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MORPHOLOGY AND HISTOPATHOLOGY OF THE CEREAL CYST  
NEMATODE (*HETERODERA AVENAE* WOLL.)  
ATTACKING WHEAT, OATS AND BARLEY IN ITALY

by  
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*Heterodera avenae* Wollenweber, 1924<sup>(1)</sup> is widely distributed in the Italian cereal growing areas where severe attacks have been reported on wheat (*Triticum durum* Desf.), oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) by Inserra *et al.* (1978) and Marinari (1981). To avoid misidentification of *H. avenae* populations present in Italy and confusion with other species of the « *Heterodera avenae* group » in the mediterranean region, detailed morphological observations by light microscopy (LM) and scanning electron microscopy (SEM) have been made on a southern Italian population of *H. avenae* and compared with those reported from Australian, European and Canadian ones. The anatomical changes of infested barley roots are described and peculiarities of host-parasite relationships of *H. avenae* are discussed.

*Materials and Methods*

Females and cysts used in this study were collected directly from wheat and barley roots and were measured in water. Newly hatched

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(1) Comments of Sturhan (1982) and Stone and Hill (1982) on the identification of European cereal and grass cyst nematodes have been taken into account in the identification of the Italian population.

second stage juveniles (J<sub>2</sub>) and males were killed and fixed in hot aqueous solution of 4% formaldehyde + 1% propionic acid and mounted in dehydrated glycerin (Seinhorst, 1966). SEM observations were made on J<sub>2</sub> and males killed and fixed in formalin-propionic acid 4:1; transferred to 1% osmium tetroxide solution for 12 h, infiltrated with Spurr's resin and mounted on SEM stubs (De Grisse, 1973). Cyst perineal and neck portions were placed in 1% osmium tetroxide for 6 h, transferred to lactophenol, then mounted on SEM stubs and dried for 2 days at room temperature. Specimens were coated with gold and observed by SEM at 5 kV accelerating voltage. For histopathological observations, segments of naturally infested roots were fixed for 48 h in formalin-acetic acid-ethanol solution, embedded in paraffin, sectioned 10 µm thick, stained with safranin and fast green and mounted for microscopic examination (Johansen, 1940).

