Biology of Heterodera mediterranea

N. Vovlas[1] and R. N. Inserra[2]

Abstract: Heterodera mediterranea completes embryogenic development in 15–18 days at 24 ± 2 C. On olive and pistachio seedlings the postembryogenic development was completed in 42–50 days at 24–30 C. Juveniles and adults have semiendoparasitic habits and do not penetrate completely into the root tissue. This cyst forming nematode has been detected only on *Olea europaea, Pistacia lentiscus,* and *P. vera.* Syncytia formation and disorder of root stelar structure are the main anatomical changes induced by the parasite. *Key words:* cyst nematode, embryogenesis, histopathology, *Olea europaea, Pistacia lentiscus, P. vera,* host range.

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Eight cyst-forming nematode species belonging to *Heterodera, Globodera,* and *Sarisodera* are reported as parasites on woody plants (2,3,4,6,8,13). A ninth species, *Heterodera mediterranea* Vovlas, Inserra, and Stone, 1981, has been described on the woody plant lentisc (*Pistacia lentiscus* L.) from the Adriatic coast in southern Italy (12). There is a dearth of knowledge on the biology of this nematode. This paper reports some aspects of the life cycle and the host range of *H. mediterranea* and illustrates the histopathological effects caused by the nematode on two newly identified hosts.

MATERIALS AND METHODS

The embryogenic development of *H. mediterranea* was studied in petri dishes, using single-celled eggs removed from the gonad of the nematode white females, washed in distilled water, mounted in 2% water agar, and maintained in an incubator at 24 ± 2 C.

The duration of postembryogenic development was determined on pistachio (*Pistacia vera* L.) and olive (*Olea europaea* L.). Pregerminated seeds of pistachio and rooted cuttings of olive were transferred into 25-cm clay pots containing pasteurized sand; 3 days later 1,500 second-stage juveniles (J_2) were added to the pots. Plants were maintained in a glasshouse at 24–30 C. Nematode penetration and the developmental phases were studied on infected roots stained with acid fuchsin in lactophenol and observed with a stereomicroscope at 3–5 day intervals after the transplanting.

Host studies were made in a glasshouse at 24–30 C. The following herbaceous plants were tested: broad bean (*Vicia faba* L.), carrot (*Daucus carota* L.), corn (*Zea mays* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.), sorghum (*Sorghum vulgare* Pers.), tobacco (*Nicotiana tabacum* L.), and tomato (*Lycopersicon esculentum* Mill.). The following fruit tree species were tested: almond (*Prunus communis* Arc.), apple (*Malus domestica* L.), apricot (*P. armeniaca* L.), fig (*Ficus carica* L.), grape (*Vitis vinifera* L.) loquat (*Eriobotrya japonica* Lindl.), pistachio (*P. vera* L.), and walnut (*Juglans regia* L.). An ornamental shrub, oleander (*Nerium oleander* L.), was also included in this test. Ten pregerminated seeds of each herbaceous plant and 2-month-old fruit tree seed-

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[1]Nematologists, Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy.

[2]Present address of second author: USDA Crops Research Laboratory, Utah State University, UMC 63, Logan, UT 84322.

lings were planted into 50 × 50 × 70-cm bins containing sand infested with *H. mediterranea* cysts and J_2. Ten 2-month-old *P. lentiscus* seedlings were included as susceptible control. Six weeks after transplanting, all plants were harvested and roots were washed, stained with acid fuchsin, and examined microscopically for nematodes. The temperatures used in these tests occur in the Adriatic Coast of south Italy during the time of lentisc root invasion by the nematode J_2. However, lower temperature values than those used may also be suitable to parasite development.

The histological changes induced by *H. mediterranea* were studied on nematode-infected olive and pistachio roots at 3–5-day intervals. Infested root segments were fixed for 48 hours in formalin-acetic acid-ethanol (FAA) solution, embedded in paraffin, sectioned 10 μm thick, stained with safranin and fast-green, and mounted in dummar xylene for microscopic examination (5).

