Life Cycle, Pathogenicity, Histopathology, and Host Range of Race 5 of the Barley Root-Knot Nematode[1]

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Abstract: The optimum temperature for development of race 5 of *Meloidogyne naasi* was 26 C. A life cycle was completed in 34 days. Growth of sorghum was suppressed when inoculated with *M. naasi*. Observations of *M. naasi*-infected sorghum roots demonstrated that roots were penetrated just behind the root cap; giant cells were generally initiated either in the procambial region or in very young phloem. When giant cells developed in the cortex, corresponding areas of the vascular system did not have an endodermis, pericycle, or phloem fibers. Nineteen plant species were tested for suitability as hosts for race 5 of *M. naasi*. Reproduction occurred on 11 of 12 monocotolydenous hosts and none of 7 dicotolydenous hosts. Reproduction often occurred without gall development. *Key Words: Meloidogyne naasi,* sorghum, barley.

The occurrence of *Meloidogyne nassi* Franklin has been reported in the United States (1, 5, 6, 8, 13) and in several European countries (3, 4, 7, 9). Michell et al. (10, 11) demonstrated that the species contained at least five races separable by hosts. Race 5, from Kansas, was the only race to reproduce on sorghum. The life cycle, pathogenicity, histopathology, and host range of race 5 of *M. naasi* are described in this paper.

MATERIALS AND METHODS

Inoculum for all experiments was reared on 'RS 610' hybrid grain sorghum [*Sorghum bicolor* (L.) Moench] in a greenhouse. Larvae were collected within 48 h after infected roots were placed in a low-volume spray mist chamber. Experimental plants were grown in Haynie loam soil (45% silt, 40% sand, 15% clay). Experiments were fertilized as needed with a 20-20-20 soluble fertilizer.

Life Cycle: Sorghum seeds were surface sterilized with 50 μg/ml chlorohexidine acetate for 5 min (12), rinsed in sterile distilled water five times, and placed on sterilized, moist filter paper in petri plates for 3 days. Seedlings were transplanted to silica sand (ca. 5mm diam) when they were 2.5-3.0 cm tall.

After they had been surface sterilized (as described for seeds), 50-75 larvae were pipetted on and about each seedling root. All test seedlings were grown at 30 C for 48 h; then they were washed from the sand. Three seedlings with slightly swollen roots were transplanted into steam sterilized soil in each 15x15-cm pot. Plants were placed randomly in growth chambers set to maintain soil temperatures at 22, 26, 30, and 34 C (±1 C) with a 12-h photoperiod at 23,672 - 26,900 lux.

Six plants grown at each temperature were processed every 2 days. Soil was washed from the roots, which were then stained in acid fuchsin-lactophenol and cleared in lactophenol. Specimens were dissected from stained tissues and the developmental stages of *M. naasi* determined. A life cycle was considered complete when hatched second stage larvae were found in the egg masses. A similar experiment was conducted with 'Meimi' barley, *Hordeum vulgare* L., with

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infected sorghum grown at 26 C as a comparative control.

Pathogenicity: Three sorghum seedlings 2.5-3.0 cm tall were transplanted to steam sterilized Haynie loam soil in 17x17-cm pots and treated in one of three ways: *(i)* each seedling was inoculated with 100 larvae and two egg masses surface sterilized with 50 μg/ml chlorohexidine acetate for 5 min, *(ii)* each seedling was inoculated with 10 ml nematode-free water in which *M. naasi* had been collected, or *(iii)* seedlings were retained as noninoculated controls. The 15 replicates were placed in the greenhouse in a completely randomized design. Plants were fertilized and watered as needed. Soil temperatures averaged 29 C (±9 C) during the first 7 days and fluctuated from 26-28 C during the last 43 days. Plant height was measured every 5 days for 50 days and stem diam was measured at days 25 and 50. Root and top weights were measured at day 50.

Histopathology: Inoculations with *M. naasi* were as described for the life-cycle studies. Plants were grown in 12x12-cm pots in a growth chamber under conditions described under "Life Cycle." Infected roots were processed for observation 2 days after inoculation and daily thereafter for 7 days, every 2 days for an additional 10 days, and every 5 days for an additional 20 days. Roots were fixed in FAA, imbedded in Paraplast® (melting point 56-57 C), sectioned 12 μm thick, and stained with safranin-fast green.

Host Range: Nineteen plant species grown in Kansas were tested for susceptibility to race 5 of *M. naasi*. Ten seeds of each plant species were placed in steam sterilized soil in 17x17-cm pots. Seedlings were thinned to three/pot in each of five replicates. During the first 7 days after the seedlings emerged, a total of 500 larvae and two egg masses were placed about the roots in each pot. Soil temperature was 25 C (±2 C). After 75 days, roots were removed and a portion of each system was stained in acid fuchsin-lactophenol. We observed larval entrance, nematode development and reproduction, and host galling.

