Meloidogyne grahami n. sp. (Meloidogynidae), A Root-knot Nematode on Resistant Tobacco in South Carolina

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Abstract: *Meloidogyne grahami* n. sp. is described and illustrated from specimens on tobacco (*Nicotiana tabacum*) originally from Florence, South Carolina. Considered for several years to be only a race of *M. incognita*, this new species readily attacks NC-95 tobacco, a variety with resistance to the *M. incognita* group that is common in the major U.S. tobacco-producing areas. *M. grahami* n. sp. is related most closely to the three subspecies of the *M. incognita* group but differs from all of them, especially in its distinctive perineal pattern and larger larvae (av. 421 μm, vs. 385 μm or less). Also, the dorsal esophageal gland orifice of females of *M. grahami* n. sp. is further from the base of the stylet (5 μm) than in *M. i. incognita* and *M. i. acrita*. Comments are given on the distribution of this new species. *Key Words:* taxonomy, morphology, new *Meloidogyne* species, resistance-breaking, *Nicotiana tabacum*.

In his detailed account of tobacco diseases, Lucas (8) pointed out that root-knot nematodes (*Meloidogyne* spp.) are a major problem in tobacco production (*Nicotiana tabacum* L.) worldwide. Apparently most prevalent and important in the U.S., particularly in the Southeast, are forms of the species group *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, although some other species, including *M. arenaria* (Neal, 1889) Chitwood, 1949, and *M. javanica* (Treub, 1885) Chitwood, 1949, are also important. As reviewed in 1958 by Clayton et al. (2) and further discussed more recently (8, 13), efforts were begun in 1935 to develop varieties with resistance to the most common root-knot nematode on tobacco in the southeastern USA. At that time all root-knot nematodes were referred to as a single species, *Heterodera marioni* (Cornu, 1879) Goodey, 1932, and more than one species might have been used in the early research on resistance (13). Soon after Chitwood (1), in 1949, reestablished the genus *Meloidogyne* Goeldi, 1887, and described five species and one "variety" of *M. incognita* (*M. i. acrita* Chitwood, 1949), research on developing resistant tobacco varieties was concentrated on the *M. incognita* group. As a final result, the NC-95 tobacco variety with monogenic dominant resistance to the *M. incognita* group (Fig. 21) was released in 1960 by Moore et al. (9). The source of that resistance was thought to have been tobacco introduction (TI) 706. However, a recent extensive study (13) with five *Meloidogyne* species and subspecies, and several *Nicotiana* species, an interspecific hybrid, and various tobaccos indicated that the resistance source is probably *N. tomentosa* Ruiz & Pavon or possibly *N. tomentosiformis* Goodsp. not TI 706. This resistance is now used in varieties grown on more than 50% of the flue-cured tobacco hectarage in the southeastern United States (13).

Beginning in 1966, Graham (5, 6) discovered "a new pathogenic race of *Meloidogyne incognita*" which caused extensive galling on the resistant tobacco variety NC-95 in the nursery breeding plots at Florence, South Carolina. Identification of this nematode as a race was consistent with the taxonomy of root-knot nematodes at that time, although the senior author (personal notes, 1968) observed peculiarities in the female perineal pattern and longer larvae than had been described for *M. incognita*. For the past 3 years, this nematode has been further examined morphologically (3) and experimentally (12, 13). Morphological and host differences between this South Carolina root-knot nematode *M. incognita* led us to describe it here as a new *Meloidogyne* species.

Specimens used in this study were obtained from cultures originally from Florence, South Carolina, and grown on NC-95 tobacco (Fig. 22) in a growth chamber and greenhouse at Beltsville, Maryland. In addition, specimens were examined from 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) grown under the same conditions. Larvae and males were generally recovered from heavily infected

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fresh roots kept in petri dishes with a small amount of water, and females were later dissected from the roots after overnight fixation in 3% formaldehyde solution to harden the specimens. The other procedures used herein, including measuring, drawing, photomicrographing, and preparing specimens, were essentially the same as those used by Golden and Birchfield (4) except that some fixed females were cut in a drop of 3% formaldehyde fixative and then mounted directly in clear lactophenol solution.

