PATHOGENICITY, LIFE CYCLE AND HOST RANGE OF *Meloidogyne naasi* Franklin FOUND ON SORGHUM IN KANSAS

by

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INTRODUCTION

In the spring of 1966, young sorghum plants were brought to the Kansas State University nematology laboratory to be checked for nematode damage. These plants were found to be heavily infected with a nematode morphologically similar to Meloidogyne spp. Further inquiry revealed that irregular areas of the field from which the sorghum plants were taken contained stunted and infected plants. The cropping history disclosed that wheat, corn, and sorghum had been grown on this land for several years. This is contrary to the general idea that root-knot nematodes present in the area neither materially increase on nor damage such crops as sorghum.

The potential of this parasite to cause significant losses to crops basic to Kansas agriculture dictated that attention be given to species identification, life cycle, pathogenicity evaluations and host range studies. The following report deals with the above aspects of the nematode in varying degrees of thoroughness.

REVIEW OF LITERATURE

The plant disease known as root-knot, caused by nematodes, was first described by Berkeley (2) in England. Since the importance of the disease was recognized, root-knot nematodes have been the subject of continuous research. Presence of root-knot nematodes in the U.S.A. was first reported by Atkinson (1) and Neal (21).

Root-knot nematodes were considered as one species until Chitwood (5) differentiated and described 5 species and 1 subspecies. Previously they had generally been known either as Heterodera radicicola (Greeff) Muller, or H. marioni (Cornu) Goodey (17). Chitwood (5) revived the generic name Meloidogyne Goeldi.

Meloidogyne spp. as far as is known, are obligate parasites. Root damage attributable to Meloidogyne spp. ranges from hypertrophy and hyperplasis to necrosis. Symptoms such as galls, coarse root development, accelerated root branching, reduction in overall plant growth and devitalized roots are common and may be found in several combinations on a host (7).

Grasses are generally poor or immune hosts to most of the species of Meloidogyne. However, M. naasi Franklin, M. acronea Coetzee and M. graminicola Golden and Birchfield have been described from graminous hosts and do not attack many of the common hosts highly susceptible to other species of Meloidogyne. The review of literature as represented here is basically concerned with the above named species because of their morphological and host range similarities.

M. naasi was described from cereals, grasses and sugarbeets in west and southwest England and Wales (19). The first infestation was found distributed over a field on Italian ryegrass. In 1956 barley was found to be a host. Field and laboratory experiments continued on the host range and several cereals, grasses, sugarbeets, and a few weeds were found to be suitable (12). Morphological studies by Mary T. Franklin (12) demonstrated that the nematode could not be assigned to any known species of root-knot nematode. She described the species as M. naasi. Recently, M. naasi has been reported damaging barley in California (13) and bentgrass in Illinois (18).

M. acronea was described from specimens collected in South Africa from the roots of Sorghum vulgare which was reported to be a "radar" host to this species (8). Taxonomic descriptions and host range evaluations were reported by Coetzee (8).

M. graminicola was found in the roots of barnyard grass, Echinoclea colonum in a field near Baton Rouge, Louisiana by Birchfield (3). He reported that this nematode was probably a new species of Meloidogyne because of its morphological structures and differential host range. Several grasses, among them oats, were found to be good hosts in greenhouse experiments. However, a number of plants, including cotton, pepper, watermelon, corn and tomato, commonly parasitized by Meloidogyne spp. normally found in the area were not hosts for this species. M. graminicola was described as a new species by Golden and Birchfield (14).

MATERIALS AND METHODS

Soil was collected from a M. naasi infested field 2 miles west and 1-1/2 miles south of Linwood, in Leavenworth County, Kansas. The infested soil was taken from 2 to 12 in. depths at random with a shovel, placed in 6- 1.83 cu ft., containers and transferred to the greenhouse. All the nematodes used in this study came from this sample.

Identification

Extracting, fixing and processing: Eggs, larvae, and female specimens were collected from infected 'RS-610' sorghum root tissues that had been stained with acid-fuchsin for 1 to 2 minutes. To collect male specimens, galled root tissues were washed and placed on an 80 mm diameter copper sieve which was placed over a Baerman funnel in a low volume-spray moist chamber. A rubber tube placed on the bottom of the funnel was closed with a pinch-cock. Nematodes collected in the tube of the funnel were periodically drawn into a watch glass. Samples were examined under a dissecting microscope. Second stage larvae and male specimens were "picked" by a small needle and placed in water in a Bureu Plant Industry watch glass. "Picked" specimens were relaxed by holding the watch glass over an alcohol flame for a few seconds. After they were relaxed, the nematodes were transferred to a fixative; 10 ml 40% formaldehyde, 10 ml acetic acid and up to 100 ml distilled water (16). The nematodes were left in fixative overnight and then transferred to a mixture of 20 parts 95% ethanol, 1 part glycerol, and 79 parts distilled water (22). The nematodes in this mixture were kept in a

closed vessel containing an excess of 95% ethanol. They were left in this saturated atmosphere for at least 12 hours at 35–40°C. This arrangement removed most of the water and left the nematodes in a mixture of glycerol and ethanol. A mixture of 5 parts glycerol and 95 parts ethanol was added to the contents of the watch glass containing the nematodes and was placed in a partly closed petri dish to allow the mixture to evaporate for at least 4 hours or until practically all of the alcohol was gone. The BPI watch glass was placed in a desiccator to remove all remaining water and alcohol. This method was preferred because it was rapid and specimens were satisfactory for permanent mounting.

Mounting: Eggs, larva, male, female, and perineal patterns of female specimens were mounted in anhydrous glycerin on Cobb aluminum slides. In preparing the perineal patterns of mature females, the following method was used. Collected mature egg laying females were transferred into 100% glycerin solution on a piece of X-ray film under a dissecting microscope. The posterior portion of female body was cut off with a sharp knife made from sewing needle and the ends of the cuticle piece were trimmed by the knife. These perineal patterns were mounted in anhydrous glycerin on Cobb aluminum slides (24).

Measuring: Mounted specimens were examined carefully under the high dry and oil immersion objectives of the microscope. The following characteristic measurements of each specimen were taken, as described by De Man (20).

Egg: Length, width, and length-width ratio

Larva: Length, width, stylet, tail, a, $\left(a = \frac{\text{Total body length}}{\text{Greatest body width}}\right)$ and b, $\left(b = \frac{\text{Total body length}}{\text{Distance from head end to end of esophagus}}\right)$

Male: Length, width, stylet, lateral field, a and b as given above. Female: Length (including neck), stylet, location of excretory pore, and a as given above. The measurements and the calculation of the degree of magnification were made by the use of a stage micrometer and a camera lucida. The camera lucida consisted of a split beam prism on the right eyepiece, a 10x16 in first surface mirror held by a separate support that could be moved into desired position and a drawing board inclined at right angles to the axis of the microscope eyepieces.

