Morphological Comparison of Head Shape and Stylet Morphology of Second-stage Juveniles of *Meloidogyne* Species[1]

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Abstract: Head shape and stylet morphology of second-stage juveniles of one population each of *M. incognita, M. javanica, M. arenaria,* and *M. hapla* were compared by light microscopy. Excised stylets of each species were also compared by scanning electron microscopy (SEM). Differences in head morphology were observed only between *M. hapla* and the other three species. In SEM, differences in stylet size, shape, and relative distance of the dorsal esophageal gland orifice to the base of the stylet were evident. Differences in stylet morphology between *M. incognita* and *M. javanica* could not be detected by light microscopy, but *M. arenaria* and *M. hapla* could be distinguished from each other and from the other two species. Head shape and stylet morphology of second-stage juveniles are considered useful taxonomic characters. *Key words: Meloidogyne incognita, M. javanica, M. arenaria, M. hapla,* scanning electron microscopy, taxonomy, root-knot nematodes, morphology.

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Identification of the four most common species of root-knot nematodes using light microscopy of second-stage juveniles is highly desirable. Second-stage juveniles are the infective stage usually present in the soil and generally the only stage that can be recovered in nematode assays. Because different species attack different crop plants and resistance is effective only against certain species, effective control of these pathogens depends upon correct identification of the species present in the field. If second-stage juveniles can be confidently identified to species, then crop rotation and resistant varieties can be better utilized on the basis of simple soil assays.

The scanning electron microscope (SEM) has proved valuable in elucidating reliable morphological characters which can be used as diagnostic characters with light microscopy (LM) (3,4,5). New characters of particular value are the stylet morphology of the male and female and the shape of the male head. Also, the morphology of the head of second-stage juveniles has been shown to be of taxonomic value (2).

The present study compares by LM the head shape of second-stage juveniles of one population of each of the four common root- knot nematode species: *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. hapla* Chitwood. In addition, the morphology of the stylets of second-stage juveniles of each species are compared by LM and SEM.

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MATERIALS AND METHODS

One population each of *M. incognita, M. javanica, M. arenaria,* and *M. hapla* was selected from the *Meloidogyne* collection at North Carolina State University. All populations were characterized by their ability to reproduce on five host differentials (6), by cytology (7), and by morphology of perineal patterns and head shape and stylet morphology of males (4). Populations were designated by the collection number, an abbreviated word indicating geographical origin, and chromosome number in parentheses as follows: *M. incognita* 68-NC (41–43) from North Carolina; *M. javanica* 76-GA (44) from Georgia, *M. arenaria* 351-FLA (54) from Florida, and *M. hapla* 48-NC (45) from North Carolina. All populations were cultured on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in a greenhouse maintained at 22–28 C. Newly hatched second-stage juveniles were obtained by incubating egg masses on wet tissue paper in a moist chamber at room temperature. Light microscope observations were made from temporary water mounts of specimens gently killed in hot water and mounted on ringed slides. Specimens were observed immediately after preparation, and only specimens in exact lateral position were critically examined. At least 100 specimens from each population were observed by LM. The stylets of the second-stage juveniles were excised for SEM observation by adapting the

technique developed for the removal of stylets from the adult males and females (4, 5). Because of the technical difficulty encountered in stylet removal, only 3–4 stylets were examined by SEM from each population.

OBSERVATIONS AND DISCUSSION

The basic morphology of the anterior region of second-stage juveniles of *Meloidogyne* species, revealed by SEM and LM, consists of a head cap, head region, and the adjoining body region which has regular annulations. In the SEM, the head cap is further resolved into a labial disc, medial lips, and lateral lips (2). The head region may be smooth or marked by 1–4 or more annulations which are usually irregular and incomplete. The cephalic framework provides internal support for the head region.

As revealed by SEM, differences exist in the head shape of *M. incognita, M. javanica, M. arenaria,* and *M. hapla,* but they are not extreme except in the case of *M. hapla* (2). In the LM (Fig. 1), *M. hapla* (Fig. 1D) can be distinguished from the other three species by the relatively narrow head cap which makes the head appear more rounded. Also, head annulations have not been observed in *M. hapla,* but may be present or absent in the other species.

