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THE RELATIONSHIP OF NUMBERS OF HELICOTYLENCHUS MICROLOBUS TO NITROGEN SOIL AMENDMENTS.

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THE RELATIONSHIP OF NUMBERS OF HELICOTYLENCHUS MICROLOBUS TO NITROGEN SOIL AMENDMENTS

by

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INTRODUCTION

Corn ranks as the most important crop in Iowa's highly agricultural economy. The plant pathologist, in concert with other scientists, must be prepared to deal with all present and potential corn pathogens. Plant parasitic nematodes are found abundantly in corn fields throughout Iowa. Yet today, we know little of the affects that these parasites have on this crop, or of the synecology of nematodes in this environment. This study was initiated to investigate these affects and relationships.

Previous studies revealed that numbers of certain plant parasitic nematodes were higher in plots that had received 160 lb nitrogen per acre year since 1915, while other species were higher in plots that had not received nitrogen since 1915 (5). The primary purpose of this investigation was to learn more about these differences and to answer these questions: 1) how do numbers of plant parasitic nematodes relate to intermediate nitrogen levels? 2) do these relationships, if existent, occur at other locations in Iowa? 3) how do nitrogen treatments favor one species and not another?

Zea mays L. and Melilotus officinalis (L.) Lamarck are associated often with large numbers of the spiral nematode, Helicotylenchus microlobus Perry, 1959. This nematode was selected, for this reason, for an investigation of its pathogenicity to these crops and of its temperature relationships.

LITERATURE REVIEW

Soil Amendment Studies

Studies on the effect of soil amendments to nematode populations have dealt primarily with the relationship of potassium and phosphorus to a root-knot nematode. Little is known if or how nitrogen affects populations of these or other nematodes. Oteifa (21) reported that the greater reproductive rate of Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 on lima beans (Phaseolus limensis Macf.) at intermediate, and high potassium levels, as compared with low levels, might not be related solely to the associated greater root density. He cautioned, however, that differences in nematode populations between intermediate and high potassium levels may be related more to available root area than to the added potassium per se. Later (20), he reported an increased M. incognita reproductive rate when the potassium level was increased from deficient to optimum or excessive levels for lima bean growth. Plant damage was reduced as potassium levels were increased. He commented that although the year's lima bean crop may not be injured by a nematode population build-up, the higher numbers may damage the succeeding crop. He (22) found that M. incognita produced fewer egg masses in lima bean roots with ammonium ion than with nitrate ion. Oteifa attributed this difference to the inhibitory effects of released ammonia gas.

Shands and Crittenden (27) found that the number of M. incognita acrita Chitwood, 1949 galls on resistant and susceptible soybeans

(Glycine max L.) increased as the amount of nitrogen and potassium applied was increased. Bird (3) found that when tomato (Lycopersicon esculentum Mill.) infected with M. javanica (Treub, 1885) Chitwood, 1949 was grown in mineral solutions deficient in nitrogen, magnesium, iron or potassium, the nematode numbers increased more rapidly than when it was grown in full mineral solution. He postulated that changes in the plant's metabolism might produce or release metabolites beneficial to the nematodes. Kirkpatrick et al. (16) made correlations between nematode soil populations and single element content of sour cherry (Prunus cerasus L.) leaves over three seasons and found that Pratylenchus sp. numbers were highest when potassium was higher than 0.15% of leaf weight and that Helicotylenchus dihystra (Cobb, 1893) Sher, 1961 numbers were highest when the potassium content of the leaf was over 1.25%. Marks and Sayre (18) found that a low potassium level retarded the development of M. incognita on cucumber (Cucumis sativa L.), while a high potassium level accelerated it. The developmental rates of M. javanica and M. hapla Chitwood, 1949 were unchanged.

To reduce the losses caused by plant parasitic nematodes, there has been some interest in determining the role of soil amendments on crop yields in land heavily infested with plant parasitic nematodes. Bessey (2) found that potassium amendments were generally beneficial to crops, enabling them to produce good yields in spite of the presence of large numbers of nematodes. He observed that ammonium sulfate applications to cowpeas increased yields, while phosphorus applications had no effect. No nematode population estimates were given. He suggested that since

most parasitic nematodes resided in the top 12-16 inches of soil that the head start provided by these amendments would enable the roots to grow past the zones of maximum nematode concentration. Duggan (8) found that ammonium sulfate dressings of 0.5, 1 and 2 cwt per acre in oat fields heavily infested with Heterodera major O. Schmidt, 1930 did not benefit the plants. Eno et al. (11) injected anhydrous ammonia into the soil and reported significant decreases in numbers of bacteria, fungi and nematodes. Generally, the higher the ammonia retention around the injection site, the fewer the nematodes. They found that when ammoniacal nitrogen retention measured 136-741 ppm nematode numbers were reduced significantly; at 608 ppm the decrease was estimated at 99%. Plant parasitic nematode numbers were greatly reduced and in some instances entirely exterminated. All parasitic nematode numbers appeared to be reduced at the same rate. They concluded that concentrations of 300-600 ppm would be necessary before nematocidal or fungicidal action could be expected. Castaner (5) observed that H. microlobus numbers were significantly higher in corn plots that had not received nitrogen for 48 years than in plots that had received 160 lb nitrogen per acre year for 48 years. Pratylenchus spp. numbers were higher in the plots that had received 160 lb nitrogen per acre year than in plots that had not. Xiphinema americanum Cobb, 1913 numbers were higher in plots that had received lime for 48 years than in plots that had not.

Pathogenicity of *Helicotylenchus*

Spiral nematodes in the genus *Helicotylenchus* usually feed ectoparasitically in the cortex of roots (6, 13, 24, 32). Feeding also has been observed in root hairs (29) and phloem tissue (23). Plant damage is characterized by local necrosis, cell wall damage and exposure of plant tissue to secondary infections (13, 14, 24). Golden (13) made the first thorough examination of the pathogenicity of a *Helicotylenchus* species. He demonstrated that *H. buxophilus* (Golden, 1956) Perry, 1959 fed throughout the length of boxwood (*Buxus sempervirens* L.) feeder roots causing small local necrotic lesions in the cortex. The color of these lesions was attributed to the deposition of substances along the ruptured cell walls and to the deterioration of cellular protoplasm. The nematodes evidently fed in the deep cortical parenchyma cells. No evidence of vascular feeding was reported. Weight losses of boxwood attributed to the nematodes were significant at .05. Sledge (29) observed *H. nannus* Steiner, 1945, through micro-observation chambers, feeding primarily on corn root hairs and secondarily on the primary and secondary roots. He also reported significant stunting of corn in test pots, but no data were given. Perry (23) found that Kentucky bluegrass (*Poa pratensis* L.) infected with *H. digonicus* Perry, 1959 was stunted, while roots were browned and the cortex prematurely sloughed off. He related these injuries to the summer dormancy disease of Kentucky bluegrass in Wisconsin. The nematodes were commonly observed with their heads buried in the cortex, but only occasionally in the phloem. Steiner (31) illustrated a spiral nematode, *Helicotylenchus* sp., feeding on a rootlet of sweet

clover (Melilotus alba L.). The necrotic lesions resembled those produced by H. buxophilus on boxwood. Sledge (28) found that prior to feeding on a corn root hair, H. nannus secreted a droplet of material through the stylet into the cell. Perry et al. (24) showed that H. digonicus caused severe losses in Kentucky bluegrass under controlled conditions. Root necroses, leaf chloroses and stunting were typical symptoms. Jensen et al. (14) observed H. nannus partially embedded into sugarcane roots (Saccharum officinarum L.). Discoloration of cells was associated with penetration and was attributed in part to secondary infections. Taylor (33) tested 127 crop plant varieties and found 94 were hosts of H. microlobus Perry, 1959. A plant was considered a host if after 3 months, 100 nematodes were recovered from pots originally infested with 100 nematodes. Zea mays, Lycopersicon esculentum and Melilotus alba were among the hosts; Pisum sativum L., Medicago sativa L. and Linum usitatissimum L. were among the non-hosts. Taylor (32) found that H. microlobus caused small light-to-dark lesions on corn root surfaces. These lesions encompassed 4-10 epidermal cells and were 4 cells deep into the cortex. Whole cotton-blue stained mounts of infected corn roots revealed nematodes completely embedded in the cortex. Infected areas were characterized by cortical cell wall damage. No vascular feeding was observed.

Ruehle and Sasser (25) reported that high numbers of H. nannus were associated with decline of pine in the American Southeast. Apt and Koike (1) reported losses of Saccharum officinarum were related to high numbers of H. nannus. The losses were further increased in the presence of

Pythium graminicola Subr. Zuckerman and Strich-Harari (35) observed H. multicinctus (Cobb, 1893) Golden, 1956 partially embedded in the cortex of banana roots (Musa sapientum (L.) Kuntze). Tissues associated with feeding nematodes were usually discolored. Khera and Zuckerman (15) attempted to observe H. erythrinae (Zimmermann, 1904) Golden, 1956 feeding in in-vitro cultures but were unsuccessful. Only occasional probing by the nematodes were seen.

MATERIALS AND METHODS

Nematode Separation

A modified Christie and Perry (7) technique for the separation of nematodes from the soil was used in all experiments. The nematode and soil mixture residue was collected after processing the soil sample, agitated in water, through a 32, 60 and 270 mesh sieve. This residue was then poured onto a muslin cloth supported between two plastic cylinders over the bottom half of a dish containing water up to the soil residue level. The live nematodes migrated through the muslin to the water below. The muslin and plastic cylinders were removed after 40 hours and the nematodes were poured into a counting dish. The numbers of nematodes within the counting dish was determined with 45X magnification. Genera and species were determined with 100X magnification.

Field Experiments

Five experiments were conducted to determine the effects of different amounts of nitrogen soil amendments on plant parasitic nematode numbers. These tests were conducted at the Iowa State Agronomy Farm, Ames, Iowa, during 1963 and 1964; the Carrington-Clyde Experiment Farm, Independence, Iowa, during 1964; the Southern Iowa Experiment Farm, Bloomfield, Iowa, during 1964.

The treatments were applied in randomized block designs at all 3 locations.

Soil samples (500 ml) were taken at the 4-9 inch depth and 6 inches

from the corn plants. Each sample was agitated thoroughly and a 100 ml subsample was removed and processed as described above.

Ames 1963

Soil samples were taken at Ames from plots in which corn has been grown continuously since 1915 and which received 0-160 lb nitrogen per acre year (10). Plots 906-1, 907-1, 909-1 received no nitrogen. Plots 906-2, 907-2 and 909-2 received 40 lb nitrogen per acre year. Plots 906-3, 907-3 and 909-3 received 80 lb nitrogen per acre year. Plots 906-4, 907-4 and 909-4 received 160 lb nitrogen per acre year. Four samples per plot were taken on June 13, July 24 and September 20, 1963.

Ames 1964

Soil samples were taken at Ames from plots in which corn has been grown continuously since 1915 and which received 0-160 lb nitrogen, 26 lb potassium and 50 lb phosphorus per acre year. Plots 906-5, 907-5 and 909-5 received no nitrogen. Plots 906-6, 907-6 and 909-6 received 40 lb nitrogen per acre year. Plots 906-7, 907-7 and 909-7 received 80 lb nitrogen per acre year. Plots 906-8, 907-8 and 909-8 received 160 lb nitrogen per acre year. Four soil samples per plot were taken on June 7, July 3, August 3 and September 25, 1964.

