

Helicotylenchus oleae n. sp. and *H. neopaxilli* n. sp. (Hoplolaimidae), Two New Spiral Nematodes Parasitic on Olive Trees in Italy

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Abstract: *Helicotylenchus oleae* n. sp. and *H. neopaxilli* n. sp., from olive roots and soil in Italy, are described and illustrated. *Helicotylenchus oleae* can be distinguished from the related species *H. canadensis* and *H. tunisiensis* especially by the smaller stylet, its distinctive tail shape, and a tail longer than one anal body width. *Helicotylenchus neopaxilli* differs from the close species *H. paxilli* by having a conical, anteriorly truncated labial region, shorter stylet, and phasmids always anterior to level of anus. Also illustrated and discussed are histopathological changes within feeder roots of olive caused by the feeding activity of the semi-endoparasitic *H. oleae*.
Key Words: taxonomy, histopathology.

Three *Helicotylenchus* species have been reported in association with olive (*Olea europaea* L.) trees: *H. dihystra* (Cobb) Sher, in Egypt and Rhodesia (1, 6); *H. erythrinae* (Zimmermann) Golden, in Italy (2); and *H. tunisiensis* Siddiqi, in Israel (6). This paper describes two undescribed species recently found in soil from around roots of olive trees in southern Italy. Also discussed and illustrated are histopatholog-

ical changes in olive roots caused by one of them (*H. oleae* n. sp.).

MATERIALS AND METHODS

Nematodes were extracted by the Cobb decanting and sieving method from soil samples containing olive feeder roots. Specimens were killed and fixed in a hot aqueous solution of 4% formaldehyde + 1% propionic acid, dehydrated slowly in an alcohol-saturated chamber, and mounted in dehydrated glycerin (5).

Small root segments with nematodes attached on the surface were used for histopathological studies of feeder roots. These were washed free of soil, fixed in FAA (formalin, acetic acid, alcohol) for 48 h,

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dehydrated in tertiary butyl alcohol and embedded in paraffin. They were sectioned at 10 to 15 μm , stained in safranin fast green, mounted in Permount and examined with a compound optical microscope (4). Nematode-infected root segments, 4–5 mm long, were fixed in hot (55 C) lactophenol, washed and dehydrated in acetone, dried for 3 h at room temperature, metalized with gold, and observed with a scanning electron microscope (SEM) (3).

SPECIES DESCRIPTION

Helicotylenchus oleae n. sp.

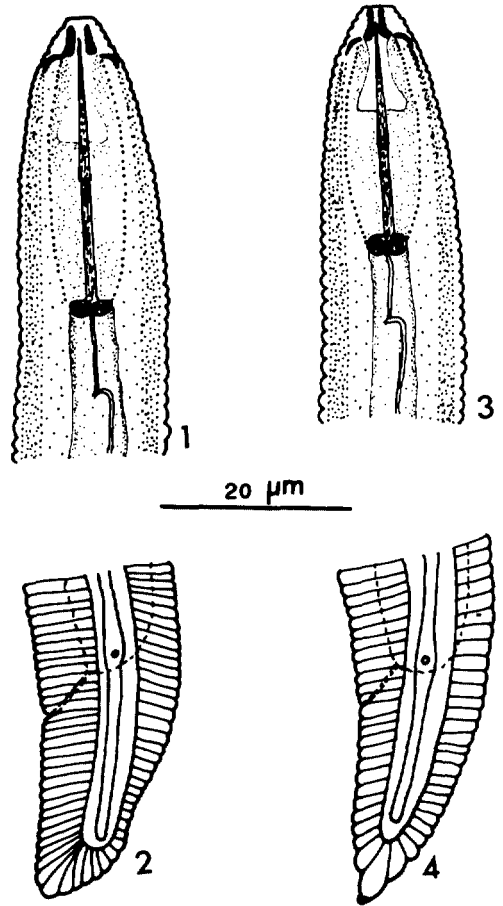
Paratypes (20 females): L = 0.80 mm (0.67–0.93); a = 34 (33–36); b = 5.5 (4.6–5.7); b' (body length/distance from anterior end to posterior end of esophageal glands) = 4.6 (3.9–5.0); c = 44 (37–45); c' (tail length/body width at anus) = 1.2 (1.1–1.7); V = 61% (58–65); stylet = 30 μm (29–31); m (length of anterior part of stylet \times 100/total stylet length) = 48 (46–51); O (distance from stylet base to dorsal esophageal gland outlet \times 100/total stylet length) = 30 (27–38).

