THE INCIDENCE AND HOST PARASITE RELATIONSHIPS OF SELECTED NEMATODES AND FORAGE GRASSES

By

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Bachelor of Science

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1968

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1975

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ACKNOWLEDGEMENTS

I wish to express my appreciation to my major advisor Dr. Charles C. Russell for his guidance, encouragement, and assistance in the preparation and conduct of these experiments. Acknowledgement is also due to Dr. John E. Thomas and Dr. George L. Barnes for their assistance, cooperation, and critical review of this manuscript.

I am very grateful to Dr. Robert M. Ahring for bringing this problem to our attention and for his invaluable assistance and cooperation in the conduct of these experiments.

I also wish to thank Mr. Lou S. Morrison, Mr. Harold A. Spiegel, and Mr. Arlin L. Bostain for their assistance in portions of this study, and Ms. Barbara M. Reed for her review of this manuscript.

I wish to extend my very special appreciation to my wife, Laverna, for her patience, encouragement, assistance, and understanding in the course of this study.

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CHAPTER I

INTRODUCTION

The history of grazing in Oklahoma has been one of intensified grazing pressure due to diversion of acreage to other uses. As the production of grain-fed beef becomes less lucrative, and the need for grain for direct human consumption intensifies, further emphasis will be placed on the production of high quality pasturage. As the pressure on grazing lands increases and available area decreases, factors limiting productivity must be more clearly understood. It is therefore imperative that problems associated with growth and reproduction of forage grasses be investigated.

One problem area was observed at the Agronomy Field Research Station, El Reno. When fertility experiments were discontinued and blanket fertility was applied to improved pasture grass stands, many plots failed to respond in the period of time normally anticipated. Initial sampling indicated the presence of high populations of plant parasitic nematodes and several genera of potentially plant pathogenic fungi in the grass rhizospheres. The highest population levels of plant parasitic nematodes were recovered from an old stand of sideoats grama (Bouteloa curtipendula (Michx.) Torr.). The species most commonly recovered from this stand were Helicotylenchus digonicus Perry from which populations in excess of 5000 per 100 cc of soil were recovered, and *Criconemoides beljaevae* Kirjanova of which populations in excess of 200 per 100 cc of soil were recovered.

Based on the initial observations made at the El Reno station, research was undertaken to determine the effects nematodes have on seed and forage production, and whether the incidence and distribution of plant parasitic nematodes would justify a major research undertaking.

CHAPTER II

LITERATURE REVIEW

Surveys and Host Range Studies

Many associations of a descriptive or taxonomic nature have been made between nematodes and forage, range, and pasture grasses. A few surveys have been performed, most of them concerning native range.

Norton (21) surveying small grains and native grasses in Texas found *Helicotylenchus nannus* and *Xiphinema americanum* to be the two species most commonly associated with native grasses. He found *H. nannus* to be associated with several different grasses and 27.5% of all sideoats grama samples.

Undisturbed native range in Kansas was surveyed by Orr (22). He found *Helicotylenchus* and *Xiphinema* to be the two most common plant parasitic genera. Of the samples taken, the appeared in 78.7% and 67.2%, respectively. *Tylenchorhynchus* spp. was well represented, appearing in 59.0% of the samples. *Tylencholiamellus* was present in 22.6% of the samples.

Schmitt and Norton (29), surveying native Iowa prairie, also found *Helicotylenchus* to be the most common genus followed by *Xiphinema* and *Tylenchorhynchus*. *Helicotylenchus pseudorobustus* was the most common species of *Helicotylenchus*; however, *H. digonicus* was also found.

Jenkins, Taylor, and Rohde (14) found Xiphinema spp. to be the most common known plant parasites of pasture, forage, and cover crops in Maryland. Tylenchorhynchus spp. and Pratylenchus spp. were also commonly recovered; however, Helicotylenchus spp. was not.

Very few host range studies have been conducted within the Graminae. In early work, McBeth (20) tested the susceptibility of 18 southern grasses to Meloidogyne sp. and found several of the grasses susceptible. Jatala et al. (13), performed host range studies on Ditylenchus radicicola and found that 17 of 27 species tested were hosts. Potter et al. (25), found several previously unreported grass hosts for Meloidogyne incognita. Two Pratylenchus species were studied by Jensen (15). He found that P. penetrans infected graminaceous hosts while P. vulnus did not. Riggs (26) tested several pasture and turf breeding lines and cultivars of bermuda grass for susceptability to four species of Meloidogyne. He found one cultivar upon which none of the species tested would reproduce.

Pathogenicity and Control

Although there are numerous reports of pathogenicity of nematodes to ornamental grasses (9, 10, 18, 28, 27, 33), they will not be treated in any detail here. Burton, McBeth and Stephens (7) showed that when Meloidogyne sp. resistant bermudagrass was planted with Kobe lespedeza in infested soil, both the grass and the legume yielded better than when susceptible bermudagrass was used in combination with Kobe lespedeza. Johnson (16) noted the pathogenicity and interaction of Criconemoides ornatus, Belonoliamus longicaudatus, and Tylenchorhynchus martini on six cultivars of bermudagrass. He found that all three species were pathogenic; separately, in combination on one cultivar, and in varying species combinations on four of the remaining five cultivars. The pathogenicity of *Paratylenchus projectus* to tall fescue was demonstrated by Coursen and Jenkins (9).

Satisfactory chemical control of plant parasitic nematodes of ornamental grasses has been achieved (2, 17, 26, 30, 39), but little attention has been devoted to chemical control of nematodes of range and pasture grasses. Benedict (3), working in southern Ontario, attempted to determine the effect of *Pratylenchus* sp. and *Rotylenchus* sp. by transplanting potted seedlings of several grasses into treated and untreated soil. He was able to show very few differences, and concluded that plant parasitic nematodes were not important contributors to forage crop failures in southern Ontario. No mention was made of initial, post treatment, or final population levels.

Research at South Dakota State University (1) increased harvestable herbage by as much as 59% with application of an unnamed nematicide to Western wheatgrass. No mention was made concerning the population levels of the nematodes involved or the techniques used. The dagger nematode was reported to be the most important nematode involved.

Host Attractiveness

Linford (19), in 1939, showed that *Meloidogyne* sp. were attracted to excised roots of several plant species. He was also able to demonstrate the attractiveness and non-attractiveness of several other plant parts to the nematodes.

