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TYLENCHORHYNCHUS NUDUS AND OTHER NEMATODES ASSOCIATED WITH TURF IN SOUTH DAKOTA

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BY

JAMES D. SMOLIK

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Plant Pathology, South Dakota
State University

1969

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TYLENCHORHYNCHUS NUDUS AND OTHER NEMATODES ASSOCIATED WITH TURF IN SOUTH DAKOTA

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser   Date

Head, Plant Pathology Dept.   Date

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INTRODUCTION

In 1963, it was estimated that the cost of maintaining turf grass in the Midwestern United States was 300 to 500 million dollars per year (51). With the exception of the work of Perry (34) in Wisconsin, the role of plant parasitic nematodes in relation to turf decline in the Midwestern United States has received little study. The decline of Kentucky bluegrass (Poa pratensis L.) turf in South Dakota is not uncommon during hot, dry periods of the summer months. Nematode injury in South Dakota may also affect yield in Kentucky bluegrass seed-producing areas. Nematode damage might also be assumed to increase maintainence costs of home-owner lawns and golf courses due to increased water and fertilizer applications necessary to maintain vigorous turf.

A stylet nematode, Tylenchorhynchus nudus (Allen) has been frequently associated with Kentucky bluegrass turf in northeastern South Dakota, but its effects on turf have not been established. Stylet nematodes feed ectoparasitically and obvious damage to root systems is usually not evident, although plant growth may be reduced.

Objectives of the present study were to determine the distribution of Tylenchorhynchus nudus in South Dakota, to investigate reproductive ability of T. nudus on certain grasses and to determine effects of T. nudus on growth of Kentucky bluegrass under greenhouse conditions. Influence of temperature on the reproduction and gross morphology of T. nudus was also studied. In addition, field experiments were conducted to determine the effect of nematicide treatments on the growth of turf grass and the ability of the nematicides to control nematodes in turf.

REVIEW OF LITERATURE

Kelsheimer and Overmann (19) in 1953 were among the first to attribute turf decline to plant parasitic nematodes. In 1954, Troll and Tarjan (49) noted high populations of Tylenchorhynchus spp. in the area of declining golf greens. The following year Tarjan and Cheo (42) associated Tylenchorhynchus spp. with a yellow-tuft symptom in golf greens. Good et al. (9) in 1956 found high populations of Belonolaimus sp. and Hoplolaimus sp. associated with declining turf in Florida and Georgia. A survey of Georgia turf nurseries by Good et al. (10) in 1959 revealed high numbers of Xiphinema sp. and Trichodorus sp. Perry et al. (34) in 1959 demonstrated pathogenicity of Helicotylenchus digonicus to Kentucky bluegrass in Wisconsin. In 1962, Rhodes (38) found Belonolaimus longicaudatus and Trichodorus christei to be important in the decline of St. Augustine grass.

A survey of Illinois golf greens by Taylor et al. (45) in 1963 indicated Helicotylenchus and Tylenchorhynchus were the most common genera of plant parasitic nematodes present. In 1964, Powell (36) associated Tylenchorhynchus maximus with declining turf in Georgia. Greenhouse and field tests conducted by Winchester and Burt (52) demonstrated the pathogenicity of Belonolaimus longicaudatus to Ormund Bermuda grass. Radewald et al. (37) in 1966 found Meloidogyne naasi able to parasitize the roots of bluegrass, bentgrass and 23 other Graminae species. Orr and Golden (30) associated Hypsoperine graminis with poor stands of turf in Texas. The same nematode was shown by Heald (13) to be pathogenic to Tifdwarf Bermuda grass. Johnson and Powell (17) in 1968 found Criconemoides lobatum capable of reproduction on several species of grasses, but were unable to demonstrate pathogenicity.

The effect of nematodes on grasses used as forage or for seed production has also been investigated. Courtney and Howell (6) in 1952 found Anguina agrostis to be an important factor in the reduction of bentgrass seed yield. Greenhouse experiments conducted by Havertz (12) demonstrated the pathogenicity of Tylenchorhynchus cylindricus to crested wheat grass. In 1958, Coursen and Jenkins (5) found high populations of Paratylenchus projectus associated with a stunting of tall fescue. Several strains of bluegrass were found to be susceptible to Meloidogyne incognita var. acrita by Gaskin (8). In 1965, Franklin (7) found Meloidogyne naasi capable of galling the roots of several genera of grasses in England and

Wales. Hodges and Taylor (15) found a Meloidogyne sp. able to heavily gall the roots of bentgrass but did not obtain severe above ground symptoms.

The effects of ectoparasitic nematodes on wheat has also received some study. Norton (29) in 1959 found Tylenchorhynchus brevidens and T. acutus to be commonly associated with wheat in Texas. In 1961, Langden et al. (21) demonstrated a stunting of small grains by T. brevidens. Schlehuber et al. (40) in 1965 found T. brevidens capable of seriously reducing wheat yields in Oklahoma. T. brevidens has also been associated with wheat in Nebraska by Kerr (20) in 1967.

Allen (1) in 1955 published a review of the genus Tylenchorhynchus (Cobb) describing Tylenchorhynchus nudus among other new species. T. nudus was first reported from Canada and in 1958 Taylor et al. (42) found the nematode in Minnesota. T. nudus has since been reported from Ceylon (22), West Pakistan (47), Wisconsin, North Dakota, Iowa, and South Dakota (32, 34, 43, 46). The nematode has most commonly been associated with grasses, cereal crops and legumes in North America.

Temperature is an important environmental factor in the development of plant parasitic nematodes. Wallace (50) in 1963 reviewed the literature concerned with the effects of temperature on plant parasitic nematodes. He concludes that the optimum temperature for the development of most plant parasitic nematodes lies between 15° and 30°C. Most of the temperatures listed by Wallace are for either endoparasitic or cyst nematodes. Subsequent papers dealing with ectoparasitic forms include that of Lownsberry and Maggnetti (23) who obtained the highest populations of Xiphinema americanum at 21°C. Malek and Jenkins (26) report 25°C. as the optimum temperature for reproduction of Criconemoides curvatum and Trichodorus cristiei. The optimum temperature for egg production of Hemicycliophora arenaria is 24°C., according to McElroy and Van Gundy (28). Patel and MacDonald (31) obtained the greatest numbers of Tylenchorhynchus martini at 30°C. Young and Struble (55) found 27°C. the optimum temperature for increase of Trichodorus cristiei on wheat.

The effect of temperature on nematode morphology has received very little work. Rhode and Jenkins (39) in 1957 found Trichodorus cristiei maintained at 22°C. were larger than those held at 30°C. Malek and Jenkins (26) also found T. cristiei to be larger at lower temperatures, but they were unable to establish any correlation between temperature and size for Criconemoides curvatum. Gysels (11) has found Panagrellus silusiae to decrease in length with increasing temperature.

A number of attempts have been made to control nematodes in turf. In 1955, Tarjan and Cheo (42) found Heptachlor and VC-13 to significantly reduce nematode populations in bentgrass turf. Injections of VC-13 gave good nematode control in golf greens, according to Manzelli (27). Perry et al. (34) found VC-13 superior to Nemagon in control of nematodes in Kentucky bluegrass plots. Control of Anguina agrostis was achieved by Apt et al. (2) by application of a herbicide which depressed heading of the bentgrass host for one year. Madison (25) has found Nemagon to significantly reduce the incidence of Rhizoctonia solani root rot in turf grass. Winchester and Burt (54) obtained good control of Belonolaimus longicaudatus on Ormand Bermuda grass with Bayer 25141. Sarolex has been found to give good nematode control on turf in Florida, according to Perry (33) and Winchester (53). In 1966, Streu and Vasvary (41) found Ethion, Diazinon and Carbophenothion gave good control of Tylenchorhynchus spp. in turf. An increase of clipping and root weights of bluegrass and red fescue was obtained by Troll and Rhode (48) following application of Bayer 25141. Brodie and Burton (3) obtained excellent nematode control following application of Phorate, Bayer 25141 or Disulfton to Bermuda grass turf. Hot water treatments were used by Heald and Wells (14) to obtain control of both endo-and ectoparasitic nematode in Bermuda grass sprigs. Perry and Miller (35) have found MoCap, Sarolex, NIA 10242, and U. C. 21149 to be effective as soil drenches for control of *Belonolaimus longicaudatus* and *Hoplolaimus coronatus* on turf grasses in Florida.