Holotype (female): L = 0.70 mm; a = 35; b = 5.6; b' = 4.5; c = 40; c' = 1.3; V = 65%; stylet = 30 μm ; m = 50; O = 30.

Population from Chiatona (Taranto), Italy (15 females): L = 0.77 mm (0.75–0.80); a = 35 (31–39); b = 5.7 (5.4–6.0); b' = 4.8 (4.7–5.0); c = 40 (36–45); c' = 1.4 (1.3–1.6); V = 61% (60–61); stylet = 30 μm (29–31); m = 49 (47–51); O = 29 (27–32).

Female body usually in spiral shape (Fig. 5). Labial region continuous, bluntly conical, flattened anteriorly, and with 4–5 annules (Figs. 1, 6–9). Stylet knobs flattened and indented anteriorly. Lateral field with four lines (Figs. 2, 35, 36). Spermatheca often well defined, but without sperm. Phasmids 4–7 annules anterior to anus. Tail length more than one anal body width, 17 μm (15–21) long and with 16 to 21 annules. Tail on dorsal side at or near anus becomes smaller, tapering in a characteristic manner and continuing to the terminus, which is curved much more dorsally (Figs. 2, 34, 10–15).

Male: Unknown.



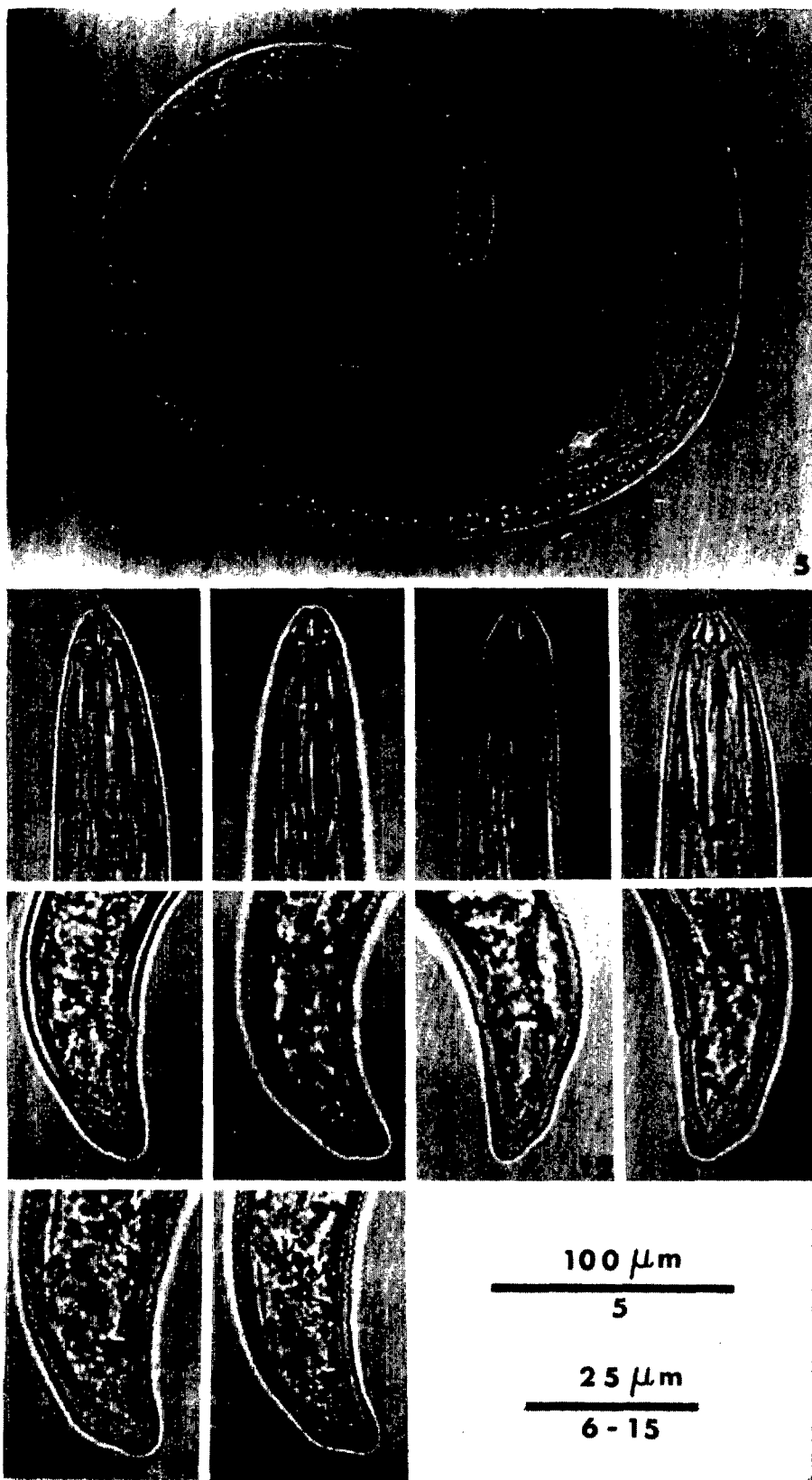
FIGS. 1-4. 1, 2) Drawings of anterior and posterior portions of adult females of *Helicotylenchus oleae* n. sp. 3, 4) *H. neopaxilli* n. sp.

