SELECTED VARIETIES OF BERMUDAGRASS AS HOSTS FOR *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES IN OKLAHOMA INCLUDING REPRODUCTION OF *X. AMERICANUM* IN PURE POPULATIONS

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CHAPTER I

INTRODUCTION

Oklahoma ranks sixth among the states in cattle production, supplying 6.5 million head valued at $1.462 billion as of January, 1979 (39). Most of the livestock is grazed on improved pastureland planted to introduced grasses. The majority of the improved grasses are bermudagrass (53). Of the 6 million acres of bermudagrass throughout the state, about 25%, i.e., 1.5 million acres, are in 'Midland' (53). Despite the vast importance of forage grasses in this state, our knowledge of the effects of nematodes on range and pasture grasses, including bermudagrass, is limited, at best.

Annual nematode-induced losses in grass production were estimated at $437 million nationally (59). This figure is based on the average farm value for forage and grain crops. It does not, however, include silage, pasture, or hay production losses.

The above losses are estimated on nematode damage alone. It is believed by many nematologists and plant pathologists that the role of nematodes in complex disease situations is of even greater economic importance than the effects caused by nematodes alone (54,60). Therefore, the above economic losses are exceedingly conservative.

Bermudagrass, introduced into the United States about 1751 from Africa is said to be the most important pasture grass in the southern states (44). Under good moisture and adequate fertilization it will support one cow per acre per year (53). Although it spreads rapidly when properly maintained; it generally loses vigor in a few years (44). Increased applications of nitrogen alone will not retard the competition from weedy species in declining pastures (53). Soon undesirable grasses invade the pasture resulting in a large decrease in forage production, i.e., requiring six acres per cow per year (53).

Midland, a hybrid bermudagrass obtained by crossing an Indiana strain of common with 'Coastal' (24), is popular in Oklahoma because of its high production of forage, its excellence for erosion control, and its winter hardiness (24,53).

Little is known about the effects of nematodes on forage production (3) perhaps because the value per acre of forage grasses has not been considered sufficient to warrant intensive investigation (55). Several nematode species were reported to multiply on and in some instances cause damage to grasses. Pratylenchus projectus originally discovered by Jenkins (28) associated with decline of pasture grasses was again reported (11) as a parasite on tall fescue. Jenkins, Taylor, and Rhodes (30) reported that out of 368 fields of clover, pasture, and forage crops, the most important frequently found nematode genera were: Xiphinema spp., 29%; Tylenchorhynchus spp., 24%; Pratylenchus spp., 17%; Paratylenchus spp., 10%; Rotylenchus spp., and Helicotylenchus spp., 8%. Norton (37) reported Tylenchorhynchus spp. as the most common nematode associated with small grains and grasses with H. nannus and X. americanum the next most prevalent; and P. projectus and R. robustus also associated.

Turf grasses also are subject to nematode attack and due to their higher value per acre have been more intensively investigated. Perry

(41) reported that spiral nematodes were found associated with "summer dormancy" of 'Kentucky' bluegrass in Wisconsin. The following year, Perry, Darling, and Thorne (43) proved that H. digonicus incites root necrosis, leaf chlorosis, and stunting of plant growth which leads to thin strands in the summer, i.e., "summer dormancy" of Kentucky bluegrass. Belonolaimus longicaudatus reduced growth of St. Augustine grass by 62 to 69% and Tricodorus christiei reduced growth by 25% (49). Heterodera leuceilyma was reported to be a severe pathogen of St. Augustine grass in Florida (13). Potter, Townshend, and Davidson (46) reported that Meloidogyne incognita formed galls on 30 species of wild and cultivated grasses, reproduced on 27 species, while four species exhibited no galling and no evidence of invasion.

Bermudagrass germplasm has been reported to range from immune to highly susceptible to Southern root-knot nematode (36). Riggs, Dale, and Hamblen (52) reported that Midland bermudagrass was immune to M. arenaria arenaria, M. hapla, and M. javanica, but susceptible to M. incognita incognita and M. incognita acreta. 'Tiflawn', 'Tiffine', and 'Tifgreen' bermudagrasses were hosts for Meloidogyne spp. while 'Ormond' bermudagrass was not (57). Criconemoides lobatum was able to multiply on Tifgreen bermudagrass, zoysia, centipede, and St. Augustine grasses (31).

Tarjan (61) reported a mixed population of X. diversicaudatum and X. coxi around native roots and X. vulgare around grass roots. Homeyer (27) reported the presence of Xiphinema, Belonolaimus, Pratylenchus, Criconemoides, and Trichodorus spp. on turf. Mixed populations of Xiphinema, Tylenchorhynchus, Criconemoides, and Helicotylenchus spp.; and less frequently Pratylenchus spp. were associated with forage grasses in northeast United States (16). In a South Dakota grassland biome study (58), X. americanum was found in all samples from depths of 5 to 60 cm; and under the grazed range areas nematodes consumed nearly twice as much forage as did the cattle.

An initial sampling of a declining Midland bermudagrass pasture in Oklahoma revealed the presence of seven different genera of plant parasitic nematodes. Xiphinema americanum was present in sufficiently high numbers to warrant investigation of its association with the host.

Xiphinema americanum was the focus of this study. The American dagger nematode is an active, cosmopolitan, and relatively large (1.5 to 2.0 mm) plant parasitic nematode possessing a long (120 to 140μ) stylet (65). It was originally described by Cobb (8) and has been associated with extensive necrosis and destruction of feeder roots of oak, pecan, and azaleas (7); maple decline (14); poor growth and premature decline of cottonwood and green ash (34); maple dieback and blight (51); poor alfalfa growth (68); unthrifty wheat, declining orchards and strawberries, and dieback of Wisconsin maple forests (65). Thorne classified it as one of the four major nematode pests in the southeastern states; and he believed that the American dagger nematode may be the "most destructive plant parasitic nematode in America", p.489.

In Oklahoma, X. americanum was found in numbers as high as 1484 nematodes per 100 cc of soil in the rhizosphere of sorghum roots (54).

Thorne (65) considered Xiphinema spp. to be a nematode of deep soil profiles. He usually found dagger nematodes below 30 cm in cultivated fields. However, in an Oklahoma vertical distribution sampling of the soil in the rhizosphere of sorghum, the greatest numbers were recovered from a depth of 13 cm even though some were found as deep as 100 cm (54). On cottonwood plots previously in roses (containing many weeds) *Xiphinema* spp. was found to a depth of 45 cm with the highest populations again recovered from the 10 to 15 cm depth sampling (54).

The failure of *X. americanum* to reproduce under artificial conditions is well recognized (19). In controlled environments, *X. americanum* is known to be intolerant to moisture and temperature extremes (10, 15, 22, 33). A soil moisture of 21% has been reported as optimum for greenhouse survival of the nematode (33). Soil temperatures of 28 C with cucumber as the host (15) and 21 C with strawberry as the host (33) have also been found to be conducive to greenhouse survival of *X. americanum*. A mixture of sand, peat moss, and commercial fertilizer also was used successfully as a survival medium for the American dagger nematode (64). Soils with extremes in pore size and/or high organic content are deleterious to survival of the nematode (45). Because methods to maintain the parameters critical for its survival have not been successfully devised, no one has been able to rear a pure population of *X. americanum* under greenhouse conditions in sufficient numbers for use as inoculum.

The objectives of this study were to elucidate some of the effects of *X. americanum* on bermudagrass, to determine what abiotic factors delimit the greenhouse manipulation of the nematode, and to evaluate systems which might allow rearing and maintenance of populations for nematode and disease complex studies on bermudagrass in the future.

CHAPTER II

GREENHOUSE REPRODUCTION OF *XIPHINEMA AMERICANUM* UNDER CONTROLLED ABIOTIC ENVIRONMENTAL FACTORS

Introduction

Xiphinema americanum is known not to flourish under artificial culture conditions (19). In controlled environments, the American dagger nematode is intolerant of moisture and temperature extremes (10, 15, 22, 33). Consequently field infested soil has been used for inoculum in studies requiring more than a few *X. americanum* (9, 10, 18, 22, 23, 33, 34, 42, 45, 68). The objective of this portion of the study was to develop a system in which *X. americanum* would reproduce in quantities sufficient for use as experimental inoculum.

Materials and Methods

Containers were fashioned of 6 mm thick polyvinyl chloride (PVC) pipe (11 cm I.D.), Figure 1. Each was 45 cm high, cut in half lengthwise, marked at each 5 cm increment, and retaped with 5 cm wide duct tape (Sekisui, Japan). A 17 X 17 cm piece of plastic window screen secured to the cylinder by a narrow band of PVC pipe served as the porous container bottom. An 11 cm diameter filter disc, Whatman #1, was placed on top of the screen bottom to prevent loss of soil through the screen. A copper heat-sink fashioned of 7 mm O.D. copper tubing bent into a U-shape and painted with two coats of white marine epoxy

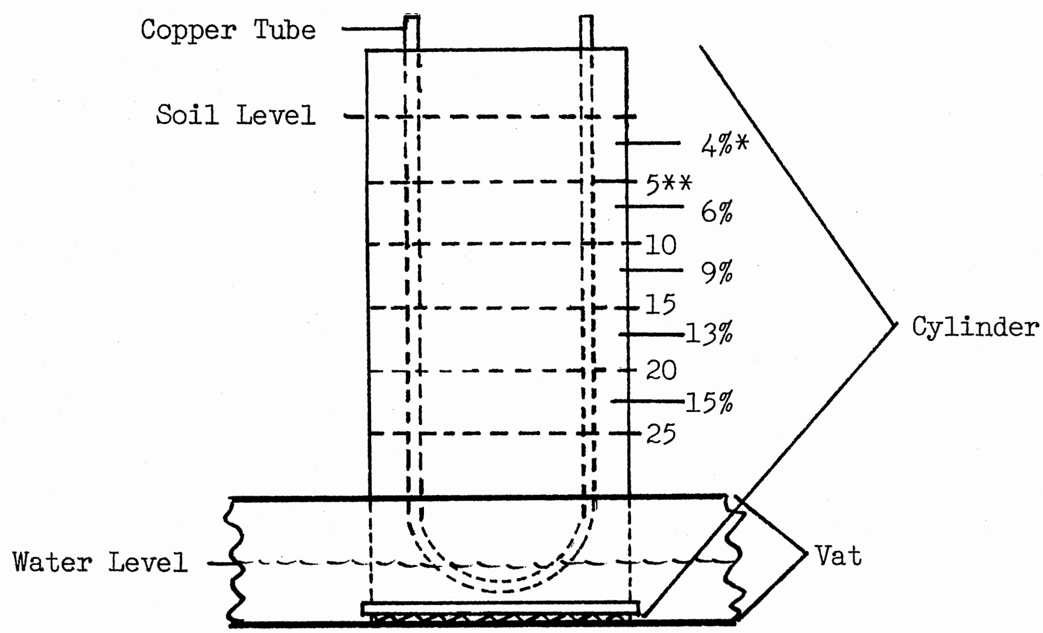

Figure 1. Schematic of Experimental Unit used in Greenhouse Reproduction Study.
* Percent Figures refer to Percent Moisture by Weight observed in Cylinders maintained at 18 C (mean of 4 observations).
** Soil Depths (cm).

paint was placed inside the container to a depth of 5 cm from the bottom. A mixture of sterile [1.35 kg methyl bromide (MB)/ 0.76 m^3 sand, untarped after 2 d] 50% Lincoln fine sand and 50% builders sand (by volume) with a maximum partical size of 841μ was poured into the container to within 5 cm from the top. The contents was compacted slightly by gently dropping each container 5 times from a height of 5 cm. Additional sand mixture was used to bring the soil surface to the original level.

The experiment was a split-plot consisting of 4 replicates with 3 main plots per block. The main plots were treatments arranged in a randomized complete block design and the 5 cm depth increments were the subplots. Midland bermudagrass was the host plant. Inoculum was 50 *X. americanum* per container. Treatments were soil temperatures of 18, 24, and 30 C. Griffin and Barker (22) reported 24 C as optimum for *X. americanum* survival while Cohn and Mordechai (10) reported 28 C as optimum. Lownsbery and Maggenti (33) found that the American dagger nematode did not survive at 32 C while it did at 16, 21, and 27 C. Twenty-four degrees C was selected for testing as it corresponds to optimum for *X. americanum* survival (22). Since 32 C was not conducive to American dagger nematode survival (33), 30 C was selected as the highest temperature for testing; 18 C, six degrees lower than 24 C and still within the range of temperature for *X. americanum* survival (33), was selected as the lowest temperature for testing. The heat sinks for each soil temperature were connected in series with 5 mm I.D. PVC tubing, then each to its own constant temperature system (53).

The containers were placed into a 244 X 123 X 10 cm (length, width, and height; respectively) vat constructed of 2 X 4 sides and 5/8" exterior plywood bottom. The vat was painted with several coats of white marine epoxy paint to make it waterproof. White was selected to prevent radiant heat absorption. A drain fitted with an overflow tube to maintain constant water level of 5 cm was used. The inflow tube was located in the opposite corner. A submersible pump (CA5 Beckett Co., Dallas, Texas) pumped the water in through a 2 mm I.D. tube and the excess flowed into the small vat containing the pump and water supply. To offset evaporation, water was added continually to this pumping vat from the greenhouse water supply.

The vat was filled with water to a depth of 5 cm and the water level maintenance system was activated. After a 2-week equilibration period, a 2-node length cutting of Midland bermudagrass (obtained from greenhouse stock) was planted in the center of each container. No further surface irrigation was used. The plants were allowed 1-wk to root at which time each container was inoculated.

Fifty hand-picked adult and fourth stage larval female X. americanum contained in a vial with 20 ml of deionized distilled water were gently poured into a 5 cm deep depression in the root zone of each container. Fresh sand-mixture was gently poured into the inoculation site to refill the depression.

Four additional cylinders of sand-mixture were prepared in the same manner without plants along with those to be tested as a control in order to determine handling and recovery losses and to calculate a recovery factor (RF). Immediately after inoculation, these four control cylinders were dismantled. The volume of soil from each 5 cm soil depth increment was placed into a prelabeled plastic bag, stored in an ice chest, and transported to the laboratory where it was assayed for nematodes by the modified Christie-Perry method (2) using a 325 mesh screen. After a 24-hr recovery period, the *X. americanum* were identified and counted using a stereoscopic microscope. A recovery factor was calculated based on the numbers of *X. americanum* recovered: $RF = \frac{\text{Inoculum Number}}{\text{Recovery Number}}$. The nematode populations recovered at the termination of the study were multiplied by the RF before being analyzed and presented in this chapter.

Soil moistures and temperatures were monitored for 9 mo at which time the study was terminated. No fertilizers were used since nutrient solutions even at the normal concentration can drastically suppress nematode populations (10). Normal greenhouse foliar spray procedures for aphids (10 ml of 50% Malathion per gallon of water) were carried out as needed, since these procedures have been found to have no adverse effects on nematode populations (10).

At termination of the study, forage per cylinder was clipped at the soil surface, weighed and oven-dried at 105 C for 1 wk. It was then cooled to room temperature and reweighed. Each cylinder was then split vertically through the center, the heat-sink gently removed, and the volume of soil from each 5 cm soil depth increment was placed into a prelabeled plastic bag and stored in an ice chest until assayed for nematodes as indicated above (immediately after study dismantling). In addition, a 15 cc soil sample from each increment was placed into a covered 8 cm O.D. glass petri dish and weighed (Metler analytical balance) as above. Soil moistures were calculated from the weights:
$\frac{\text{Wet Weight - Dry Weight}}{\text{Dry Weight}} \times 100 = \%$ Moisture.

Roots from each increment were carefully extracted from the soil, combined for each cylinder, and weighed (Metler analytical balance).

All moistures are reported as % by weight, but the partial pressures for each moisture increment can be determined by use of the standard curve specifically derived for this soil texture, Figure 2. Although variations up to ± 3.0 C were found at each soil temperature, 18, 24, and 30 C will be used for the sake of consistency.

The data were subjected to analysis of variance. Comparisons between pairs of means were made using LSD figures.

Results and Discussion

Table I shows the results of the study. *Xiphinema americanum* reproduced on Midland bermudagrass during the 9 mo of study at soil temperatures of 18, 24, and 30 C. Since 15 cc of each soil volume were used for moisture determination, the nematode counts presented are conservative. For the sake of discussion it is assumed that the nematodes recovered from a particular depth reproduced at that depth. Nearly a 17 fold increase was seen in the total *X. americanum* counts recovered from the cylinders kept at 24 and 30 C over those kept at 18 C, Table I. Reproduction at 24 and 30 C substantiate the population increase peaks of *X. americanum* occurring in nature, Chapter IV, which occurred at soil temperatures of 23 and 26 C. The reproduction data also substantiate the survival data of Griffin and Barker (22) who reported 24 C as optimum for *X. americanum* survival, Cohn and Mordechai (10) who reported 28 C, and Lownsbery and Maggenti (33) who reported survival at 21 and 27 C.