Life cycle

Sixty small pots were filled with non-treated infested soil. Each pot was seeded with 'RS-610' sorghum seeds and placed in the greenhouse at 30° C. Plants were removed from 4 pots every 2 days. Root systems were washed, stained, and stored in lactophenol. Stained roots were examined under a dissecting microscope. The infection by nematodes and the results of their injury to the plants were followed in every stage of the nematode development. Selected samples were mounted in anhydrous glycerin on Cobb aluminum slides.

Pathogenicity

One-third of the soil sample was treated with D-D(1-3 dichloropropene, 1-2 dichloropropane) mixture at a rate of 20 gal/A. The amount of fumigant for the sample was calculated according to Elmer (11) at a rate of 1.7380 ml per linear or square ft. The containers containing fumigated soil were covered and sealed with plastic for 9 days.

To set up the pathogenicity test, 10 washed plastic pots were filled with D-D treated soil, and 10 were filled with nontreated soil. Each pot

was seeded with several sorghum seeds and was placed in the greenhouse at 28-30°C. Watering was generally applied at 2 day intervals. Plants were through the soil in 5 days and were thinned to 5 plants/pot at this time. Starting at the 5th day after emergence, top measurements of the sorghum plants were taken in cm for 60 days at 5 day intervals.

On the 65th day after emergence (70th day after planting), diameter of stems was measured in mm at the second node above the soil level.

On the 74th day after planting, plants were removed from the pots and the roots washed under low pressure of water spray to free them of adhering soil particles. Excess water was removed by blotting the roots with paper towels. Blotted roots were weighed. One gr of infected roots was taken from each of the 10 infected pots. Each sample was placed in boiling 0.05% acid fuchsin in lactophenol solution for 1-1/2 to 2 minutes to stain and relax the nematodes. Excess surface stain was rinsed off by low pressure of tap water. Stained roots were left in lactophenol for at least 3 days to clear acid fuchsin from the unstained root tissues. Stained roots were examined under the dissecting microscope and the number of females, males, larvae, and egg masses were counted. When nematode concentrations were so high that direct counting was impossible, the number was approximated to determine the rate of infection.

Fresh weights were taken of roots and tops of plants grown in treated and non-treated soil and then they were transferred to paper sacks. These paper sacks with their contents were placed in an oven at 110°C and allowed to dry. Seventy-two hours later dry weights were taken in gr.

Host range

To determine the infection possibilities of *M. naasi* on different host

plants, 18 kinds of plants were selected as listed in Table 1. Infested soil was placed in 72, 4-inch diameter pots, and 8-10 seeds of each kind of plant were sown in each of 4 pots. Plants were thinned after emergence so that each pot contained no more than 5 plants. On the 68th and 95th day after planting, plants were carefully removed from 2 pots of each plant species. After the plants were washed, foliage was removed and roots were stained in the laboratory by using the same acid fuchsin in lactophenol method as described above. Stained roots were kept in small jars for at least 3 days before they were examined under the dissecting microscope.

For resistance or susceptibility rating of tested plants the method described by Southney (23) was used. He suggested that plant resistance to Meloidogyne sp. include; (1) Those whose roots which were not penetrated by the larvae, (2) Those into which larvae entered freely but soon died, (3) Those in which development started but was limited, (4) Those in which development was completed by only a few individuals and which there was an abnormally long time with a very low final reproductive rate. He also suggested that, in some resistant plants galls may be present, containing dead nematodes, while susceptible may be gall-free and yet carry a thriving nematode population. For this reason, estimates of resistance cannot be based on the degree of galling alone without reference to the reproductivity of the nematode.

Table 1. List of plants tested for host range.

Common name	Scientific name
Barley 'Meimi'	Hordeum vulgare L.
Beet 'Detroit red'	Beta vulgaris L.
Buffalograss 'W2F2'	Buchloe dactyloides (Nutt.) Engelm.
Corn 'Pioneer 321'	Zea mays L.
Fescue 'K. 31'	Festuca elatior Huds.
Indiangrass 'SYN^{3} 2'	Sorghastrum nutans L. Nash
Kaw big bluestem 'KG-1579'	Andropagan gerardi Vitman.
Blue grass 'Kentucky'	Poa paratensis L.
Oat 'Lanka'	Avena sativa L.
Peanut '647 Early Spanish'	Arachis hypogaea L.
Pepper "California wonder'	Capsicum frutescens var. grossum Sendt.
Rye 'Bulboa'	Secale cereale L.
Sorghum 'RS 610' Hybrid sorghum combine Kafir 60 x combine 7078	Sorghum vulgare Pers.
Soybean 'Clark 63'	Glycine Max (L.) Merr.
Tomato 'Rutgers 885'	Lycopersicum esculentum Mill.
Watermelon 'Charleston gray 62'	Citrullus vulgaris Schaad.
Wheat 'Bison'	Triticum aestivum L.
Zoysia grass 'Meyers'	Zoysia japonica Steud.

RESULTS

Identification

Identification was made by means of morphological structures including measurements of eggs, larvae, male, and female specimens, characteristics of perineal patterns of adult females, and host range. Taxonomically, the species studies was classified as:

Phylum: Nemata

Class: Secernentea (Phasmidia)

Order: Tylenchida

Superfamily: Tylenchoidea

Family: Heteroderidae

Genus: Meloidogyne

Species: M. naasi

Morphology: Female; 25 specimens, stained with acid fuchsin in lactophenol (Fig. 1). Length 0.478-0.690mm (0.564mm); width 0.314-0.436mm (0.368mm); a 1.57-1.601 (1.53); stylet 9.11-12.3 μ (11.7 μ). Female pear shaped with small neck compared to the other known Meloidogyne sp. Cuticle annulations seen clearly around neck and in anus and vulva region. Head slightly offset. Stylet small and knobbed. Distinct excretory pore located between the 9th-15th annule about one-half stylet length behind the head and in front of median bulb (observations of 13 specimens). Esophagus cylindrical and mascular median bulb large and rounded with large sclerotized valve. Three irregular lobes overlayed intestine latero-ventrally. Vulva and anus terminal. Ovaries two. Eggs deposited in gelatinous matrix. Perineal

