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# BIOLOGY, IDENTIFICATION AND CONTROL OF ROOT-KNOT NEMATODES
## (Meloidogyne Species)



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INTERNATIONAL MELOIDOGYNE PROJECT

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# of ROOT-KNOT NEMATODES
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DEPARTMENT of PLANT PATHOLOGY
NORTH CAROLINA STATE UNIVERSITY
*and the*
UNITED STATES AGENCY for
INTERNATIONAL DEVELOPMENT

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# PREFACE

The primary purpose of this book is to aid in accomplishment of the principal goals of the International *Meloidogyne* Project. These include: 1) to increase production of economic food crops in developing nations; 2) to improve crop protection capabilities of developing nations; 3) to advance knowledge about one of the world's most important groups of plant-parasitic nematodes.

The accomplishment of the above goals will be largely dependent on the more than 70 cooperators working in 7 major geographical regions of the world. We believe this compilation will help to unify research efforts on this major group of plant pests.

Obviously this book is not an in-depth coverage of the subject; we have only tried to give the essentials, based on the literature and our own research experience, and have presented our assessment of the relative importance of the various described species to world agriculture and their distribution. Considerable emphasis has been placed on identification, variability, and practical control measures. We recommend that users of the book desiring more detail study the specific references cited and make use of various excellent reviews dealing with root-knot nematodes.

To facilitate identification, we have attempted to bring under one cover what is known concerning the morphology of the various species. Numerous illustrations from the original descriptions are reproduced, and characters considered useful in routine species identification are emphasized in the text. In addition, we have stressed the importance of using all available information in arriving at an identification. This includes location of collection, differential host response including type galls produced, morphology and cytogenetics.

We believe the book can be helpful to the Project and to our cooperators as they endeavor to train new students. We also hope it will be useful to scientists of other disciplines in the cooperative development of control strategies in conformity with resources easily available in developing countries.

Literature cited is only a part of the vast amount published on the genus *Meloidogyne*, and we have drawn primarily from those papers which deal with the specific points we wish to illustrate and the several general areas we wish to emphasize.

We hope that this work will lead to greatly expanded research which will provide better answers to the numerous unsolved problems which will be apparent to readers, and that it will be the foundation for a much more complete and definitive book in the future.

Thanks are due to the nematologists and other scientists who have contributed directly and indirectly to the information presented. Also, to the various officers of the Agency for International Development who have made the International *Meloidogyne* Project and this book possible.

Special thanks are due to Mrs. Josephine Taylor who did the preliminary typing and to Mrs. Joyce Denmark who organized the literature files and did much of the typing of the final manuscript.

We wish to especially thank Dr. J. L. Starr, North Carolina State University, for reading the entire book and for making many helpful suggestions with reference to its organization and content. The authors, however, assume full responsibility for any errors of fact or interpretation.

# CONTENTS

# The Genus *Meloidogyne** (Root-Knot Nematodes)

## I.  Introduction

### A.  Historical

One hundred years ago, in August 1877, Jobert (1878) observing diseased coffee trees in the Province of Rio de Janeiro, Brazil, found fibrous roots with numerous galls, some terminal and some on the axis of the root, or more rarely on lateral roots. The terminal galls were pyriform, pointed, and frequently recurved (see Fig. 9.12). The largest were about the size of a very small pea and contained "cysts" with hyaline walls. There were also elliptical eggs enclosed in hyaline membranes and containing little "nematoid" worms. He noted that the worms hatched from the eggs, escaped from the roots, and were in large numbers in the soil. But apparently he did not have time to study the problem further before writing his report.

Ten years later, Goldi** (1887) investigated the same problem and published a paper of 105 pages on the disease of coffee trees. He named a root-knot nematode, *Meloidogyne exigua*, as the cause of the disease and as the type species of a new genus.

These were the first investigations of a *Meloidogyne* species as the cause of an important disease of an economic plant. Later, the species and genus were synonymized, first with *Heterodera radicicola* and then with *Heterodera marioni*, until revived by Chitwood (1949), who also described or redescribed the four most common and widely distributed species of the genus, *M. incognita, M. javanica, M. arenaria* and *M. hapla*.

Chitwood's (1949) paper was published at a time when nematicides were being developed and tested. Experiments with nematicides provided conclusive evidence that *Meloidogyne* species (root-knot nematodes) and other nematodes were economically important plant parasites, and that their control was often followed by large increases in crop yields. There was a large increase in the number of plant nematologists and a corresponding increase in publications on *Meloidogyne* species.

An important part of this research has shown that in addition to direct damage to crops, *Meloidogyne* species and the fungal and bacterial diseases which they predispose are a major cause of preventable plant disease and crop yield loss in the warmer regions of the world, with less widespread loss in cool climates (Sasser, 1977).

Since the establishment of the International *Meloidogyne* Project, progress has been made toward cooperative world-wide research on *Meloidogyne* species. An important accomplishment of the Project was presented at the Sixteenth Annual Meeting of the Society of Nematologists on August 19, 1977 as a paper by Sasser and Triantaphyllou (1977). The authors reported results of North Carolina Differential Host Tests (see Appendix 1) of 180 populations collected by cooperators of the Project in various countries of North and South America, Africa, Asia and Europe. A remarkable uniformity of host response was found for populations of *M. incognita, M. javanica, M. arenaria* and *M. hapla* in addition to similarity of the perineal patterns of the respective species. Cytogenetic studies of mode of reproduction, chromosome numbers, and chromosome behavior during maturation of oocytes also indicated uniformity of the species on a world-wide basis. The essential research results of this work are given in Chapter 5 of this book.

Especially important was the confirmation of the existence of only four widespread races of *M. incognita*, two races of *M. arenaria*, one race of *M. javanica*, and one race of *M. hapla*. Eventually this confirmation of species uniformity will lead to greatly simplified rotation schedules for control of root-knot nematodes; to simplified procedures for breeding root-knot-resistant crop plant cultivars; and to a much better understanding of root knot, one of the most widespread plant diseases of the world.

### B.  Crop Losses Caused by Plant-parasitic Nematodes and Associated Organisms

Excellent data on crop losses due to nematodes and associated soil-borne pests have been collected for flue-cured tobacco (*Nicotiana tabacum*) in North Carolina (USA) by the Extension Service from experimental and demonstration plots over a period of about 20 years. In 1976 there were 15 full-scale field trials and demonstrations in "problem fields." The trials consisted of replicated plots treated with various nematicides and compared with control plots which received no nematicide. In each experiment a

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*Note on pronunciation and derivation: Mel'oid o gyne from Greek *melon* (apple or gourd) + *oeides, oid* (resembling) + gyne (woman or female) = gourd-like female. The accent is on the second syllable. See Webster's Unabridged Dictionary.

**Correct spelling of the German-Swiss name usually spelled Goeldi in nematological literature.

comparison could be made between the average yield of the four replicated plots of the treatment with the highest yield and the control plots. Averages of controls in all 15 experiments were 75.5% of the highest yield plots. In other words, lack of treatment to control nematodes and associated pests would cost the farmer 24.5% of his potential yield.

These being problem fields, it cannot be argued that they were representative. Nevertheless, it is interesting to note that in a state where nematicides were used on 85% of the land planted to tobacco in 1976, it was possible to find fields where yields were only 75.5% of the potential. Tobacco growers in North Carolina spent an estimated $19,000,000 for chemical soil treatment in 1976 (chemicals $13,900,000 and application $5,100,000) with full expectation of making a good profit (Todd, 1976a).

Such data encourage belief in "estimates" of crop losses due to Meloidogyne and other nematodes on a world basis of about 5%. This would not be of great significance if evenly distributed, but it is not; the greater part of the loss is borne by those least able to afford it, namely, the small farmers of undeveloped countries. Their losses may be as much as 25% to 50% over wide areas of the available farm land of the country.

## II. Zoological Classification of the Genus *Meloidogyne*

*Meloidogyne* species are a small part of the Phylum Nemata (or Nematoda) which includes parasites of man and animals; parasites of plants; and species which live in the soil, in fresh water, and in the sea. Their Class is Secernentea, Order Tylenchida, Superfamily Tylenchoidea, and Family Meloidogynidae (Wouts, 1973).

## III. Species of the Genus *Meloidogyne* Goeldi, 1887

### A. List of Described Species

Up to the end of 1976, at least 36 species of the nematode genus *Meloidogyne* have been named and sufficiently well described to fulfill the requirements of the International Rules of Zoological Nomenclature (Table 1.1). These are probably only a small part of the *Meloidogyne* species which will eventually be described. Only a few regions of the world have been thoroughly surveyed for *Meloidogyne* species in farm fields; most forests and other areas of uncultivated land have not been explored. It is reasonably certain that most of the widespread economically important species are known. These

are, roughly in order of distribution and crop damage: *M. incognita, M. javanica, M. hapla* and *M. arenaria*.

Table 1.1. Species of *Meloidogyne*[1]

1. *M. acrita* (Chitwood, 1949) Esser, Perry and Taylor, 1976
2. *J. acronea* Coetzee 1956
3. *M. africana* Whitehead, 1960[2]
4. *M. ardenensis* Santos, 1968[3]
5. *M. arenaria* (Neal, 1889) Chitwood, 1949
6. *M. artiellia* Franklin, 1961
7. *M. bauruensis* (Lordello, 1956) Esser, Perry and Taylor, 1976
8. *M. brevicauda* Loos, 1953
9. *M. coffeicola* Lordello and Zamith, 1960
10. *M. decalineata* Whitehead, 1968
11. *M. deconincki* Elmiligy, 1968
12. *M. ethiopica* Whitehead, 1968
13. *M. exigua* Goeldi, 1887
14. *M. graminicola* Golden and Birchfield, 1965
15. *M. graminis* (Sledge and Golden, 1964) Whitehead, 1968
16. *M. hapla* Chitwood, 1949
17. *M. incognita* (Kofoid and White, 1919) Chitwood, 1949
18. *M. indica* Whitehead, 1968
19. *M. inornata* Lordello, 1956
20. *M. javanica* (Treub, 1885) Chitwood, 1949
21. *M. kikuyensis* de Grisse, 1960
22. *M. kirjanovae* Terenteva, 1965
23. *M. litoralis* Elmiligy, 1968
24. *M. lordelloi* da Ponte, 1969
25. *M. lucknowica* Singh, 1969
26. *M. mali* Itoh, Ohshima and Ichinohe, 1969
27. *M. megadora* Whitehead, 1968
28. *M. megriensis* (Pogosyan, 1971) Esser, Perry and Taylor, 1976
29. *M. microtyla* Mulvey, Townshend and Potter, 1975
30. *M. naasi* Franklin, 1965
31. *M. oteifae* Elmiligy, 1968
32. *M. ottersoni* (Thorne, 1969) Franklin, 1971
33. *M. ovalis* Riffle, 1963
34. *M. poghossianae* Kirjanova, 1963[4]
35. *M. spartinae* (Rau and Fassuliotis, 1965) Whitehead, 1968
36. *M. tadshikistanica* Kirjanova and Ivanova, 1965
37. *M. thamesi* (Chitwood, 1952, in Chitwood, Specht and Havis) Goodey, 1963

1. As of January 1, 1977.
2. Printed in Nematologica 4(4):272-278, dated December, 1959, but not actually published until January 15, 1960. See "Dates of Publication of Nematologica," Nematologica 6(1):88. 1961.
3. This was published in 1968. See original publication, Nematologica 13(1967):593-598 (published in 1968).
4. *Species inquirenda.*

### B. Taxonomy of *Meloidogyne* Species

Unfortunately, the taxonomy of the genus *Meloidogyne* is confused, mostly because of the dif-

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ficulty of identifying the species. Triantaphyllou and Hussey (1973) discussed this subject, pointing out that study of morphology and anatomy has not been adequate to explain the relationships within the genus. "Characterization of morphological forms has not provided an objective definition of what constitutes a species in *Meloidogyne*. Recent experimental and cytological studies demonstrated that many members of the genus *Meloidogyne* reproduced by parthenogenesis (Triantaphyllou, 1970). This means that the 'biological species' concept cannot be applied to *Meloidogyne*, at least not without certain clarifications." Only an undescribed species from North Carolina (USA) is known to reproduce exclusively by amphimixis. A few others reproduce by both amphimixis and meiotic parthenogenesis. The biological species concept can be applied to species which do reproduce by amphimixis without many theoretical implications. Research is needed to determine whether or not the species mentioned are potentially reproductively isolated or not.

Parthenogenesis in *M. incognita, M. javanica, M. arenaria* and sometimes in *M. hapla* is of the mitotic type, with no meiosis during oogenesis; the somatic (2n) number of chromosomes is maintained during maturation of the oocytes. Parthenogenesis is obligatory. Since there is no definite species concept for organisms with obligatory parthenogenesis, the species are subjective entities, based on morphology and, to some extent, on host response. From the practical standpoint, each of these parthenogenetic species consists of a large number of field populations which share some common characteristics of taxonomic value. The basic chromosome number of the genus is 18, and *M. hapla* populations with haploid chromosome numbers n = 15, 16 and 17 have been found. The somatic numbers for *M. javanica* are 2n = 43, 44, 46 and 48. *M.arenaria* has 2n = 36 and 3n = 54, and *M. incognita* has 2n = 41 to 44.

Due to present lack of a better classification, and for convenience, we will refer to all of the forms named in Table 1.1 as species of *Meloidogyne*, even though it is already evident that taxonomists will eventually synonymize or divide some of them and place others in different genera.

One of the objectives of the International *Meloidogyne* Project is to collect the specimens and information needed to clarify the taxonomy of the genus. With better descriptions of morphology of the species, more knowledge of host ranges, and additional cytological research, it will be possible to correlate host ranges and morphology for the most common root-knot nematodes.

In the meantime, workers with root-knot nematodes should consider that mistakes in identification are possible. The great bulk of literature using the names *Heterodera marioni* (Cornu, 1879) Goodey, 1932 and *Caconema radicicola* (Greef, 1872) Cobb, 1924 and published before 1949 is of little value because the authors believed that there was only one root-knot nematode species. Undoubtedly, similar mistakes have been made since 1949 in the belief that there are only five or six *Meloidogyne* species.

## IV. World Distribution of *Meloidogyne* Species

Original habitats of *Meloidogyne* species are unknown. Widespread distribution of vegetative planting stock infected with root-knot nematodes makes it difficult to distinguish between species originating in a region and adapted to long continued existence there, imported species adapted to a climate and capable of existing indefinitely, and imported species able to survive only for a few months or a few years. Enough is known to make certain statements highly probable, though with reservations and exceptions.

In cool climates where the average temperature of the coldest month of the year is near or below 0°C and the average temperature of the warmest month is about 15°C or above, the most common *Meloidogyne* species is *M. hapla*. Present information indicates that *M. hapla* is adapted to long time existence in northern United States and southern Canada in North America, in northern Europe and in northern Asia. In South America, *M. hapla* is found south of about 40°S latitude and in the mountainous regions of the western part of the continent. In Africa, it may be adapted to continued existence in altitudes above 1500 meters. In Australia, it is common in Victoria, the southernmost state.

In the tropic zone, the most common *Meloidogyne* species are *M. incognita* and *M. javanica*. In North and South America, *M. javanica* is seldom found above 30°N and 35°S latitude and becomes more common as the equator is approached. In many parts of tropical Africa, Australia and southern Asia, *M. javanica* is probably the most common *Meloidogyne* species. *M. incognita* and *M. arenaria* are common and widespread in the same regions. In the United States, the northern limit for continued existence of *M. incognita* is a few hundred miles farther north of the limit for *M. javanica*. *M. arenaria* is found in much the same regions as *M. incognita*.

Thus, the part of the world between 35°S and 35°N latitudes is widely infested by three species of *Meloidogyne* adapted to continuous existence in

warm countries, namely, *M. javanica*, *M. incognita* and *M. arenaria*. North of 35° latitude in the northern hemisphere the most common *Meloidogyne* species is *M. hapla*. These four species, as presently identified by taxonomists, are the most widespread and common *Meloidogyne* species of the world and very probably cause more damage to farm crops than all the other *Meloidogyne* species combined (Sasser, 1977).

## V. Morphology and Development of *Meloidogyne* Species

### A. Life Cycle—Preparasitic

The life cycle of *Meloidogyne* species starts with an egg, usually in the one-celled stage, deposited by a female which is completely or partially embedded in a root of a host plant. The eggs are deposited into a gelatinous matrix which holds them together in "egg masses' or "egg sacs." More than 1000 eggs have been found in one egg mass, and it may be larger than the female body. Egg development begins within a few hours after deposition, resulting in 2 cells, 4, 8, and so on, until a fully formed larva with a visible stylet lies coiled in the egg membrane. This is the first larval stage (Fig. 1.1,A-T). It can move in the egg but is not very active. The first molt takes place in the egg, and

it is not difficult to see the separated first-stage cuticle protruding beyond the head of the second-stage larva (Fig. 1.1,U). Shortly after, the larva hatches, emerging through a hole made in the end of the flexible egg shell by repeated thrusting with the stylet.