The nematodes were inoculated 5 cm from the top surface of the

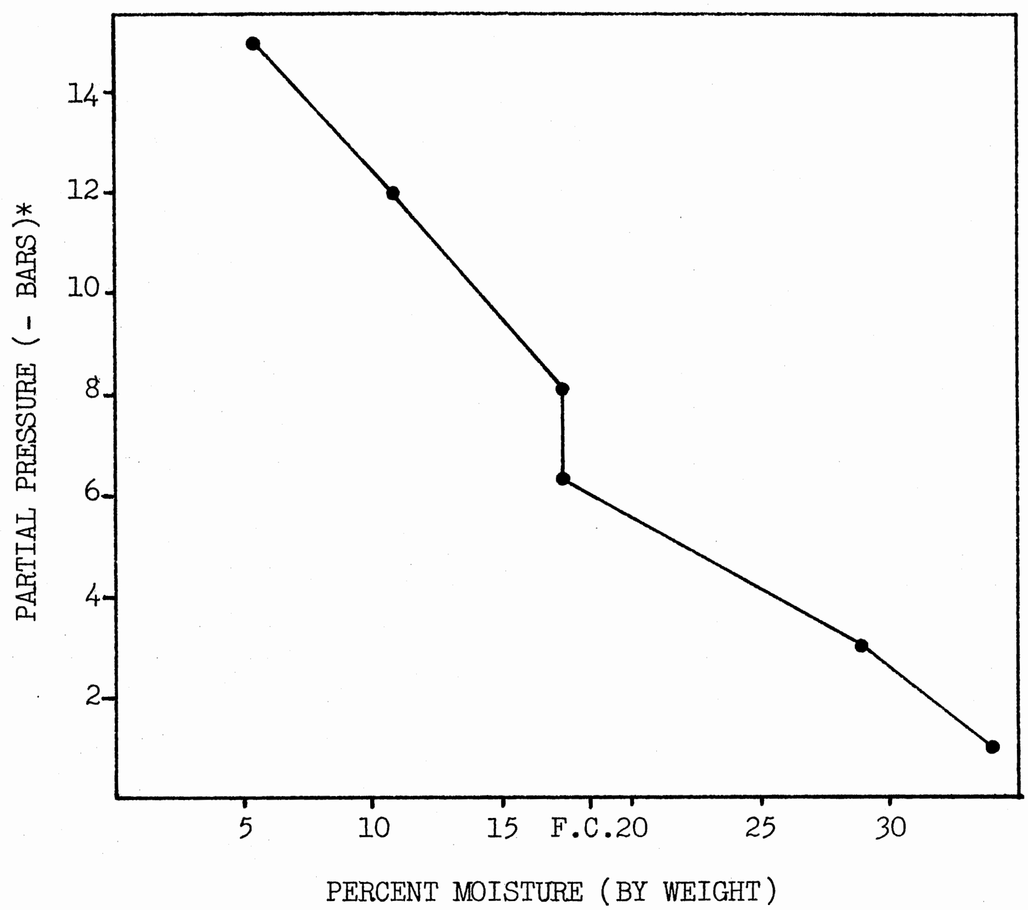

Figure 2. Standard Curve of Partial Pressures (-bars) Versus % Moisture (by Weight) for a Mixture of 50% Lincoln fine and 50% Builders Sand, by Volume, Determined by the Pressure Membrane Apparatus (4).
*Data mean of 4 observations.

TABLE I

NEMATODE COUNTS AND MOISTURES BY DEPTH FOR 18, 24, AND 30 C

	18 C		24 C		30 C	
Depth[a]	Nematodes	Moisture**	Nematodes	Moisture	Nematodes	Moisture
5	22 *	3.99	581	5.48	191	5.21
10	31	6.16	798	7.37	806	6.93
15	63	8.55	656	9.87	709	8.20
20	38	13.44	575	13.53	934	11.91
25	13	15.36	197	15.71	209	14.73
30	0	16.87	41	17.10	34	15.70
35	0	16.41	0	16.85	0	16.12
40	0	16.27	0	15.07	0	15.94
Total	167		2848		2882	

LSD ns

a Depth: 5 cm is 5 cm from the top of the soil level, etc.

* Data mean of 4 observations.

** Moisture is given in % by weight but can be converted to -bars partial pressure from Figure 2.

soil. At termination of the study, *X. americanum* were recovered from soil depths: 5 to 25 cm at 18 C and 5 to 30 cm at 24 and 30 C. No surface irrigation was utilized throughout the study. Therefore it is assumed that the *X. americanum* moved from their original inoculation site to the sites from which they were recovered as other nematodes have done in previous investigations (32, 35).

Low root weights, Table II, from the plants grown at 24 and 30 C correspond to the high counts of *X. americanum* recovered from cylinders kept at these temperatures, Table I. Twenty-four and 30 C also seemed to be more conducive to Midland bermudagrass growth since the plants grown at these more favorable temperatures had more forage than did the plants grown at 18 C, Table II.

Although there were roots growing in the 35 and 40 cm soil depths, no nematodes were recovered from these low depth increments probably due in part to lack of sufficient oxygen to sustain life (66).

The soil moisture in each cylinder increased with depth, Table I. However, the analysis of variance of data presented in Figure 3 showed no significant treatment by depth interaction. The lines for 18, 24, and 30 C are essentially parallel. The analysis of variance shows a significant linear ($P = 0.0001$), quadratic ($P = 0.0001$), and cubic ($P = 0.0001$) effect due to depth. No linear or quadratic effects due temperature treatment were found.

The log nematode counts by depth for each treatment are shown in Figure 4. The linear ($P = 0.0001$), quadratic ($P = 0.0001$), and cubic ($P = 0.0001$) effects due to depth were highly significant indicating a curvilinear effect due to depth as shown in Figure 4. As the depth increased there was a sharp increase in nematode populations which

TABLE II

ROOT AND FORAGE WEIGHTS FOR PLANTS
GROWN AT 18, 24, AND 30 C

	18 C	24 C	30 C
Root Weight (g)	4.91 *	1.91	2.48
Fresh Forage (g)	6.53	8.83	10.43
Dry Forage (g)	2.76	3.36	4.47

LSD ns

* Data mean of 4 observations.

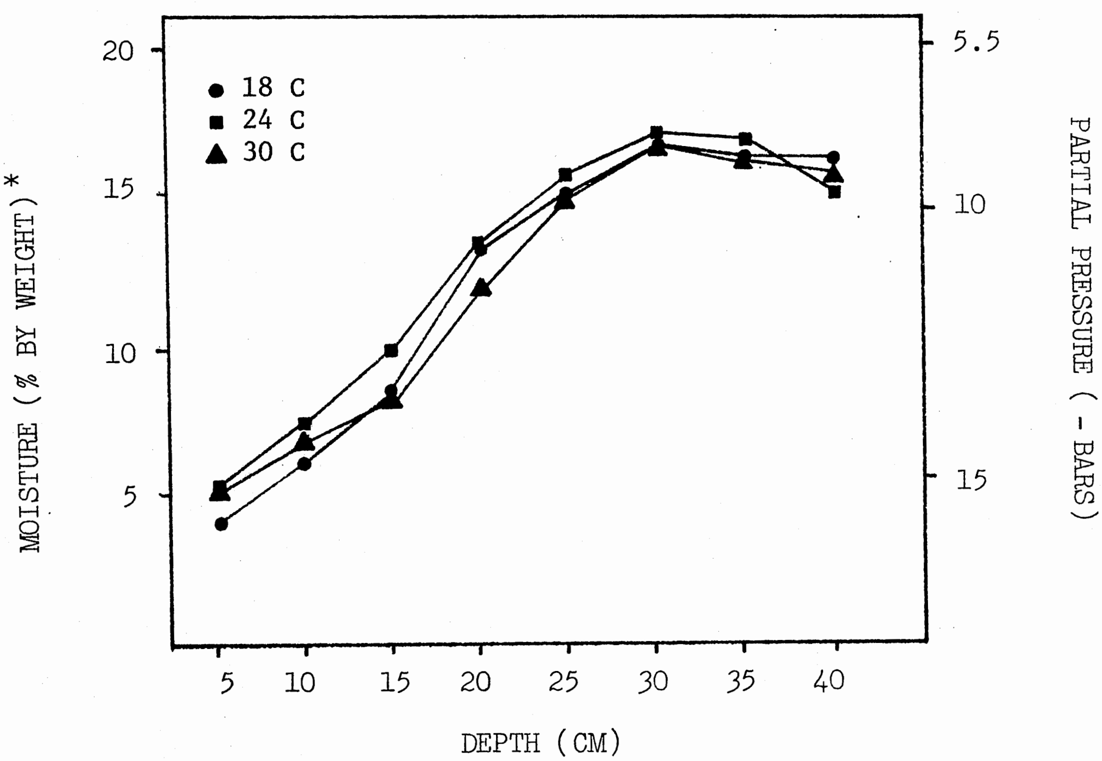

Figure 3. Moisture Response by Depth for Each Treatment in Greenhouse Reproduction Study.
* Data mean of 4 observations.

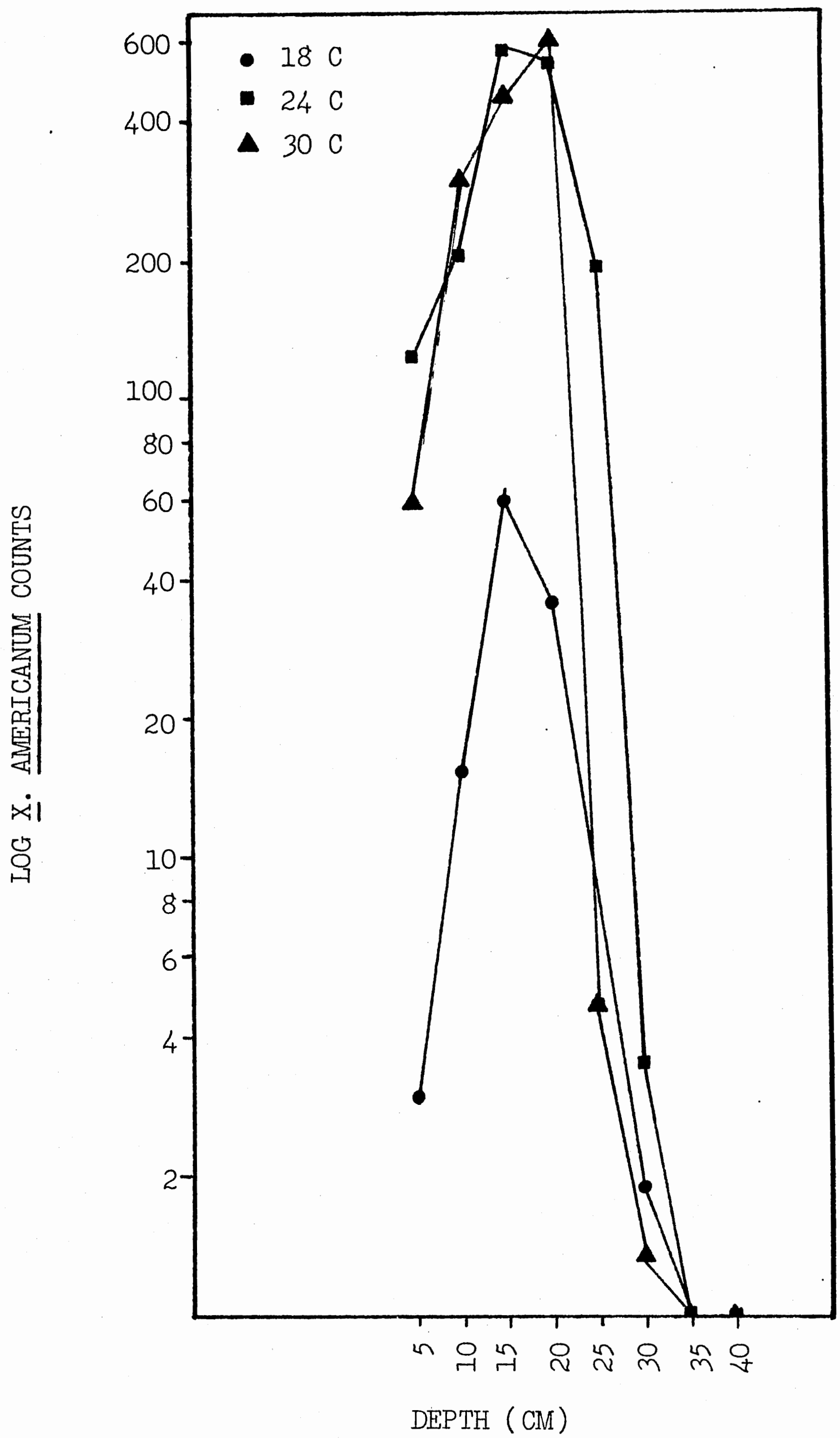

Figure 4. Log Xiphinema americanum Counts by Depth for Each Treatment in Greenhouse Reproduction Study.
* Data mean of 4 observations.

reached a peak of 633 and then decreased sharply as the depth approached 40 cm. There was no significant treatment by depth interaction for the nematode populations. However, there was a linear log effect due to treatments and very little curvature. Moisture significantly correlates to log nematode counts: -0.2559.

It is noteworthy that X. americanum reproduced at such a high rate at the 30 C soil temperature which was lethal to X. diversicaudatum larvae (12).

Summary and Conclusions

Twenty-four and 30 C were much more conducive to X. americanum reproduction on Midland bermudagrass under the conditions of this study than was 18 C. The cylinders kept at the two higher temperatures each had a nematode population approximately 17 fold that found in the cylinders kept at the low temperature. Xiphinema americanum, then, may be similar to other species in the genus Xiphinema that were found to reproduce more rapidly as the temperature increased from 16 to 28 C (10).

The sub-irrigated cylinder system allowed a range of soil moistures in each cylinder for 18, 24, and 30 C. There was a negative correlation between log nematode counts and moisture levels. Essentially as the moisture increased, the nematode counts decreased. No nematodes were found in the soil increments having less than 3% or more than 16% moisture. This corresponds to previous work regarding the moisture requirements of X. americanum (22). Maximum nematode populations were found in soil with 7.37% moisture kept at 24 C and in soil with 11.91% moisture kept at 30 C. The bulk of the populations in the cylinders at both temperatures were found in soil having moistures of 5.48 to 15.71% and 5.21 to 14.73% kept at 24 and 30 C, respectively.

The soil pore sizes achieved by the use of the sand-mixture were conducive to movement and reproduction. They were within the range utilized successfully for previous studies (45).

The results of this study strongly indicate that *X. americanum* completed its life cycle in 9 mo or less. Under laboratory conditions, *X. index* completed its life cycle in 22 to 36 d (47, 48, 62). Studies by Flegg (21) in Kent, England, showed other species of *Xiphinema* to have one reproduction peak per year in nature with the nematodes completing their life cycle in less than 2 to 3 yr. In Chapter IV and in other studies in the United States (1, 21, 38), two population peaks were found per year in nature for *X. americanum*. This suggests that the American dagger nematode may reproduce twice a year in Oklahoma, since summer soil temperatures stayed above 20 C for as long as 6 mo, (Chapter IV). The life cycle of the American dagger nematode has not been elucidated due to the inability to maintain pure populations under artificial conditions (19). Several attempts were made to observe *X. americanum* complete its life cycle on agar plates in the laboratory. However, the larvae died several days after emerging from the eggs in each instance. The greenhouse sub-irrigated cylinder system with bermudagrass as a host in soil maintained at 24 to 30 C may allow in future investigation the determination of the complete life cycle of *X. americanum*.

Xiphinema americanum has been reported to parasitize perennial, non-herbaceous and herbaceous plants as well as annuals (29). The list of its associations with declining and unthrifty plant growth is exhaustive (7, 14, 34, 51, 65, 68). However, experimental evidence of the American dagger nematode's association with disease is lacking (29) due in part to the inability to rear X. americanum under cultural conditions in sufficient quantities for use as inoculum. In controlled environments, X. americanum is known to be intolerant to moisture and temperature extremes (10, 15, 22, 33). Consequently field-infested soil has been used for an inoculum source for X. americanum in studies requiring more than a few nematodes (1, 9, 10, 18, 21, 22, 23, 33, 34, 42, 45, 68). Perhaps X. americanum can be reared on bermudagrass variety 71-X 8-4 found to be the best host for the American dagger nematode, (Chapter III) in the sub-irrigated cylinder system with soil temperatures maintained at 24 to 30 C in sufficiently large populations for use as inoculum in pathogenicity trials.