The basic morphology of the excised stylet and attached cuticular lining of the lumen of the esophagus of the second-stage juvenile (Fig. 2) closely resembles that of the female (5). In all four species, the stylet opening is often marked by a small ventral protuberance posterior to the opening, near the tip of the cone (Fig. 3D). The cone gradually increases in width posteriorly, and the junction with the shaft is irregular. The shaft gradually increases in width posteriorly, and the three stylet knobs are either set off from the shaft in varying degrees or they gradually merge with the shaft. The cuticular lumen lining in the procorpus is round in cross section, and the orifice of the dorsal esophageal gland (DGO) branches off into several channels less than one shaft-length from the base of the stylet. As in the female (5), the lining becomes triradiate in the metacorpus and the outside edges of the lining of the enlarged lumen of the median bulb are thick and rigid, while the inner portions are thin

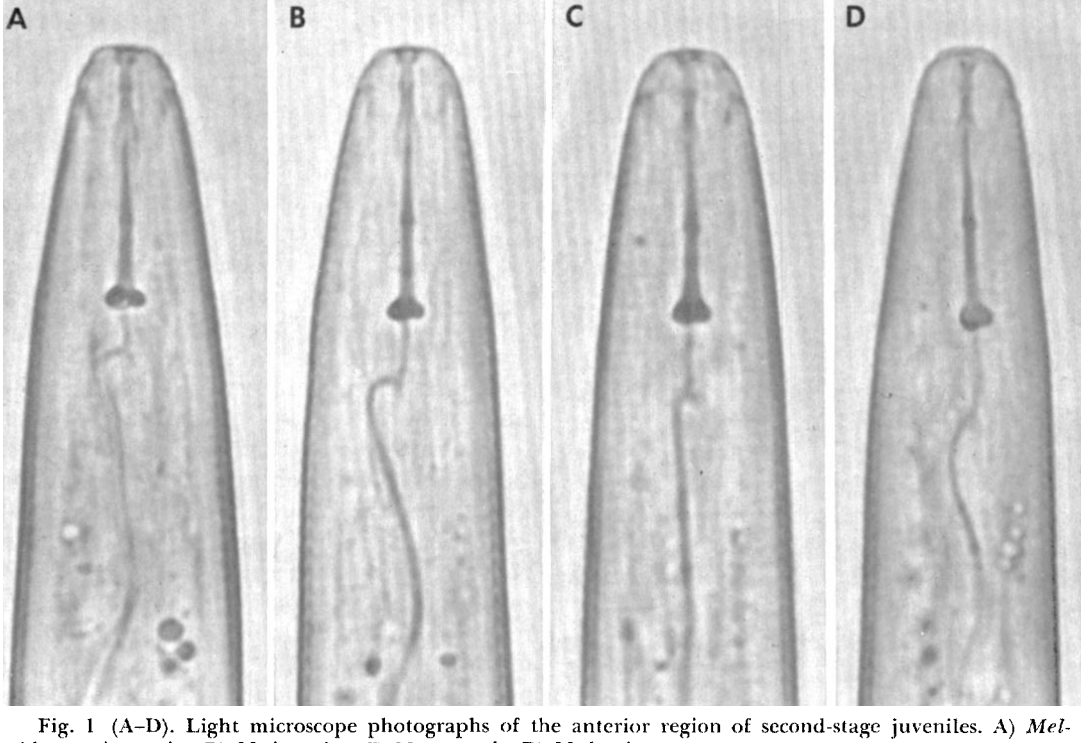

Fig. 1 (A–D). Light microscope photographs of the anterior region of second-stage juveniles. A) *Meloidogyne incognita.* B) *M. javanica.* C) *M. arenaria.* D) *M. hapla.*