Meloidogyne grahami n. sp.

FEMALES (30): Length 592–826 μm (mean 694 μm, standard deviation (SD) 52 μm); width 388–571 μm (483 μm, SD 48); L/W ratio 1.2–1.9 (1.5, SD 0.17); stylet 14.6–16.2 μm (15.0 μm, SD 0.4); width of stylet knobs 3.4–4.7 μm (4.1 μm, SD 0.4); dorsal esophageal gland orifice (DGO) 4.3–6.5 μm (5.0 μm, SD 0.6) from base of stylet; excretory pore 23–41 μm (30.9 μm, SD 4.9) from anterior end; vulval slit length 21–27 μm (25 μm, SD 1.5); distance from vulval slit to anus 15–21 μm (18 μm, SD 1.4).

HOLOTYPE (female): Length 714 μm; width 439 μm; L/W ratio 1.6; stylet 14.8 μm; stylet knob width 4.3 μm; DGO 4.5 μm from base of stylet; vulval slit length 24 μm; distance from vulval slit to anus 17.9 μm; excretory pore 31 μm from anterior end.

Description: Body pearly white, globular to pear-shaped, and without posterior protuberance. Distinct neck situated anteriorly on a median plane with the terminal vulva and anus. Esophageal and anterior region commonly as illustrated (Figs. 5 and 15–18). Head not offset from neck, bearing a labial cap and generally two cephalic annules. Cephalic framework present but weak. Stylet delicate, with small rounded knobs commonly sloping posteriorly only slightly. Excretory pore distinct and variable in exact position, although usually posterior to base of unprotruded stylet. Perineal pattern (Figs. 6–14) with rather rounded arch, possessing fine, closely spaced lines which may be wavy to straight and discontinuous. Several lines often extend near to one or both ends of vulva (Fig. 6) and just above the anus the lines form a characteristic squarish area which sometimes shows a prominent whorl within (Fig. 11).

MALES (25): Length 1492–2274 μm (1906 μm, SD 199); a = 41–60 (48, SD 4.9); b = 6.6–9.5 (8, SD 0.9); c = 126–179 (150, SD 15.1); stylet 24.1–25.8 μm (25 μm, SD 0.42); stylet knob width 4.7–5.6 μm (5.4 μm, SD 0.28); DGO 3.4–4.7 μm (3.7 μm, SD 0.4) from base of stylet; spicules 30–36 μm (32 μm, SD 1.8); gubernaculum 8.6–9.9 μm (8.9 μm, SD 0.5); tail 11–14 μm (12.8 μm, SD 0.9).

ALLOTYPE (male): Length 1977 μm; a = 49; b = 8.5; c = 161; stylet 24.6 μm; stylet knob width 4.6 μm; orifice of dorsal esophageal gland from base of stylet 3.5 μm; spicules 32.5 μm; gubernaculum 8.7 μm; tail 11 μm.

Description: Body slender, vermiform, tapering slightly toward both ends. Head only slightly offset, typical of the genus with massive labial annule (head cap) and with 2 or 3 faint post-labial annules, although these are often difficult to see (Figs. 19–20). Cuticular annulation prominent, with midbody annules measuring about 3 μm. Lateral field (Fig. 3) with 4 lines and without areolation except in posterior portion, and about 1/5 of body width, the latter at widest part being 31–48 μm (40 μm, SD 4.7). Stylet, knobs, cephalids, and anterior portion appearing generally as illustrated (Fig. 4). Excretory pore located 2–10 annules posterior to prominent hemizonid. Center of median bulb 93–110 μm (101 μm, SD 4) from anterior end. Testis 1 or 2. Spicules arcuate, with rounded tips. Phasmids rather prominent, usually anterior to cloaca (Fig. 3). Tail short, rounded, slightly swollen, and often appearing as illustrated (Fig. 3).

