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LIFE STAGES IDENTIFICATION AND FEEDING BEHAVIOR OF *OGMA CIVELLAE*

by
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Summary. *Ogma civellae* life stages are illustrated and differentiated on the basis of ovary development, body length, stylet length, cuticular ornamentation and arrangement of scales. Second-stage juveniles have body annules without spines, and body, ovary and stylet 138-177, 8-10, and 34-39 µm long, respectively. The third-stage juveniles have body annules with 4-6 palmate and spinate scales, ending with a variable number and form of projections disposed in eight longitudinal rows. This stage has body, ovary, and stylet 203-263, 16-19, and 45-60 µm long, respectively. The fourth-stage juveniles each have body annules with 8 scales similar to those of J3, disposed in 8 longitudinal rows and body, ovary, and stylet 368-385, 60-85, and 62-69 µm long, respectively. Adult females maturity can be distinguished by body, ovary and stylet lenght of 381-531, 282-382, and 70-84 µm, respectively, and their unique cuticular ornamentation. Each of the anterior 60-63 body annules bears a continuous fringe with 66-80 spines showing simple or bifurcated tips, while 8-10 terminal annules bear palmate projections in alternate rows. Histological examination of *Ipomoea batatas* roots infected by *O. civellae* indicated that the nematode feeds ectoparasitically on the epidermal and hypodermal cells. Feeding tubes surrounding the stylet were observed in these cells.

Ogma civellae was described by Steiner in 1949 from juvenile specimens collected from *Citrus grandis* (Steiner, 1949). Morphometrics of *O. civellae* females were reported in a redescription of the species using nematode specimens collected from the locality of the original type (Golden and Friedman, 1964). Although *O. civellae* is one of the most widely distributed criconematid species there is little information about the characteristics of its developmental stages and its parasitic habit. This paper describes the morphological characters of *O. civellae* motile stages and illustrates the anatomical alterations induced by this parasite on sweet potato *Ipomoea batatas* (L.) Lam. roots.

Material and methods

A population of *O. civellae* reared on sweet potato, *Ipomoea batatas* (L.) Lam., in a glasshouse (24±2 °C temperature) was used in this study. Specimens for light microscopy observations were extracted from soil by centrifugation. After extraction live motile stages of the nematode were separated in water agar on the basis of body and stylet lenght. Specimens of each life stage were fixed in hot formaldehyde 4% solution + 1% propionic acid and transferred to glycerin, by Seinhorst's rapid method.

Specimens for scanning electron microscope (SEM) were prepared by Wergin's methods (1981), coated with gold, and observed with a JEOL 50 A stereoscan at 10 kV accelerating voltage. Histological observations were made on sweet potato roots infected by *O. civellae*. Root segments, 5 mm long, were fixed in FAA, dehydrated in a tertiary butyl alcohol series, embedded in paraffin, cut in 8-12 µm sections, stained with safranin-fast green, mounted permanently in Dammar xylene, and examined microscopically (Johansen, 1940).

Abbreviations used are defined in Siddiqi (1986). All measurements are in micrometers (µm) unless otherwise stated.

Results and discussion

Morphological characteristics of motile life stages: The embryogenic development was not studied and it is assumed that the second-stage juvenile (J2) (Fig. 2A) emerges from the egg. Three motile juvenile stages J2, J3, J4 and the adult stage, represented only by the female, were observed in the rhizosphere of sweet potato. Morphometric and allometric characters of diagnostic value of these life-cycle stages are reported in Table I. The body length of *Ogma civellae* increased from 138-177 µm for the newly hatched J2 to 389-531 µm of the female. The body dimensions of each motile stage increased by 35, 72, and 20% compared to that of previous stage after the moults of J2, J3, and J4, respectively.

The number of body annules of juvenile stages (J2, J3, J4) progressively decreased 68, 64, 51 with each stage but their widths increased 2.4, 4.2, 6.8 µm. However, the number of annules was constant in J4 and females, but annule width increased considerably (2+1/2 times) after the fourth moult, reaching 16-18 µm in females.

Stylet length increased from 34-39 µm for J2 to 70-84

TABLE I - *Morphometrics of the juveniles and females of an Italian population of* Ogma civellae.