The hatched second-stage larva (Fig. 1.2,A) may or may not leave the egg mass immediately. Usually there are some hatched larvae in the egg mass along with eggs in various stages of development. After leaving the egg mass, the larva moves through the soil in search of a root on which to feed. The search seems to be random until the larva comes within a few centimeters of a root, then guided by some substances emanating from the root, it moves more directly toward the root tip.

### B. Life Cycle—Parasitic

#### 1. Penetration of Roots

Second-stage infective larvae usually penetrate roots just above the root cap. They move mostly between undifferentiated root cells, and finally come to rest with heads in the developing stele near the region of cell elongation and bodies in the cortex (Fig. 1.2,B). Cell walls are pierced with stylets, and secretions from the esophageal glands are injected. These secretions cause enlargement of cells in the vascular
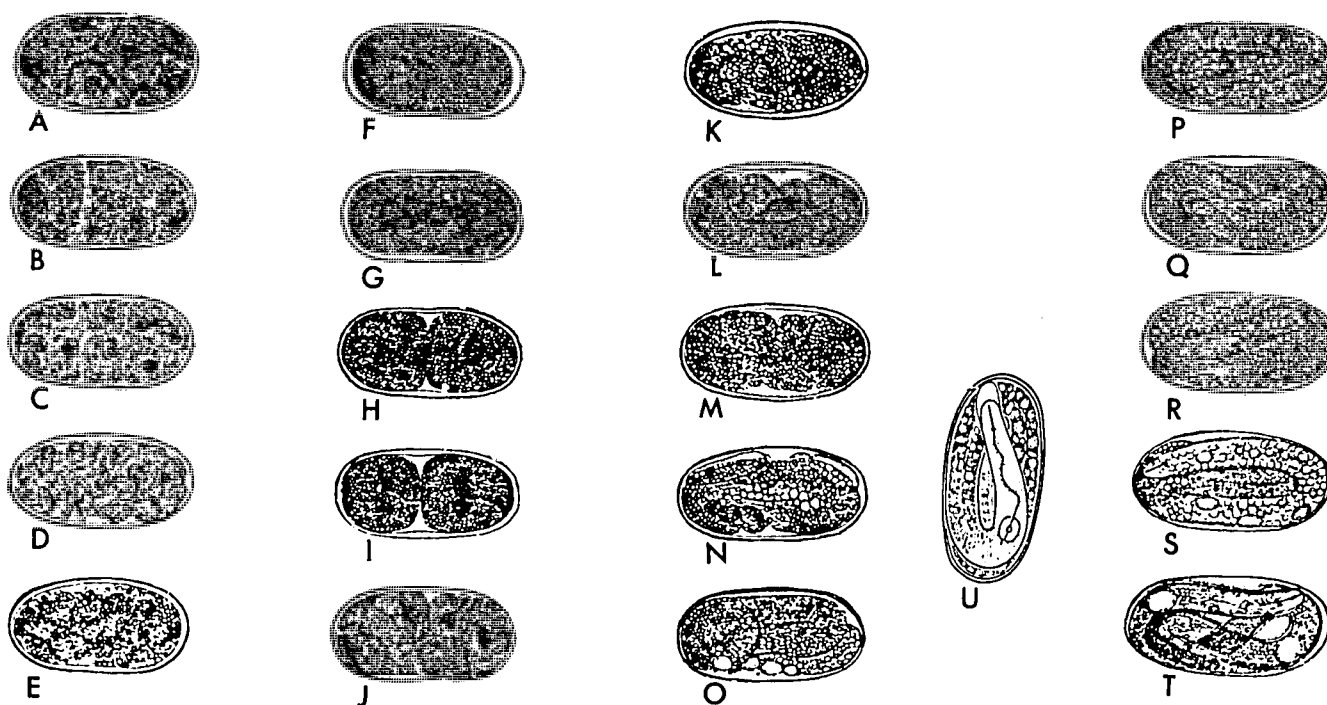


Fig. 1.1. Development of eggs of a *Meloidogyne* species. A-S: Stages in development from one cell to second-stage larva. T: Second-stage larva. U: Second-stage larva showing molted cuticle. A-T from Saigusa, 1957; U from Christie and Cobb, 1941.

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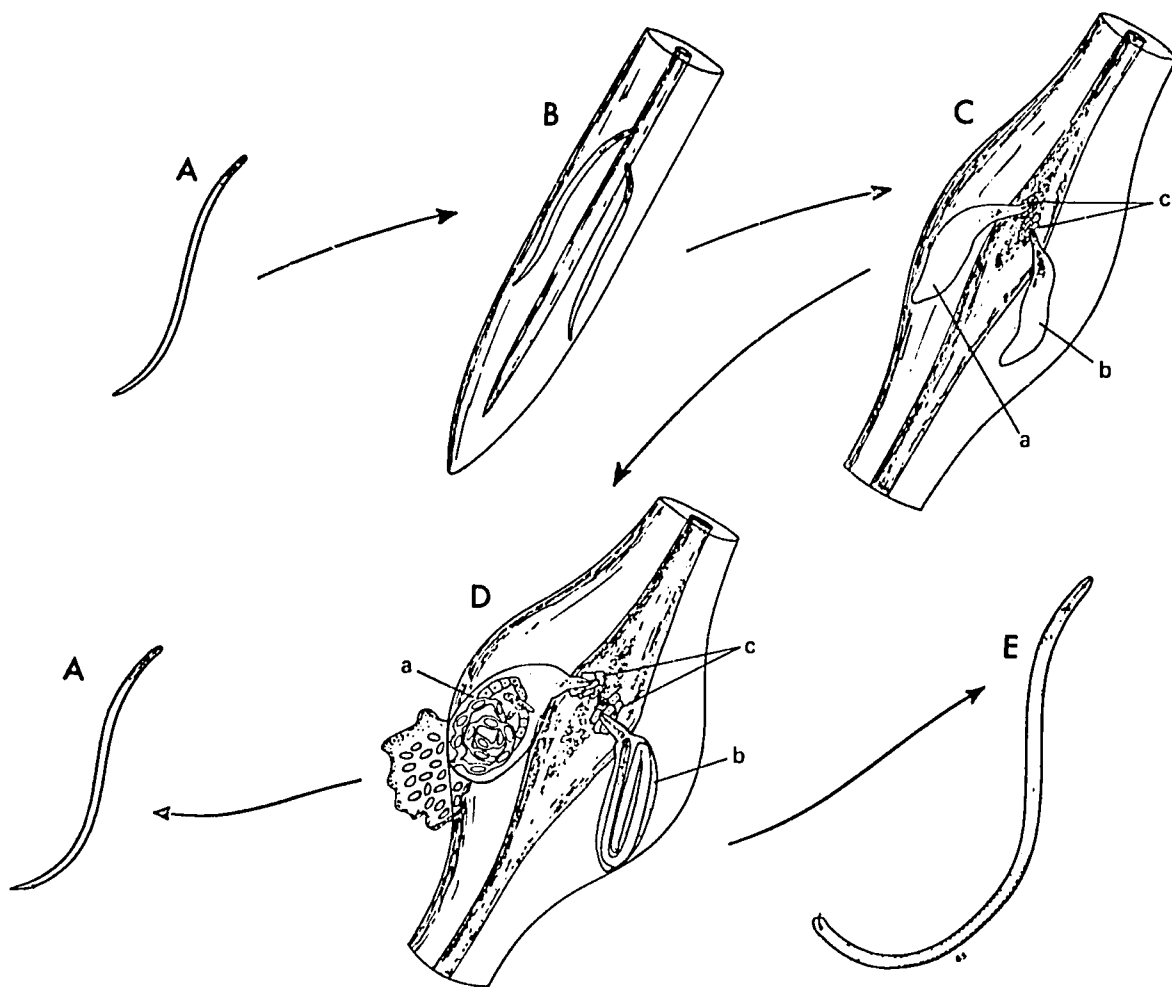
Fig. 1.2. Life cycle of a *Meloidogyne* species (schematic). A: Preparasitic second-stage larva. B: Two larvae which have entered a root, become stationary, and started to feed. C: Start of gall formation, development of larvae (a,b) and giant cells (c). D: Gall with mature female and egg mass (a), male after metamorphosis (b), and giant cells (c). E: Male free in soil. De Guiran and Netscher, 1970.

cylinder and increased rates of cell division in the pericycle (Fig. 1.2,C). This leads to formation of giant cells (also called syncytia) formed by enlargement of cells (hypertrophy), possible dissolution of cell walls, enlargement of nuclei, and changes in the composition of the cell contents. At the same time, there is intense cell multiplication (hyperplasia) around the larval head. These changes are usually, but not invariably, accompanied by enlargement of the root to form distinct galls (Fig. 1.2,D). On small roots, galls containing only one female are round to fusiform and may be one to three millimeters in diameter.

## 2. Development of Parasitic Stages

While the giant cells and galls are forming, width of larvae increases as shown in Fig. 1.3, B, and there

is considerable enlargement of the esophageal glands. The cells of the genital primordium divide and the genital primordium enlarges, becoming distinctly two pronged in the female, or forming an elongated body in the male (Fig. 1.3, C and D). Six rectal glands begin to enlarge in the nearly hemispherical posterior part of the female body (Fig. 1.3, C). As the second-stage larvae continue feeding, body size increases, bodies become flask-shaped, and the gonads lengthen (Fig. 1.3, D, E).

With the second and third molts completed by the female, as shown by the two loose cuticles (Fig. 1.3,F), the stylet and median esophageal bulb disappear. Shortly after the fourth molt, the stylet and median bulb are regenerated, the uterus and vagina are formed, and a perineal pattern is visible (Fig. 1.3,G). Further development of the two female

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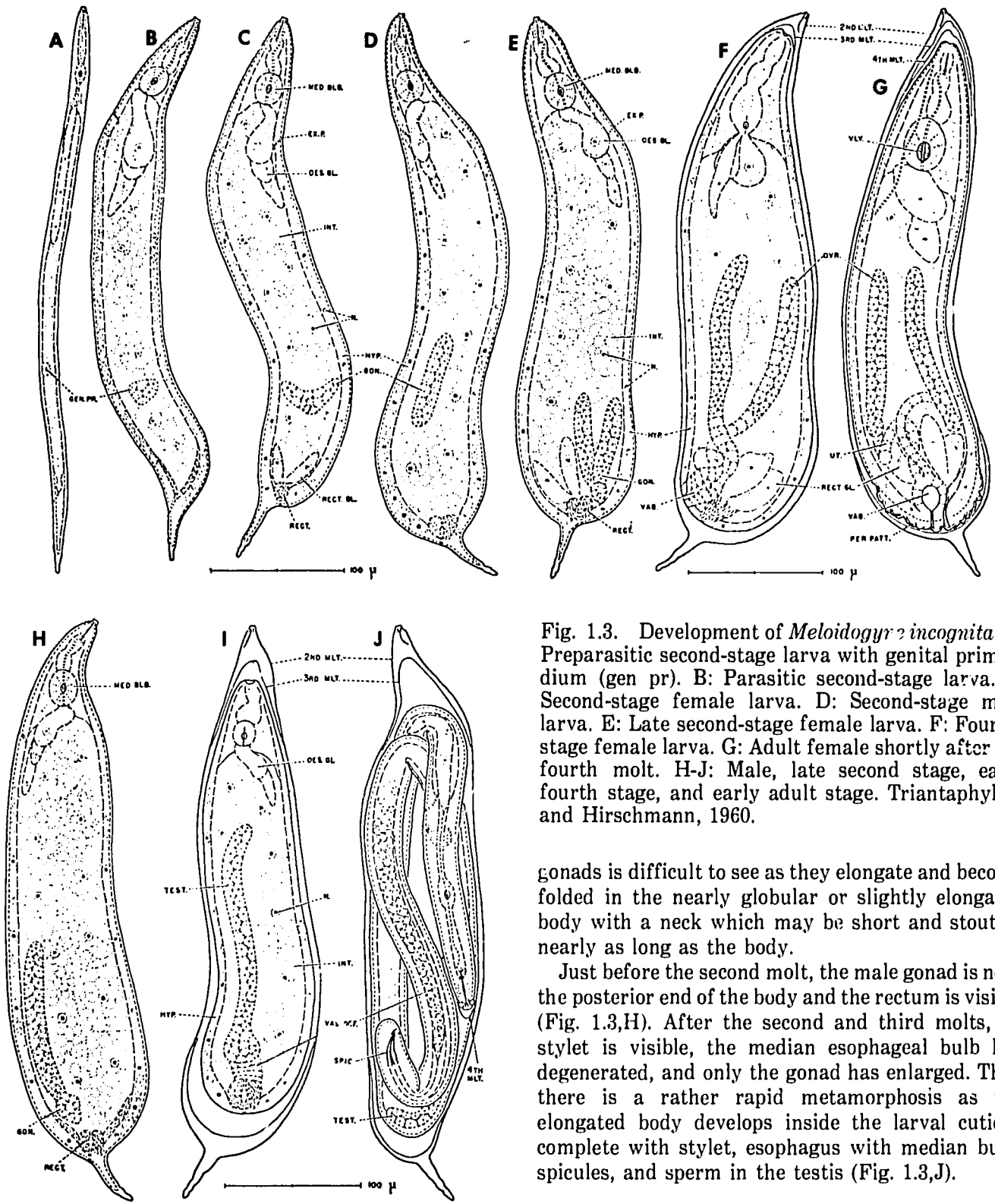
Fig. 1.3. Development of *Meloidogyne incognita*. A: Preparasitic second-stage larva with genital primordium (gen pr). B: Parasitic second-stage larva. C: Second-stage female larva. D: Second-stage male larva. E: Late second-stage female larva. F: Fourth-stage female larva. G: Adult female shortly after the fourth molt. H-J: Male, late second stage, early fourth stage, and early adult stage. Triantaphyllou and Hirschmann, 1960.

gonads is difficult to see as they elongate and become folded in the nearly globular or slightly elongated body with a neck which may be short and stout or nearly as long as the body.

Just before the second molt, the male gonad is near the posterior end of the body and the rectum is visible (Fig. 1.3,H). After the second and third molts, no stylet is visible, the median esophageal bulb has degenerated, and only the gonad has enlarged. Then there is a rather rapid metamorphosis as the elongated body develops inside the larval cuticle, complete with stylet, esophagus with median bulb, spicules, and sperm in the testis (Fig. 1.3,J).

## C. Morphology—Adults

Adult females of *Meloidogyne* species range in median length from about 0.440 to 1.300 mm, and median width range is about 0.325 to 0.700 mm. Females of most species have symmetrical bodies as shown in
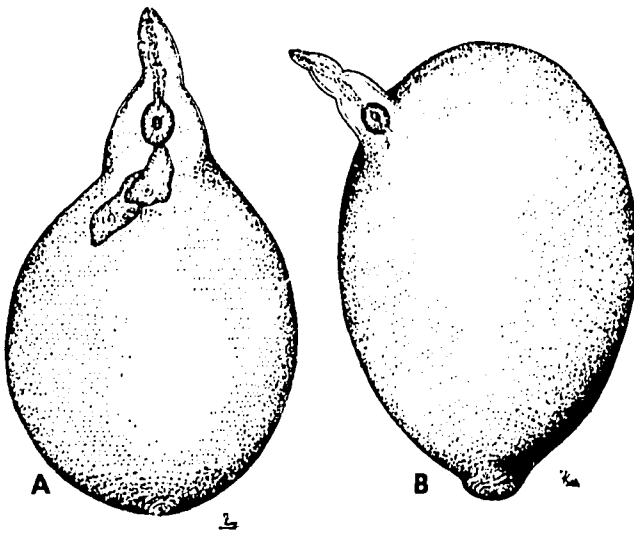
Fig. 1.4. Body shapes of *Meloidogyne* females. A: The bodies of most *Meloidogyne* females are pyriform and the axis is a nearly straight line from anus to stylet. B: Females of some species are oval with a posterior protuberance, and necks are at an angle to the body axis. The angle varies from about 15° to more than 90°. Esser, Perry and Taylor, 1976.

Fig. 1.4,A; that is, a line from vulva to stylet passes through the middle of the body. Many females illustrated in the original descriptions of *M. ottersoni* (Fig. 8.12), *M. spartinae* (Fig. 9.20), *M. acronea* (Fig. 9.21), *M. graminis* (Fig. 9.22) and *M. megriensis* (Fig. 9.28) have bodies of the general shape shown in Fig. 1.4,B. The female body is not symmetrical; that is, the neck is not on or near the center line of the body but distinctly to one side, so that center lines of neck and body make an angle of from at least 15° to more than 90° in some specimens. Figure 1 of the original description of *Hypsoperine graminis* (Fig. 9.22,B) shows the esophageal gland of *H. graminis* on the ventral side, indicating that the placement is ventral (Sledge and Golden, 1964). Siddiqui and Taylor (1970) present a photograph of a mature female of *M. naasi* apparently in lateral view (Fig. 8.11). The axis of the neck makes an angle of nearly 90° with the axis of the body, and the vulva is nearer to the neck than the anus, again indicating ventral displacement. If the displacement is ventral, it would be at an apparent maximum with the body in lateral view, and less visible in ventral or dorsal view.