*Description of an Italian population of Heterodera avenae  
from Gravina, Bari*

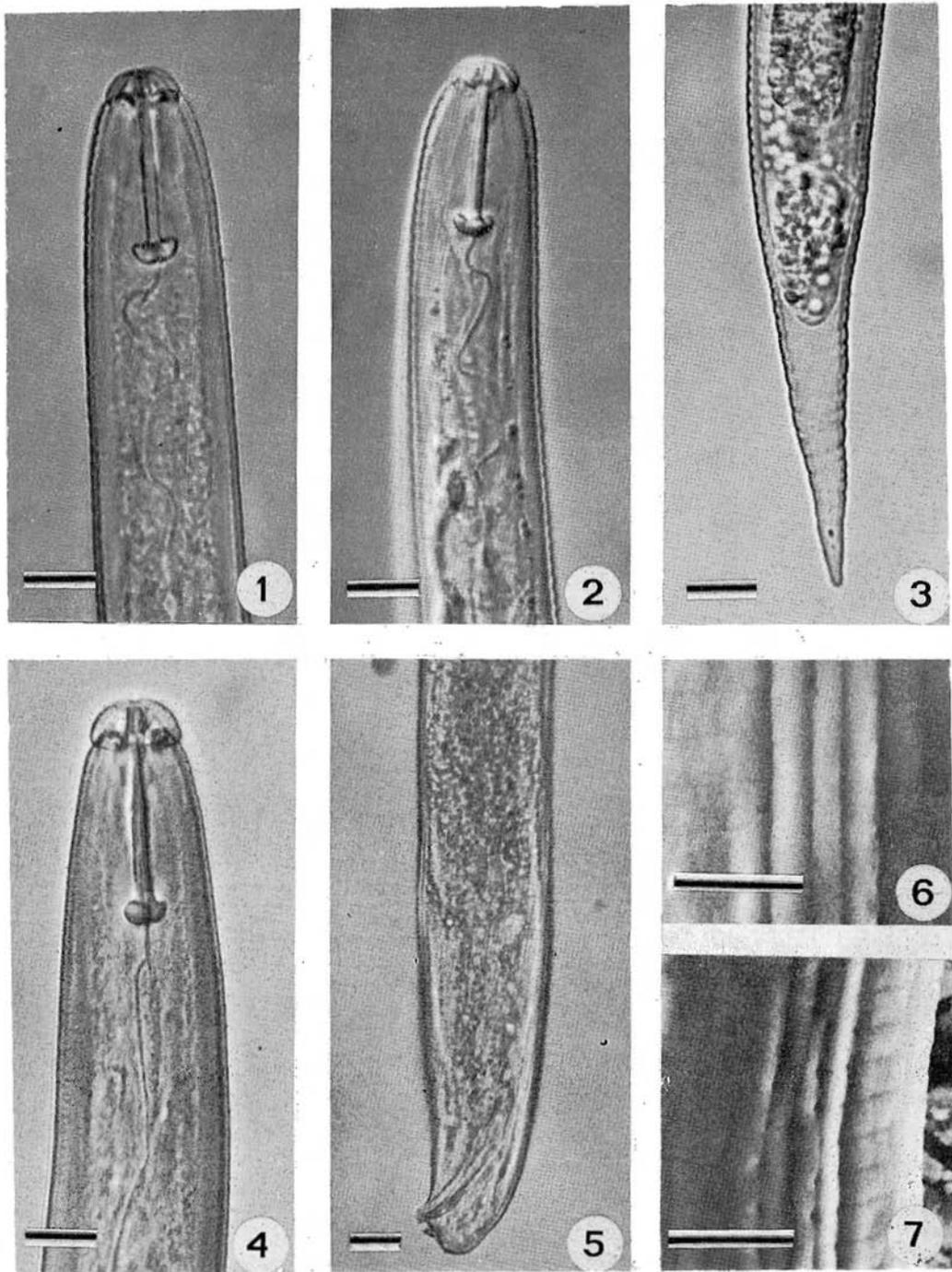
Eggs: (n = 75). Length = 132 (120-142) µm; width = 42 (40-44) µm; egg shell unsculptured and hyaline. J<sub>2</sub> folded three or four times within the egg shell.

*Second stage juveniles:* (n = 50). L = 541 (515-570) µm; a = 23 (21-25); b' (to the end of oesophageal lobe) = 2.5 (2.3-2.8); c = 8.8 (8.6-9.0); stylet length = 27 (26-28) µm; tail length = 60 (58-63) µm; hyaline tail length = 41 (40-42) µm; ratio hyaline tail/stylet = 1.55 (1.46-1.57).