LARVAE (65): Length 391–459 μm (421 μm, SD 16); a = 27–34 (31, SD 1.3); b = 2.2–2.7 (2.4, SD 0.12); c = 7.1–8.8 (7.9, SD 0.29); stylet 10.1–11.8 μm (10.9 μm, SD 0.29); DGO 2.2–3.4 μm (2.9 μm, SD 0.27) from base of stylet; center of median bulb 52–63 μm (57 μm, SD 2.9) from anterior end; head width 5.0 μm; head height 2.2–2.8 μm (2.6 μm, SD 0.25); hw/hh ratio 1.8–2.3 (1.9, SD 0.2); tail length 47–61 μm (53 μm, SD 2.6); hyaline tail terminal 8.4–12.9 μm (10.7 μm, SD 0.9); caudal ratio A 2.1–3.3 (2.7, SD 0.3); caudal ratio B 3–5.8 (4.5, SD 0.7).

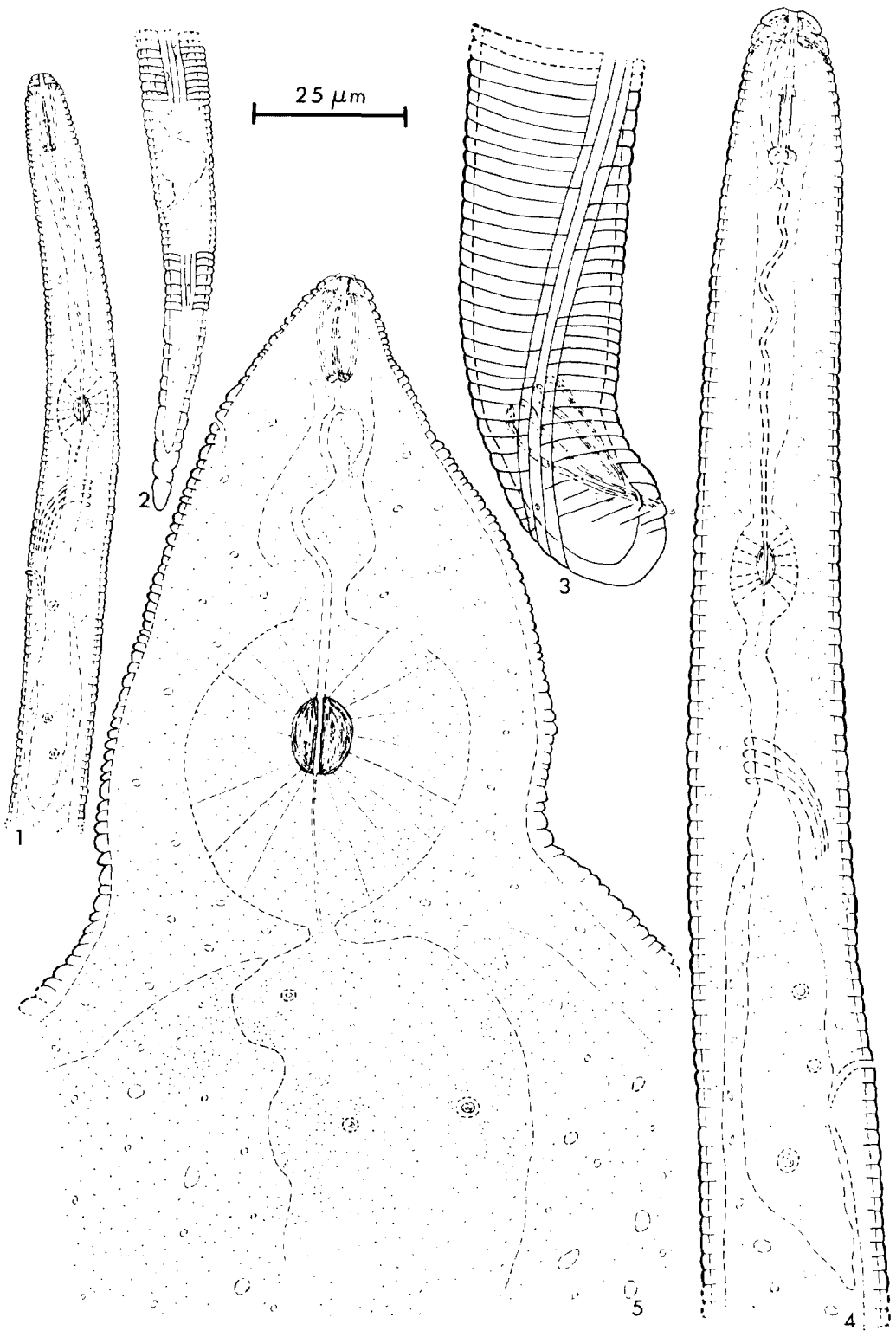

FIG. 1–5. Drawings of *Meloidogyne grahami* n. sp. 1–2) Larva. 3–4) Male. 5) Anterior region of female.

