

Parasitism of Walnut, *Juglans regia*, by *Cacopaurus pestis*

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Cacopaurus petis Thorne was considered a monophagous sessile nematode parasite of Persian walnut (*Juglans regia* L.) roots in California (10). Recently *C. pestis* has been reported from France (7,8), Iran (9), Italy (4) and Spain (1) on hosts such as lilac (*Syringa vulgaris* L.), poplar (*Populus nigra* L.), rose (*Rosa indica* L. cv. Major), and sour orange (*Citrus aurantium* L.). Although the biology of *C. pestis* has been reported (3,7,10), many aspects of the life cycle of this species remain unclear. In this study biological observations of *C. pestis* were conducted on 3-yr-old walnut seedlings. Single seedlings were grown in pots containing 3 liters of soil inoculated with 3,000 second-stage juveniles and males collected in a walnut orchard in Campania, Italy. The inoculum in water suspension was obtained by incubation (11) of nematode infested walnut roots and was poured into 10 holes spaced evenly around the base of each seedling. After inoculation the plants were maintained in a screenhouse for 2 yr. Small root segments of infected secondary roots were used for histopathological studies of secondary feeder roots. These were washed free of soil, fixed in FAA (formalin, acetic acid, alcohol) for

48 h, dehydrated in tertiary butyl alcohol, and embedded in paraffin. The embedded tissue was sectioned at 10 or 15 μm , stained with safranin and fast green, mounted in Permount, and examined with a compound optical microscope (6).

Parasitic habits and histopathological observations: *C. pestis* primarily parasitizes the walnut secondary roots. In walnut the phellogen differentiation occurs in the first year. The nematode formed colonies between the epidermis and the multiple layers of cork (phellem) originated by phellogen (Figs. 1-2). The colonies extended 1-3 cm along the root axis. Mature swollen females and vermiform males, together with eggs and juveniles, congregated in groups and were embedded in a white felt-like mass (Figs. 2-3) produced by the phellogen in response to nematode feeding. The eggs adhered to the felt mass with a mucoid substance secreted during the egg laying (2).