In the generic diagnosis of *Hypsoperine* Sledge and Golden, 1964, it is stated that the female has a "well defined protruding neck usually situated to one side," and in the description of the holotype female: "Body white and oval with protruding neck usually situated well to one side of median plane through vulva."

Descriptions of some of the other species mentioned previously also make reference to the asymmetrical neck. The significance of this difference in body shape is not yet clear, but it is interesting to note that the diploid number of chromosomes of *M. graminis*, *M. ottersoni* and *M. naasi* is 36 and that all are primarily parasites of Gramineae (Triantaphyllou, 1971, 1973).

## D. Reproduction

The female reproductive system of *Meloidogyne* species consists of two ovaries, each with a germinal zone, growth zone, oviduct, spermatotheca and uterus. The uteri lead to a common vagina (Fig. 1.5). The reproductive system is formed from the four-celled genital primordium of the second-stage larva (Fig. 1.3,A) and develops through the third and fourth larval stages as shown in Fig. 1.3, B, C, E, F, and G. At the distal end of the adult reproductive system (Fig. 1.5,E), there are cells which divide many times forming oogonia with the somatic (2n) chromosome number. The most advanced oogonia cease dividing and become oocytes which pass through a long growth zone (Fig. 1.5,C), becoming larger and moving one by one through the oviduct and spermatotheca. Another mitotic division takes place; the eggs become oval and form a flexible shell (Fig. 1.5, D, E). Finally, they pass through the vagina and are deposited in the egg mass in the one-celled stage (Triantaphyllou, 1962).

This kind of reproduction is called parthenogenesis (mitotic) and is usual in *M. incognita*, *M. javanica*, *M. arenaria*, some populations of *M. hapla* and other species. The diploid chromosome number is preserved. Sperm are not necessary for egg development, and fertilization does not occur, even when sperm are present in the spermatotheca.

All of the species reproducing by parthenogenesis have males in numbers which vary with the food supply and other factors. Generally when food is plentiful, most larvae develop to females. When food is less plentiful as with heavy infections or old plants, a large percentage of the larvae may become males.

Production of sperm follows much the same pattern as production of eggs, except that there is a reduction of chromosomes to the 1n number. The sperm are, of course, much smaller.[*]

Facultative reproduction by amphimixis in addition to reproduction by meiotic parthenogenesis has been observed in some populations of *M. graminis*, *M.*

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[*]Details of reproduction are discussed by Triantaphyllou (1963, 1966, 1969, 1970 and 1973.) Methods for study are also given in these publications.

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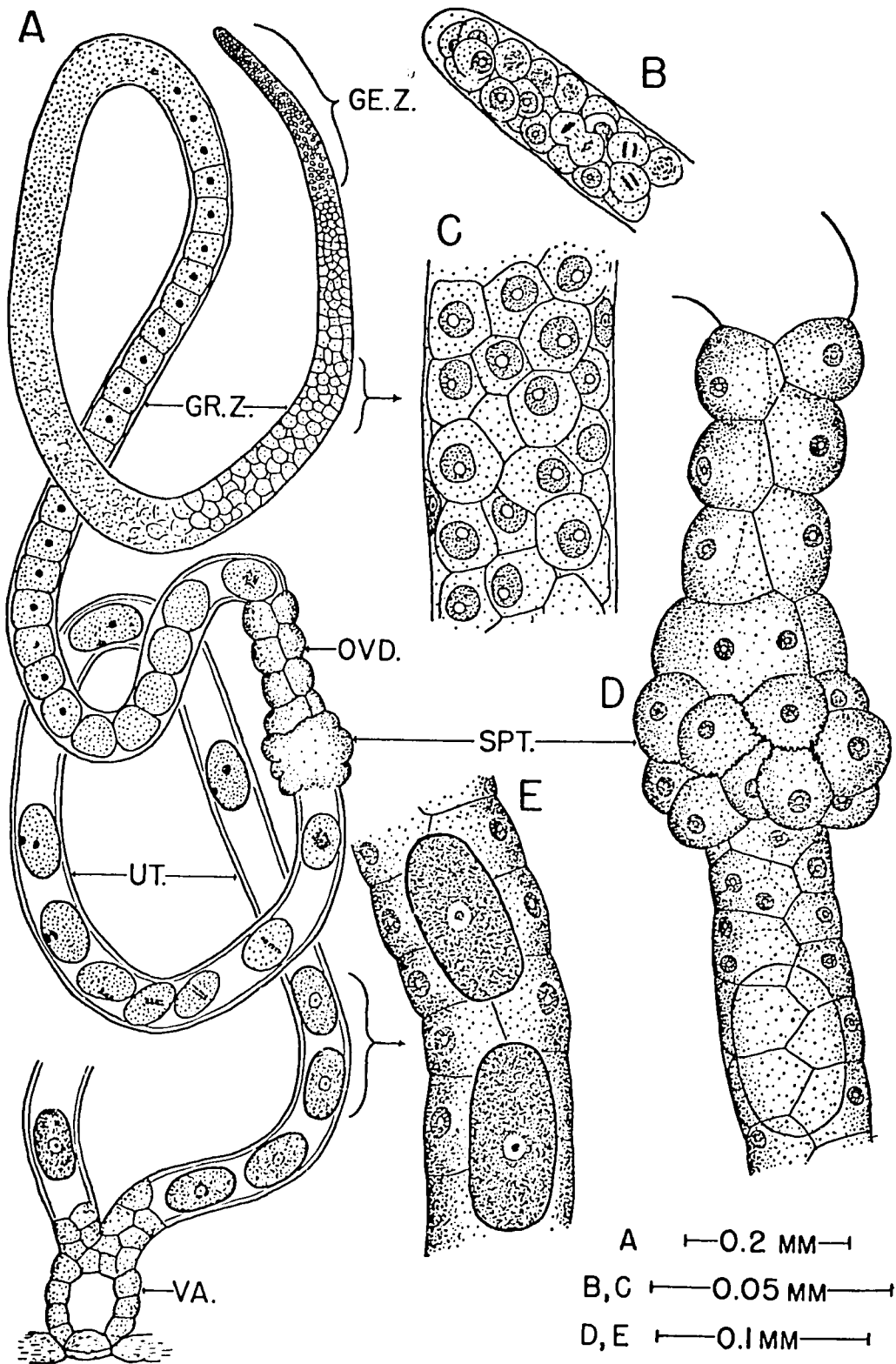
Fig. 1.5. Female reproductive system of *M. javanica*. A: One of the two gonads showing various regions and the process of oogenesis. B: Distal end of germinal zone of ovary with mitotic oogonial divisions. C: Growth region of ovary. D: oviduct and spermatotheca. E: Lower region of uterus and oocytes with pronucleus. Ge.Z. = germinal zone. Gr.Z. = growth zone. Ut. = uterus. Va. = vagina. Spt. = spermatotheca. Ovd. = oviduct. Triantaphyllou, 1962.

*graminicola, M. naasi, M. ottersoni* and some populations of *M. hapla*. Only one undescribed species is known to reproduce exclusively by amphimixis. (Triantaphyllou and Hussey, 1973.)

## E.  Anatomy

Males, females and larvae of *Meloidogyne* species (Fig. 1.6,A-D) all have stylets which consist of a tapering point, a straight shaft and three knobs. The stylet can be protruded by means of muscles attached to the knobs; it is used to puncture plant cells. The stylet has an opening near the tip which leads to a lumen continuous with the lumen of the esophageal tube attached to the knobs. Close behind the knobs, the tube has a short branch called the dorsal gland orifice (dgo). The esophageal tube leads to a valve enclosed in the median esophageal bulb. Muscles attached to the valve alternately expand and contract it so that it functions as a pump, transferring food to the intestine. Posterior to the median bulb there are three large glands in the esophagus, one dorsal and two subventral. The duct of the dorsal gland leads to the dorsal gland orifice, and the ducts of the subventral glands open into the esophageal tube in the median bulb.

In feeding, the nematode pushes the point of the stylet into a plant cell. Secretions from the dorsal esophageal gland flow out through the stylet opening into the plant cell. This secretion and possibly also secretions from the two subventral glands have important effects on the plant, as will be discussed later.

The esophageal glands of larvae and females are well developed and are used in feeding. Males apparently do not feed and lack well developed esophageal glands.

Males have well developed stylets and slender bodies which taper anteriorly and are rounded posteriorly (Fig. 1.6,A,B,E). The two spicules are used in copulation, being protruded through the cloaca which combines the functions of anus and sex opening. The cuticle of the male body has numerous annules which are interrupted at the sides of the body by lateral fields with four or more lines. The annules and lateral lines are usually less conspicuous on larvae and females.

The female body is white, and details of the ovaries are difficult to see. The neck is more transparent, the stylet, esophageal bulb and excretory canal are usually visible.

## VI.  Time of Life Cycle

## A.  Influence of Temperature

Length of the life cycle in root-knot nematodes is greatly influenced by temperature. Papers on the influence of temperature on various activities of *Meloidogyne* species reviewed by Wallace (1964) indicated that *M. hapla* and other cool climate species have lower minimum, optimum and maximum requirements for hatching, mobility, invasion of roots, growth, reproduction and survival than *M. incognita, M. javanica* and *M. arenaria* which occur in warmer climates. Optimum temperatures range from 15° to 25° for *M. hapla* and related species, and 25° to 30°C for *M. javanica* and related species. There is very little activity by any *Meloidogyne* species above about 40°C, or below 5°C.

In South Africa, 56 days were required for the life cycle of *M. javanica* at a mean temperature of 14°C, compared with only 21 days at 26°C (Milne and Du Plessis, 1964).

The first molt of *M. naasi* in wheat seedlings was in 8½ to 11 days at 22° to 26° C, and infective second-stage larvae hatched in 15 to 17 days. Large numbers of larvae penetrated roots in 24 hours and were sedentary in feeding position in 2 or 3 days. Body width started to increase about 6 days after penetration, and sex differentiation was visible after 12 days. The second molt was at 18 days, followed by the third and fourth molts between 18 and 24 days. The female grew rapidly between the 24th and 30th day, and the gelatinous matrix was seen after 27 to 30 days. Eggs were deposited starting the 30th to 40th day (Siddiqui and Taylor, 1970).

## B.  Length of Life

The length of life of female root-knot nematodes has not been studied. Field observations indicate that females may continue to produce eggs for two or three months and live for some time after egg production stops. Old females which are still alive but not producing eggs, as shown by transparent bodies, are common late in the season. Males probably live only for weeks rather than months.

Length of life of hatched larvae varies from a few days to a few months. Many larvae hatched under favorable conditions find a root and start development in a few days. Others, hatched late in the fall when temperatures are low, may live over winter and
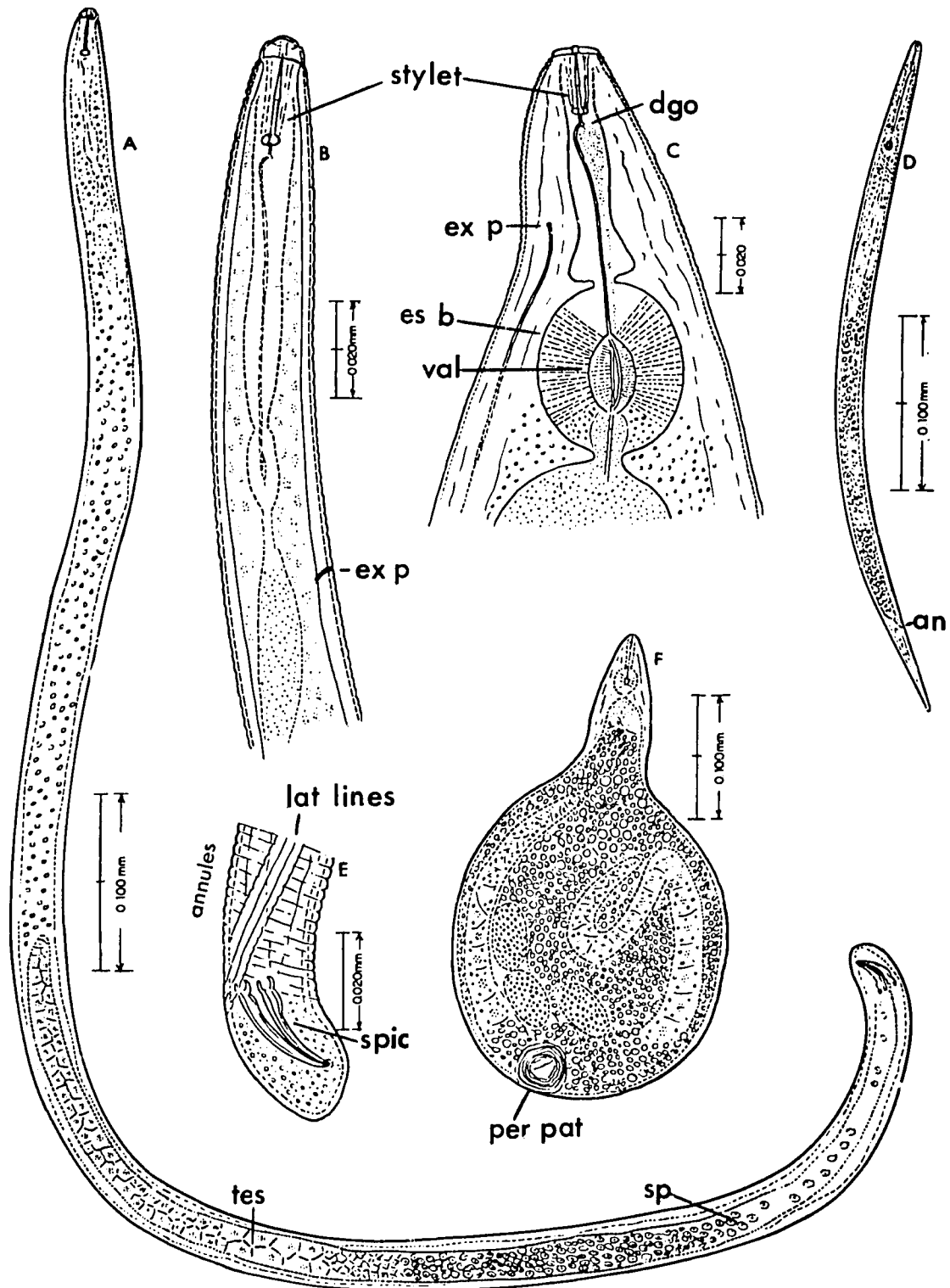
Fig. 1.6. Anatomy of *Meloidogyne* species. A: Male, full length, showing stylet, testis (tes), sperm (sp), and spicules. B: Male anterior, showing stylet, esophagus and excretory pore (ex p). C: Female anterior, showing stylet, dorsal gland orifice (dgo), excretory pore (ex p), esophageal bulb (es b), and valve (val). D: Larva, showing stylet, esophageal bulb, and anus (an). The tail is the portion of the body posterior to the anus. E: Male posterior, showing lateral (lat) lines, annules, and spicules, F: Female, showing esophagus, ovaries, and perineal pattern. Taylor,1967.

complete their life cycle the next spring. Most evidence indicates that hatched larvae live only a few weeks in moist soil at summer temperatures.

## C. Effect of Moisture

In the field, hatching is dependent on two principal factors, namely, soil temperature and soil moisture. Very low soil moisture is important only in irrigated fields or in regions where there are dry seasons with little or no rain alternating with rainy seasons. As the soil dries at the beginning of the dry season, *Meloidogyne* eggs are subjected to osmotic stress. Hatching ceases, but development in the egg continues, so all living eggs soon contain second-stage larvae. If they become too dry, the larvae die; but if they survive until the beginning of the rainy season, they can hatch and infect plants (Dropkin, Martin and Johnson, 1958).

Peacock (1957) held sandy loam soil naturally infested with an unidentified *Meloidogyne* species but without undecayed plant material for 2 to 5 days, then planted indicator plants. Results are given in Table 1.2.

In a similar experiment with soil containing undecayed infested roots, there was nearly complete control at 1.3% moisture (5% saturation), but no control at 3.2% to 27% moisture (13% to 100% saturation).

In field experiments (Gold Coast, Africa), it was found that simple cultivation of the soil during the dry season (November to February) was sufficient to obtain a practical measure of control as determined by the severity of attack on the following crop.