RESULTS

Life Cycle: The optimum soil temperature for *M. naasi* to complete a life cycle on sorghum and barley was 26 C. Completion at this temperature required 34 days (Table 1). At 30 C, the nematode required 36 days to complete a life cycle on sorghum and 38 days on barley. At 34 C, completion of a life cycle required 40 days on sorghum and 46 days on barley. At 22 C, the nematode required 44 days on sorghum and 50 days on barley to complete a life cycle. Comparatively, both sorghum and barley grew well at 26 and 30 C. Growth of sorghum was suppressed at 22 C and growth of barley was suppressed at 34 C.

The young female stage of *M. naasi* was reached 10 days sooner on sorghum than on barley at both 22 and 34 C. However, at completion of the life cycle (larvae to larvae), the maximum difference between the two hosts at these same temperatures was six days. Except at the optimum temperature for development (26 C), race 5 of *M. naasi* developed faster on sorghum than on barley.

Pathogenicity: Growth of sorghum inoculated with race 5 of *M. naasi* was less

TABLE 1. Developmental stages of *Meloidogyne naasi* on sorghum and barley.

Developmental Stages	Temperature (C) 22		26		30		34	
	Sorghum	Barley	Sorghum	Barley	Sorghum	Barley	Sorghum	Barley
3rd-stage larvae	18[a]	26	10	12	12	16	12	24
Adult	24	34	16	18	18	20	22	32
Egg deposition	30	40	22	24	24	30	28	38
2nd-stage larvae	44	50	34	34	36	38	40	46

[a]Average number of days from infection that the most advanced stage of *M. naasi* occurred, in six replications. The greatest variation was ± 2 days.

than that of noninoculated plants (Table 2). Compared to controls, plants inoculated with *M. naasi* (P = 0.01) had lower shoot and root weights, shorter heights, and smaller diameters. Plants receiving supernatant water (water in which nematodes were collected) as inoculum were as large and larger than the noninoculated controls (Table 2). Infected plants were pale green with some yellowing.

Histopathology: Second-stage larvae of *M. naasi* penetrated roots just behind the rootcap and then moved through tissues inter- and intra-cellularly. When nematodes began to feed, plant cells around the head divided anticlinally and periclinally to form a hyperplastic area. Cells in the hyperplastic area had dense cytoplasm and prominent nuclei and nucleoli. Some cells were multinucleate. Three to five giant cells formed in the hyperplastic area; smaller than normal cells surrounded them (Fig. 1-A). Feeding generally started in the procambial region or in very young phloem. When nematodes and giant cells developed in the tip of a root, the root ceased to elongate.

Most giant cells were in the phloem. Endodermis bordering the giant-cell area lacked a casparian strip. When giant cells protruded into the cortex, endodermis minus the casparian strip also bordered the giant cell complex (Fig. 1-A). Less frequent, but distinctive, was the development of giant cell complexes in the cortex (Fig. 1-B). In such instances, the corresponding area of the vascular system did not have an endodermis or pericycle and was devoid of phloem fibers (Fig. 1-B). All nematodes that developed in cortical sites were males. Regardless of infection site, vessel-like elements (identifiable by pitting), developed around the giant-cell complex by the 6th day after initiation. When the giant-cell complex was in the vascular cylinder, the abnormal vessels connected with phloem fibers; when the giant-cell complex was in the cortex, vessels were independent of surrounding cells.

Host Range: Susceptibility of plants tested as hosts for *M. naasi* fell into three categories: *(i)* roots severely galled and prolific reproduction—*Sorghum bicolor* 'RS 610', *Hordeum vulgare* L. 'Meimi', *Secale cereale* L. 'Balboa', and *Sorghastrum nutans* (L.) Nash. 'Syn^{3}2'; *(ii)* little root swelling and reproduction--*Zea mays* L. 'Pioneer 321', *Festuca elatior* Huds. 'K31', *Andropogon gerardi* Vitman. 'KG 1579', *Poa pratensis* L. 'Kentucky', *Triticum aestivum* L. 'Bison', *Avena sativa* L. 'Tonka', and *Zoysia japonica* Steud. 'Meyer'; *(iii)* no galling nor reproduction—*Beta vulgaris* L. 'Detroit Red' and an unknown variety of sugar beet, *Buchloe dactyloides* (Nutt.) Engelm. 'W2F2', *Arachis hypogaea* L. '647 Early Spanish', *Capsicum frutescens* var. *grossum* Sendt. 'Calif. Wonder', *Lycopersicum esculentum* Mill. 'Rutgers 885', *Citrulus lanatus* Schaad. 'Charleston Gray 62', *Glycine* max (L.) Merr. 'Clark 63'.