Character	Juveniles			Adults
	J2 (n = 16)	J3 (n = 12)	J4 (n = 16)	(n = 20)
Body length	161±13 (138-177)	218±21 (203-263)	376±7 (368-385)	453±61 (389-531)
Body width	17±1 (16-18)	26±4 (23-29)	54±2 (51-56)	66±5 (59-78)
Annule width	2.4±0.3 (2.1-2.8)	4.2±2 (3.9-5.9)	6.8±0.3 (6.3-7.2)	16±2 (14-17)
Stylet length	36±1.4 (34-39)	47±4 (45-60)	65±3 (62-69)	81±4 (70-84)
Oesophagus length	66±3.1 (63-71)	84±8 (79-89)	100±4 (96-105)	128±11 (101-142)
Female gonad length	8±1 (7.6-9.7)	17±2 (16-19)	71±10 (60-85)	60-85% of body length
Ratios				
a	9.3±07 (8.9-10.3)	8.3±2 (6.6-8.8)	7.0±0.3 (6.5-7.2)	7.5±1 (6.6-9.5)
b	2.4±0.2 (2.1-2.6)	2.6±0.8 (2.8-2.8)	3.7±0.1 (3.6-3.9)	3.7±0.5 (3.2-3.9)
R	68±1.5	64±3	51±0.8	51±3
(total body annules)	(66-71)	(56-65)	(48-52)	(46-55)

µm for the females with a growth rate of 30%, 38%, and 24% for the respective stages J3, J4, and female. The reproductive system (representing 4-5% of the body length in J2 increased progressively reaching 70% of the body length in adult females), showing a growth rate of 112, 317, and 320% respectively after the second, third and fourth moult.

The oesophagus, criconematoid in all stages, increased in length progressively with an increment of 27% between J2 and J3, 19% from J3 to J4 and 28% from J4 to female.

Ratios: The values of body length / body width ratios for J4 and female were similar indicating that J4 and female are closer in dimensions than J2 and J3 to J4 and female (Table I). Body length/oesophagus length ratio increased with nematode development and reached the greatest value with the appearence of J4, concomitantly with the greatest increment (72%) of body lenght. The ratio between body length/tail length was not calculated since the anal opening was not detectable in juveniles.

Cuticular changes occur after each moult. J2 have scale-like ridges (Figs. 1 - 3), J3 and J4 scales with spines (Fig. 1 - 3) and females simple or bifurcated spines anterior to the vulva and palmate scales with terminal projections on the last 8-10 annules. LM and SEM demonstrate that each stage can be distinguished by cuticular ornamentation. Scale shape and arrangement, in fact, are of diagnostic importance for the separation of *O. civellae* life stages. The following key differentiates juveniles and the female of *O. civellae*, based on morphometric values and cuticular ornamentations of each developmental stage.

KEY TO JUVENILES AND FEMALE OF OGMA CIVELLAE (measurements in µm)

1 - Variable number of ridges or scales with spines per body annule; 8-85 ovary length; 138-385 body length; 34-69 stylet length.....2

- Simple or bifurcated spines along the body, palmate scales with spined projections in alternate rows on the posterior (8-10) body annules. Ovary 60-85% of body length; 389-531 body length and 70-84 stylet length. (R = 46-55).....*Female.*

2 - Ridges or scales with spines per body annule; 8-19 ovary length; 161-263 body length; and 36-60 stylet length.....3

- Eight palmate scales with 4-6 spined projections per annule, in 8 longitudinal rows; 60-85 ovary length; 368-385 body length; and 62-69 stylet length; (R = 48-52) ..... *Fourth-stage juvenile.*