If this study were to be repeated, more replicates are recommended. Several replicates could be terminated at monthly intervals up to 9 mo in order to more closely ascertain the time required for X. americanum to complete its life cycle. A concomitant population dynamics study could be done in which X. americanum would be counted by its various life cycle stages. The presence of oviferous females would be sought in order to determine the number of life cycles that occur per year in nature.

CHAPTER III

XIPHINEMA AMERICANUM - BERMUDAGRASS HOST SUITABILITY TRIALS

Materials and Methods

A 12 X 90 m area at the Agricultural Experiment Station near Perkins, Oklahoma, was selected as the study site. The field area was disced several times, raked, then staked before planting. The plots were 3 X 3 m in size arranged in a randomized complete block design. There were 10 replicates.

Eleven improved and/or experimental varieties of bermudagrass: 'Hardie', 71-X 8-3, 'Coastal', 71-X 8-4, 'Oklan', 'Stratford Common', 71-X 6-7, 'Alicia', 71-X 11-5, SS-16, 'Greenfield', and Midland were selected for the host suitability study. Midland plants were started from greenhouse stock originally obtained from a pastureland at the Scannell Ranch, Ringwood, Oklahoma. Each plant was grown in a 5 X 5 X 5 cm plastic pot containing sterile Lincoln fine sand in the greenhouse. All other plant materials were obtained from the forage breeding program in the Agronomy Department, Oklahoma State University. All materials obtained were grown in 5 X 5 X 5 cm peat pots containing a sterile soil mixture under greenhouse conditions for 1 mo. Each pot was fertilized weekly with 10 ml of "Peters soil test fertilizer", 20-20-20. Ten plants of similar size were selected from each variety to establish plots for the field study.

Xiphinema americanum infested soil from a sorghum field on the Scannell Ranch was collected for use as an inoculum source in this study. The soil was transported to Stillwater in 80 liter plastic containers and mixed in a cement mixer for 15 sec to augment uniformity of nematode distribution. After mixing, four 177 g (100 cc) aliquants were removed and assayed for the number of *X. americanum* per unit soil. Although *X. americanum* was the predominant species present, other genera including: *Pratylenchus*, *Leptonchus*, and *Quinisulcius* spp. were also present. After mixing and sampling the remaining soil was placed into 80 liter plastic containers, covered, and stored for 30 hr under air-conditioned laboratory conditions to prevent overheating. The containers of soil were transported to the study site and the plots were inoculated after sunset to prevent loss of nematodes from overheating.

A 5 X 15 cm (diameter and depth, respectively) cylindrical hole was cut into the center of each plot using a soil sampling probe. One hundred cc of the soil from each plot was removed and placed into a plastic bag for background field nematode population analysis. One hundred and fifty cc of the sorghum-field soil inoculum containing 390 *X. americanum* was volumetrically measured and was gently poured into the hole. Immediately after plot site preparations were complete, the assigned plant was transplanted into the site. Additional soil from the surrounding area was used to refill the hole. To determine nematode mortality due to handling, four separate 150 cc aliquants of inoculum soil were taken after plot installation. Each was placed into a plastic bag and returned to the laboratory along with the 100 cc soil samples taken at each plot site to determine the background nematode populations. The soil samples were immediately assayed, as previously described.

Each plant was hand watered with 500 ml of water at planting. The entire study site was watered as needed thereafter. Weeds were controlled with Roundup in the alleys and hand rogued in the plots.

Plots were not allowed to grow together. Alleys 90 cm wide were maintained with a hand driven motorized cultivator throughout the study.

The growth rate of each plant by plots was measured in late October of the first year of the study.

Soil samples were taken from the inoculation sites with a soil sampling probe 15 mo after study initiation. One hundred cc of soil was removed from a 15 cm depth, and assayed for nematodes. When possible, roots were extracted to ascertain nematode damage.

An analysis of variance was performed on the *X. americanum* populations recovered from each plot. Comparisons between pairs of means were made using LSD figures.

Results and Discussion

Table III shows the results of the host suitability trials. The highest *X. americanum* populations found were associated with varieties; 71-X 8-4, Stratford Common, Alicia, Midland, Greenfield, Coastal, Hardie, and 71-X 8-3. As nematode population levels decreased, the incidence of winterkill increased, with the exception of Alicia bermudagrass which supported *X. americanum* populations of 16 to 116/100 cc of soil at the end of the study but sustained 100% winterkill. However, no relationship can be assumed between nematode infestation

TABLE III

NEMATODE COUNTS FROM BERMUDAGRASS VARIETY SUITABILITY TRIALS

Variety	*X. americanum* **	% Winterkill
71-X 8-4	124 *	10
Stratford Common	73	0
Alicia	66	100
Midland	55	10
Greenfield	42	0
Coastal	36	0
Hardie	31	10
71-X 8-3	31	40
Oklan	12	80
71-X 6-7	4	80
SS-16	4	90
71-X 11-5	4	80

LSD
.05 = 64
.01 = 85

** *X. americanum*/100 cc soil.
* Data mean of 10 observations.

level and percentage winterkill. Winterkill appears to be independent of X. americanum infestation levels.

One criterion for a suitable host is that it stays in a viable condition. A second is that it support a nematode population. Thirdly, a suitable host is one that allows the nematode to reproduce by making the environment conducive to nema reproduction (63). Evidently varieties 71-X 8-4 with a final X. americanum population of 124/100 cc of soil and Coastal with a final X. americanum population of 36/100 cc of soil were suitable hosts for the American dagger nematode. Variety 71-X 8-4 had the least amount of growth in the 3½ mo period prior to the onset of the first hard freeze. It sustained 10% winterkill, but the final X. americanum populations in two replicates of that variety were 388 and 524/100 cc of soil, well above the initial inoculum level of 260/100 cc of soil. Roots were very sparse and appeared to be necrotic at the termination of the study suggesting that extensive nematode feeding had occurred during the study. Cohn (9) found grape roots to be similarly parasitized in his study with Xiphinema spp. on grape roots. DiSanzo and Rohde (14) noticed a general darkening pattern of sugar maple roots parasitized by X. americanum. Similarly, Perry (42) reported a darkening of strawberry roots parasitized by X. americanum and X. chambersi. Coastal not only remained viable, but also grew well during the study. It grew 4 times faster than variety 71-X 8-4 in the first 3½ mo, and supported a X. americanum population as high as 241/100 cc of soil. The portion of the main root system that was examined at the termination of the study showed little visible nematode damage and no discoloration. The growth pattern of bermudagrass suggests that nematodes may move from the inoculation site to new growth areas of rooting stolons as the plant grows radially from a central inoculation site. DiSanzo and Rohde (14)observed that *X. americanum* moved to the root tips of sugar maple seedlings to feed. Limber (32) found that *Anguina tritici* larvae moved 7 to 19 cm to reach a host while Marcinowski (35) reported that the larvae moved 30 cm to infest their host. Movement to new feeding sites would account for: 1) the lower-than-inoculum-levels of final populations recovered from the inoculation site at the termination of the study and 2) the initial root system showing little nematode damage.

Small populations of *X. americanum*: 12, 4, 4, and 4/100 cc of soil were recovered from varieties; Oklan, 71-X 6-7, SS-16, and 71-X 11-5, respectively. Since there was a concomitant increase in the amount of winterkill sustained by these varieties; 80, 80, 80, and 90%, respectively, resistance - or low suitability - could not be distinguished from nematode population reductions due to the absence of hosts.

Decreases in *X. americanum* populations were found in most plots. As shown in Figure 6 (Chapter IV), the seasonal population fluctuation of *X. americanum* in Oklahoma peaks in June and September. Thereafter, populations decrease considerably and fluctuate throughout the rest of the months. Norton (38) also found two seasonal population peaks for *X. americanum* in Iowa. One occurred in early spring, the other in late summer. Wallace and MacDonald (64) reported finding the highest populations of *X. americanum* on apple in Minnesota in June; the lowest in September. Final *X. americanum* population samples were taken in mid-October at the termination of the study. Sampling at this time would normally give low X. americanum populations, so it is not unnatural to have considerably fewer nematodes in plots inoculated with a two fold or greater population.

Variety 71-X 8-4 was the most suitable host in this study. In order to be a suitable nematode host, the variety must support and allow for nematode reproduction (63). Variety 71-X 8-4 did so. In two of the ten replicates, the X. americanum populations were 388 and 524/100 cc of soil, well above the inoculum level populations of 260/100 cc of soil. Reproduction is thereby assumed to have occurred. Also, none of the plots were void of X. americanum at the termination of the study when a mean X. americanum population of 124/100 cc of soil was recovered. So variety 71-X 8-4 was maintaining an American dagger nematode population even when the seasonal density was at a low, Figure 6 (Chapter IV).

Summary and Conclusions

Varieties; 71-X 8-4 and Coastal were suitable hosts for X. americanum because each variety remained viable, supported American dagger nematode populations, and allowed X. americanum to reproduce. These varieties can be used in laboratory host attraction studies (2), as a screening procedure prior to their use in other field trials. If X. americanum is attracted to a growing bermudagrass plant of variety 71-X 8-4 or Coastal; then further use can be made of the varieties as host plants to rear large quantities of American dagger nematodes for future studies. If large populations of X. americanum can be reared, then pathogenicity trials can be conducted. Since it is generally not economical to fumigate pastureland due to the cost/benefit ratio, it would be advisable to not plant variety 71-X 8-4 or Coastal in soil that contains populations of *X. americanum*.

If this study were to be repeated, the following changes in procedure are recommended:

a. Initiate the study in April or May in order to allow the plants to become established before winter.

b. Reduce replicates to 6 or 4 and include uninoculated hosts to check for possible nematode-associated winterkill. If some of the varieties exhibiting winterkill in this study do not show winterkill normally, then perhaps the presence of *X. americanum* allowed for conditions to be unfavorable enough to lead to plant death. "Winterkill...of perennials", (according to Gerald Thorne), "frequently is associated with nematode infestations" (65), p. 24.

c. Assay nematode populations during the end of May or the beginning of June to coincide with the *X. americanum* population peak in Oklahoma (Figure 6, Chapter IV). Sample inoculation sites at several depths that bear roots. Heald and Thames (26) and Raski et al. (50) reported that the majority of the plant parasitic nematodes are recovered from the root zones. Also, sample sites extending radially along the host's growth pattern at several root zone depths for possible nematode movement data.

d. Harvest forage at nematode assay time and correlate results.

e. Excavate at least the central root from each plant at the termination of the study to ascertain possible nematode damage. The root weight may be correlated to (c) and (d) above.

Except for Alicia bermudagrass, varieties Oklan, 71-X 6-7, SS-16, and 71-X 11-5 had lower *X. americanum* populations than did the other varieties and concomitantly higher incidents of winterkill. Therefore resistance - or low suitability - could not be distinguished from nematode population reductions due to the absence of a host. Perhaps a host suitability trial run in the greenhouse would eliminate the winterkill effect and would allow for determination of resistance- or low suitability - to *X. americanum*. Repeat field host suitability trials with the appropriate modifications in procedure as recommended in the preceding paragraph may also provide reliable resistance - or low suitability - data.

CHAPTER IV

POPULATION DYNAMICS OF *XIPHINEMA AMERICANUM*, *CRICONEMOIDES XENOPLAX** AND *QUINISULCIUS* SP. ON AN ESTABLISHED MIDLAND BERMUDAGRASS PASTURE

Materials and Methods

A 7 yr old pasture of Midland bermudagrass located at the Scannell Ranch, Ringwood, Oklahoma, was selected for nematode population dynamics sampling. The 28 check plots utilized in the chemical control trials, Chapter VII, were sampled monthly for 14 mo. No sampling was done in January of 1977 due to a combination of inclement weather and frozen soil conditions. Soil samples were taken at three different similar randomly selected sites per plot per sampling date with a 3 cm diameter motorized auger to a depth of 20 cm. The soil samples for each plot were combined, mixed, and a 200 cc subsample was extracted and placed into a plastic bag. The bags containing the subsamples were placed into ice chests for storage and transportation to the laboratory. The residual soil was returned to the sampling holes and sufficient sterile Lincoln fine sand was added to fill the depressions at each sampling date. Immediately on return to the laboratory,

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* *Macroposthonia xenoplax* of some authors.

100 cc of soil from each plot was assayed for nematodes as previously described.

Soil temperatures taken at each sampling date at a depth of 18 to 20 cm were recorded as the mean of 6 observations.

Soil moisture was determined for all samples at each sampling date as previously described.

A composite soil sample taken from the first month's samples was sent to the soil and water service laboratory, Oklahoma State University Agronomy Department, for soil type analysis and pH determination. The soil was sandy loam with a pH of 7.4.

Soil moisture is reported as percent (%) by weight, but the partial pressures for each moisture increment can be determined by use of the standard curve specifically derived for this soil texture, Figure 5.

Results and Discussion

Figure 6 shows the results of the population dynamics study. When the X. americanum populations are viewed on a monthly basis over a 14- wk period, at least two peaks are seen. In 1977, the spring peak occurred in May in soil with an average moisture content of 6% and an average temperature of 18 C. The second peak in 1977 occurred during late summer : September, in soil with a moisture content of 7% and a temperature of 26 C. These results coincide with those of Norton (38) who also found two seasonal population peaks for X. americanum in Iowa. One occurred in March-May, in soil with an average moisture content of 21% and an average temperature of 0 C; the other in July-September, in soil with an average moisture content of 16%

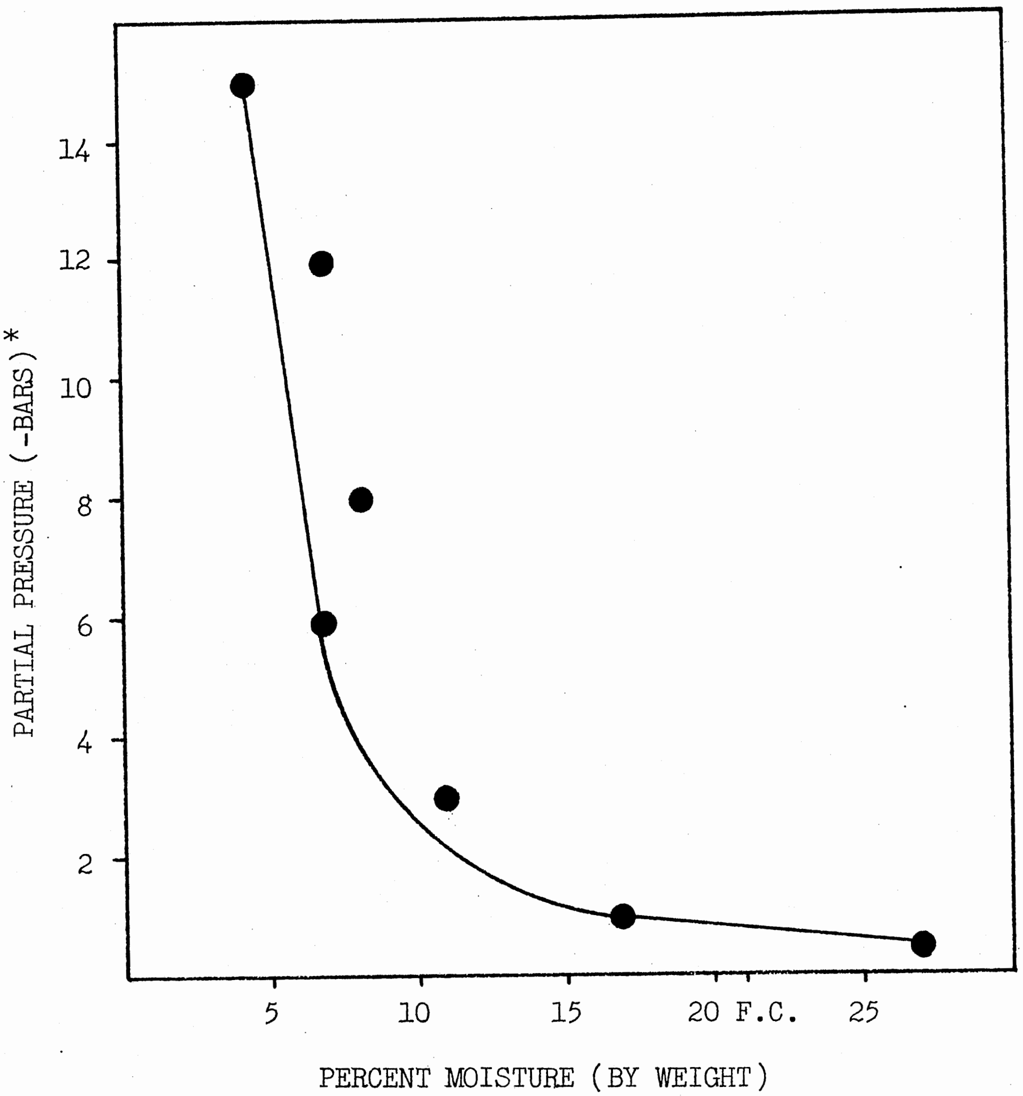

Figure 5. Standard Curve of Partial Pressures (-bars) Versus % Moisture (by Weight) for Scannell Soil Determined by the Pressure Membrane Apparatus (4).