MATERIALS AND METHODS

Certain procedures, unless otherwise stated, were followed throughout the investigations. Nematodes were extracted from soil by the method of Christie and Perry (4). Nematode numbers were determined by counting the number present in each of three 1 ml. aliquots of a 100 ml. suspension in a Scott slide hookworm larvae counter. Populations of *Tylenchorhynchus nudus* used in greenhouse experiments were obtained from greenhouse colonies maintained on red clover, *Trifolium pratense* (L.). Soil used in greenhouse experiments was freed of nematodes by steam treatment, or fumigation. Steam treatment consisted of placing two gallons of soil in a cloth sack and steaming for three hours without pressure in an autoclave. One cubic foot of soil to be fumigated was placed in a container and the fumigant was sprinkled over the soil surface. The container was then sealed and the soil thoroughly mixed by shaking; 24 hours later the soil was removed and spread over a clean surface to allow escape of the fumigant. Table 1 lists the dosages and formulations of fumigants and nematicides used in the various experiments. Host preference and pathogenicity tests were conducted in a greenhouse cooled during the summer

Table 1. Fumigant and nematicide formulations used in certain greenhouse and field studies.

Chemical	Formula	% Active ingredient	Dosage per 1000 ft.^{2}
Dasinit	0,0-Diethyl 0-p-methylsulfinyl-phenyl phosphorothionate	63.5	1.3 pt
MoCap	0-Ethyl S,S-dipropyl phosphorodithioate	10 (Gran.)	6.98lb
Sarolex	0,0-Diethyl 0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate	46.2	2.5 pt
Thimet	Phorate; 0,0-Diethyl S-(ethylthio-methyl) phosphorodithioate	66	2 pt
Zinophos	0,0-Diethyl 0-2-pyrazinyl phosphorothioate	46	2 pt
Nemagon	1,2-Dibromo 3-chloropropane	67.2	1.5 pt
D-D	1,3-Dichloropropane and 1,2-Dichloropropane	100	7.3 pt
Telone	1,3-Dichloropropane	100	7.3 pt

months with a Trane[1] air-conditioner. The greenhouse temperature was maintained at $75^{o}\pm7^{o}$F., throughout the experiments. A completely soluble 20-20-20 analysis fertilizer was applied every four weeks to pots in greenhouse experiments.

Survey samples were collected by means of a probe 13/16 inch in diameter to a depth of six inches. Ten to twelve borings were taken at each location and were stored at 5^{o}C. until processed. The borings were thoroughly mixed, and a 400 cc. aliquot was removed and nematodes were extracted and counted as described.

The nine types of grasses[2] used in the host suitability test were: Common and Merion Kentucky bluegrass, Poa pratensis (L.), red fescue, Festuca rubra (L.), colonial bentgrass, Agrostis tenuis (Sibth), creeping bentgrass, A. palustris (Huds.), annual rye grass, Lolium multiflorum (Lam.), perennial rye grass L. perenne (L.), Canada bluegrass, Poa compressa (L.), and redtop, Agrostis alba (L.). An equivalent volume of seeds within each species was scattered evenly over the surface of 1800 grams of steam-treated Vienna loam in six-inch clay pots.

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[1]Trane Company, La Crosse, Wisconsin.

[2]Seed obtained through courtesy of Mr. Norm Evers, Horticulture Department, South Dakota State University.

A suspension of approximately 1,000 Tylenchorhynchus nudus was then poured over the seeds and covered with 200 grams of the same soil. Each treatment was replicated four times with a tenth treatment consisting of nematodes alone. The pots were arranged on the greenhouse bench, and the grass was clipped and fertilized every four weeks.

Another greenhouse experiment was designed to determine the ability of Tylenchorhynchus nudus to survive and reproduce in non-sterile soil. Two gallons of soil were obtained from Kentucky bluegrass turf in the Plant Pathology Department lawn. The soil was mixed and 1600 grams were placed in each of ten six-inch clay pots. Five of the pots were seeded with equal volumes of Kentucky bluegrass and all pots were inoculated with 1,000 T. nudus. An additional 200 grams of soil were then added to each pot. An 1800 gram sample of soil was processed to determine the number of nematodes present initially.

Several greenhouse experiments were designed to determine the effects of Tylenchorhynchus nudus on the growth of Kentucky bluegrass. Test 1 was designed as a 2^4 factorial with the effects and interactions of soil type, water, nutrients and nematodes being measured. Six-inch clay pots containing 1800 grams of steam-treated

Vienna loam or Dickie sandy loam were seeded with bluegrass at an equivalent rate of 1 lb/600 ft^2. One-half of the pots of each soil type received 5,000 T. nudus at time of seeding. Each treatment was replicated six times, and the pots were arranged in a randomized complete block design on the greenhouse bench. Two months after seeding, the grass was clipped to a height of two inches with the aid of a clipping guide and the water and nutrient treatments initiated. Prior to this time the pots were watered regularly, but were not fertilized. One-half of the pots were watered regularly, usually every four days, while the remaining half received water only when the plants were obviously wilted, usually once a week. Nutrients were completely withheld from another one-half of the pots, and the remainder received fertilizer every four weeks. The grass was clipped to a height of two inches every three weeks, and the clippings were oven dried at 60°C. for five days before weighing.

Test II was designed to compare the effects of Tylenchorhynchus nudus in fumigated versus non-fumigated soil. One-half of the pots received 1800 grams of fumigated Dickie sandy loam and the remainder received non-fumigated Dickie sandy loam. All pots were seeded with bluegrass and one-half in each soil treatment were inoculated with 5,000 T. nudus. Each treatment was replicated six times

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and the pots were arranged in a randomized complete block design. An 1800 gram sample of both fumigated and non-fumigated soil was processed to determine the number of nematodes present initially. The pots were watered and fertilized regularly and the grass was clipped, dried and weighed at three week intervals as in test I.

Test III was conducted in an Isco[3] environmental control chamber to eliminate some of the variation inherent in most greenhouse experiments. Plastic pots, 3 1/2 inches in diameter, each containing 210 cc. of five hour steamed Blendon loamy sand, were seeded with surface-sterilized bluegrass seed. One-half of the pots received a washed suspension of 1,000 *Tylenchorhynchus nudus*, and the remainder received an equivalent volume of wash water. Each treatment was replicated ten times, and the pots were randomly arranged in each of two flats and placed in the chamber. Each week an equal amount of sterile, White's (24) nutrient solution was added to each pot. The grass was clipped and weighed as in tests I and II. A constant temperature of 74°F. and 50% relative humidity were maintained with a 16 hour photoperiod.

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[3]Instrumentation Specialties Company, Inc. Lincoln, Nebraska.

The effect of *Tylenchorhynchus nudus* on the growth of spring wheat, *Triticum aestivum* (L.), was also determined. Five-inch clay pots containing 900 cc. of fumigated Vienna loam or Blendon loamy sand were seeded with Manitou spring wheat. One-half of the pots of each soil type were inoculated with 20,000 *T. nudus* at the time of seeding. Each treatment was replicated six times in a randomized complete block design. Following emergence, the plants were thinned to two per pot. Eleven weeks after planting, the plants were clipped at soil level and the total oven dry weight was recorded. Immediately after clipping, the pots were again seeded and as before the plants were thinned to two per pot. Thirteen weeks after reseeding, the mature plants were removed and oven dry weights of roots, straw and heads were recorded. The pots were watered regularly throughout the test and fertilizer was applied two weeks after emergence in each part of the experiment. Supplemental lighting was supplied from 6 to 11 p.m. during the late fall and winter months.

An experiment to determine the effects of various nematicides (Table 1) on the growth of turfgrass and in control of nematodes was conducted on the Plant Pathology Department lawn. A level area consisting largely of Kentucky bluegrass was selected, and prior to application of the nematicides the area was mowed, raked twice, aerated, and over a three day period 1 1/2 inches of water was applied. A Latin square design was used with six replications of each treatment. The plots were 6 x 12 feet with two foot alleyways. Emulsifiable formulations were applied in two gallons of water and were sprinkled evenly over the plot. A granular formulation was applied with the aid of a fertilizer spreader. All of the nematicides were applied at the maximum recommended dosages. Two inches of water were applied to the treated areas by means of an overhead irrigation system immediately after treatment. A similar system was also used throughout the experiment to apply supplemental water when needed. The plots were mowed every three to four weeks using a reel type mower equipped with a grass catcher. The clippings from each plot were weighed and recorded immediately after mowing. Nematode populations were determined by randomly taking twelve borings from each plot, mixing the borings thoroughly and processing a 250 cc. aliquot. On September 29, 1967, a 16-20-0 analysis fertilizer was applied at an equivalent rate of 330 lbs. per acre.

Four weeks after application of the nematicides the Zinophos and Thimet treated areas showed considerable growth stimulation. To determine which nematicide or if the combination was best, an experiment consisting of two replications of each of three treatments was conducted adjacent to the original experiment. The plot areas were prepared, treated, mowed, and sampled as previously described.

