Parasitism of Rotylenchulus reniformis onSoybean Root Rhizobium Nodules in Venezuela

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Rotylenchulus reniformis Linford and Oliveira parasitizes soybean *Glycine max* (L.) Merr. in Venezuela and is often associated with *Pratylenchus brachyurus*. In the tropical climate of Venezuela, *R. reniformis* reproduces rapidly on soybean roots; the nematode also infects the *Rhizobium* root nodules. Since there is no report of *R. reniformis* infection of soybean *Rhizobium* root nodules, a histological study was made to determine the effect of parasitism of nodules by a Venezuelan population of *R. reniformis*.

Soybean cv. Jupiter roots infected with *R. reniformis* were collected from a field in Portuguesa State. Roots were washed, and some were stained with acid fuchsin in hot lactophenol and examined microscopically. Other roots with *Rhizobium* nodules infected by mature nematode females were fixed in FAA (23% formalin, 3% acetic acid, 30% ethyl alcohol, 44% distilled water), dehydrated in TBA (tertiary butyl alcohol), and embedded in paraffin. Cross sections 10–15 μm thick were stained with safranin fast-green, mounted in Dummar xylene (3) and observed with a compound microscope.

Rotylenchulus reniformis parasitized both the roots and the *Rhizobium* nodules of Jupiter soybean. The swollen posterior portion of the females and egg masses protruded from the root nodule surface (Fig. 1). The female nematodes were observed most frequently in the portion of the bacterial nodule in contact with the root, but

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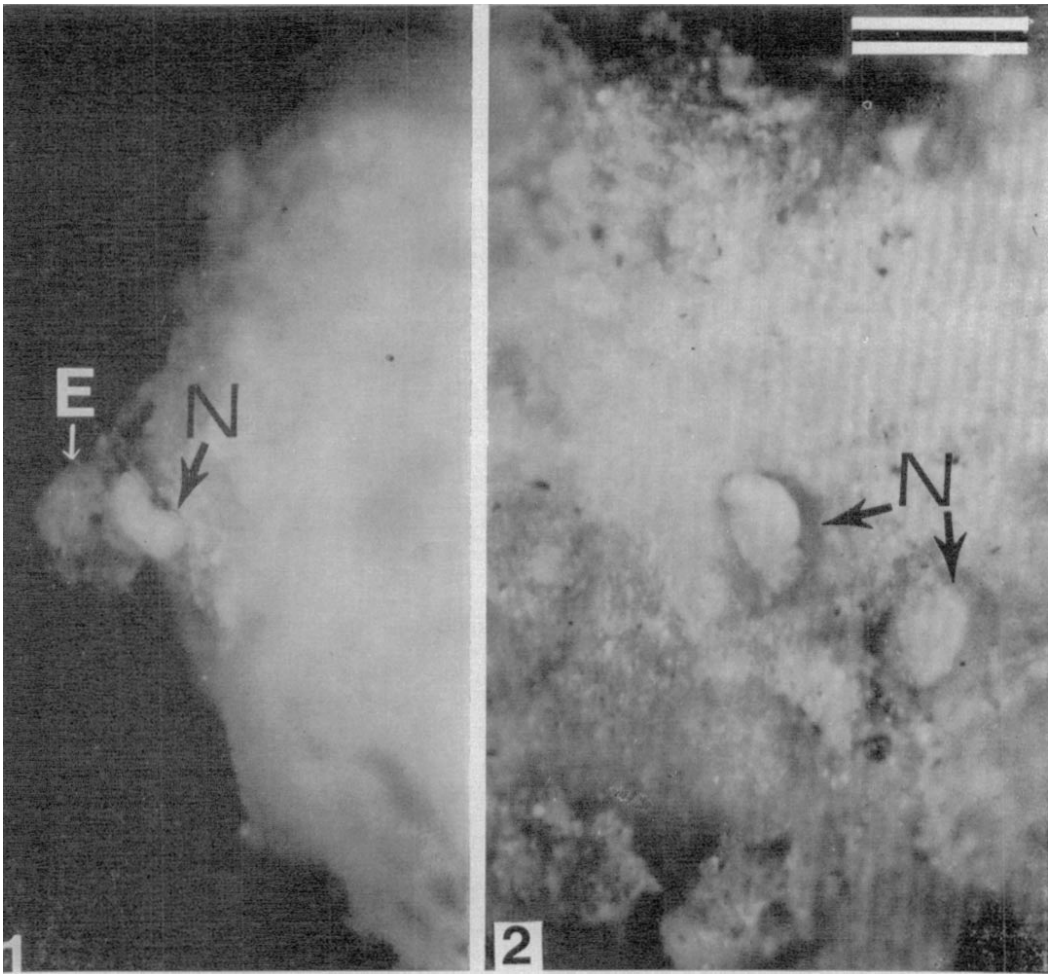

