Pathogenicity and Histopathology of Rotylenchulus reniformis Infecting Cantaloup

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Abstract: Rotylenchulus reniformis was pathogenic to cantaloup (*Cucumis melo* 'Perlita') under greenhouse conditions. These findings confirm field symptoms of cantaloup infected with *R. reniformis*. Histopathological studies show that the nematode penetrates the cortex perpendicular to the vascular system and comes to rest with the head against the endodermis in young roots. Feeding stimulated the pericycle to either side of the endodermal feeding cell and caused cell hypertrophy with enlargement of the nucleoli and granular thickening of the cytoplasm. In older roots where the endodermis had collapsed, the nematode fed directly into the pericycle and caused similar symptoms. Nematode development was more rapid at 27 C than at 21 C. *Key Words:* reniform nematode, cantaloup, pathogenicity, histopathology.

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) has become a major problem in some field crops, especially cotton (3, 9) in the lower Rio Grande Valley of Texas. Often fields that have been in continuous cotton for several years are planted to other crops to avoid the nematode problem. I have observed that several such fields planted to cantaloup showed very poor plant growth and virtually no melon production. Injury to cotton and cantaloup can be observed very soon after seedling emergence.

The purposes of these investigations were to study the pathogenicity of the reniform nematode to cantaloup (*Cucumis melo* L. 'Perlita') under greenhouse conditions, to examine histopathologically, penetration and feeding in roots, and to study the temperature effects on rate of development.

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MATERIALS AND METHODS

Sixteen metal pots 15 cm diam were filled with 2,000 g of a steam-sterilized Hidalgo sandy clay loam, and two cantaloup seeds were planted to each pot. Nematodes were collected from an infested cantaloup field and increased on cowpea (*Vigna unguiculata* (L.) Walp. 'Blackeye') in a greenhouse. Nematodes were extracted from soil and roots by a Baermann funnel technique over a 48-h period. Twenty-five thousand pre-adult *R. reniformis,* which had been washed five times in sterile distilled water, were added to each of eight pots in 40 ml of water in four 0.6-cm (diam) holes 10-cm deep equally spaced 2.5 cm from seed location.

After 54 days, the experiment was ended. Fresh top weights were recorded, and length of central stems and secondary branches were measured for all plants. Roots were washed free of soil, fixed in FAA (formalin-acetic acid-alcohol) for at least 48 h, dehydrated in a tertiary butyl alcohol, embedded in Tissuemat® (melting point, 56-58 C), sectioned 12-15 μm thick, stained in safranin

and fast green, and examined microscopically (4).

Temperature: To determine the effect of temperature on rate of nematode reproduction, 200-ml waxed paper cups were filled with 180 g of acid-washed fine sand. Two Perlita cantaloup seeds were planted per cup, and 80 cups were placed in each of two growth chambers programmed at 21 and 27 C. Twenty days after seeding, 6,000 pre-adult *R. reniformis* in 50 ml of water were added to each cup. Twenty-four hours later, and daily for 40 days, two cups were removed from each chamber. Then root systems were washed, stained in hot lactophenol-anilin blue, and examined microscopically for nematode development. The experiment was repeated to confirm earlier results.

RESULTS AND DISCUSSION

Pathogenicity and histopathology: After 54 days significant ($P = 0.01$) growth increases of average top weight for noninoculated plants was 91.9 g compared to 55.8 g for nematode-inoculated plants. Growth differences in the greenhouse, as in the field, appeared very early.

The length of the central stems and secondary branches 40 days after seeding averaged 57 cm for noninoculated plants and 28 cm for inoculated plants. After 54 days lengths were 156.3 cm for noninoculated plants and 117.7 cm for inoculated plants, a difference statistically significant, $P = 0.01$. These findings support observations that *R.*

