Biological Relationship of Rotylenchulus borealis on Several Plant Cultivars

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Abstract: The embryogenic development of *Rotylenchulus borealis*, at 24–26 C, was completed on corn, in 12–15 days, and the life-cycle of the nematode from egg to egg required 35–40 days at 20–25 C. Juveniles remained in the soil as preinfective stages for 17–19 days before becoming adults. Only immature vermiform and swollen egg-laying females were found attached to corn roots. Eggs were laid in a gelatinous matrix on the root surface; the number of eggs per egg mass was 45 ± 28 on corn roots. Bean, green pea, potato, sorghum, and sweet potato were also found to be hosts of *R. borealis*. The nematode established a permanent feeding site on corn root in an endodermal cell that became hypertrophied. Pericyclic cells close to the feeding site showed granular cytoplasm and nuclei with hypertrophied nucleoli. A cell wall ingrowth was also noted around the area of stylet penetration into the endodermal cell. *Key words:* embryogenesis, host list, histopathology.

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Rotylenchulus borealis Loof & Oostenbrink (=*R. variabilis* Dasgupta, Raski & Sher) is widely distributed in Europe and Africa (4). It has been found mainly infecting corn (*Zea mays* L.) (3), but has also been reported on grasses (9), several vegetable crops (4), field crops such as cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogea* L.) (4), and fruit crops such as *Citrus* spp. (1) and grape (*Vitis* spp.) (3). There is, however, a lack of information on the biology and pathogenicity of *R. borealis*. This paper reports on the life cycle of this nematode with additional details on its host range and histological changes in the roots of corn.

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MATERIALS AND METHODS

Development: The embryogenic development of *R. borealis* was studied on 20 freshly deposited eggs washed in distilled water and mounted in water-2% agar in small Petri dishes at the room temperature of 24–26 C. The postembryongenic development was determined on 30 corn plants grown in soil infested with second-stage juveniles. Glasshouse temperature during these tests ranged from 20 to 25 C. Penetration and the postembryogenic development phases were observed at 2–5-day intervals by staining corn roots with acid fuchsin in lactophenol. The secretion of the gelatinous matrix by the nematode females was observed by microscope examination of egg-laying females removed from the roots and mounted in water in small Petri dishes.

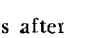
Host range: The host range of *R. borealis* was studied by sowing bean (*Phaseolus vulgaris* L. cv. Harvester), corn (cv. Dekalb-XL-41), cotton (cv. Deltapine 16), green pea (*Pisum sativum* L. cv. Progress), peanut (cv. Florunner), pepper (*Capsicum annuum* L. cv. Yolo wonder), potato (*Solanum tuberosum* L. cv. Alfa), tomato (*Lycopersicon esculentum* Mill. cv. Roma), sweet potato (*Ipomea batatas* L.), sorghum (*Sorgum vulgare* L. cv. N 12), and wheat (*Triticum durum* Desf. cv. Creso) in three glasshouse bins containing soil infested with *R. borealis*. Three months after sowing, plants were harvested and the roots were washed, stained with acid fuchsin in lactophenol, and examined microscopically (15×) for nematodes.

Histological changes in corn roots were studied by sampling infested roots at 10-day intervals. The roots segments were fixed in FAA (formalin, acetic acid, ethyl alcohol), dehydrated in TBA (tertiary butyl alcohol), and embedded in paraffin. Cross sections, 10–15 μm thick, were stained with safranin- fast green, mounted in Permount (7), and observed with a compound microscope.