Figs. 1–2. *Rotylenchus reniformis* on soybean (*Glycine max*) root nodule. Scale bars = 480 μm. 1) Mature female (N) with the body posterior portion protruding from the root nodule surface; E = egg mass. 2) Mature females (N) in the interstitial fissures of ruptured nodule surface.

any point of the root nodule surface was susceptible to the nematode penetration. Some females with egg masses were located in the interstitial fissures of the ruptured nodule surface (Fig. 2). The number of mature females with egg masses averaged from one to three per nodule.

Sections of nematode infected soybean nodules showed that *R. reniformis* penetrated into the epidermis, root cortex, and nodule cortex and established a permanent feeding site in the nodule endodermis and the bundle endodermis (Figs. 3,4). The most common reaction to the nematode in the nodule vascular bundles was safranin positive stained bundle endodermal cells and accentuated hypertrophy of the bundle sheath cells compared with the bundle

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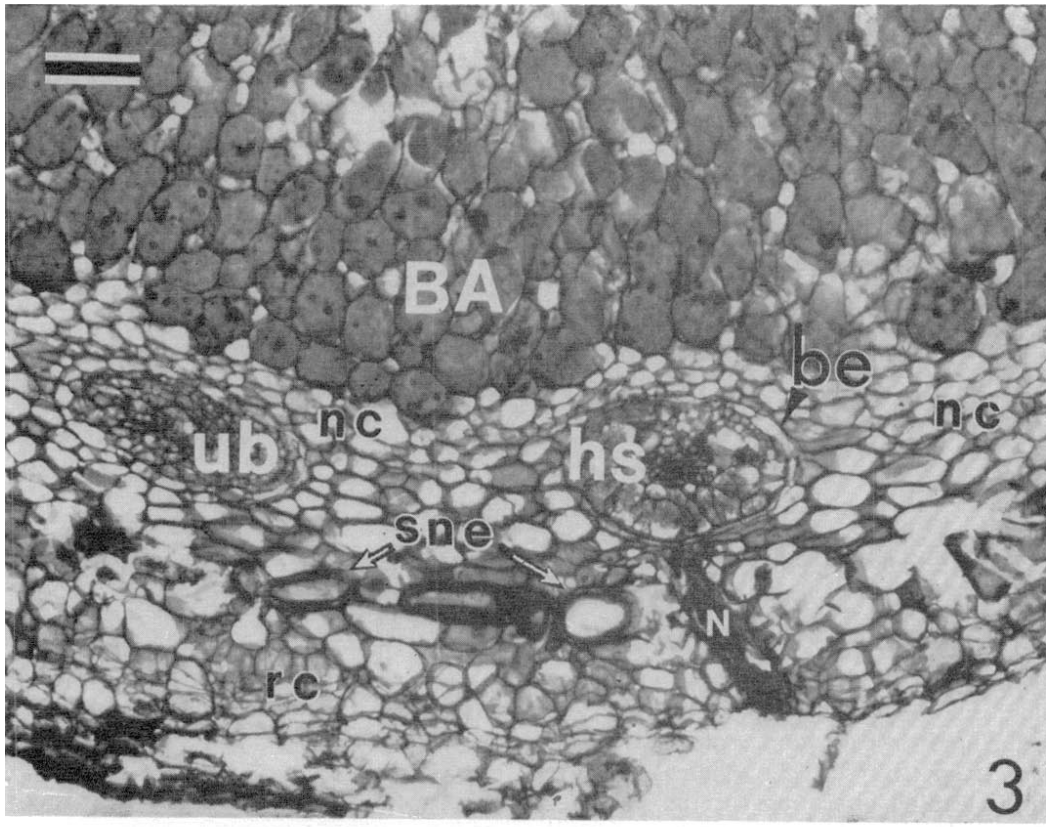
Figs. 3–5. Histological alterations caused by *Rotylenchulus reniformis* in soybean root nodules. Scale bars = 71 μm. 3) Cross section showing a nematode (N) penetrated into the root cortex (rc) and feeding in the bundle endodermis (be) of a vascular bundle. Note the hypertrophy of the bundle sheath cells (hs) compared to those of uninfected bundle (ub); BA = bacteroid region, nc = nodule cortex, sne = suberized nodule endodermis. 4) Cross section showing a nematode (N) feeding in the nodule endodermis (nen). Note the necrosis (ne) of nodule endodermal (nen) and nodule cortical (nc) cells at the nematode feeding site; BA = bacteroid region, rc = root cortex. 5) Cross section showing a nematode (N) feeding in a nodule cortical (nc) cell adjacent to the bacteroid region (BA). Necrosis (ne) is evident in the nodule cortical cell at the feeding site.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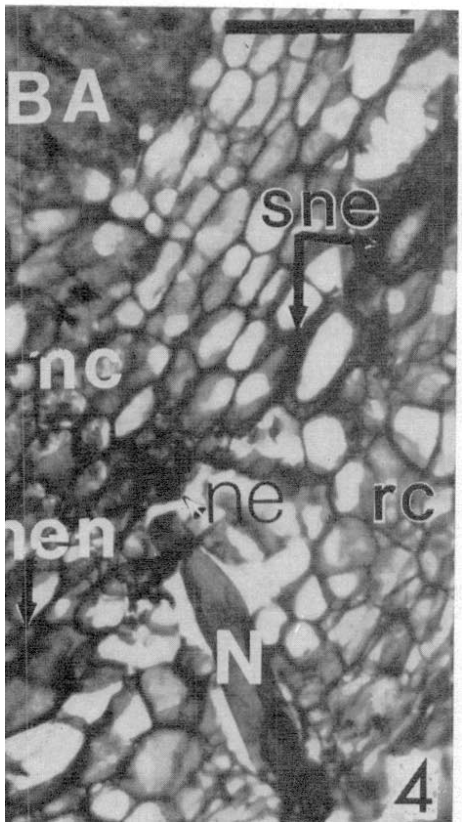