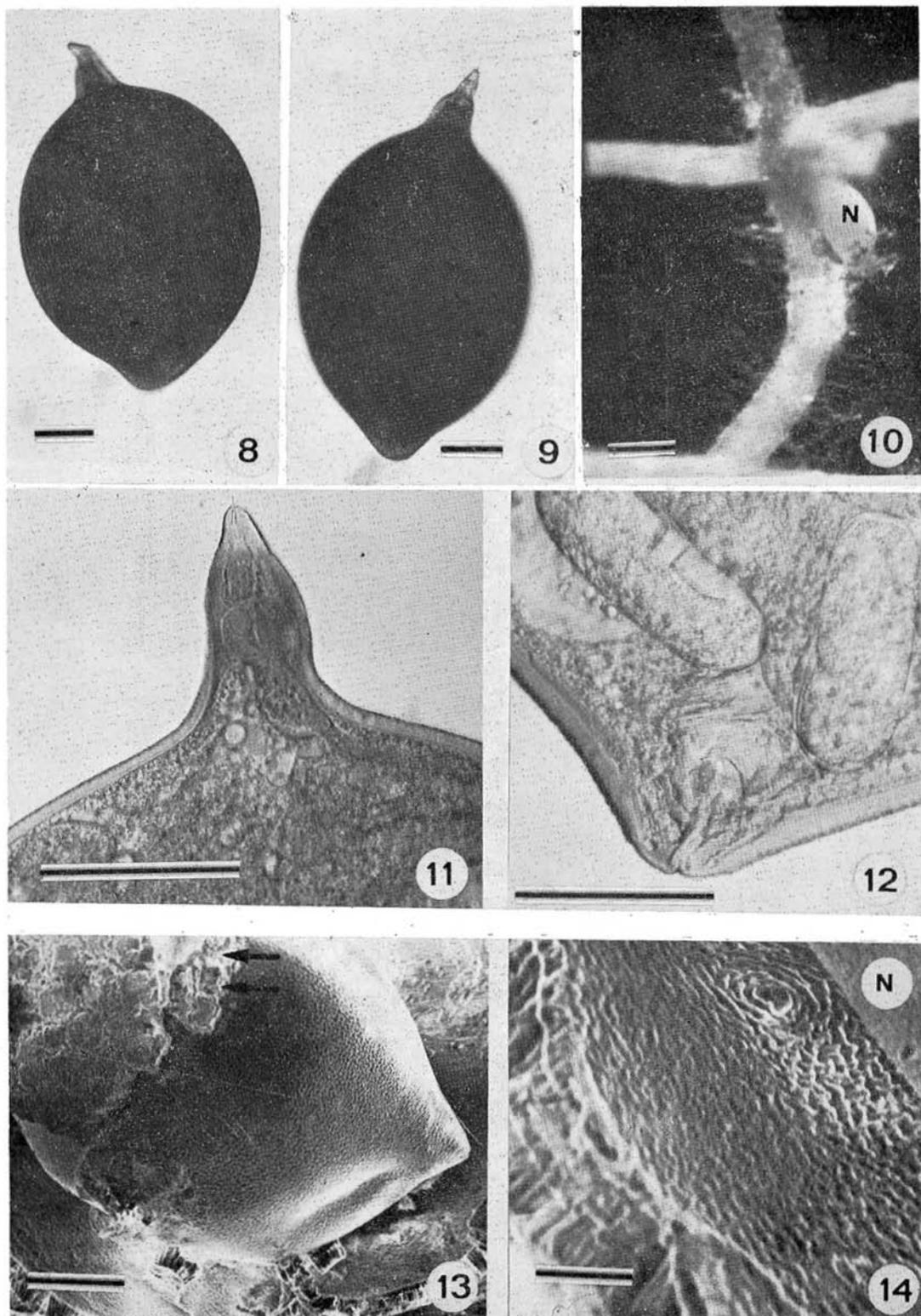
Head hemispherical, slightly offset 4 µm high and 9-10 µm wide with massive skeleton (Figs 1, 2). Stylet well-developed with concave anterior surfaces of knobs 4-5 µm wide. The dorsal oesophageal gland opens 6-7 µm behind stylet base. Head tip to excretory pore distance 110 (106-112) µm. The oval genital primordium 10 x 20 µm is situated at 59% of the body length. Cuticular annulations distinct, about 1.6 µm apart. Lateral field with four incisures (Fig. 7) 20% of body width, with outer bands areolated.

Tail regularly annulated tapering uniformly with a terminal hyaline portion 67 (64-68)% of tail length.

*Gravid females:* (n = 30). L = (body length excluding neck) 604



Figs. 1-7 - Second stage juveniles ( $J_2$ ) and male of an Italian population of *Heterodera avenae*: 1, 2)  $J_2$  anterior body portion; 3)  $J_2$  tail; 4) male head; 5) male tail; 6) male mid-body lateral field; 7)  $J_2$  mid-body lateral field. Scale bar of Figs 1-5 = 10  $\mu$ m and of Figs 6-7 = 5  $\mu$ m.



