The Biology of Rotylenchulus macrodoratus

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Abstract: Rotylenchulus macrodoratus completed its embryogenic development in about 16–19 days at 18–32 C. On olive seedlings the life-cycle from egg to egg was completed in 45–55 days. The first molt occurred in the egg while the other three were superimposed, with retention of successive larval cuticles. Only immature vermiform and swollen egg-laying females were found attached to olive roots. Eggs are laid in a gelatinous matrix on the root surface. The maximum number of eggs seen was 55. Males were not parasitic. *Dianthus barbatus, Parietaria officinalis,* and *Eriobotrya japonica* were found to be hosts of the parasite. Observed in all infested hosts was an uninucleate giant cell that expanded from the endodermis toward the center of the stele in primary roots, and from the secondary vascular tissue toward the periphery of the cortex in secondary roots. *Key Words:* Embryogenesis, ontogenesis, host list, histopathology, mononucleate giant cell.

Among the species of *Rotylenchulus* of major economic importance, *R. reniformis* Linford & Oliveira and *R. parvus* (Williams) Sher are worldwide in distribution (6, 9). *Rotylenchulus macrodoratus* Dasgupta, Raski & Sher occurs only in the Mediterranean region, particularly in France (8), Greece (12), Israel (1), Italy, and Malta (12).

This nematode is common in southern Italy, where it mainly parasitizes root systems of various fruit trees (12). Symptoms and histological changes caused by *R. macrodoratus* in infested roots have been described on soybean (*Glycine hispida* var. Lee) (1,2) and olive (*Olea europaea* L.) (11). Information is lacking, however, on the biology of this nematode. This paper reports on the life cycle of *R. macrodoratus,* with additional details on its host range and histophathological changes in the roots of some previously known and newly identified hosts.

MATERIALS AND METHODS

Embryogenesis was studied in freshly deposited eggs washed and mounted in distilled water in shallow cavity slides. The slides were enclosed in petri dishes lined with moist filter paper to retard evaporation and maintained at 18–32 C. Postembryogenic development was studied in a glasshouse at 20–26 C. Olive seedlings were inoculated with 100 second-stage juveniles 12 h after hatching from the egg. Every 5 days the penetration and the postembryogenic development phases were determined by staining seedling roots with acid fuchsin in lactophenol.

Additional information on the host-range of this species was obtained by transplanting pellitory (*Parietaria officinalis* L.), a weed common in fruit orchards in southern Italy, large-flowered sweet william (*Dianthus barbatus* L.) and loquat (*Eriobotrya japonica* Lindl.) seedlings into a glasshouse bin containing soil infested with *R. macrodoratus.* Six months after transplanting, the roots of the plants were stained and examined microscopically for nematodes.

Histological changes in grape (*Vitis* sp.), fig (*Ficus carica* L.), and large-flowered sweet william were studied by sampling infested roots. The root segments were fixed for 48 h in FAA (formalin, acetic acid, alcohol), dehydrated in TBA (tertiary butyl alcohol), and embedded in paraffin. Cross and longitudinal sections 15μm thick were stained with safranine-fast green, mounted in Permount, and observed with a compound microscope (7).

RESULTS AND DISCUSSION

Embryogenesis: Embryogenic development was basically the same in *R. macrodoratus* as in *R. parvus* (3) (Fig. 1 A–I). Single-cell *R. macrodoratus* eggs measured 111 μm (98–119) × 44 μm (40–49), about twice as long as eggs of *R. parvus* (56–59 × 30–38 μm). The first cleavage was equatorial and resulted in two blastomeres of equal size in 10–12 h. The four-cell stage was attained in 2 days and the gastrula stage 8–10 days after egg laying. The first-stage juvenile appeared after 11–14 days, and the second-stage juvenile after 14–17 days, and hatching occurred 16–19 days after egg deposi-