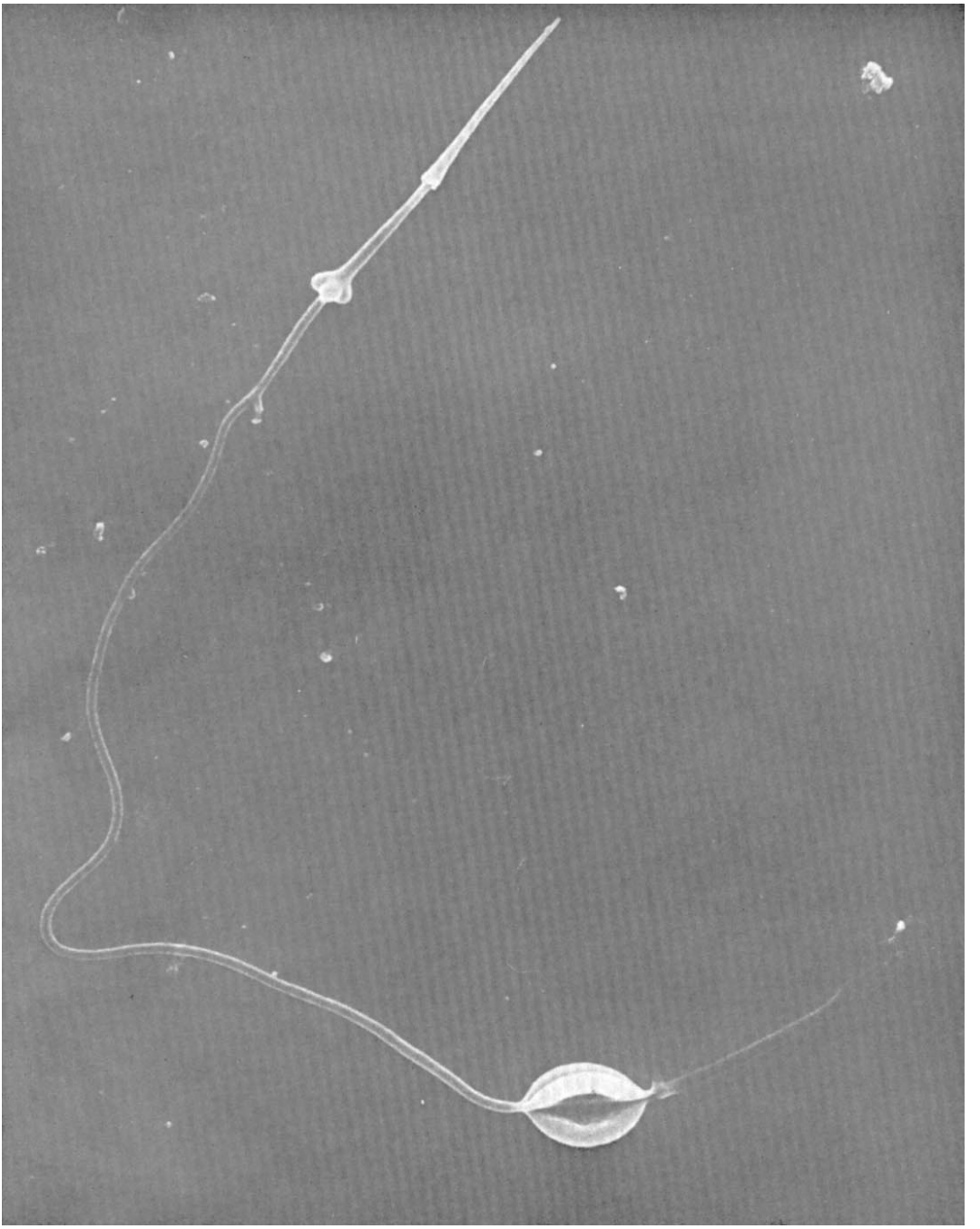

Fig. 2. SEM photograph of an excised stylet and attached cuticular lumen lining of the esophagus of a *Meloidogyne hapla* second-stage juvenile.

and flexible. The branched orifices of the subventral glands are located immediately posterior to the enlarged lumen of the median bulb. The lining of the posterior part of the metacorpus and isthmus is very thin.

In the SEM comparisons (Fig. 3), differences in stylet morphology were observed among the four common species. Differences in stylet size, distance of the DGO to the stylet base, and the shape of the stylet knobs are important distinguishing characters. In *M. incognita,* the DGO is short and the stylet knobs are rounded, set off from the shaft, and generally slope only slightly pos-