* Data mean of 4 observations.

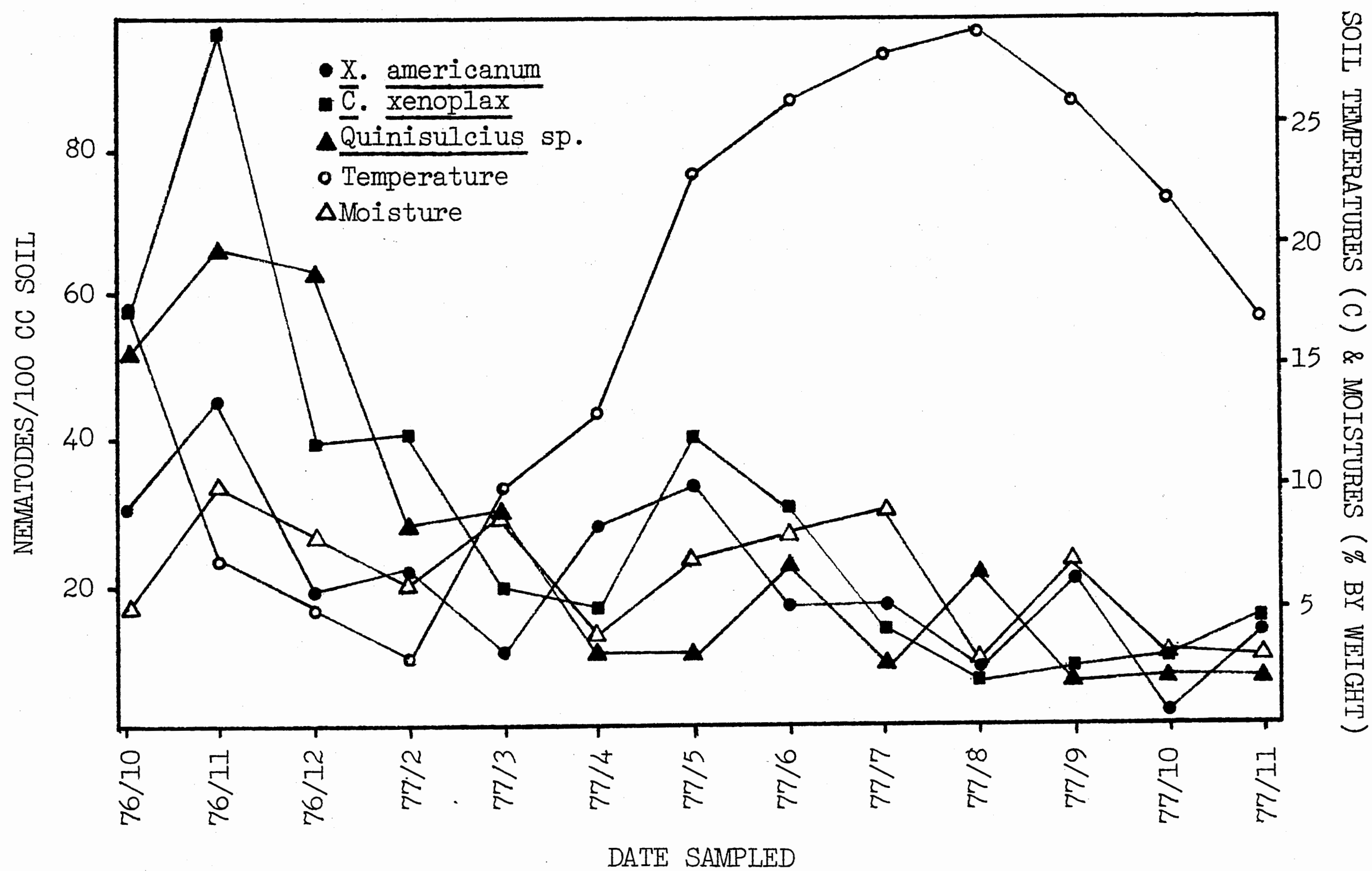

Figure 6. Xiphinema americanum, Criconemoides xenoplax, and Quinisulcius sp. Population Dynamics on Midland Bermudagrass (number of nematodes/ 100 cc soil, mean of 28 observations; Soil Temperatures (mean of 6 observations); Soil Moistures (mean of 28 observations).

and an average temperature of 17 C.

In Oklahoma, the greatest *X. americanum* population peak occurred in November of 1976 with 45 *X. americanum*/100 cc of soil recovered from soil with a moisture content of 10% and a temperature of 7 C. This peak had 1.5 times more *X. americanum* as the fall of 1977. It is unusual to have nematode populations decrease on a forage host (17, 38) from one year to the next. The reverse is generally the rule. However, the hot and dry Oklahoma summer of 1977 may have been responsible for the decrease in the American dagger nematode populations for 1977. Apparently the soil conditions occurring in November of 1976 closely approximate optimum conditions for *X. americanum* population increases. The 10% moisture content falls within the range reported by Griffin and Darling (21) for good *X. americanum* survival. However, Griffin and Barker (22) reported the optimum soil temperature for reproduction of *X. americanum* on tomato and strawberry plants was 20 and 24 C, respectively. Norton (38) reported a high population peak of 70 *X. americanum* in fall samples of sandy loam soil with a moisture content of 16% and a temperature of 17 C.

The two other nematodes found in significant numbers along with *X. americanum* were *C. xenoplax* and *Quinisulcius* sp. Although these species were not frequently found associated with Midland bermedagrass in survey 2, (Chapter V), they were recovered from this pasture. Both peaked in November 1976 as did *X. americanum*. *Criconemoides xenoplax* populations were 68% higher than the previous month while populations of *Quinisulcius* sp. were 26.5% higher. *Xiphinema americanum* populations in November 1976 were 46% higher than the previous month. The soil temperature and moisture conditions of 7 C and 10%, respectively, may have been close to optimum for population increases of both genera.

The spring peak in 1977 occurred in May for *C. xenoplax* and in June for *Quinisulcius* sp. The soil moisture and temperature conditions may have been too extreme for both of these genera to attain population peaks in late summer or fall of 1977, since even the *X. americanum* population peak was small.

Soil moistures peaked in November of 1976 (10%), March (9%), and July (9%) of 1977. The lowest soil moisture of 3% was recorded in August and corresponds to the highest temperature reading of 29 C. However, the lowest nematode counts of 2, *X. americanum*, 7, *Quinisulcius* sp., and 10, *C. xenoplax*, were found in October 1977.

Summary and Conclusions

Xiphinema americanum populations on Midland bermudagrass in Oklahoma peaked twice a year; in May and in September. These data substantiate those of Norton (38) who found similar peaks for the American dagger nematode on alfalfa in Iowa. Knowing that there are two population peaks per year and approximately when they occur can be an invaluable tool for Oklahoma Agriculture. For example, this information can be useful in planning fumigation measures for *X. americanum* infested fields of perennials. Populations of *X. americanum* on annuals may also follow similar monthly fluctuations with two peaks per year, as Thorne (65) reported that there is a marked population fluctuation of *X. americanum* in nature. However, further population dynamics studies will need to be done in order to ascertain if the American dagger nematode has a comparable population fluctuation pattern for other hosts.

If the results of this study were known when the chemical control trials, Chapter VII, were being planned, their initiation time may have been different. A spring treatment timed with the population peak may have been a more suitable time for nematicide application in order to aim at the largest populations of the American dagger nematode.

Until large quantities of *X. americanum* can be reared in the greenhouse, inoculum has to come from infested field soil. *Xiphinema americanum* inoculum can be gathered under field conditions during the time populations peak.

A 10% soil moisture content combined with a soil temperature of 7 C and a pH of 7.4 in sandy loam soil seemed to be conducive to *X. americanum* population increases on Midland bermudagrass in Oklahoma. In Iowa, Norton (38) also found the highest populations of the American dagger nematode in his fall samples of a sandy loam soil with a moisture content of 16% and a temperature of 17 C. Knowing the soil conditions under which the greatest populations of *X. americanum* were found can be useful in setting up controlled greenhouse studies.

Criconemoides xenoplax and *Quinisulcius* sp. populations peaked in November. Soil moisture of 10% combined with a soil temperature of 7 C seemed to be good for population increases of these two species, also. These data can be utilized for planning future studies on these two nematode species.

CHAPTER V

INCIDENCE AND VERTICAL DISTRIBUTION OF *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES ON SELECTED MIDLAND BERMUDAGRASS PASTURES IN OKLAHOMA

Materials and Methods

Nine geographical areas throughout Oklahoma were selected for survey with the aid of Odos Henson, State Agronomist with the United States Department of Agriculture Soil Conservation Service (USDA-SCS). The first survey was taken to find three areas in the state in which Midland bermudagrass was extensively grown so that incidence and vertical distribution data on *X. americanum* and associated species could be collected. Nine District Conservationists (USDA-SCS): Eden Matheny, Otho W. LaMar, George Brown, Walter L. LaMar, Steve York, Jr., John Worthy, Leroy Brown, Jr., Douglas Drain, and Alvin Trissell were asked the largest total acreage in Midland by county in their respective districts, along with the number of new spriggings for that county. From these data, three counties having the greatest acreage percentage of Midland were selected for detailed study, survey 2.

Survey 2 consisted of sampling for nematodes in three unthrifty and/or declining pastures per county selected by the District Conservationist. The experimental design was a split-plot in which site selection was the main plot and depth was the subplot. Three sites were selected in each pasture on the basis of plant vigor : poor, good, and best. Soil samples were taken with a spade at a depth of 15 and 30 cm in each hole from each of three locations per vigor site. The three samples at each depth were mixed and a subsample of about 300 cc was taken and placed into a plastic bag. Each bag was labeled and sealed before placing into an ice chest for storage and transportation to the laboratory. Immediately upon return to the laboratory, 100 cc of soil from each depth per site was assayed for nematodes as previously described.

Soil temperatures were taken at a depth of 18 cm and reported as the mean of three observations. Soil moistures were determined for all samples as previously reported. Twenty-five ml of 0.01 M $CaCl_2$ was mixed with 1 g of soil for 12 hr on a shaker prior to taking pH readings. The pH of each soil sample was determined by the use of a Beckman meter.

Each landowner was asked to complete a questionaire regarding past, present, and future usage of his pasture. A nematode analysis report was sent to each owner with a duplicate to the District Conservationist for that area.

Results and Discussion

Love, Stephens, and Logan counties, with 17.7, 9.2, and 5.2% respectively, of the total acreage in each county in Midland bermudagrass, were selected for survey 2. They also had the largest number of new Midland plantings per county: Love, 2,400; Stephens, 1,300; Logan, 1,000; Table IV.

TABLE IV

MIDLAND BERMUDAGRASS PASTURELAND AND ACREAGE ON COUNTY BASIS FOR SELECTED OKLAHOMA COUNTIES: SURVEY 1

County	County Size in Acres	Acres of Pastureland	% Pastureland/ County Acreage	Acres of Midland	% Midland/County Pastureland	Midland Plantings/ County	% Midland/County Acreage
Greer	408,320	16,450	4.0%	12,500	76.0%	312	3.1%
Logan	481,280	98,730	20.5%	25,000	25.3%	1,000	5.2%
Love	338,560	56,906	16.8%	56,906	100.0%	2,400	17.7%
Major	616,320	71,380	11.6%	3,000	4.2%	45	0.5%
Okmulgee	449,920	100,243	22.3%	7,188	7.2%	321	1.6%
Stephens	572,160	95,658	16.7%	52,600	55.0%	1,300	9.2%
Washita	646,400	19,131	3.0%	14,348	75.0%	359	2.2%

The results of survey 2 are shown in Tables V-VII. Nematodes were recovered from every sampling site ranging from 4 to 256/100 cc of soil. Xiphinema americanum, "Tylenchorhynchus", Criconemoides, Leptonchus, Pratylenchus, Trichodorus, Heterodera, Hoplolaimus, Helicotylenchus, Belonolaimus, Longidorus, and "Tylenchus" spp. were found along with the usual abundance of free-living forms. Xiphinema americanum was found in all of the soil types and at each depth sampled, but not at each vigor site. Many previous investigators have reported recovering American dagger nematodes from a variety of soil types (21, 25, 33, 38) and from various depths (21, 38, 65). Some vigor sites may not have had recoverable levels of American dagger nematodes present due to the fact that survey 2 was taken in August when X. americanum populations are low on Midland bermudagrass in Oklahoma, Figure 6 (Chapter IV). Sampling in September or in May during peak X. americanum population periods may have revealed the presence of the American dagger nematode at all vigor sites. Griffin and Darling (21) reported that X. americanum does not survive in soil with a moisture content under 3% by weight. However, in this study the American dagger nematodes were recovered from sites having a moisture content (% by weight) ranging from 1.5 to 3.8%, soil temperatures of 23.5 to 29.0 C, and a pH range of 4.2 to 7.5 which substantiates Burns' (6) findings that X. americanum can be found in soils with a pH of 4.0 to 8.0.

Xiphinema americanum was recovered from 52% of the samples with only Helicotylenchus spp. being recovered more frequently (80%), Table VIII. Alby (2) found both genera to occur quite frequently on range grasses in Oklahoma. In a Kansas study on native range, Orr (40)

TABLE V

LOGAN COUNTY MIDLAND PASTURE SURVEY FOR XIPHINEMA AMERICANUM AND ASSOCIATED SPECIES IN SOIL OF VARIOUS TYPES, TEMPERATURES, MOISTURES, AND PH

County	Pasture Owner	Vigor	Depth (cm)	X. americanum **	"Tylenchorhynchus" spp.	Criconemoides spp.	Leptonchus spp.	Pratylenchus spp.	Trichodorus spp.	Heterodera spp.	Hoplolaimus spp.	"Tylenchus" spp.	Helicotylenchus spp.	Belonolaimus spp.	Longidorus spp.	Total nematodes/100 cc soil	pH	Temp. (C) @ 18 cm depth	Moisture (% by wt.)	Soil Type
Logan	R. Coffin	poor	15	--	--	--	--	--	--	--	--	--	8	--	--	8	4.4	27	4.7	Derby loam fine sand 8-20% slopes.
Logan		poor	30	12*	--	--	--	8	--	--	--	--	8	--	--	28	5.1	27	4.3	
Logan		good	15	--	--	--	--	--	--	--	--	--	8	--	--	8	4.7	27	2.2	
Logan		good	30	--	--	--	--	--	--	--	4	--	28	--	--	32	4.9	27	3.3	
Logan		best	15	--	8	--	--	--	4	--	--	--	28	--	--	40	5.3	27	2.9	
Logan		best	30	--	--	--	--	--	--	--	--	--	--	4	--	4	5.0	27	3.1	
Logan	H. Halsey	poor	15	--	--	4	--	8	--	--	--	--	--	--	--	12	7.2	27	4.8	Port soils.
Logan		poor	30	4	--	--	--	32	--	--	--	--	--	--	--	36	7.2	27	4.2	
Logan		good	15	--	4	--	--	12	--	--	--	--	20	--	--	36	5.9	27	4.7	
Logan		good	30	20	--	--	--	--	4	--	--	--	--	--	--	24	5.6	27	4.7	
Logan		best	15	--	--	--	--	12	--	--	--	--	--	--	--	12	6.6	27	10.4	
Logan		best	30	4	--	--	--	--	--	--	12	--	8	--	--	24	6.9	27	8.1	
Logan	B. Knopfel	poor	15	--	4	--	--	12	--	--	--	--	56	--	--	72	7.2	27	4.5	Mixed alluvial land.
Logan		poor	30	4	12	--	--	8	--	--	4	--	68	--	--	96	7.5	27	6.2	
Logan		good	15	--	--	4	12	8	--	--	20	--	92	--	--	136	7.4	27	8.0	
Logan		good	30	24	--	--	--	24	--	--	8	--	48	--	--	104	7.3	27	7.3	
Logan		best	15	8	--	--	36	--	--	--	12	--	84	--	--	140	7.4	27	6.2	
Logan		best	30	60	--	--	12	--	--	--	8	--	32	--	--	112	7.4	27	5.9	

-- For entry = 0 population recovered.