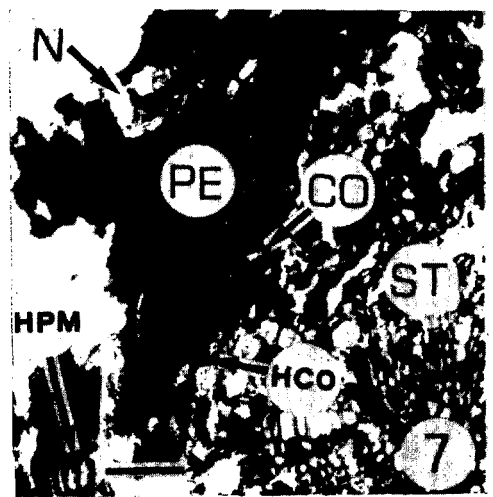
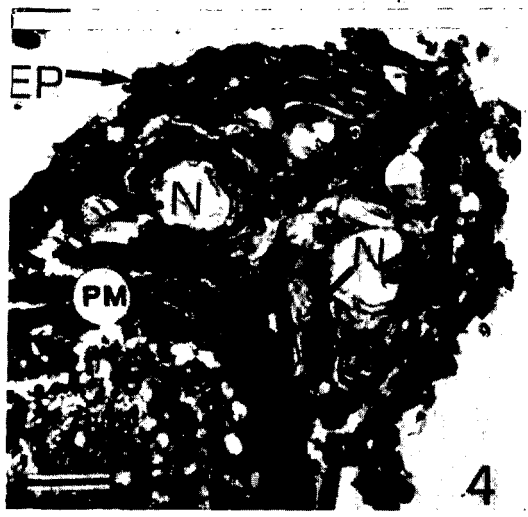
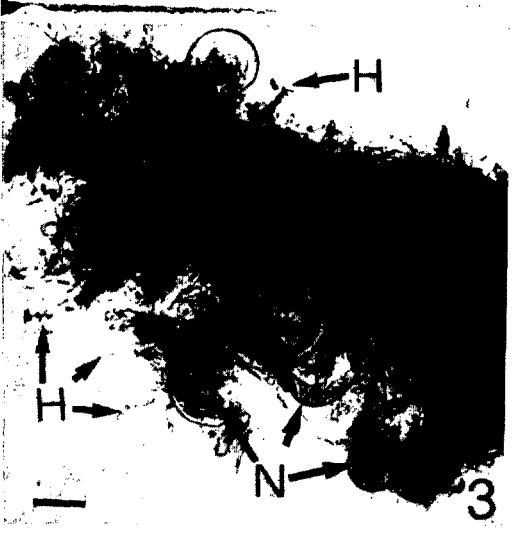
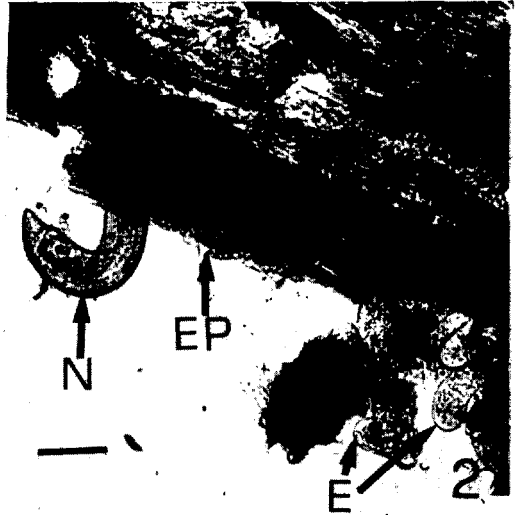
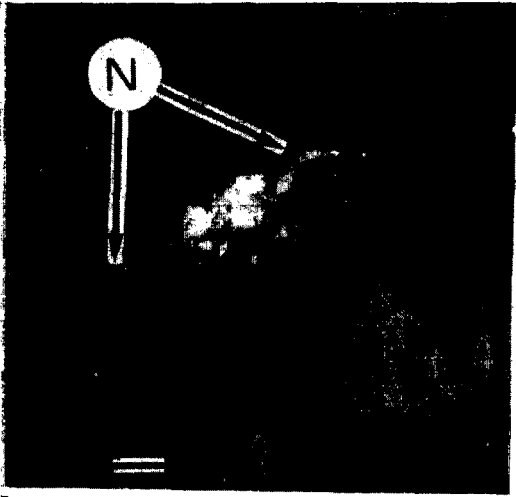
Observations of cross sections of infected roots showed that the epidermis was partially or completely detached from the phellem by the nematode colonies (Figs. 4-5). The long stylets of the females were inserted into the phellem (Fig. 6) where feeding caused necrosis (as indicated by safranin positive staining). Necrosis was also observed in the phellogen, phelloderm, and outer cortical parenchyma. These tis-

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Figs. 1-7. *Cacopaurus pestis* on secondary roots of walnut (*Juglans regia*). 1) Females (N) embedded in a white felt mass. 2) Female (N) and eggs (E) covered by the felt mass dislodged from beneath the epidermis (EP). 3) Nematodes (N) embedded in the felt mass of curled hairs (H). 4) Cross section of root showing a colony of *C. pestis* females (N) wedged between the epidermis (EP) and the phellem (PM). 5) Cross section of root showing a *C. pestis* female (N) between the epidermis (EP) and the phellem (PM); spear (SP). 6) Cross section of root showing a *C. pestis* female (N) with its spear (S) inserted into the phellem (PM). 7) Cross section of root showing accentuated necrosis of the periderm (PE) and outer cortex (CO) in relation to the nematode (N) feeding site. Note the healthy phellem (HPM) and cortex (HCO) cells in the root portion not affected by the nematode feeding; stele (ST). Scale bare (Fig. 1) = 100 μm ; (Fig. 2-7) = 50 μm .



sues formed a necrotic pit surrounded by healthy root tissue (Fig. 7). No evidence of damage to stelar tissue was detected.

Scotto La Massese (7) reported that *C. pestis* females became attached to the root surface by their stylets in a manner similar to *Gracilacus peratica* Raski, a species which also forms colonies on the root surface of its hosts. *G. peratica* females become swollen and remain permanently attached on the root surface by inserting the spear into the epidermis and the outer cortex (5). However, in the case of *C. pestis* on walnut roots, the nematode remains concealed beneath the epidermis of the secondary roots and feeds on periderm and outer cortex.

The initial damage caused by *C. pestis* on walnut roots seems to be limited, but it predisposes the infested plants to dieback (10). The extent of the nematode infestation, with the resultant necrosis of phellogen and phelloderm induced by the parasite feeding activity, reduces the protective function of the periderm and favors invasion by other pathogens.

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