RESULTS

Embryogenesis: Mean dimensions of single-celled eggs were 42 × 96 μm (Fig. 1). The first cleavage was equatorial and the two-blastomeres stage occurred in 12–18 hours (Fig. 2). The second and third divisions were also transverse (Figs. 3, 4) and the four-cell stage (Fig. 4) was obtained in 60 hours. Forty-eight hours later, rapid cell division resulted in the formation of multicell eggs (Fig. 5). The gastrula stage (Fig. 6) was observed during days 9–12. First- and second-stage juveniles appeared in 11–13 and 14–16 days, respectively, (Figs. 7,8,9) and were coiled three or four times within the egg shell. Second-stage juveniles hatched within 1–3 days after the first molt. The duration of embryogenic development from a single cell to hatch of the second-stage juveniles averaged from 15 to 18 days at 24 ± 2 C. The embryogenic development was basically the same as in *Heterodera schachtii* (7); however, there is no report on the duration of the embryogenic phases of *Heterodera* species.

Postembryogenic development: Three days after inoculation, second-stage juveniles were partially embedded into the cortex of both primary and secondary olive and pistachio roots (Figs. 10, 11). Eight days after penetration, the majority of the juveniles had attained the third stage (Fig. 12). The head and neck region penetrated only a few cortical cell layers; the nematode then reached maturity in a semiendoparasitic feeding position (Fig. 14). The swollen posterior portion of the sexually mature nematode protrudes from the root surface (Figs. 14, 15). The semiendoparasitic habit of *H. mediterranea* differentiates this species from the other *Heterodera* species.

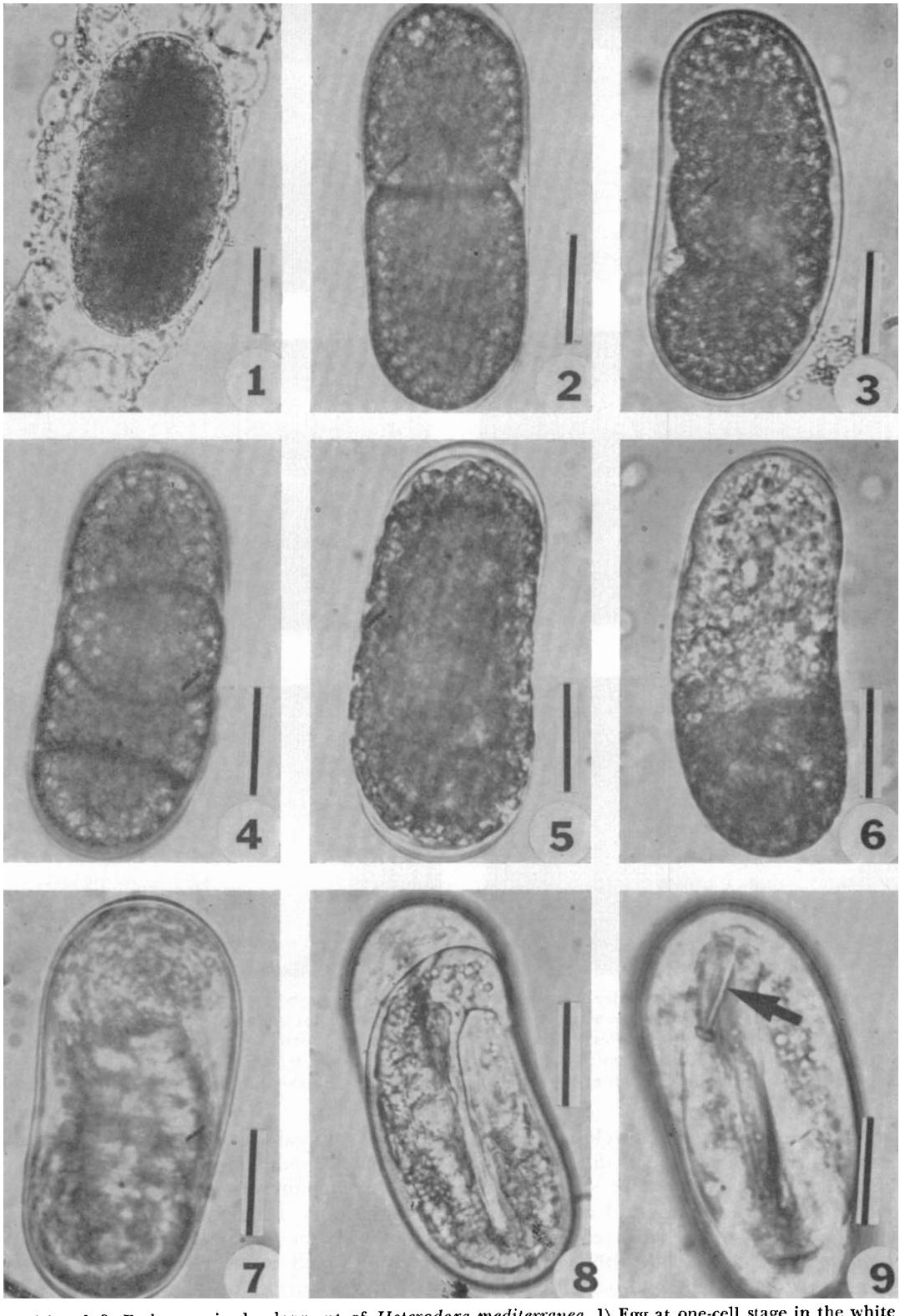
Development of second and third stages of *H. mediterranea* is longer than that reported for other *Heterodera* species (9). Fourth-stage juveniles were observed 15–18 days after penetration of pistachio roots and spheroidal to lemon-shaped white adults were obseved 6–8 days later. The production of egg-sac or gelatinous matrix began when the females were white; ovideposition began during the white or white-yellow phase and terminated when females became brown. This compares to *H. schachtii* (7). *H. mediterranea* postembryogenic development (from second-stage juvenile to brown cysts) was 42–50 days at 24–30 C.