3 - Four palmate scales with 4-6 spined projections per

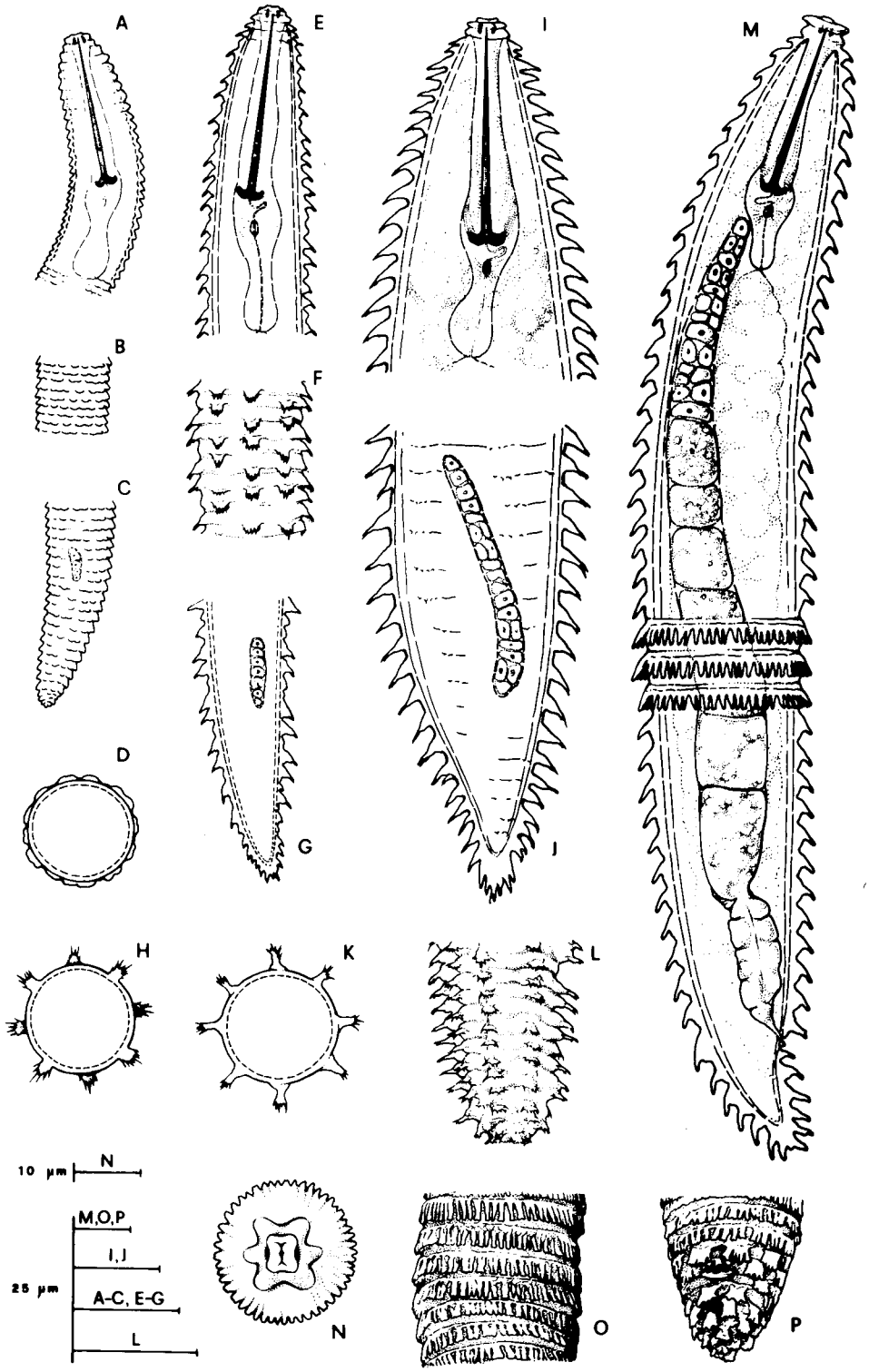

Fig. 1 - Juveniles and female of *Ogma civellae*: A - D, anterior end, posterior and cross section through midbody of a 2nd-stage juvenile; E - H, anterior end, posterior, and cross section through midbody of 3rd-stage juvenile; I - K, anterior, posterior ends, cuticular ornamentation and cross section through midbody of 4th-stage juvenile; M - P, entire body, face view, midbody spines, and tail in ventral view of adult female.