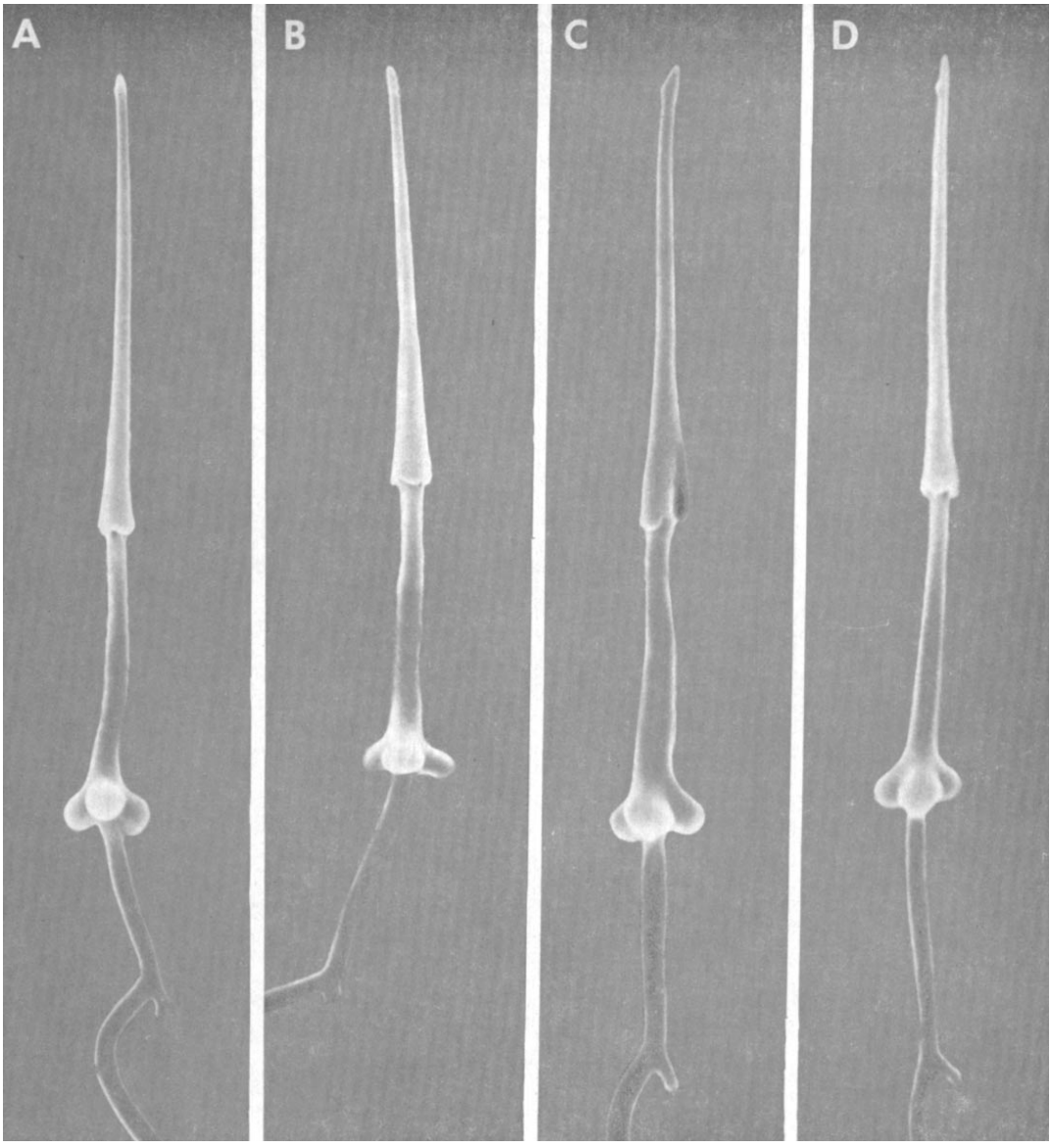

Fig. 3 (A–D). SEM photographs of excised stylets of *Meloidogyne* second-stage juveniles. A) *M. incognita*. B) *M. javanica*. C) *M. arenaria*. D) *M. hapla*.

teriorly. The DGO of *M. javanica* is moderately long and the knobs are rounded, low, and set off from the shaft. In *M. arenaria* the entire style is slightly more robust, the DGO is moderately long, and the knobs gradually merge with the shaft. The DGO of *M. hapla* is long, and the knobs are rounded and set off from the shaft.

The differences in stylet morphology revealed by SEM are also visible by LM (Fig. 1), except *M. incognita* and *M. javanica* are too similar to be distinguished. However, *M. arenaria* and *M. hapla* can be identified with some certainty on the basis of second-stage juvenile head shape and stylet morphology. Our findings confirm many of Chitwood's (1) earlier descriptions as illustrated in his figures 5I (*M. incognita*), 2I (*M. javanica*), 1G (*M. arenaria*), and 3S (*M. hapla*).

Although it may be possible to identify certain root-knot nematode species by the head shape and stylet morphology of second-stage juveniles, it would be unwise to base an identification on just one character, par-

ticularly with this life stage. Second-stage juveniles are extremely small and difficult to observe, even when they lie in a level, lateral position. Also, populations of *Meloidogyne* are quite variable, and certain measurements such as distance of the DGO to the base of the stylet may not be stable or always reliable. Likewise, many natural field populations are mixtures of two or more species and may be difficult or impossible to distinguish on the basis of second-stage juvenile morphology alone. Instead, several different kinds of characters should be used in conjunction to ensure an accurate identification. Of particular importance are host differential tests (6); perineal patterns; stylet morphology of females, males, and second-stage juveniles; and head shapes of males. Cytological and biochemical characters also may be of value, provided facilities and expertise are available (7).

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