Table 1.2. Survival of *Meloidogyne* species in soil as influenced by moisture content.

| Moisture content of soil | | Mean number of knots on indicator plant roots (10 replications) | |
|---|---|---|---|
| Percent moisture | Percent saturation | Number | Percent |
| 2.9 | 10.7 | 0.0 | 0.0 |
| 3.7 | 13.6 | 17.4 | 23.0 |
| 4.9 | 18.3 | 70.6 | 93.3 |
| 7.8 | 29.0 | 75.6 | 100.0 |
| 19.1 | 70.5 | 47.6 | 62.9 |
| 27.0 | 100.0 | 22.9 | 30.3 |

# 2.
# Relationships of *Meloidogyne* Species and Plants

## I. Susceptible and Resistant Host Plants

*Meloidogyne* species are obligate plant parasites. Reproduction occurs only when second-stage infective larvae enter roots or other underground parts of a suitable host plant, initiate giant cells on which to feed, and develop to egg-laying females. The eggs hatch, giving rise to a new generation of infective second-stage larvae. The plant on which the nematode feeds is a host plant. If the nematode species can reproduce on it, it is a susceptible host plant.

Host plants have many degrees of susceptibility. The most important are the highly and moderately susceptible host plants in which reproduction of the nematode is normal; a large percentage of the larvae which enter the roots develop and produce many eggs. These plants are most likely to be damaged by root-knot nematodes in the field; populations in-
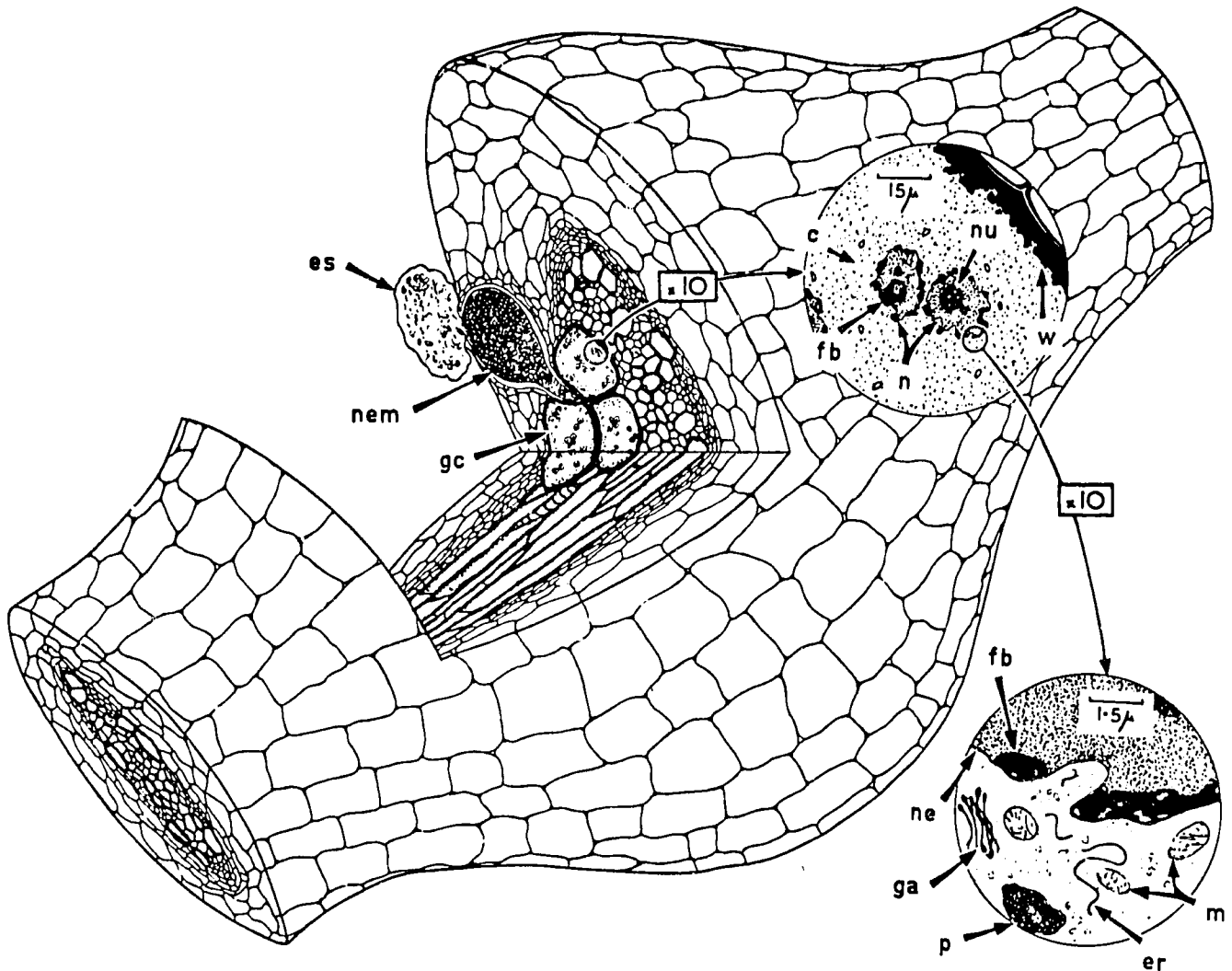


Fig. 2.1. Semi-diagrammatic drawing of a tomato root gall containing a single *Meloidogyne* female (nem) with egg sac (es) outside the gall. Three giant cells (gc) with thick walls are shown. The enlarged views (circles) show details of the giant cells, including large nuclei surrounded by nuclear envelopes (ne) and containing Feulgen positive bodies (fb). The giant cell cytoplasm is dense and contains mitochondria (m), proplastids (p), endoplasmic reticulum (er) and Golgi apparatus (ga). Bird, 1961.

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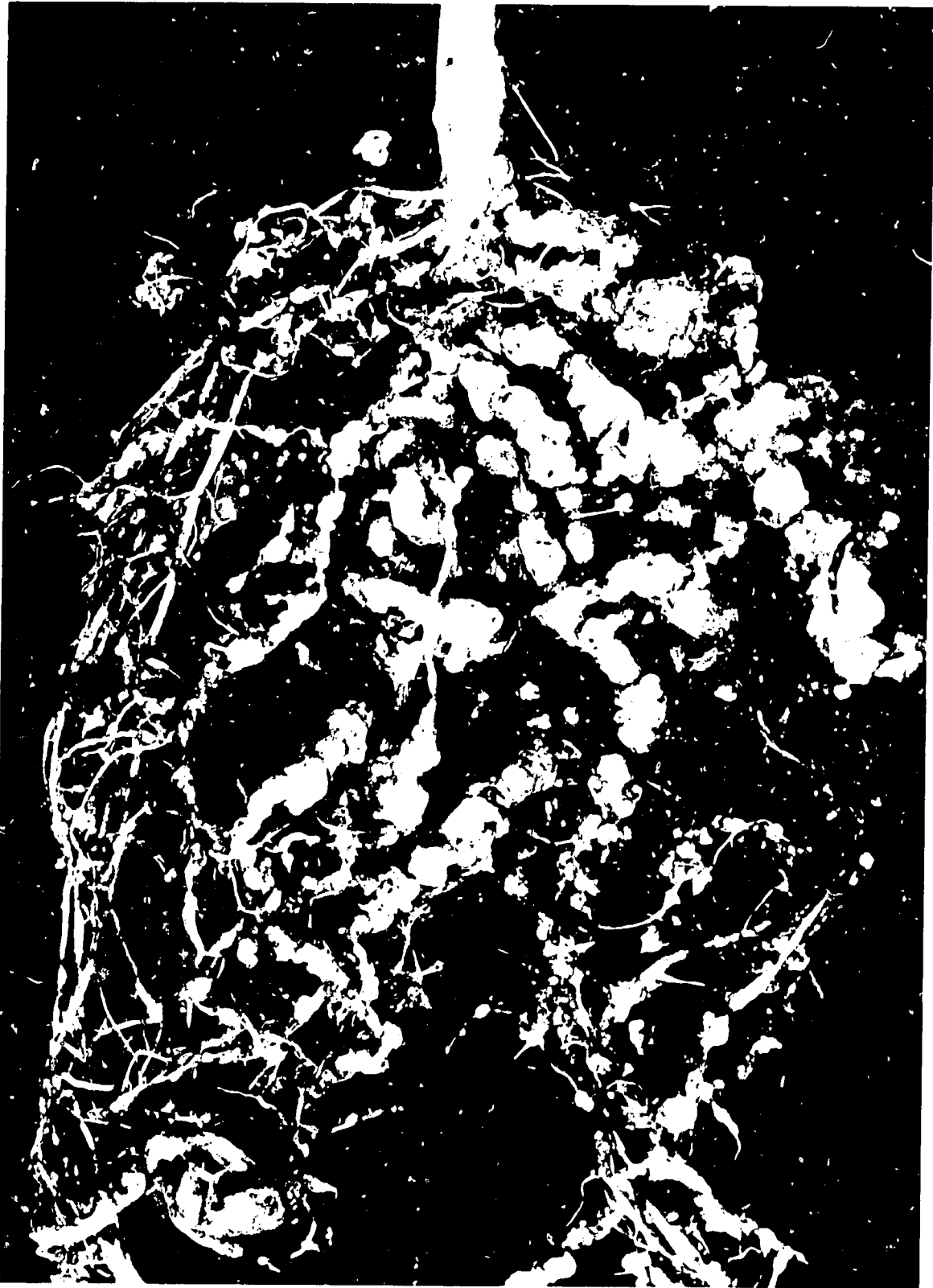
Fig. 2.2. *Meloidogyne* galls on bean roots. About natural size.

crease rapidly, and a small infestation in the soil early in the growing season can become a heavy infestation by mid-season, resulting in severe damage to plant growth, with reduced yields and quality of crop. Root-kr nematodes multiply logarithmically for several ,erations during the growing season. Theoretica if as many as 5% of 500 eggs produced by female ve to reproduce, the numbers will be 25, 625, 15,625, 390,625 in only four generations.

For the great majority of highly susceptible crop plants, the most common and conspicuous symptom of infection by *Meloidogyne* species is the presence of distinct enlargements of the roots, called galls or knots (Fig. 2.1). On the very small roots these may be as small as 1 or 2 millimeters in diameter; on larger roots, 1 centimeter or more (Fig. 2.2). Large galls usually contain several females (Fig. 2.2); small galls may contain only one female (Fig. 2.3). Galls are a symptom of nematode attack, but presence of a *Meloidogyne* species in galled roots cannot always be taken for granted; species of other nematode genera also cause gall formation (*Nacobbus, Meloidodera, Ditylenchus* and others). Root galls are also caused by other organisms such as *Plasmodiophora brassicae* (club root of Crucifers). Nitrogen nodules on legumes can be mistaken for root-knot nematode galls. Exact diagnosis can only be made by microscopic examination and identification of nematodes dissected from the galls.

Plants less than moderately susceptible are called "resistant," with a qualifying adjective, slightly resistant, moderately resistant, highly resistant or immune. Resistance is defined by reference to reproduction. In a highly resistant plant, reproduction is less than 2% of the reproduction of a susceptible plant with comparable soil infestation; in a moderately resistant plant, 10% to 20%; and in a slightly resistant plant, up to 50%.

## II. Host Specificity of *Meloidogyne* Species

Each species of *Meloidogyne* has plant species and cultivars which are highly susceptible, moderately



Fig. 2.3. A single female *Meloidogyne* and egg mass on a small root with minimum gall formation.

susceptible, slightly susceptible, and immune.

Susceptible hosts of *M. arenaria, M. hapla, M. incognita* and *M. javanica* are numerous and belong to many plant families. Other widely distributed species have hosts which belong to only a few plant families. Most of the hosts of *M. naasi* and *M. graminis* are Gramineae. *M. exigua* is a serious pest of *Coffea arabica* in South and Central America; few other hosts have been reported.

The most interesting resistant plants are those closely related to highly susceptible plants. These can be related species of the same botanical family or genus and are sometimes cultivars of the same species. Such closely related plants provide a source of resistance genes for breeding resistant cultivars.

For the great majority of the species named in Table 1.1, little is known of hosts and resistant plants.

# Histology and Pathogenesis

## I. Formation of Giant Cells and Galls

When second-stage *Meloidogyne* larvae enter roots, they move through the growing point of the root and region of cell elongation. Possibly the larvae feed on some cells; certainly there are rapid changes in root growth. In a tomato root entered by several larvae at the same time, Christie (1936) found that root cap cells were lacking in 24 hours. A group of cells near the root tip had moderately dense protoplasm but were not dividing.* Cells immediately behind these were enlarged, and the partially differentiated central cylinder ended abruptly.

In 48 to 60 hours after the larvae had stopped moving, retardation of normal differentiation was noted in cells near their heads. Many of these cells are those which would normally become conducting elements, leaving a break in continuity of phloem elements. The abnormal cells are the beginning of the giant cells, which apparently are formed by cell wall dissolution resulting in coalescence of contents of adjacent cells, and a series of synchronized endomitotic divisions (Rohde and McClure, 1975).** The nuclei of giant cells are large and have large nucleoli. About 6 days after inoculation, giant cells are filled with a dense cytoplasm different in appearance from the adjacent cells. Cross sections of a root show the nematode head closely surrounded by five or six giant cells (Fig. 3.1). The cells next to the giant cell have divided and form a distinct ring of small cells. Giant cells are always elongated and lie more or less parallel to the axis of the root.

### A. Nuclei in Giant Cells

In addition to enlargement, nuclei of giant cells have abnormalities of chromosomes. The broad bean (*Vicia faba*) has a diploid chromosome number of 2n = 12. In galls caused by *M. javanica*, chromosome numbers of 4n, 8n, 16n, 32n and 64n were found, derived from repeated mitoses of the plant cells without normal formation of cells by division of the protoplasm and formation of cell walls. The numbers of chromosome sets per nucleus are highly variable even in the same giant cell, due to irregularities in the mitotic apparatus (Huang and Maggenti, 1969).***

### B. Cytoplasm of Giant Cells

Females of *Meloidogyne* species feed on the cytoplasm of giant cells. It has a granular texture which increases in density as the cell matures, and contains many mitochrondria and such usual cellular
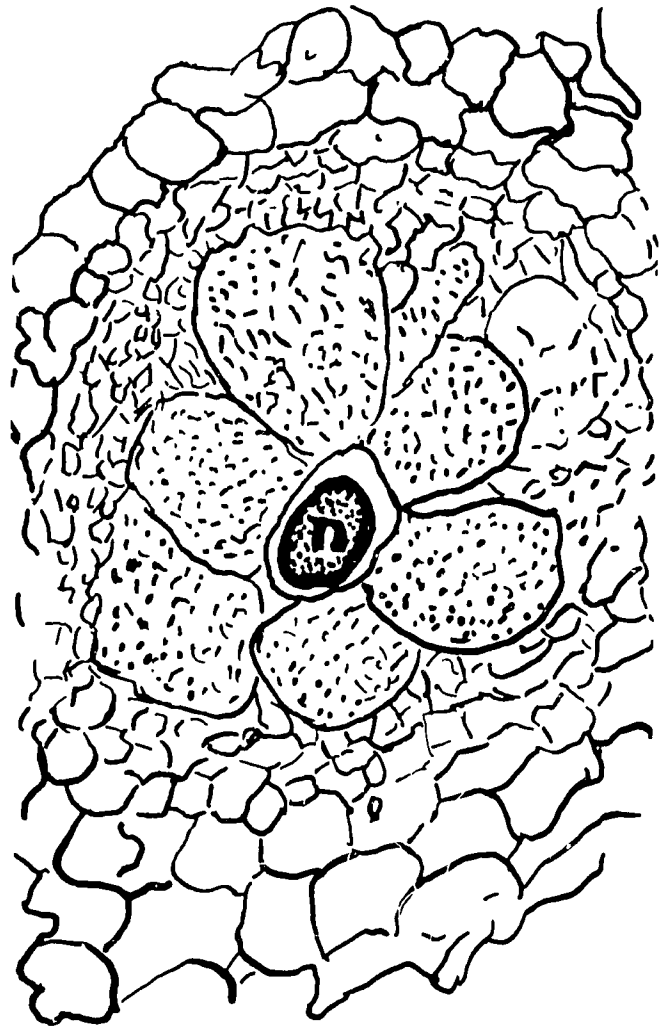


Fig. 3.1. Giant cells surround a root-knot nematode (n) near its head. Adjacent cells have been stimulated to divide, resulting in a ring of small cells. Jones and Northcote, 1972.

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*Possibly the quiescent center (Thomas, 1967).

**These authors review and discuss evidence for and against the view that cell wall dissolution has no part in formation of syncytia (giant cells).

***Evidence that multinucleosis results from repeated endomitoses within a single cell is reviewed.

components as Golgi bodies, proplastids, and a large amount of endoplasmic reticulum. Giant cell cytoplasm has 10 times more protein than normal cell cytoplasm and also traces of carbohydrates and fats. Maintenance of the giant cell appears to depend on continual stimulus from the nematode; when females were killed by pricking with a needle or by heating to 44°C, the giant cells collapsed and the space was occupied by normal plant cells (Bird, 1962).

There are recent reviews on giant cell formation by Endo (1971) and Bird (1974.)