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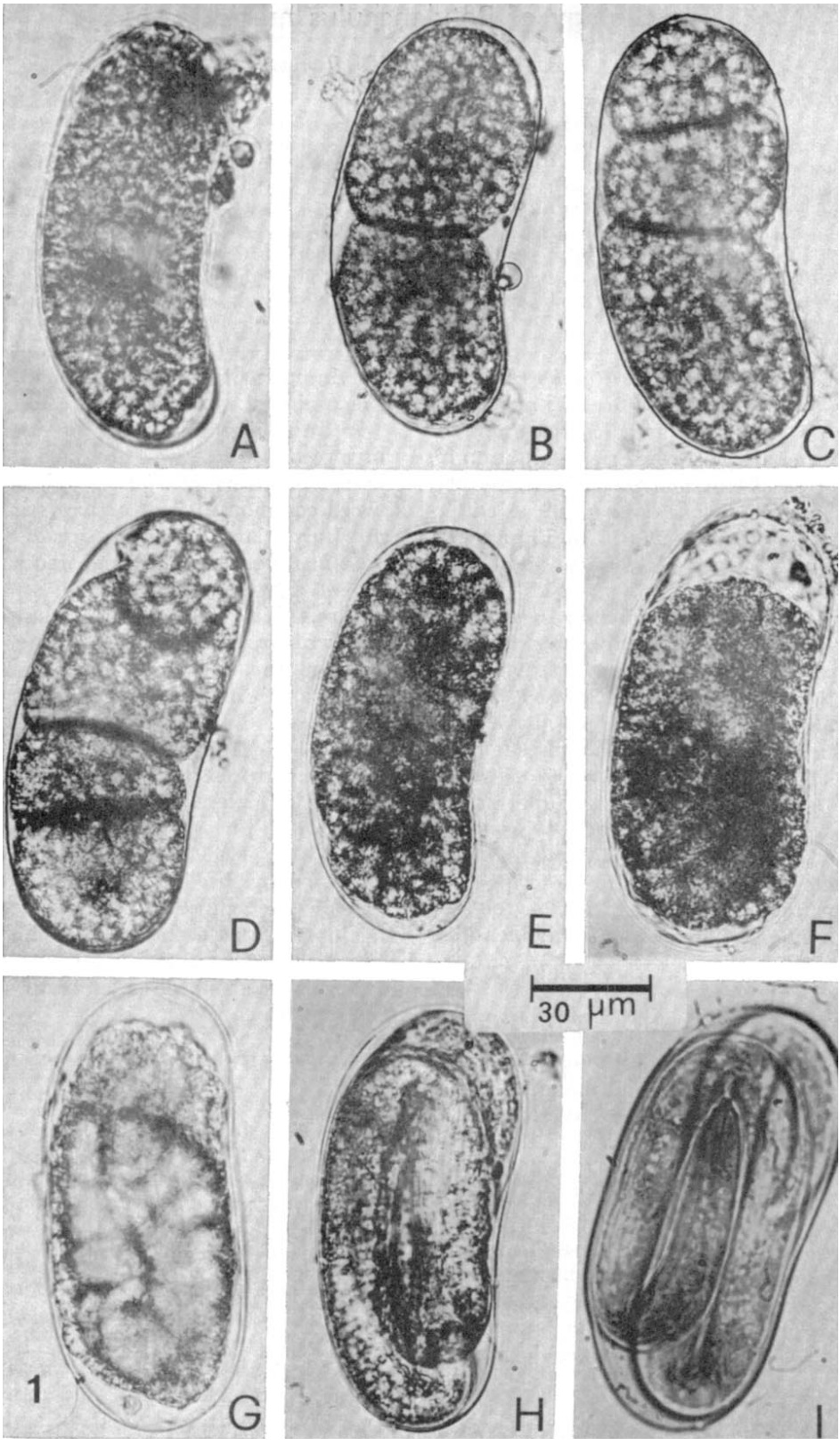

Fig. 1. Embryogenic development of *Rotylenchulus macrodoratus*. A) One-cell stage. B) Two-cell stage. C) Three-cell stage. D) Four-cell stage. E) Multicellular stage. F) Gustrula. G) "Tadpole" stage. H) First-stage juvenile. I) Second-stage juvenile.

tion. Dasgupta and Raski (3) reported that *R parvus* eggs developed faster (11–14 days), but that was at 24–27 C.