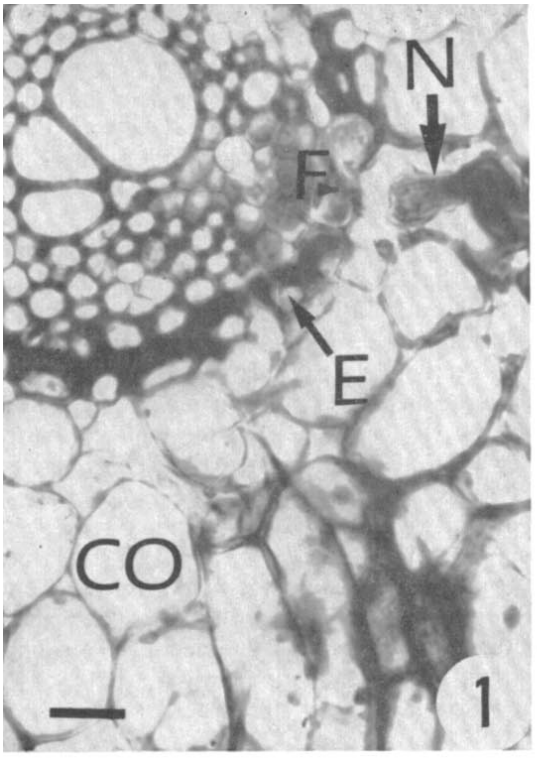
RESULTS AND DISCUSSION

Development: Embryogenic development was basically the same for *R. borealis* as other *Rotylenchulus* species (2,6). Single cell *R. borealis* eggs (n = 10), measured 90 μm (88–102) × 42 μm (38–46), shorter than those of *R. macrodoratus* Dasgupta, Raski & Sher (98–119 × 40–49 μm) and longer than those of *R. parvus* (Williams) Sher (56–59 × 30–38 μm). The first cleavage with the formation of the two blastomeres occurred in 9–12 h, the four-cell stage was attained in 2.5 days, the gastrula stage 7–8 days, and the tadpole stage 8–11 days after egg laying. The first juveniles stage appeared 11–12 days and the second-stage juvenile 12–15 days after egg deposition. The length of the embryogenic development of *R. borealis* (12–15 days at 24–26 C) was intermediate between that of *R. macrodoratus* (16–19 days at 18–32 C) (6) and that of *R. parvus* (11–14 days at 24–27) (2).

Like *R. macrodoratus, R. parvus,* and *R. reniformis* Linford and Oliveira, only immature females of *R. borealis* penetrated into the roots. There was no evidence of root penetration by the second-stage juveniles. The immature vermiform females were observed in the root 17–19 days after inoculation with second-stage juveniles. Swollen semiendoparasitic egg laying females were found 23–25 days after inoculation. The complete life-cycle from egg to egg took about 35–40 days and was shorter than that of *R. macrodoratus* (45–55 days at 20–26 C) (6) and somewhat longer than that of *R. parvus* (27–36 days at 24–28 C)



Figs. 1–4. Histological alterations induced by *Rotylenchulus borealis* in corn roots, 10–15 days after nematode penetration (scale bar = 15μm). 1) Cross section showing a specimen of *R. borealis* (N) penetrated in the cortex (CO) and feeding in the endodermis (E) (F = feeding site). 2) An enlargment of the nematode feeding site illustrated in Fig. 1. Apparently nonaffected thick-walled endodermal cells (*) are adjacent to endodermal cells without thickened wall (F) on which the nematode (N) fed (p = pericycle). 3) Cross section showing a nematode (N) feeding in an endodermal cell (E). The pericyclic cell (p) adjacent to the feeding site shows granular cytoplasm and hypertrophic nucleolus (n). 4) Cross section showing nematode (N) feeding in an endodermal cell. A cell wall ingrowth or feeding peg (I) surrounds the portion of the nematode stylet (ST) penetrated into the endodermal cell. Asterisks indicate adjacent normal endodermal cells.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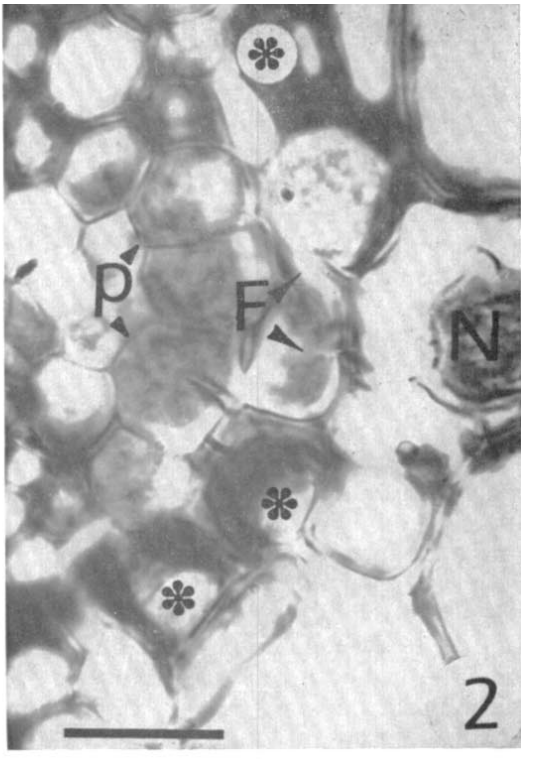
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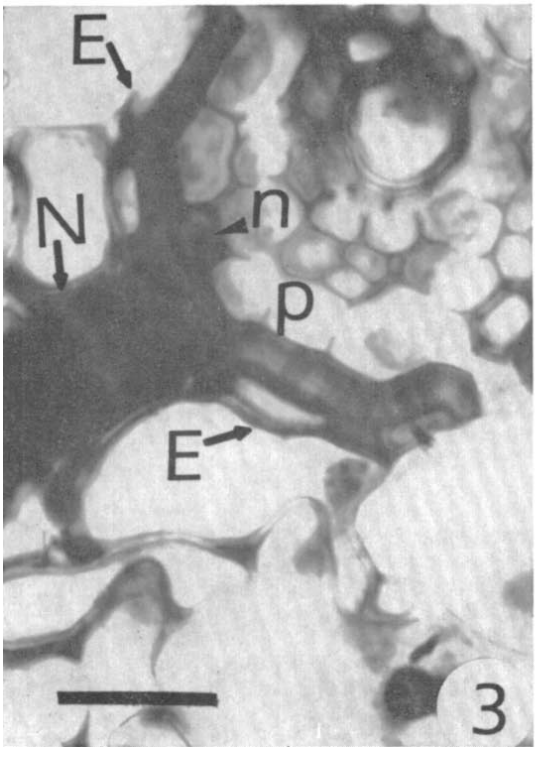
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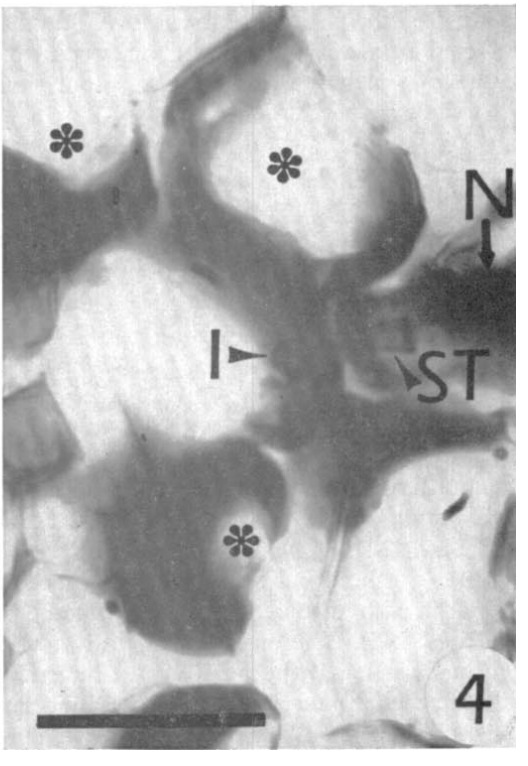
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(2). It was also longer than that of *R. reniformis* (25 days at room temperature) (8). Eggs were laid by mature females in a gelatinous matrix secreted through the vulvar aperture concurrent with the egg deposition. On corn roots the maximum number of eggs per egg mass was 45 ± 28 after 53 days from nematode inoculation.