Wisconsin (18) type temperature tanks were used to study the effects of constant temperature on the reproduction and morphology of Tylenchorhynchus nudus. Number ten cans were lined with a plastic bag, and one inch of coarse gravel was then placed in the bottom of the can. Four-inch clay pots were rested on the gravel and then buried to within 1/2 inch of the pot rim in autoclaved coarse sand. Each pot contained 450 cc. of steam treated Vienna loam or Blendon loamy sand and was seeded with Manitou spring wheat. The plants were thinned to one per pot and the cans were placed in the water baths. Each treatment was replicated five times. Three days after being placed in the tanks all of the pots were inoculated with a suspension of 1,000 T. nudus. Temperatures were maintained at 15°, 20°, 25°, 30°, and 32.5° ± 1°C. Water of the same temperature as that of the respective tank was applied when necessary and all pots were fertilized once. Supplemental lighting was applied during the late fall and winter months. Twelve weeks after inoculation the wheat was clipped at soil level. Nematodes were then extracted from the soil and sand, since in some cases roots had grown through the bottom of the pots into the sand.

The effect of temperature on morphology of the nematodes was determined after the population of each pot was recorded. Twenty adult females were picked at random from the population of each pot. The nematodes were carefully heat relaxed and mounted in FAA on glass slides with ten nematodes per slide. Morphological determinations were made from tracings obtained with the aid of a camera lucida.

Nematodes were extracted from root balls of potted plants by placing the roots and soil in two quarts of water and allowing them to soak for one to two hours. As much soil as possible was then gently removed from the roots and the remaining soil was washed from the roots beneath a tap into a bucket. In all tests, except I and II of Kentucky bluegrass pathogenicity experiments, the entire soil suspension was processed for nematodes. In tests I and II the soil suspension was raised to a two-gallon level and thoroughly mixed. A two-quart aliquot was then removed and processed.

The generalized mechanical analysis (52) of the soil types used in the various experiments was: Vienna loam, sand - 43.0%, silt - 34.3%, clay - 22.7%, Dickie sandy loam, sand - 63.3%, silt - 11.4%, clay - 15.3%, Blendon loamy sand, sand - 86.6%, silt - 7.5%, clay - 5.9%.

RESULTS

Survey

The following nematodes were identified from the survey samples: Aphelenchoides hamatus Thorne and Malek, 1968; Aphelenchus avenae Bastian, 1865; Aorolaimus baldus Thorne and Malek, 1968; Boleodorus thylactus Thorne, 1941; B. acutus Thorne and Malek, 1968; Criconemoides permistus Raski and Golden, 1965; C. xenoplax Raski, 1952; Ditylenchus microdens Thorne and Malek, 1968; D. obesus Thorne and Malek, 1968; Helicotylenchus bradys Thorne and Malek, 1968; H. digonicus Perry, 1959; H. exallus Sher, 1966; H. hydrophilus Sher, 1966; H. leiocephalus Sher, 1966; H. pseudorobustus (Steiner, 1914) Golden, 1945; Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935; Nagelus aberrans Thorne and Malek, 1968; Paraphelenchus intermedius Thorne and Malek, 1968; Paratylenchus pesticus Thorne and Malek, 1968; P. vexans Thorne and Malek, 1968; Pratylenchus scribneri Steiner, 1943; P. tenuis Thorne and Malek, 1968; Psilenchus hilarulus de Man, 1921; Trichodorus californicus Allen, 1957; T. proximus Allen, 1957; Tylenchorhynchus acutus Allen, 1955; T. nudus Allen, 1955; T. maximus Allen, 1955; T. robustoides Thorne and Malek, 1968; Tylenchus davainei Bastian, 1865; T. exiguus de Man, 1876; and Xiphinema americanum Cobb, 1913.

Figure 1 shows a map of the areas sampled in the state-wide survey and the occurrence of *Tylenchorhynchus nudus* in South Dakota. The location and number of *T. nudus* and selected phytophagous nematode genera found during the state-wide and Brookings, South Dakota surveys are shown in Tables 2 and 3 respectively. The occurrence and number of *T. nudus* and selected nematode genera found in a survey of South Dakota Kentucky bluegrass seed-producing areas is shown in Table 4. The locations shown in Table 4 were in Kentucky bluegrass for at least five years.

Host suitability

The results of the host suitability experiment conducted in a greenhouse are shown in Table 5. All of the grasses used in the experiment were established within 10 days of seeding and all had well-developed root systems at the conclusion of the experiment.

The results of the experiment to determine the ability of *Tylenchorhynchus nudus* to survive and reproduce in non-sterile field soil transported to the greenhouse are shown in Table 6.

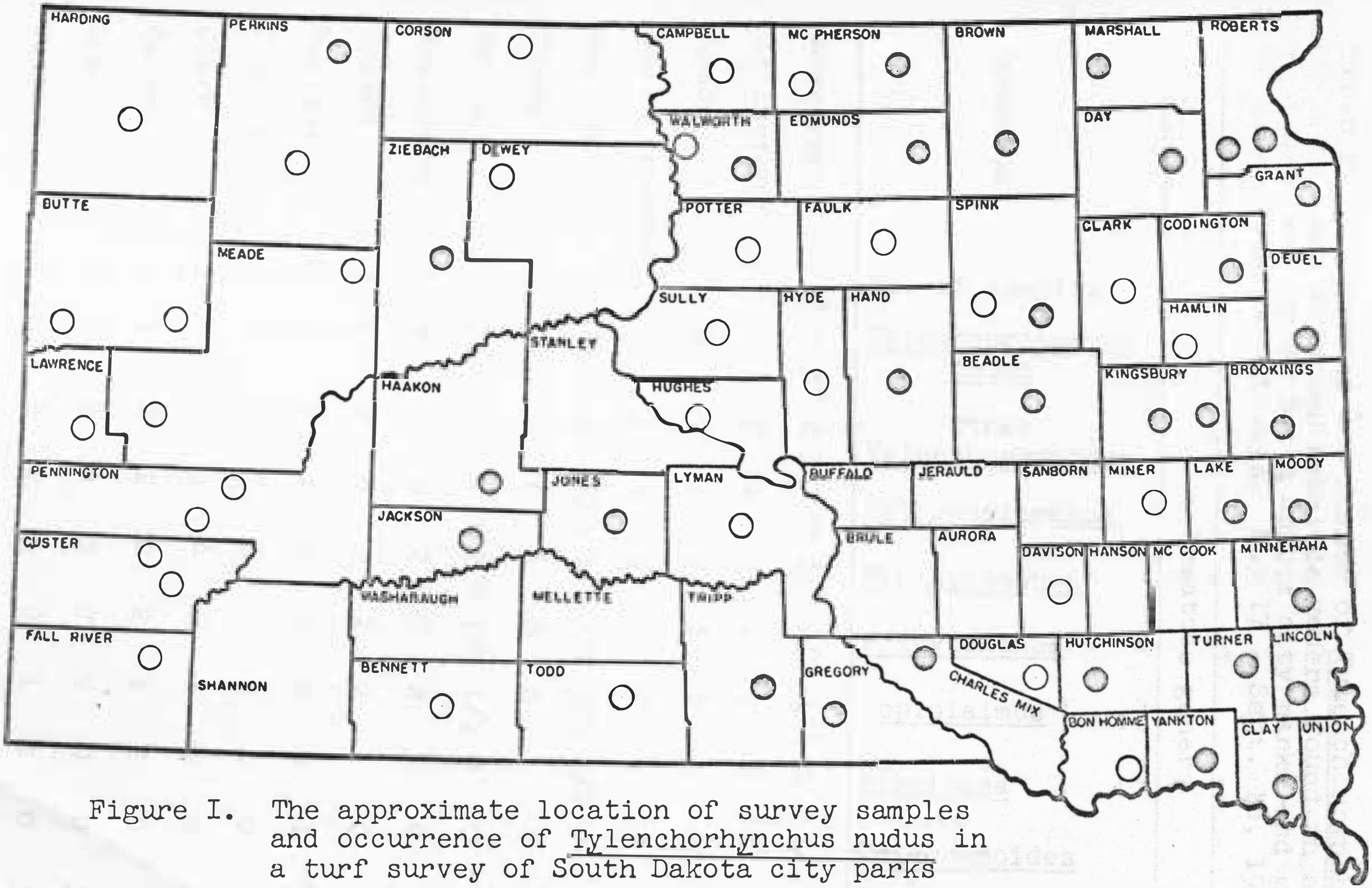

Figure I. The approximate location of survey samples and occurrence of Tylenchorhynchus nudus in a turf survey of South Dakota city parks and golf course fairways.