Postembryogenic development: The infective stages of *R. macrodoratus* were the immature females (Fig. 2), as reported for *R. parvus* (3) and *R. reniformis* (9). There was no evidence of root penetration by second-stage juveniles 5 days after olive seedlings were inoculated. However, immature females were found in the roots 14–16 days after inoculation. Swollen semi-endoparasitic females without eggs were observed 25–31 days after inoculation, and 4–5 days thereafter fully developed females with the first eggs were found. The complete life-cycle from egg to egg took about 45–55 days, somewhat longer than that of *R. parvus* (27–36 days) (6) and more than twice that for *R. reniformis* (17–23 days)

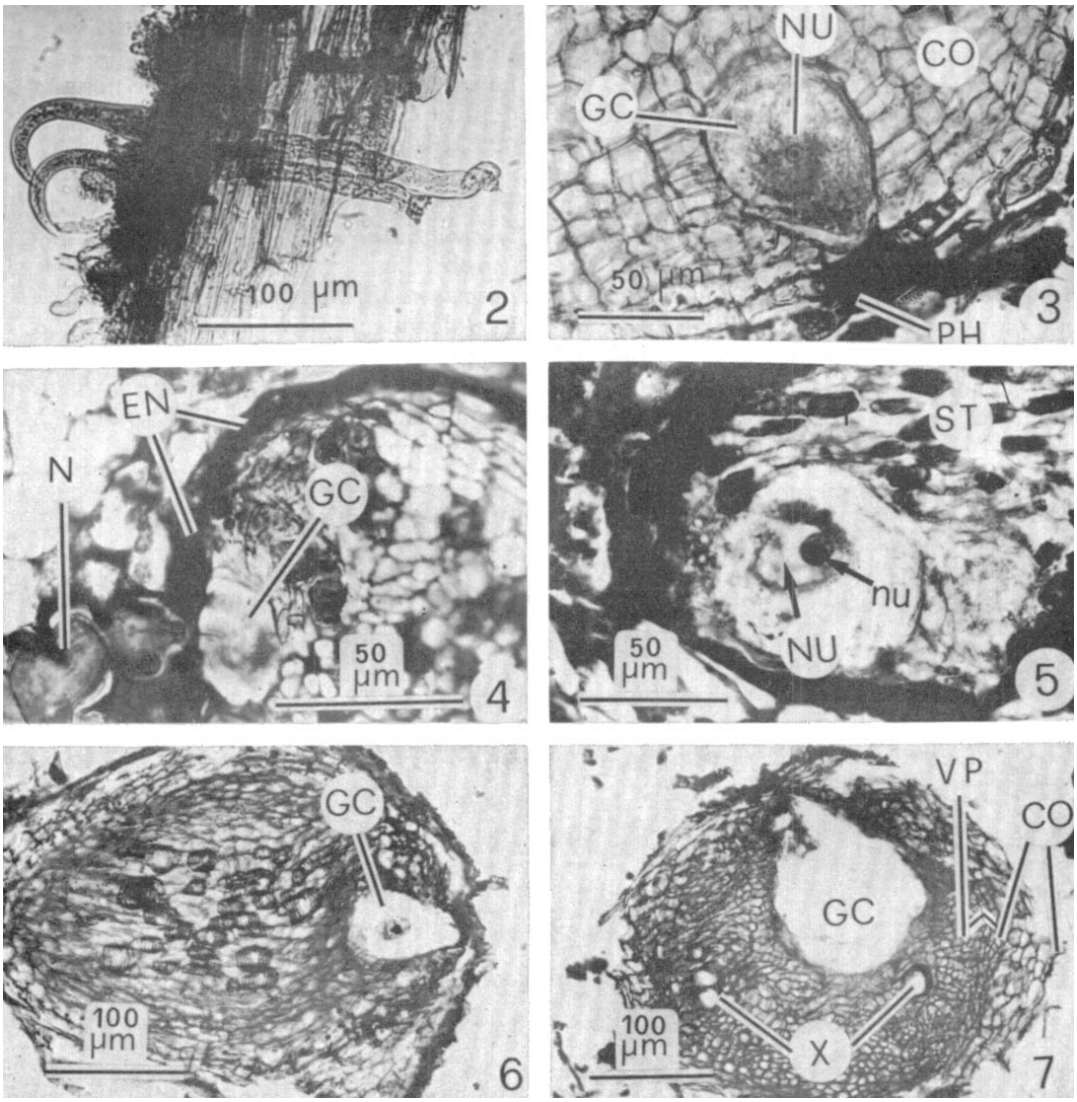

Fig. 2–7. Histopathological changes induced by *R. macrodoratus* in roots of different hosts. 2) Longitudinal section of olive root showing *R. macrodoratus* infective immature females penetrated partially into the root tissues. 3) Cross-section of a secondary fig root with a giant cell (GC) in the cortex (CO) and necrosis of phellogen cells (PH), (NU = nucleus) induced by *R. macrodoratus*. 4) *R. macrodoratus* (N) feeding in a giant cell (GC) originating from the endodermis (EN) and expanding to the stele of a grape root. 5) Cross-section of grape root infested with *R. macrodoratus*, showing a giant cell with hypertrophic nucleus (NU) and nucleolus (nu) in the stele (ST). 6–7) Cross-sections of large-flowered sweet william secondary root infested with *R. macrodoratus*, with giant cell (GC) expanding from secondary vascular parenchyma (VP) toward the periphery of the cortex (CO) (X = xylem elements).

(9). When juveniles were maintained in water at 20 C the postembryogenic development from hatching of second-stage juveniles to adults took longer (about 26–30 days).