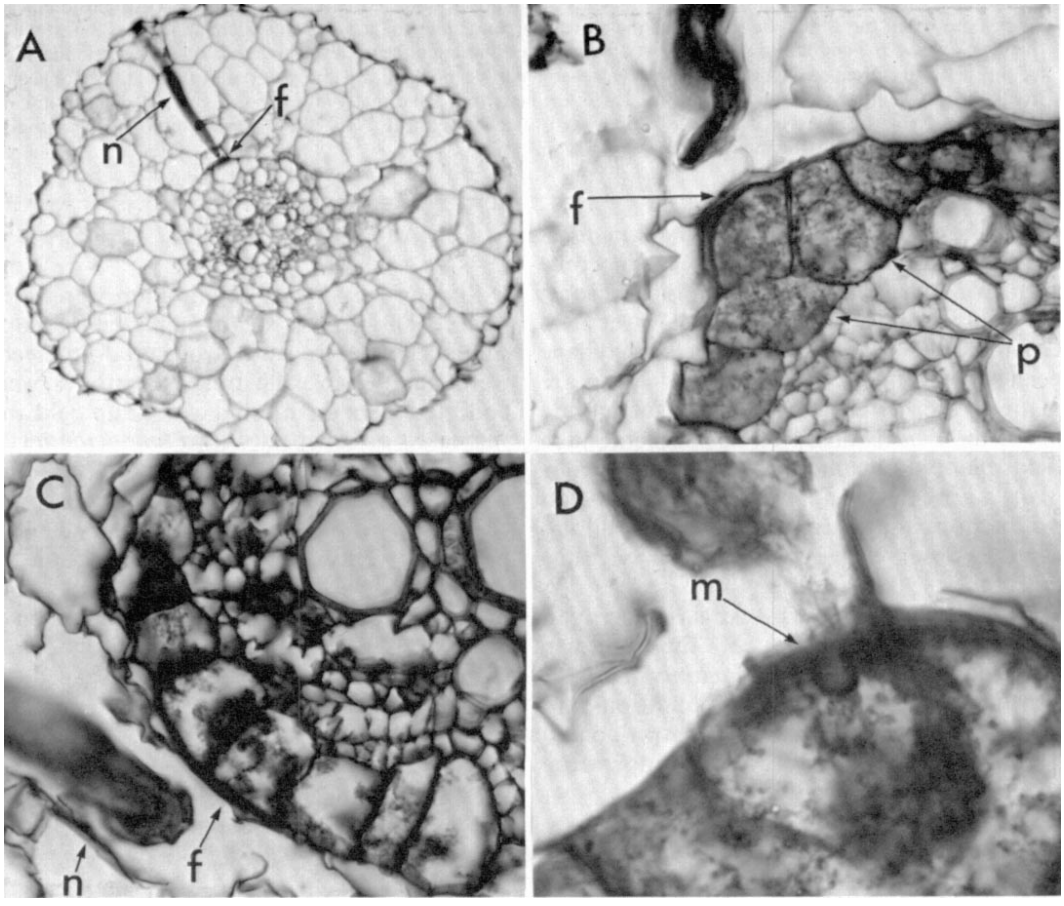

FIG. 1. *Rotylenchulus reniformis* feeding in roots of cantaloup. **A)** Penetration of young female (n) into root with head resting against endodermis (f). **B)** Developed female showing hypertrophied pericycle (p) joining endodermal feeding cell and thickened endodermal cell wall (f) at feeding site. **C)** Developed female (n) feeding directly in pericycle (f). **D)** Feeding site of developed female with digit-like indentation at point of stylet penetration (m).

reniformis is a severe pathogen to cantaloup under field conditions.

The nematode penetrated the root at various locations without any preferred feeding site. In new roots, the vermiform female usually penetrated the root perpendicular to the vascular system and came to rest with its head against the endodermis (Fig. 1-A). The endodermal cell on which the nematode chose to feed became wedge-shaped (Fig. 1-A). A substance appeared to be translocated from the nematode to the pericycle, because several pericycle cells to either side of the endodermal "feeding cell" became hypertrophied, and the contents took on a granular appearance when stained (Fig. 1-B). As the root matured, the other endodermal cells were crushed, but the feeding cell remained rigid and the cell wall next to the nematode head thickened and took the safranin stain (Fig. 1-B). All hypertrophied cells of the pericycle were uninucleate with prominent nucleoli. In older roots, the vermiform female penetrated the roots and fed directly in the pericycle (Fig. 1-C). Damage to the pericycle was the same as in younger roots. In many of the feeding cells, a small digit-like indentation, observed in front of the stylet, was surrounded by a thin membrane (Fig. 1-D). As the female matured, cortical cells adjacent to the nematode's body were destroyed, probably crushed by the increase in size of the neck and body of the maturing female (Fig. 1-D).

Rebois et al. (7) found that external root cells of soybean ruptured as *R. reniformis* females began to swell. They also noted that cortical cells around the nematode body thickened slightly and stained darker than other cortical cells. They concluded that mechanical damage to the cortex resulted from direct penetration and growth of the nematode.

Birchfield (1, 2) studied the host-cell responses of *R. reniformis* to several plant species, and found that the nematode preferred the pericycle in all plants studied except corn, where it fed only in the cortical parenchyma. He noted, as did I, signs of a toxin translocated from point of feeding.

Oteifa (5) found that *R. reniformis* caused hypertrophy of pericycle cells in cotton roots and noted that this symptom was the major histological response for the debilitated root system. The reniform nematode also has been reported (7) to feed in pericycle tissue of soybean roots adjacent to the outermost xylem vessels of the protoxylem poles. However, I found that the nematode fed at random around the vascular system and did not seem to prefer any one location.

Temperature: The nematode developed more rapidly at 27 C than at 21 C. At both temperatures 24 h after inoculation, juvenile females were found attached to roots of plants. Maturing females were observed in 6 days at 27 C and in 9 days at 21 C. At 27 C, females began to secrete a gelatinous matrix on the 7th day, and were completely enveloped with eggs deposited in the matrix on the 10th day. At 21 C, they secreted a matrix on the 11th day and began to deposit eggs on the 15th day. Newly hatched larvae were found in the matrix on the 13th day at 27 C and on the 18th day at 21 C.

Rebois (6) reported that *R. reniformis* made its most rapid development at 29.5 C and concluded that the female life cycle was completed in 19 days at that temperature. Sivakumar and Seshadri (8) reported that *R. reniformis* required 24-29 days to complete its life cycle. However, they did not state the temperature of their experiment. Before this study, the life cycle of *R. reniformis* was suspected to be accelerated at temperatures near 21 C, because of early plant stunting in the field. However, this study showed that as many nematodes infected cantaloup root at 21 C as at 27 C. Thus, early stunting is accounted for, even though 27 C was more conducive to nematode development.

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