Weischer (37) was able to show that when *Heterodera schactii* larvae came in contact with root leachates, they "became excited" and moved more rapidly. They did not respond in this manner when only water was used. When the leachate was more than 3 cm away from them, they were not responsive. *Ditylenchus dipsaci* did not respond even when it came into direct contact with the leachate.

Weiser (38) showed that some excised roots were more attractive to *Meloidogyne hapla* than other roots of the same host species.

Griffin (11) found that *Ditylenchus dipsaci* was more strongly attracted to the root diffusates of susceptable alfalfa than to the diffusates of resistant alfalfa.

Viglierchio (36) leached water across plant roots through a membrane placed at the end of a section of aluminum channeling filled with quartz sand. He found that nematodes in an abutting section of channeling responded to non-hosts, but not as strongly as to host plants.

CHAPTER III

SURVEYS

Materials and Methods

Collections were made in three areas: The Agronomy Field Research Station at El Reno, Oklahoma, the Wichita Mountains Wildlife Refuge, and selected sites in Payne County, Oklahoma.

The area sampled at the El Reno station is a grass seed production area where several grasses are planted in pure stands. The soil type is Brewer clay loam (Packic Arguistolls). Grass species sampled here were: buffalograss (Buchloe dactyloides 'Texoka'), Asiatic bluestem (OWB) "S" and "T" strains (Bothriochloa ischaemum and Bothriochloa intermedia, respectively), big bluestem (Andropogon geradii 'Kaw'), smooth bromegrass (Bromus inermis 'Southland'), sideoats grama (Bouteloa curtipendula 'Coronado'), switchgrass (Panicum virgatum 'Caddo'), weeping lovegrass (Eragrostis curvula 'Ermelo'), and indiangrass (Sorghastrum nutans).

The samples taken in Payne County came primarily from three areas: the Oklahoma State University Ecology Preserve, the Lake Carl Blackwell area, and patches of native grass within and near the Oklahoma State University Intramural Field. Grasses sampled were big bluestem, blue grama (Bouteloa gracilis), little bluestem (Andropogon scoparius), indiangrass, buffalograss, sideoats grama, and switchgrass.

The Wichita Mountains Wildlife Refuge has been protected since territorial settlement, and features vegetation in an almost native state. The predominant soil types from which samples were taken are Tillman clay loam (Typic Paleustolls), and Lawton gravelly clay (Udic Argruistolls). Samples from this area were taken from the rhiosphere of indiangrass, little bluestem, sideoats grama, buffalograss, blue grama, and switchgrass.

Each soil sample contained in excess of 300 cc of soil. Samples were immediately placed in polyethylene bags and carefully stored to prevent dessication or overheating in route to the laboratory. Within 24 hours of being taken, samples were processed using a modification of the Christie-Perry technique (8). This technique differs from the standard Christie-Perry procedure in that plastic cottage cheese tubs with a volume of approximately 450 cc are substituted for the funnels, and facial tissues are substituted for the muslin. When the sample is to be concentrated, the wire mesh and the facial tissue are removed, the sample is allowed to settle for approximately one hour, and either serially decanted to a volume of approximately 10 milliliters, or concentrated upon a screen. Due to the heavy soil type, all samples taken from the El Reno station were placed in individual containers, covered with water and allowed to soak for one hour prior to processing. It was found that the handling time for extremely heavy soils could be reduced by eight minutes per sample using this method. No differences in recovery were found when the soaking method was compared to the standard processing method.

Nematode identification was to genus in most cases, and only known or suspected plant parasites were recorded.

Results and Discussion

Data from initial samplings taken at the El Reno station are presented in Table I (page 10).

The most frequently recovered nematode genus was *Tylenchus* which occurred in 75% of the samples taken. Less frequent genera of plant parasites found at the El Reno station included *Criconemoides*, *Pratylenchus*, *Meloidogyne*, *Paratylenchus*, *Neotylenchus*, *Nothotylenchus*, *Heterodera*, and *Xiphinema*. The predominant species of *Helicotylenchus* was *H. digonicus*, and the predominant species of *Criconemoides* was *C. beljaevae*.

High populations of *C. beljaevae* were found in small areas of the sideoats grama planting. *Pratylenchus* sp. populations were high in some areas of the weeping lovegrass planting and in the smooth bromegrass planting. Although they were recovered in the initial sampling, isolated large populations of *Leptonchus* sp. were found in subsequent samplings in the switchgrass and sideoats grama plantings.

Data from the samples taken in Payne County are presented in Table II (page 11) . It is of interest that relatively few samples taken from this area contained *Helicotylenchus* spp., and only one large population was recovered. *Tylenchorhynchus* spp. was recovered from a relatively large proportion of these samples (84%). Other genera of known or suspected plant parasitic nematodes represented in lesser numbers were *Criconemoides*, *Pratylenchus*, *Paratylenchus*, *Trichodorus*, *Neotylenchus*, *Xiphinema*, and *Tylencholiamellus*.

The results of the Wichita Mountains Wildlife Refuge sampling are presented in Table III (page 12). The most commonly encountered genus

TABLE I

GENERA OF PLANT PARASITIC NEMATODES COMMONLY ASSOCIATED WITH GRASSES AT THE AGRONOMY FIELD RESEARCH STATION AT EL RENO

Number of Samples	Host	Nematode Genus and Number Recovered						
		<u>Helicotylenchus</u>	<u>Tylenchus</u>	<u>Tylenchorhynchus</u>	<u>Tylencholiamellus</u>	<u>Xiphinema</u>	Other Plant Parasitic Genera	All Genera
2	Buffalograss	732[a] (1296)[b]	60 (92)	8 (12)	0	6 (12)	6 (12)	812[c] (1412)[d]
2	OWB "S" strain	1123 (2452)	154 (184)	68 (80)	0	0	0 (224)	1345 (2940)
4	OWB "T" strain	7 (28)	379 (962)	359 (1408)	217 (304)	0	0 (4)	
2	Smooth bromegrass	4 (8)	80 (116)	470 (684)	20 (40)	4 (4)	170 (116)	748 (748)
9	Sideoats grama	1957 (5000)	50 (250)	36 (200)	14 (108)	7 (17)	14 (20)	2068 (5436)
10	Switchgrass	124 (764)	58 (208)	17 (88)	70 (640)	28 (140)	11 (140)	308 (1076)
8	Weeping lovegrass	12 (40)	18 (94)	5 (8)	78 (108)	1 (2)	111 (424)	225 (890)

[a]Average recovery per 100 cc of soil.

[b]Maximum recovery per 100 cc of soil.