** Each entry represents nematodes/cc soil.
* Data mean of 3 observations.

TABLE VI

LOVE COUNTY MIDLAND PASTURE SURVEY FOR *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES IN SOIL OF VARIOUS TYPES, TEMPERATURES, MOISTURES, AND PH

County	Pasture Owner	Vigor	Depth (cm)	*X. americanum* *	"*Tylenchorhynchus*" spp. **	*Criconemoides* spp.	*Leptonchus* spp.	*Pratylenchus* spp.	*Trichodorus* spp.	*Heterodera* spp.	*Hoplolaimus* spp.	"*Tylenchus*" spp.	*Helicotylenchus* spp.	*Belonolaimus* spp.	*Longidorus* spp.	Total nematodes/100 cc soil	pH	Temp.(C) @ 18 cm depth	Moisture (% By wt)	Soil Type
Love	L. Embry	poor	15	16	16	--	--	4	--	--	--	--	8	--	--	44	5.5	29	7.6	Durant loam 1 -3% slopes.
Love		poor	30	24	--	4	4	--	--	--	--	--	--	--	--	32	6.4	29	13.8	
Love		good	15	--	--	--	--	4	--	--	--	8	--	--	--	12	5.2	29	5.8	
Love		good	30	4	--	--	--	--	--	--	--	--	--	--	--	4	5.8	29	9.4	
Love		best	15	4	--	--	--	--	--	--	--	--	8	--	--	12	5.5	29	8.5	
Love		best	30	4	--	--	--	--	--	--	--	--	16	--	--	20	6.6	29	9.4	
Love	L. Meeks	poor	15	--	20	--	8	--	--	--	--	--	--	--	--	36	5.9	29	0.7	Eufaula fine sand, undulating.
Love		poor	30	--	--	4	4	--	--	--	--	--	92	--	--	100	5.8	29	1.1	
Love		good	15	--	--	4	--	--	--	--	--	4	80	--	--	88	6.5	29	1.0	
Love		good	30	--	--	4	4	--	--	--	--	--	32	--	4	44	6.5	29	2.4	
Love		best	15	--	--	--	--	--	--	--	--	--	52	--	--	52	5.6	29	0.9	
Love		best	30	4	4	--	--	--	--	--	--	--	36	--	--	56	5.9	29	1.5	
Love	W. Choate	poor	15	--	--	--	--	--	--	--	--	8	52	--	--	60	5.8	29	5.0	Stephenville fine sandy loam, 1 -5% slopes eroded.
Love		poor	30	--	--	--	8	--	--	4	--	--	--	--	--	12	6.0	29	6.5	
Love		good	15	8	--	4	4	--	--	--	--	--	--	--	--	16	5.2	29	4.2	
Love		good	30	--	8	--	--	--	--	--	4	--	20	--	--	32	5.5	29	4.3	
Love		best	15	56	--	8	--	--	8	--	4	4	176	--	--	256	5.2	29	2.9	
Love		best	30	80	--	8	--	--	4	--	--	--	32	--	--	124	5.3	29	5.0	

-- For entry = 0 population recovered.

** Each entry represents nematodes/cc soil.

* Data mean of 3 observations.

TABLE VII

STEPHENS COUNTY MIDLAND PASTURE SURVEY FOR XIPHINEMA AMERICANUM AND ASSOCIATED SPECIES IN SOIL OF VARIOUS TYPES, TEMPERATURES, MOISTURES, AND PH

County	Pasture Owner	Vigor	Depth (cm)	X. americanum	"Tylenchorhynchus" spp.	Criconemoides spp.	Leptonchus spp.	Pratylenchus spp.	Trichodorus spp.	Heterodera spp.	Hoplolaimus spp.	"Tylenchus" spp.	Helicotylenchus spp.	Belonolaimus spp.	Longidorus spp.	Total nematodes/100 cc soil	pH	Temp. (C) @ 18 cm depth	Moisture (% by wt)	Soil Type
Stephens	G. Littleton	poor	15	4*	--	--	--	--	--	--	--	--	20	40	--	64	4.3	23.5	3.1	Port sandy/ loam.
Stephens		poor	30	--	--	--	--	--	--	--	--	--	16	48	--	20	4.4	23.5	5.1	
Stephens		good	15	--	--	12	--	--	--	--	--	--	8	--	--	20	4.3	23.5	3.5	
Stephens		good	30	12**	--	4	--	--	4	--	--	--	8	56	--	84	4.3	23.5	5.4	
Stephens		best	15	84	--	8	--	--	8	--	--	--	8	20	--	128	4.2	23.5	3.2	
Stephens		best	30	32	--	--	--	4	4	--	--	--	56	4	--	100	4.3	23.5	4.5	
Stephens	J. Graham	poor	15	--	--	--	--	--	--	--	--	--	48	--	--	48	6.0	24.5	2.5	Port fine sandy loam.
Stephens		poor	30	--	--	--	--	--	--	--	--	--	52	--	--	52	5.8	24.5	4.7	
Stephens		good	15	72	--	--	--	4	--	--	--	--	24	--	--	100	6.3	24.5	3.4	
Stephens		good	30	12	--	--	--	--	8	--	--	--	48	--	--	68	6.1	24.5	5.8	
Stephens		best	15	16	--	--	--	--	--	--	--	--	24	--	--	40	6.0	24.5	3.1	
Stephens		best	30	20	--	--	--	12	4	--	--	--	100	--	--	136	6.1	24.5	5.5	
Stephens	E. Graham	poor	15	--	--	--	--	--	--	--	--	--	84	--	--	84	5.9	26	4.9	Nash-Noble fine sandy loam 3 -5% slope.
Stephens		poor	30	--	--	--	--	--	--	--	--	--	8	--	--	8	6.2	26	4.3	
Stephens		good	15	16	--	--	8	8	--	--	--	--	48	--	--	80	5.5	26	3.9	
Stephens		good	30	--	--	--	--	4	--	--	--	--	8	--	--	12	6.0	26	7.0	
Stephens		best	15	12	--	--	--	4	--	--	--	--	16	--	--	32	5.9	26	6.9	
Stephens		best	30	16	--	--	16	--	--	--	--	--	24	--	--	56	5.9	26	3.2	

-- For entry = 0 population recovered.

** Each entry represents nematodes/cc soil .
* Data mean of 3 observations.

TABLE VIII

INCIDENCE AND VERTICAL DISTRIBUTION OF *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES ON MIDLAND BERMUDAGRASS FOR SELECTED OKLAHOMA COUNTIES

Nematode	Incidence	Vertical Distribution 15 cm	30 cm
X. americanum	52 *	41 *	63 *
"Tylenchorhynchus" spp.	15	19	11
Criconemoides spp.	22	26	19
Leptonchus spp.	20	19	22
Pratylenchus spp.	32	37	26
Trichodorus spp.	17	11	22
Heterodera spp.	2	0	4
Hoplolaimus spp.	17	11	22
"Tylenchus" spp.	7	15	0
Helicotylenchus spp.	80	82	78
Belonolaimus spp.	11	7	15
Longidorus spp.	2	0	4

* Data are the % of samples from which the nematodes were recovered rounded to the nearest whole number.

also found Xiphinema sp. and Helicotylenchus sp. to occur most commonly (with frequencies of 90 and 86%, respectively). Norton (37) found both X. americanum and H. nannus on small grains and grasses in Texas. However, he recovered Tylenchorhynchus spp. most frequently. The reverse frequencies of occurrence were found by Jenkins et al. (30) in a study of clover, pasture, and forage crops in Maryland. They found Xiphinema spp. to occur with a frequency of 29% and Helicotylenchus spp. with a frequency of 8%. In these studies Pratylenchus spp. was the next most commonly encountered plant parasitic nematode occurring with a frequency of 32%. Jenkins (28) reported members of this genus to be associated with the decline of pasture grasses and to be parasitic on tall fescue (11). Criconemoides, Leptonchus, Trichodorus, Hoplolaimus, "Tylenchorhynchus", Belonolaimus, "Tylenchus", Heterodera, and Longidorus spp. were also recovered with frequencies of: 22, 20, 17, 18, 15, 11, 7, 2, and 2%, respectively. It is of interest that only 7% of the samples contained "Tylenchus" spp., which was the most commonly encountered plant parasitic nematode recovered from range grasses in Oklahoma with a frequency of 74% (2).

The vertical distribution of X. americanum for Logan, Love, and Stephens counties is shown in Figure 7. Logan and Love counties had fewer X. americanum at the 15 cm depth than at the 30 cm depth; while Stephens county had more X. americanum at the 15 cm depth than at the 30 cm depth. Stephens county's greater X. americanum populations at 15 cm depth were based on two sites out of nine and they were only established for about 1 yr each. Perhaps the majority of the root systems were still at the upper 15 cm depth and therefore the X. amer-

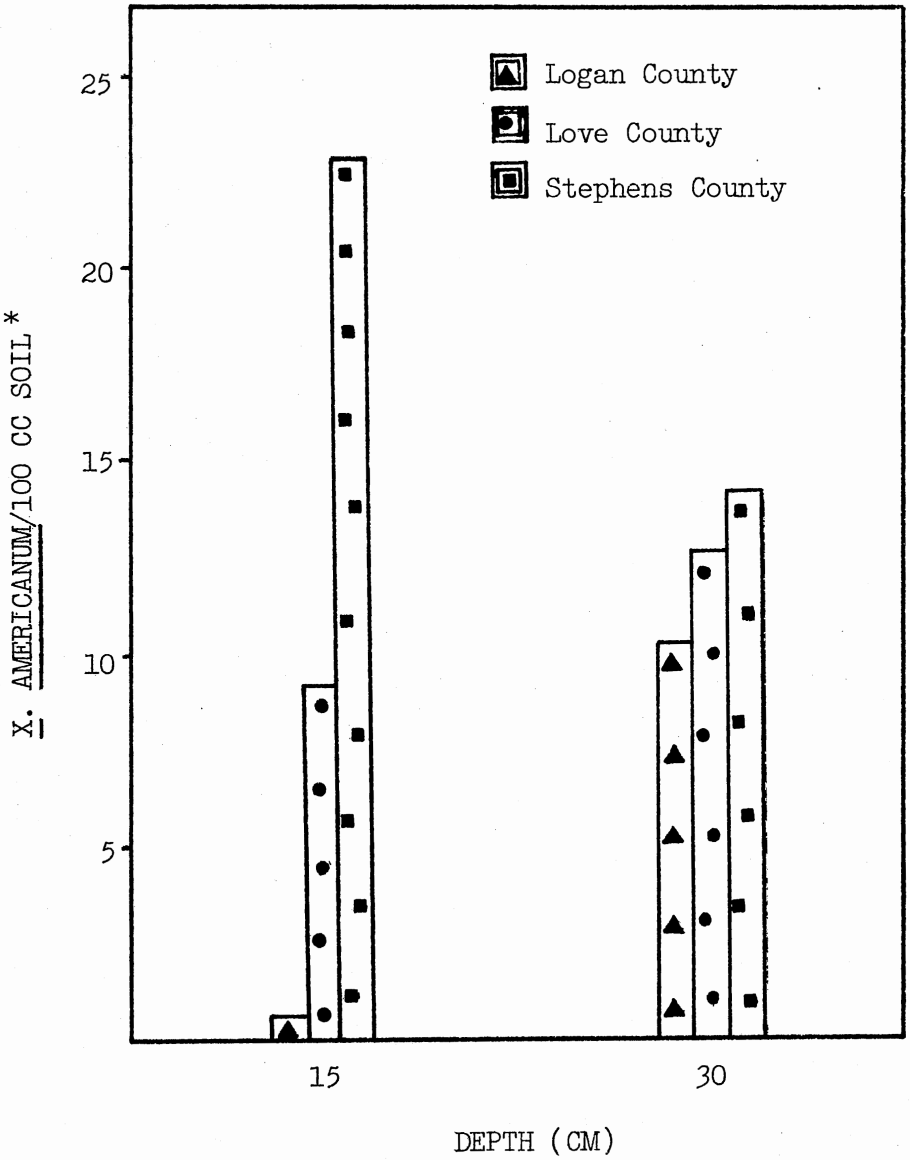

Figure 7. Vertical Distribution of *Xiphinema americanum* for Logan, Love, and Stephens Counties.

* Data mean of 3 observations.

icanum populations built-up at this depth to a greater degree than at the 30 cm level. Xiphinema spp. are considered to be a nematode of deep, 30 cm, soil profiles (65). Logan and Love counties substantiate this with 78% of the nematodes recovered in Logan county from the 30 cm depth being X. americanum, Table IX. Only 11% of the nematodes recovered from the 15 cm depth were X. americanum. Likewise in Love county, 56% of the nematodes recovered from the 30 cm depth were X. americanum, while only 44% were recovered from the 15 cm depth, Table X. Stephens county, however, had fewer X. americanum, 56%, at the 30 cm depth as compared to 67% at the 15 cm depth, Table XI.

The nematodes found in Stephens county are shown in Table XI. Xiphinema americanum occurred with a frequency of 61% while Helicotylenchus spp. was found in all samples. Pratylenchus spp. occurred with a frequency of 33% and Trichodorus and Belonolaimus spp. were found in 28% of the samples. Only two other genera were encountered: Criconemoides spp. with a frequency of 17% and Leptonchus spp. with a frequency of 11%. Hoplolaimus, Heterodera, "Tylenchorhynchus", and Longidorus spp. were not found in Stephens county.

Table X shows the nematodes recovered in Love county. Xiphinema americanum and Helicotylenchus spp. again were the most frequently encountered species, occurring in 50 and 67% of the samples, respectively. Criconemoides and Leptonchus spp. followed with 39 and 33% frequency of occurrence, respectively. "Tylenchorynchus" and "Tylenchus" spp. occurred in 22% of the samples, while Pratylenchus Trichodorus, and Hoplolaimus spp. had a frequency of occurrence of 11%. Heterodera and Longidorus spp. were found in 6% of the samples. This was the only county from which Longidorus spp. was recovered. It was found in

TABLE IX

INCIDENCE AND VERTICAL DISTRIBUTION OF *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES ON MIDLAND BERMUDAGRASS FOR LOGAN COUNTY

Nematode	Incidence	Vertical Distribution 15 cm	30 cm
X. americanum	44 *	11 *	78 *
"Tylenchorhynchus" spp.	22	33	11
Criconemoides spp.	11	22	0
Leptonchus spp.	17	22	11
Pratylenchus spp.	50	56	44
Trichodorus spp.	11	11	11
Heterodera spp.	0	0	0
Hoplolaimus spp.	39	22	56
"Tylenchus" spp.	0	0	0
Helicotylenchus spp.	72	78	67
Belonolaimus spp.	6	0	11
Longidorus spp.	0	0	0

* Data are the % of samples from which the nematodes were recovered for Logan County rounded to the nearest whole number.

TABLE X

INCIDENCE AND VERTICAL DISTRIBUTION OF XIPHINEMA AMERICANUM AND ASSOCIATED SPECIES ON MIDLAND BERMUDAGRASS FOR LOVE COUNTY

Nematode	Incidence	Vertical Distribution 15 cm	30 cm
X. americanum	50 *	44 *	56 *
"Tylenchorhynchus" spp.	22	22	22
Criconemoides spp.	39	33	44
Leptonchus spp.	33	22	44
Pratylenchus spp.	11	22	0
Trichodorus spp.	11	11	11
Heterodera spp.	6	0	11
Hoplolaimus spp.	11	11	11
"Tylenchus" spp.	22	44	0
Helicotylenchus spp.	67	67	6
Belonolaimus spp.	0	0	0
Longidorus spp.	6	0	1

* Data are the % of samples from which the nematodes were recovered for Love County rounded to the nearest whole number.

TABLE XI

INCIDENCE AND VERTICAL DISTRIBUTION OF XIPHINEMA AMERICANUM AND ASSOCIATED SPECIES ON MIDLAND BERMUDAGRASS FOR STEPHENS COUNTY

Nematode	Incidence	Vertical Distribution	
		15 cm	30 cm
X. americanum	61 *	67 *	56 *
"Tylenchorhynchus" spp.	0	0	0
Criconemoides spp.	17	22	11
Leptonchus spp.	11	11	11
Pratylenchus spp.	33	33	33
Trichodorus spp.	28	11	44
Heterodera spp.	0	0	0
Hoplolaimus spp.	0	0	0
"Tylenchus" spp.	0	0	0
Helicotylenchus spp.	100	100	100
Belonolaimus spp.	28	22	33
Longidorus spp.	0	0	0

* Data are the % of samples from which the nematodes were recovered for Stephens County rounded to the nearest whole number.

Eufaula fine sand, undulating, pH 6.5, at a depth of 30 cm. Belonolaimus spp. was not found in samples from Love county.