Host studies: Potential hosts were limited to species with agricultural importance or to plants which were known hosts for *H. mediterranea* or other *Heterodera* spp. Nematode infection was detected only in *P. lentiscus* L., *P. vera* L., and *Olea europaea* L., all of which are fruit trees.

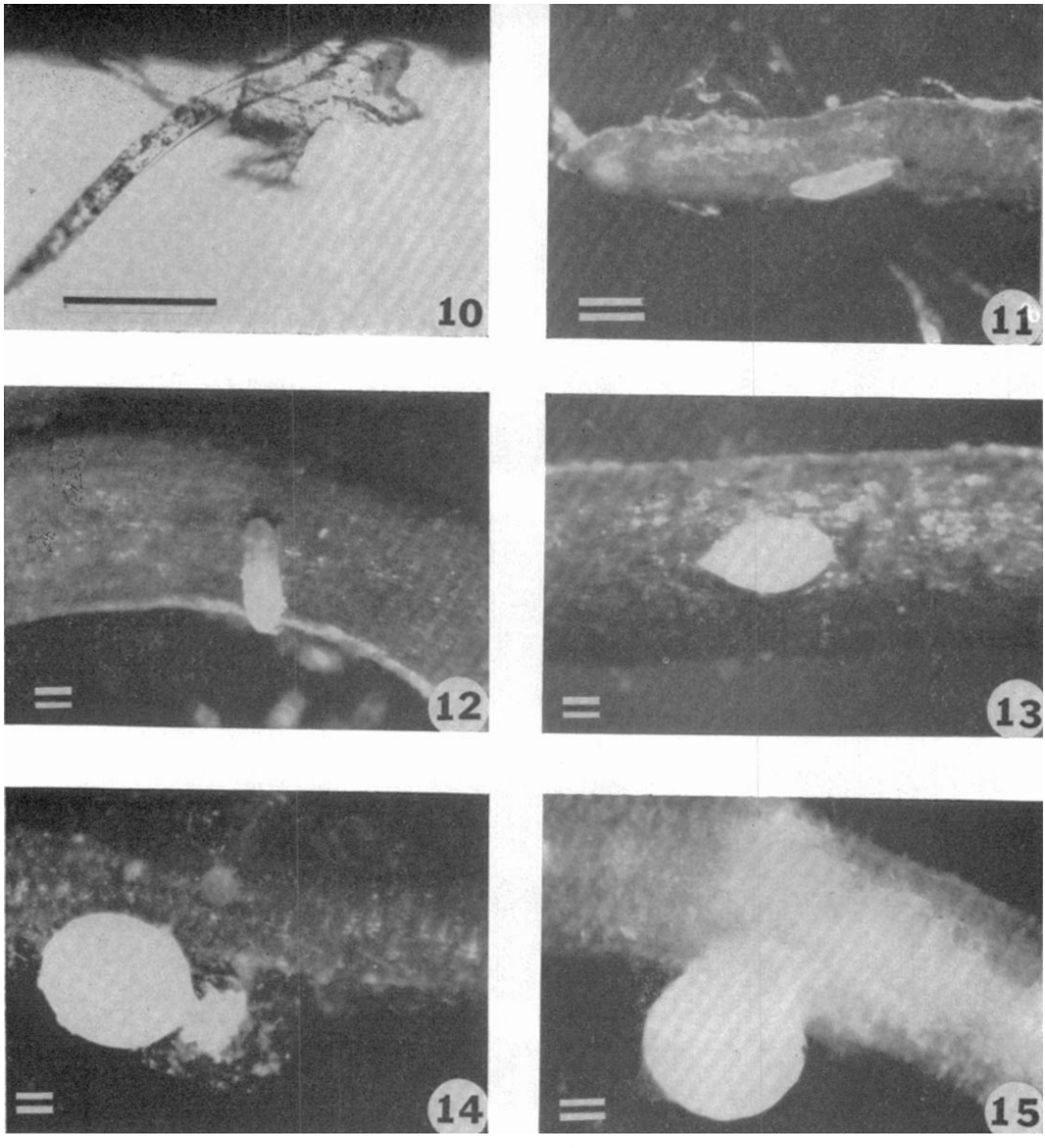
Histology: H. mediterranea induced cellular alterations in cortical, endodermal, pericyclic, and vascular parenchyma tissues of infected olive and pistachio roots. In olive roots, the anterior body portion of the J_2 penetrated into the cortex, and stylets were inserted into enlarged synctial cells (Figs. 16, 17) 3–5 days after inoculation. The cytoplasm of active syncytia was dense (dark staining) alveolar and contained small globules and numerous hypertrophied nuclei and nucleoli (Fig. 18).