[c]Average recovery per 100 cc of soil of all plant parasitic genera (includes genera not listed in table).

[d]Maximum recovery per 100 cc of soil from a single plot (includes genera not listed in table).

TABLE II

GENERA OF PLANT PARASITIC NEMATODES COMMONLY ASSOCIATED WITH GRASSES AT SELECTED SITES IN PAYNE COUNTY, OKLAHOMA

Number of Samples	Host	Nematode Genus and Number Recovered				
		Helicotylenchus	*Tylenchus*	*Tylenchorhynchus*	*Tylencholiamellus*	All Genera
4	Big bluestem	44[a] (64)[b]	10 (24)	24 (48)	13 (20)	91[c] (300)[d]
4	Blue grama	0	12 (12)	52 (100)	0	64 (112)
7	Little bluestem	62 (44)	30 (152)	31 (62)	20 (48)	89 (292)
7	Indiangrass	6 (32)	19 (56)	6 (24)	12 (52)	62 (120)
4	Buffalograss	62 (120)	40 (64)	0	12 (16)	114 (200)
4	Sideoats grama	12 (48)	12 (20)	76 (204)	4 (4)	104 (220)
8	Switchgrass	12 (24)	27 (200)	53 (64)	0	92 (216)

[a]Average recovery per 100 cc of soil.

[b]Maximum recovery per 100 cc of soil.

[c]Average for the four genera only.

[d]Maximum recovery per 100 cc of soil from a single plot (includes genera not listed in table).

TABLE III

GENERA OF PLANT PARASITIC NEMATODES COMMONLY ASSOCIATED WITH GRASSES AT THE WICHITA MOUNTAINS WILDLIFE REFUGE

Number of Samples	Host	Nematode Genus and Number Recovered						
		Helico-tylenchus	Tylenchus	Para-tylenchus	Tylencho-liamellus	Neo-tylenchus	Other Plant Parasitic Nematodes	All Genera
8	Indiangrass	22[a] (48)[b]	6 (16)	12 (24)	6 (16)	12 (44)	4 (8)	62[c] (116)[d]
7	Little bluestem	19 (52)	6 (12)	14 (68)	7 (12)	17 (64)	11 (8)	74 (154)
6	Sideoats grama	37 (172)	28 (48)	8 (32)	5 (12)	28 (60)	17 (36)	123 (256)
12	Buffalograss	85 (456)	7 (12)	66 (384)	13 (32)	9 (28)	16 (204)	196 (540)
7	Blue grama	13 (28)	2 (4)	25 (124)	5 (16)	12 (48)	2 (8)	65 (148)
4	Switchgrass	30 (80)	5 (20)	10 (28)	9 (28)	1 (4)	73 (174)	128 (300)

[a]Average recovery per 100 cc of soil.

[b]Maximum recovery per 100 cc of soil.

[c]Average recovery per 100 cc of soil of all plant parasitic genera (includes genera not listed in table).

[d]Maximum recovery per 100 cc of soil from a single plot (includes genera not listed in table).

was Helicotylenchus occuring in 82% of the samples. Three species of Helicotylenchus were recovered; H. digonicus, H. pseudorobustus, and H. bradys. The frequency with which Paratylenchus spp. were recovered in this survey (70%) was surprising in light of the surveys mentioned in the literature review. Tylenchorhynchus spp. and Xiphinema spp. occurred in a relatively low number of the samples in this survey, 26% and 20%, respectively. Tylenchus spp. occurred in 59% of the samples, Neotylenchus in 65% and Tylencholiamellus in 70% of the samples. Helicotylenchus spp. and Paratylenchus spp. occurred in quite high populations in a number of samples taken from buffalograss. Rotylenchus spp. also reached a rather high population in one instance on buffalograss. The peak populations of the genera Helicotylenchus, Rotylenchus and Paratylenchus were obtained from a prairie dog town where a practically pure stand of buffalograss was highly disturbed by the prairie dog activity. Samples obtained from the rhizosphere of buffalograss in an abandoned prairie dog town yielded much lower populations. Other genera of plant parasitic nematodes recovered in this survey were Criconemoides, Hoplolaimus, Pratylenchus, Trichodorus, Heterodera, and Longidorus.

The incidence of all plant parasitic nematodes recovered from all areas taken together is presented in Table IV (page 14). The most commonly recovered genera were Tylenchus and Helicotylenchus. Both genera are represented in samples taken from the rhizosphere of all but one species of grass each. Tylencholiamellus, Tylenchorhynchus, Paratylenchus, Xiphinema, and Neotylenchus were also recovered from many samples. The relatively high frequency of recovery of Neotylenchus and Tylencholiamellus is quite interesting since the biology and parasitic

TABLE IV

SUMMARY OF PLANT PARASITIC NEMATODES RECOVERED FROM THE RHIZOSPHERE OF SELECTED RANGE GRASSES

Nematode Genus	Grass											
	Buffalo grass	OWB "S"	OWB "T"	Big Bluestem	Little Bluestem	Smooth Brome	Side-oats grama	Switchgrass	Love grass	Indian grass	Blue grama	All grasses*
Tylenchus	81	100	75	100	50	100	79	63	0	73	82	74
Helicotylenchus	89	100	0	100	50	50	100	72	88	60	43	71
Tylencholiamellus	83	0	25	40	71	100	42	27	0	60	27	44
Tylenchorhynchus	27	100	50	60	42	0	32	37	50	13	36	33
Paratylenchus	61	100	25	10	26	50	10	23	0	46	27	31
Xiphinema	28	0	50	100	21	0	26	60	38	40	0	31
Neotylenchus	55	0	0	40	42	0	32	23	0	46	27	30
Pratylenchus	5	0	0	0	0	100	5	23	100	0	0	13
Criconemoides	5	0	0	0	21	0	37	4	0	13	0	12
Rotylenchus	28	0	0	0	7	0	10	9	0	0	27	8
Trichodorus	0	0	0	0	14	0	0	14	38	27	0	7
Heterodera	11	0	0	0	0	0	10	0	12	0	0	4
Hoplolaimus	11	0	0	0	0	0	0	4	0	0	9	3
Longidorus	11	0	0	0	14	0	0	0	0	0	0	3
Meloidogyne	0	0	0	0	0	0	0	0	12	0	0	1
Nothotylenchus	0	0	0	0	0	0	0	4	0	0	0	1

Numbers to the right of the nematode genera indicate the percentage of samples from which that genus was recovered.