The data from Logan county are presented in Table IX. Xiphinema americanum was the third most commonly encountered nematode, occurring in 44% of the samples. Helicotylenchus spp. was most commonly found, 72%, while Pratylenchus spp. was the second most commonly occurring plant parasitic nematode in Logan county, 50%. Other genera found were Hoplolaimus, "Tylenchorhynchus", Leptonchus, Criconemoides, Trichodorus, and Belonolaimus spp. with frequencies of occurrence of: 39, 22, 17, 11, 11, and 6%, respectively, Heterodera, "Tylenchus", and Longidorus spp. were not found in Logan county.

Vertical distribution data for the nematodes recovered from all counties sampled are presented in Table VIII. Most frequently found at 15 cm were: "Tylenchorhynchus", Criconemoides, Pratylenchus, "Tylenchus", and Helicotylenchus spp. The nematodes most frequently found at 30 cm were: X. americanum, Leptonchus, Trichodorus, Heterodera, Hoplolaimus, Belonolaimus, and Longidorus spp.

Table IX shows the incidence and vertical distribution results for Logan county. "Tylenchorhynchus", Criconemoides, Leptonchus, Pratylenchus, and Helicotylenchus spp. were most often recovered from 15 cm, while X. americanum, Hoplolaimus, and Belonolaimus spp. were found most frequently at the 30 cm depth. Trichodorus spp. were found at each depth in 11% of the samples.

Love county data on incidence and vertical distribution are presented in Table X. The most frequently encountered genera at 15 cm were: Pratylenchus, "Tylenchus", and Helicotylenchus spp. Xiphinema americanum, Criconemoides, Leptonchus, Heterodera, and Longidorus spp. were most often found at 30 cm. "Tylenchorhynchus", Trichodorus, and Hoplolaimus spp. were found at both depths with each genus occurring at the same frequency.

Incidence and vertical distribution of nematodes for Stephens county are shown in Table XI. Xiphinema americanum and Criconemoides spp. were found most often at 15 cm while Trichodorus and Belonolaimus spp. were most frequently encountered at 30 cm. Leptonchus, Pratylenchus, and Helicotylenchus spp. were found at both depths, each genus with the same frequency. Helicotylenchus spp. were recovered from every sampling site in Stephens county.

Summary and Conclusions

Love, Stephens, and Logan counties were found to be the three areas in Oklahoma in which Midland bermudagrass was most extensively grown, Table IV. They were selected for nematode sampling, survey 2.

Survey 2 revealed the presence of nematodes ranging from 4 to 256/100 cc of soil in every sampling site. Listed in order of frequency of occurrence, the genera recovered were: Helicotylenchus spp., X. americanum, Pratylenchus, Criconemoides, Leptonchus, Hoplolaimus, Trichodorus, "Tylenchorhynchus", Belonolaimus, "Tylenchus", Heterodera, and Longidorus spp., Table VIII. Xiphinema americanum was recovered from 52% of the samples with only Helicotylenchus spp. being recovered more frequently, 80%. Pratylenchus spp. was recovered with a frequency of 32%. These latter three genera were found by other investigators (2, 11, 16, 28, 30, 37, 40) on range, grasses, pasture, and forage crops with high frequencies.

Xiphinema americanum was recovered from all soil types encountered and at each depth sampled, but not at each vigor site. It was found at 77% of the best vigor sites, at 44% of the good vigor sites, and at 33% of the poor vigor sites; primarily at the 30 cm depth in each instance. American dagger nematodes were recovered from sites having a moisture content of 1.5 to 3.8% (by weight), soil temperatures of 23.5 to 29.0 C, and a pH range of 4.2 to 7.5, Tables V-VII, in populations as high as 84/100 cc of soil. These population estimates are conservative, however, in light of the population dynamics of X. americanum in Oklahoma, Chapter IV. Had survey 2 been done in April-May or in September, more accurate population densities may have been found, as these are the months when X. americanum populations peak in Oklahoma on Midland bermudagrass.

The vertical distribution of X. americanum varied with soil type. Logan and Love counties had more X. americanum at the 30 cm depth which substantiate Thorne's findings that X. americanum is a nematode of deep, 30 cm, soil profiles (65); while Stephens county had more of the American dagger nematode at the 15 cm depth.

The results of survey 2 clearly indicate that X. americanum is associated with Midland bermudagrass as other plant parasitic nematodes have been reported (16, 28, 30, 37) to also be associated with other grasses. Pathogenicity trials are needed to determine if the American dagger nematode is a pathogen of Midland bermudagrass. The results of Chapter II indicate that X. americanum can reproduce on Midland. Variety 71-X 8-4 proved to be the best host for X. americanum in the host suitability trials, Chapter III. Therefore, theoretically X. americanum may now be reared on variety 71-X 8-4 in sufficient quantities for use as inoculum for a pathogenicity trial on Midland. Determination of the American dagger nematode's pathogenicity on Midland bermudagrass and its associations with other varieties of bermudagrass in nature are suggested for future investigations.

CHAPTER VI

ESTABLISHMENT AND GROWTH OF MIDLAND BERMUDA-GRASS FIELD PLOTS IN THE PRESENCE OF *XIPHINEMA AMERICANUM* POPULATIONS

Materials and Methods

A fallow 30 X 30 m study site of sandy loam soil at the Agricultural Experiment Station near Perkins, Oklahoma, was selected because it was naturally infested with nematodes; primarily *X. americanum*. The study site was disced several times, raked smooth, then divided into 3 X 3 m plots. The experimental design was a split plot in which the main plot was a 3 X 5 factorial laid out in a randomized complete block; and time was considered as a subplot. The 3 Midland bermudagrass treatments consisted of planting: 1) cuttings in non-fumigated soil, 2) rooted plants in non-fumigated soil, and 3) rooted plants in MB fumigated soil. There were 5 inoculum levels: 0, 150, 300, 450, and 600 *X. americanum*; and 6 replicates. Five plots per replicate were covered with a plastic tarp and fumigated with MB soil fumigant (0.56 kg/9.29 m^2 plot, uncovered after 2 d).

Inoculum was sandy loam soil heavily infested with *X. americanum* from the Scannell Ranch, Ringwood, Oklahoma. To obtain uniformity, freshly dug soil was mixed in a cement mixer for 15 sec. To determine the amount of soil to use for each inoculum level, four 177 g (100 cc) aliquants were taken and assayed for nematodes as previously described.

Although X. americanum was the predominant species present, other plant parasitic genera including Leptonchus, Pratylenchus, Tylencholaimellus, Dipthorophora, Criconemoides, Quinisulcius, and Trichodorus spp. were identified to the generic level and counted. Tylenchus spp. and other closely related genera were identified only to subfamily to facilitate counting and were reported as "Tylenchus". On the basis of the number of X. americanum recovered from these initial 177 g aliquants, the grams of mixed field soil required to provide: 150, 300, 450, and 600 X. americanum was determined. Plastic bags containing 375 g of sterile Lincoln fine sand were provided for each check plot. Soil subsamples of the appropriate weight for each inoculum level were weighed on a Metler scientific balance and immediately placed into a plastic bag. Extra bags of soil for each inoculum level were included to be used to determine inoculum mortality due to handling. In order to avoid overheating, all bags of inoculum were kept in an air-conditioned laboratory after weighing for 4 hr until they were transported after sunset to the study site.

The bermudagrass used in this study was obtained from greenhouse stock material established from cuttings taken from the Scannell Ranch. In order to ascertain differences in plant establishment and/or growth, single healthy 15 cm cuttings were used along with 3½- wk old rooted plants established from 15 cm cuttings in the greenhouse in individual plastic pots (2.5 X 5 X 5 cm - bottom diameter, top diameter, and height; respectively) and grown in sterile Lincoln fine sand. The rooted plants were each fertilized in the greenhouse 1 wk prior to transplanting into the field with 25 ml of "Peters soil test fertilizer", 20-20-20.

A 5 X 15 cm (diameter and depth, respectively) cylindrical hole was cut in the center of each plot using a standard golf course cup cutter. One-half of the soil volume removed was placed into a plastic bag for background field nematode population analysis. The inoculum was gently poured into the hole, then a cutting or a rooted plant was set into the inoculum. The second half of the extracted plug was added to the site with additional soil from the surrounding area to refill the hole. The inoculum subsamples to be used to determine nematode mortality due to handling and the samples taken to determine the background nematode population at each site were returned to the laboratory after the study installation and assayed for nematodes.

The plants were uniformly hand-watered as needed. Growth was monitored monthly using a 150 X 150 cm metal grid. The grid which was blocked into 30 X 30 cm sections, was placed over the plot and centered by placing the center block directly over the plant. The square meter coverage was measured using the grid.

Forage yields, 3 cm above soil level, were taken with the aid of an electric hedge trimmer (Black and Decker model #8114) from the center 30 X 30 cm area of each plot at the end of the second and the third growing seasons (16 and 24 mo, respectively, after plot initiation). The forage was placed into prelabeled drying bags and sealed. Fresh and dry weights were determined as previously described. Weeds were hand rogued as needed to keep the study site weed-free.

A 5 cm diameter soil core sample was taken at a depth of 15 cm from the center of the inoculation site for neamtode analysis. Residual soil from the probe was returned to the sampling hole and sufficient sterile Lincoln fine sand was added to fill the depression. The soil samples were transported to the laboratory in ice chests. The entire study site was mowed at the end of each growing season after forage yields were taken.

Nematode inoculum numbers were adjusted for mortality due to handling but are referred to as: 0, 150, 300, 450, and 600 in this discussion for the sake of consistency.

Statistical analysis: A preliminary analysis was made on each response variable by considering all treatments by all periods of time. In the analysis presented, a separate analysis was made on each response variable for each time period. The sources of variation in the analysis were:

Source	df
Replication (R)	5
Treatment (T)	2
Inoculum Level (I)	4
T * I	8
Error (R * T|I)	70

All differences were studied by using the error term having 70 df.

Least significant difference analyses were used for statistical comparisons.

Data were also examined for linear and quadratic effects due to inoculum level.

Results and Discussion

Cuttings vs Rooted Plants in Non-fumigated Soil

Tables XII-XIII show the growth of Midland bermudagrass at 2 and 3 mo. No statistically significant differences were found. However, for the majority of the inoculum levels, plants established by cuttings had slightly more ground coverage in the first 3 mo and throughout the first growing season. Although simple linear and quadratic effects due to inoculum levels were not found and no interactions were present, different ground coverage trends corresponding to inoculum level were seen by the end of the first growing season, Figure 8. At month 5, coverage for the plots started from cuttings essentially decreased with inoculum increases. The nematodes initially present in the plots, Figure 9, which may have reproduced, Chapter IV, could account for less growth rate in terms of coverage in the: 0, 150, and 300 inoculum level plots. Essentially, the reverse trend is seen in the month 5 coverage for the plots started from rooted plants, Figure 8. The small amount of ground coverage attained by the check plots could be due to reproduction by nematodes initially present in the plots, Figure 9.

Simple linear, quadratic effects, and significant interactions due to inoculum level were not found for forage yields. Forage yields taken at the end of the second growing season did not show the same trends, Table XIV and Figure 10, as the first cut. Instead, the forage from the plots started from cuttings essentially corresponds to the final nematode populations taken at 21 mo, Figure 9. The final nematode populations for the 150 inoculum level plots were

TABLE XII

EFFECT OF XIPHINEMA AMERICANUM INOCULUM LEVELS ON MIDLAND BERMUDAGRASS GROWTH AT 2 MO (M^2)

Inoculum Levels	Treatments: Cuttings, Non-Fumigated Soil	Rooted Plant, Non-fumigated Soil	Rooted Plants, Fumigated Soil
0	0.58 *	0.45	0.68
150	0.35	0.41	0.72
300	0.36	0.33	1.00
450	0.62	0.39	0.61
600	0.37	0.48	0.77
LSD			
.05	ns	0.28	0.28
.01	ns	0.37	0.37

* Data mean of 6 observations.

TABLE XIII

EFFECT OF XIPHINEMA AMERICANUM INOCULUM LEVELS ON MIDLAND BERMUDAGRASS GROWTH AT 3 MO (M^2)

Inoculum Levels	Treatments: Cuttings, Non-Fumigated Soil	Treatments: Rooted Plants, Non-fumigated Soil	Treatments: Rooted Plants, Fumigated Soil
0	1.89 *	1.69	1.94
150	1.24	1.42	1.88
300	1.38	1.20	2.21
450	1.70	1.51	1.92
600	1.64	1.63	1.90
LSD			
.05	ns	0.55	0.55
.01	ns	0.73	0.73

* Data mean of 6 observations.

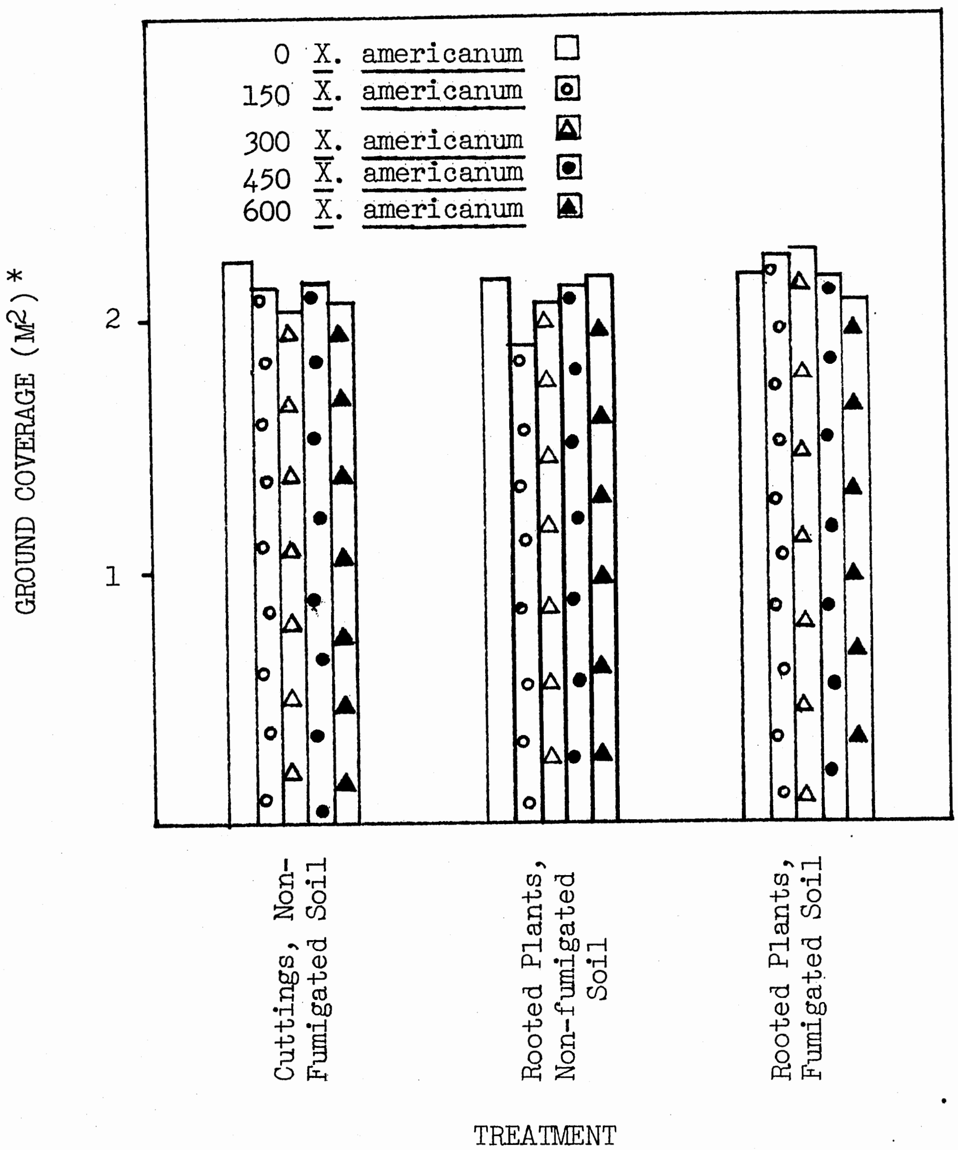

Figure 8. Midland Bermudagrass Growth at 5 Months by Treatments and Inoculum in the Field Plot Establishment Study.