○ - Area sampled

◎ - Tylenchorhynchus nudus present

Table 2. Occurrence and number of *Tylenchorhynchus nudus* and selected nematode genera found in a turf survey of South Dakota city parks and golf course fairways. May 17 - Sept. 18, 1967.

Location	No. of samples	*Tylenchorhynchus nudus*	Other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Criconemoides*	*Tylenchus*	*Boleodorus*
	Nematode genera										
Aberdeen	3[a]	2/3[b]	2/3	2/2	3/3	2/2	2/1	3/1	1/1	2/2	2/2
Arlington	1	1	2	2	0	1	1	1	0	0	3
Armour	1	0	1	1	2	3	0	1	0	2	2
Belle Fourche	1	0	1	2	2	1	0	1	0	3	2
Beresford	2	2/3	2/1	2/3	2/3	1/2	2/1	2/2	0	2/2	1/2
Bison	1	0	0	0	4	0	0	1	0	3	0
Britton	2	1/3	2/3	2/4	2/4	1/1	1/1	0	0	2/3	1/2
Brookings	1	4	2	3	2	0	2	1	0	2	2
Bryant	1	0	2	4	3	1	2	1	0	2	3
Buffalo	1	0	2	1	0	0	0	0	1	3	2
Clear Lake	1	2	1	1	5	0	1	1	0	3	2
Clark	1	0	2	1	4	0	1	0	0	1	0
Colman	1	3	2	2	2	2	1	2	0	3	0
Craven	1	3	3	4	3	2	0	0	0	3	0
Custer	1	0	2	2	5	0	1	1	0	3	0

Table 2. Continued.

Location	No. of samples	*Tylenchorhynchus nudus*	other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Criconemoides*	*Tylenchus*	*Boleodorus*
	Nematode genera										
Deadwood	1	0	2	0	5	0	2	0	0	5	2
De Smet	1	3	0	2	5	0	0	1	0	2	0
Dupree	1	2	4	1	3	0	0	2	0	2	0
Eureka	1	0	2	2	4	0	0	0	0	1	2
Faith	1	0	3	1	0	0	0	0	0	5	0
Faulkton	1	0	4	1	3	0	1	0	0	3	1
Frankfort	1	4	2	2	2	0	2	1	0	1	2
Gettysburg	1	0	2	1	3	0	0	1	0	0	3
Gregory	1	2	2	2	4	1	0	0	0	2	2
Highmore	1	0	2	1	3	1	0	1	0	3	2
Hill City	1	0	0	0	5	0	0	0	0	4	2
Hot Springs	1	0	4	2	3	1	0	1	0	1	5
Howard	1	0	1	4	2	1	1	0	0	4	4
Huron	1	2	2	4	3	0	1	1	0	3	3
Isabel	1	0	2	0	3	0	0	2	0	2	0
Kadoka	1	1	0	0	2	0	0	0	0	2	0
Lemmon	1	2	2	0	2	0	0	1	0	2	0

Table 2. Continued.

Location	No. of samples	*Tylenchorhynchus nudus*	other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Criconemoides*	*Tylenchus*	*Boleodorus*
	Nematode genera										
Leola	1	5	0	2	2	0	1	0	0	4	0
Madison	1	1	2	0	3	1	0	1	0	5	5
Martin	1	0	3	0	4	0	0	0	0	3	0
McIntosh	1	0	3	0	1	0	0	0	0	3	0
Midland	1	1	2	0	3	0	0	0	0	1	0
Milbank	2	1/1	2/2	2/2	2/3	0	1/1	1/1	0	2/3	0
Miller	1	5	3	2	2	0	1	0	0	3	2
Mission	1	0	2	2	3	0	0	0	1	2	0
Mitchell	1	0	2	1	1	1	1	1	0	1	0
Mobridge	1	0	2	4	2	1	0	1	0	5	3
Mound City	1	0	4	3	3	1	2	0	0	4	0
Murdo	1	5	3	3	5	0	0	1	0	3	0
Newell	1	0	2	5	1	0	0	4	0	2	0
Onida	1	0	2	2	3	5	0	1	0	5	0
Ortley	1	3	4	5	4	0	2	1	0	3	1
Parker	1	1	2	3	5	0	1	0	0	2	4
Parkston	2	1/2	2/1	2/4	2/2	1/3	0	1/1	0	2/3	2/4

Table 2. Continued.

Location	No. of samples	*Tylenchorhynchus nudus*	other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Criconemoides*	*Tylenchus*	*Boleodorus*
		Nematode genera									
Pierre	1	0	1	1	4	1	0	0	0	4	3
Platte	1	1	1	4	2	4	0	0	0	3	0
Presho	1	0	1	3	2	0	0	1	0	4	2
Pringle	1	0	2	3	2	0	0	1	0	5	0
Rapid City	6	0	4/2	6/4	4/4	3/2	1/1	1/1	0	4/4	4/5
Redfield	1	0	3	1	3	0	0	0	0	2	2
Selby	1	1	2	0	2	2	0	0	0	2	0
Sioux Falls	4	3/3	3/2	4/3	4/3	3/2	3/1	1/1	1/3	4/3	2/4
Sturgis	1	0	3	5	3	2	2	0	0	5	4
Tabor	1	0	2	3	3	0	0	2	0	2	2
Vermillion	1	1	1	4	3	2	0	1	0	3	0
Watertown	2	2/2	2/2	2/2	2/2	1/3	2/2	0	0	2/3	0
Webster	2	2/2	2/2	2/4	2/5	1/2	1/1	1/1	0	2/3	2/1
Wilmont	1	1	1	5	3	0	1	0	0	3	1
Winner	1	1	0	4	4	0	0	0	0	2	2
Yankton	1	0	3	1	3	1	1	1	0	2	0

Table 2. Continued.

	Nematode genera										
	No. of samples	*Tylenchorhynchus nudus*	other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Criconemoides*	*Tylenchus*	*Boleodorus*
Total	81	39	71	69	76	34	33	40	4	76	43
% Occurrence of Total		48	88	85	94	41	41	50	5	94	53
Nematode Number[c]		273 / 1200	202 / 1000	386 / 1330	615 / 4480	143 / 880	44 / 200	50 / 800	78 / 270	452 / 3630	365 / 1900

[a]Number of areas sampled within each city.

[b]Upper figure indicates the number of occurrences and lower figure is the average number of nematodes when present. Population rating scale: 0 (not present); 1 (1-50); 2 (51-200); 3 (201-500); 4 (501-1000); 5 (1000+).

[c]Upper figure is average nematode number per sample and lower figure is highest population encountered.

Table 3. Occurrence and number of Tylenchorhynchus nudus and selected nematode genera found in a survey of Brookings, S. Dak. lawns. Sept. 12, 1967.

Nematode genera									
Sample number	Tylenchorhynchus nudus	other Tylenchorhynchus	Helicotylenchus	Paratylenchus	Pratylenchus	Hoplolaimus	Xiphinema	Tylenchus	Boleodorus
1	4[a]	3	2	3	2	2	2	3	2
2	5	3	0	5	0	1	2	3	5
3	4	2	2	5	0	1	1	3	3
4	2	2	2	5	0	1	1	3	3
5	3	2	4	4	2	2	2	3	4
6	3	0	4	4	0	3	2	3	3
7	3	1	4	3	2	1	1	3	5
8	5	1	4	5	1	3	1	3	4
9	2	2	2	4	0	1	2	4	5
10	2	1	3	3	0	1	1	2	3
11	0	2	3	2	0	1	0	2	2
12	0	3	4	3	2	1	1	3	5
13	2	2	3	5	3	1	0	4	5
14	1	0	3	3	0	0	0	1	2
15	4	2	3	3	0	1	1	5	3
16	0	1	1	5	1	1	2	3	5

Table 3. Continued.

	Nematode genera								
Sample number	*Tylenchorhynchus nudus*	other *Tylenchorhynchus*	*Helicotylenchus*	*Paratylenchus*	*Pratylenchus*	*Hoplolaimus*	*Xiphinema*	*Tylenchus*	*Boleodorus*
17	2	0	5	1	0	0	0	3	4
18	0	0	1	4	0	0	1	3	3
19	2	1	1	5	0	4	2	3	5
20	2	1	1	4	0	2	2	3	4
No. of occurrences	16	15	19	20	6	17	15	20	20
% Occurrence of Total	80	75	95	100	30	85	75	100	100
Nematode Number[b]	434/1770	103/500	395/1270	1071/3570	110/370	104/570	57/170	372/1070	750/1830

[a]Population rating scale: 0 (not present); 1 (1-50); 2 (51-200); 3 (201-500); 4 (501-1000); 5 (1000+).

[b]Upper figure is average nematode number per sample and lower figure is highest population encountered.