## II. Differences in Giant Cell and Gall Formation

### A. Susceptible Plants

In susceptible plants, no differences in giant cell formation have been reported which can be attributed to different *Meloidogyne* species. There are considerable differences in gall formation of various plants infected by different species of *Meloidogyne*. On tomato and cucurbits infected by *M. incognita*, galls may be 1 centimeter or more in diameter and often contain many females completely embedded in the root tissue. On pepper (*Capsicum frutescens*) infected by this species, galls are about 2 mm in diameter, females are partly exposed, and egg masses are external to roots. In other plants, heavily infected roots may show no trace of gall formation. These are normal reactions of susceptible plants.

### B. Reaction of Resistant and Immune Plants

Highly resistant and immune plants are also invaded by second-stage larvae of *Meloidogyne* species.

Often in comparative experiments as many larvae invade immune plants as invade highly susceptible plants. These larvae have various fates.

### 1. Larvae and Roots Are Unchanged

Alfalfa (*Medicago sativa*) cultivars Africa, Moapa and Sonora, resistant to *M. incognita*, and cv Lahontan, susceptible, were compared in a study of penetration, development and migration. Larvae entered roots of all cultivars in about equal numbers. In roots of Lahontan, larvae developed normally with egg production 18 days after penetration. A few larvae did not become sedentary and left the roots after about 4 days. In the three resistant cultivars, numbers of larvae in the roots decreased in 4 days and was nearly zero in 10 days. There were no symptoms and neither giant cells nor necrotic cells developed in the resistant cultivars (Reynolds et al., 1970).

### 2. Various Reactions

There were no significant differences between average numbers of *M. incognita* larvae invading 2 susceptible cultivars, 11 resistant breeding lines and 1 resistant cultivar of tomato. Within 72 hours after penetration, 3 to 12% of larvae in the roots of susceptible cultivars and 0 to 67% in the resistant roots did not produce galls. Average number of larvae per root was 3.7 to 13.2 in susceptible roots, and 1.0 to 9.3 in resistant roots. There was no necrosis of cells near the larvae in susceptible roots, but light to severe necrosis in 10 of the 12 resistant roots (Fig. 3.2,B). Three apparently independent reactions occurred in the resistant roots: 1) necrosis, 2) lack of galling, and 3) reduced larval penetration (Dropkin and Webb, 1967).



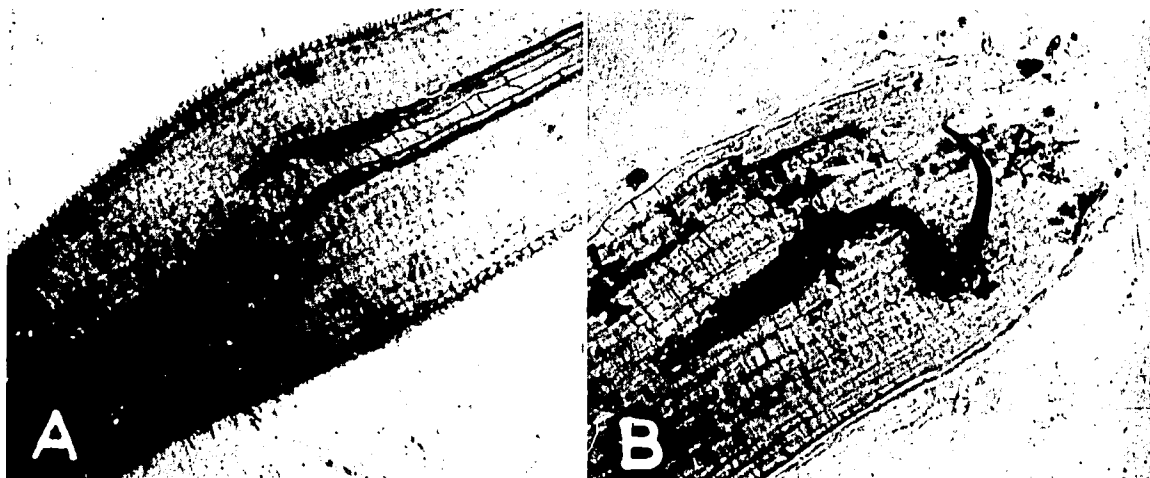Fig. 3.2. A: Absence of necrotic reaction in tomato 72 hours after inoculation. B: Necrotic reaction after 72 hours. Dropkin and Webb, 1967.

### 3. Histological Comparisons of Susceptible and Resistant Cultivars

A study of histological responses of 19 cultivars of soybean (*Glycine max*) to *M. incognita* produced four types of response:

1) Formation of five to nine large, thick-walled multinucleate giant cells with granular, dense cytoplasm and cell walls of two layers. These cells had as many as 150 nuclei; some were very large (21 x 16 micrometers) and others much smaller. This type of giant cell is optimal for reproduction of the nematodes.

2) Giant cells were large, but cells walls thinner and the cytoplasm less dense than in type 1. This giant cell is much less than optimal for reproduction.

3) Giant cells are formed but are small and have many inclusions, such as spirals, strands or lobed matter; all these are colored by fast-green stain and give positive results when tested for cellulose and pectin. Such cells are associated with very poor nematode reproduction.

4) There is little cell enlargement and no formation of giant cells, but marked necrosis of cells around the head of the larva. This reaction indicates immunity; larvae die without development (Dropkin and Nelson, 1960).

### 4. Galls on Immune and Highly Resistant Plants

Galls can be produced by *Meloidogyne* species on immune plants. *M. javanica*, *M. incognita*, and *M. hapla* produced galls on roots of citrange (*Citrus sinensis x Poncirus trifoliata*), and *M. incognita* produced galls on sour orange roots. The galls contained numerous swollen second-stage larvae but no adult females or egg masses after 8 weeks. Roots of citrange in a nursery were heavily galled by larvae of *M. javanica* from a nearby tamarisk hedge (*Tamarix gallica*) (Van Gundy et al, 1959).

In corn (*Zea mays*) cultivar Coker 911, development

of giant cells and *M. incognita* females was normal; in cultivar Pioneer 309B, giant cells were collapsed and often associated with apparently dead larvae; only a few females produced eggs (Baldwin and Barker, 1970a).

## III.  Summary

*Meloidogyne* larvae are attracted to and apparently have little difficulty in finding and entering actively growing roots. In the roots, their development and reproduction are determined by their ability to interact compatibly with the host.

It is clear that normal development of nematodes and abundant reproduction depends on the formation of giant cells by a large proportion of larvae which enter the root. If the host and the nematode are not compatible, a smaller proportion of giant cells are formed, and probably an even smaller proportion of larvae develop to the adult stage. Such plants are resistant to the particular species of *Meloidogyne* concerned. From the practical standpoint, growth of a highly resistant plant can make a very large difference in the *Meloidogyne* population of a field in a few nematode generations, perhaps reducing it below the damage threshold for a subsequent crop.

Larvae which invade roots of resistant plants may: 1) develop to maturity as females, but produce no eggs or defective eggs; 2) develop to maturity as males; 3) have development arrested before completing the second, third, or fourth molt; 4) be killed by an immune reaction (Fig. 3.2,B); or 5) leave the root, and still in the second stage be able to enter another root. None of these reactions is entirely positive or negative; with most combinations of nematode species and plant species, some individual nematodes will be able to reproduce, even if only one in 10,000. Partial development may leave visible galls on the roots, and the immune reaction may leave distorted or broken tissue in the root (Fig. 3.2,A).

# Effects of *Meloidogyne* Infection on Plant Growth

## I.  Physical Effects

### A.  Reduction and Deformation of Root Systems

In addition to galls and giant cell formation, *Meloidogyne* species have other important effects on plant roots. Heavily infected roots are much shorter than uninfected roots, have fewer branch roots and fewer root hairs. The root system does not utilize water and nutrients from as large a volume of soil as an uninfected root system (Fig. 4.1). Vascular elements are broken and deformed in root-knot galls and normal translocation of water and nutrients is mechanically hindered.

### B.  Decreased Root Efficiency

Deformity of roots and their inefficiency causes stunting of growth, wilting in dry weather, and other symptoms of shortage of water and nutrients, even when these are plentiful in the soil. Growth of plants is reduced.

In a glasshouse experiment, cotton plants heavily infected with *M. incognita* in soil with moisture automatically maintained at field capacity had dry weights only 10.4% less than uninfected plants. Dry weights of infected plants grown with irregular irrigation, that is, with soil moisture alternating between 50% of field capacity and 100%, were reduced by 78.6%, indicating that when soil moisture is alternately low and high, efficiency of a root system galled by *Meloidogyne* species is very much reduced. Reduction of root efficiency explains the wilting of infected plants often seen in fields during hot, dry weather. On the other hand, the results with automatic irrigation show that heavily infected plants can grow fairly well if irrigated frequently (O'Bannon and Reynolds, 1965).

In fields of plants heavily infected by *Meloidogyne* or other species of nematodes, growth is often uneven
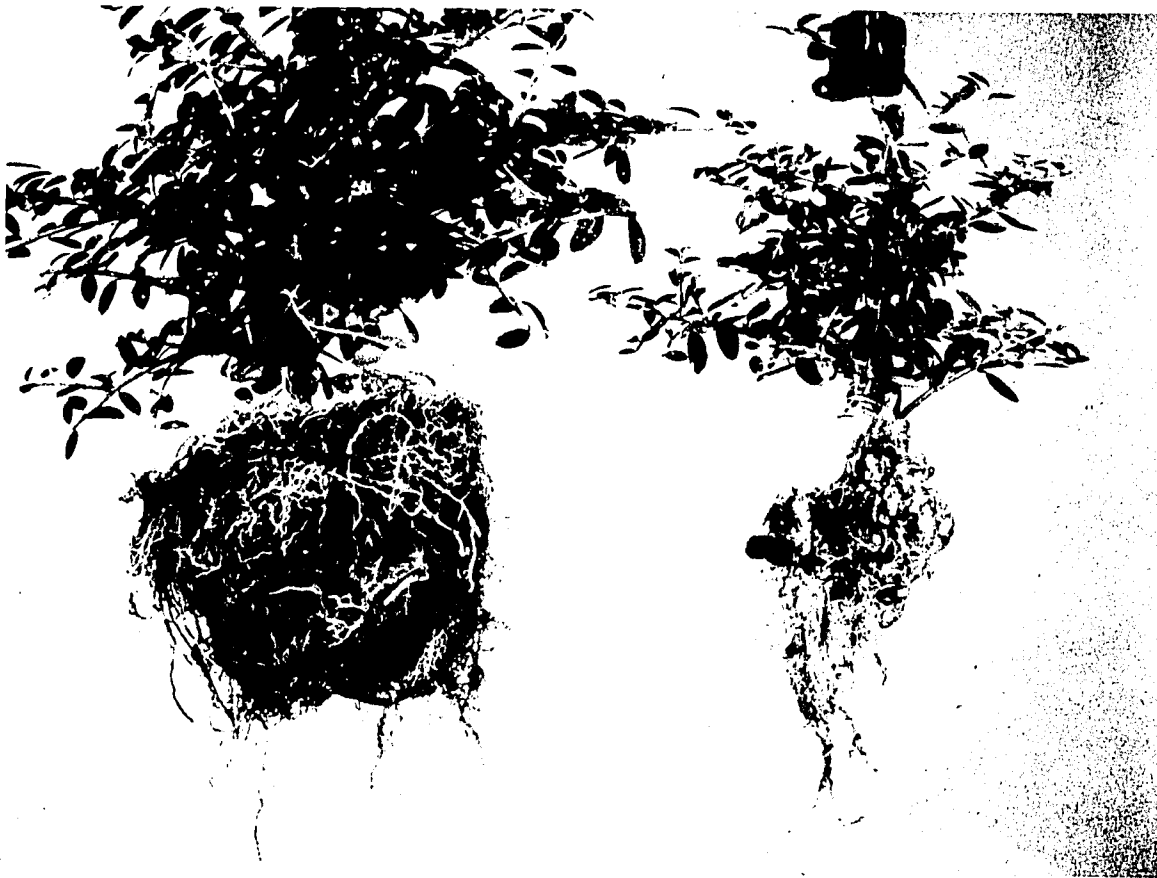


Fig. 4.1.  Differences in size of root system. Left, uninoculated; right, inoculated with *Meloidogyne*. Top growth is proportional.

Fig. 4.2. A peanut field in North Carolina (USA) shewing uneven growth on the right side due to infection by *Meloidogyne hapla*. This side of the field was planted the previous year to a soybean cultivar susceptible to *M. hapla*. The left side of the field was planted to cotton which is immune to *M. hapla*. Growth is better and more uniform.

(Fig. 4.2). This alone is not a definite symptom of *Meloidogyne* infection since it is difficult or perhaps impossible from a study of top growth and symptoms to distinguish between root knot and damage caused by other kinds of nematodes, soil insects, bacteria and fungi. If root systems are also examined, it is easy to demonstrate a correlation between galling by *Meloidogyne* species and reduction of top growth (Fig. 4.1).

## II. Physiological Effects

Loss of root efficiency and part of the consequent reduction of growth and yield can be accounted for by reduction and deformation of the root system. In addition, changes in physiology of plants when giant cells and galls are formed contribute to reduced growth.

Dropkin (1972) reviewed literature on the effects of infection by nematodes on host physiology: "Mature galls in comparison to ungalled tissue from the same plants had about one-third as much carbohydrates, pectins, cellulose and lignins, but more hemicellulose, organic acids, free amino acids, protein, nucleotides, nucleic acids, lipids and minerals. Increases were especially marked in protein, free amino acids, RNA and DNA. Some kinds of sugars were present in galls but not in healthy tissues; the proportions of free amino acids changed; the rates of intermediary metabolism were accelerated in galls, especially in pathways leading to synthesis of proteins and nucleic acids. A number of reports show that nitrogen, phosphorus and potassium accumulated in roots, but not in leaves of infected plants. Brueske and Bergeson (1972) found that gibberellin and cytokinin transport was reduced from roots of *Meloidogyne*-infected plants. They also found a qualitative difference in the types of gibberellins transported out of roots. The general picture seems to be that roots with galls shift their metabolism in the direction of increased protein synthesis and reduced transport of substances to the rest of the plant. In part, this probably reflects the reduced root surface. . . . we may hypothesize that infection with *Meloidogyne* brings about increased synthesis of proteins in galls, and the attendant disturbance of transport of growth regulators and other compounds between roots and stem results in profound disturbance of top growth."

Wallace (1974) found that incorporation of $^{14}CO_2$ into tomato plants inoculated with 250, 500, 1,000 or 2,000 *M. javanica* larvae was markedly less in infected plants than in uninfected plants, whatever the inoculum level. This seems to indicate a decrease of photosynthesis due to infection by the nematodes.

# III. Predisposition: *Meloidogyne* Species Prepare Plants for Infection by Fungi and Bacteria

In farm fields, infection of plants by *Meloidogyne* alone is improbable; bacteria, fungi and viruses are always present and often interact with the nematodes. Interaction between *Meloidogyne* and other plant-parasitic nematodes and other disease-causing agents was reviewed by Powell (1971) who referred to physiological changes in plant tissues caused by nematodes and other organisms as "predisposition."

## A. *Fusarium*

Both *Fusarium* wilt-susceptible and wilt-resistant tobacco cultivars showed significant increases in wilt development in the presence of *M. incognita*, *M. javanica* and *M. arenaria*, with no large differences between nematode species. Wilt was more severe when nematode inoculation was 2 to 4 weeks before fungus inoculation (Porter and Powell, 1967). Giant cells and nearby vessel elements of cultivar Dixie Bright 101 were heavily invaded by hyphae of *Fusarium*, and giant cell protoplasm disappeared soon after (Melendez and Powell, 1967).

## B. *Fusarium* and *Alternaria*

Tobacco plants inoculated with *M. incognita*, followed in 3 weeks by *Fusarium oxysporum* f. *nicotianae*, and in another 3 weeks by *Alternaria tennis* (brown spot of tobacco) had as much as 70% of leaf area destroyed. There was no reaction unless plants were inoculated with *Meloidogyne*, which predisposed plants to *A. tennis* (Powell and Batten, 1969).

## C. *Phytophthora*

Interactions of *M. incognita* and black shank caused by *Phytophthora parasitica* f. *nicotianae* in black shank resistant tobacco were reported to be very similar to those with *Fusarium*; the disease developed only with combined infections of nematodes and fungus, not with either alone, or with mechanical injury and fungus (Sasser et al., 1955). Black shank was more severe in root-knot susceptible than in root-knot resistant breeding lines when inoculated with both nematodes and fungus, but not when inoculated with the fungus alone. The fungus invaded hypertrophied and hyperplastic galled tissue more readily than adjacent normal tissue, and giant cells invaded by fungi lost their protoplasm within 72 hours (Powell and Nusbaum, 1960). A combined infection of *M. hapla* and *Phytophthora megasperma* var. *sojae* in soybean produced results about equal to the sum of the effects of either alone (Wyllie and Taylor, 1960).