* Data mean of 6 observations.
LSD ns

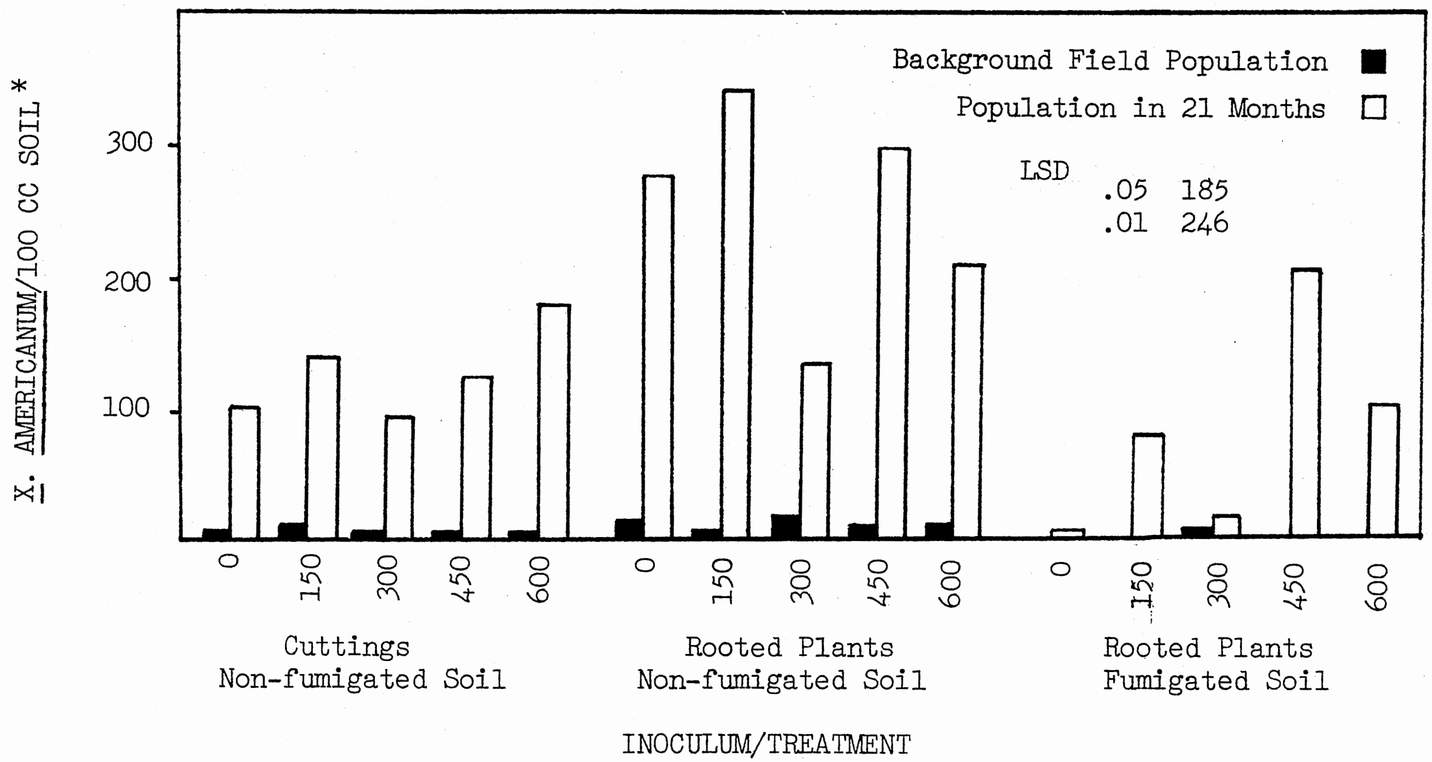

Figure 9. Background Field Populations and Final Populations of *Xiphinema americanum* for all Treatments and all Inoculum Levels in the Field Plot Establishment Study.

* Date mean of 6 observations.

TABLE XIV

EFFECT OF <u>XIPHINEMA</u> <u>AMERICANUM</u> INOCULUM LEVELS ON FORAGE YIELDS OF MIDLAND BERMUDAGRASS AT 16 MO (G)

Inoculum Levels	Treatments		
	Cuttings, Non-Fumigated Soil	Rooted Plants, Non-fumigated Soil	Rooted Plants, Fumigated Soil
0	77.24 *	71.88	102.52
150	64.58	86.26	95.80
300	73.72	69.78	87.49
450	58.58	78.99	91.20
600	72.63	79.45	72.70

LSD
.05 21
.01 27

* Data mean of 6 observations.

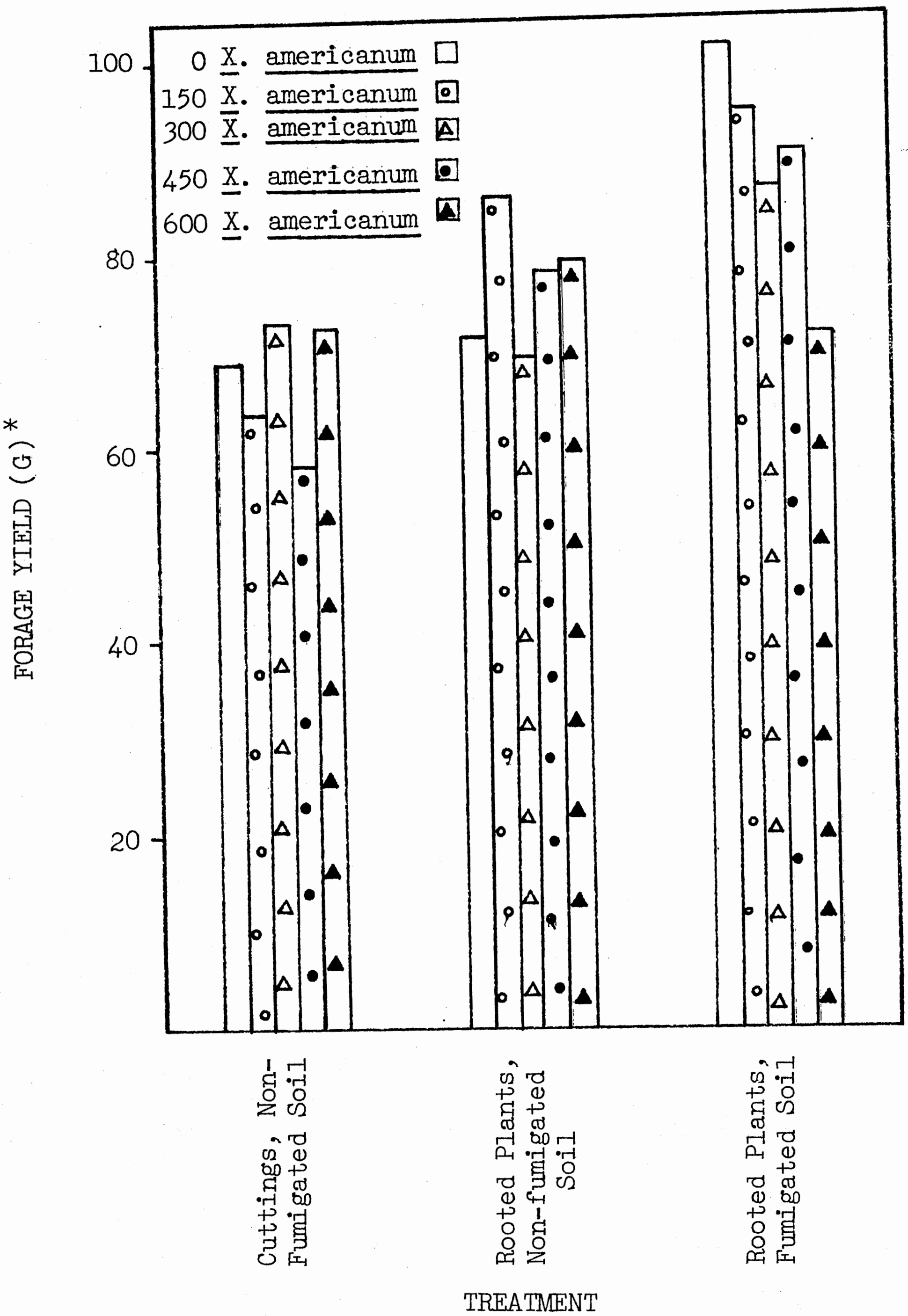

Figure 10. Forage Yield Taken at the end of the Second Growing Season, 16 Months After Initiation of the Field Plot Establishment Study.

* Data mean of 6 observations.

LSD .05 21 / .01 27

greater than those from the check plots. Likewise, the forage yield from the 150 inoculum level plots was less than the check plots. Whereas the final *X. americanum* populations for the 300 inoculum level plots decreased. However, *X. americanum* populations increased as the forage yield decreased for the 450 inoculum level plots. At the 600 inoculum level, this trend did not exist. No trend was evident between the final *X. americanum* populations and forage yields taken at the end of the second growing season for the plots started from rooted plants, Figures 9-10.

The forage yield from the 150 inoculum level plots started from rooted plants was greater ($P < 0.05$) than the yield from the 150 inoculum level plots started from cuttings, Table XIV. A concomitant decrease in *X. americanum* populations in plots started from rooted plants would explain this greater forage yield. However, there was an increase in *X. americanum* populations at 21 mo in the plots started from rooted plants, Figure 9. The *X. americanum* populations from the 150 inoculum level plots started from rooted plants were also significantly ($P < 0.05$) greater than the *X. americanum* populations from the 150 inoculum level plots started from cuttings, Figure 9. Since the forage yields were taken in October preceding the final *X. americanum* population sampling, it is possible that the *X. americanum* populations remained low until October accounting for a large forage yield, then increased, Chapter IV, and remained high at the nematode sampling date in March. However, it is not probable that only 1 out of 15 units in the study would behave in this way. They are merely anomalous results.

Forage yields taken at the end of the third growing season essentially followed the same trend of the previous year, Table XV and

TABLE XV

EFFECT OF XIPHINEMA AMERICANUM INOCULUM LEVELS ON FORAGE YIELDS OF MIDLAND BERMUDAGRASS AT 27 MO (G)

Inoculum Levels	Treatments: Cuttings, Non-Fumigated Soil	Treatments: Rooted Plants, Non-fumigated Soil	Treatments: Rooted Plants, Fumigated Soil
0	30.36 *	27.25	48.09
150	26.46	33.48	38.16
300	30.02	28.98	38.73
450	24.04	32.72	40.22
600	28.93	30.85	29.02
LSD			
.05	ns	20	20
.01	ns	26	26

* Data mean of 6 observations.

Figure 11. Yields from the plots started from cuttings were similar to the yields from the plots started from rooted plants. However, yields taken at the end of the third growing season were about half of the yields of the previous year, Table XIV and Figure 10. Lack of rain and higher temperatures than usual for the summer months in 1978 may in part account for the decrease in forage yields.

Attempts were made to sample each plot for a horizontal distribution of X. americanum at set increments from the center (inoculation site) of each plot at the end of the third growing season. However due to the arid conditions, the soil was too hard to sample more than 8 to 10 cm deep even with a motor-driven auger. In the few plots that could be sampled, very few X. americanum were found. Harrison and Murad (25) found the lowest populations of X. americanum in the fall; and Norton (38) recovered the fewest American dagger nematodes from soil with low moisture contents and high temperatures. Whether X. americanum populations died, entered a state of anabiosis, or moved downward under these dry conditions is speculative and for future investigators to determine. Perhaps this was just a summertime low population period prior to the fall population peak, Chapter IV, for X. americanum in Oklahoma. Nematode assays taken 1 mo later may have given answers to these questions.

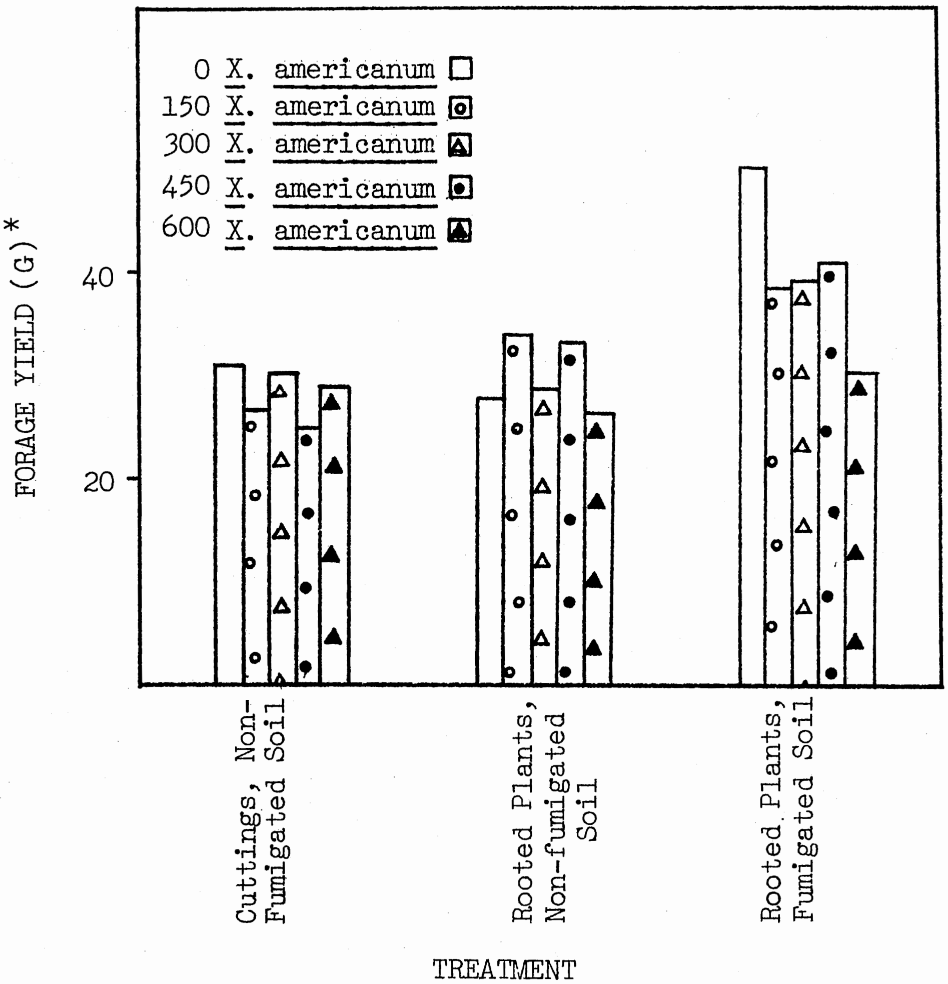

Figure 11. Forage Yield Taken at the end of the Third Growing Season, 27 Months after Initiation of the Field Plot Establishment Study.

* Data mean of 6 observations.

LSD	.05	20
	.01	26

Rooted Plants in Fumigated vs Non-fumigated Soil

Two months after study initiation, the Midland bermudagrass ground cover in the 150, 300, and 600 inoculum level fumigated plots was significantly ($P < 0.05$) greater than the coverage in the non-fumigated plots, Table XII. At 3 mo only the 300 inoculum level fumigated plots had significantly ($P < 0.01$) greater coverage than the non-fumigated plots, Table XIII. All plots were beginning to outgrow the measuring grid and to grow together at 4 mo No significant differences in coverage were found at 4 mo or for the remainder of the study. Most of the fumigated plots had more coverage than did the non-fumigated plots during the first growing season, Figure 8.

When the data were examined for linear and quadratic effects due to inoculum levels and for interactions, none were found. However, by month 5, a trend in coverage by inoculum levels was seen. In the fumigated plots, Figure 8, essentially, as the inoculum level increased, coverage decreased. Forage yields taken at the end of the second growing season, Figure 10, showed the same trend. The non-fumigated plots, however, showed a reverse trend, Figure 8. With the exception for the check plots which had nearly the same level coverage as did the 600 inoculum level plots, as the inoculum level increased, the coverage increased, Figure 8.

Xiphinema americanum were present at each site at study initiation, Figure 9. Forage yields, Figure 10, taken at the end of the second growing season showed the same trend, for the most part. *Xiphinema americanum* populations had the time and opportunity, Chapter IV, to increase and readjust levels at each inoculation site. By 21 mo, essentially a decrease in nematode populations was seen with an increase in original inoculum levels, Figure 9. This decreasing trend for X. americanum populations also coincides with the coverage at 5 mo, Figure 8, and the forage yields, Figure 10, for the most part. No significant simple linear or quadratic effects due to inoculum level and no interactions were found.

Final X. americanum populations, Figure 9, showed that the American dagger nematodes increased over the 21 mo period of the study. Thirteen X. americanum/ 100 cc of soil were recovered from the check plots which had no X. americanum background field populations at study inoculation time. Surrounding field populations had three opportunities to reproduce, Chapter IV. The growing bermudagrass plants had the opportunity to attract X. americanum to the new root feeding sites. The X. americanum populations increased at the various inoculation sites by the end of the study, however not in linear proportions to the inoculum levels.

Summary and Conclusions

Ground coverage and forage yields, Figure 10 and Table XIV, for plots established from cuttings in non-fumigated soil was the same as plots started from 3½ wk old rooted plants in non-fumigated soil.