Holotype (female): Slide 3/1/1, Laboratorio Nematologia agraria, CNR, Bari, Italy.

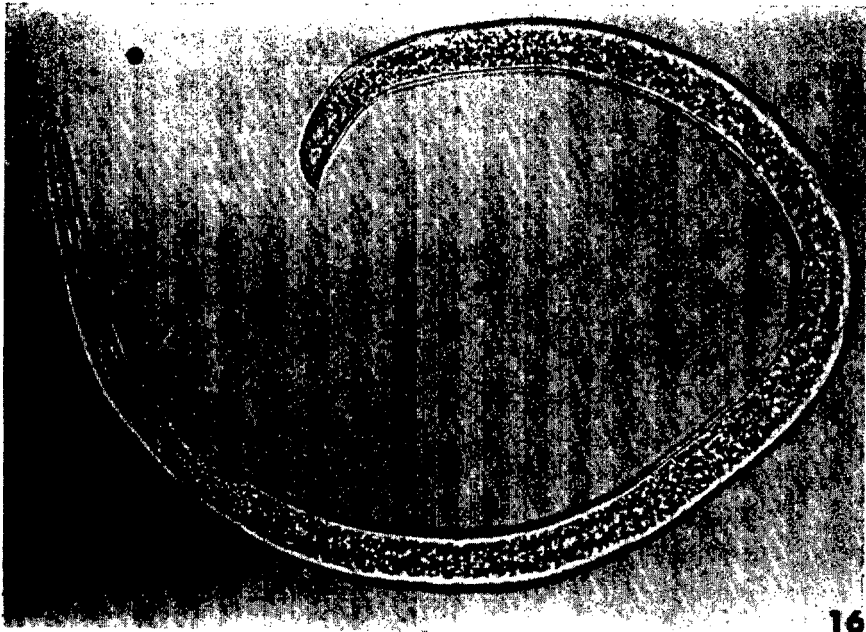
Paratypes distributed as follows: Slides 3/1/2-21 at Laboratorio Nematologia agraria, CNR, Bari, Italy; 5 females each at: Plantenziektenkundige Dienst, Wageningen, The Netherlands; Nematology Department, Rothamsted Experimental Station, Harpenden, United Kingdom; University of California Nematode Survey Collection, Davis, California, USA; the United States Department of Agriculture Nematode Collection, Beltsville, Maryland, USA; and the Indian Agricultural Research Institute, New Delhi, India.

Type habitat and locality: Soil and roots of olive trees, Palo del Colle (Bari), Italy.