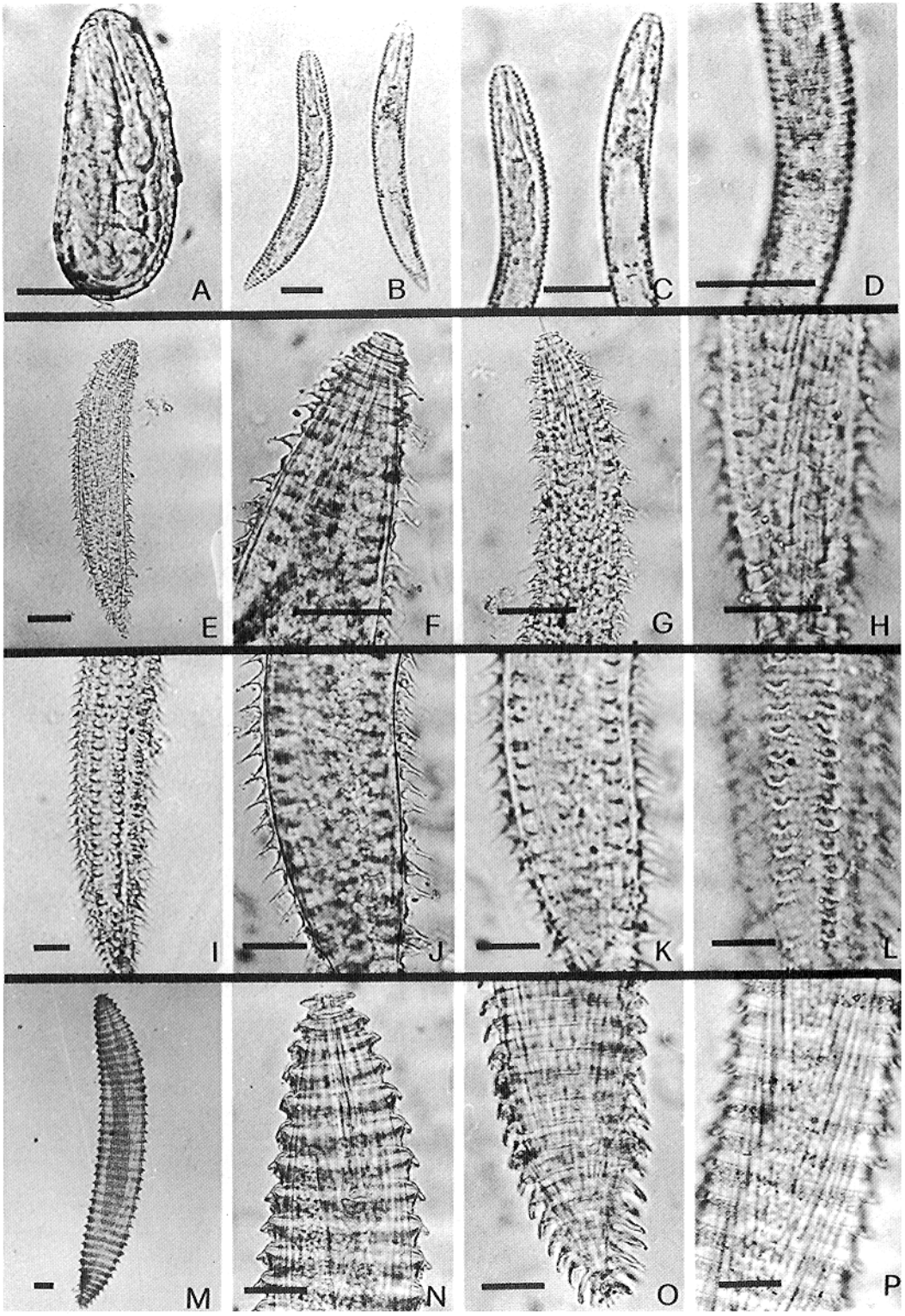

Fig. 2 - Photomicrographs of life stages of *Ogma civellae*: A, embryonated egg; B - D, 2nd-stage juvenile; E - H, 3rd-stage juvenile; I - L, 4th-stage juvenile; M - P, female. (Scale bars = 25 µm).