*Average incidence based on all samples taken. The apparent discrepancy in average is due to unequal sample numbers for the grasses.

habits of both genera, and even the families to which these genera belong, are poorly understood.

The large populations of *H. digonicus* and *C. beljaevae* found associated with the sideoats grama and the low productivity of this grass strongly suggested pathogenicity of the nematodes to sideoats grama.

CHAPTER IV

PATHOGENICITY TRIALS

Materials and Methods

Populations of *Helicotylenchus digonicus* and *Criconemoides beljaevae* from the El Reno station were tested to determine their pathogenicity to sideoats grama. Pure populations, reared on sideoats grama, were used in both trials.

Prior to setting up the experiments, the growth room to be used was tested for uniformity. Forty milliliters of water was poured into 50 ml beakers which were placed in various locations about the room in order to measure rates of evaporation. The beakers were allowed to remain for 24 hours and the water remaining in them was measured. This procedure was repeated 4 times. Two areas of uniformity were noted. Air temperature and light intensity measurements were then taken in various locations about these two areas and it was determined that wide variation did not exist within them. All trials were conducted in one or the other of these two areas. The growth room was set for 14 hour 25 C days and 10 hour 20 C nights. Light intensity at plant level was approximately 450 foot candles.

In the *H. digonicus* trial 24 glazed crocks were filled with 1300 cc of methyl bromide treated Lincoln fine sand. A 25 ml vial was inserted into the center of the soil to a depth of 6 cm below the soil level in order to retain an inoculation port in the root zone for later use. Approximately 25 seeds of sideoats grama planted within 15 cm of the center of the crock. The first nine seedlings to appear were allowed to grow for one month. All other seedlings were removed from the crocks as they emerged. At the end of one month the two largest and the two smallest plants were removed from each crock. The four crocks exhibiting the best growth and the four crocks exhibiting the poorest growth were then removed from the trail in order to reduce variability.

Inoculum was extracted from the stock colony via the Seinhorst ellutriation technique. Initial inoculum counts were made by aliquot. After an estimate of the total number of nematodes was obtained, a determination was made of the volume of a water suspension of the inoculum required for each inoculum level. Inoculum for each crock was measured and placed in a test tube. The total volume of the suspension in all test tubes was brought to 20 ml and the test tubes were stoppered.

Inoculations were made after the final selection of the crocks to be used in the trial. Crocks were watered and the vials were removed from the inoculation ports. The inoculum was poured from the test tubes into the inoculation ports, the test tubes were rinsed with 10 ml of water which was also poured into the inoculation port, and the port was filled with Lincoln fine sand. The crocks were then placed in a completely randomized design. Four replications were used.

The C. beljaevae trial was similar in design to the H. diagonicus trial; however, due to space limitations, smaller 850 ml plastic containers were substituted for the glazed crocks.

After 120 days, the containers were removed from the growth rooms. The containers were inverted and the contents tapped out into a plastic bucket. The plants were removed from the soil and all soil adhering to the roots was removed by gentle washing. The soil was thoroughly mixed by hand, a 100 cc aliquot of soil was withdrawn, and nematodes were extracted from the soil by the modified Christie-Perry technique described earlier. The plants were oven dried for 72 hours at 80 C and weighed.

Results and Discussion

Since the results of both the *H. digonicus* trial and the *C. beljaevae* trial were quite similar, both trials will be discussed together. The oven dried plant weights and final nematode populations and the results of a Duncan's Multiple Range Test performed on the final plant weights are presented in Table V (page 19).

Plant growth as indicated by dry weights was significantly greater at the zero population level. None of the final weights for the other population levels could be separated statistically in either trial.

It may be noted that in the *C. beljaevae* trial the final nematode populations seem to indicate a reduction in population at the 20,000 inoculum level. This is considered to be an aberration that may be attributable in part to initial inoculum mortality.

There were no observable differences in the tops of the plants in either trial. The roots of the plants for the 500, 1000, and 20,000 inoculum levels exhibited marked differences at the end of the trial (Figure 1). In general, a lack of lateral branching and a reduction in root mass was observed. Most of the root growth occurred around

TABLE V

PATHOGENICITY OF *H. digonicus* AND *C. beljaevae* ON SIDEOATS GRAMA

Nematode	Inoculation Level	Final Population	Total Plant Weight (grams)
H. digonicus	0	0	4.2 a
	500	48,100	2.8 b
	1000	72,800	1.6 b
	20,000	59,800	2.2 b
C. beljaevae	0	0	5.6 a'
	500	4267	2.9 b'
	1000	5338	3.3 b'
	20,000	8570	2.2 b'

Plant weights and final populations are the mean of four replications. Plant weights which do not have a common letter to the right are significantly different at the .01.

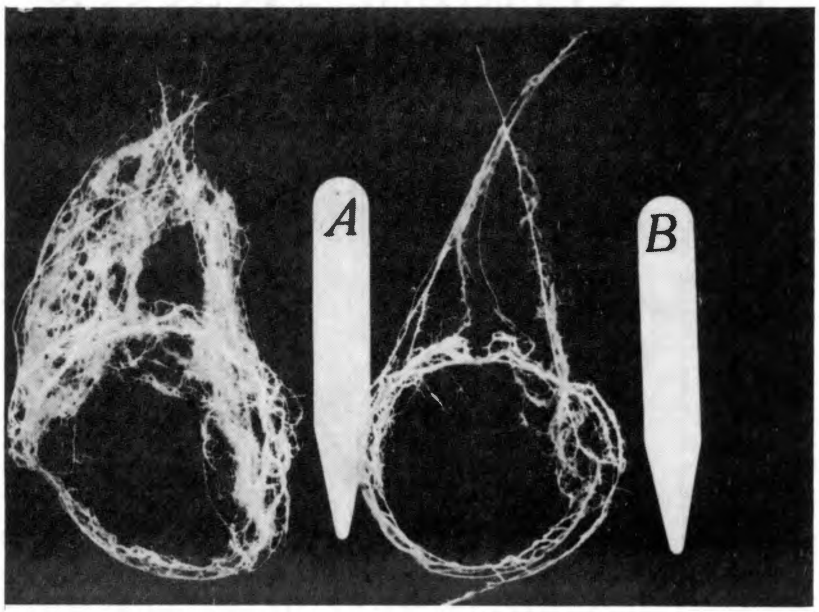

Figure 1. Effect of C. beljaevae on the roots of sideoats grama. A = control, B = 20,000 inoculum level

the periphery of the containers at the 500, 100, and 20,000 inoculum levels. Presumably, this pattern of growth was largely due to the unfavorable conditions for the nematodes created by the rapidly fluctuating temperatures near the outside of the containers.