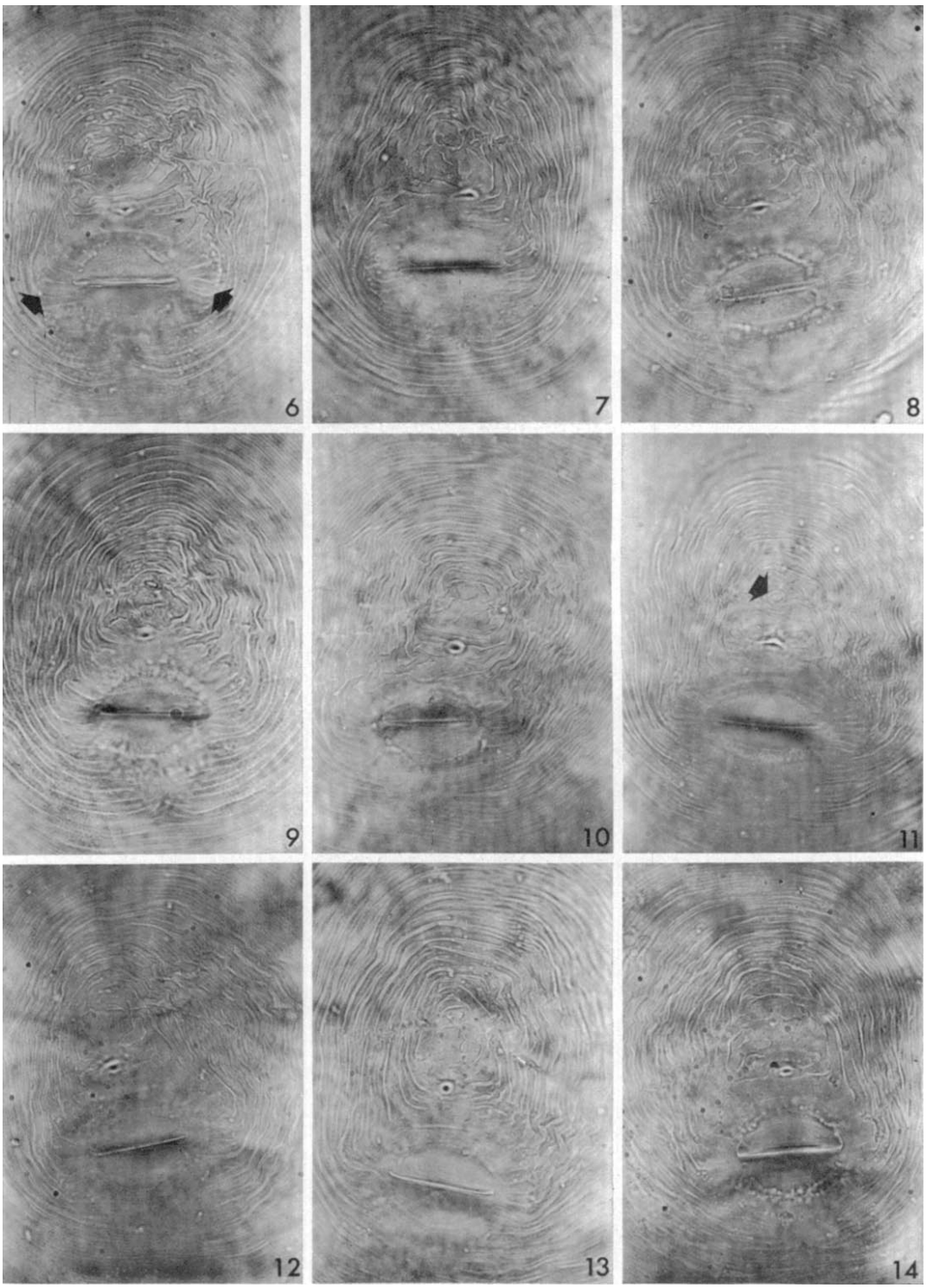

FIG. 6–14. Photomicrographs of representative perineal patterns of nine different females of *Meloidogyne grahami* n. sp. (Arrows in Fig. 6 point to typical lines extending to vulva, and in Fig. 11 indicate a whorl.)