Table 4. Occurrence and number of Tylenchorhynchus nudus and selected nematode genera found in a survey of Kentucky bluegrass seed-producing areas. June 28, 1968.

Location	Tylenchorhynchus nudus	other Tylenchorhynchus	Helicotylenchus	Paratylenchus	Pratylenchus	Hoplolaimus	Xiphinema	Tylenchus	Boleodorus
1 mile E. De Smet	0[a]	4	1	2	0	2	1	2	0
1 mile E. Harrold	4	0	4	0	0	0	1	4	0
4 mile N. Highmore	4	4	0	2	1	0	1	4	0
12 miles S. Onida	0	4	5	5	0	0	2	2	0
1/2 mile E. Ree Heights	3	2	2	2	0	0	2	4	3
3 mile E. St. Lawrence	0	5	3	4	0	0	1	4	0
Number of occurrences	3	5	5	5	1	1	6	6	1
% Occurrence of Total	50	83	83	83	17	17	100	100	17
Nematode Number[b]	657/970	798/1630	578/1730	516/1570	10/10	70/70	62/100	528/930	230/230

[a]Population rating scale: 0 (not present); 1 (1-50); 2 (51-200); 3 (201-500); 4 (501-1000); 5 (1000+).

[b]Upper figure indicates average nematode number per sample and lower figure represents the highest population encountered.

Table 5. Population levels of *Tylenchorhynchus nudus* obtained 28 weeks after inoculation of various treatments in the host-suitability test.

Treatment	Number of *T. nudus*[a]
Common Kentucky bluegrass	37,276
Creeping red fescue	21,100
Redtop	18,176
Colonial bentgrass var. Exetor	14,667
Kentucky bluegrass var. Marion	14,533
Creeping bentgrass var. Penncross	13,533
Canadian bluegrass	13,100
Annual ryegrass	7,533
Perennial ryegrass	5,787
Fallow	300

[a]Each value represents the mean of three replications.

Table 6. Number of nematodes recovered from field soil 25 weeks after seeding with Kentucky bluegrass or fallowed.

	Nematode genera								
	Tylenchorhynchus nudus	Tylenchorhynchus acutus	Tylenchorhynchus maximus	Helicotylenchus	Paratylenchus	Pratylenchus	Boleodorus	Tylenchus	Hoplolaimus
Initial population of 1800 grams of soil	(1000)[a]	30	800	2830	470	830	230	200	30
Seeded with Kentucky bluegrass	1400[b]	136	6457	4712	306	486	340	2388	0
Fallow	194	0	20	1566	126	338	92	20	0

[a]1,000 T. nudus were added at the initiation of the experiment since none were originally present in the field soil.

[b]Each value is the mean of five replications.

Pathogenicity tests

The first clippings from Kentucky bluegrass pathogenicity test I obtained June 20, 1967, were not included in Table 7 or 8. A statistical analysis of these initial clippings revealed a significant difference between inoculated and non-inoculated treatments. The clipping weight data shown was obtained at three week intervals from July 27, 1967, through March 15, 1968. Percent reductions were calculated from the formula:

% Reduction=Non-inoculated-Inoculated/Non-inoculated X 100

The analyses of variance for Kentucky bluegrass test I, spring wheat pathogenicity test and the temperature test are presented in the appendix. Appendix tables are designated by the letter A.

Final nematode population levels from Kentucky bluegrass pathogenicity test I are shown in Table 9. No nematodes were recovered from non-inoculated treatments.

Results of the second Kentucky bluegrass pathogenicity test were confounded by a lack of nematode control in the fumigated soil portion of the experiment. Apparently the fumigant did not destroy the egg stage of a *Paratylenchus* sp. since an examination of the fumigated soil at the initiation of the experiment indicated that all larval and adult stages of the various nematodes present had been controlled. At the conclusion of the experiment ten months later, however, the non-inoculated fumigated soil was found to contain an average of 180,000 *Paratylenchus* sp. per pot. The fumigated soil inoculated with *Tylenchorhynchus nudus* contained 162,000 *T. nudus* and 18,000 *Paratylenchus* sp. per pot. The inoculated field soil contained an average of 24,000 *T. nudus* per pot and both field soil treatments had nearly equal *Paratylenchus* sp. populations of about 11,000.

Table 7. Effect of *Tylenchorhynchus nudus* on the growth of Kentucky bluegrass grown in Vienna loam in the greenhouse. April 28, 1967 - March 15, 1968.

			Dry clipping weight (g)[a]	% Reduction[b]	Dry root and crown weight (g)[c]	% Reduction
Nutrients applied	Watered regularly	Inoculated	18.81	9	38.8	27
		Non-inoculated	20.69		52.8	
	Water withheld	Inoculated	17.01	18	38.7	4
		Non-inoculated	20.68		40.2	
Nutrients withheld	Watered regularly	Inoculated	3.41	35	33.3	22
		Non-inoculated	5.27		42.7	
	Water withheld	Inoculated	3.76	21	28.7	20
		Non-inoculated	4.73		35.8	

[a]Each value is the mean of the total of all clippings within a treatment.

[b]All reductions are statistically significant. The analysis of variance is presented in Tables A. 1 and A. 2.

[c]Each value is the mean of six replications.

Table 8. Effect of Tylenchorhynchus nudus on the growth of Kentucky bluegrass grown in Dickie sandy loam in the greenhouse. April 28, 1967 - March 15, 1968.

			Dry clipping weight (g)[a]	% Reduction[b]	Dry root and crown weight (g)[c]	% Reduction
Nutrients applied	Watered regularly	Inoculated	17.37	7	33.4	13.5
		Non-inoculated	18.72		38.6	
	Water withheld	Inoculated	16.29	14	25.2	23
		Non-inoculated	19.05		32.8	
Nutrients withheld	Watered regularly	Inoculated	1.84	44	17.6	35
		Non-inoculated	3.27		27.2	
	Water withheld	Inoculated	1.65	40	17.2	36
		Non-inoculated	2.76		26.7	

[a]Each value is the mean of the total of all clippings within a treatment.

[b]All reductions are statistically significant. The analysis of variance is presented in Tables A. 1 and A. 2.

[c]Each value is the mean of six replications.

Table 9. Number of *Tylenchorhynchus nudus* recovered from inoculated treatments in the Kentucky bluegrass pathogenicity test I.

Dickie sandy loam				Vienna loam			
Nutrients applied		Nutrients withheld		Nutrients applied		Nutrients withheld	
watered regu-larly	water with-held	watered regu-larly	water with-held	watered regu-larly	water with-held	watered regu-larly	water with-held
171,565[a]	200,760	96,576	127,448	146,768	132,908	178,228	159,948

[a]Each value is the mean of six replications.

There were no statistically significant clipping or root weight reductions among any of the treatments.

The experiment conducted in the environmental control chamber (Table 10) was designed to measure more closely the effects of Tylenchorhynchus nudus on the growth of Kentucky bluegrass. No attempts were made to place the plants under a water or nutrient stress. The mean number of T. nudus recovered from inoculated pots at the conclusion of experiment was 8,093.

Table 10. The effect of Tylenchorhynchus nudus on the growth of Kentucky bluegrass grown in an environmental control chamber. December 16, 1967 - April 20, 1968.

	Inoculated	Non-inoculated	F values
Cumulative clipping weight (g)[a]	0.60	0.83	52.80**
% Reduction	28%		
Root and crown weight (g)[b]	0.105	0.164	22.08**
% Reduction	36%		

[a]Each value is the total mean dry weight of all clippings.

[b]Each value is the mean dry weight of ten replications.

**Significant at the .01 level.

The spring wheat pathogenicity test was conducted in two parts. The results of part I, in which the effects of *Tylenchorhynchus nudus* on total, above ground dry weight of spring wheat were determined, are shown in Table 11. The pots remaining at the conclusion of part I were reseeded with spring wheat. The results of part II are shown in Table 12. The mean number of *T. nudus* recovered from the inoculated pots at the conclusion of part II was 29,574 in Vienna loam and 23,707 in Blendon loamy sand.

Table 11. The effect of *Tylenchorhynchus nudus* on the growth of Manitou spring wheat grown under various soil treatments in the greenhouse.

Part I.

	Vienna loam		Blendon loamy sand	
	Inoculated	Non-inoculated	Inoculated	Non-inoculated
Weight[a]	3.69	4.26	3.16	3.47
% Reduction	13%		9%	

[a]Each value is the mean dry weight in grams of plants clipped at soil level. The analysis of variance is presented in Table A. 3.

Table 12. The effect of Tylenchorhynchus nudus on the weight of heads, straw and roots of Manitou spring wheat grown in the pots remaining at the conclusion of part I.

Part II.