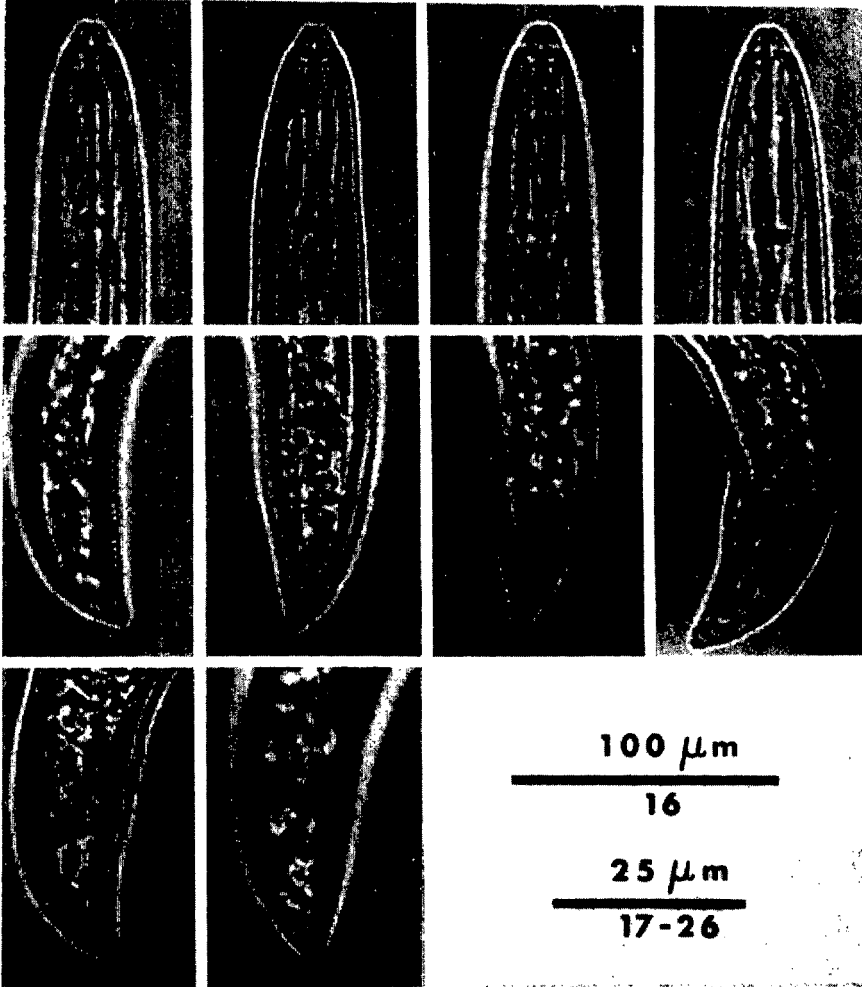
Diagnosis: *Helicotylenchus oleae* n. sp. is closely related to *H. canadensis* Waseem,