Independence 1964

Soil samples were taken at Independence from plots in which corn has been grown continuously since 1952 and which received 30-120 lb nitrogen per acre year. Plots 11b and 20b received 30 lb nitrogen per acre year. Plots 17c and 20c received 60 lb nitrogen per acre year. Plots 11d and

20d received 120 lb nitrogen per acre year. Six soil samples per plot were taken on June 24, July 27, August 11 and September 30, 1964.

Bloomfield 1964

Soil samples were taken from Bloomfield plots in which corn has been grown continuously since 1952 and which received 0-240 lb nitrogen and 50 lb phosphorus per acre year. Plots 704-3, 712-3 and 718-3 received no nitrogen. Plots 702-5, 711-5 and 717-5 received 30 lb nitrogen per acre year. Plots 702-6, 711-6 and 717-6 received 60 lb nitrogen per acre year. Plots 702-7, 711-7 and 717-7 received 120 lb nitrogen per acre year. Plots 702-8, 711-8 and 717-8 received 240 lb nitrogen per acre year. Four soil samples per plot were taken on June 17, July 17, August 7 and September 13, 1964.

Short range field experiment Ames 1963

The short range effect of a high nitrogen amount on plant parasitic nematode numbers was studied at Ames. Approximately 160 lb nitrogen per acre was applied to 3 blocks of 2 corn rows in plot 910 (no amendments since 1915). Ammonium nitrate was selected as the nitrogen source, since it is the nitrogen form used at the three experiment stations. The ammonium nitrate was broadcast in a 2 foot wide band on June 1, 1963. Four soil samples were taken from each block and from 3 adjacent check blocks on June 15, July 11, August 13 and October 7, 1963.

Greenhouse Experiments

The effect of nitrogen on Helicotylenchus microlobus numbers was studied in 3 greenhouse experiments. This nematode was selected for study because it is a common habitant of Iowa corn fields. Iowa 4570 corn was used in all the experiments. The H. microlobus was obtained from cultures of hand-picked specimens grown on tomatoes. These cultures became contaminated with saprobic nematodes, but no other stylet bearing nematodes were present. Experimental pots whether or not infested with nematodes received nematode-free soil-washings from these culture pots.

First greenhouse experiment

The first greenhouse experiment was conducted to determine the effects of nitrogen on H. microlobus numbers in unsterilized field soil. Field soil with a high H. microlobus concentration from plot 906-1 (no amendments since 1915) was distributed into 40 six inch clay plots and planted with Iowa 4570 corn. Fourteen days after planting, the pots were arranged into five treatment groups of 8 pots each. Group one received no treatment; groups two, three, four and five received 0.03, 0.3, 1.5 and 3 grams of 33.5-0-0 ammonium nitrate, respectively. These levels approximate 0, 5, 50, 250 and 500 lb nitrogen per acre in the field. No severe burning of the corn was observed. After 102 days the tops and roots were dried and weighed and the number of H. microlobus present was determined.

Second greenhouse experiment

The second greenhouse experiment was conducted to determine if H. microlobus numbers are affected by different nitrogen amounts and if this nematode is responsible for corn dry weight losses in sterilized soil. Soil from plot 906-1 (no amendments since 1915) was steam sterilized, placed into 70 six inch clay pots and planted with Iowa 4570 corn. After 7 days, 2500-2700 H. microlobus were added to 35 of the 70 pots. Fourteen days after planting, the pots were arranged into five treatment groups of 14 pots each: 7 pots with nematodes and 7 pots without. The first treatment group received no nitrogen; the second, third, fourth and fifth groups received 0.03, 0.3, 1.5 and 3 grams of ammonium nitrate, respectively. Because of severe damage to the corn plants at some of the high nitrogen treatments, a new planting of corn was made 20 days after the original planting. When the new seedlings were 5-7 inches tall, the old tops were removed. Helicotylenchus microlobus numbers were determined and top and root dry weights taken 127 days after the original planting.

This experiment was performed during the winter months. Supplementary lighting of one 250 watt bulb per 12 plants was used to increase intensity and photoperiod.

To determine if any anatomical differences existed that were related to nitrogen treatments and which might affect the feeding habits of H. microlobus, root sections from plants grown under all treatments were examined. Root pieces were taken 5-20 mm from the root cap 50 days after planting. These were fixed in a Craf III solution; dehydrated in an

ethyl alcohol series; infiltrated and embedded with paraffin in a xylene-wax series. The roots were sectioned at 12 microns, stained with safranin O and fast green FCF and compared. These methods are explained in detail by Sass (26).

Third greenhouse experiment

The third greenhouse experiment was designed primarily to determine the effect of ammonium nitrate to H. microlobus numbers when applied at high levels over several weeks rather than at once as in the previous two experiments. Because of the extreme stunting of corn encountered in the second greenhouse experiment, soil was taken from plot 909-4 (no nitrogen, but 50 lb phosphorus and 26 lb potassium per acre year since 1915) to provide the plants with a better balanced substrate in hopes of lessening the stunting. The soil was steam sterilized, distributed to 70 pots and then planted with Iowa 4570 corn. After 5-7 days, 3300-3600 spiral nematodes were added to 35 of the 70 pots. The pots were then arranged into five treatment groups of 14 pots each: 7 pots with nematodes and 7 pots without. Fourteen days after planting, the first group received no treatment; the second, third, fourth and fifth received 0.03, 0.3, 0.5 and 1 gram of ammonium nitrate, respectively. Twenty-eight days after planting, group four and five received an additional 0.5 and 1 gram, respectively. Fifty-two days after planting, group four and five received a final 0.5 and 1 gram, respectively. No damage to the plants was visible. After 113 days, H. microlobus numbers and top and root weights were determined.

Feeding of Helicotylenchus microlobus

To determine the nature of feeding of H. microlobus on corn and yellow sweet clover, seeds were planted in 3 inch waxed paper cups filled with steam sterilized soil. Water containing a large number of H. microlobus was poured around the seedlings as they emerged from the soil. When the corn plants were 6 inches high and the yellow sweet clover plants were 2 inches high, the soil was saturated with a hot (60-80° C) solution of formalin-propionic acid fixing agent. This solution is composed of .70 95% ethyl alcohol, .05 propionic acid, .05 formalin (37-40% formaldehyde solution in water) and .20 water (13). The nematodes were killed and fixed in-situ. The pieces of roots with nematodes attached were embedded in paraffin, as described previously, and microtomed at 12 microns. The sections were stained with safranin O and fast green FCF (26).

Pathogenicity of Helicotylenchus microlobus

Yellow sweet clover was selected to study the temperature relationship and pathogenicity of H. microlobus, because growing corn is difficult in the greenhouse. Sweet clover has been known to harbor large numbers of spiral nematodes. In one field of stunted sweet clover (near Ida Grove, Iowa) extremely high numbers were found.

Steam sterilized soil was distributed into 72 water proof 4 inch plastic pots and planted with about twelve Madrid yellow sweet clover seeds. Twenty days after planting, 4200-4600 H. microlobus were placed

into 36 of the 72 pots. The pots were then arranged into four treatment groups of 18 pots each: 9 pots with nematodes and 9 pots without. The 4 treatment groups were then placed into 1 of 4 temperature controlled tanks set at 15, 20, 25 and 30° C ± 1° C. Temperatures were determined by inserting a thermometer into the center of the pots. The number of plants per pot was reduced to five 30 days after planting. Seventy-eight, and 108 days after planting, the tops were cut, dried and weighed. After 138 days, all the tops and roots were dried and weighed. Numbers of H. microlobus per pot were determined and compared.

RESULTS

Field Experiments

Plant parasitic nematode numbers in plots receiving different nitrogen amounts are compared over the entire growing season and are reported in nematodes per liter. The regression form of nematode numbers to nitrogen levels was determined by methods described by Snedecor (30) and Li (17).

Ames 1963

The Ames plots which received 0-160 lb nitrogen per acre year since 1915 contained high numbers of Helicotylenchus microlobus, Pratylenchus penetrans (Cobb, 1917) Chitwood and Oteifa, 1952, Pratylenchus hexincisus Taylor and Jenkins, 1957, and Xiphinema americanum; low numbers of Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935; and low numbers of Tylenchorhynchus nudus Allen, 1955. Numbers of T. nudus are not recorded.

Over the entire growing season, there was a significant negative linear regression of H. microlobus (Fig. 1) and H. galeatus (Fig. 3) numbers to nitrogen amounts. There was a significant positive linear regression of Pratylenchus spp. numbers to nitrogen amounts (Fig. 2). Numbers of Xiphinema americanum were only weakly related to nitrogen amounts (Fig. 4). These data are summarized in Table 1.

The numbers of plant parasitic nematodes averaged over all treatments were generally lowest during July and highest in September (Table 2).

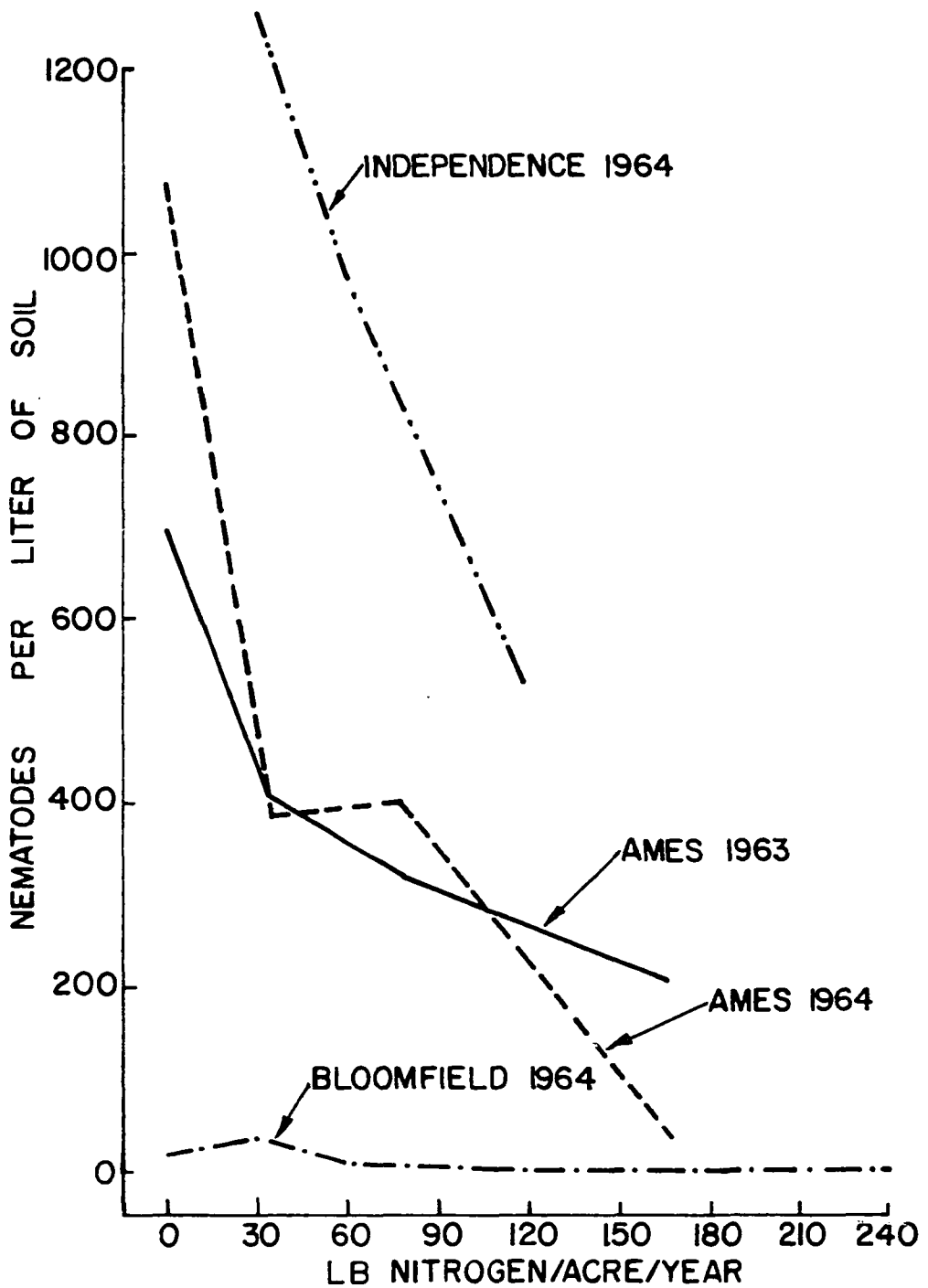


Fig. 1. The relative number of Helicotylenchus microlobus in continuous corn plots receiving 0-240 lb nitrogen per acre year at three locations in Iowa