Figs 8-14 - Gravid females and cysts of an Italian population of *H. avenae*: 8, 9) lemon-shaped gravid females; 10) gravid female (N) attached to a barley root. Note proliferation of lateral roots caused by nematode infestation; 11, 12) neck and terminal cone of a gravid female; 13) SEM photomicrograph of a female body partially covered by subcrystalline layer (arrows); 14) replication of cuticular pattern of inner surface of the subcrystalline layer at the excretory pore area. Scale bar = 100  $\mu$ m.

(504-780)  $\mu\text{m}$ ; w = (body width) 341 (350-490)  $\mu\text{m}$ ; neck = 108 (95-120)  $\mu\text{m}$ ; stylet = 29 (28-30)  $\mu\text{m}$ ; L/W ratio = 1.4 (1.2-1.6).

Female body lemon shaped (Figs 8, 9, 10), with well defined neck and prominent terminal cone (Figs 11, 12, 13). A thick white subcrystalline layer covers the entire body (Figs 13, 14). No egg sac present. Vulva, terminally positioned as a transverse slit (Figs 22, 23). Anus subterminal located in a small depression of the cuticle 8-10  $\mu\text{m}$  in diameter (Fig. 24). Vulva-anus distance 40-57  $\mu\text{m}$ . Wall ornamentation with irregular zig-zag ridges (Fig. 21).

*Cysts*: (n = 20). L = (excluding neck) 710 (590-830)  $\mu\text{m}$ ; W = 512 (420-580)  $\mu\text{m}$ ; L/W ratio = 1.35 (1.30-1.38).

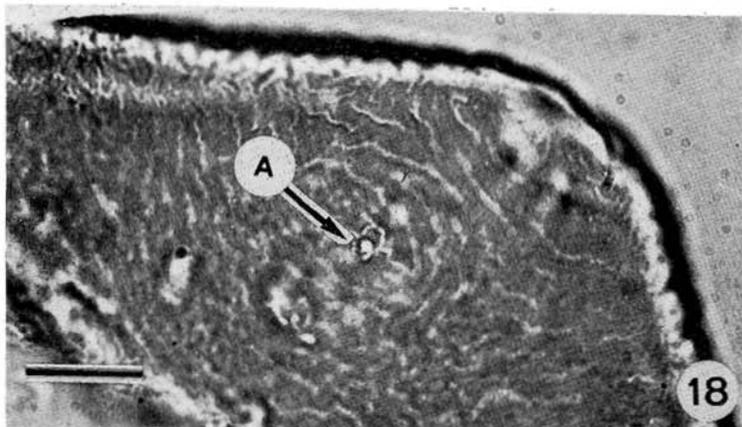
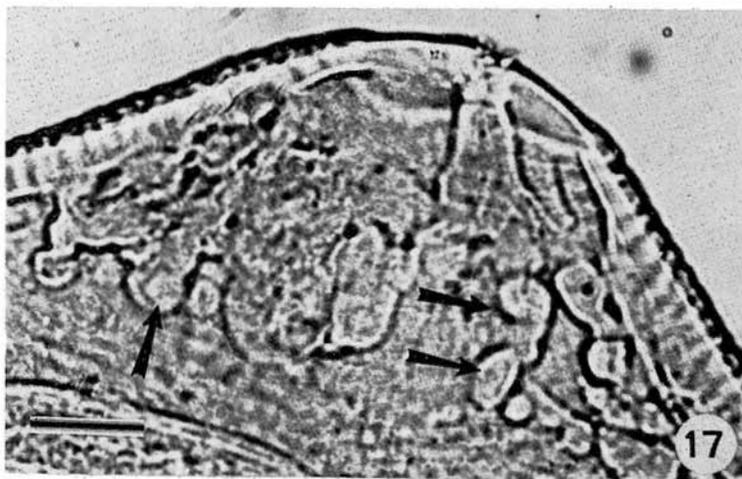
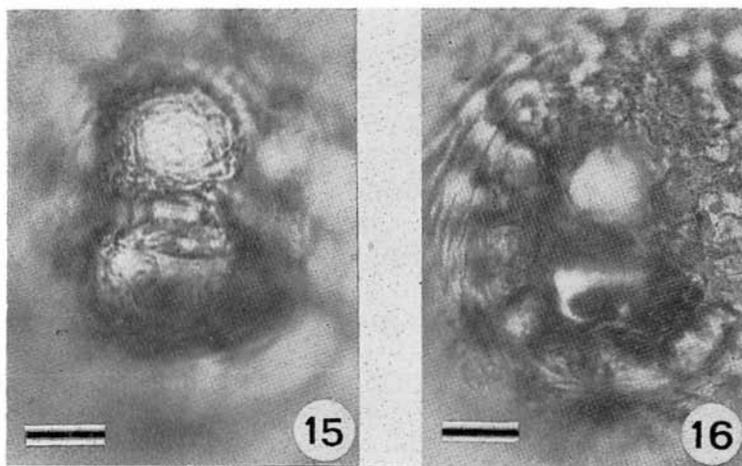
Predominantly lemon-shaped body with prominent neck and vulval cone. Conspicuous subcrystalline layer also covers the dark brown cysts. The external pattern of cyst wall consists of irregular zig-zag ridges (Fig. 21). Ambi-fenestration (Figs 15, 16) with circular semifenestrae. Numerous dark brown bullae are present irregularly distributed at the periphery of the vulval cone (Fig. 17).