In both olive and pistachio secondary roots, the syncytia extended from the stelar area into the cortical parenchyma 40 days after inoculation (Fig. 19). Compression of xylem elements and disorder of stelar structure were common in infected roots of both hosts (Fig. 19).

These results indicate that embryo-



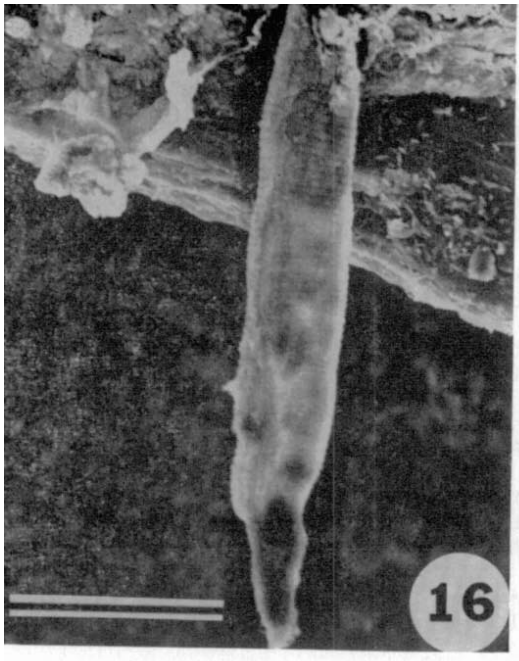
Figs. 1–9. Embryogenic development of *Heterodera mediterranea*. 1) Egg at one-cell stage in the white female gonad. 2) Two-cell stage. 3) Three-cell stage. 4) Four-cell stage. 5) Multicellular stage. 6) Gastrula. 7-8) First-stage juvenile. 9) Second-stage juvenile, note the stylet (arrow). (Scale bar = 25 μm.)