## D. *Verticillium*

In untreated plots of a chemical control experiment in Victoria, Australia, strawberry plants had 48.6% *Verticillium* wilt at the end of harvest in February compared to 15.0% in plots treated with the nematicide ethylene dibromide (EDB). The plots were also infested with *M. hapla*; root-knot index of control plots was 2.5, for EDB plots it was 0.1. Since EDB has no effect on *Verticillium* in soil, this is the first report of interaction between *M. hapla* and *Verticillium* in strawberry (Meagher and Jenkins, 1970). Pre-

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planting treatment of soil in tomato experiments in Florida (USA) reduced *Verticillium* wilt on susceptible cultivars and also reduced root galling by *M. incognita*. It was concluded that *Verticillium* resistance in tomato was essential to crop survival in soil not treated with nematicide, but not in soil treated with nematicide (Overman et al. 1970.)

Tomato cultivars Gilat 38 (*Verticillium* resistant) and Rehovot 13 (susceptible) were inoculated with *M. javanica* and *Verticillium dahliae*. Resistance of Gilat 38 was not broken, but leaf symptoms and vascular discoloration of Rehovot 13 were increased by the combination of nematodes and fungus over the fungus alone (Orion and Krikun, 1976).

## E. *Rhizoctonia*

Experiments with *Rhizoctonia solani* and *M. incognita* on cotton (Carter, 1975 a and b), tobacco (Batten and Powell, 1971), okra *(Hibiscus esculentus)* and tomato (Golden and Van Gundy, 1975), all indicated that severity of fungus infection was increased by inoculation with fungus after the nematodes had invaded roots and galls and giant cells had been formed.

## F. *Helminthosporium*

No significant interaction was found in experiments with oat (*Avena sativa*) cultivars resistant and susceptible to *M. incognita* and *Helminthosporium victoriae* after simultaneous inoculation with both, or after inoculation with one before the other (Stavely and Crittenden, 1967).

## G. *Pythium, Curvularia, Botrytis, Aspergillus, Penicillium* and *Trichoderma*

*Pythium ultimum* causes seedling damping-off of tobacco but is of little or no importance after plants pass the seedling stage. *Curvularia trifolii, Botrytis cinerea, Aspergillus ochraceus, Penicillium martensii* and *Trichoderma harzianum* are not considered to be pathogenic to tobacco in the usual sense of the term, that is, they cause no significant damage to tobacco when inoculated alone. In experiments in root observation boxes, tobacco plants were grown for 50 days after fungal inoculation and 78 days after inoculation with *M. incognita*. There were plants inoculated with each of the fungi, plants inoculated with *M. incognita* alone, plants inoculated with one of the fungi 4 weeks after inoculation with *M. incognita*, and uninoculated controls. Disease ratings were made on root necrosis on a scale with zero for no necrosis, 5 for 76-100%

necrosis, and 1, 2, 3 and 4 representing the intermediate percentages. Averages were multiplied by 100 and had a range from zero to 100. Inoculations with *M. incognita* alone gave average disease indexes of 13 and 5 in two independent experiments. Disease indexes after inoculation with fungi alone were always zero. Inoculation with fungi of tobacco plants infected 4 weeks previously with *M. incognita* gave the following average disease indexes: *P. ultimum* 57, *T. harzianum* 71, *C. trifolii* 60, *B. cinerea* 75, *A. ochraceus* 50, and *P. martensii* 65 (Powell, Melendez and Batten, 1971).

The authors point out that the usually non-pathogenic fungi became pathogenic only after the root systems were infected by *M. incognita* and galls and giant cells were formed. They concluded that "root-knot nematode infections on certain hosts effectively predispose these roots to subsequent invasion by a range of other organisms present in the rhizosphere." They quote Gäumann (1950): "The primary pathogen not only breaks down the host's resistance to penetration but also its resistance to spread, thus making possible for the secondary parasites not only entry but also affording them, by a local change of substrate, a start for their further extension." The nematodes as primary pathogens predispose the host to a nonspecific secondary pathogen, and disease damage is greatly increased.

## H. Bacteria

Results parallel to those with fungi have been reported for combinations of *M. incognita* and *Pseudomonas solanacearum* by Johnson and Powell (1969) and Lucas et al., (1955); for *M. javanica* and *Agrobacterium tumefaciens* by Orion and Zutra (1971); for *M. hapla* and *A. tumefaciens* by Griffin et al., (1968); and for *M. hapla* and *Corynebacterium insidiosum* by Norton (1969) and Griffin and Hunt (1972).

## I. Microflora

Infection by *M. incognita* of tomato plants grown in sterile (gnotobiotic) culture reduced dry weight of plants by 12.3%. Inoculation with crude extract of soil from around tomato plants (microflora) reduced dry weight 13.9%. Inoculation with both nematodes and microflora reduced dry weight 71.4%. The microflora were apparently mostly bacteria (Mayol and Bergeson, 1970).

In a similar study with *M. hapla* on celery, only *Pythium polymorphon* was found to cause root decay

of *M. hapla*-infected celery roots. None of the other microflora components of nematode-infected roots were able to induce root decay (Starr and Mai, 1976).

## J. *Summary.*

Predisposition was summarized by Powell (1971). Plants growing in farm fields or in other places are constantly exposed to infection by a large variety of organisms, and multiple infections of root systems are common rather than exceptional. Very often plant-parasitic nematodes are a component of double or multiple infections, with considerable evidence that they are the predisposing agent. This may be due to the fact that plant-parasitic nematodes are actively moving components of soil life; they penetrate plant roots and cause physiological changes in root tissue.

Physiological changes in the host due to nematode infection may be responsible for changes in plant susceptibility to pathogens. Root tissue altered by nematode activity is more extensively colonized by fungi than adjacent comparable tissue. Such tissue has been physiologically changed, and the change influences fungal growth and development. In some cases the fungus invades tissue changed by nematode activity and then extends beyond to tissue not visibly affected by nematodes.

In other cases, modification of the plant tissue permits invasion by fungi and bacteria which do not colonize nematode-free roots, with predisposition reaching a maximum only after the nematodes have been in the host plant for several weeks.

He concludes that interactions with nematodes may be a major factor in diseases due to bacteria and fungi, and that the nematodes have only a part, but a very important part in root decay complexes. Three biological systems are involved in these interactions, nematodes, the plant, and the fungus or bacteria. It is logical to assume that metabolic activities of any one part of the complex influence those of the other components.

# 5
# Physiological Variation in *Meloidogyne* Species

## I. Definitions and Terminology of Biological Races

As pointed out by Sturhan (1971), the term "biological race" as it has been used in literature on plant nematology includes: 1) Sibling species, physiologic races that are morphologically nearly or completely indistinguishable. 2) Geographic races, subdivisions of species found in geographic regions and presumably the result of environmental peculiarities. 3) Distinct phenotypes within single populations, nematodes having a particular detectable difference, usually a host preference. 4) Host races, which are the true biologic or physiologic races, namely, biotypes distinguished by host preferences within a taxonomic group. "The term biological (or physiological) race is most commonly used to designate groups of individuals which have *several important host preferences* and other biological characters in common. However, high intrapopulation variability, overlapping of host ranges, interbreeding, etc., make evident the difficulty of defining, naming and fixing the limits of races—if one would not name every population differing in some respect a special race."

In this book, the words "race" or "biologic race" will be used only for populations of *Meloidogyne* species which have been shown by numerous experiments to have host preferences significantly different from those which have been established as normal for the species concerned. Following the precedent set for *Heterodera glycines* (Golden, et al., 1970), we have not named or designated races until it was evident that they have wide geographical distribution and are of sufficient significance to be considered in crop rotation or plant breeding programs.

## II. Variation between *Meloidogyne* Populations

Host-range studies conducted by Sasser (1954) revealed that there were one or more crop plants which were not attacked by some root-knot nematode species and that the non-hosts varied with the nematode species. Thus, a set of differential hosts*

was available for use in separating the species, based on host reaction. This method, based on the assumption that species and populations within a species will always react on a given host according to previous tests, has been used successfully in the United States for over 20 years in distinguishing between the four common species, namely, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. When populations of the various species were obtained from widely separated geographical regions, host responses were more variable and the existence of widely distributed races of *M. incognita* and *M. arenaria* was confirmed.

Sasser (1972a) reported reactions of 11 populations of *M. incognita*, 15 populations of *M. javanica*, and 10 populations each of *M. hapla* and *M. arenaria*.

None of the 11 *M. incognita* populations from South America, Asia, North America and Africa reproduced on peanut or strawberry. All reproduced on cultivar All Gold sweetpotato and watermelon when it was included in the test. Only one population collected in Peru reproduced on root-knot resistant tobacco (cv NC 95) (Fig. 5.1), and this same population did not reproduce on pepper. Two populations from Peru, two from the United States, and one from the Ivory Coast reproduced on cotton with a medium rating; four others from Taiwan (2), Greece and Nigeria did not reproduce on cotton, and two from the United States and Belgium had very low reproduction.

Response of the *M. javanica* populations from Europe, Africa, Asia, Australia and North America was uniform. None of the 15 populations reproduced on cotton, peanut, pepper, strawberry or sweetpotato (cv Porto Rico). All reproduced on tobacco and watermelon, and all but one (from Netherlands) reproduced on sweetpotato cv All Gold. Reproduction on corn was variable, mostly very low.

Reaction of *M. hapla* populations was also mostly uniform, with no reproduction on corn, cotton or watermelon. Reaction on sweetpotato (cv Porto Rico was not included in all tests) was variable. All ten populations reproduced on peanut. Nine populations reproduced on tobacco, one had no reproduction on pepper, and four had no reproduction on strawberry.

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*Differential hosts and varieties now used include: Tobacco, NC 95; Cotton, Deltapine 16; Pepper, California Wonder; Watermelon, Charleston Grey; Peanut, Florrunner; Corn, Minn. A401; Strawberry, Allbritton; Sweet-

potato, All Gold and Porto Rico; Tomato, Rutgers. More recently, corn, strawberry and sweetpotato have been dropped from the list.

All ten of the *M. arenaria* populations reproduced on tobacco and watermelon. None reproduced on cotton, strawberry or sweetpotato (cv Porto Rico). Three reproduced on peanut and seven did not.

## III. Additional Characterization of Populations from Widely Separated Geographical Regions

Data on 70 additional populations of *Meloidogyne* species collected by cooperators of the International *Meloidogyne* Project became available after the preliminary presentation of data by Sasser and Triantaphyllou in August, 1977. This makes a total of 250 populations which have been characterized on the basis of host response, morphology, and cytogenetics. This new data adds substantial additional information, but does not significantly change the percentages of populations of the principal species reported at that time.

Of the 250 populations studied thus far, 150 (or 60%) were identified as *M. incognita*, 60 (24%) as *M. javanica*, 22 (8.8%) as *M. hapla*, and 14 (5.6%) as *M. arenaria*. Other species studied (usually not more than one or two populations) include *M. microtyla*, *M. naasi* and *M. exigua*. These collections obviously represent only a small part of the agricultural land of the world and, for the most part, were collected from cultivated fields. Collections from other habitats will undoubtedly result in the finding of more of the other described species and perhaps some new ones.

Evidence now available strongly indicates that the so-called common species, namely, *M. incognita*, *M. javanica*, *M. hapla*, and *M. arenaria*, account for most of the damage to farm crops caused by root-knot nematodes.
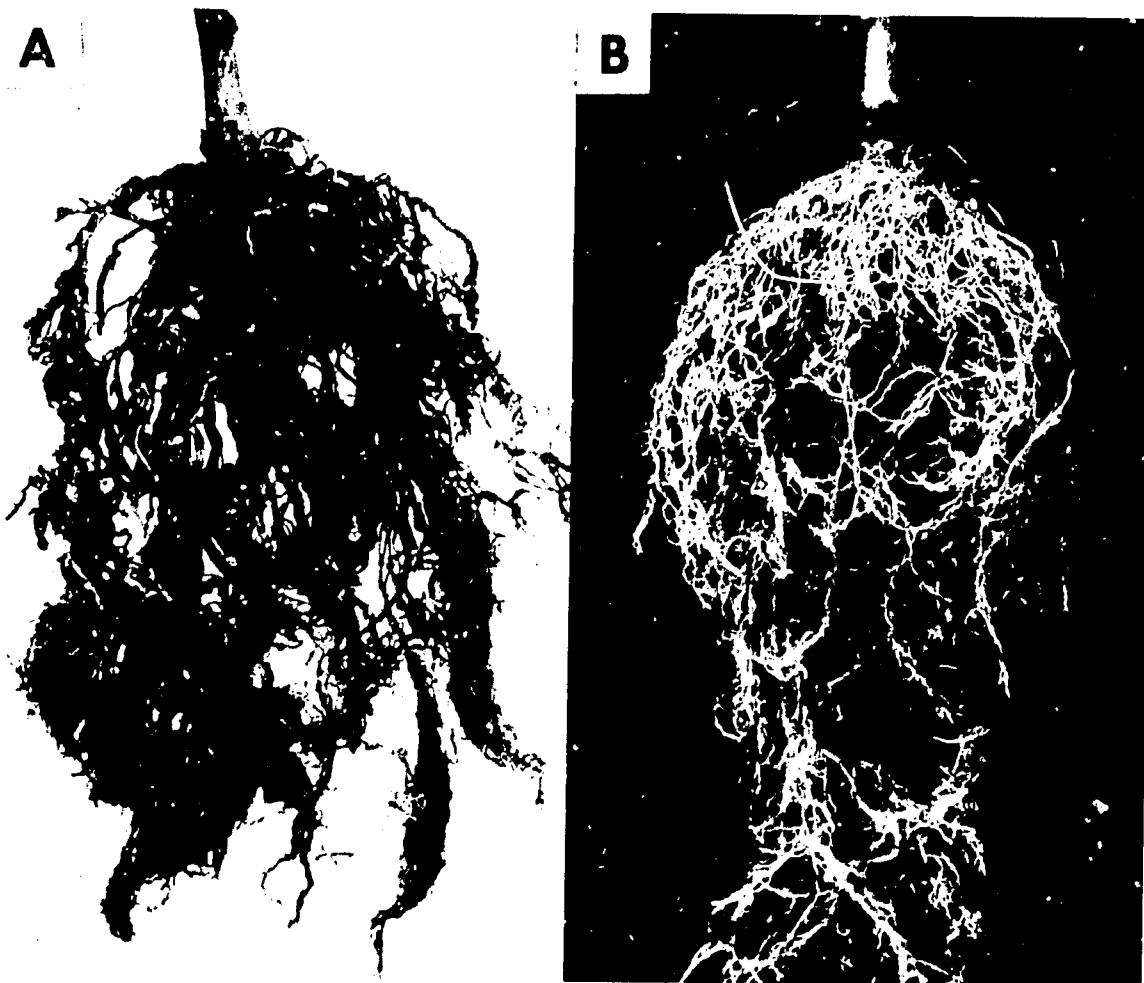


Fig. 5.1. Pathogenic variation in *Meloidogyne* populations. Roots of tobacco cultivar NC 95 (resistant to *M. incognita* Races 1 and 3). A: Inoculated with a population of *M. incognita* Race 4 from Peru. B: Inoculated with a population of *M. incognita* Race 1 from North Carolina (USA).

## A. *M. incognita*

Approximately 150 populations of *M. incognita* have been studied, and all of these reproduced on pepper and watermelon but failed to reproduce on peanut. One hundred populations did not reproduce on root-knot resistant tobacco (cv NC 95) or cotton (Race 1). Thirty-three populations reproduced on tobacco but not on cotton (Race 2). Thirteen reproduced on cotton but not on tobacco (Race 3). Eight reproduced on both cotton and tobacco (Race 4). Distribution of the four races was as follows: Race 1, 16 populations from Africa, 42 from Southeast Asia, 25 from Central and South America, 2 from Europe, and 14 from North America; Race 2, 4 populations from Africa, 12 from Southeast Asia, 12 from Central and South America, 3 from Europe, and 2 from North America; Race 3, 2 from Southeast Asia, 4 from Central and South America, 1 from Europe, and 6 from North America; Race 4, 1 from Africa, 3 from South America, and 4 from North America.

## B. *M. javanica*

Sixty populations of *M. javanica* reproduced normally on tobacco and watermelon but lightly or not at all on cotton, pepper and peanut. Seventeen populations were from Africa, 17 from Southeast Asia, 15 from Central and South America, 7 from North America, and 1 each from France, Israel, Cyprus and Japan.

## C. *M. hapla*

Twenty-two populations of *M. hapla* were studied. All reproduced on peanut but not on watermelon and cotton. Sixteen reproduced normally on tobacco, and 6 reproduced lightly or not at all. Nineteen reproduced normally on pepper, and 3 had light or no reproduction. Two *M. hapla* populations came from Canada, 4 from Chile, 2 from Kenya, 3 from Korea, and 1 from Colombia. Ten were collected in the United States, 5 in North Carolina and 1 in each of the states, California, Maryland, Virginia, Tennessee and Ohio.