Rotylenchulus macrodoratus juveniles completed three superimposed molts and were enveloped by shed cuticles until the last molt (Fig. 10). All the second-stage juveniles had a well-developed stylet that was shorter (20–22 μm) than that of active vermiform females (24–26 μm). After the second molt, sexual dimorphism between female juveniles and preadult males was recognizable in the tail shape. The tail was broader and more swollen in preadult males than in juvenile females of the same age (Fig. 10–11). The third- and fourth-stage juveniles were inactive and without a developed stylet. The vermiform males, like those of other *Rotylenchulus* species, were not parasitic. The egg masses usually contained only one to three males. In infested soil, the number of males was 7 to 10% that of juveniles and infective females. Males emerged at the same time as the young females.

Eggs were laid by mature swollen females in a gelatinous matrix. The gelatinous matrix was secreted through the vulvar aperture concurrent with egg deposition. On olive roots the maximum number of eggs per egg mass was 55. Olive roots ranging in diameter from 1.5 to 9 mm were infested by the nematode. The female population density was higher in feeder roots of 1.5 mm diameter (150 females/g of root) than in larger roots of 9 mm diameter (1–7 females/g of root). The juvenile population density usually detected in infested olive orchards ranged from 150 to 900 juveniles and males/g of fresh feeder root.

Host list: Woody host plants of *R. macrodoratus* include *Ceratonia siliqua* L. (carob), fig, grape, *Hedera helix* (ivy), *Laurus nobilis* L. (laurel), *Nerium oleander* L. (oleander), olive, *Prunus amygdalus* L. (almond), *P. armeniaca* L. (apricot), *P. domestica* L. (plum), *Quercus calliprinos* Webb, and *Q. farnetto* Ten. (oak) (2,4,8,