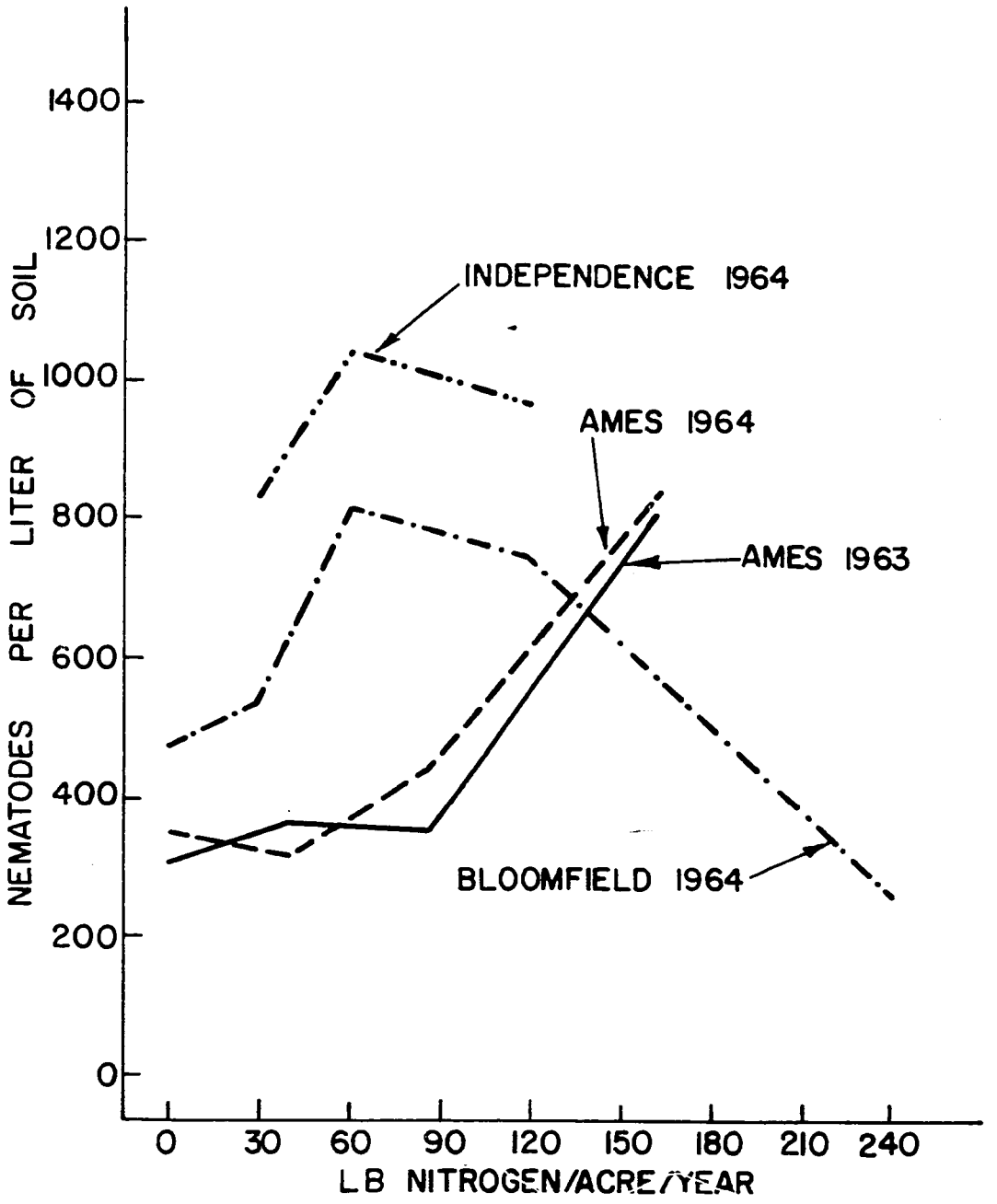


Fig. 2. The relative number of Pratylenchus spp. in continuous corn plots receiving 0-240 lb nitrogen per acre year at three locations in Iowa

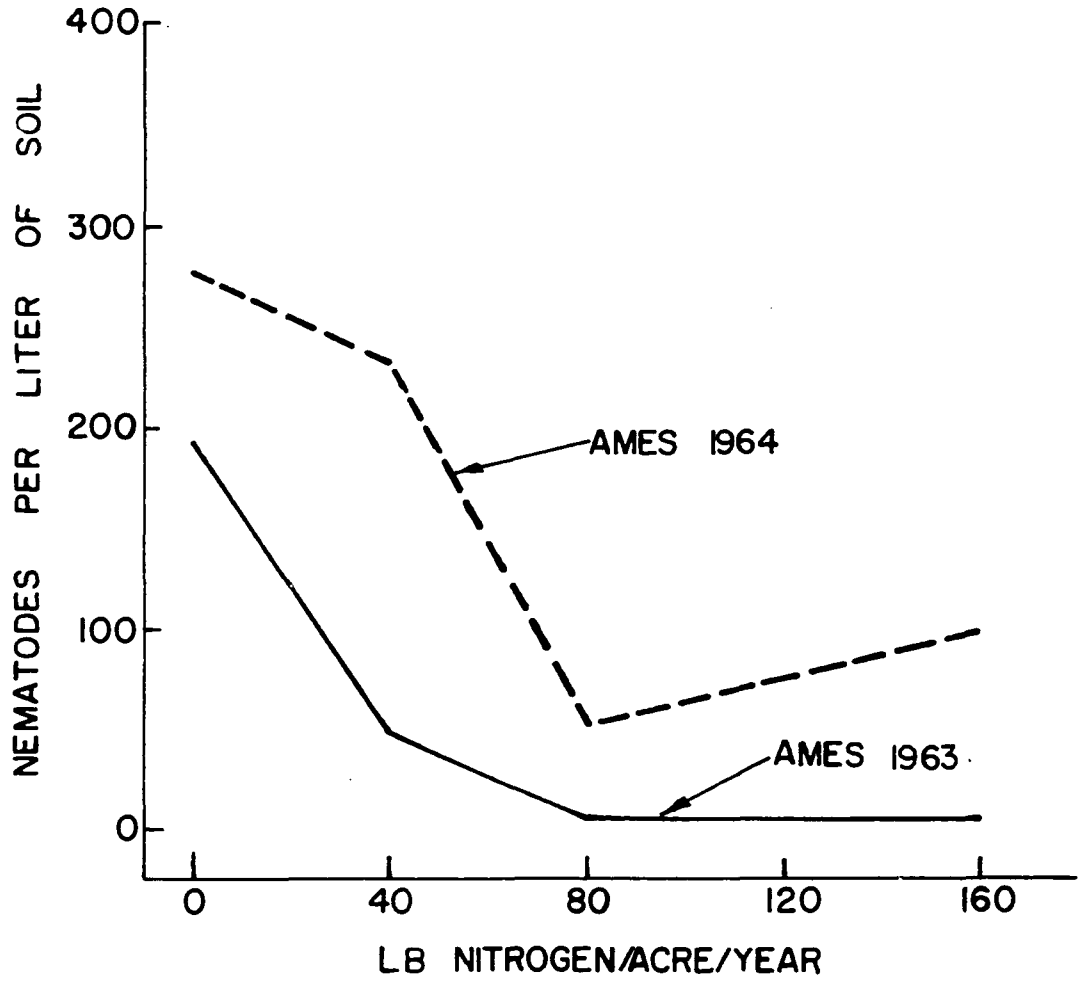


Fig. 3. The relative number of Hoplolaimus galeatus in continuous corn plots receiving 0-160 lb nitrogen per acre year at Ames, Iowa