## D. *M. arenaria*

Fourteen populations of *M. arenaria* were studied. All reproduced on tobacco and watermelon, and none reproduced on cotton. Four reproduced well on peanut, and 10 did not. Reaction on pepper was variable. With the exception of 1 population from Colombia, all populations that attacked peanut were from the United States (Virginia, Georgia, Florida and Texas). Those which did not reproduce on peanut were from Brazil, Philippines, Nigeria and 4 from the United States (North Carolina 3 Ohio 1).

## E. Discussion

Several aspects of this summary of behavior of species and populations within a species are of interest. The *M. incognita* group appears to be the most variable of the species tested. The variability is primarily with reference to reproduction on cotton and resistant tobacco.

Using results of host response on these two crops, the *M. incognita* group can be separated into 4 host races. Race 1 which does not reproduce on cotton or resistant tobacco is dominant throughout the world. Race 2 which reproduces on "root-knot resistant" NC 95 tobacco, and Race 3, attacking Deltapine 16 cotton, were less common in the populations tested. Populations which attack both cotton and tobacco, Race 4, were fewest in number. Peanuts were not attacked by any of the four races.

*M. arenaria* populations that attack peanut were usually from peanut growing areas, and those which failed to reproduce on peanut were from areas where peanuts are not grown.

There was no evidence of host races among the *M. hapla* and *M. javanica* populations studied. All populations of *M. hapla* reproduced on peanut regardless of origin or the production of peanut in the area and failed to reproduce on watermelon and cotton. *M. javanica* appears stable with reference to its reaction on the various differentials, always attacking resistant tobacco, watermelon and tomato, and failing to attack cotton, pepper and peanut.

The long series of experiments with differential host tests provide the most convincing available evidence that *Meloidogyne* species in many parts of the world have a small number of definite physiological variations, and not a large number of random variations as suggested by unduplicated experiments with single egg-mass populations.

## IV. Physiological variation within *Meloidogyne* populations.

The following three reports indicate that there is individual physiological variation within field populations of *Meloidogyne*.

## A. *M. incognita*

Seventeen single egg-mass isolates of *M. incognita* from nine counties in Tennessee (USA) were tested with tomato (*Lycopersicon esculentum* cv Rutgers), tobacco (*Nicotiana tabacum* cv NC 95), watermelon

(*Citrullus vulgaris* cv Dixie Queen) and pepper (*Capsicum frutescens* cv California Wonder). Root-knot indexes on a 1 to 10 scale ranged from 6.6 to 9.9 for tomato and were uniformly 1.0 for tobacco. On pepper they ranged from 4.1 to 7.7 for 16 isolates and 1.0 for the seventeenth. Range on watermelon was 4.2 to 7.9 except for one isolate rated 1.0. In a second test using cotton (*Gossypium hirsutum* cv McNair 1032) and cowpea (*Vigna sinensis* breeding line M57-13N), index range on cowpea was 1.0 to 2.5. Eleven isolates had ratings of 1.0 or 1.3 on cotton, others were 2.1, 2.6, 2.8, 3.4, 3.9 and 4.2. Three isolates originally from cotton had ratings of 2.8, 3.4 and 4.2 (Southard and Priest, 1973).

## B. *M. hapla*

Isolates of three egg masses from each of 11 collections of *M. hapla* from various locations in Idaho, Oregon and Washington (USA) were used to inoculate 12 test plants. Five variants were found (Ogbuji and Jensen, 1972).

## C. *M. naasi*

Five populations of *M. naasi* from England and California, Illinois, Kentucky and Kansas (USA) were obtained and an isolate from a single egg mass established for each. The isolates were used to inoculate 22 species of plants, mostly Gramineae. Reactions of all isolates to all species were similar with five exceptions, no two of which were identical (Michell et al., 1973).

## D. Persistence of Aberrant Populations

Giles and Hutton (1958) found that a root-knot resistant tomato (H.E.S. 4242) (*Lycopersicon esculentum*) gradually lost its resistance when grown in the same plot of infested soil for 5 years. When grown in a plot which had been repeatedly planted to a susceptible tomato cultivar (Pan American), H.E.S. 4242 had a high degree of resistance. They suggested that resistant hybrids should be used only in a crop rotation which does not change the infectivity pattern of the natural nematode population.

Riggs and Winstead (1959) inoculated root-knot resistant Hawaii 5229 tomato plants and transferred the populations three times at 3-month intervals. Root-knot index for *M. incognita* was near 1.0 initially, and 3.8 (scale 0 to 4) after the third transfer.

Triantaphyllou and Sasser (1960) found that most single egg mass or single larva isolates of 15 *M. incognita* populations reproduced slightly on root-knot resistant tobacco (*Nicotiana tabacum*) and tomato

cultivars. With 3 to 5 successive transfers on resistant plants, clones of isolates from resistant plants had root-knot index ratings of 4.0 (scale 0 to 5) on resistant tobacco and tomato plants compared with 2.0 for the original isolate. Clones with increased infectivity on resistant tobacco had no similar increase on resistant tomato.

Graham (1968) reported that the root-knot resistant tobacco cultivar NC 95 was not susceptible to an *M. incognita* population from the susceptible cultivar Hicks. It was highly susceptible to a population of this species from a plot where cv NC 95 had been grown every year for 6 to 8 years.

Nishizawa (1971) found the root-knot resistant sweetpotato (*Ipomoea batatas*) cv Norin No. 2 severely infected by *M. incognita* in pots where this cultivar had been planted continuously for 10 years.

## E. Discussion

All three of the experiments with populations from single egg masses (Sections IV, A,B,C) demonstrated a few differences and many similarities in the populations sampled. Reactions of *M. incognita* on tobacco and cotton were those of Races 1 and 3 of the species, as would be expected in a region where cotton is extensively grown.

The general reactions of *M. hapla* and *M. naasi* in the other two experiments were also like those reported for other populations of those species. The differences can be explained as differences of individuals within the populations, not as population differences since the samples were inadequate for this purpose.

The experiments on persistence of aberrant populations (Section D) indicate that percentages of individuals originally present in small numbers in a population can increase to become dominant after several generations of reproduction on the resistant host plant.

This may happen with monoculture of annual crops in farm fields, and in glasshouses used for monoculture. In perennial plantings such as orchards, vineyards and coffee plantations, there is a possibility that increasing adaptation of *Meloidogyne* populations to perennial plants may force growers to change locations.

## V. Summary

The word "race" should be used only for populations of *Meloidogyne* which have been shown by numerous experiments to have host preferences significantly different from those established as

"normal" for the species concerned, and also have wide geographical distribution. This follows the precedent set for *Heterodera glycines* (soybean cyst nematode) (Golden et al., 1970).

Standardized host tests with *Meloidogyne* populations from many parts of the world have revealed only four races of *M. incognita*. These have been found repeatedly to give the same response, without differences correlated with geographical relation or host plant from which the population was collected. Two races of *M. arenaria* have also been found. Tests of *M. javanica* and *M. hapla* populations have not revealed more than one race.

This is the best available evidence that a large and indefinite number of "resistance-breaking races" do not occur in cultivated fields. It implies that any crop cultivar resistant to one or more of the four races of *M. incognita*, two races of *M. arenaria*, one race of *M. javanica* or one race of *M. hapla* will be useful in all parts of the world. If such a cultivar is not grown in monoculture, but rotated with other crops, it can be grown repeatedly in the same field without significant loss of resistance.

If a resistant cultivar is grown in monoculture for several years, a resistance breaking strain of a *Meloidogyne* species can become dominant in that field.

Experiments with populations derived from single egg masses selected from populations indicate that it is easy to find individual variations in infectivity, but such experiments reveal little about the composition of the population from which it was selected.

# 6
# Ecology of *Meloidogyne* Species

## I. Survival of Eggs and Larvae in Soil

*Meloidogyne* populations of most economic importance are inhabitants of farm soils. If the field is used for susceptible annual crops, their distribution in the soil is about the same as that of the crop plant roots. The majority of the population is 5 to 30 cm beneath the soil surface, with decreasing numbers to a depth of one meter. In soil used for perennial plants, extreme depth may be 5 meters or more.

Where susceptible host plants are present, the most important factor in the lives of nematodes is soil temperature, which is largely determined by climate. Climate depends on latitude, elevation above sea level, geographic location, and seasonal variation.

The second most important factor is soil moisture which depends on rainfall or irrigation. In agricultural soil, sufficient soil moisture for nematode activity is present if there is sufficient moisture for crop growth.

Soil texture has an important influence on density of nematode populations.

## A. Soil Temperature

For *Meloidogyne* eggs and larvae, two temperature ranges are important because they determine: 1) survival time of eggs and larvae in cold soil (about 0° to 5°C), and 2) infectivity in warm soil (about 35° to 40°C). These are the approximate upper and lower temperatures for survival and reproduction.

At 0°C 41% of eggs of *M. hapla* survived for 90 days in soil and were infective when used as inoculum. Eggs of *M. incognita* and *M. javanica* were not infective as inoculum after 11 days. Larvae of *M. hapla* survived and were infective at 0°C for 16 days; *M. incognita* larvae were non-infective in 7 days.

At 4.6°C about 27% of *M. hapla* larvae were infective after 28 days, but no *M. incognita* larvae survived for 14 days.

In soil at 10°C, some *M. incognita* larvae were infective after 12 months. At 15.6°C and 26.7°C, all were non-infective in 4 months. At 26.7°C decline in infectivity was very rapid during the first 2 months (Bergeson, 1959).

Temperatures for normal activities of some *Meloidogyne* species, embryogenesis, hatch, mobility, invasion of plant roots, growth, and reproduction are approximately known. The lowest is 5°C as minimum for invasion of roots by *M. hapla*, 15° to 20°C is optimum, and 35°C is maximum. Minima for growth and reproduction of *M. hapla* are 15° to 20°C, optima 20° to 25°C, and maximum about 30°C. Corresponding temperatures for *M. javanica* are about 5°C higher (Bird, 1972; Bird and Wallace, 1965; Thomason and Lear, 1961).

Life cycle time of *M. javanica* was measured outdoors in South Africa 23 times with average temperatures ranging from 14.3° to 26.1°C. At 14.3°C, 56 days were required, or 9136 centigrade degree hours above 7.44°C, the calculated threshold temperature. At 26.1°C, life cycle time was 21 days or 9361 centigrade degree hours. For all 23 life cycles observed, the minimum was 8105 degree hours, the maximum 10,937 degree hours, and the average 9261 degree hours (Milne and Du Plessis, 1964).

Larvae of *M. javanica* are hatched with a food reserve equal to about one-third of their body weight. At 15°C, about half of this was lost between 4 and 16 days in storage; and all was lost after 16 days at 30°C when the larvae were no longer motile or infective (Van Gundy et al., 1967).

Information on soil temperatures at depths where most root-knot nematodes are found, 15 to 100 cm, is not available, but can be approximated by study of air temperatures. In this layer of soil, there is little daily variation; but average maxima and minima for the warmest and coldest months of the year approach those of air temperature shown on climatic maps. Below 1 to 3 meters, depending on location, soil temperature remains constant during the year (Kellog, 1941).

Climatic maps (Figs. 6.1 and 6.2) and the imperfectly known distribution of certain species indicate that:

a) The northern limit of *M. incognita* is about the 30°F (-1.1°C) isotherm of average January temperature (Fig. 6.1,A). The northern limit of *M. javanica* is probably near the 45°F (7.2°C) isotherm (Fig. 6.1,B).

b) *M. hapla* can survive in frozen soil and can reproduce from about 15°C to about 28°C. It is not established outdoors in the United States beyond the 80°F (26.7°) isotherm for average July temperature (Fig. 6.2,B). Its northern limit is near the 65°F (18°C) isotherm (Fig. 6.2,A).

AVERAGE JANUARY TEMPERATURE (°F.)
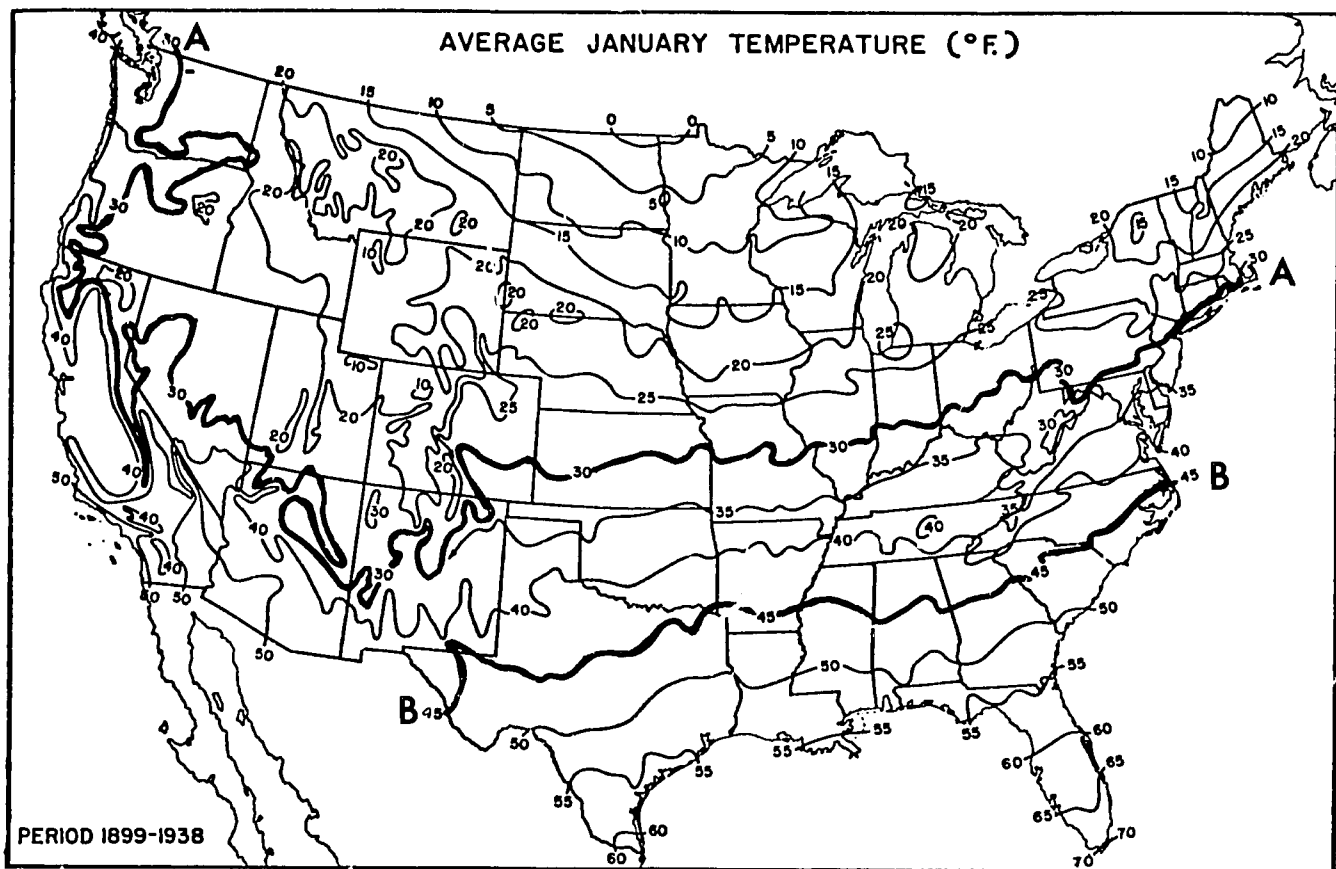
PERIOD 1899-1938

Fig. 6.1. Line A is the approximate northern limits of permanent establishment of *M. incognita* and *M. arenaria* in the United States. This line is the 30°F (-1.1°C) isotherm for average January temperature. The 45°F (7.2°C) isotherm, Line B, is the approximate northern limit of permanent establishment of *M. javanica* in the United States. Note complicated lines in western states due to mountainous terrain. Map from U.S.D.A. Yearbook of Agriculture, 1941, p. 704.

## B. Soil Moisture

*Meloidogyne* species are dependent on soil water for continued life and all activities. Larvae and eggs die in dry soil but can survive so long as there is enough moisture to maintain the soil air at nearly 100% humidity (Peacock, 1957). Larvae hatch readily and move freely through the pores of the soil (spaces between soil particles) when there is enough water to form thin films on the soil particles (Wallace, 1964, p. 114). At lower water content, hatching is inhibited because some water has been removed from the eggs, and movement of larvae is more difficult. In very wet soils, hatching may be inhibited and larval movement slowed by lack of oxygen.

## C. Soil Texture

Nematode larvae must move through soil pore spaces. Size of pore spaces depends on the size of soil particles. Movement is impossible if the pore spaces are too small for the nematodes to squeeze through, and mobility is apparently at a maximum when the ratio of particle diameter to nematode length is about 1:3 (Wallace, 1964, pp. 110-112).