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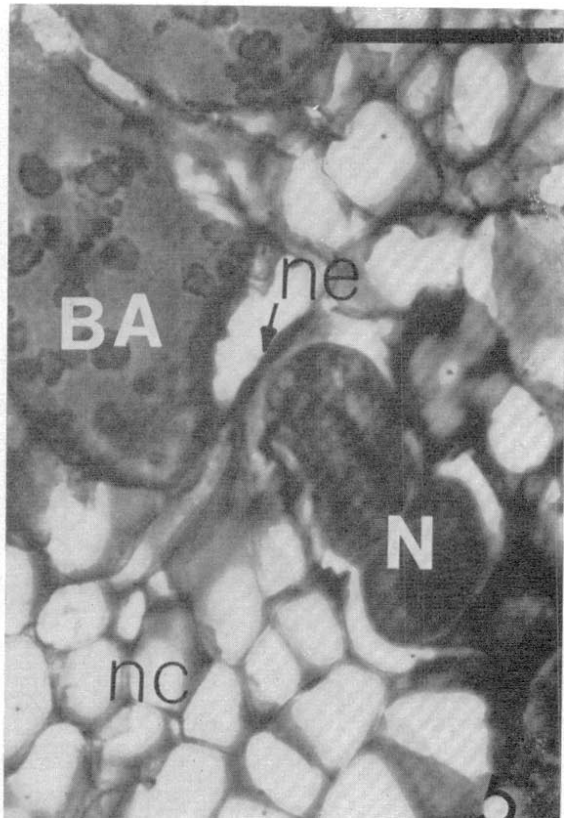
ne
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sheath cells of healthy vascular bundles (Fig. 3). Accentuated necrosis (safranin stained tissue) was also observed in the nodule endodermal cells in direct contact with the nematode head (Fig. 4). In some cases, nematode specimens penetrated the root and nodule cortex and reached the innermost layer of nodule cortical cells free of rhizobial infections and adjacent to the bacteroid region (Fig. 5). Considering that all the sectioned nodules were infected with mature females only, this would indicate that *R. reniformis* was able to develop by feeding in the nodule cortical cells of soybean. However, there was no evidence of nematode penetration into the bacteroid region.

In addition to *R. reniformis, Meloidogyne incognita* is able to reproduce in soybean root nodules (2). But soybean root nodules are unfavorable for the development of *Heterodera glycines* (1). *H. glycines* and *M. incognita* both attack the vascular bundles, damaging the vascular elements by inducing syncytium and giant cells formation, respectively (1,2), whereas *R. reniformis* caused alterations of the periphery of vascular bundles, damaging the bundle endodermis and inducing hypertrophy of bundle sheath cells.

The adverse effect of *R reniformis* on *Rhizobium* nodule production has been reported in cowpea (4), but from our observations on soybean root nodules there was no evidence of bacteroid disruption by *R. reniformis*. *R. reniformis*-infected soybean root nodules did not differ in size and shape from healthy ones. The necrosis induced by the nematode in the endodermal layers and also in the nodule and root cortex may predispose the nodular tissues to the infections of other pathogens and to their consequent premature breakdown.

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