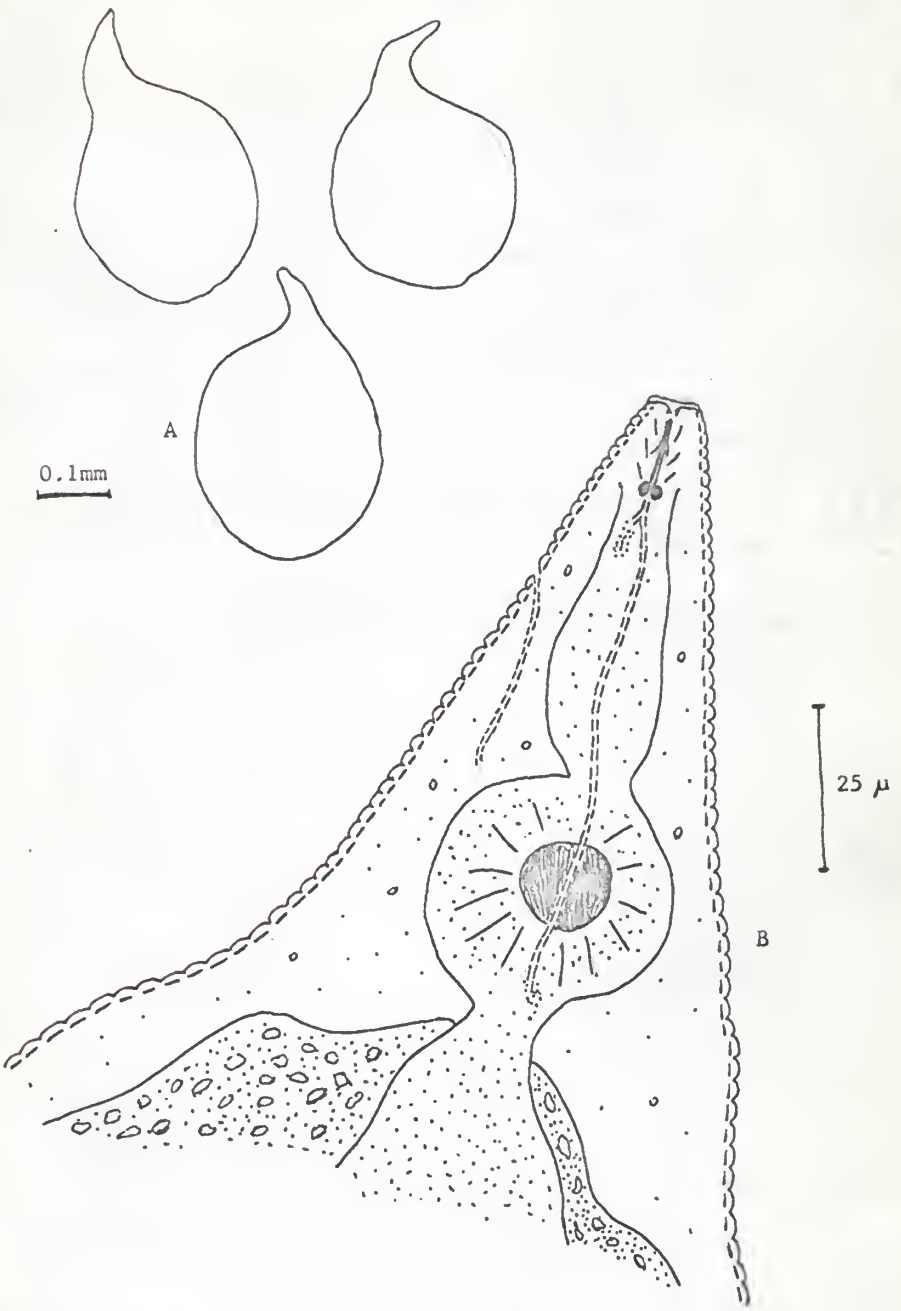

Figure 1. M. naasi, A- Mature females; B- Anterior portion of female.

pattern of female showed two large phasmids (Fig. 2).

Male; 10 specimens, processed by glycerin-ethanol method (Fig. 3C). Length 1.080-1.528mm (1.187mm); width 0.290-0.363mm (0.302mm); a 37.2-42.5 (39.8); lateral field width 6.9-7.8 μ (7.3 μ). Male body cylindrical with slightly tapering neck and blunt tail. Head not offset. Cuticle annulated. Esophagus cylindrical and muscular median bulb spherical with a sclerotized valve. Glandular lobes overlayed intestine latero-ventrally. Lateral field distinct with 4-8 incisures depending on its location (Fig. 3B,C). Spicules arcuate.

Larvae; 50 second stage larvae, processed by glycerin-ethanol method (Fig. 3D). Length 0.398-0.504mm (0.436mm); width 19.7-23.4 μ (21.8 μ); a 21.91-26.52 (23.21); b 2.7-3.8 (2.74); stylet 11.6-13.7 μ (11.97 μ); tail 69-73.5 μ (71.2 μ). Body slender and the anterior portion resembled that of the male. Tail conical with rounded terminus.

Egg; 25 eggs, stained with acid-fuchsin in lactophenol method (Fig. 4). Length 98-103.5 μ (99.3 μ); width 39.5-47.3 μ (44.3 μ); a = 2.24.

The diagnostic characteristics of the species were found to be almost the same as described for M. naasi. The morphological structure, measurements, and host range of M. naasi differed to some extent from all known related species of Meloidogyne but were closest to M. naasi and M. graminicola. Although some of the observed characteristics such as location of excretory pore and the number of incisures in the lateral field created some doubt, the presence of 2 large phasmids in the perineal patterns of females (which is a primary diagnostic character of M. naasi) confirmed the identification as M. naasi.

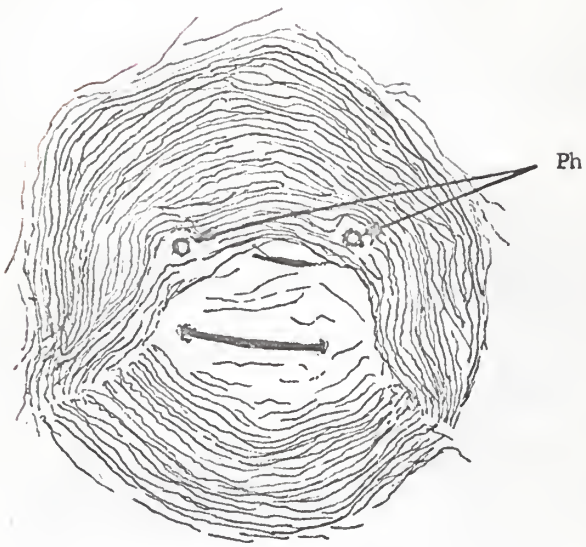

Fig. 2. Diagram of perineal pattern of female; note relatively large phasmids.