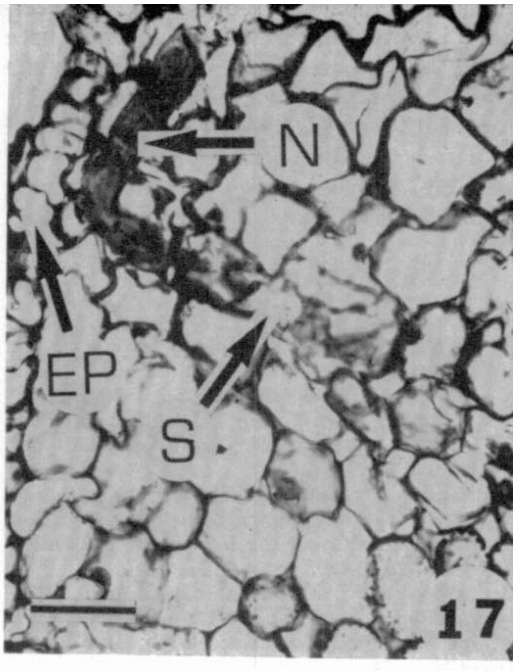


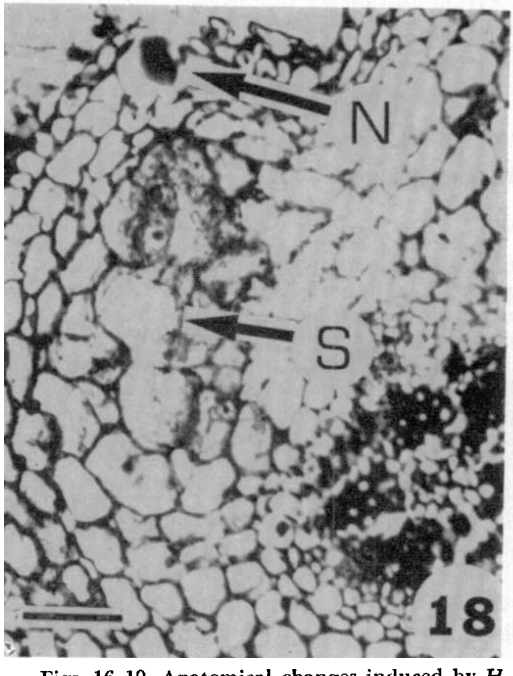
Figs. 10–15. Postembryogenic development of *H. mediterranea* on pistachio (*Pistacia vera* L.) and olive (*Olea europaea* L.) roots. 10–11) Second-stage juveniles on pistachio and olive roots 5 days after inoculation. 12) Third-stage juveniles 8 days after penetration on pistachio roots. 13) Fourth-stage juveniles just before the last molt on pastachio root. 14) Mature white female on pistachio roots 56 days after juvenile penetration. 15) Mature white female on olive roots 40 days after juvenile penetration. (Scale bar = 100 μm.)

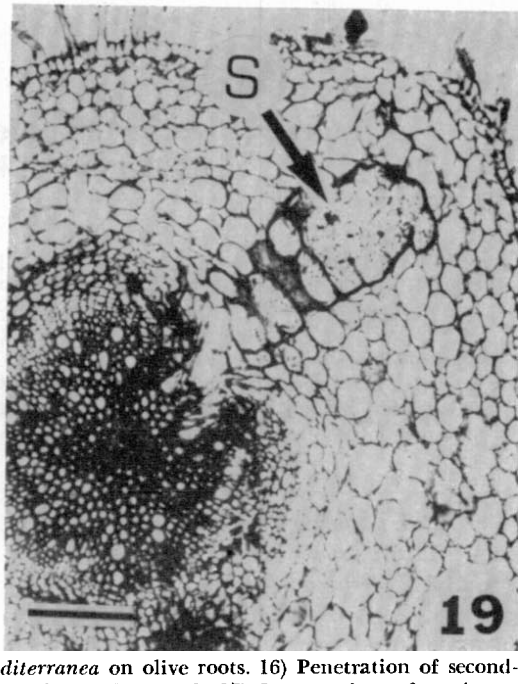
genesis, postembryogenic development, and the parasitic habits of *H. mediterranea* are similar to other *Heterodera* species. The nematode induces anatomical changes and syncytia formation as reported for other *Heterodera* spp. Strubell (10) and Steele (9) reported some semiendoparasitic development of *H. schachtii* on small roots of beet and tomato; however, the majority of externally developing nematodes became males. Crump and Kerry (1) and Unny (11) also report semiendoparasitic development of *H. avenae* on oat roots and *H. sacchari* on rice roots, respectively. Semiendoparasitic development did not occur with all specimens and appeared to be associated with development in fine roots.

H. mediterranea differs from the other cyst-forming nematodes in the semiendoparasitic habits of all nematode juveniles









Figs. 16–19. Anatomical changes induced by *H. mediterranea* on olive roots. 16) Penetration of second-stage juveniles of *H. mediterranea* in olive feeder roots. SEM micrograph. 17) Cross sections of a primary root 3 days after inoculation, showing the infective juvenile (N) near a syncytium at initial stage (S). EP = epidermis. 18) Cross section of 15-day infested root showing enlarged cortical syncytium (S) with granular cytoplasm and hypertrophied nuclei. Syncytium wall near the nematode (N) feeding site is slightly thickened. 19) Cross sections of olive secondary root 40 days after inoculation showing enlarged syncytium (S) extending into cortex and secondary vascular tissues. Note the stelar structure disorder. (Scale bar = 50 μm.)

and adult. The host range of *H. mediterranea* is apparently restricted to only three woody host plant species belonging to the families *Anacardiaceae* and *Oleaceae*.

So far *H. mediterranea* has been found only in Italy. Under natural conditions, it has been detected only on lentisc growing in deep coastal sands.

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