Pathogenicity and Histopathology of *Meloidogyne graminis* Infecting 'Tifdwarf' Bermudagrass Roots[1]

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Abstract: In a greenhouse experiment *Meloidogyne graminis* was pathogenic to 'Tifdwarf' bermudagrass, causing significant reduction in plant weight. Roots and tops of inoculated grass weighed 28.4% less than non-inoculated grass 8 months after inoculation. Clipping weight of nematode-infected turf weighed 68.9% less than clippings from non-infected turf. Histopathological studies showed that the head of the female nematode penetrated the vascular system and resulted in giant cell formation in the feeding area. The nematode body remained in the cortex parallel to the vascular system. Eggs were deposited at the posterior of the nematode in a gelatinous matrix in the cortex. *M. graminis* fed with its anterior end oriented toward the growing root tip. *M. incognita* had no set body orientation pattern when feeding on bermudagrass.

In 1965, a new bermudagrass variety, 'Tifdwarf' [*Cynodon dactylon* (L.) Pers.], was released for turf uses by the U. S. Department of Agriculture at the University of Georgia's Coastal Plain Experiment Station, Tifton. Early unpublished reports referred to the grass as nematode-resistant, probably because it was first found growing among 'Tifgreen' bermudagrass heavily damaged by nematodes. In turf nurseries, I have observed injury to 'Tifdwarf' caused by high populations of the pseudo-root knot nematode of turf [*Meloidogyne graminis* (Sledge and Golden, 1964) Whitehead, 1968]. Turf damage was especially evident in periods of moisture stress.

The presence of *M. graminis* in turf has been reported in several states (2, 3, 6, 7, 9, 10). Mirza and Perry (8) reported on the histopathology of 'St. Augustine' grass roots infected with *M. graminis*. Van Weerdt, et al. (12) reported a new, grass-root knot nematode causing a marked yellowing and a general decline of 'St. Augustine' grass in Florida. This nematode was later described as *Hypsoperine graminis* (11) and recently synonymized as *M. graminis* by Whitehead (13). Dickerson (2) found that *M. graminis* caused stunting and chlorosis of 'Meyer' zoysia. Bell and Krusberg (1) observed damage on zoysia and bermudagrass to occur as circular areas of dead and dying grass 1 to 15 ft in diam.

The present study was undertaken to determine the pathogenicity of *M. graminis* to 'Tifdwarf' bermudagrass under greenhouse conditions and to investigate root injury by histopathological procedures.

Materials and Methods

Cores of 'Tifdwarf' bermudagrass were taken from plots on the Coastal Plain Experiment Station with a soil probe 2.5 cm in diam to a depth of 8 cm. These cores were placed in water at 55 C for 15 min to free the roots of nematodes (4). Five cores were planted in each of 24, 15-cm pots filled with steam-sterilized Tifton fine sandy loam. Two weeks later 20 ml of water containing 300 *M. graminis* larvae was added to each of 12 pots, and 12 remained as controls. The pots were maintained in a greenhouse at temperatures of 21 to 32 C. Approximately 400 ml of a liquid fertilizer solution (20-20-20) was added weekly through irrigation water with

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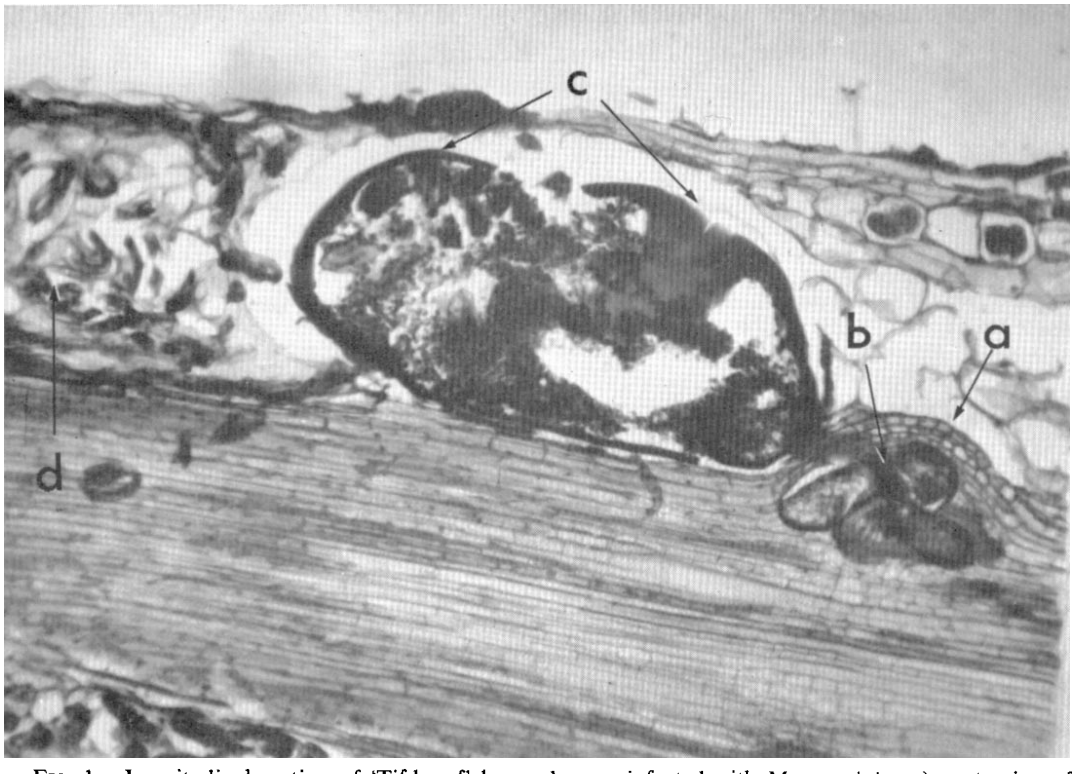