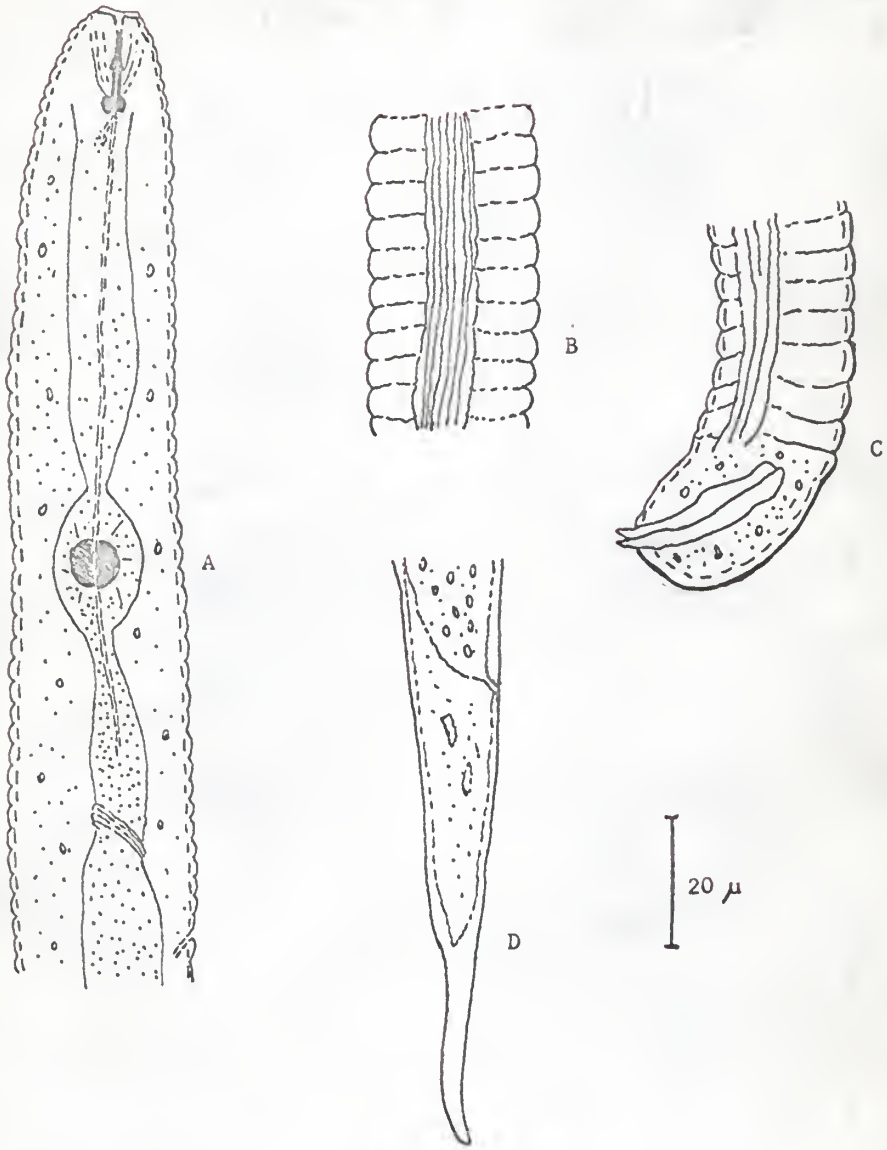

Fig. 3. Adult male and larva, A- Anterior portion of male, B- Lateral field of male, C- Posterior portion of male, D- Tail portion of second stage larva.

Fig. 4. M. naasi eggs before hatching.

Life cycle

Infected root tissues of sorghum stained at 2 day intervals were examined under the dissecting microscope. The first sample was taken 9 days after planting (2 days after emergence). Roots stained from the 9th to 17th day had many second stage and a few third stage larvae which were usually located near the central cylinder (Fig. 5). By the 21st day the nematodes had enlarged (diameter about 1/5 of length) and many had undergone the second moult (Fig. 6). Several of them had completed the third and fourth moults by the 27th day (Fig. 7). The females continued to enlarge in girth, and to some extent in length, until they became pear-shaped with a protruding neck region (Fig. 8). A few females were depositing eggs and a small number of eggs were hatching by the 35th day. The number of egg laying females increased with each successive sampling period. Eggs were deposited in a gelatinous matrix that was around the vulva region. Each egg laying female deposited from 350 to 500 eggs (Fig. 9). Based on the above observation *M. naasi* can complete a life cycle within 35 days after seeding at 30°C on sorghum.

Very few males were found in the root tissues. Males go through a metamorphosis at about the 4th moult and revert from saccate to ellworm shape.

During the development of *M. naasi* histological changes were observed in the sorghum root tissues such as "giant" cell formation and gall production. As a rule, histological changes in the plant tissues from the nematode activity are related to the feeding habits of the nematodes. The root-knot nematodes feed from the contents of "giant" cells which developed around the head of the larvae in the central cylinder portion of the host

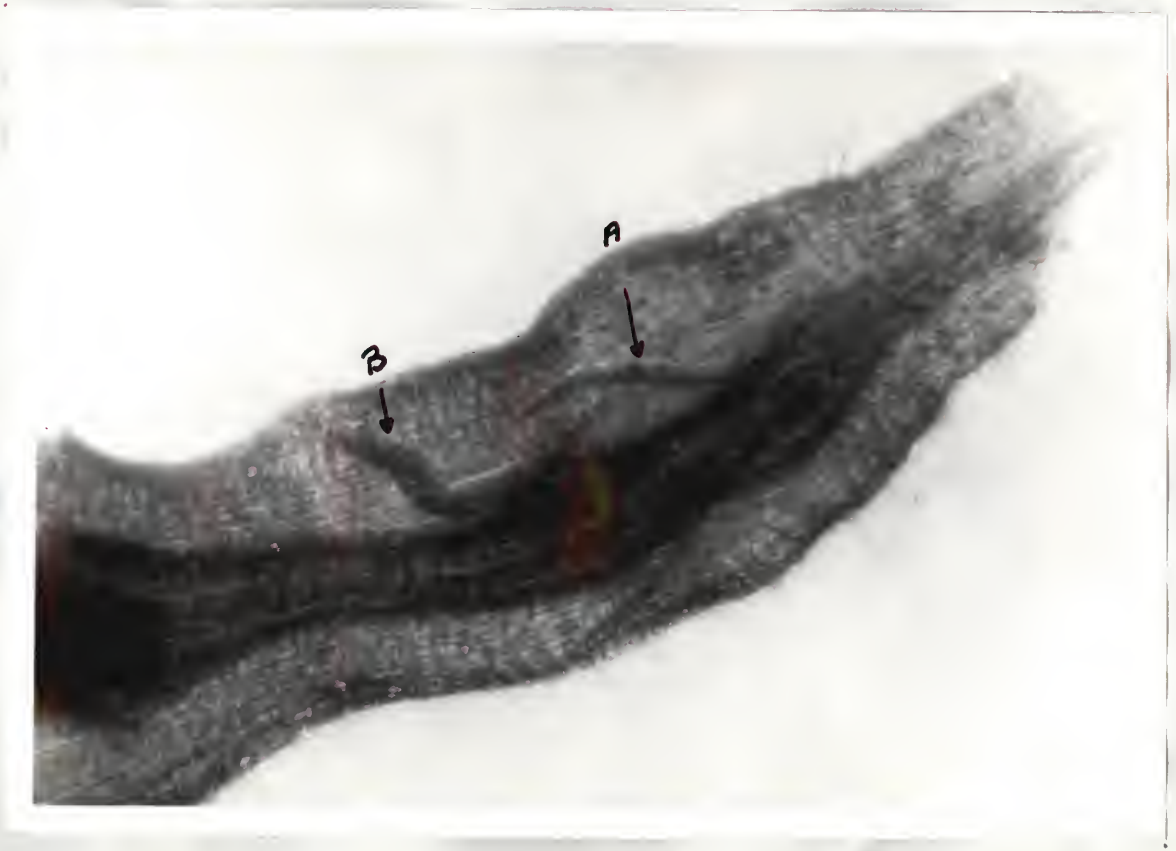

Fig. 5. Second and third stage larvae feeding around the central cylinder of a sorghum root tissue. A- Second stage larvae, B- Third stage larvae.