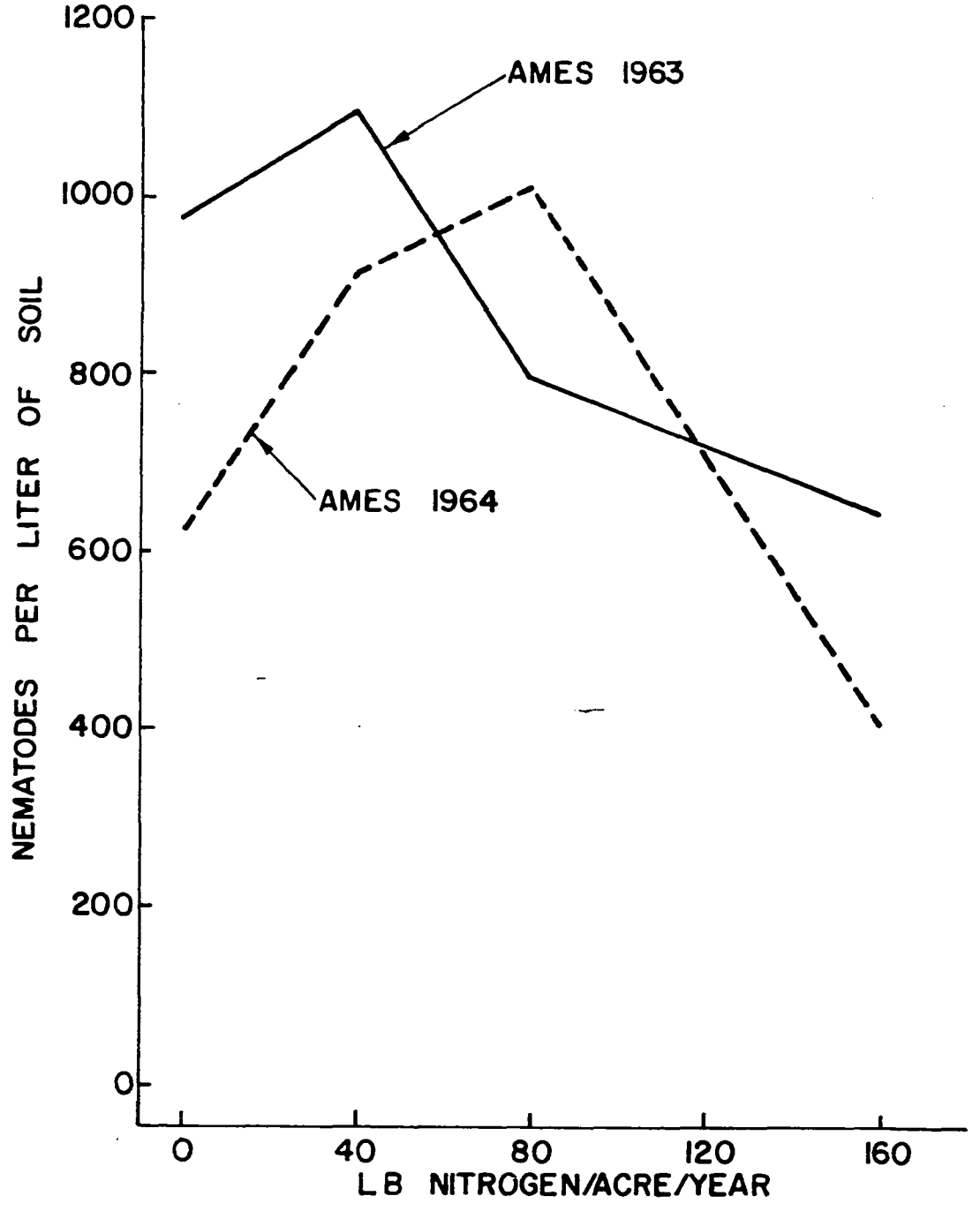


Fig. 4. The relative number of Xiphinema americanum in continuous corn plots receiving 0-160 lb nitrogen per acre year at Ames, Iowa

Table 1. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots receiving 0-160 lb nitrogen per acre year since 1915; Iowa State Agronomy Farm, Ames, Iowa, 1963

Treatments[a] N-P-K	Bu/acre	Helicotylenchus microlobus	Pratylenchus spp.	Hoplolaimus galeatus	Xiphinema americanum
		Nematode numbers[b]			
0-0-0	30	694	300	192	975
40-0-0	64	404	351	45	1091
80-0-0	87	321	341	7	797
160-0-0	111	202	807	7	641
Regression form		linear	linear	linear	linear
Significance		.005	.01	.005	.10

[a]Pounds per acre year since 1915.

[b]Average of 36 determinations taken on 3 sampling dates.

Table 2. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots; Iowa State Agronomy Farm, Ames, Iowa, June-September 1963

Dates	Helicotylenchus microlobus	Pratylenchus spp.	Hoplolaimus galeatus	Xiphinema americanum
	Nematode numbers[a]			
June 13	326	187	87	640
July 24	115	218	19	544
September	774	943	65	1445

[a]Average of 48 determinations; averaged over all nitrogen treatments.

Ames 1964

The Ames plots which received 0-160 lb nitrogen, 26 lb potassium and 50 lb phosphorus per acre year since 1915 contained high numbers of H. microlobus, P. penetrans, P. hexincisus and X. americanum. Numbers of H. galeatus and T. nudus were only moderate.

There was a significant negative linear regression of H. microlobus numbers to nitrogen amounts (Fig. 1). There was a significant positive linear regression of Pratylenchus spp. numbers to nitrogen amounts (Fig. 2). Numbers of H. galeatus (Fig. 3) and X. americanum (Fig. 4) were only weakly related to nitrogen amounts. These data are summarized in Table 3.

The numbers of plant parasitic nematodes averaged over all the treatments were generally lowest during July and highest during September (Table 4).

Independence 1964

The Independence plots which received 30-120 lb nitrogen per acre year since 1952 contained high numbers of H. microlobus, P. penetrans and Tylenchorhynchus agri Ferris, 1963.

There was a significant negative linear regression of H. microlobus numbers to nitrogen amounts (Fig. 1). Numbers of Pratylenchus penetrans (Fig. 2) and T. agri (Fig. 5) were not related to nitrogen amounts. These data are summarized in Table 5.

The numbers of plant parasitic nematodes averaged over all the treatments were generally lowest in June and highest in September (Table 6).

Table 3. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots receiving 0-160 lb nitrogen per acre year since 1915; Iowa State Agronomy Farm, Ames, Iowa, 1964

Treatments[a] N P K	Bu/A	Helicotylenchus microlobus	Pratylenchus spp.	Hoplolaimus galeatus	Xiphinema americanum	T.[b] nudus
		Nematode numbers[c]				
0-26-50	38	1070	345	276	619	41
40-26-50	68	388	285	231	911	13
80-26-50	93	399	439	51	1015	262
160-26-50	114	35	829	93	401	65
Regression form		linear	linear	linear	quadratic	
Significance		.01	.025	.25	.10	

[a]Pounds per acre year since 1915.

[b]T. nudus = Tylenchorhynchus nudus.

[c]Average of 48 determinations taken on 4 sampling dates.

Table 4. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots; Iowa State Agronomy Farm, Ames, Iowa, June-September 1964

Dates	Helicotylenchus microlobus	Pratylenchus spp.	Hoplolaimus galeatus	Xiphinema americanum	T.[a] nudus
	Nematode numbers[b]				
June 7	366	206	144	907	128
July 3	313	320	148	419	59
August 3	252	496	113	398	80
September 25	983	878	246	1223	115

[a]T. nudus = Tylenchorhynchus nudus.

[b]Average of 48 determinations; averaged over all nitrogen treatments.

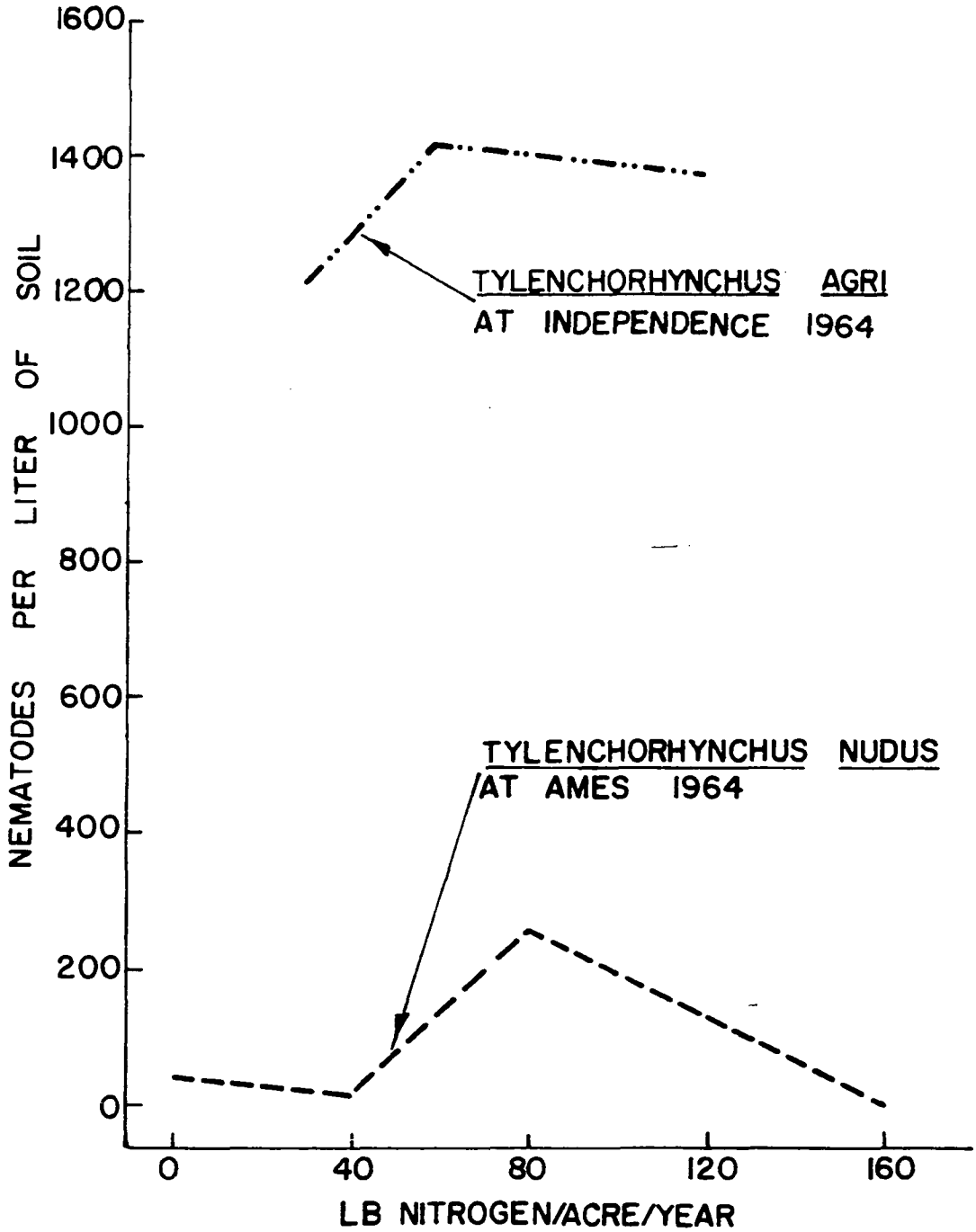


Fig. 5. The relative number of Tylenchorhynchus spp. found in continuous corn plots receiving 0-160 lb nitrogen per acre year at two locations in Iowa

Table 5. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots receiving 30-120 lb nitrogen per acre year since 1952; Carrington-Clyde Experiment Farm, Independence, Iowa, 1964

Treatments[a] N-P-K	Bu/acre	Helicotylenchus microlobus	Pratylenchus spp.	Tylenchorhynchus agri
		Nematode numbers[b]		
30-0-0	80	1259	922	1205
60-0-0	97	967	1049	1415
120-0-0	92	525	962	1333
Regression form		linear		
Significance		.05		

[a]Pounds per acre year since 1952.

[b]Average of 48 determinations taken on 4 sampling dates.

Table 6. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots; Carrington-Clyde Experiment Farm, Independence, Iowa, June-September 1964

Dates	Helicotylenchus microlobus	Pratylenchus spp.	Tylenchorhynchus agri
	Nematode numbers[a]		
June 24	681	386	899
July 27	565	429	1210
August 11	980	1123	1022
September 30	1442	1838	1239

[a]Average of 36 determinations; averaged over all nitrogen treatments.

Bloomfield 1964

The Bloomfield plots which received 0-240 lb nitrogen and 50 lb phosphorus per acre year since 1952 contained high numbers of Pratylenchus penetrans and P. hexincisus, while H. microlobus numbers were low. Occasional H. galeatus and X. americanum were found; these data were not recorded.

There was a significant negative linear regression of H. microlobus numbers to nitrogen amounts (Fig. 1). Pratylenchus spp. were only weakly related to nitrogen amounts (Fig. 2). These data are summarized in Table 7.

The numbers of plant parasitic nematodes averaged over all the treatments were generally higher during June and July and lower the rest of the season (Table 8).

Short range field experiment Ames 1963

In the test to determine the short range effect of a high nitrogen amount on nematode numbers, the equivalent of 160 lb nitrogen per acre was applied to plots containing high numbers of H. galeatus, X. americanum, H. microlobus, P. penetrans and P. hexincisus.