	Vienna loam			Blendon loamy sand	
	Inoculated		Non-inoculated	Inoculated		Non-inoculated
Head[a]	2.48		2.95	1.93		2.57
% Reduction[b]		16%			25%	
Straw[a]	2.26		2.77	2.02		2.45
% Reduction		18%			17.5%	
Roots[a]	0.75		1.02	0.69		0.97
% Reduction		26.5%			30%	

[a]Each value is the mean dry weight in grams of six replicates.

[b]All reductions are statistically significant. The analysis of variance is presented in Table A. 3.

Control

The following nematodes were identified from the Kentucky bluegrass field plots: Boleodorus thylactus Thorne, 1941; Helicotylenchus digonicus Perry, 1959; H. leiocephalus Sher, 1966; Hoplolaimus galeatus (Cobb, 1913) Thorne, 1935; Paratylenchus vexans Thorne and Malek, 1968; Pratylenchus scribneri Steiner, 1943; Psilenchus hilarulus de Man, 1927; Tylenchorhynchus acutus

Allen, 1955; T. nudus Allen, 1955; T. maximus Allen, 1955; Tylenchus exiguus de Man, 1876; and Xiphinema americanum Cobb, 1913.

Boleodorus, Helicotylenchus, Paratylenchus, Tylenchorhynchus and Tylenchus were present in all of the plots. Helicotylenchus was the most prevalent genera and frequently one thousand or more were recovered from the 250 cc. soil samples. Hoplolaimus was present in about 22% of the plots and Pratylenchus was found in 33%. Tylenchorhynchus nudus was found infrequently and in low numbers in the field plots.

The effect of nematicide treatments on the growth of Kentucky bluegrass in field plots is shown in Table 13. The influence of the treatments on the stylet-bearing nematode populations in the field plots is shown in Table 14.

Zinophos and Thimet comparison

An assumed phytotoxic effect was observed in the Zinophos and Thimet treated areas seven days after treatment. The upper 1/2 to 1 inch of the grass blades in these areas became brittle and shrunken and turned brown two to three days later. The affected areas seemed to be confined to the upper one inch of the grass blades and were not observed to progress below this level. Similar symptoms

Table 13. The effect of nematicide treatments on the growth of Kentucky bluegrass turf in field plots.

Clip-ping date	Clipping weight (g)[a]						
	Zinophos & Thimet	Sar-olex	Das-init	Nem-agon	Mo-Cap	Check	Dunnett's "t" .05
7/28/67	564*	399*	410*	321	276	294	104
8/26/67	490*	319*	291	233	188	199	111
9/20/67	219*	163*	130	99	94	83	48
5/5/68[b]	62	71	75	65	65	61	23
5/25/68	889	1186	955	1251	1092	1088	421
6/22/68	619	681	687	856	636	620	262
7/11/68	294	313	336	392	322	329	75
8/2/68	187	205	236	339	223	296	125

[a]Each value is the mean fresh weight of six replicates.

[b]The clippings obtained May 5, 1968 were oven dried five days at 60°C. before weighing.

*Indicates a significant difference at the .05 level when compared to the check.

Table 14. The effect of nematicide treatments on nematode populations in Kentucky bluegrass field plots.

Sampling date	Number of stylet-bearing nematodes[a]						
	Zinophos & Thimet	Sar-olex	Das-init	Nem-agon	Mo-Cap	Check	Dunnett's "t" .05
8/11/67	547	2062	1498	2295	2022	3120	797
10/28/67	120	1395	1308	2047	1852	3312	896
5/24/68	75	1173	488	1070	708	1985	599
7/30/68	372	3718	1743	2155	2247	3858	1696

[a]Each value is the mean of six replicates.

were again observed in the Zinophos and Thimet areas following the first two clippings. Subsequently an experiment was conducted adjacent to the original plot area to determine which chemical was responsible for the phytotoxic effect and also to compare their effects on the growth of Kentucky bluegrass and ability to control nematodes. The effect of Zinophos and Thimet alone or in combination on the growth of Kentucky bluegrass and in production of the phytotoxic effect is shown in Table 15. A comparison of nematode control achieved by the treatments is presented in Table 16.

Table 15. The effect of Zinophos and Thimet alone or in combination on the growth of Kentucky bluegrass turf in field plots.

Clipping date	Clipping weight (g)[a]			
	Zinophos & Thimet	Thimet	Zinophos	lsd .05
8/26/67	716*[c]	538[c]	676	163
9/20/67	364*[c]	285[c]	195	129
10/28/67	245*	180	107	81
5/5/68[b]	94	62	71	63
5/25/68	1158	835	1169	695
6/22/68	661	587	625	414
7/11/68	284	279	332	121
8/2/68	192	158	207	102

[a]Each value is the mean fresh weight of two replicates.

[b]The clippings obtained May 5, 1968 were oven dried five days at 60°C. before weighing.

[c]Indicates presence of phytotoxic effect.

*Indicates a significant difference at .05 level when compared to Zinophos or Thimet.

Table 16. The effect of Zinophos and Thimet alone or in combination on nematode populations in Kentucky bluegrass field plots.

Sampling date	Number of stylet-bearing nematodes[a]				
	Zinophos & Thimet	Thimet	Zinophos	lsd .05	Check[b]
7/24/67[c]	2120	2845	3310		
9/7/67	250	145*	1215	1016	2390
10/28/67	70*	180*	1265	1016	2320
5/24/68	30*	30*	345	265	1160
7/30/68	15	100	2680	3365	4800

[a]Each value is the mean of two replicates.

[b]The check samples were obtained from the alleyways between the treated plots and are not statistically comparable.

[c]The experiment was initiated on this date and the values presented represent the initial populations.

*Indicates a significant difference at the .05 level when compared to Zinophos.

Temperature study

The effect of constant temperature on the reproduction of Tylenchorhynchus nudus on spring wheat in the greenhouse was determined. The population levels attained by T. nudus eleven weeks after inoculation of Manitou spring wheat grown in two soil types are shown in Table 17. The effect of temperature on the length of adult, female T. nudus was also determined (Table 18).

Table 17. The effect of temperature on the reproduction of Tylenchorhynchus nudus in two soil types in constant temperature tanks.

Temperature degrees Centigrade	Vienna loam		Blendon loamy sand	
	Final[a] numbers	% Adult	Final numbers	% Adult
10	356	60.3%	354	58.9%
15	388	63.9%	226	55.8%
20	2792	28.6%	1260	21.7%
25	27398	27.1%	13380	31.4%
30	52054	26.7%	25714	25.1%
32.5	33726	31.5%	22840	29.0%

[a]Each final population value is the mean of five replicates. The analysis of variance is presented in Table A. 4.

Table 18. The effect of constant temperature on the length of adult, female *Tylenchorhynchus nudus* on spring wheat in the greenhouse.

Temperature degrees centigrade	Length in mm.[a]	
	Vienna loam	Blendon loamy sand
10	.808	.802
15	.848	.842
20	.858	.858
25	.876	.863
30	.835	.837
32.5	.856	.865

[a]The analysis of variance is presented in Table A. 5.

DISCUSSION

Survey

Figure 1 shows *Tylenchorhynchus nudus* was found more frequently in the eastern half of South Dakota, but in lesser numbers throughout the remainder of the state. Table 2 shows *Tylenchus* and *Paratylenchus* were encountered in 94% of the samples. *Tylenchorhynchus,* including *T. nudus,* occurred in 93% of the samples and *Helicotylenchus* was found in 85% of the samples. Several species of *Tylenchorhynchus* and *Helicotylenchus* have been commonly associated with turf grasses and *H. digonicus* has been shown to be pathogenic to Kentucky bluegrass (34). The remaining genera were found comparatively infrequently and usually in low numbers (Table 2). A comparison of nematode numbers within or among genera may be misleading since environmental conditions varied considerably with locations and sampling dates.

The results of the Brookings, South Dakota, survey (Table 3) show *Tylenchus, Boleodorus* and *Paratylenchus* were present in all of the samples and often in high numbers. *Helicotylenchus, Hoplolaimus, Tylenchorhynchus* and *Xiphinema* were also found frequently; and a wide variety of hosts, including several grasses, are known to be attacked by certain members of these genera. Table 3 also shows

Tylenchorhynchus nudus was encountered more frequently and in higher numbers than in the state-wide survey.

Table 4 shows *Tylenchus* and *Xiphinema* were found in all of the samples from bluegrass seed-producing areas and *Helicotylenchus, Paratylenchus* and *Tylenchorhynchus* were of nearly equal occurrence. The average nematode numbers were usually higher than those obtained in the two previous surveys and the earlier sampling date may have been responsible.