Fig. 6. M. naasi late second stage larva about to moult.

Fig. 7. M. naasi male just after fourth moult.

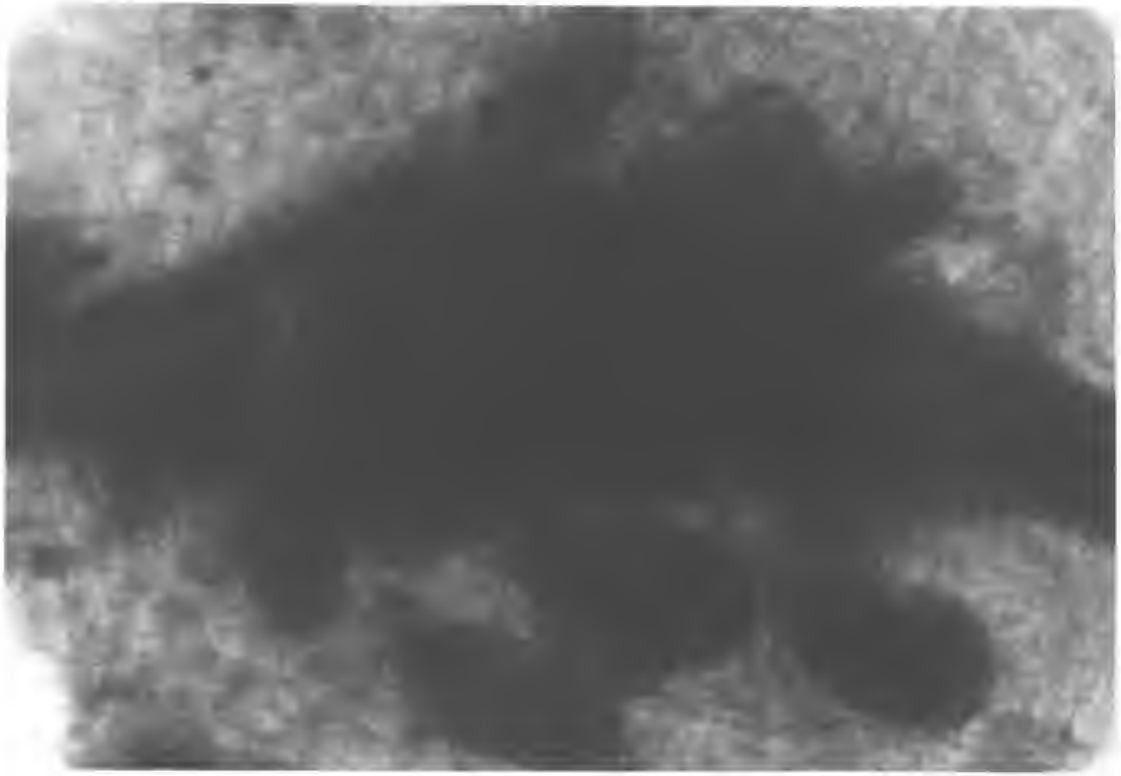

Fig. 8. Pear shaped females around the central cylinder of sorghum root.

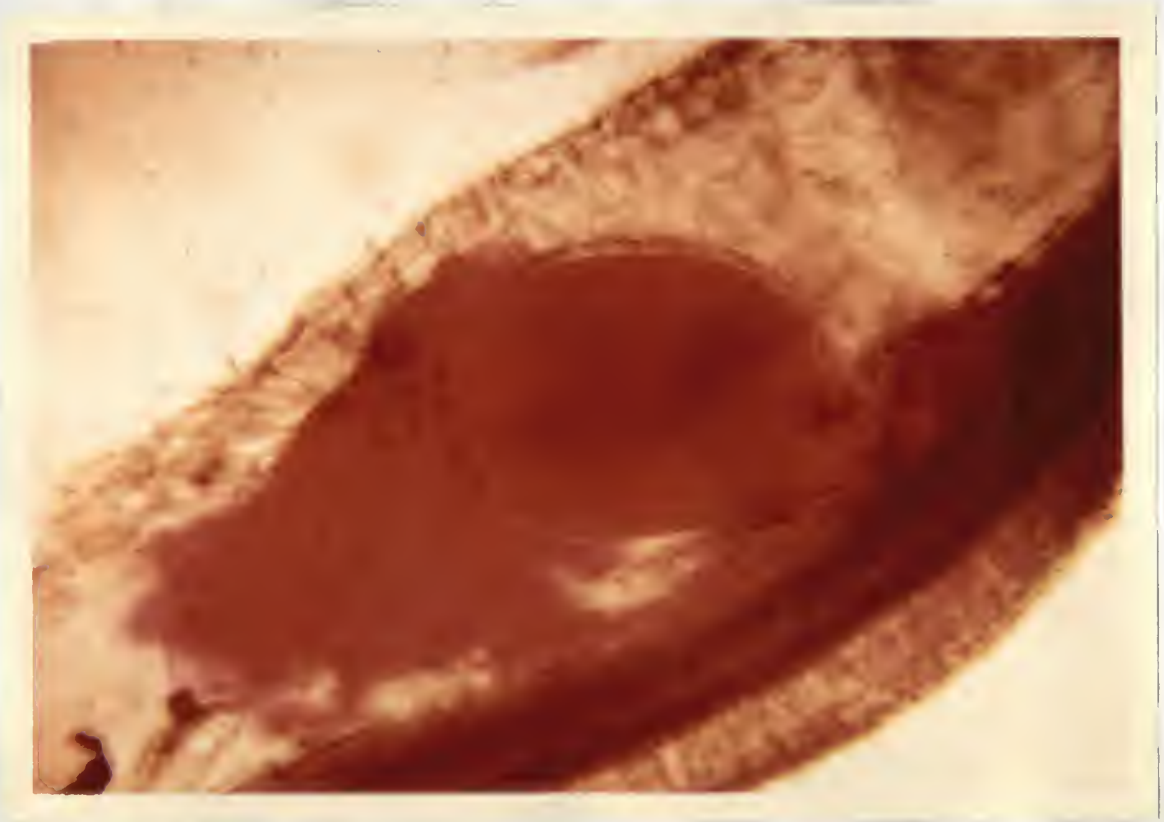

Fig. 9. Egg laying female in the sorghum root tissue.

roots. These "giant" cell developments occurred after 15 to 17 days in the sorghum tissues and were observed by their multinucleate, granulated protoplasm and coalescence of the neighboring cells.

Pathogenicity

The pathogenicity test was designed to measure the difference in plant growth in M. naasi-infested field soil and soil treated with a nematicide (D-D at 20 gal/A) as control. Differences were measured by plant height at different time intervals, stem diameters and fresh and oven dry weights of both above ground parts and roots.