*Vulval cone structures*: fenestral length = 43 (40-45)  $\mu\text{m}$ ; semi-fenestral length = 22 (20-24)  $\mu\text{m}$ ; vulva slit length = 10-12  $\mu\text{m}$ ; vulva-anus distance = 45-58  $\mu\text{m}$  (Fig. 18).

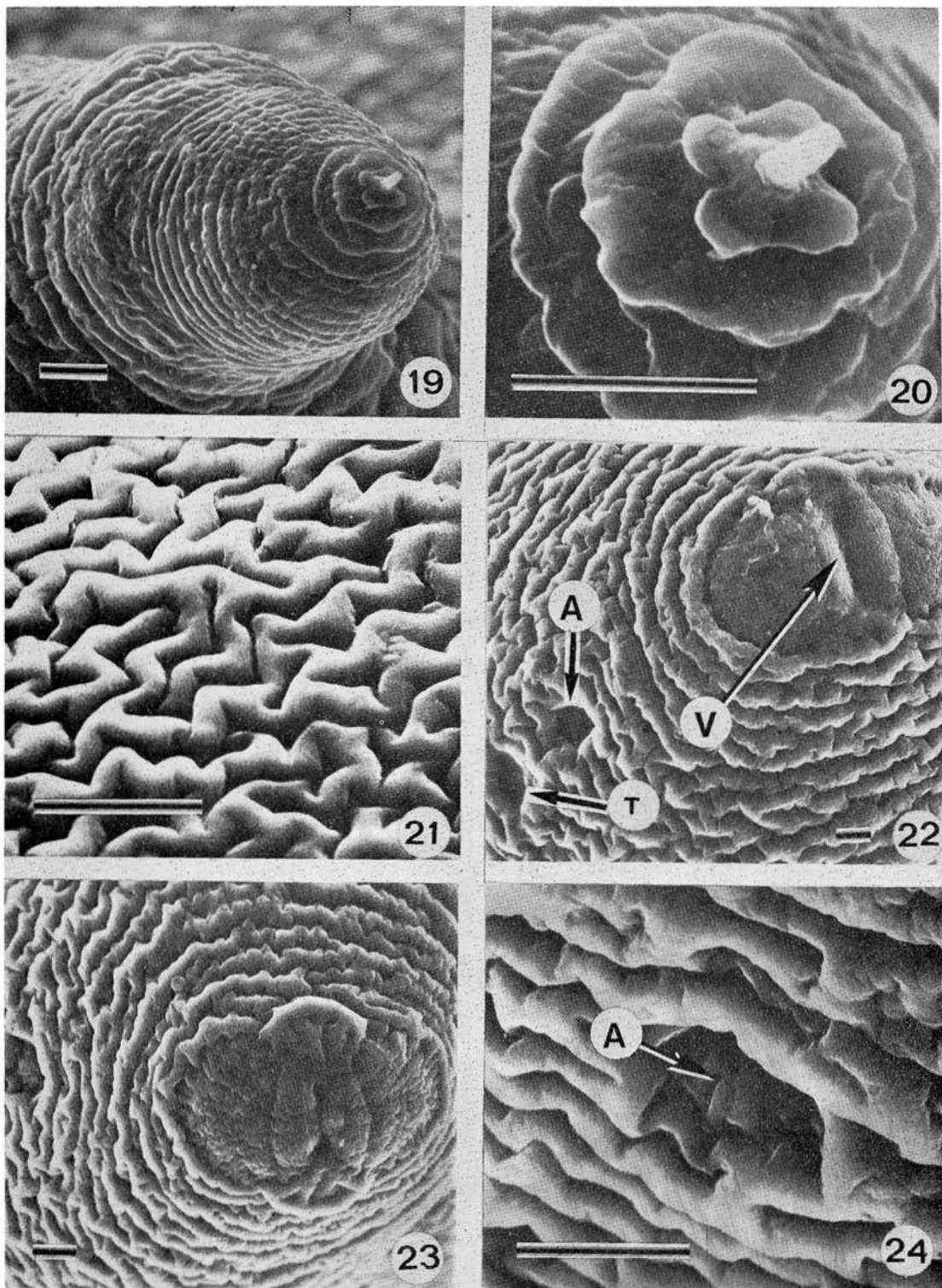
*Male*: (Figs 4, 5, 6). (n = 25). L = 1.46 (1.35-1.61) mm; a = 51 (48-55); b' = (end of lobe) 5.6 (5.0-6.4); T = 52 (41-60); stylet = 29.5 (29-30)  $\mu\text{m}$ ; spicules = 35-36  $\mu\text{m}$ ; gubernaculum = 10-12  $\mu\text{m}$ .

Body cylindrical, tending to twist at the posterior portion. Head set off 12-13  $\mu\text{m}$  wide, 5-6  $\mu\text{m}$  high. Cuticular annulation distinct, annules about 1.5-1.7  $\mu\text{m}$ . Lateral field marked by four lines (Fig. 6). Excretory pore at 158-170  $\mu\text{m}$  from the anterior end of body. Stylet strong with round knobs 4-5  $\mu\text{m}$  wide and directed slightly anteriorly. DGO opens 5-6  $\mu\text{m}$  behind the base of stylet.