Host suitability

Common Kentucky bluegrass was the most favorable host for *Tylenchorhynchus nudus* as indicated by population levels presented in Table 5. All of the grasses tested were considered as hosts for *T. nudus* since the minimum increase was five-fold for the poorest host, perennial ryegrass. The necessity of a grass host for the reproduction of *T. nudus* is apparent when the number of nematodes recovered from the fallowed soil is compared with other treatments.

The results shown in Table 6 indicate *Tylenchorhynchus nudus* was unable to compete well with indigenous nematode populations. One possible reason for the low populations of *T. nudus* may be the assumed bisexual nature of this nematode, whereas *T. maximus* and several species of

Helicotylenchus have been described as monosexual. The ability of a plant to support only certain numbers of nematodes as well as the effects of other soil microorganisms are other factors that should be recognized in the interpretation of Table 6. The presence of Kentucky bluegrass appears to be necessary for the reproduction of Tylenchus, Helicotylenchus and the three species of Tylenchorhynchus. The decline of Paratylenchus, Pratylenchus and Hoplolaimus numbers may be due to the lack of suitable hosts or a seasonal reproductive cycle.

Pathogenicity tests

Results presented in Tables 7 and 8 indicate Tylenchorhynchus nudus was more pathogenic to Kentucky bluegrass under stress conditions. When plants were subjected to a nutrient or water stress, or both, pathogenicity was significantly increased as indicated by the greater percentage reduction in clipping weights. The nematode was also pathogenic with regular watering and fertilizer applications as indicated by a 9% reduction of clipping weights in Vienna loam and a 7% reduction in Dickie sandy loam. The analysis of variance (Table A. 1) revealed a significant interaction involving water, nutrients and nematodes. The investigation of the simple effects of these factors indicated the comparatively low

21% reduction obtained in the Vienna loam, nutrient, and water stress treatment to be responsible for the interaction.

Effects of various treatments were not as evident in reduction of root and crown weights (Table 7 and 8). The plants had grown for 7 1/2 weeks prior to initiation of nutrient and water stress treatments, which may account for the lack of effect of these treatments. Soil type may also have influenced results since in Dickie sandy loam a reduction in root and crown weights similar to clipping weights occurred, whereas in Vienna loam no similar pattern was apparent. No obvious reduction in feeder roots or other signs of nematode injury were observed in examinations of the root systems. The analysis of variance (Table A. 2) revealed a significant interaction. Subsequent investigation indicated the 4% reduction obtained in the Vienna loam, regular nutrients, and water stress treatment to be responsible. All reductions due to nematodes were statistically significant and the greatest reduction in both clipping and root and crown weights occurred in those treatments in which nutrients were withheld.

The number of <u>Tylenchorhynchus nudus</u> recovered at the conclusion of Kentucky bluegrass pathogenicity test I

(Table 9) was considerably higher than those in the host suitability test. The longer duration and higher initial inoculum levels of the pathogenicity test would doubtless account for the higher nematode numbers.

Results of the environmental control chamber experiment (Table 10) show a 28% reduction of clipping weights and a 36% reduction of root and crown weights following inoculation with Tylenchorhynchus nudus when compared with the non-inoculated treatment. Pots in the environmental chamber were not subjected to the sun and the accompanying hot spots, unequal drying and shrinkage of the root ball as was observed in some greenhouse pots. Therefore, a greater percentage of the roots, a majority of which are usually found adjacent to the pot wall, would be available to the nematodes. This may explain the greater degree of pathogenicity in the environmental chamber test when compared to those treatments not under stress in the Kentucky bluegrass pathogenicity test I.

Pathogenic capabilities of Tylenchorhynchus nudus on the growth of Manitou spring wheat were determined. The total above ground weight of the wheat plants was reduced by 13% in Vienna loam and by 9% in Blendon loamy sand in part I (Table 11). The analysis of variance (Table A. 3) revealed a significant difference between inoculated and non-inoculated treatments. Lack of a significant interaction indicated that effects of the nematode were the same in both soil types even though overall plant growth was significantly less in Blendon loamy sand.

The effects of *Tylenchorhynchus nudus* on the dry weight of heads, straw and roots were measured in part II, (Table 12). The analysis of variance (Table A. 3) indicated that all reductions in inoculated treatments were significant. As in part I, no significant interactions occurred indicating that the effects of the nematode were the same in both soil types. The weights of heads and straw were significantly reduced in Blendon loamy sand although no significant reduction due to soil type occurred for root weights. The high initial inoculum level of *T. nudus* (20,000) was used because of the shorter period of time in which spring wheat becomes established when compared to Kentucky bluegrass, and also the limited three to four month period of growth of spring wheat. The number of *T. nudus* recovered from the inoculated treatments at the conclusion of Part II was only slightly higher than the initial inoculum level, with 29,574 recovered from Vienna loam and 23,707 in Blendon loamy sand. These slight increases indicate that the initial inoculum levels were near the optimum number of nematodes that could be supported by spring wheat under the conditions of the test.

Control

The Zinophos and Thimet combination appears to have been the most favorable nematicide treatment as indicated by the clipping weights (Table 12). However, the Zinophos and Thimet treated areas differed significantly from the check areas only for the three clipping dates in 1967. Clipping weights of the Sarolex treated areas also were significantly greater than the check areas during 1967, although they were consistently less than the Zinophos and Thimet treated areas during the same period. Dasinit also significantly increased the weight of the initial clipping, but not in the remainder of the experiment. Nemagon and MoCap did not significantly increase clipping weights. None of the treatments differed significantly from the check in clipping weights in 1968. The previously described phytotoxic effect did not slow the growth rate of the grass as indicated by clipping weights. The 1968 clippings were secured earlier in the season, and the accompanying cooler temperatures and adequate rainfall may have accounted for the lack of significant differences in clipping weights during this period.

All of the nematicide treatments significantly reduced the stylet-bearing nematode populations in the Kentucky bluegrass field plots (Table 13). The Zinophos and Thimet combination was consistently superior to the other treatments in nematode control for the duration of the experiment. Six weeks after treatment the nematode populations in the Zinophos and Thimet areas were approximately 1/6 of those in the check areas. Thirteen months after treatment the nematode populations were about 1/10 of the check. The remaining nematicide treatments significantly reduced nematode populations through May 24, 1968. However, thirteen months after treatment nematode populations in the Sarolex and MoCap treated areas did not differ significantly from the check and nematode control appeared to be deteriorating in the Nemagon and Dasinit treated areas. As noted earlier, Dasinit, Nemagon, MoCap, Sarolex and Thimet (Phorate) have been used successfully to control nematodes in turf. With the possible exception of Nemagon, the majority of the work concerned with these nematicides has been conducted in the Southern states, notably Florida. Sand content of Florida soils is reportedly considerably higher than that of South Dakota soils, and this may account for the comparatively poor response, due to poor soil penetration, of the majority of the nematicides used in the present study.

Zinophos and Thimet comparison

Clipping weights of the Zinophos and Thimet treated areas differed significantly from the Thimet treated areas only for the first clipping date (Table 14). These data also show that the Zinophos and Thimet combination was significantly superior to Zinophos alone at the second and third clippings. None of the 1968 clippings differed significantly. The phytotoxic effect was again apparent in the Zinophos and Thimet and Thimet alone treated areas. The symptoms and duration of the effect were similar to those noted in the original experiment and thus it would appear that Thimet was responsible for the phytotoxic effect.

Zinophos and Thimet in combination and Thimet alone were superior in nematode control to Zinophos alone (Table 15). These data also indicate that Thimet was largely responsible for the nematode control obtained in the areas treated with a Zinophos and Thimet combination. If the high clay content of the soil in the experimental area was a factor in the response of the nematicides, the systemic action of Thimet would be advantageous, since the thorough soil penetration required of the other nematicides would not have been as essential for Thimet.

Temperature study

Results of temperature tests (Table 16) indicate 30°C. was the optimum temperature for reproduction of Tylenchorhynchus nudus. Table 16 also shows that little or no reproduction occurred at 10° or 15°C. and that the lower threshold for reproduction of T. nudus probably lies between 15° and 20°C. The increase in nematode numbers from 20° to 25°C. was about tenfold and from 25° to 30°C. the numbers nearly doubled. At 32.5°C. the number of T. nudus recovered from Vienna loam showed a significant decrease and in Blendon loamy sand a slight decrease occurred. The analysis of variance (Table A. 4) revealed that in Vienna loam a significant difference existed between nematode numbers for all temperatures except 10° versus 15°C. In Blendon loamy sand similar differences occurred with the exception that the nematode population reduction from 30° to 32.5°C. was not significant.