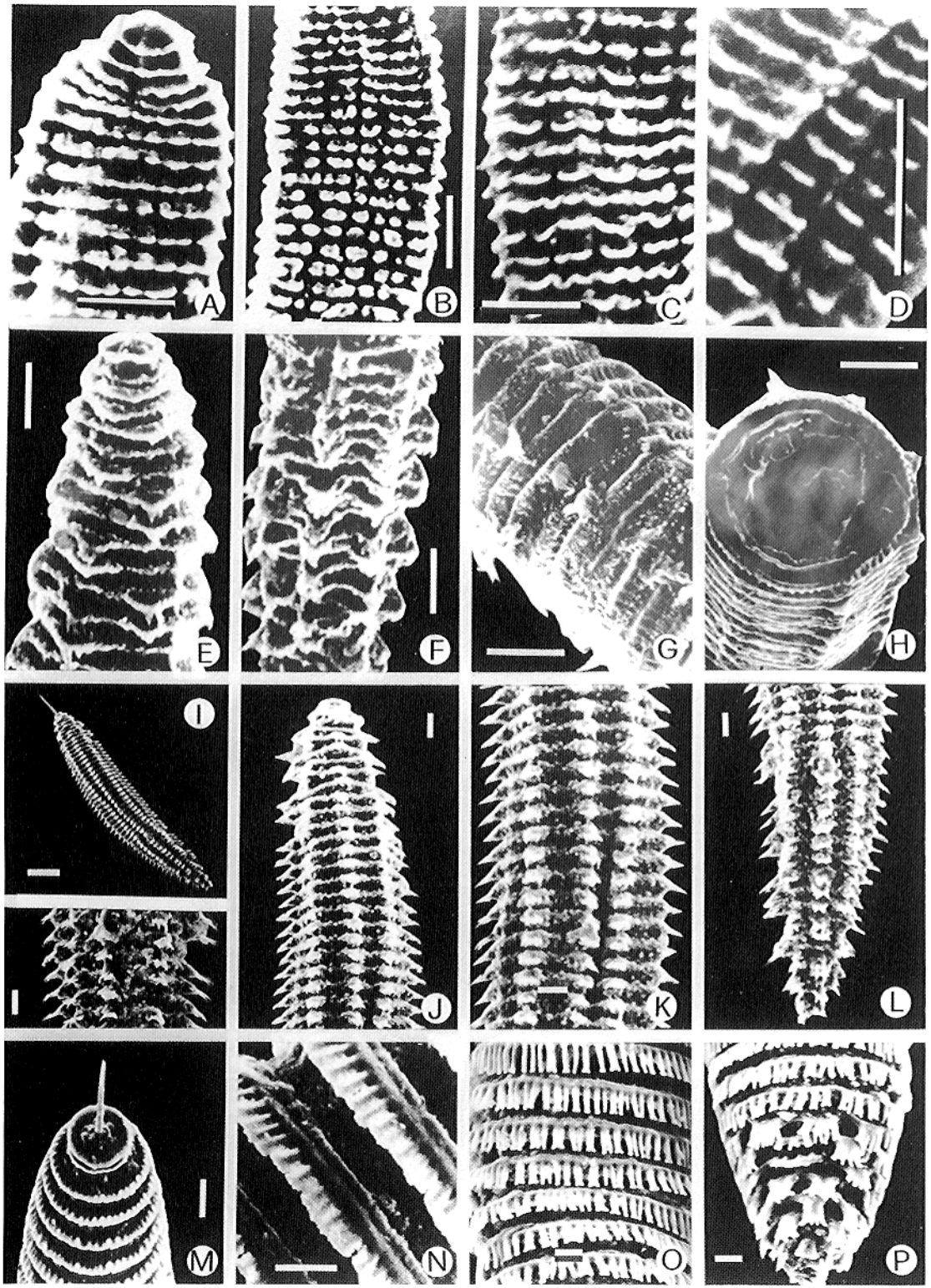

Fig. 3 - SEM micrographs of juveniles and female of *Ogma civellae*: A - D, 2nd-stage juvenile; E - H, 3rd-stage juvenile; I - L, 4th-stage juvenile; M - P, female. (Scale bars = 50 µm in Fig. I, 10 µm in all others).