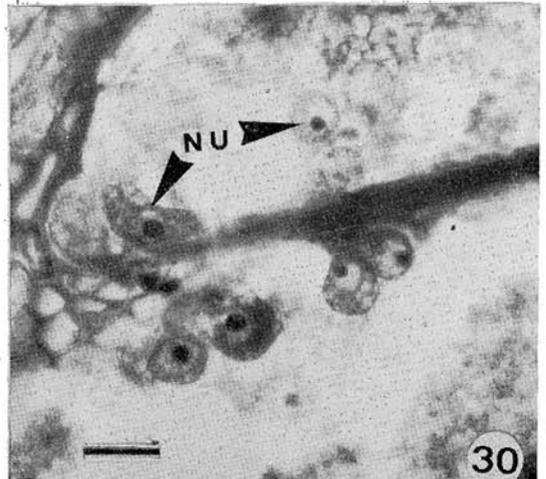
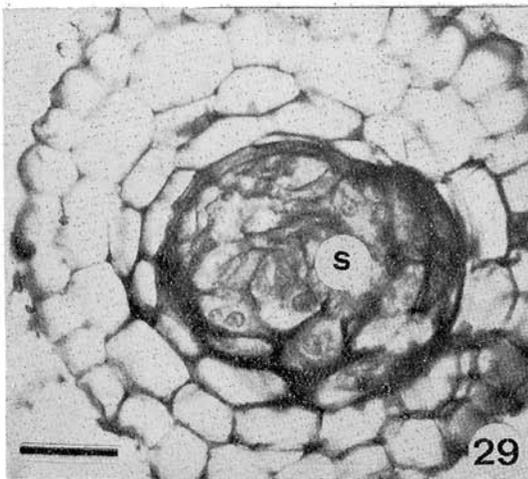
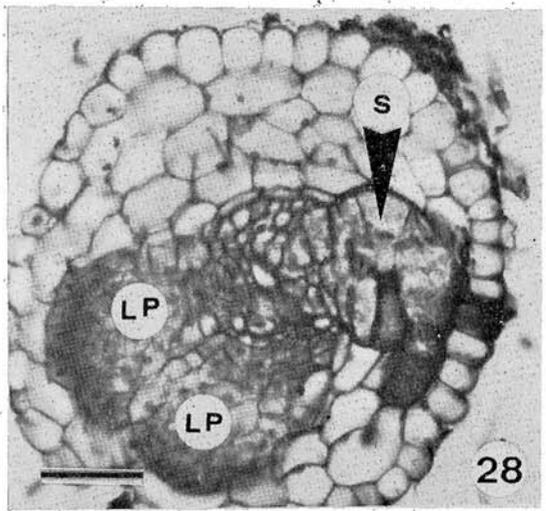
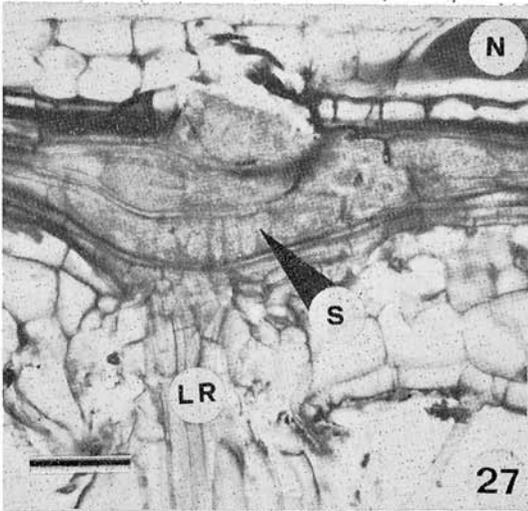
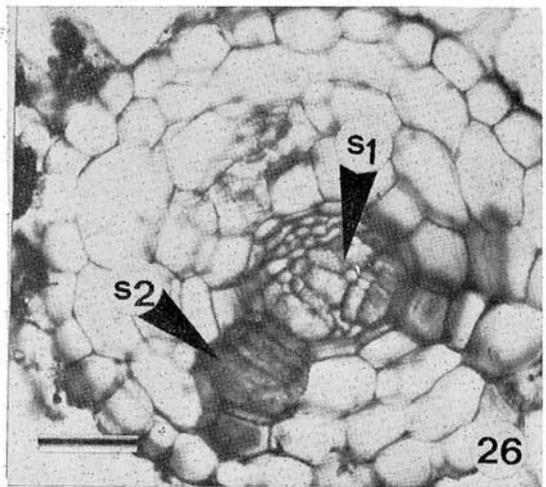
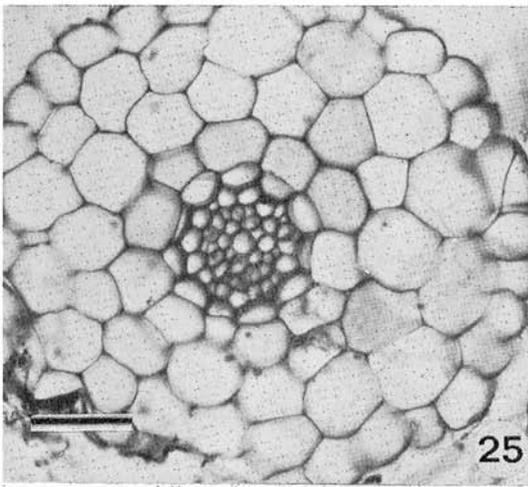
*Remarks*: *Heterodera avenae* with dark brown lemon-shaped, bullate and ambifenestrate cysts can readily be distinguished from the other species of *Heterodera* attacking cereals and grasses with the exception of *H. mani* and *H. hordecalis*. In the original description of *H. mani*, Mathews (1971) used several characters to distinguish *H. mani* from *H. avenae*. One of these was the presence of four incisions in the lateral field of J<sub>2</sub> of *H. mani* compared with the two lines described by Franklin (1951 and 1969) for *H. avenae*. My SEM observations of J<sub>2</sub> show that the Italian population of *H. avenae* also possesses four lines in the lateral field (Fig. 7) which agrees with the