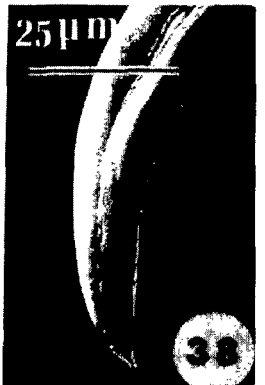
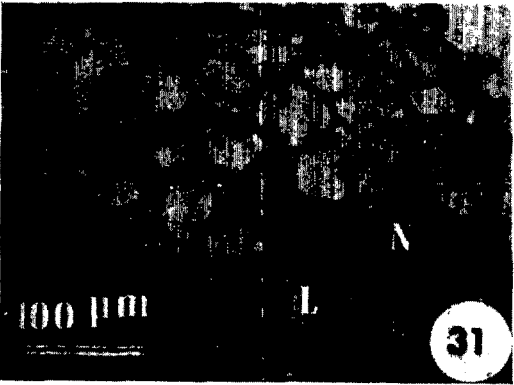
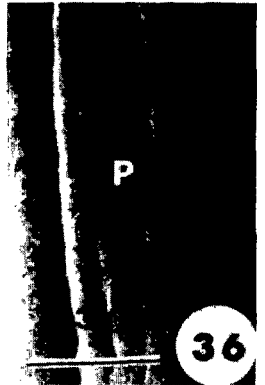
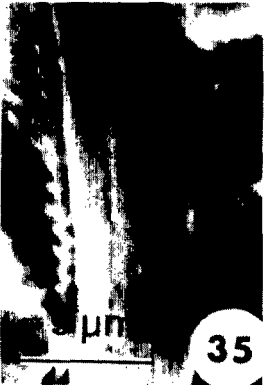
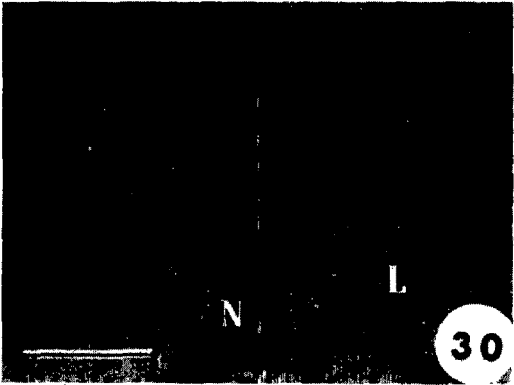
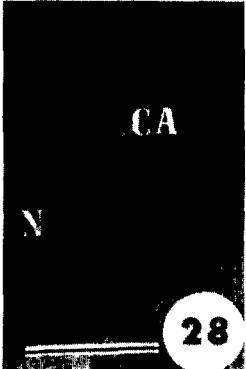
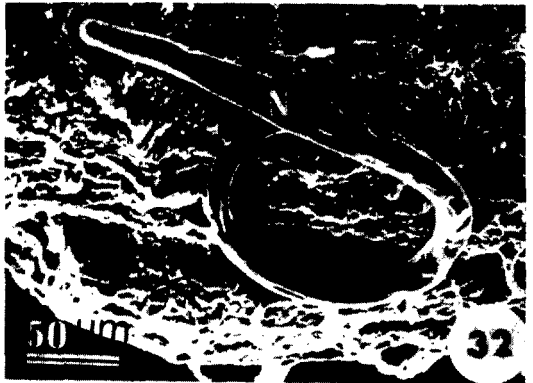
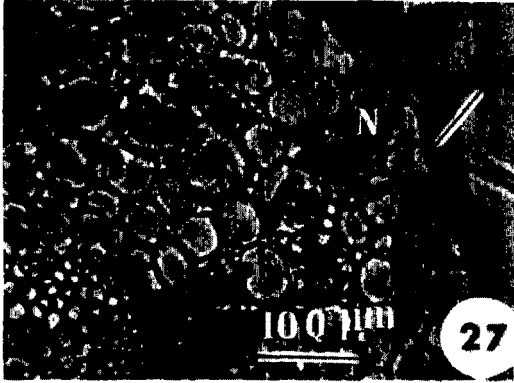
FIGS. 5-15. Photomicrographs of females of *Helicotylenchus oleae* n. sp. 5) Whole specimen. 6-9) Anterior region of four different females. 10-15) Tail region of six different females.



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FIGS. 16-26. Photomicrographs of females of *Helicotylenchus neopaxilli* n. sp. 16) Whole female. 17-20) Anterior portion of four different specimens. 21-26) Tail region of six different females.



1961, and *H. tunisiensis* Siddiqi, 1963. *Helicotylenchus oleae* n. sp. can be distinguished from the former especially by its distinctive tail shape and its greater tail length, being over one anal body width (less than one anal body width in *H. canadensis*). It differs further from *H. canadensis* in having a shorter stylet (29–31 μm vs. 31–33 μm) and a higher O value (27–32 vs. 21–29). *H. oleae* n. sp. can be differentiated from *H. tunisiensis* by its smaller stylet (29–31 μm vs. 32–36 μm) and its distinctive tail shape. Also, *H. oleae* n. sp. has a typically conical, truncated head, whereas in *H. tunisiensis* the head appears trapezoid in lateral view and with sides slightly concave but truncate anteriorly.

Although Waseem (7) gave a stylet length of . . . "30 (28–30) μ " . . . for *H. canadensis*, Yuen (8) established the length as 31–33 μm in a redescription of the species, and Sher (6) reported it as being 30–33 μm . Also consistent with Yuen's report are observations of specimens by the third author of this paper.