As with other *Rotylenchulus* species, the vermiform males and all the juvenile stages were never observed to parasitize roots. A maximum number of males found in an egg mass surrounding a female was nine.

Host: Vegetable and field crops are the preferred hosts of *R. borealis*. They include bean, corn, green pea, potato, sorghum, and sweet potato. In this study, corn and potato appeared to be more susceptible than sweet potato, bean, green pea, and sorghum, to the nematode attack. Cotton, peanut, pepper, tomato, and wheat were not hosts of the nematode population used. The nematode also has been reported from vineyard soil in north Italy and France (2). Field observations in several localities of north Italy revealed no evidence that this nematode parasitizes grapes. The immature females detected in vineyard soil probably resulted from corn grown before or in association with the grape.

Host-parasite relations: Only the histological alterations caused by *R. macrodoratus* and *R. reniformis* have been previously studied. Both nematodes feed on endodermal tissue, causing the formation of a mononucleate giant cell and a syncytium (6,11,12). Histopathological alterations of corn roots infected by *R. borealis* are similar to those induced by *R. reniformis* on roots of cantaloupe, soybean, and sunflower (5,11,12). The nematode penetrates the epidermis and cortex establishing a permanent feeding site in an endodermal cell (Figs. 1–3). Hypertrophy of endodermal feeding site and adjacent pericyclic cells was induced by the nematode feeding activity (Figs. 1–2). The endodermal cell at the feeding site lacked the portion of thickened wall that characterizes this tissue in corn roots (Figs. 1–2). A cell wall ingrowth was observed around the stylet penetrated into the endodermal cell (Fig. 4). This appears similar to feeding peg associated with *R. reniformis* feeding on cotton (10). Usually, the pericyclic cells (1 to 3) immediately centripetal to the feeding site were hypertrophic and contained granular cytoplasm and enlarged nucleoli (Figs. 2–3). Positive safranin staining of cortical cells close to the nematode body was common in all examined infested roots and probably indicated necrosis.

The results of these observations indicate that *R. borealis*, like *R. reniformis* on cantaloupe (5), sunflower (12), and soybean (11), feeds primarily on the endodermis but also damages pericyclic cells. The described alterations of corn root tissues would indicate that *R. borealis* is more closely related to *R. reniformis* than to *R. macrodoratus*. However, field and glasshouse observations indicate *R. borealis* is a less destructive parasite than *R. reniformis*. The nematode population densities detected on corn roots from infested Italian soils have been usually low (4–6 nematode females per gram of fresh roots) in comparison of those of *R. reniformis* (> 40 per gram of fresh bean roots). Further studies are needed to determine the influence of *R. borealis* population densities on host-plant growth.

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