Description: Body vermiform, tapering at both ends but more so posteriorly. Head not offset, conically rounded, with weak cephalic framework, and bearing two cephalic annules. Body cuticular annulation fine but distinct. Lateral field (Fig. 2) with 4 lines, not areolated. At widest part body measures 12.9–14.6 μm (13.7 μm, SD 0.4). Stylet, knobs, hemizonid, excretory pore, and anterior portion appearing as illustrated (Fig. 1). Phasmids small, located a little more than one anal body width posterior to level of anus (Fig. 2). Rectum generally much inflated (Fig. 2), often shaped like an egg or football. Tail commonly appearing as illustrated (Fig. 2), with rounded terminus.

EGGS (25): Length 87–99 μm (93 μm,

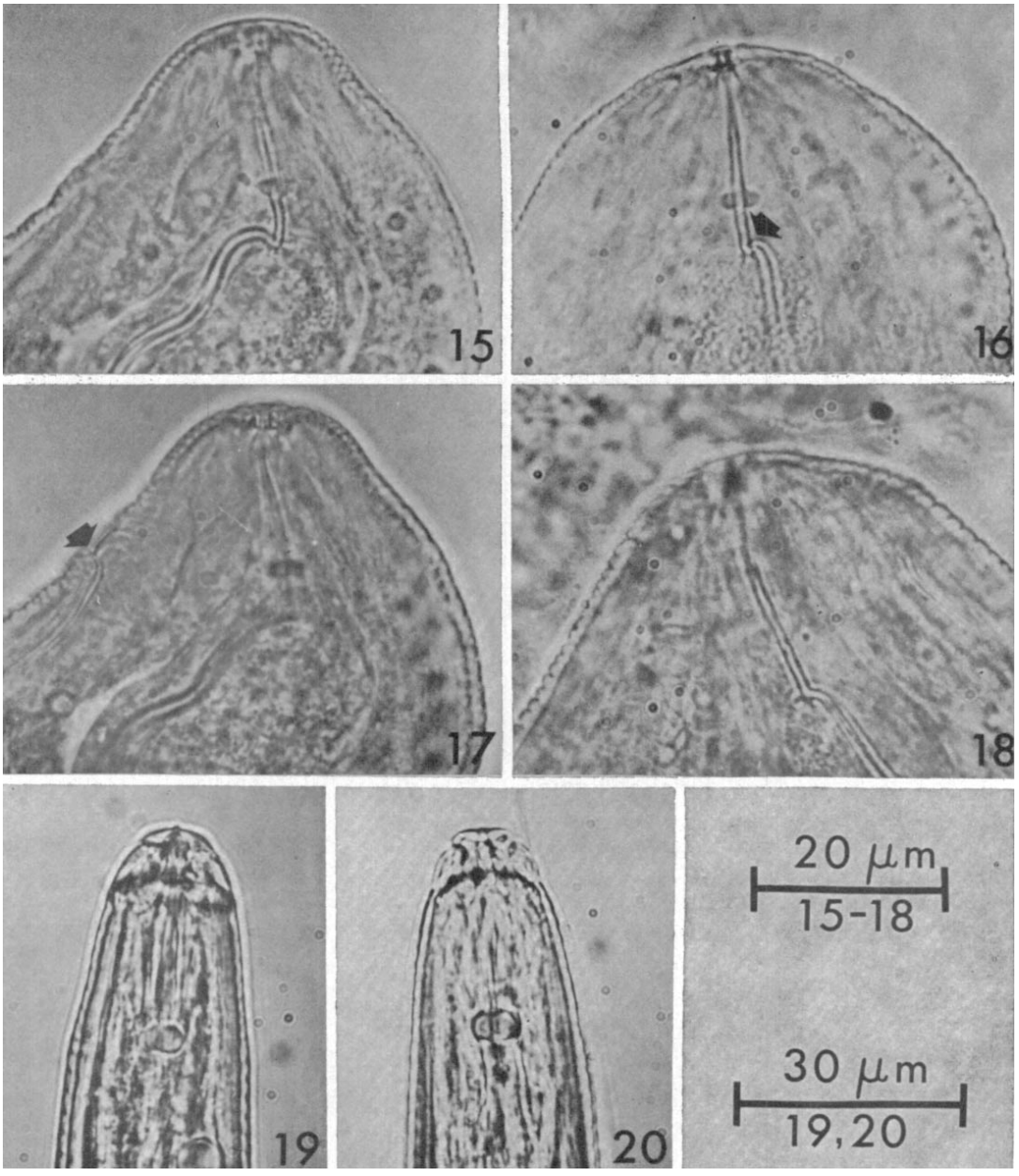

FIG. 15–20. Photomicrographs of extreme anterior region of specimens of *Meloidogyne grahami* n. sp. 15–18) Four different females. (Arrow in Fig. 16 indicates DGO, and in Fig. 17, the excretory pore.) 19–20) Two different males without visible cephalic annules.

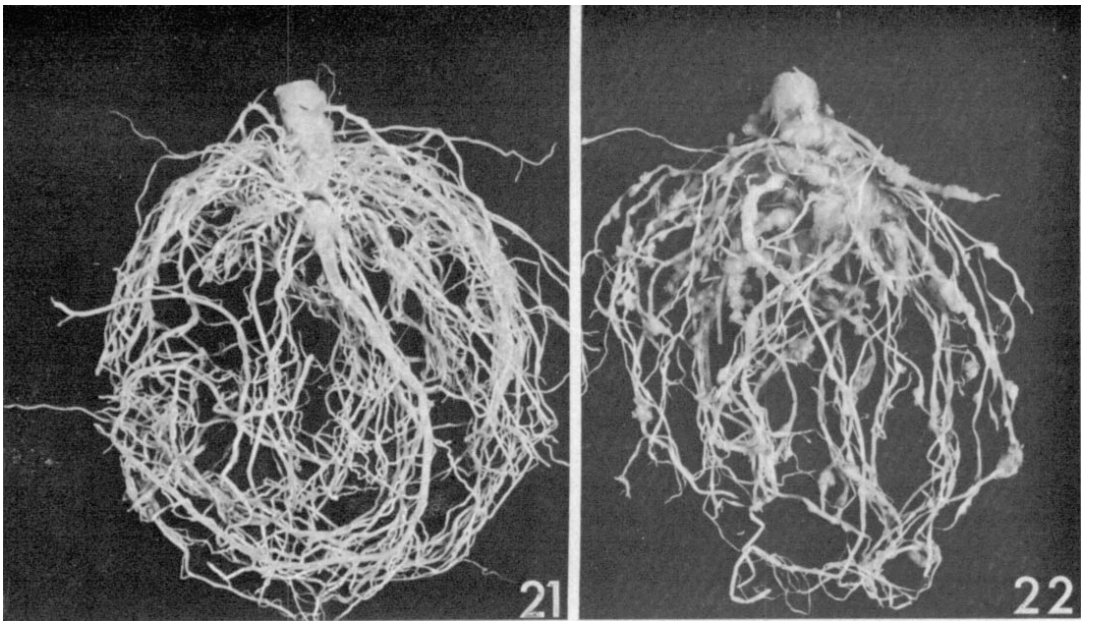

FIG. 21–22. Photographs of NC-95 tobacco inoculated with two root-knot nematodes. 21) *Meloidogyne incognita acrita* (*M. i. incognita* produced a similar response of essentially no galling). 22) *M. grahami* n. sp.

SD 3); width 40–48 μm (43 μm, SD 2.3); L/W ratio 1.8–2.4 (2.2, SD 0.13). Egg shell hyaline, without visible markings by optical microscopy.