Table A. 4 also showed a significant difference in nematode numbers between soil types. At 20°, 25° and

30°C. the number of Tylenchorhynchus nudus recovered from Vienna loam was significantly higher than those recovered at corresponding temperatures in Blendon loamy sand. The host plant used in the temperature test was Manitou spring wheat. As in the pathogenicity test the wheat grew substantially better in Vienna loam,and at 20°, 25° and 30°C. the total dry weight of wheat plants grown in Vienna loam was 30 to 50% higher than wheat grown in Blendon loamy sand. The improved wheat growth in Vienna loam may account for the higher numbers of T. nudus recovered from this soil type. At 10° and 15°C. about 60% of the nematodes were adults which seems to indicate that the adult stage is most resistant to adverse conditions and may be the principal overwintering stage of T. nudus. The true optimum constant temperature for reproduction of T. nudus would seem to lie between 25° and 30°C. since the sharp drop in nematode numbers between 30° and 32.5°C. indicates that nematode numbers were declining rapidly at temperatures above 30°C.

Results presented (Table 17) indicate that temperature did influence the morphology of Tylenchorhynchus nudus. The length of adult, female T. nudus increased with increasing temperature up to 30°C. at which point a significant decrease in length occurred, however, at 32.5°C.

nematode length significantly increased. The analysis of variance (Table A. 5) revealed that increases in length from 10° to $15^{\circ}C$. in both soil types were significant and in Blendon loamy sand the increase in length from 15° to $20^{\circ}C$. was also significant. The difference in length occurring from 20° to $25^{\circ}C$. was not significant in either soil type. Table A. 5 also shows the number of observations at each temperature. The original number of 100 observations at each temperature was not obtained due to a poor cover slip seal on some of the slides and subsequent loss of the specimens.

SUMMARY

Tylenchorhynchus nudus was generally distributed throughout South Dakota, but was more prevalent in the eastern portion of the state. No correlation was found between generalized soil associations of South Dakota and distribution of *T. nudus.* The nematode was also found frequently in lawns in Brookings, South Dakota and in South Dakota Kentucky bluegrass seed-producing areas.

The greatest reproduction of *Tylenchorhynchus nudus* occurred on Common Kentucky bluegrass, although species tested of *Agrostis, Festuca* and *Lolium* were also parasitized by *T. nudus.* In greenhouse experiments *T. nudus* was found to be more pathogenic to Kentucky bluegrass under nutrient and water stress conditions, although significant clipping weight reductions also occurred with regular water and nutrient applications. The root and crown weight of bluegrass was also reduced by *T. nudus.* Clipping and root and crown weights of bluegrass plants grown under controlled conditions in an Isco environmental chamber were significantly reduced by *T. nudus.* The growth of Manitou spring wheat was reduced by *T. nudus* in greenhouse experiments.

Treatment with Zinophos and Thimet in combination resulted in the greatest increase in turfgrass growth. Dasinit and Sarolex also increased turf growth, but no significant increases resulted following application of MoCap or Nemagon. No significant growth increases were observed the following season. Initially all of the nematicides significantly reduced nematode populations in turf. Thirteen months after treatment nematode control was not observed in the MoCap and Sarolex areas and appeared to be deteriorating in the Dasinit and Nemagon plots. The Zinophos and Thimet treatment was consistently superior in nematode control throughout the experiment; and thirteen months after treatment, nematode populations were 1/10 of those in check areas. Thimet was found to be responsible for a phytotoxic effect in areas treated with a Zinophos and Thimet combination, but also was primarily responsible for the nematode control achieved in these areas. The optimum, constant temperature for reproduction of T. nudus on wheat was 30°C. Temperature also appeared to influence the morphology of T. nudus.

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APPENDIX

Table A. 1. The analysis of variance for clipping weight data obtained in the Kentucky bluegrass pathogenicity test I, conducted in the greenhouse.

Source	d.f.	s.s.	m.s.	F
Treatments	15	5751.39		
Soil Type (A)	1	67.49	-	73.28**
Nutrients (B)	1	5573.73	-	6051.82**
Water (C)	1	4.47	-	4.85**
Nematodes (D)	1	84.54	-	91.79**
AB	1	1.36	-	1.48
AC	1	0.11	-	0.12
AD	1	1.12	-	1.22
BC	1	1.03	-	1.12
BD	1	6.94	-	7.54**
CD	1	1.50	-	1.63
ABC	1	0.95	-	1.03
ABD	1	0.50	-	0.54
ACD	1	0.02	-	0.02
BCD	1	7.30	-	7.93**
ABCD	1	0.33	-	0.36
Error	73	67.21	0.921	

Due to the significant BD and BCD interactions the simple effects of D for B and C were investigated.

			F
S.S. of D within C_1 for B_1[a]	15.62	-	16.96**
S.S. of D within C_1 for B_2	16.14	-	17.52**
S.S. of D within C_2 for B_1	62.05	-	67.37**
S.S. of D within C_2 for B_2	6.47	-	7.02**

[a]Two levels of each factor were used: The upper level of B indicates nutrients were applied regularly and the upper level of C indicates water was applied regularily.

**Significant at the .01 level.

*Significant at the .05 level.

Table A. 2. The analysis of variance for root and crown weight data obtained in the Kentucky bluegrass pathogenicity test I, conducted in the greenhouse.

Source	d.f.	s.s.	m.s.	F
Treatments	15	7657		
Soil Type (A)	1	3267	-	134.33**
Nutrients (B)	1	1838	-	75.58**
Water (C)	1	551	-	22.67**
Nematodes (D)	1	1552	-	63.81**
AB	1	37	-	1.52
AC	1	40	-	1.64
AD	1	1	-	0.04
BC	1	63	-	2.59
BD	1	15	-	0.62
CD	1	60	-	2.47
ABC	1	40	-	1.64
ABD	1	6	-	0.25
ACD	1	103	-	4.24*
BCD	1	29	-	1.19
ABCD	1	55	-	2.26
Error	73	1775	24.32	

Due to the significant ACD interaction the simple effects of D for A and C were investigated.

	s.s.		F
S.S. for D at A_1 for C_1[a]	345	-	14.19**
S.S. for D at A_1 for C_2	442	-	18.17**
S.S. for D at A_2 for C_1	817	-	33.59**
S.S. for D at A_2 for C_2	113	-	4.65*

[a]The upper level of A indicates Dickie sandy loam. The upper level of C indicates water was applied regularly.

**Significant at the .01 level.

*Significant at the .05 level.

Table A. 3. The analysis of variance for data obtained in the spring wheat pathogenicity tests conducted in the greenhouse.

Part I Source	d.f.	s.s.	m.s.	F
Treatments	3	3.85	1.28	
Nematodes (A)	1	1.16	-	11.048**
Soil Type (B)	1	2.58	-	23.571**
AB	1	0.11	-	1.048
Error	15	1.58	0.105	

Part II Source	d.f.	s.s.	m.s.	F
		Heads		
Treatments	3	3.21	1.07	
Nematodes (A)	1	1.86	-	29.523**
Soil Type (B)	1	1.30	-	20.634**
AB	1	0.05	-	0.794
Error	15	0.94	0.063	
		Straw		
Treatments	3	1.72	0.57	
A	1	1.18	-	
B	1	0.53	-	26.222**
AB	1	0.01	-	11.778**
Error	15	0.67	0.045	
		Roots		
Treatments	3	0.49	0.163	
A	1	0.47	-	58.75**
B	1	0.02	-	2.50
AB	1	-	-	
Error	15	0.12	.008	

**Significant at the .01 level.

Table A. 4. The analysis of variance for nematode population data obtained in the temperature test.

Vienna loam	t values	Blendon loamy sand	t values
10° vs. 15°	0.250	10° vs. 15°	0.150
15° vs. 20°	12.265**	15° vs. 20°	11.663**
20° vs. 25°	7.390**	20° vs. 25°	16.066**
25° vs. 30°	4.565**	25° vs. 30°	5.660**
30° vs. 32.5°	2.566*	30° vs. 32.5°	0.989

Vienna loam vs. Blendon loamy sand	
10°	1.060
15°	2.269
20°	4.042**
25°	6.344**
30°	4.891**
32.5°	1.907

**Significant at the .01 level.

*Significant at the .05 level.

Table A. 5. The analysis of variance for nematode length data obtained in the temperature test.

Vienna loam	t values	Blendon loamy sand	t values
10°(83)vs. 15°(54)[a]	3.721**	10°(59)vs. 15°(57)	3.153**
15° vs. 20° (20)	0.271	15° vs. 20° (76)	2.041*
20° vs. 25° (41)	1.140	20° vs. 25° (60)	0.543
25° vs. 30° (60)	4.300**	25° vs. 30° (91)	3.315**
30° vs. 32.5° (59)	2.041*	30° vs. 32.5° (70)	3.636**

[a]The figure in parenthesis indicates the number of of observations for the temperature.

**Significant at the .01 level.

*Significant at the .05 level.