Although there was little inhibition of top growth in these trials, similarly parasitized plants might suffer more severe grazing damage, and drought stress would likely be more acute.

Due to the use of different types of containers in each of the two trials, no attempt will be made to determine the comparative pathogenicity of the two nematodes, however, pathogenicity was demonstrated for both species at population levels approximating those found in the field.

CHAPTER V

FIELD TRIALS

Materials and Methods

All 11 year old stand of sideoats grama (Bouteloa curtipendula 'Coronado') was selected for study. The stand had previously been used to study the effects of soil fertility and management upon seed yield. A blanket fertility treatment consisting of 55 kg of nitrogen and 35 kg of P_2O_5 per ha had been applied for each of the previous three years. Soil analysis indicated adequate nutrient levels with a soil pH of 7.4. The study area consisted of nine rows which had been established on 1 m centers. The inter-row areas were clean cultivated.

Plots were established and treatments were applied on the even numbered rows in a randomized complete block design. Plots were 6 m long with 1.5 m buffers between all plots and at the end of each row.

Soil samples were taken to a depth of 22.5 cm in each plot. Subsamples were taken every 60 cm within each plot. Subsamples for each plot were consolidated, thoroughly hand mixed, and a single 100 cc aliquot of this soil was withdrawn for nematode extraction. Extraction was by the modified Christie-Perry technique described earlier. All soil samples were allowed to soak for one hour prior to processing. Samples of root tissue were taken from selected plots for fungal isolations. Samples of root tissue were taken from all plots for the extraction of endoparasitic nematodes in each of the first three samplings.

Treatments consisted of four nematicides, a soil fungicide, and a fungicide-nematicide combination. Nematicides were carbofuran [2,3dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate], oxamyl [methyl N',N'-Dimethyl-N-[(Methylcarbamoyl)oxy]-1-thioxamimidate], ethroprop [O-Ethyl S,S-Dipropyl Phosphorodithioate], and DBCP [1,2-Dibromo-3-Chloropropane]. The fungicide was PCNB [Pentachloronitrobenzene]. Carbofuran, oxamyl, and ethroprop were a 10% granular form, PCNB a 15% granular form, and DBCP an 84.21% emulsifiable concentrate. The granular materials were applied with a Gandy 901 Jr. granule applicator. The DBCP was applied via a small hand injection apparatus at 30 cm spacings to a depth of 22.5 cm. Application rates and dates of application are presented in Table VI. Chemical applications were continued 30 cm into the buffer zone at the end of each plot. The first chemical application was made on May 9. The second application was made on July 19.

Forage was harvested with a tractor-mounted flail mower. Total green weight per plot was recorded. A subsample was immediately withdrawn from each plot sample and weighed. The subsample was later oven-dried and reweighed. Dry weight percentages derived for each subsample were used in calculating the dry weight per plot. Seed harvesting was performed by removing the seed heads from each plot with a hand sickle. The seeds were mechanically cleaned and weighed. The pure seed content was determined for each plot by the method described by Harlan and Ahring (12). The first harvest was performed on July 9, 1973. The second harvest, which did not include a seed harvest, was performed on November 12, 1973.

TABLE VI

RATES OF CHEMICAL APPLICATION

Chemical	May 9 Application	July 19 Application
Carbofuran	5.4	7.3
Oxamyl	5.4	7.3
Ethroprop	7.3	7.3
PCNB	9.1	11.0
DBCP	65.5 L/ha	None

All rates except the DBCP rates are given as kg active ingredient/ha.

Results and Discussion

Helminthosporium, *Fusarium*, *Pythium*, *Curvularia*, and *Rhizoctonia* were among the fungal genera isolated. All of these genera contain known plant pathogenic species. Plant parasitic nematode genera recovered were *Helicotylenchus*, *Criconemoides*, *Pratylenchus*, *Paratylenchus*, *Tylenchorhychus*, *Tylenchus*, *Tylencholiamellus*, and *Xiphinema*. *Helicotylenchus digonicus* and *Criconemoides beljaevae* were the two most common species. Helicotylenchus digonicus far outnumbered all other plant parasitic species on all plots.

Average population levels of H. digonicus over the course of the growing season are presented in Table VII (page 26). A steady decline in the population levels until the final sampling may be noted on the control plots. This was probably attributable to rapid fluctuations in soil temperature and moisture throughout the summer months.

Although Carbofuran did achieve an initial population reduction of approximately 90%, the level of control after the first application was somewhat disappointing for all other nematicides. The other nematicides achieved approximately equal levels of control. It should be noted, however, that all of the granular materials were applied topically and were exposed for two weeks to extremely warm sunny days preceeded by heavy morning dews before the materials were finally watered in.

Yield data are presented in Table VIII (page 27). Poor late season growth is characteristic of sideoats grama. Even though great increases over the control plot were noted in the fall forage harvest and in the seed harvest, no statistical significance could be assigned to them due to variation within treatments. Increases in both forage and seed yield were realized with PCNB and Carbofuran, but the greatest increases in yield were obtained with the PCNB-Carbofuran combination. The results of this trial clearly indicate that both nematodes and fungi constitute significant factors in sideoats grama seed and forage production and their control results in greater yields.

TABLE VII

POPULATION LEVELS OF *H. digonicus*

Treatment	Sampling Dates				
	May 9	May 29	July 9	August 7	November 12
Carbofuran + PCNB	394 a	38 a	13 a	12 a	41 a
Carbofuran	455 a	49 a	118 b	34 a	53 a
PCNB	205 a	263 c	121 b	96 b	164 ab
Oxamyl	713 a	173 bc	204 bc	92 b	188 b
Ethroprop	378 a	141 b	133 b	92 b	95 ab
DBCP	508 a	110 b	129 b	80 b	103 ab
Control	780 a	503 d	288 c	202 c	368 c

Each number is an average of 4 replicates. Means in each column followed by a common letter are significantly different at the .05 level from means not followed by that letter. No sifnificant differences could be found for May 9.