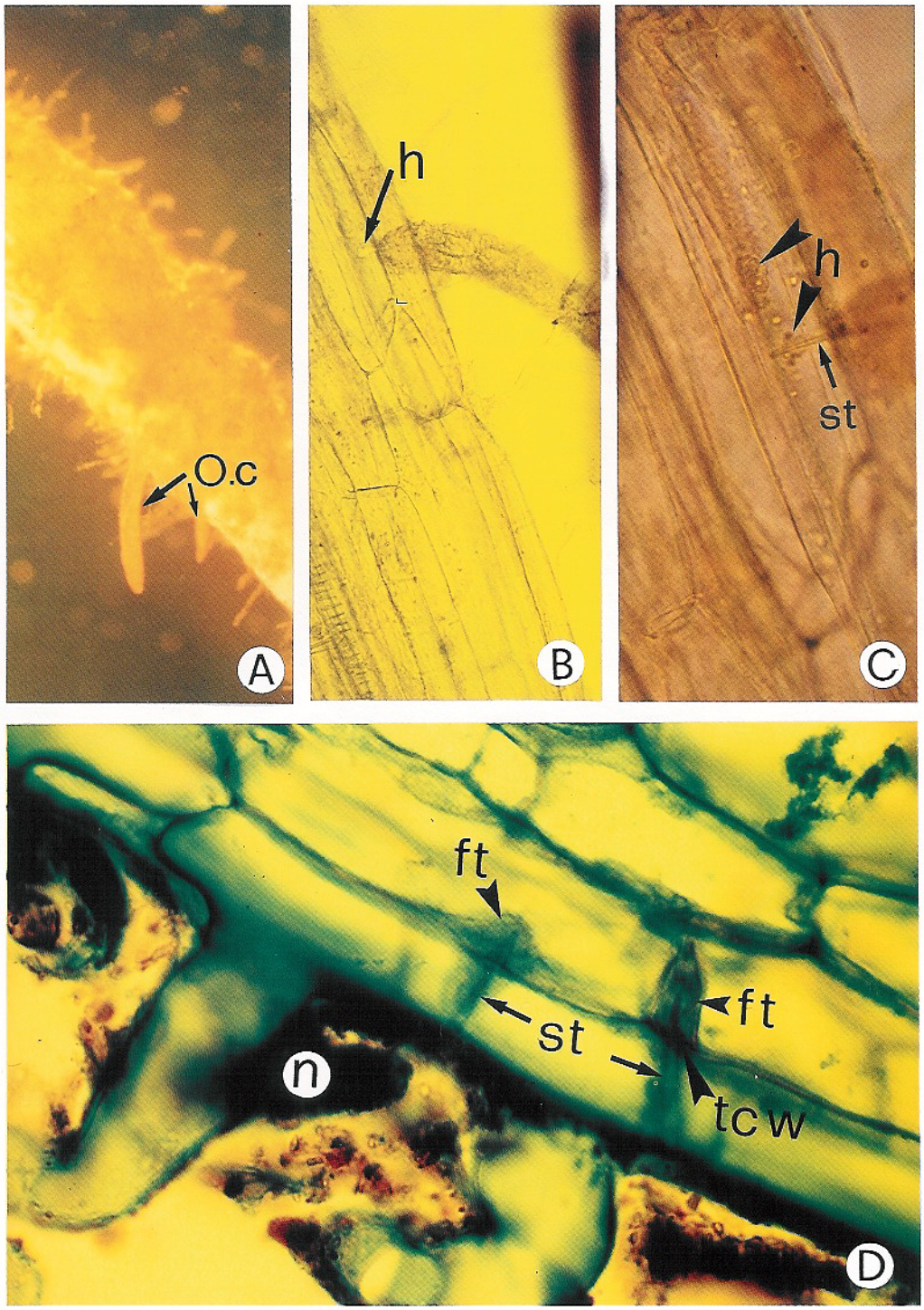

Fig. 4 - Sweet potato roots infected by *Ogma civellae*: A, B, females (O.c) attached to the root axis; C, longitudinal section showing the nematode stylet (st) inserted through the epidermis in a hypodermal cell. Globules of hardened saliva (h) are visible around the stylet; D, longitudinal section of sweet potato root showing a female nematodes (n) attached to the root with the stylet (st) which is inserted through the epidermis into the hypodermis; finger shaped feeding tubes (ft) surround the stylets; note thickened cell wall (tcw) in proximity of the nematode stylet insertion point.

annule, in eight longitudinal rows in alternating (every two annules) position 16-19 ovary length; 203-263 body length; and 45-60 stylet length; (R = 56-65)..... *Third-stage juvenile*

- 12-16 smooth cuticular ridges, slightly protruding, per annule; 8-10 ovary length; 138-177 body length; 34-39 stylet length; (R = 66-71)..... *Second-stage juvenile.*

DESCRIPTION OF LIFE STAGES

Embryonated eggs: (n = 16). L = 71±4.3 (68-81); width = 29±3.6 (26-30); L/W ratio = 2.5±0.3 (1.9-2.9); stylet length of second-stage juvenile inside the egg shell = 36±0.5 (34-38). Second-stage juvenile folded once, or rarely twices within the egg shell (Fig. 2A).