Helicotylenchus oleae n. sp. is widespread in olive orchards in Italy.

Helicotylenchus neopaxilli n. sp.

Paratypes (20 females): L = 0.65 mm (0.61–0.75); a = 33 (28–37); b = 4.9 (4.8–5.8); b' = 3.8 (3.6–4.8); c = 39 (32–42); c' = 1.6 (1.2–1.9); V = 60% (55–64); stylet = 25 μm (23–26); m = 52 (49–55); O = 30 (27–33).

Holotype (female): L = 0.73 mm; a = 29; b = 5.3; b' = 4.5; c = 36; c' = 1.6; V = 60%; stylet = 25 μm ; m = 52; O = 31.

Female body usually in spiral shape (Fig. 16). Labial region continuous, conical, and truncated anteriorly, with 4–5 annules (Figs. 3, 17–20). Stylet with rounded knobs; dorsal esophageal gland orifice 1/3 or less

of stylet length from base of stylet. Lateral field with four lines (Fig. 4). Spermatheca visible, without sperm; vulva distinct (Fig. 37). Phasmids 3–5 annules anterior to anus. Tail with pronounced ventral extension, which sometimes possesses a small mucron, and with 10–14 annules (Figs. 4, 21–26, 38).

Male: Unknown.

Holotype (female): Slide 3/2/1, Laboratorio Nematologia agraria, CNR, Bari, Italy.

Paratypes distributed like those of *H. oleae* n. sp.: Slides 3/2/2–21 at Laboratorio Nematologia agraria, CNR, Bari, Italy.

Type habitat and locality: Soil from olive rhizosphere, Catania, Sicily.

Diagnosis: *Helicotylenchus neopaxilli* n. sp. is closely related to *H. paxilli* Yuen, 1964. It can be distinguished from *H. paxilli* especially by its conical, anteriorly truncated labial region (vs. "Head bulbous" or hemispherical); shorter stylet (23–26 μm vs. 29–32 μm) with rounded knobs; greater O value (27–33 vs. 22–27); and phasmids always anterior to level of anus. In addition, the head of *H. neopaxilli* n. sp. is continuous and with 4–5 annules, but is offset and with 6 annules in *H. paxilli*.

This new species has been found so far only in Sicily.

Feeding habits of H. oleae: *Helicotylenchus oleae* has been observed in semi-endoparasitic feeding positions (Figs. 32, 33) on olive feeder roots. The anterior portion of the body of this species penetrates the cell walls of the epidermis and 5 or 6 layers of the cortex (Figs. 30, 31). Coiled specimens were sometimes observed within the epidermis and 2 or 3 cells into the cortex (Figs. 27, 29). Brown lesions were formed in the cell walls adjacent to the nematode body (Fig. 31) and cavities remained in the cortex when the parasite



FIGS. 27–38. 27) Cross section of olive roots showing a coiled specimen (N) of *H. oleae* in the epidermis (EP) and cortex (CO). 28) Head of *H. oleae* (N) in a cavity (CA) of the cortex of olive feeder root. 29) A particular portion of Fig. 27 at greater magnification. 30–31) Cross sections of olive feeder roots showing *H. oleae* (N) in semi-endoparasitic feeding position and brown lesions (L) of cell walls adjacent to the nematode's body. 32) *Helicotylenchus oleae* (N) feeding on olive rootlet (SEM micrograph). 33) A portion of Fig. 32 at greater magnification. 34) Female tail of *H. oleae* (SEM micrograph). 35) Lateral field of *H. oleae* female (SEM micrograph). 36) Lateral field of *H. oleae* at the phasmids (P) level. 37) *Helicotylenchus neopaxilli* vulva area (SEM micrograph). 38) Female tail of *H. neopaxilli* (SEM micrograph).

withdrew from the feeder roots (Figs. 28, 30). *Helicotylenchus oleae* fed on a selected site on the root for only a limited time and then moved to other feeding sites. The nematode's feeding activity appeared to affect only epidermal cells and cortical tissue. No evidence of vascular damage was detected.

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