TABLE VIII

FORAGE AND SEED YIELDS FROM SIDEOATS GRAMA CHEMICAL TRIALS

Treatment	First Forage Harvest		Second Forage Harvest		Total Forage Harvest		Seed Harvest	
	Yield kg/ha	% of check	Yield kg/ha	% of check	Yield kg/ha	% of check	Yield kg/ha	% of check
Carbofuran + PCNB	9464 a	129	3187 a	160	12,652 a	137	38.72 a	139
Carbofuran	8607 a	118	2530 a	127	11,089 abc	120	28.24 a	102
PCNB	9424 a	129	2172 a	109	11,636 ab	125	32.98 a	119
Oxamyl	8089 a	111	1992 a	100	10,081 bc	109	30.77 a	111
DBCP	8727 a	120	1657 a	83	10,421 bc	113	38.91 a	104
Ethroprop	7890 a	108	1853 a	93	9,763 c	106	27.40 a	99
Check	7312 a	100	1992 a	100	9,245 c	100	27.67 a	100

All yield figures are an average of four replicates. Means in the column for total forage harvest followed by a common letter are significantly different at the .05 level from means not followed by that letter. No significant differences could be found for any of the other harvests.

CHAPTER VI

HOST SUITABILITY TRIALS

Materials and Methods

Fifteen plant species were tested in two separate studies to determine their suitability as hosts for the El Reno Station population of *Helicotylenchus digonicus*. The species selected for the first study were: 'Coronado' sideoats grama, 'Southland' smooth bromegrass, bermudagrass (*Cynodon dactylon* 'Midland'), 'Caddo' switchgrass, "T" strain bluestem, and 'Texoka' buffalograss. The species used in the second study were: creeping bentgrass (*Agrostis palustris* 'Seaside'), 'Kaw' big bluestem, 'Capitan' blue grama, indiangrass, 'Alduous' little bluestem, 'Ermelo' weeping lovegrass, rye (*Secale cereale* 'Elbon'), wheat (*Triticum aestivum* 'Danne'), western yarrow (compositae: *Achillea lanulosa*), and sideoats grama. Varietal selections were used wherever possible in order to increase uniformity. Crocks were prepared for planting in the same manner as those used in the pathogenicity trials described in Chapter IV. Six crocks were prepared for each species. Twenty-five seeds of each species except for buffalograss, bermudagrass, and creeping bentgrass were planted in each crock. Bermudagrass was vegetatively propagated by allowing stolons from a stock source to root at the nodes in the crocks to be used for the trial. The first 10 seedlings to emerge in each crock were allowed to grow in all crocks except for bermudagrass and creeping bentgrass. Due to the extremely fine nature of the creeping bentgrass plants, the first 20 plants were allowed to grow. All other seedlings were removed from the crocks upon emergence. Plants were allowed to grow until all crocks became root bound. The four crocks of each species exhibiting the most uniforn plant growth were then selected for use in the trial. Crocks were inoculated with 700 nematodes in the same manner as for the pathogenicity trials. The trials were maintained for 90 days in the growth room at the same settings used in the pathogenicity trials. At the end of this period the contents of each crock were removed and the soil and root mass cut in half. The soil was gently shaken from the roots and hand mixed. Nematodes were extracted from a 100 cc aliquot. The tops were removed from all plants, the roots were thoroughly washed, oven dried, and weighed.

Results and Discussion

The trials will be considered separately since the space limitations of the growth room precluded the conduct of both studies simultaneously. The results of the first trial are shown in Table IX. Root mass variations between species were analyzed and it was determined that this was not an important source of variation in nematode counts. Root mass was therefore disregarded in the subsequent analyses. Since no reproduction was noted in any of the replications for switchgrass, it was removed from further analysis.

Analysis of variance for nematodes per 100 cc of soil indicated that buffalograss was a significantly better host than any other grass species included in this trial. Analysis using the logarithms of the number of nematodes per 100 cc of soil indicated that buffalograss and sideoats grama were better hosts than all other hosts, but did not distinguish between the two.

TABLE IX

SUITABILITY OF SELECTED CANDIDATE HOSTS FOR *H. digonicus*: STUDY ONE

Candidate Host	Nematodes/ 100 cc of soil	Log Nematodes/ 100 cc of soil
Switchgrass	0	--
"T" strain bluestem	65 a	1.0540 a
Smooth bromegrass	104 a	1.5600 a
Bermudagrass	143 a	2.0080 a
Sideoats grama	1716 a	3.1988 b
Buffalograss	4673 b	3.6593 b

All figures are the mean of four replications. Numbers in each column which do not bear a common letter to the right are significantly different. Confidence is to the .01.

The high rate of reproduction noted for buffalograss appears to reflect field conditions since large populations were recovered from the rhizosphere of buffalograss in the Wichita Mountains Wildlife Refuge and at the El Reno Station.

The suitability of sideoats grama as a host is verified at the El Reno station, but is not borne out elsewhere. It must be noted, however, that only very small isolated clumps of sideoats grama were found at other sampling sites.

Large populations of _H. digonicus_ were not found in the rhizosphere of other grasses in the field.

The results of the second study are presented in Table X. Variability of nematode counts due to interspecific variation of root mass was determined to be an unimportant source of variation in this study. Root weights were not considered in further statistical analysis of this trial.

TABLE X

SUITABILITY OF SELECTED CANDIDATE HOSTS FOR _H. digonicus_: STUDY TWO

Candidate Host	Nematodes/ 100 cc of soil	Log Nematodes/ 100 cc of soil
Big bluestem	1 a	.1747 a
Western yarrow	1 a	.1747 a
Creeping bentgrass	5 a	.4823 a
Weeping lovegrass	11 a	.5779 a
Little bluestem	17 a	.9049 a
Indiangrass	22 a	.9070 a
Wheat	41 a	1.6129 a
Rye	77 a	1.7436 a
Blue grama	110 a	2.0329 a
Sideoats grama	328 b	2.4333 a

All figures are the mean of four replications. Numbers which do not bear a common letter to the right are significantly different. Confidence is to the .01.

The apparent low degree of suitability indicated for creeping bentgrass in this trial is suspect since extremely high populations of Helicotylenchus spp. have been recovered from bentgrass turf in many parts of the state. The species of Helicotylenchus found in the rhizosphere of these greens is not known.

The low populations recovered from indiangrass, weeping lovegrass, and little bluestem appear to be in line with the situations noted in the field surveys presented earlier.