Second-stage juvenile: (Figs. 1 A-D; 2 B-D; 3 A-D). Body ventrally arcuate, tapering toward both extremities, especially posterior end. Body annules retrorse, bearing 12-16 ridges on each annule irregularly disposed (not in rows). The genital primordium in the newly hatched juvenile is four-celled, oval, at 106-148 from anterior end or 73-80% of body length. Additional morphometric date are reported on Table I.

Third-stage juvenile: (Figs. 1 E-H; 2 I-L; 3 E-H). Body fusiform with 64 annules and 8 longitudinal rows of scales. Each annule bears 4 spined scales in alternating position (every two annules). Additional morphometric data in Table I.

Fourth-stage juvenile: (Figs. 1 I-K; 2 I-L; 3 I-L). Body fusiform tapering toward both extremities. The number of J4 body annules is similar to that of female (48-52 vs. 46-55). J4 annules are ornated with eight longitudinal rows of spined (6-8 spines) scales. Additional morphometrics in Table I.

Female: (Figs. 1 M-P; 2 M-P; 3 M-P). Morphometrics in Table I. Body stout, almost straight with 46-55 annules, each bearing a continuous fringe of spines varying from simple or bifurcate in consecutive rows anterior to vulva, to palmate projections in alternate rows at and behind vulva.

The results of this study indicate that in the original description Steiner (1949) used J4 specimens for his description instead of adults. Measurements provided by Steiner refer to the female for the stylet and to J4 for body length, while the number of body annules refers to J2 or to J3. In general morphology as well as measurements, the Italian specimens fit weel with previous data of *O. civellae* and SEM observations agree with those of Ebsary (1981), Castillo et al., (1990), Chitambar (1992), and Decraemer and Geraert (1992).

FEEDING BEHAVIOR

Ogma civellae feeds ectoparasitically on epidermal and hypodermal cells. Juveniles and adult females penetrate the epidermal cell layer of sweet potato roots with their stylets. The head region then adheres to the root surface and nematodes remain attached to the roots by their stylets (Fig. 4). The root portion most commonly infected by juveniles and females was between the apical meristem and the beginning of the root hair zone which is also frequently attacked. Although no distinct phases of feeding were detected, the formation of feeding tubes enveloping the stylet tips was clearly observed (Fig. 4D) in epidermal and hypodermal cells. When the stylet was withdrawn the feeding tubes remained visible as a finger-like structure (Fig. 4D). Similar feeding tubes probably formed by hardened secretum from the oesophageal gland have been reported for ectoparasitic species such as *Trichodorus similis* on tomato (Wyss, 1975) and *Gracilacus peratica* on olive (Inserra and Vovlas, 1977). As in these examples, it was not also possible with *O. civellae* to ascertain if the feeding tubes were formed by hardened saliva or by coagulated cytoplasm. In 18 observations of nematode feeding sites by cross and longitudinal sections, stylet penetration only was observed without any evidence of nematode body penetration. Feeding tubes were variable in length. Slightly thickened cell walls were also observed proximally to the feeding tube or at the point of stylet penetration (Fig. 4D).

The aggregation of cytoplasm around the nematode stylet in *O. civellae* parasitized cells appears identical to that described for *Criconemella xenoplax* (Golden and Friedman, 1964) during its feeding on tomato or carnation roots.

Although histological studies were made only by light microscopy, cellular modifications induced by *O. civellae* on a single food cell of the host tissue appear not to be particularly elaborate. Observations on feeding sites of the nematode show that its parasitism does not induce cellular destruction of sweet potato roots although food cells provide nutrients for the nematode.

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