Plant height: Plant heights were measured in centimeters starting 5 days after emergence and continuing for 60 days at 5 day intervals. Plants were not mature at this time but it appeared that growth potential had passed the maximum possible for a relatively small pot experiment. Average differences between replicates of plants grown in infested and control plots were tabulated (Table 2) and are graphically presented in Figure 10. Figure 11 illustrates the overall difference in plant growth.

Differences in height were statistically significant at each of the intervals of measurement (Table 3). Variations within the treatments were significant through the 10th day and leveled off to no appreciable differences until the plants had begun to reach their growth potential in the experiment. Height measurements on the 60th day demonstrated significant variation within the treatments although calculated "T" values remained relatively high. The lack of significant variation within the treatments indicated that treatment affected all the plants equally and that there was an even distribution of nematodes.

Calculated "T" values ranged from 3.23 on the 5th day to a high

Table 2. Height comparison between sorghum plants planted in D-D treated infested soil and sorghum plants planted in nontreated infested soil

Sample	Sampling period after emergence	Replicates										
		1	2	3	4	5	6	7	8	9	10	Average
Treated	5th day	17.20[a]	16.60	18.00	16.74	16.78	17.40	16.84	17.38	16.58	16.42	16.99
Nontreated	" "	15.66	14.32	13.99	14.10	17.06	17.22	16.50	16.50	15.56	15.82	15.66
Treated	10th day	34.56	35.00	36.70	33.38	36.80	34.98	32.54	33.34	35.91	33.10	34.58
Nontreated	" "	28.26	28.00	27.92	32.48	30.75	27.72	31.88	28.87	21.33	23.46	28.06
Treated	15th day	45.38	45.78	42.05	47.72	41.26	41.64	46.18	42.06	44.92	45.20	44.21
Nontreated	" "	30.95	33.24	31.95	34.98	38.56	30.82	34.52	34.37	30.23	24.80	32.44
Treated	20th day	49.20	52.92	52.90	49.14	57.24	51.76	53.26	48.30	57.48	50.78	51.79
Nontreated	" "	38.20	39.82	41.75	41.54	42.47	40.48	40.14	40.62	33.06	31.63	39.00
Treated	25th day	58.76	59.30	58.50	56.20	61.34	51.16	58.50	56.34	54.92	50.22	56.55
Nontreated	" "	42.35	44.62	45.92	44.94	43.32	41.76	41.48	43.25	37.60	32.70	42.28
Treated	30th day	61.24	62.85	61.54	61.17	64.30	61.68	58.28	61.78	66.14	63.24	61.62
Nontreated	" "	42.90	45.58	47.22	45.28	44.10	43.63	43.68	46.02	37.40	45.06	44.05
Treated	35th day	65.14	64.57	63.60	61.85	67.26	63.70	62.82	62.84	63.08	64.48	64.20
Nontreated	" "	44.60	47.95	48.45	46.34	45.34	45.52	47.87	47.66	39.13	48.45	45.76
Treated	40th day	66.20	66.05	63.76	63.06	68.68	65.32	63.96	64.20	63.22	65.50	64.94
Nontreated	" "	45.82	48.80	48.60	47.16	47.74	46.26	48.15	48.77	44.90	48.77	47.55
Treated	45th day	65.58	67.43	65.68	64.58	68.98	67.22	64.14	66.22	64.24	69.44	66.31
Nontreated	" "	46.97	49.25	49.85	47.83	48.24	48.29	49.75	49.70	48.07	49.50	48.13

Table 2 (Concluded).

Sample	Sampling period after emergence	Replicates										Average
		1	2	3	4	5	6	7	8	9	10	
Treated	50th day	68.02	67.80	66.81	67.98	65.42	67.24	66.82	67.08	65.88	69.96	67.02
Nontreated	" "	48.20	49.85	53.86	49.74	51.26	50.07	49.77	53.70	49.64	52.76	50.98
Treated	55th day	68.70	68.20	67.52	68.30	66.46	69.48	68.98	68.08	68.58	70.26	68.25
Nontreated	" "	49.70	50.97	53.00	53.90	51.52	51.25	53.30	53.76	51.24	53.82	52.24
Treated	60th day	69.94	69.47	70.70	68.16	69.16	68.18	68.90	70.50	71.44	68.74	69.39
Nontreated	" "	50.65	51.62	53.33	50.46	50.82	55.43	56.57	55.32	53.76	54.32	53.34

[a]Each figure is the average number of five plants in cm.

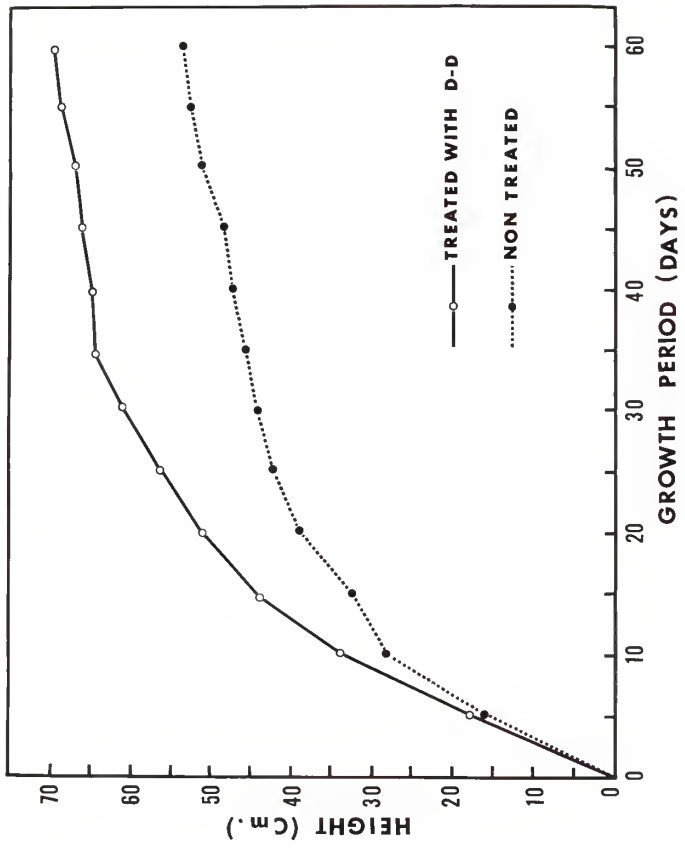

Fig. 10. Graphic illustration of height comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Fig. 11. Comparison of sorghum plants grown in D–D treated infested and nontreated infested soil on the 23rd day after planting.

Table 3. Results of testing equality of means of height at different time intervals.