Helicotylenchus microlobus and Pratylenchus spp. numbers were almost twice as large in the rows that received nitrogen as in the control rows. Numbers of X. americanum were similar in both nitrogen treated and control rows. Numbers of H. galeatus were significantly smaller in nitrogen treated rows than in control rows. The H. microlobus and Pratylenchus spp. numbers were related more to bushels per acre yield than to the nitrogen treatments (Table 9).

Table 7. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots receiving 0-240 lb nitrogen per acre year since 1952; Southern Iowa Experiment Farm, Bloomfield, Iowa, 1964

Treatments[a] N--P-K	Bu/acre	Helicotylenchus microlobus	Pratylenchus spp.
		Nematode numbers[b]	
0-26-0	28	15	469
30-26-0	60	34	526
60-26-0	95	5	818
120-26-0	144	0	742
240-26-0	144	0	257
Regression form		linear	linear
Significance		.01	.10

[a]Pounds per acre year since 1952.

[b]Average of 48 determinations taken on 4 sampling dates.

Table 8. The relative number of plant parasitic nematodes per liter of soil from continuous corn plots; Southern Iowa Experiment Farm, Bloomfield, Iowa, June-September 1964

Dates	Helicotylenchus microlobus	Pratylenchus spp.
	Nematode numbers[a]	
June 17	13	1115
July 17	15	285
August 7	7	359
September 13	8	492

[a]Average of 60 determinations; averaged over all nitrogen treatments.

Greenhouse Experiments

Helicotylenchus microlobus numbers in pots that received different nitrogen amounts are compared by absolute numbers and nematodes per gram dry root weight. If dry root weights were significantly affected by the nitrogen treatments, then a covariance analysis was performed according to methods described by Snedecor (30) to eliminate the variance in nematode numbers due to root weights and to determine significant differences in final nematode numbers between treatments. Where dry weights were not significantly affected by nitrogen treatments a standard analysis of variance was performed to determine significant differences between treatment means.

First greenhouse experiment

In the first greenhouse experiment, the average number of H. microlobus per liter of natural field soil (no amendments since 1915) was related closely to the average total dry weights in the 0, 0.03, 0.3 and 1.5 gram ammonium nitrate treatments (Fig. 6). In the 3 gram treatment, however, the average number of H. microlobus was not related to average dry weights and was significantly different from the other treatment nematode numbers at the .01 level. There were also fewer nematodes per liter/gram of root in the 3 gram treatment than in the other 4 treatments (Table 10).

The plants were stunted, but most tasselled although only a few plants set fruit.

Table 9. The relative number of plant parasitic nematodes in continuous corn plot soil that received 0 and 160 lb nitrogen per acre in 1963; Iowa State Agronomy Farm, Ames, Iowa, 1963

lb nitrogen[a] added/acre	Helicotylenchus microlobus	Pratylenchus spp.	Hoplolaimus galeatus	Xiphinema americanum
	Average number of nematodes per liter of soil[b]			
0	621	417	697	523
160	1205	864	296[c]	583
	Average number of nematodes per liter/bushels per acre			
0	16.3	11.0	18.3	13.8
160	16.7	12.0	4.1	8.1

[a]Applied June 1963 to plots that received no soil amendments since 1915.

[b]Average of 48 determinations taken on 4 sampling dates.

[c]296 is significantly different from 697 at the .025 level.

Table 10. The relative number of Helicotylenchus microlobus per pot of unsterilized field soil in which corn was grown for 102 days with 0-3 grams of ammonium nitrate; first greenhouse experiment

Ammonium nitrate g	Dry weights[a] Tops g	Roots g	Total g	Helicotylenchus microlobus[a] Per liter of soil	Per liter/g root
0	2.14	2.54	4.68	446	176
0.03	2.96	2.57	5.53	654	254
0.30	4.41	2.90	7.31	669	231
1.50	6.34	3.34	9.68	949	284
3.00	4.65	3.16	7.81	280[b]	89

[a]Average of 8 determinations.

[b]280 is significantly different from 654, 669 and 949 at the .01 level.

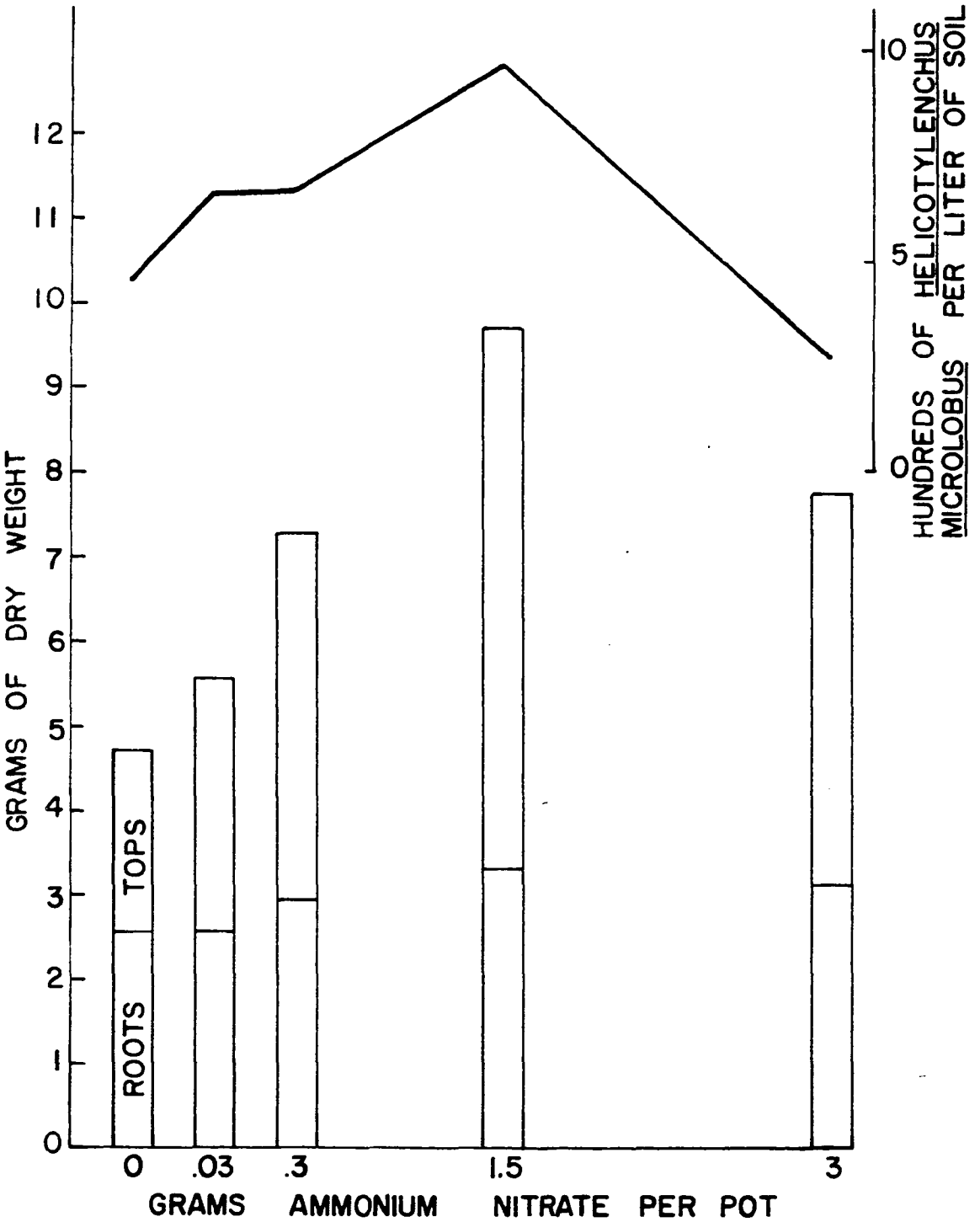


Fig. 6. A comparison of Helicotylenchus microlobus numbers with 0-3 gram ammonium nitrate treatments and dry weights of Iowa 4570 corn grown for 102 days; first greenhouse experiment

Second greenhouse experiment

In the second greenhouse experiment, the average number of H. microlobus per pot of steam sterilized soil (no amendments since 1915) was related closely to total dry weights in the 0, 0.03 and 0.3 gram ammonium nitrate treatments (Fig. 7). Helicotylenchus microlobus numbers were not related to total dry weights in the 1.5 and 3 gram treatments, however. The lowest number of spiral nematodes were found in the 3 gram treatments; this number was significantly different from the other treatment numbers at the .01 level. The fewest H. microlobus numbers per pot/gram of root also was found in the 3 gram treatment (Table 11).

The plants were extremely stunted despite supplementary light during short winter days. None of the plants tasselled.

Plants grown in pots without spiral nematodes weighed more than plants grown in pots with spiral nematodes (Fig. 7). These differences were not significant.

A comparison of roots revealed no anatomical differences between roots grown with or without nitrogen treatments that might affect the feeding of H. microlobus. Mature corn roots are characterized by a layer of sclerenchyma tissue beneath the epidermis (26). In the roots examined, this layer was not yet formed, whether or not the roots had received nitrogen amendments.

Third greenhouse experiment

In the third greenhouse experiment, the average number of H. microlobus per liter of steam sterilized soil (no nitrogen, but 50 lb phosphorus per acre year since 1915) was not related to the total dry weights.