Although the Midland bermudagrass growth on the non-fumigated plots at 2 and 3 mo was essentially greater in the plots started from rooted plants, the ground coverage in the fumigated plots started from rooted plants was significantly better at the 150, 300, and 600 inoculum levels at 2 mo ($P < 0.05$) and at 3 mo ($P < 0.01$), Tables XII-XIII. However, by the end of the first growing season, all plots essentially had the same coverage, although trends due to inoculum level were seen, Figure 8.

Forage yields taken at the end of the second growing season for the check plots started from rooted plants in fumigated soil were significantly ($P < 0.01$) higher than the yields from the plots started from cuttings in non-fumigated soil and the plots started from rooted plants in non-fumigated soil, Table XIV. Build-up of background field populations of X. americanum, Figure 9, along with the X. americanum populations moving into the inoculation/sampling site might account for the yield differences in the check plots. Likewise for the results at the 150 inoculum level where all plots started from rooted plants showed greater fresh forage yields than did the plots started from cuttings, Table XIV, and at the 450 inoculum level where only the plots started from rooted plants planted in fumigated soil showed a significant ($P < 0.01$) fresh forage yield over the plots started from cuttings in non-fumigated soil.

If this study were to be repeated, the following changes are recommended:

a. Use cuttings only, as no significant advantage was derived from use of 3½ wk old rooted plants. This would thereby reduce the treatments.

b. Since initial field populations of X. americanum clouded the results of this study, all plots should be fumigated prior to inoculation. Perhaps two or more fumigants can be utilized for comparative results.

c. Begin to monitor coverage sooner, since more differences were evident at 2 mo than at any other time. A weekly growth/coverage monitoring, with soil samples taken at sites where new roots are set down might show whether populations from the original inoculum move to new root zones.

d. Take soil samples at a minimum of 2 depths: 15 and 30 cm. *Xiphinema americanum* is considered to be a nematode of deep soil profiles (65). In the vertical distribution survey, Chapter V, 78% of the nematodes recovered from Midland bermudagrass in Logan and Love counties at the 30 cm depth were *X. americanum*.

e. Take a forage yield at the end of the first growing season in order to ascertain differences in yield due to inoculum nematode populations.

f. Plan to irrigate the study in order to be able to take all of the soil samples needed.

CHAPTER VII

CHEMICAL CONTROL TRIALS

Materials and Methods

A declining Midland bermudagrass pasture, Scannell Ranch, Ringwood, Oklahoma, established for about 7 yr was selected for the study. An analysis of the sandy loam soil indicated adequate nutrient levels except potassium which was deficient by 35 lbs/A. The soil pH was 7.4.

A split-plot design with adjacent checks was utilized to give a more accurate estimate of nematode population/variation within the plots. The treatments laid out in a randomized complete block design were the main plots while the adjacent checks were the subplots. There were 4 replicates.

Plots were 150 X 300 cm arranged in one continuous row (300 cm wide X 120 m long). There were 11 treatments utilizing 2 nematicides, 2 fungicides, 4 combinations of nematicide + fungicide, and a H_2O 'Nema Jet' application. The nematicides were: (1) DBCP (1, 2-dibromo-3-chloropropane) an 84% emulsifiable concentrate applied at rates of 98.8 kg ai/ha, or 49.4 kg ai/ha and (2) fensulfothion (O, O-diethyl O-[p-(methylsulfinyl)phenyl] -phosphorothioate) 15% granular applied at rates of 19.6 kg ai/ha or 9.8 kg ai/ha. The fungicides were: (1) benomyl [methyl-1-(butylcarbamoyl)-2-benzimidazole = carbamate] a 50% wettable powder applied at a rate of 3.9 kg ai/ha and

(2) PNCB-ETMT [penta-chloronitrobenzene 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole] 10% granular applied at a rate of 48.9 kg ai/ha. The combinations of nematicide + fungicide consisted of: (1) DBCP at 98.8 kg ai/ha + benomyl at 3.9 kg ai/ha, (2) DBCP at 98.8 kg ai/ha + PCNB-ETMT at 48.9 kg ai/ha, (3) fensulfothion at 19.6 kg ai/ha + benomyl at 3.9 kg ai/ha, and (4) fensulfothion at 19.6 kg ai/ha + PCNB-ETMT at 48.9 kg ai/ha. DBCP was applied with a 'Nema Jet' at 135 kg pressure. The granular materials were applied as a top dressing with a Scott # 4209 chemical spreader, while benomyl was applied as a drench. The H_2O treatment applied with a 'Nema Jet' consisted of applying the same volume of H_2O to the plots under 135 kg pressure as used in application of DBCP. It was another treatment included in the randomization and was included as an extra check for the DBCP treatment plots to determine the effect of the pressure injection alone on the plots. The presentation in this chapter reflects the H_2O 'Nema Jet' treatment results subtracted from each of the treatments applied with the 'Nema Jet'.

After all the treatments were applied, the plots were irrigated with 6 cm of water in order to allow the treatments to permeate the soil. The fall treatments were applied on October 11, 1976. Spring treatments applied June 6, 1977, consisted of: (1) DBCP an 84% emulsifiable concentrate applied at a rate of 98.8 kg ai/ha and (2) a H_2O treatment applied with a 'Nema Jet'. Both were applied with like volumes of H_2O at 135 kg pressure.

Nematode populations were monitored monthly by soil sampling as previously reported except in January, 1977, when weather conditions prevented it.

The data were converted in the following manner:

$$\frac{\text{Xiphinema americanum Populations (Treated Plot) + 1}}{\text{Xiphinema americanum Populations (Check Plot) + 1}} \times 100$$

which gave the final figures in % of check. Statistical comparisons used in the analysis of variance were LSD values.

Forage was harvested in June, 1977, from the center 100 X 250 cm area of each plot with a Mott # 964 tractor-mounted flail mower equipped with a catcher. Total fresh forage weight from each plot was recorded. A random forage subsample was immediately withdrawn from each sample and weighed. These subsamples were subsequently oven-dried and reweighed for dry forage determination as previously reported.

Forage yield data was converted as follows:

$$\frac{\text{Forage Weight in Treated Plot}}{\text{Forage Weight in Check Plot}} \times 100$$

which gave the final figures in % of check. Statistical comparisons were made using LSD values.

Samples of root tissue were taken from each plot for endoparasitic nematode assays and for fungal isolations.

Results and Discussion

No endoparasitic nematodes were extracted from the root tissue. Although soil samples contained plant parasitic nematode genera : *Xiphinema*, *Quinisulcius*, *Criconemoides*, *Pratylenchus*, *Trichodorus*, *Leptonchus*, and "Tylenchus", *X. americanum* was present in greatest numbers.

Fensulfothion at 9.8 kg/ha showed a 59% reduction in *X. americanum* populations 2 mo after fall application, Table XVI, with a concomitant fresh forage yield increase, 34.1% of check, Table XVII.

TABLE XVI

XIPHINEMA AMERICANUM POPULATIONS (% OF CHECK)[a] FOR EACH TREATMENT AND EACH SAMPLING

		Month After Fall Application												
Treatment	Rate**	0	1	2	4	5	6	7	8	9	10	11	12	13
Fensulfothion	19.6	-14	-37	-8	-8	4	-24	10	-9	-4	2	-4	0	-3
Fensulfothion	9.8	-29	1	-46	-15	0	10	28	-8	0	0	7	5	1
Benomyl	3.9	9	39	4	23	9	7	3	1	-1	10	-12	2	17
PCNB-ETMT	48.9	-10	-32	-17	-26	-3	-11	-2	-1	-11	1	-1	-1	8
Fensulfothion Benomyl	19.6 3.9	-22	36	-7	1	4	-22	20	-2	5	2	17	-2	6
Fensulfothion PCNB-ETMT	19.6 48.9	-11	10	-29	4	3	-39	26	6	-3	3	0	-1	-8
DBCP	98.8	53	51	15	30	23	30	3	9	14	16	6	1	-4
DBCP	49.4	52	53	20	62	21	48	9	5	31	12	3	2	15
DBCP Benomyl	98.8 3.9	49	33	25	12	20	41	3	0	6	16	-6	0	-6
DBCP PCNB-ETMT	98.8 48.9	40	12	12	18	14	35	2	5	24	12	-10	-2	-7
DBCP (SP)	98.8	X	X	X	X	X	X	X	-6	-11	26	9	-1	7

LSD ns

a % of check = $\frac{\text{X. americanum Population in Treated Plot} + 1}{\text{X. americanum Population in Adjacent Check Plot} + 1} \times 100$.

** Kg ai/ha .

* Data mean of 4 observations .

TABLE XVII

FORAGE YIELDS FROM MIDLAND BERMUDAGRASS CHEMICAL CONTROL TRIALS

Treatment	Rate**	Fresh Forage Yield (% of Check)[a]	Dry Forage Yield (% of Check)
Benomyl	3.9	261.4 *	141.7
Fensulfothion Benomyl	19.6 3.9	198.9	27.4
DBCP	49.4	102.3	28.9
Fensulfothion	9.8	34.1	-24.3
Fensulfothion	19.6	-39.8	34.3
DBCP Benomyl	98.8 3.9	-96.6	-30.2
PCNB-ETMT	48.9	-193.2	-149.7
DBCP	98.8	-289.6	-199.2
Fensulfothion PCNB-ETMT	19.6 48.9	-306.8	-91.5
DBCP (Spring)	98.8	-312.5	-118.6
DBCP PCNB-ETMT	98.8 48.9	-653.4	-314.7
LSD .05		545	ns
.01		720	ns

a $(\% \text{ of Check}) = \frac{\text{Yield in Treated Plot}}{\text{Yield in Check Plot}} \times 100$.

** Kg ai/ha.

* Data mean of 4 observations.

Fensulfothion at 19.6 kg/ha showed a 164% reduction in X. americanum populations 1 mo after fall application, Table XVI. However, fresh forage yield data indicate that the higher rate of the nematicide was phytotoxic to the Midland bermudagrass as a -39.8% of check yield was recorded, Table XVII.

Two months after fall application, the populations treated with DBCP showed a 49 to 72% reduction in X. americanum from the pretreatment levels, Table XVI. Populations treated with DBCP at 98.8 kg/ha, DBCP at 98.8 kg/ha + PCNB-ETMT at 48.9 kg/ha, and DBCP at 98.8 kg/ha + benomyl at 3.9 kg/ha did not recover for the duration of the study while populations treated with DBCP at 49.4 kg/ha and populations treated with DBCP at 98.8 kg/ha (spring) recovered in 4-3 mo , respectively. However, only the plots treated with DBCP at 49.4 kg/ha showed a consequent increase in fresh forage; 102.3% of check, Table XVII. All other plots showed forage decreases indicative of phytotoxicity. Therefore, in future fumigation of bermudagrass pastures with DBCP, a rate of 49.4 kg/ha or somewhere in between 49.4 and 98.8 kg/ha should be effective.

Fusarium, Helminthosporium, and Tricoderma spp. were the fungus genera isolated from root tissue which are known to contain plant pathogenic species (56).

The fall application of benomyl at 3.9 kg/ha allowed for a 56% reduction in X. americanum populations 2 mo afterwards, Table XVI.

Thereafter X. americanum populations recovered. The 333% increase in X. americanum populations 1 mo after fall application of benomyl at 3.9 kg/ha, Table XVI, may be due to the action of benomyl on endozoic fungi that were maintaining a natural control over X. americanum populations in the soil rhizosphere. Although no significance can be attributed to them due to variation within the treatments, the action of benomyl at 3.9 kg/ha allowed for a 261.4% increase in fresh forage, while the action of benomyl at 3.9 kg/ha + fensulfothion at 19.6 kg/ha allowed for a 198.9% increase, Table XVII.

Fall application of PCNB-ETMT at 48.9 kg/ha allowed for a 220% decrease in X. americanum populations 1 mo after application with the populations recovering after 5 mo, Table XVI. Yield data, Table XVII, however indicates phytotoxicity for the plots treated with PCNB-ETMT at 48.9 kg/ha, PCNB-ETMT at 48.9 kg/ha + DBCP at 98.8 kg/ha, and PCNB-ETMT at 48.9 kg/ha + fensulfothion at 19.6 kg/ha. Therefore future studies with PCNB-ETMT on bermudagrass pastures should utilize a rate lower than 48.9 kg/ha.

Summary and Conclusions

DBCP at 98.8 kg/ha, fensulfothion at 19.6 kg/ha, and PCNB-ETMT at 48.9 kg/ha were phytotoxic to Midland bermudagrass under the conditions of this study, Table XVII.

Fresh forage yields of plots treated with DBCP at 49.4 kg/ha, fensulfothion at 9.8 kg/ha, fensulfothion at 19.6 kg/ha + benomyl at 3.9 kg/ha, and benomyl at 3.9 kg/ha were 102.3, 34.1, 198.9, and 261.4% of check, respectively, 8 mo after their fall application, Table XVII. Most also resulted in corresponding reductions in X. americanum populations, Table XVI. Therefore X. americanum is a factor in the decline of this 7 yr old Midland bermudagrass pasture.

The use of benomyl revealed the presence of a natural control by endozoic fungi over X. americanum in operation in the rhizosphere of the pasture. The chemical control trials added a short term reduction in *X. americanum* populations because of their limited dispersal range even under optimum soil moisture and temperature conditions. However, natural control organisms such as the endozoic fungi have fewer limitations to seeking and attacking *X. americanum* in deep as well as in shallow root zones in the soil rhizosphere. Therefore future investigations into control in this 7 yr old Midland bermudagrass pasture ought to be directed to enhancing the biological control of *X. americanum* with treatments to increase the populations of the natural control organisms to a maximum level.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

An initial sampling of a declining Midland bermudagrass pasture in Oklahoma revealed the presence of seven different genera of plant parasitic nematodes. *Xiphinema americanum* was present in sufficiently high numbers to warrant investigation of its association with the host.

The plots treated with DBCP at 49.4 kg/ha, fensulfothion at 9.8 kg/ha, and benomyl at 3.9 kg/ha had increased forage yields and concomitantly reduced American dagger nematode populations. Therefore *X. americanum* is a factor in the decline of the pasture.

On the same pasture, population dynamics sampling revealed that populations of *X. americanum*, *C. xenoplax*, and *Quinisulcius* sp. peaked twice per year.

In areas of Oklahoma where Midland bermudagrass is most extensively grown, *X. americanum* were found in all nine soil types encountered in populations as high as 84 per 100 cc of soil. Although the American dagger nematode is known to be a nematode of deep soil profiles, 41% were found at a depth of 15 cm while 63% were found at a depth of 30 cm on selected Midland bermudagrass pastures. They were recovered from soil with moistures of 1.5 to 3.8% by weight, temperatures of 23.5 to 29.0 C, and having a pH range of 4.2 to 7.5.

While *X. americanum* may retard the establishment and rate of growth (coverage) of Midland bermudagrass field plots for a few months, the plots do become established by the end of the first growing season. Bermudagrass variety 71-X 8-4 was the best field host for the American dagger nematode while Stratford Common, Midland, Greenfield, Coastal, and Hardie were poorer hosts.

Under controlled soil temperature and moisture conditions, X. americanum completed its life cycle in nine months or less in the greenhouse. Population increases were more than three fold at 18 C, more than 56 fold at 24 C, and more than 57 fold at 30 C.

In conclusion, X. americanum were found on Midland bermudagrass wherever the host was grown in Oklahoma, especially in the areas of the state in which it was most extensive. Xiphinema americanum is a factor in the decline of a seven year old Midland bermudagrass pasture. Pasture treatments to increase the populations of endozoic fungi and exert a greater natural control over X. americanum ought to be investigated. Although the American dagger nematode may retard establishment and coverage of Midland bermudagrass field plots for a few months, the plots do establish in the presence of varying populations of X. americanum. Bermudagrass variety 71-X 8-4 was the best field host for the American dagger nematode. Populations of X. americanum peaked twice per year on an established Midland bermudagrass pasture. Xiphinema americanum reproduced on Midland bermudagrass under controlled abiotic environmental conditions. Since a system was developed in which X. americanum reproduced more than 57 fold in nine months, pure populations can be reared for use as inoculum in future pathogenicity trials. The greenhouse sub-irrigated cylinder system can also be utilized for the determination of the life cycle of X. americanum.

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VITA

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Doctor of Philosophy

Thesis: SELECTED VARIETIES OF BERMUDAGRASS AS HOSTS FOR *XIPHINEMA AMERICANUM* AND ASSOCIATED SPECIES IN OKLAHOMA INCLUDING REPRODUCTION OF *X. AMERICANUM* IN PURE POPULATIONS

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