Sampling period	"T," values	Rejection region of "T"	% 95 Confidence interval
5th day	3.234	T > 2.260000	No confidence interval ($\sigma_1 \neq \sigma_2$)
10th day	5.473	T > 2.260000	No confidence interval ($\sigma_1 \neq \sigma_2$)
15th day	8.6448013	-2.1000 > T > 2.1000	8.916 < DIFF. < 14.638
20th day	8.6396464	-2.1000 > T > 2.1000	10.088 < DIFF. < 16.566
25th day	8.7513263	-2.1000 > T > 2.1000	11.195 < DIFF. < 18.265
30th day	16.8466382	-2.1000 > T > 2.1000	15.874 < DIFF. < 20.396
35th day	17.5413690	-2.1000 > T > 2.1000	15.672 < DIFF. < 19.934
40th day	24.8983846	-2.1000 > T > 2.1000	16.022 < DIFF. < 18.974
45th day	26.1581498	-2.1000 > T > 2.1000	16.193 < DIFF. < 19.019
50th day	22.5171268	-2.1000 > T > 2.1000	14.885 < DIFF. < 17.947
55th day	28.2905614	-2.1000 > T > 2.1000	15.007 < DIFF. < 17.413
60th day	20.737	-2.1000 > T > 2.1000	No confidence interval ($\sigma_1 \neq \sigma_2$)

of 28.29 on the 55th day. Calculated "T" values were down on the 60th day and it was obvious that plants were not going to grow much more in either the infested or the control plots and the experiment was stopped.

Stem diameters: Measurements of stem diameters were taken on the 60th day. Differences between plants grown in infested soil and treated soil were statistically different (Table 4).

Top weights: Fresh top weights were found to be different at the 5% level (Table 5). However oven dry weights were not different at this high of a confidence level (Table 6).

Root weights: Fresh and oven dry root weights were taken (Tables 7 and 8). In both cases the infected root weights were significantly heavier (5% level) than the controls.

Infecting rate: On the 68th day after seeding 1 gr of sorghum roots was taken from each of the 10 infected pots. After they were stained and cleared in lactophenol solution, they were examined under the dissecting microscope. The number of larvae, adult females, egg laying females, and adult males were counted and the eggs in each gelatinous matrix were approximated (Table 9). Those females which were reproducing had deposited approximately 350 to 500 eggs. Some females failed to produce eggs even though they had a gelatinous matrix. The number of adult males was very low with none in most of the samples.

Host range

The stained roots of 18 different kinds of plants grown in M. naasi infested soil were examined under the dissecting microscope. Their resistance or susceptibility to this nematode species was based on entrance, degree of galling, development of the larval stages, and reproduction

Table 4. Diameter of stem comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Sample	Diameter of stems Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Treated	12.46	12.16	13.40	12.94	12.74	11.42	11.52	12.08	11.42	12.62	12.27
Nontreated	8.82	8.95	9.15	9.80	10.36	9.30	8.61	9.06	8.95	8.66	9.10

[a]Each replicate represented the average diameter of 5 stems in mm.

Calculated "T" value: 11.3191781

Rejection region of "T": $T < -2.1000$ or $T > 2.1000$

% 95 confidence interval: $2.533 < \text{"DIFF."} < 3.687$

Table 5. Fresh top weight comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Sample	Fresh top weights Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Treated	45	42	43	44	69	73	85	72	65	80	61.80
Nontreated	37	29	45	43	51	42	38	54	60	39	43.80

[a]Each replicate represents the average fresh top weight of 5 plants in gr.

Calculated "T" value: 2.9948282

Rejection region of "T": $T < -2.1000$ or $T > 2.1000$

% 95 confidence interval: $5.378 < \text{"DIFF."} < 30.622$

Table 6. Dry top weight comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Sample	Dry top portions weights Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Treated	19	17	13	14	23	28	31	27	24	25	22.1
Nontreated	19	16	18	14	17	19	28	23	18	12	18.4

[a]Each replicate represented the average oven dry weight of 5 plants in gr.

Calculated "T" value: 1.5433049

Rejection region of "T": $T < -2.1000$ or $T > 2.1000$

% 95 confidence interval: $-1.335 < \text{"DIFF."} < 8.735$

Table 7. Fresh root weight comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Sample	Fresh root weights Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Treated	64	61	57	55	85	62	64	60	43	49	60.00
Nontreated	69	72	83	108	68	70	110	83	85	89	83.70

[a]Each replicate represented the average weight of 5 plants roots in gr.

Calculated "T": -3.9408279

Rejection region of "T": $T < -2.1000$ or $T > 2.1000$

% 95 confidence interval: $11.166 < \text{"DIFF."} < 36.234$

Table 8. Dry root weight comparison between sorghum plants grown in D-D treated infested soil and sorghum plants grown in nontreated infested soil.

Sample	Dry root weights Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Treated	21	20	19	35	20	22	23	18	19	19	21.60
Nontreated	22	26	28	29	32	23	26	39	31	30	28.60

[a]Each replicate represented the average root weight of 5 plants in gr.

Calculated "T" value: -3.1774445

Rejection region of "T": $T < -2.1000$ or $T > 2.1000$

95% confidence interval: $2.374 < \text{"DIFF."} < 11.626$

Table 9. Infecting rate of *M. naasi* in sorghum root tissues.

The stage of nematode	Number of nematode found in the root tissues Replicates[a]										Average
	1	2	3	4	5	6	7	8	9	10	
Larva[b]	18	13	16	12	29	15	21	16	25	19	18.4
Adult male	--	--	--	--	--	--	1	--	1	--	0.2
Adult female	23	18	29	19	20	31	19	21	12	22	21.4
Egg laying adult female[c]	9	5	11	7	3	12	10	14	8	6	8.5

[a] Each replicate represented a gr of infected sorghum root tissue.

[b] Total number of second, third and fourth stage larvae.

[c] Eggs were in gelatinous matrix and each matrix contained from 350 to 500 eggs.

(Table 10).

These results showed that, among these different kinds of plants, sorghum was highly susceptible, barley, corn, fescue, Indian grass, Kaw big bluestem, and zoysia grass were moderately susceptible, and wheat, buffalo grass, Kentucky bluegrass, oats, beets, peanut, pepper, rye, soybean, tomato and watermelon were resistant to M. naasi.

Table 10. Resistance or susceptibility of tested plants to *M. naasi*.

Tested plants	Entrance	Development	Galling	Reproduction
Sorghum	+	+	++	++
Corn	+	+	+	+
Kaw Big bluestem	+	+	+	+
Zoysia	+	+	+	+
Indiangrass	+	+	±	+
Fescue	+	+	o	+
Barley	+	+	o	+
Rye	+	+	o	o
Wheat	+	+	o	o
Pepper	+	+	o	o
Buffalograss	+	o	o	o
Kentucky bluegrass	+	o	o	o
Watermelon	+	o	o	o
Peanut	o	o	o	o
Beets	o	o	o	o
Oats	o	o	o	o
Soybean	o	o	o	o
Tomato	o	o	o	o

DISCUSSION AND CONCLUSION

Many field and laboratory studies have been made on Meloidogyne spp. There are many similarities among these species, but they differ in their morphological structure, host and nematode physiological response or host range. Even in the same species it is possible to find variability in their morphology and physiologic behavior.

Although M. naasi had been studied and described, another detailed study of the species was deemed feasible because M. naasi is a new species to Kansas, sorghum is a new host and there was very little information on its biology.