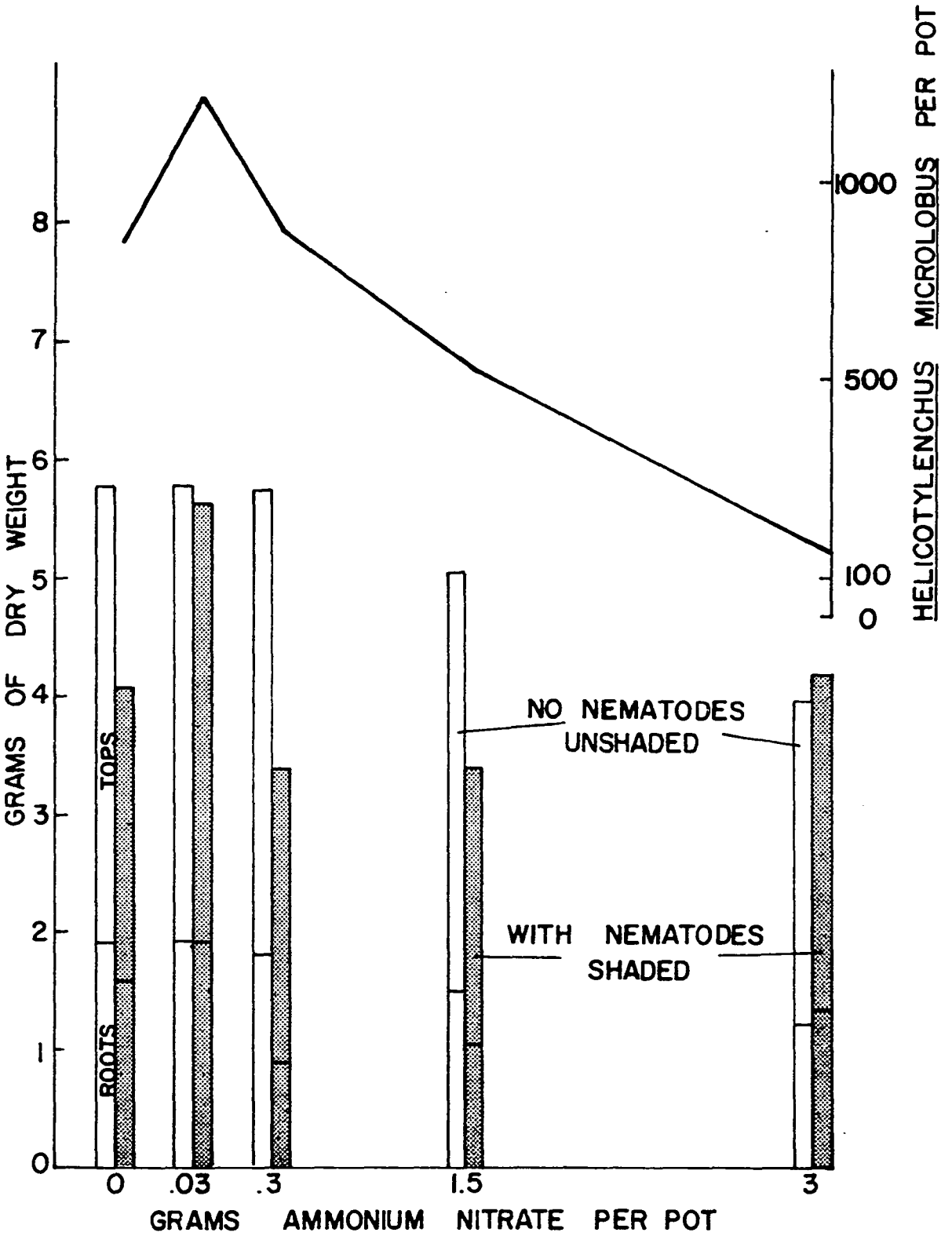


Fig. 7. A comparison of Helicotylenchus microlobus numbers with 0-3 gram ammonium nitrate treatments and dry weights of Iowa 4570 corn grown for 127 days; second greenhouse experiment

Table 11. The relative number of Helicotylenchus microlobus per pot of steam sterilized soil in which corn was grown for 127 days with 0-3 grams of ammonium nitrate and 0-2500 Helicotylenchus microlobus; second greenhouse experiment

Treatments		Dry weights[a]			Helicotylenchus microlobus[a]	
Nematodes added/pot	Ammonium nitrate	Tops	Roots	Total	Per pot	Per pot/g root
	g	g	g	g		
0	0	3.84	1.93	5.77	0	0
0	0.03	3.80	1.92	5.72	0	0
0	0.30	3.84	1.81	5.65	0	0
0	1.50	3.61	1.43	5.04	0	0
0	3.00	2.74	1.24	3.98	0	0
		17.83	8.33	26.16		
2500	0	2.56	1.51	4.07	849	562
2500	0.03	4.64	1.91	6.55	1217	637
2500	0.30	2.47	0.92	3.39	860	935
2500	1.50	2.29	1.06	3.35	517	488
2500	3.00	2.90	1.29	4.19	183[b]	142
		14.86	6.69	21.56		

[a]Average of 7 determinations.

[b]183 is significantly different from 849, 1217, 860 and 517 at the .01 level.

The H. microlobus number per liter in the 0.3, 1.5 and 3 gram treatments were significantly different from the number per liter in the 0 and 0.03 treatments but not from each other (Fig. 8). These differences were significant at the .05 level. The lowest number of spiral nematodes were found in the 3 gram treatment. This number was not significantly different from the average number found in the 0.3 and 1.5 treatments (Table 12).

The plants were slightly stunted, but all tasselled and set some fruit.

Plants grown without nematodes weighed more than plant grown with nematodes. These differences were not significant.

Feeding of Helicotylenchus microlobus

Stained root sections indicate that the feeding of spiral nematodes in corn (Iowa 4570) and yellow sweet clover (Madrid) is limited to the cortex outside the endodermis. Feeding may occur from the root tip back to the zone of lateral root formation. The nematodes feed ectoparasitically with the anterior portion inserted into the root while the posterior portion coils into the familiar spiral (Fig. 9 and 10). Epidermal and cortical cell walls are penetrated and ruptured by the nematode's body (Figs. 11, 12 and 13). The cell walls along the length of the nematode stain more darkly than do other undisturbed cortical cells, especially cortical cell walls associated with the head or anterior portion of the nematode (Figs. 13 and 14). The darker staining is not apparent in sweet clover roots as it is in corn roots (Figs. 15 and 16).

The nematodes can feed with their heads in the cells or they may protrude their stylets through the cell walls into the adjacent cells (Figs. 11 and 15). No nematodes were found in the stele or endodermis of corn or sweet clover, although one was found with its head buried in the cortical cell row adjacent to the endodermis of a corn root.

Pathogenicity of *Helicotylenchus microlobus*

In the test to determine temperature relationships and pathogenicity of *H. microlobus* to yellow sweet clover, it was found that *H. microlobus* numbers increased only at 30° C (+282%) and decreased at 15, 20 and 25° C (-96, -94 and -26%, respectively). Overall top weights were not significantly affected by *H. microlobus* infestations; at 30° C, however, infected plant top weights were less than control plants at each cutting. These differences were not significant. The overall root weights of *H. infected plants* were reduced slightly (significance of .10). Most of this reduction came from the weight differences of plants growing at 30° C, which were infected by substantially more spiral nematodes than plants at the other temperatures. These data are summarized in Table 13.

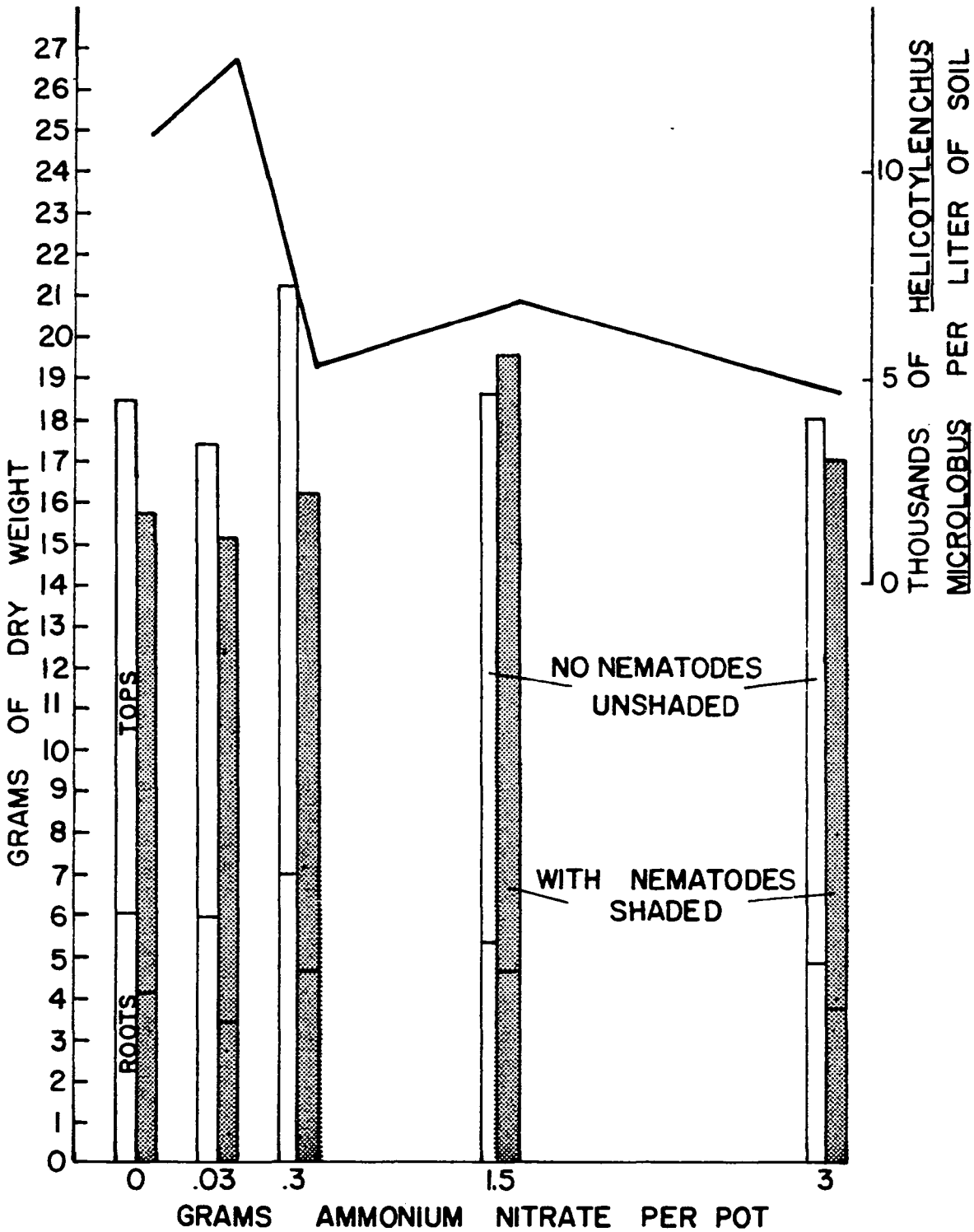


Fig. 8. A comparison of Helicotylenchus microlobus numbers with 0-3 gram ammonium nitrate treatments and dry weights of Iowa 4570 corn grown for 113 days; third greenhouse experiment

Table 12. The relative number of Helicotylenchus microlobus per liter of steam sterilized soil in which corn was grown for 113 days with the addition of 0-3 grams of ammonium nitrate and 0-3000 Helicotylenchus microlobus; third greenhouse experiment

Nematodes added/pot	Ammonium nitrate	Dry weights[a]			Helicotylenchus microlobus[a]	
		Tops	Roots	Total	Per liter	Per liter/g root
	g	g	g	g		
0	0	12.4	6.03	18.4	0	0
0	0.03	11.4	5.96	17.4	0	0
0	0.30	14.3	6.95	21.3	0	0
0	1.50[b]	13.2	5.29	18.5	0	0
0	3.00[c]	13.1	4.83	17.9	0	0
		64.4	29.06	93.5		
3000	0	11.7	4.15	15.8	10,933	2,634
3000	0.03	11.7	3.43	15.2	12,774	3,715
3000	0.30	11.5	4.67	16.2	5,183[d]	1,110
3000	1.50[b]	14.9	4.62	19.5	6,892[d]	1,492
3000	3.00[c]	13.2	3.79	17.0	4,666[d]	1,231
		63.0	20.66	83.7		

[a]Average of 7 determinations.

[b].5 grams applied 14, 28 and 52 days after planting.

[c]1 gram applied 14, 28 and 52 days after planting.