HOLOTYPE (female): Originally obtained from T. W. Graham, Florence, South Carolina, 30 January, 1974 (and subsequently grown on tobacco in an isolated greenhouse area). Slide T-277t, USDA Nematode Collection (USDANC), Beltsville, Maryland, USA.

ALLOTYPE (male): Slide T-278t. Same data as holotype. USDANC, Beltsville, Maryland, USA.

PARATYPES: Males, females, larvae, and eggs: USDANC, Beltsville, Maryland; University of California Nematode Survey Collection (UCNSC), Davis, California, USA; Nematology Department, Rothamsted Experimental Station, Harpenden, Herts., England; Canadian National Collection of Nematodes, Ottawa, Canada.

TYPE HOST AND LOCALITY: Roots of tobacco (*Nicotiana tabacum* L.) in field breeding plots at the Pee Dee Experiment Station, Florence, South Carolina, USA.

DIAGNOSIS: *Meloidogyne grahami* n. sp. is related most closely to the forms of the *M. incognita* group [*M. i. incognita* (Kofoid & White, 1919) Chitwood, 1949; *M. i. acrita* Chitwood, 1949; and *M. i. wartellei* Golden & Birchfield, 1978] but differs from all of those especially by: 1) a distinctive perineal pattern as illustrated (Figs. 6–14), having a lower, more rounded arch and generally a squarish area formed by striae just above the anus; and 2) larger larvae, generally measuring over 400 μm long (average 421 μm) compared with usually less than 400 μm long (average of 385 μm or less) in the above subspecies. Also, the female DGO is further back (5 μm average) than that in *incognita* (average 3.8 μm) and *acrita* (4 μm average). The male stylet is longer (25 μm average) than that of *wartellei* (22.4 μm average) and *acrita* (23 μm average), although about the same length as that of *incognita* (25.5 μm). The two or three faint or indistinguishable cephalic annules on the new species differ from the three or more prominent cephalic (post-labial) annules on males of *M. incognita*.

The species name honors Dr. T. W. Graham, the outstanding scientist (now retired from the United States Department of Agriculture) who discovered this nematode.

Distribution and Comments: At this time, *M. grahami* n. sp. is known definitely only from South Carolina, where T. W. Graham (personal communication, 1977)

found galled tobacco roots of varieties resistant to the "common root-knot" in several areas of the state. The nematode from those roots appeared to be "morphologically the same as the new race" from Florence. There are indications, however, that this nematode might be in other areas also. K. R. Barker, North Carolina State University, Raleigh, indicated (personal communication, 1978) a root-knot nematode attacking NC-95 tobacco and identified as *M. incognita* was "becoming fairly common" in the flue-cured tobacco-producing areas of North Carolina. In 1972, Phipps et al. (11) reported that a race of *M. incognita* taken from roots of Mimosa (*Albizia julibrissin* Durazz.) on the eastern shore of Virginia "reproduced well" on NC-95 tobacco and some other plants. In 1972 also, Perry & Zeikus (10) tested four populations of the "*Meloidogyne incognita* species group" collected in Florida and reported that one of them, obtained from sugar cane, attacked and reproduced on NC-95 tobacco. Kirby et al. (7) found three populations of *M. incognita* from different areas of Florida that attacked NC-95 tobacco. One of those populations, from Irish potato (*Solanum tuberosum* L.), caused heavy galling; and another from Irish potato and one from "*Maranta*" produced only slight galling. Kirby et al. (7) also cite reports of infection of NC-95 tobacco by populations of *M. incognita* in India, Peru, and Colombia. It would be interesting to compare those nematodes with *M. grahami*.

Species of root-knot nematodes can adapt or change over time in response to population stresses exerted by resistant varieties or other factors (8). Sometimes this is a change in food preference, accompanied by little or no morphological alteration (14). It seems unlikely however, that *M. grahami* arose so quickly from *M. incognita* in response to the presence of the resistant NC-95 in the relatively short time since introduction of that variety. It also seems unlikely that a single mutation which might have produced virulence on NC-95 tobacco would also produce the morphological differences listed here. This nematode showed essentially the same characters in 1968 when examined by the senior author as given herein. These are important morphological characters which evidently have remained stable for 10 years.

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