DISCUSSION

Race 5 of *M. naasi* reproduced well on both warm-season and cool-season crops over a 12-C range of temperature. Although race 5 is known from only one location (Kansas), it would appear that it has the capability to survive under a broad

TABLE 2. Influence of race 5 of *Meloidogyne naasi* on the growth of sorghum.

Treatment	Dry foliage wt (gm)	Dry root wt (gm)	Plant heights (cm) 25 day	Plant heights (cm) 50 day	Stem diam (cm) 25 day	Stem diam (cm) 50 day
M. naasi-inoculated	5.6**[a]	3.5**	40.5**	71.7**	3.8**	11.0**
Supernatant	22.0	12.5	61.0	104.0	10.0	20.0
Nontreated	18.0	11.2	57.5	99.3	8.3	18.3
LSD (P = 0.01)	8.4	3.7	4.13	6.73	2.09	1.93

[a]Asterisks (**) indicate a significant difference (P = 0.01), as compared to noninoculated control.

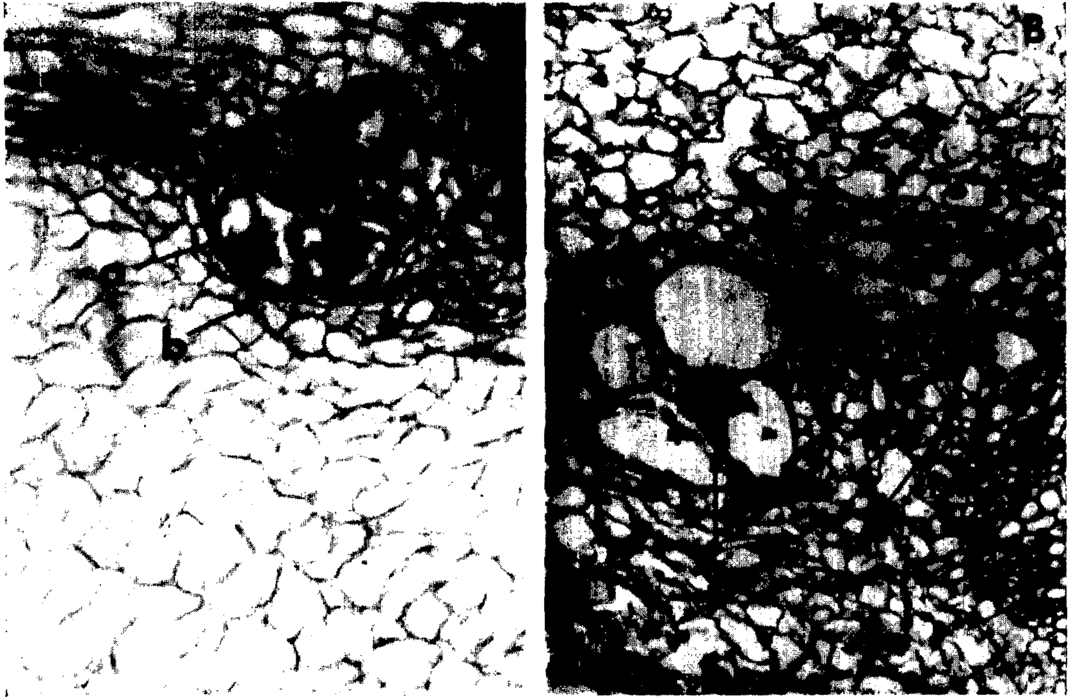

FIG. 1-(A-B). Photomicrographs of sorghum roots infected with race 5 of *Meloidogyne naasi*. *A)* Giant cell area (a) and the endodermis minus the casparian strip bordering (b). *B)* Giant cells in the cortex (a), showing the adjacent vascular system minus endodermis, pericycle (b), and phloem fibers (c).

range of conditions. The nematode did develop quicker on sorghum than on barley at temperatures other than optimum, an indication of a better host-parasite relationship with sorghum than barley. Small differences reported in the length of *M. naasi* life cycles (11, 13, 14) could be the result of nematode-host-temperature interactions.

Our results demonstrated that *M. naasi* can be an etiologic factor in a root-knot disease of sorghum. Measurable effects were losses in growth as reflected by plant heights, stem diam, and weights of foliage and roots.

Observations of *M. naasi*-infected sorghum roots indicated that histological responses to the nematode were similar to those described for *M. naasi* and other *Meloidogyne*-species-infected plants (14, 2).

Cropping sequences that include corn (a poor host) and soybeans (a nonhost) should keep race 5 of *M. naasi* populations well below those that would be economically damaging.

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