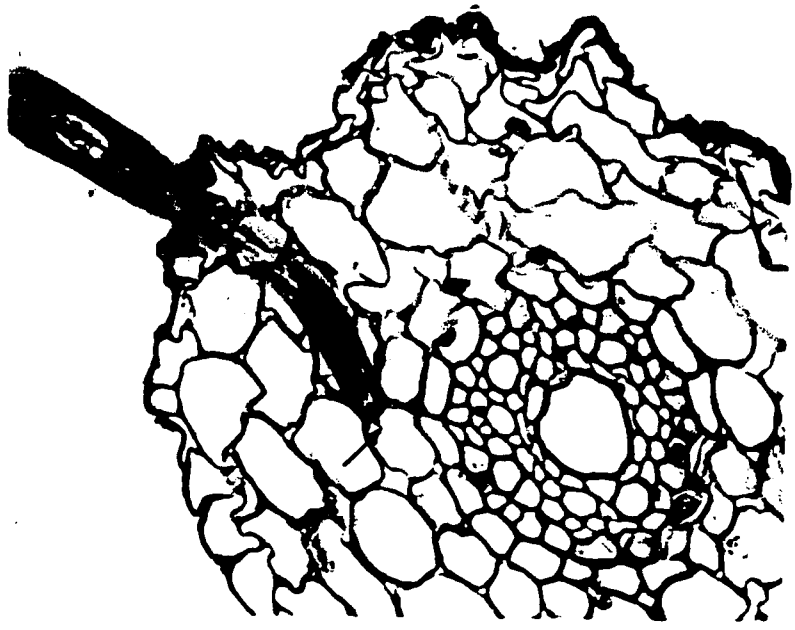
[d]5,183, 6,892 and 4,666 are significantly different from 10,933 and 12,774 at the .05 level.

Fig. 9. Photomicrograph showing five Helicotylenchus microlobus embedded into the root hair zone of a corn root (36X)

Fig. 10. Photomicrograph showing anterior portion of Helicotylenchus microlobus embedded into a corn root (190X)

Fig. 11. Photomicrograph of Helicotylenchus microlobus feeding in cortex of corn root (240X)

Fig. 12. Photomicrograph of Helicotylenchus microlobus feeding in cortex of corn root showing damage to cell walls in adjacent areas (240X)



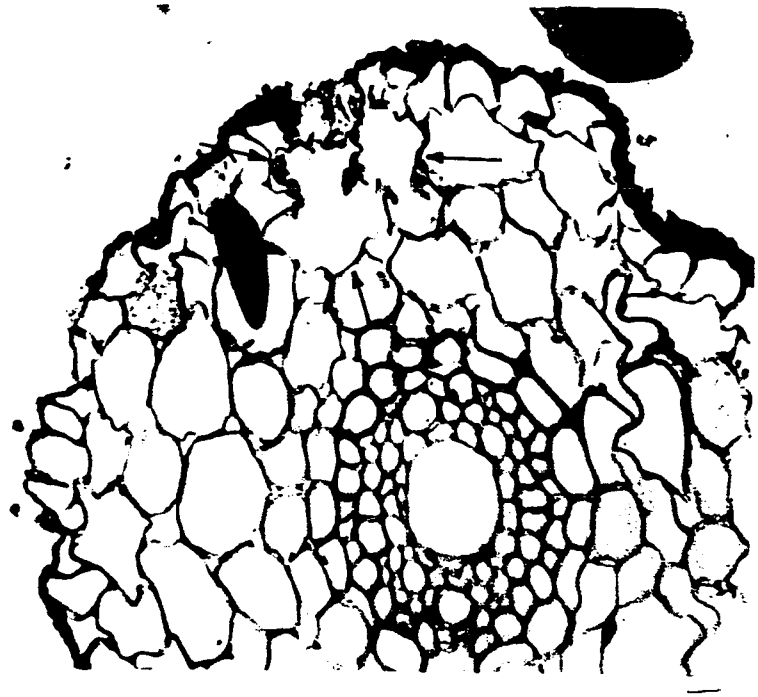
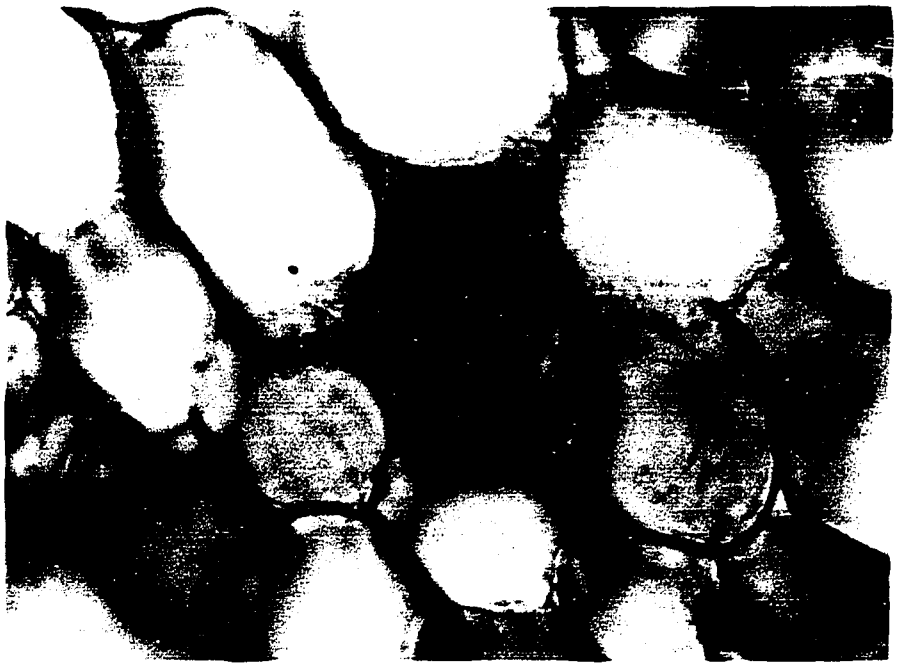


Fig. 13. Photomicrograph of Helicotylenchus microlobus showing head in cortical cell with darkly staining walls; compare with adjacent undisturbed cell walls (1500X)

Fig. 14. Photomicrograph of Helicotylenchus microlobus embedded in corn root cortex showing cell wall damage and darkly staining cell walls associated with body of nematode (240X)



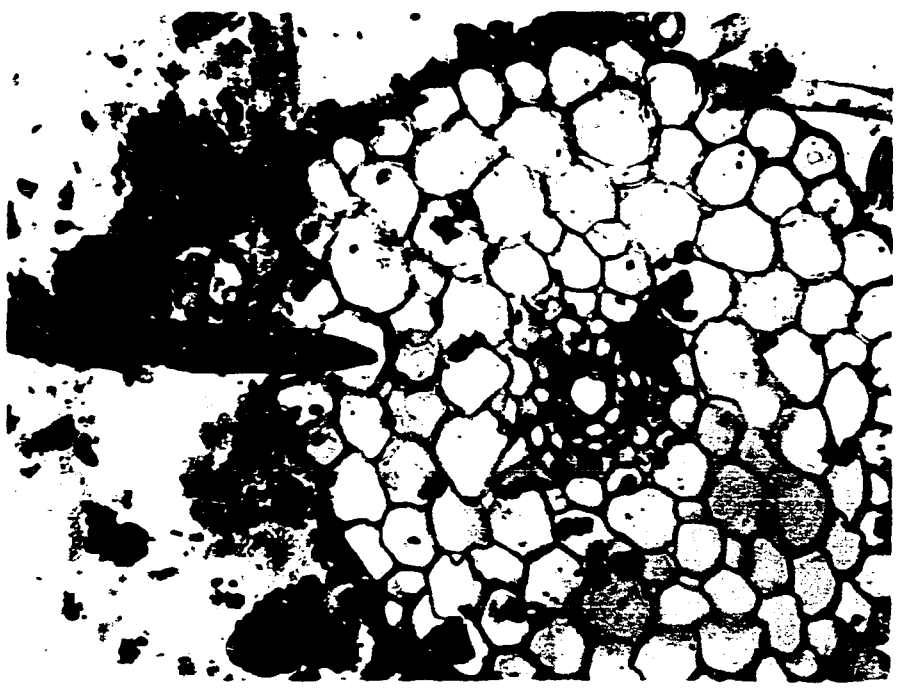


Fig. 15. Photomicrograph of Helicotylenchus microlobus with stylet protruding into an adjacent cortical cell of a yellow sweet clover root (240X)

Fig. 16. Photomicrograph of Helicotylenchus microlobus penetrated into the cortex of a yellow sweet clover root (240X)

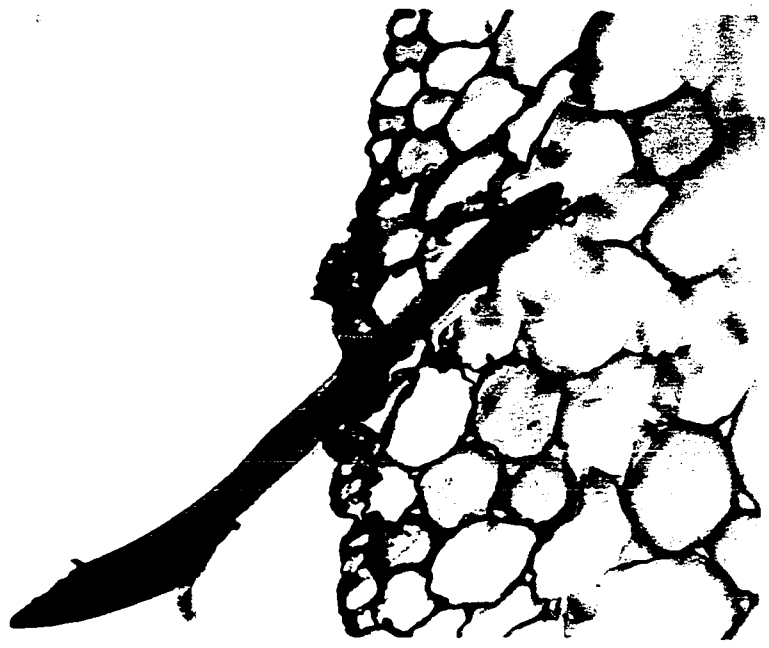


Table 13. The relative dry weights of Madrid yellow sweet clover and Helicotylenchus microlobus numbers from pots initially infested with 0-4600 Helicotylenchus microlobus and placed at 15, 20, 25 and 30° C for 138 days

Treatments[a]		Tops[b]			Roots[b]	Total[b]	Helicotylenchus[b]	Change
		g	g	g	g	g	microlobus	
Temp.	H. microlobus	78 days	108 days	138 days	138 days		Per pot	%
15° C	0	6.95	10.37	21.23	9.67	48.22	0	
15° C	4200-4600	7.61	10.69	21.48	8.42	48.20	176	-96
20° C	0	9.19	11.56	24.14	9.95	54.84	0	
20° C	4200-4600	10.65	12.82	25.69	10.11	59.27	264	-94
25° C	0	12.68	12.10	23.31	8.00	56.09	0	
25° C	4200-4600	11.18	11.88	23.63	6.94	53.63	3,117	-26
30° C	0	11.91	7.52	16.45	5.01	40.89	0	
30° C	4200-4600	10.24	7.01	13.42	3.58	34.25	16,064	+282
Totals								
All	0	40.73	41.55	85.13	32.63	200.04		
All	4200-4600	39.68	42.40	84.22	29.05[c]	195.35		

[a]Treatments applied to each pot 20 days after planting.

[b]Average of 9 determinations.

[c]29.05 is significantly different from 32.63 at the .10 level.