Distinct characteristics were found to be common for both M. naasi and M. graminicola, i. e., excretory pore was observed on the 9th to 15th annule behind the head, and the number of incisures in the lateral field varied from 4 to 8. The excretory pore was reported on the 7th to 11th annule behind the head for M. naasi and the number of incisures were reported as 4 (12). M. graminicola was described with an excretory pore located from the 11th to 18th annule behind the head and 6 to 8 incisures in the lateral field (14). Both were reported on graminous hosts. With this information the species could be identified as either M. naasi or M. graminicola. The nematode in this study had 2 large phasmids in the perineal patterns. This is one of the main characteristics for identification of M. naasi. Therefore, even though there was some variation in certain morphological characters and host range it seemed plausible to designate this nematode as M. naasi.

The following strict scientific principles, known as Koch's postulates are normally applicable to given disease situations. (1) Constant association with the disease, (2) Isolation and study of the organism in pure culture, (3) Production of the disease by inoculation of the host with a pure culture of the organism, (4) Reisolation of the disease from the inoculated diseased host confirmation of its identity with the original inoculent. However, these principles are not quite applicable to many nematodes, because they are obligate parasites. Distinct improvement of plant development in the soil treated with a nematicide like D-D indicated that nematodes were cause of disease (23). Accordingly, the difference in growth of plants in D-D treated soil and field soil was interpreted as damage attributed to nematodes.

Differences in height were significant at each of the intervals measured. The magnitude of this difference, i.e. calculated "T" values ranged from 3.23 on the 5th day after emergence to a high of 28.29 on the 55th day. Extrapolating from the data in Table 3, it could be assumed that from the time of germination until the 15th day after emergence there was a relatively high source of infectious larvae and that as new roots were produced they became infected. From the 15th through the 25th day intervals the "T" values remained essentially the same indicating little remaining inoculum potential in the soil. Between the 25th and the 55th day "T" values became progressively larger. This would coincide with the hypothesis that at least the first larvae to infest the roots had completed a life cycle and infectious larvae were again present. When roots grown in infested soil were stained at 2 day intervals it was observed that on the 35th day after seeding some larvae were present in the egg masses. It was concluded

that this nematode can complete its life cycle in 35 or less days at 30°C on 'RS-610' Sorghum.

It was observed that each egg laying female deposited from 350-500 eggs. By the end of the experiment (68 days) there were an average of 8.5 egg laying females/gr of root. Each plant had an average of 16.75 gr of roots. This would mean a potential of at least 49,831 infectious larvae in each plant after the second generation. The maturity span of 'RS-610' sorghum is about 100 days which means that there would be time for a third generation. If only half of the larvae penetrated the roots and reproduced at the same rate there would be 8,720,425 larvae to attack the roots at the time the sorghum was maturing. It is unlikely that such an inoculum potential often develops in the fields because of lower soil temperatures, moisture restrictions, nematode parasites and a limited number of roots available for larvae to enter. However, it does serve to demonstrate that under optimum conditions plenty of inoculum to inflict severe damage to the plants can and does develop.

The number of males was very low with none in most of the samples. Tyler (25) suggested that reproduction without males appeared to be regular and normal and demonstrated that as many as 12 generations could occur in the absence of males. Dropkin (9) also reported that many generations of root-knot nematodes could be produced without males.

There were very striking differences in the degree of susceptibility and resistance to different hosts (Table 10). In one group (sorghum, corn, big bluestem and zoysia), the nematodes entered, developed, reproduced, and swelling or galls were induced. In another group consisting of rye, wheat, and pepper, the larvae entered and developed past the second stage,

but no galls were found and there was no reproduction. Larvae entered watermelon but did not develop past the second stage. This indicates that giant cells were not produced and the nematodes could not feed. There was one group consisting of peanut, beets, oats, soybean, and tomato in which the larvae apparently did not enter.

SUMMARY

A root-knot nematode identified as Meloidogyne naasi was found to be destructive on sorghum in Kansas. Because of the economic potential of this parasite to graminous crops attention was given to identification, life cycle, pathogenicity and host range studies.

Pathogenic effects of M. naasi on 'RS-610' sorghum were determined by comparing the plant growth between sorghum plants grown in D-D treated infested soil and sorghum plants grown in non-treated infested soil. The results showed that M. naasi infected sorghum plants were decreased in height, stem diameters were smaller and fresh and oven dry foliage weights decreased. Fresh and oven dry root weights were increased compared to the sorghum plants grown in D-D treated infested soil.

Roots of the infected sorghum plants were taken every 2 days after emergence (7 days after seeding) for 30 days. The development of the nematode were observed stage by stage and M. naasi, completed its life cycle in, at most, 35 days at 30°C. As a result of nematode attack giant cells were formed in the infected sorghum root tissues associated with the central cylinder and around the head of the larvae.

Eighteen different kinds of plants were included in the host range study. Among these plants, sorghum was highly susceptible, barley, corn, fescue, Indian grass, Kaw big bluestem, and zoysia grass were susceptible and wheat, rye, buffalo grass, Kentucky bluegrass, oats, beets, peanut, pepper, soybean, tomato, and watermelon were resistant to M. naasi.

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PATHOGENICITY, LIFE CYCLE AND HOST RANGE OF *Meloidogyne naasi* Franklin FOUND ON SORGHUM IN KANSAS

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Meloidogyne naasi Franklin, was found on stunted sorghum plants in the spring of 1966 in Kansas. Because of the economic potential of this parasite to graminous crops, attention was directed toward pathogenicity, histopathology, life cycle, and host range studies.

The nematode identification as M. naasi was based on perineal pattern characteristics, especially phasmid size and location, male, female, and larvae measurements and host range tests.

Pathogenicity evaluations were made by comparing plant growth, in the greenhouse, between sorghum plants seeded in infested field soil, and those seeded in soil fumigated with a 1-3 dichloropropene, 1-2 dichloropropane mixture. Plants grown in non-treated soil were shorter, had smaller stem diameters and less foliage dry weights. Both fresh and oven dry root weights of infected plants were significantly heavier than the controls.

Roots grown in infested soil in the greenhouse at 30°C were harvested and stained with acid fuchsin-lactophenol every 2 days after emergence (7 days after seeding) for 30 days. Larvae had penetrated and had begun feeding at the first staining. By the 21st day many had undergone the second moult. Several of them had completed the third and fourth moults by the 27th day. There were a few eggs hatching by the 35th day after seeding.

Although some swellings were observed, galls were small. Root tips were often curved in a hooked fashion. Giant cells were observed only in toto but did not appear to be different than those described for other Meloidogyne spp.

Host range tests demonstrated that sorghum was highly susceptible, barley, corn, fescue, Indiangrass, Kaw big bluestem, and zoysia grass were moderately susceptible and wheat, buffalo grass, Kentucky bluegrass, oats,

beet, peanut, pepper, rye, soybean, tomato, and watermelon were resistant to the parasite.