CHAPTER VII

HOST ATTRACTIVENESS

Materials and Methods

Plastic pots were fashioned from 30 cc individual plastic condiment serving cups by melting drain holes in the bottom of them. The pots were filled with silica sand. Ten seeds were planted in each pot and allowed to germinate and grow for two to four weeks.
A 310 mm X 45 mm section of channeling was filled with 600 cc of silica sand, a 25 ml vial (Figure 2C) was placed in the center of the channeling to a depth of 2.5 cm below the soil surface. The pots (Figure 2A) were placed on the surface of the sand at opposite ends of the channeling. Ninety milliliters of distilled water was dripped from each of two columns (Figure 2B) simultaneously onto the surface of both pots containing the plants to be tested. The water was allowed to percolate through the pot and into the sand in the channeling through the drain holes in the bottom of the pots. The water was allowed to diffuse through the channeling until a uniform sand moisture content was established. The vial in the center of the channeling was then removed and 500 nematodes in 10 ml of distilled water were pipetted into the hole. Care was taken to avoid slanting the pipette to one side or the other so the nematodes were not washed toward one pot or the other. The holes were filled with dry silica

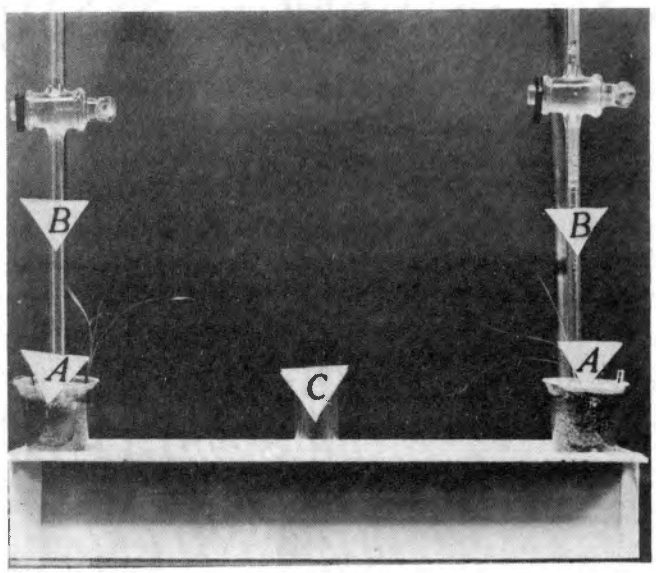

Figure 2. Host Attractiveness Testing Apparatus
A = pots with candidate hosts
B = columns containing water
C = vial

sand. The sections of channeling were covered with plastic film and placed under an inverted dishpan for a period of 48 hours. At the end of this period the pots were removed from the ends of the trays and the plants were removed from the pots. The plants were oven dried and weighed. Extraction tubs were prepared as described for the modified Christie-Perry technique discussed earlier. The pot contents were placed on the surface of the facial tissue and water was added to the tub until the sand was inundated. The sand was then removed from the channeling in 2.5 cm increments. Each increment was processed in the same manner as the pot contents. After 48 hours the nematodes recovered from each 2.5 cm segment and from the pots were counted. The nematodes recovered from the increments immediately adjacent to the inoculation point were not considered in the analysis.

Results and Discussion

The results are presented in Table XI. Of the grasses tested, sideoats grama was the most attractive. Little bluestem, switchgrass, and indiangrass appeared to be the least attractive; however, in the entire trial, only four pairings demonstrated a strong differential preference.

The nematodes appeared to be more active when a plant was used in the trial, even if it was an unsuitable host. In the empty pot trials only an average of five nematodes responded in each replication while in the sideoats grama X empty pot and switchgrass X empty pot trials, an average of 143 nametodes and 165 nematodes, respectively, responded in each replication. Some host pairings also seemed to be accompanied by a lower level of response.

TABLE XI

HOST PREFERENCES EXPRESSED BY THE EL RENO POPULATION OF *H. digonicus*

Candidate Host	# Nematodes Responding	X	Candidate Host	# Nematodes Responding
Sideoats grama	93	x	Sideoats grama	124
Sideoats grama	129	x	Empty pot	0 **
Sideoats grama	33	x	Indian grass	9
Sideoats grama	63	x	Bentgrass	22
Sideoats grama	69	x	Little bluestem	24 *
Sideoats grama	51	x	Buffalo grass	45
Sideoats grama	122	x	Switchgrass	22 **
Switchgrass	36	x	Buffalo grass	53
Switchgrass	37	x	Little bluestem	24
Switchgrass	32	x	Indian grass	24
Switchgrass	94	x	Switchgrass	75
Switchgrass	129	x	Empty pot	36 **
Empty pot	4	x	Empty pot	1

Figures presented are the average of four replications.

**Significant at the .05.

*Significant at the .10.

There appears to be some correlation with the host suitability trials reported earlier, however, it does not appear to be a strong one. Sideoats grama is a more suitable host than switchgrass and is more attractive; however, buffalograss is also more suitable than switchgrass, but is not more attractive. While the differences in attractiveness may account for some of the differences in incidence of *H. digonicus* noted in native prairie situations, it is unlikely that it is responsible for the apparent poor suitability noted for some hosts in pure plantings. It, therefore, appears that differential response to root diffusates may play a role in host selection under conditions of mixed plant populations, but it is not a controlling factor and could not function as the only resistance mechanism in monoculture.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Areas in which improved pasture grass stands failed to respond to fertilizer as anticipated were observed at the Agronomy Field Research Station, El Reno. Plant pathogenic fungi and large populations of plant parasitic nematodes were recovered from these areas. The highest populations of plant parasitic nematodes were recovered from sideoats grama. The two species most commonly recovered from the rhizosphere of the sideoats grama were *Helicotylenchus digonicus* and *Criconemoides beljaevae*.

Determination of the occurrence and distribution of plant parasitic nematodes in selected areas within the state was undertaken. Several genera of plant parasitic nematodes were recovered from each area sampled. These nematodes often occurred in quite high numbers, even where the range was in a native or near native condition.

Pathogenicity to sideoats grama was demonstrated for both *H. digonicus* and *C. beljaevae* in a controlled environment. Chemical control of plant parasitic nematodes and plant pathogenic fungi on field plots of sideoats grama resulted in an increase in harvestable herbage of 37% for the entire growing season and 60% for the last part of the growing season, a critical period in most grazing schemes. Seed yield increases of 39% were also realized with this treatment.

Differences in the suitability of certain hosts for _H_. _digonicus_ were demonstrated in a controlled environment.

Differences in attractiveness of certain hosts were found. Many of these differences coincided with the field incidence and host suitability trial data.