DISCUSSION AND CONCLUSIONS

These data show that Helicotylenchus microlobus field populations respond negatively to nitrogen treatments at three locations in Iowa. Three possible reasons are considered: 1) high nitrogen amounts increase the host's resistance to feeding; 2) high nitrogen amounts favor the growth and development of competitive plant parasitic nematodes to the detriment of H. microlobus; 3) high nitrogen amounts per se, in the form of ammonium nitrate, are nematocidal to H. microlobus.

The comparison of roots grown under different nitrogen amounts failed to reveal any anatomical differences between roots grown with or without added nitrogen. Since the sclerenchyma layer beneath the epidermis was not well formed in young roots, whether grown with or without added nitrogen, there is no apparent anatomical barrier to feeding. The only apparent effects to the plants were greater dry weights or stunting when high amounts of nitrogen were applied. Finally, nitrogen constitutes only a small part of the cell wall make-up (12). The chemistry of lignin is still imperfectly known, but it is an organic compound high in carbon and distinct from the carbohydrates, and does not have nitrogen as an important constituent (4).

No evidence was obtained to support the theory that in the presence of high nitrogen amounts other competitive plant parasitic nematodes were favored to the detriment of H. microlobus. In the greenhouse tests, H. microlobus numbers responded negatively to nitrogen treatments in the absence of any other plant parasitic nematodes. Pratylenchus spp.

numbers, which were almost inversely proportional to H. microlobus numbers at Ames during 1963 and 1964, are probably more related to increased corn root systems as a result of the added nitrogen than to H. microlobus numbers per se.

It is believed that nitrogen treatments are themselves detrimental to H. microlobus. Numbers of H. microlobus were negatively responsive to nitrogen treatments at all three experiment stations, although soil types, species and relative numbers of accompanying plant parasitic nematodes, and growing conditions were different at all three locations.

Nitrogen (usually as ammonium nitrate) is applied at these stations in a narrow band running through the hills of corn. It is probable that in this narrow band, which receives all of the nitrogen, the ammonium nitrate concentration reaches a high enough level in places to kill spiral nematodes. The chances of reaching a killing concentration would be dependent on the pounds of nitrogen applied per acre; thus, at higher nitrogen applications more killing concentrations would occur and nematode numbers would be reduced.

In the greenhouse experiment, the ammonium nitrate was applied on the relatively small surface area of a six inch pot. When high amounts were applied, killing concentrations were frequently created and spiral nematode numbers were accordingly reduced. When the ammonium nitrate was applied in thirds at two week intervals in the third greenhouse experiment (Table 12), rather than at once as in the first and second greenhouse experiments (Tables 10 and 11), the killing concentrations were probably not so often reached and nematode numbers in the 0.3, 1.5 and 3 gram treat-

ments were not significantly different from one another, as they were in the other two experiments.

In the short range field experiment at Ames, instead of fewer spiral nematodes being found with 160 lb nitrogen per acre, higher numbers were found. It is believed that this was the result of the application method. Whereas, it is usual to apply ammonium nitrate mechanically in a narrow row band close to the hills of corn, the ammonium nitrate in this experiment was broadcast manually in a two foot wide band. Because of this, nematocidal concentrations were probably not so often reached and the increased corn root volume provided a greater food supply that increased the spiral nematode numbers in treated rows over control rows.

This theory is supported in part by work of Eno et al. and Oteifa. Eno et al. (11) found that anhydrous ammonia was a potent nematocide around injection sites at retention concentrations of 400-700 ppm ammoniacal nitrogen. The nematode numbers surviving the applications were dependent on their distance from the injection site. Since ammonium nitrate is the most common form of nitrogen applied to the soil at the three test locations, it is probable that ammonia is important in the reduction of H. microlobus numbers. Oteifa found that Meloidogyne incognita produced fewer egg masses with the ammonium ion present than with the nitrate ion (22).

How ammonium nitrate reduces spiral nematode numbers is uncertain. Major osmotic pressure changes caused by additions of high amounts of nitrogen may be fatal to nematodes. A rapid salt concentration change in the soil-water solution may cause physiological desiccation of the

nematodes. It is also possible that the ammonium ion disrupts the nematode's metabolism, or destroys the protective cuticle, leaving the nematode unprotected against pathogen invasion and minor osmotic changes.

The low Pratylenchus spp. numbers in the Bloomfield plots that received 240 lb nitrogen per acre year since 1952 and the low H. galeatus numbers in the plots that received over 80 lb nitrogen per acre year at Ames since 1915 and Bloomfield since 1952 indicate that species other than H. microlobus may be affected by high nitrogen treatments, although probably to different degrees.

Under ordinary circumstances, the use of ammonium nitrate as a control measure in Iowa soils does not appear practical. First, most Iowa soils contain more than one species of plant parasitic nematode. These species may possess varying tolerances to ammonium nitrate. For example, at Ames, those plots that received high nitrogen amounts for 48 years had low H. microlobus numbers, but the added corn root volume was also responsible for high Pratylenchus spp. numbers. Hence, nitrogen may control one nematode but favor others. Second, Iowa farmers as a rule do not apply nitrogen in the amounts necessary for nematocidal action. If future investigations reveal that a nematode species is responsible for substantial yield losses in a crop, the nitrogen level required for control of the nematode may be discounted as contrary to normal practices. Third, high nitrogen applications may be too expensive. Finally, a nitrogen level effective in reducing nematode numbers of all species may be beyond the tolerance of the host plants. This may be avoided by a

non-growing season application, but this may be impractical. In the fall, nitrogen could be applied when the stubble is chopped, but cold winters normally reduce the nematode numbers (5), and later winter and spring rains may lessen the nitrogen effect by leaching the nitrogen out. In the spring, a high nitrogen amount may cause severe damage to seedlings or predispose them to pathogens.

These data show that fluctuation patterns found during the growing season agree generally with previous findings in Iowa. Norton (19) found that X. americanum numbers were high in spring, lowest in June and highest in August or September. Castaner (5) found that fluctuation patterns of H. microlobus, Pratylenchus spp., H. galeatus and X. americanum were similar to those Norton reported for X. americanum.

Helicotylenchus microlobus was found only in the cortex of corn and yellow sweet clover roots. Sledge (29) reported that H. nannus fed on corn root hairs as well as in the cortex. The present study can not rule out this feeding method for H. microlobus since few in vitro observations were made. Perry (23) found that H. digonicus fed occasionally in the phloem of Kentucky bluegrass. In the present study, no penetration occurred in the endodermis or tissues below. The deepest penetration occurred in the layer of cells adjacent to the endodermis.

Perry (23) obtained Kentucky bluegrass weight losses with an initial infestation of only 2500 H. digonicus per pot after 2.5 months. In the greenhouse experiments reported here, no significant differences in weight were found among corn total dry weights and yellow sweet clover top dry weights after 113, 127 and 138 days with initial infestations of

2500-4200 H. microlobus (Tables 11, 12 and 13). The inability of these experiments to detect significant weight differences, if one assumes that nematode feeding involves the loss of materials and that this loss could be accurately measured by these experiments, is considered to be related to: 1) the combined effect of time and temperature and 2) the confounding of differences with greenhouse growing conditions. In preliminary work, the accumulated top dry weights from several cuttings (60-210 days) of Madrid yellow sweet clover infested initially with 2000 H. microlobus were higher than plants grown without nematodes. It was only in the last cutting (210 days) that infected plants weighed less. In the temperature controlled tanks experiment, the differences in top weights (Table 13) between treated and untreated plants were greatest at the 138 day cutting. Most of this difference came from the 30° C treated plants where the average number of nematodes per pot was 16,000. The average spiral nematode numbers at other temperatures was less than the initial infestation. This indicates that plants may sustain H. microlobus infections of less than 16,000 per pot without severe losses and that temperatures around 30° C are important in the reproduction of H. microlobus. The average temperature at 6 inches, at the Iowa State Agronomy Farm, Ames, from mid-July to mid-August is 25° C or more 70% of the time at noon and 90% of the time at 7 PM. If, in this warm period, egg production (or hatch of eggs previously produced) is initiated, this would explain then the rapid build-up of spiral nematode numbers during August and September. Thus the absence of a large spiral nematode population for a period of time after the host and nematodes

are in contact may indicate that relative losses may be small. At Ames, spiral nematodes are most abundant 2.5-3 months after corn planting. Another aspect of the combined influence of time and temperature on the pathogenicity of H. microlobus is the expendibility of cortical root tissues. Repeated observations in August has shown that little or no root growth occurs at the 6 inch depth; apparently most of the plant's food is being channelled into fruit production. If, by endodermal lignification, the cortex is eventually cut off from the stele of maturing roots, isolating the cortical cells and their contents, then damage caused by feeding H. microlobus would be considered unimportant to the plant's accumulated top weights and would serve to provide a food supply necessary for the increase in spiral nematode numbers that occur during August and September. Finally, greenhouse conditions are unlike field conditions. Corn requires a great deal of soil and usually does not grow well in the greenhouse. The corn plants grown in these experiments were far below "normal" appearance. Losses caused by spiral nematodes may have been confounded with differences between field grown and greenhouse grown plants.

SUMMARY

Field populations of Helicotylenchus microlobus were found to respond negatively to nitrogen amounts applied to experiment plots at the Iowa State Agronomy Farm, Ames, during 1963 and 1964; the Carrington-Clyde Experiment Farm, Independence, during 1964; the Southern Iowa Experiment Farm, Bloomfield, during 1964. Most H. microlobus were found in plots that received 0-40 lb nitrogen per acre year, while fewer or none were found in plots that received 160-240 lb nitrogen per acre year.

In three greenhouse experiments, 0, 0.03, 0.3, 1.5 and 3 grams of ammonium nitrate were applied to Iowa 4570 corn (Zea mays) planted in 6 inch plots and infested initially with 2500-3300 H. microlobus. After 103-127 days, the H. microlobus numbers per pot were found to be significantly lower in the 3 gram treated pots than in the other nitrogen treated pots, except when the 3 grams were applied over a four week period instead of once at the beginning of the experiment, in which case, H. microlobus numbers were not significantly lowered. No significant total dry weights between nematode infected and uninfected plants were found after 103-127 days.

It is believed that ammonium nitrate at high application amounts is nematocidal to H. microlobus. These high applications increase the chances of nematocidal concentrations occurring in the soil.

Helicotylenchus microlobus was observed only in the cortex of corn and yellow sweet clover (Melilotus officinalis) roots. Penetration caused cell wall rupture and cell necrosis. Cell walls associated with feeding nematodes stain considerably darker with fast green FCF than do

undisturbed cell walls.

Madrid yellow sweet clover plants were grown at 15, 20, 25 and 30° C with and without nematodes. After 138 days, total root dry weights of infected plants were significantly lower than total root dry weights of uninfected plants. The total top dry weights of nematode infected plants were not significantly different from those of uninfected plants, although top dry weights for infected plants grown at 30° C were consistently lower than for uninfected plants for three cuttings. The numbers of H. microlobus were reduced from initial infestation numbers at 15, 20 and 25° C by 96, 94 and 26%, respectively. At 30° C, the spiral nematode numbers increased by 282%. Seasonal field population build-ups may be related to periods of soil temperatures over 25° C.

Field losses caused by H. microlobus may be slight, if populations are low during most of the plant's active root growing period. Greenhouse losses may be undetected for the same reason, but may also be confounded with losses caused by poor greenhouse growing conditions.

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