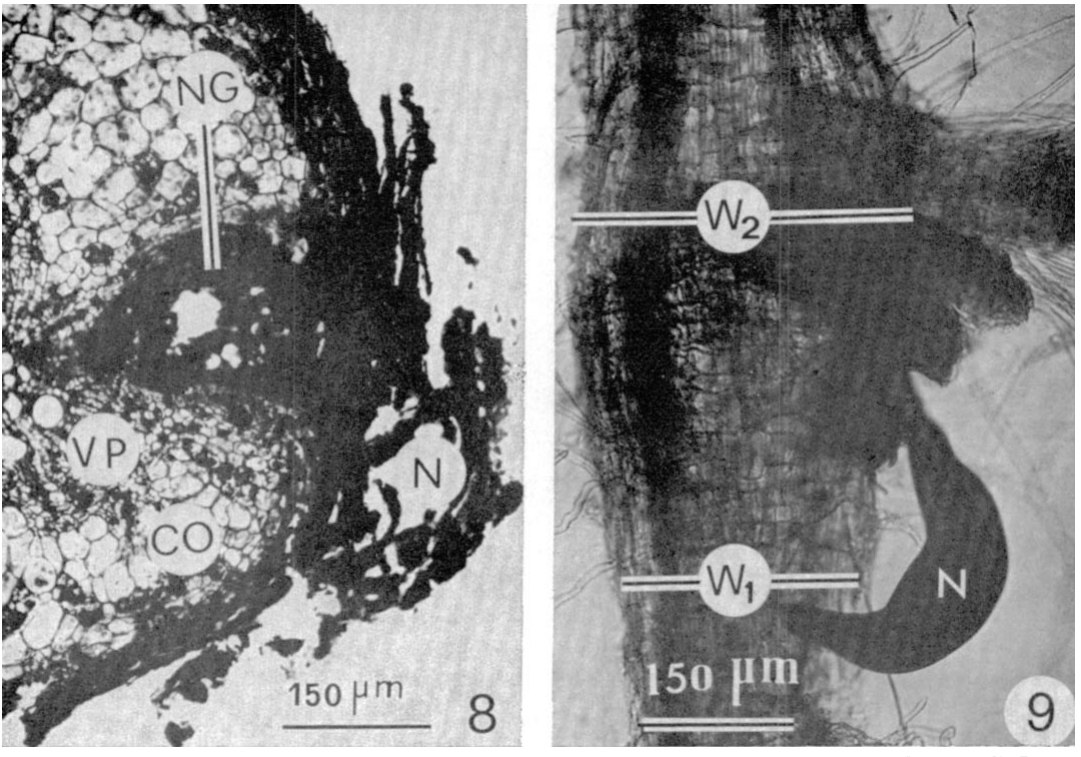

Fig. 8–9. Roots of grape and large-flowered sweet william infested with *R. macrodoratus*. 8) Large necrotic cell (NC) in the secondary vascular parenchyma (VP) and cortex (CO) of root grape (N = nematode). 9) Gall induced by *R. macrodoratus* (N) in a root of large-flowered sweet william (W1–W2 = different root diam.).

12). Herbaceous host plants include *Dianthus caryophyllus* L. (carnation), soybean, and *Phlomis fruticosa* L. (1,2,12). *Citrus sinensis* Osb. (orange) has been found infested by the nematode under experimental conditions (10), but field surveys in Italy found no evidence that *R. macrodoratus* parasitizes orange or other *Citrus* spp. grown together with heavily infested olive trees. Our test showed that *R. macrodoratus* also matures and reproduces in pellitory, large-flowered sweet william, and loquat.

Host-parasite relations: Although *R. macrodoratus* was similar to *R. reniformis* in life-cycle, histological changes caused by feeding activity were different (1,2,11). Examination of transverse and longitudinal sections of roots of fig, grape, and large-flowered sweet william infested with *R. macrodoratus* confirmed the formation of a mononucleate giant cell originating in the endodermis, where the nematode established a permanent feeding site (Fig. 4). A syncytium has been reported (9) in the roots of hosts infested with *R. reniformis*. The presence of the giant cell with an irregular hypertrophic nucleus and a thickened wall, especially in the region of stylet penetration (Fig. 4–5), appeared to be a nematode-specific rather than a host-specific response.