FIG. 1. Longitudinal section of 'Tifdwarf' bermudagrass infected with *M. graminis.* a) protrusion of giant cells into cortex; b) giant cell around nematode head; c) female body in root cortex; d) egg mass.

a Mixer-Proportioner® at the rate of 50 parts water to one part of 500 gm fertilizer in one gal of water. Two weeks before termination of the experiment, infected and non-infected grasses were clipped to the same height. At completion of the 8-month experiment the grasses were clipped again and fresh clipping weights for the infected and non-infected turf were recorded. Then the soil was washed from the grass roots, and fresh weights were recorded for the infected and non-infected tops and roots. Selected portions of infected and non-infected roots were fixed and preserved in FAA under a partial vacuum for 24 hr and then stored. A microtechnique procedure as described by Johansen (5) was used. The roots were dehydrated in a tertiary butyl alcohol, embedded in Tissuemat® (melting point 56–58 C), sectioned longitudinally 10–12 μ in thickness, stained in safranin and fast green, and examined microscopically.

Sprigs of 'Tifdwarf' were rooted in plastic petri dishes filled with coarse building sand to determine the mode of entry and development of *M. graminis* in 'Tifdwarf' bermudagrass. One week after the sprigs were placed in the dishes, 1000 larvae of *M. graminis,* in 2 ml of water, were added with an 18-gauge hypodermic needle directly to the root zone in each of six dishes. The same procedure was repeated with *M. incognita* (Kofoid & White, 1919) Chitwood, 1949. Two weeks later roots were removed and plunged in a boiling mixture of lactophenol-cotton blue for 1 min. Roots were then

removed and placed in lactophenol for cleaning and microscopic examination. Later some roots were re-stained in lactophenol-acid fuchsin and examined.

RESULTS AND DISCUSSION

Meloidogyne graminis was pathogenic on 'Tifdwarf' bermudagrass under greenhouse conditions. Fresh weights of roots and tops from the non-inoculated treatment averaged significantly more, 245 g per pot, compared with inoculated turf, 176 g per pot at the 1% level of probability. Clipping weights of non-inoculated grass were also significantly higher than weights of grass from nematode-inoculated turf, 6.52 compared with 2.03 g, respectively. This greenhouse study confirms field observations that *M. graminis* is pathogenic on 'Tifdwarf' bermudagrass. The nematode prevents normal development of the roots and suppresses vegetative growth.

Histopathological studies of 'Tifdwarf' revealed extensive damage to the vascular and cortical tissue of the roots. These studies showed that *M. graminis* fed in the vascular area and caused the formation of four to five giant cells surrounded by a thickened cell wall. Frequently, two nematodes were observed feeding in close proximity, with the resulting formation of eight to 10 giant cells. These cells were usually oblong, extending longitudinally in the vascular system. Most giant cells were located in the xylem with fewer in the phloem. Cellular contents of giant cells were more granular and more heavily stained than were non-infected cells. Giant cells contained several nuclei, and prominent nucleoli were usually concentrated in the center of the cell. At each infection site the vascular system was enlarged because of hypertrophy and hyperplasia of the vascular tissue; these enlargements protruded into the cortex of the root (Fig. 1a). Only the head and neck of the nematode extended into the vascular system (Fig. 1b). The remaining oval-shaped portion of the female was located in cortical tissue adjacent to the vascular system of the root (Fig. 1c). Egg masses were deposited in a gelatinous matrix at the posterior end of the nematode (Fig. 1d). Hatched larvae were often observed in the matrix. The nematode body and egg masses caused considerable disruption of the cells in the cortex. The females were always found with their anterior end oriented toward the growing tip of the root. My histopathological observations of *M. graminis* in the roots of 'Tifdwarf' bermudagrass are similar to those of Mirza and Perry (8) on 'St. Augustine' grass and by Dickerson (2) on 'Meyer' zoysia.

Examination of the roots from plants grown in petri dishes and stained with lactophenol-cotton blue, or acid fuchsin, showed that *M. graminis* entered just posterior to the root cap. After entry, larvae became concentrated in the cortex of the root, usually parallel to the vascular system of the plant. The head and neck were then inserted into the vascular system; the body was oriented as above. Larvae of *M. incognita* penetrated in a similar manner but were not specific in their orientation within the root. In many samples, the female was located with her anterior end oriented away from the root tip. *Meloidogyne incognita* did not lie parallel to the vascular system, as did *M. graminis*; and large galls were usually formed where the nematode penetrated the vascular system. We noticed only slight swellings where *M. graminis* initiated feeding.

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