Figs 15-18 - Fenestration in an Italian population of *H. avenae* cysts: 15) focus at surface; 16) same as in Fig. 15, but with deeper focus; 17) bullae in a dark brown cyst (arrowed); 18) ventral view of terminal cone, focus at surface (A = anus). Scale bar = 20  $\mu$ m.



Figs. 19-24 - Scanning electron photomicrographs of an Italian population of *H. avenae*: 19) female neck and head; 20) female head; 21) cyst mid-body cuticle; 22-23) unfenestrated vulval cones (A = anal depression; T = tail terminus; v = vulval slit); 24) anal area (A = anus). Scale bar = 5  $\mu$ m.



Figs 25-30 - Anatomical changes induced by *H. avenae* on barley roots compared with a noninfested root; 25) cross section of a noninfested root; 26) cross section of an infested root showing a syncytium extending from the cortex (S<sub>2</sub>) into the stele (S<sub>1</sub>); 27) longitudinal section showing a syncytium (S) induced by the nematode (N) adjacent to a lateral root (LR); 28) cross section showing a large cortical syncytium (S) extending into the stele, note proliferation of lateral root primordium (LP); 29) a large syncytium (S) within the stele; 30) enlarged syncytial cells filled with granular cytoplasm and numerous hyperthrophied nuclei (NU). Scale bar = 50 μm (Figs 25, 29); 15 μm (Fig. 30).

descriptions of Williams and Siddiqi (1972) and Meagher (1974) made respectively on English and Australian populations. However, *H. mani* J<sub>2</sub> differs from *H. avenae* for the shape of stylet knobs that are anchor-shaped in *H. mani* compared to those of *H. avenae*. In addition, *H. mani* does not reproduce on wheat, oats and barley. *H. avenae* differs from *H. hordecalis* Anderson (1975) (which also attacks cereals and grasses and morphologically resembles *H. latipons*) by the narrower vulva slit, 10-12 µm compared with 17-25 µm of *H. hordecalis*.

*Histopathology:* *Heterodera avenae* induces cellular alterations in the cortical, endodermal, pericyclic and vascular parenchyma tissues of infested barley roots (Figs 26-30). The syncytia were located in the stele, in the cortical parenchyma, or in both areas (Figs 26, 28, 29). Compression of xylem elements and disorder of the stelar structures were commonly seen in sectioned infested roots. Thus the histopathological effects caused by *H. avenae* in barley roots are similar to those observed by Endo (1964) for *H. glycines* in soybean and by Johnson and Fushtey (1966) for *H. avenae* on oats and maize.

#### S U M M A R Y

Morphometric data of *Heterodera avenae* Woll. from Italy are given. Important diagnostic features of second stage juveniles, males and gravid females and cysts are illustrated by light and scanning electron photomicrographs. The histological alterations induced by the Italian population of *H. avenae* in barley (*Hordeum vulgare* L.) roots are also discussed and illustrated.

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Accepted for publication on 7 February 1985.