At the end of this latter test, soil moisture at these temperatures was 0.63% and 0.42% respectively.

T. brevidens was not induced to reproduce on aseptic root cultures of a wide variety of plants grown on White's nutrient medium.

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