Root type and age also influenced the expansion of the giant cell. In the primary root the giant cell expanded from the endodermis toward the center of the stele and did not involve the cortical parenchyma (Fig. 5). In the secondary root, the giant cell expanded into the secondary vascular parenchyma and into the cortex toward the epidermis (Figs. 3, 6–7). The enlarged giant cell occupied more than one-third of the root section, with consequent disorganization of the root structure (Fig. 7).

Deterioration and necrosis of the giant cell were observed in cross-sections of grape roots (Fig. 8). Syncytial cell necrosis has been reported in soybean roots infested with *Heterodera glycines* when nematode feeding was reduced or stopped (5).

Small swellings in the area of nematode

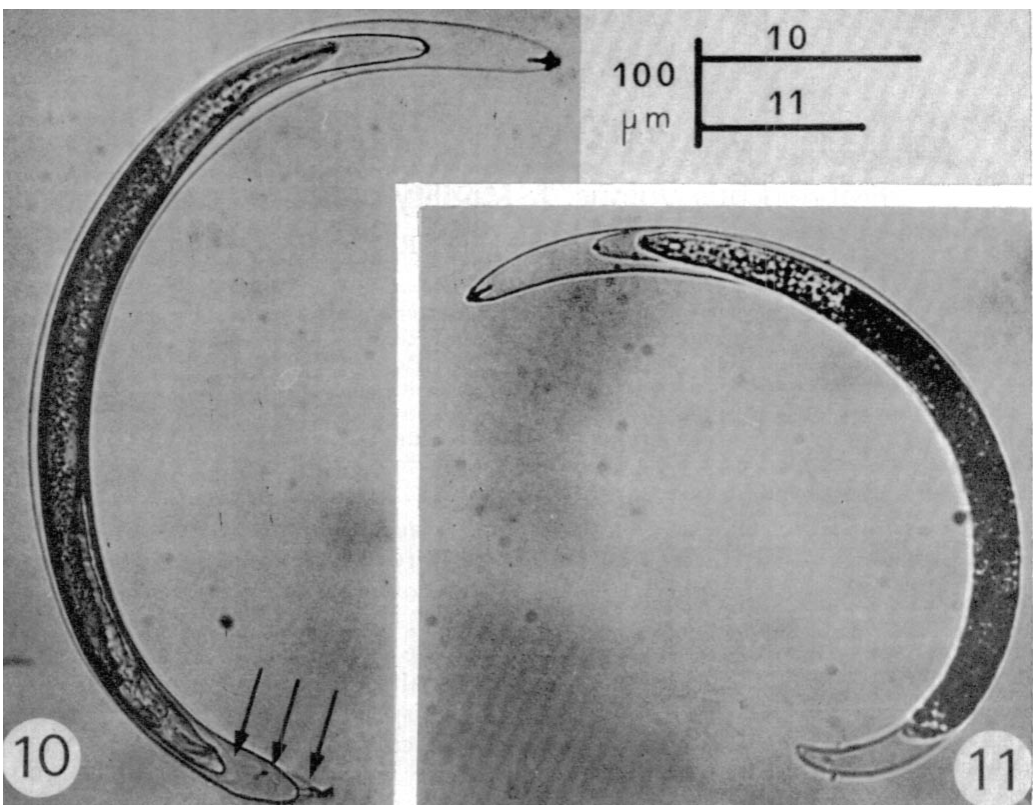

Fig. 10–11. *R. macrodoratus* molting stages. 10) Male enveloped by three shed cuticles (arrows). 11) Female fourth-stage juvenile enveloped by two cuticles.

penetration were noted in infested roots of *Dianthus* sp. (Fig. 9). The symptom was not found in other hosts tested.

R. macrodoratus is widespread in Italy but limited in distribution in France and Israel (2,8). The detrimental effects of this nematode on the growth and yield of its economic hosts are unknown. Further studies are needed on the pathogenicity, threshold limits, and influence of population densities of this nematode on host-plant growth.

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