SELECTED REFERENCES

1. ANON. 1974. Nematodes on range. Agribusiness Fieldman 3:13

2. BELL, A. A. and L. R. KRUSBERG. 1964. Occurrence and control of a nematode of the genus Hypsoperine on zoysia and bermudagrass in Maryland. Plant Dis. Rep. 48:721-722

3. BENEDICT, W. G. 1956. The effect of certain factors on forage crop failures in southern Ontario. Plant Dis. Rep. 40:564-569

4. BOYD, F. T. and V. G. PERRY. 1970. The effect of sting nematodes on establishment, yield, and growth of forage grasses on Florida sand soils. Proc. Soil and Crop Sci. Soc. Fla. 30:288-300

5. BOYD, F. T. and V. G. PERRY. 1971. Effects of seasonal temperatures and certain cultural treatments on sting nematodes on forage grasses. Proc. Soil and Crop Sci. Soc. Fla. 30:360-365

6. BOYD, F. T., V. N. SCHRODER, and V. G. PERRY. 1971. Interaction of nematodes and soil temperature on growth of three tropical grasses. Agron. J. 64:497-500.

7. BURTON, G. W., C. W. McBETH, and J. S. STEPHENS. 1945. The growth of Kobe lespedeza as influenced by the root knot nematode resistance of the bermudagrass strain with which it is associated. Agron. J. 38:651-656

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

9. COURSEN, B. W., and W. R. JENKINS. 1958. Host parasite relationships of the pin nematode, Paratylenchus projectus on tobacco and tall fescue. Plant Dis. Rep. 42:865-872

10. DiEDWARDO, A. A., and V. G. PERRY. 1963. An undescribed species of Heterodera pathogenic to St. Augustine grass in Florida. Phytopathology. 53:874

11. GRIFFIN, G. D. 1969. Attractiveness of resistant and susceptible alfalfa to stem and root knot nematodes. Jour. of Nematol. 1:9

12. HARLAN, J. R., and R. M. AHRING. 1960. A suggested method for determining the purity of certain chaffy-seeded grasses. Agron. J. 52:223-226

13. JATALA, P. H., H. J. JENSEN, and R. A. SHIMABUKUVA. 1973. Host range of the "Grass Root-Gall Nematode" Ditylenchus radicicola and its distribution in the Williamette Valley. Plant Dis. Rep. 57:1021-1023

14. JENKINS, W. R., D. P. TAYLOR, and R. A. ROHDE. 1956. Nematodes associated with cover, pasture, and forage crops in Maryland. Plant Dis. Rep. 40:184-186

15. JENSEN, H. J. 1953. Experimental greenhouse host range studies of two root-lesion nematodes, Pratylenchus vulnus and Pratylenchus penetrans. Plant Dis. Rep. 37:384-386

16. JOHNSON, A. W. 1969. Pathogenicity and interaction of three nematode species on six bermudagrasses. J. of Nematol. 2:36-38

17. JOHNSON, A. W. 1969. Control of the lance nematode Hoplolaimus galeatus on Tifdwarf bermudagrass. J. of Nematol. 2:179-181

18. JOHNSON, A. W., and W. M. POWELL. 1968. Pathogenic capabilities of a ring nematode Criconemoides lobatum on various turf grasses. Plant Dis. Rep. 52:109-113

19. LINFORD, M. B. 1939. Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helminth. Soc. Wash. 6:11-18

20. McBETH, C. W. 1935. Tests on the susceptability and resistance of several southern grasses to the root knot nematode Heterodera marioni. Proc. Helminth. Soc. Wash. 12:41-44

21. NORTON, D. C. 1959. Relationship of nematodes to small grains and native grasses in north and central Texas. Plant Dis. Rep. 43:227-235

22. ORR, C. C. 1965. Nematodes in native prairie soils of Kansas and the plants with which they are associated. Dissertation (PhD) Kansas State University

23. PERRY, V. G. 1958. A disease of Kentucky bluegrass incited by certain spiral nematodes. Phytopathology 48:397

24. PERRY, V. G., H. M. DARLING, and G. THORNE. 1959. Anatomy, taxonomy, and control of certain spiral nematodes attacking bluegrass in Wisconsin. Bull. Wis. Agric. Exp. Sta. 207:1-24.

25. POTTER, J. W., J. L. TOWNSHEND, and T. R. DAVIDSON. 1969. Wild and cultivated grass hosts of the southern root-knot nematode, Meliodogyne incognita. Nematologica 15:29-34

26. RIGGS, R. D., J. L. DALE, and M. L. HAMBLEN. 1962. Reaction of bermudagrass varieties and lines to root-knot nematode. Phytopathology 52:587-588

27. RHOADES, H. L. 1962. Effects of sting and stubby root nematode on St. Augustine grass. Plant Dis. Rep. 46:424-427

28. RHOADES, H. L. 1965. Parasitism and pathogenicity of Trichodorus proximus to St. Augustine grass. Plant Dis. Rep. 49:259-262

29. SCHMITT, D. P., and D. C. NORTON. 1972. Relationships of plant parasitic nematodes to sites in native Iowa prairies. J. of Nematol. 4:200-206

30. SECHLER, D. T., W. B. TAPPAN, and H. H. LUKE. 1967. Effect of variety and fumigation on nematode populations in oats, wheat, and rye. Plant Dis. Rep. 51:915-919

31. SHER, S. A. 1966. Revision of the Hoplolaiminae (Nematoda) VI. Helicotylenchus Steiner 1945. Nematologica 12:1-56.

32. SIDDIQI, MOHAMMED RAFIQ. 1972. On the genus Helicotylenchus (Nematoda:Tylenchida), with descriptions of nine new species. Nematologica 18:74-91

33. SLEDGE, E. B. 1962. Preliminary report on a Meloidogyne sp. parasite of grass in Florida. Plant Dis. Rep. 46:52-54

34. TARJAN, A. C. 1966. A compindium of the genus Criconemoides (Criconematidae:Nemata). Proc. Helminth. Soc. Wash. 33:109-125

35. THORNE, G. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8:261p

36. VIGLIERCHIO, D. R. 1961. Attraction of parasitic nematodes by plant root emanation. Phytopathology 51:136-142

37. WEISCHER, B. 1959. Experimentelle untersuchungen uber die wanderung vun nematoden. Nematologica 4:172-186

38. WEISER, W. 1956. The attractiveness of plants to larvae of the root knot nematodes II. The effect of excised bean, eggplant, and soybean roots on Meloidogyne hapla Chitwood. Proc. Helminth. Soc. Wash. 23:59-64

39. WINCHESTER, J. A., and E. O. BURT. 1964. The effect and control of sting nematodes on Ormand bermuda grass. Plant Dis. Rep. 48:625-628

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Master of Science

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