Numerous nematologists have reported that root knot is more severe in sandy soils than clay soils. In Arizona (USA), three soil types were compared: 1) loamy sands with about 7% clay, 6% silt, 14% coarse silt and 73% sand; 2) sandy loam with 8% clay, 8% silt, 31% coarse silt and 53% sand; and 3) silt loam with 20% clay, 20% silt, 26% coarse silt and 34% sand. In the field, soils with 50% or more sand had severe damage to cotton by root knot, and 70% yield increases after application of nematicides. In the silt loam there was little root knot and small or no increase in yields after use of nematicides (O'Bannon and Reynolds, 1961).

Other reports of occurrence and damage to crops by *Meloidogyne* species in various kinds of soil are apparently conflicting, possibly because soil texture is
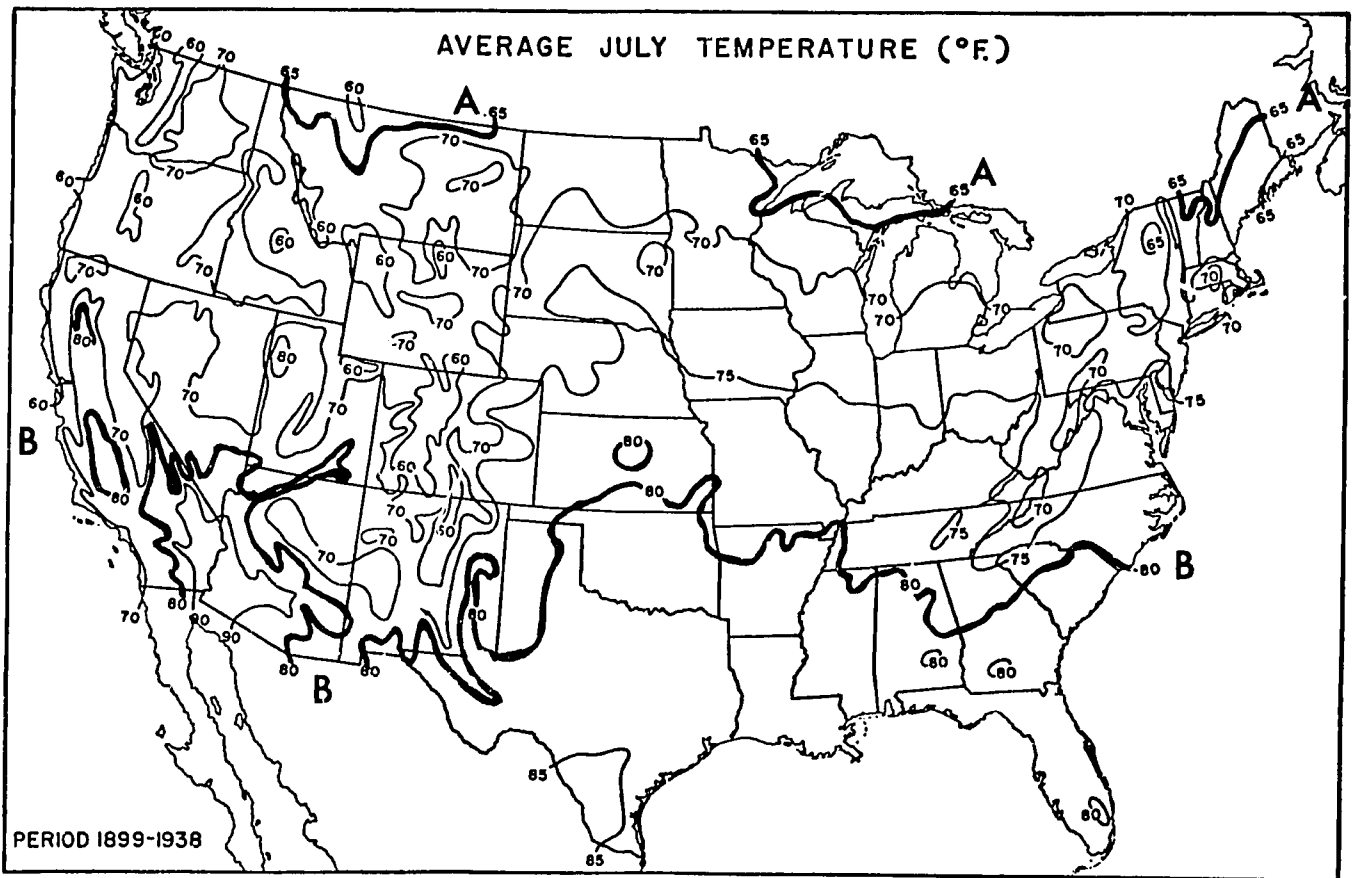
34

Fig. 6.2. Approximate northern and southern limits of permanent establishment of *M. hapla* in the United States. A: The 65°F (18°C) isotherm for July. B: The 80°F (26.7°C) isotherm for July. *M hapla, M. incognita* and *M. arenaria* all occur between line A of Fig. 6.1 and line B of Fig. 6.2. Map from U.S.D.A. Yearbook of Agriculture, 1941, p.705.

given only in general terms. But there is general agreement that damage in soils with a large percentage of clay is minimal, that it is maximal in sandy soils, and that *Meloidogyne* species inhabit a large variety of soil types.

## D. Other Soil Factors

### 1. Osmotic Effects

Laboratory experiments have shown that hatching of *Meloidogyne* eggs can be inhibited by osmotic effects of chemicals dissolved in water. As field soils lose moisture, concentration of dissolved salts increases; but osmotic pressure seldom exceeds 2 atmospheres. Wallace (1966) found little difference in hatch of *M. javanica* eggs in deionized water and solutions up to 2.5 atmospheres, but decreasing hatch up to 12 atmospheres. The eggs hatch when the soil becomes wet again, and the effect is probably not important in field soil except in climates where there is a long, dry season each year.

### 2. Soil pH

Soil pH has little direct effect on *Meloidogyne* activities over the range of 4.0-8.0. If the pH is in the range favorable for plant growth, the nematodes are active (Wallace, 1971).

### 3. Root Exudates

Hatching of *Heterodera rostochiensis* larvae can be increased several hundred percent by placing the cysts in leachings of roots of host plants. Hatching tests of *Meloidogyne* eggs in containers with tomato seedlings produced increases averaging only about 24% in 10 days (Viglierchio and Lownsbery, 1960).

### 4. Soil Oxygen

Tomato plants were grown with soil oxygen at normal concentration (21%) and reduced to 5.5%, 3.5%, 2.0%, 0.6% and 0% for 4 or 5 weeks in two experiments. Plants with no oxygen were barely alive; with

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21% oxygen growth was vigorous; and with other concentrations, intermediate. The number of galls produced by secondary infection was reduced almost in proportion to the oxygen supply at 5.5% and 3.5%, more sharply at lower concentrations. Number of larvae hatched per egg mass was reduced to 55% at 5.5% oxygen, and to 27% at 3.5%, and only a few eggs were produced at 2.0%. This was the first direct evidence that soil aeration affects nematode development (Van Gundy and Stolzy, 1961).

Wong and Mai (1973) found that numbers of *M. hapla* invading lettuce were 72% less when oxygen was reduced to 10%, and 44% less when oxygen was increased to 40%, as compared with 21%, the normal level.

# 7
# Breeding Plant Cultivars for Resistance
# to *Meloidogyne* Species

## I. Introduction

Nematologists are not usually also plant breeders but often cooperate with plant breeders who wish to include resistance to *Meloidogyne* species in crop plant cultivars. Nematologists can be of assistance in several ways: 1) Make surveys to assess the magnitude of the nematode problem and the distribution of the species involved. 2) Establish and multiply populations to be used as inoculum. 3) Devise screening techniques and cooperate with plant breeders and technicians in their use. 4) Aid in discovery and testing of accessions for sources of resistance. 5) Aid by reviewing nematology literature for information that may be useful to the plant breeder.

Extensive use of root-knot resistant cultivars has shown that so far as nematode resistance is concerned, they have indefinite longevity. The possibility of rapid and widespread development of resistance-breaking biotypes of *Meloidogyne* species has remained an apparently remote possibility; such biotypes have not been found on a large scale in farming operations.

## A. Nature of Resistance

Resistance to *Meloidogyne* species may be defined as some characteristic or characteristics of plants which inhibit reproduction of one or more *Meloidogyne* species. To be of value in practical control of root knot, a resistant cultivar must prevent a large proportion of reproduction, usually 90% or more as compared to susceptible cultivars of the same species.

Tolerant plants have characteristics which reduce damage to growth or yield of a plant infected with a *Meloidogyne* species. Tolerance usually implies a considerable increase in yield or growth over comparable plant cultivars lacking tolerance or resistance. By strict definition, tolerant plants may be highly susceptible. As generally used, "tolerance" implies low to moderate susceptibility.

The nature of resistance of plants to *Meloidogyne* species is only partly known. Failure of larvae to enter roots because of lack of attraction has not been the explanation in most plants examined. Larvae enter roots of resistant and susceptible plants in about equal numbers. In roots of susceptible plants, formation of giant cells (syncytia) is stimulated by feeding of larvae; and the larvae develop normally to maturity, producing eggs from which viable larvae hatch. In resistant plants, this sequence may be interrupted or fail at any point. Larvae may be killed by an immune reaction soon after they start to feed. No giant cell may be formed, or the giant cell may be defective. If giant cell formation is not normal, the larvae may fail to develop to maturity as adult females or males, or perhaps produce few or no viable eggs.

Endo (1971) discusses the nature of resistance, bringing together information concerning the mechanisms involved.

## B. Inheritance of Resistance to *Meloidogyne* species

Resistance to *Meloidogyne* species may be due to a single major gene (vertical resistance or race specific resistance). Plants with this kind of resistance are immune or hypersensitive. Or resistance may be due to a number of minor genes, each of which has a small effect (horizontal resistance or generalized resistance). Such resistance is quantitative, varying from high to low (Sasser, 1972c).

Resistance to *M. javanica*, *M. incognita* and *M. arenaria* has been reported in many kinds of vegetables, but resistance to *M. hapla* has been reported in only a few cases (Singh, Bhatti and Singh, 1974). Table 7.1 lists root-knot resistant cultivars of vegetables as given by Fassuliotis (1976) and Table 7.2 gives those of tree and field crops.

## II. Yield Increases Due to Breeding for Resistance

In field tests of cultivars and breeding lines of soybean (*Glycine max*) for resistance to *M. incognita* and *M. javanica*, susceptible cultivars Hood (average root-knot rating 4.6 on a 0 to 5 scale) and Hampton 266A (rating 3.2), and resistant cultivar Bragg (rating 1.5) were used for comparison with the material to be

tested. An average of three years of yield tests with four replications per year in nematode-free soil indicated that the potential yields of the three at the test location were not significantly different: Hood 2258 kg/ha, Hampton 2546 kg/ha, and Bragg 2480 kg/ha.

In a yield experiment on soil infested with *M. incognita*, yield of Hood was 209 kg/ha compared with 1641 kg/ha for Bragg, an increase of 1432 kg/ha for the highly resistant cultivar. Yields in a similar experiment were 1049 kg/ha for Hampton and 1809 for Bragg, an increase of 760 kg/ha (Kinloch and Hinson, 1972).

Table 7.1. Root-knot resistant cultivars of vegetables. (Fassuliotis, 1976.)

............................................................

Plant name, *Meloidogyne* species, and cultivars grouped by reference number[1] or VL[2]

............................................................

*Capsicum frutescens* (pepper)
  *M. arenaria*: Oakview Wonder, Red Chile (5); Burlington, California Wonder Special, Ruby King, Santanka x S (6); Nemaheart
  *M. incognita*: Red Chile (5); Santanka x S (6); Nemaheart
  *M. javanica*: Oakview Wonder, Red Chile (5); California Wonder Special, Early California Wonder, Santanka x S (6); Nemaheart
*Glycine max* (edible soybean)
  *M. incognita*: Mokapu Summer, Kailua, Kaikoo, Kahala (7);
*Ipomoea batatas* (sweet potato)
  *M. arenaria*: Heartogold (12); Acadian, Allgold, Centennial, Goldrush, Porto Rico (16); Maryland Golden (19)
  *M. hapla*: Heartogold (12)
  *M. incognita*: Nemagold, Orlis, Heartogold (12); Kandee (14); Tinian (PI 153655) (15); Jewel (16); Apache, Hopi, Sunnyside, Whitestar (18); Buster Haynes Red, Jasper, Keyline White, Red Jewel, White Bunch, White Triumph (VL)
  *M. javanica*: Tinian (PI 153655) (15); Heartogold (16); Maryland Golden (19)
*Lycopersicon esculentum* (tomato) and other *Lycopersicon* sp.
  *M. arenaria*: Nematex (20); VFN-8 (32)
  *M. hapla*: PI 270435 *Lycopersicon peruvianum* (21)
  *M. incognita*: Nematex (20); Nemared (22); Anahu R (23); Atkinson (24); Pelican (25); Beefeater, Beefmaster, Sunburst, Vine Ripe (26); Roodeplaat Albesto (27); Gawaher (Giza-1) (28); Coldset, Small Fry (30); VFN-8 (32); Better Boy, Big Seven, Bonus (H), Peto 662 VFN, Red Glow (H), Terrific (H) (33); Anahu, Florida-Hawaii Cross, Gilestar, Hawaii-55, Kalohi, Merbein Canner, Merbein Early, Merbein Mid-Season, Merbein Monarch, Monte Carlo (34); Bigset (H), BWN-21-F1, Calmart, Healani, Kewalo, Kolea, N-52 (H), Puunui, Ronita, Rossol, Tuckcross K, VFN Bush, VFN 368 (VL)

*M. javanica*: Nematex (20); Gawaher (Giza-1) (28); Atkinson, Healani, Kalohi (31); VFN-8 (32); Anahu (34)
*Phaseolus limensis* (lima bean)
  *M. incognita*: Hopi 5989, Westan (3); Nemagreen (4); White Ventura N
*Phaseolus vulgaris* (common bean)
  *M. incognita*: Alabama Nos. 1, 18, 19, Coffee Wonder, Ishell's Nematode Resistant, Springwater Half Runner, Wingard Wonder (1); Manoa Wonder (2)
*Vigna sinensis* (southern pea, cowpea)
  *M. arenaria*: Brown Seeded Local, Mississippi Crowder, Purple Hull Pink Eye (8); Iron (10)
  *M. hapla*: Iron (10)
  *M. incognita*: Brown Seeded Local, Mississippi Crowder, Purple Hull Pink Eye (3); Iron (10); Blackeye 5 and 7, Browneye 7 and 9, Chinese Red, Chino 2, Early Red, Early Sugar Crowder, Groit, Iron 3-5, 9-1, 9-10, New Era, Red Ripper, Rice, Suwanee, Victor (11); Brabham Victor, California Blackeye No. 5, Clay, Colossus, Floricream, Magnolia Blackeye, Mississippi Purple, Mississippi Silver, Zipper Cream (VL)
  *M. javanica*: Brown Seeded Local, Mississippi Crowder, Purple Hull Pink Eye (8); Iron (10)

1. Commercially developed cultivars do not have reference numbers.
2. VL indicates that cultivar was tested at the Vegetable Research Laboratory, Charleston, South Carolina, USA.
   References: 1. Blazey et al., 1964. 2. Hartmann, 1968. 3. Allard, 1954. 4. Wester et al., 1958. 5. Hare, 1956. 6. Hare, 1957. 7. Gilbert et al., 1970. 8. Choudhury et al., 1969. 9. Hare, 1959. 10. James, 1969. 11. Thomason and McKinney, 1960. 12. Cordner et al., 1954 13. Elmer, 1950. 14. Elmer, 1958. 15. Gentile et al. 1962. 16. Giamalva et al. 1960. 17. Giamalva et al. 1963. 18. Martin et al. 1970. 19. Sasser, 1954. 20. Dropkin, 1969. 21. Dropkin et al., 1967. 22. Fassuliotis and Corlet, 1967. 23. Gilbert et al., 1969. 24. Greenleaf, 1967. 25. Hernandez et al., 1972. 26. Jenkins, (no date). 27. Joubert and Rappard, 1971. 28. Moh et al., 1972. 29. Rebois et al., 1973. 30. Sidhu and Webster, 1973. 31. Sikora et al., 1973. 32. Singh and Choudhury, 1973, 33. Southards, 1973. 34. Winstead and Riggs, 1963.

Table 7.2. Root-knot resistant cultivars of field and tree crops. (Unless otherwise specified, these cultivars have been reported to be highly resistant to the *Meloidogyne* species named.)

Plants, *Meloidogyne* species and cultivars

*Arachis hypogaea* (peanut, groundnut). All cultivars are resistant to all *Meloidogyne* species except *M. arenaria* and *M. hapla*. Minton and Hammons (1975) tested 512 entries without finding significant resistance to *M. arenaria*.

List available from N. A. Minton, Georgia Coastal Plain Expt. Station, Tifton, GA 31794, USA.

*Avena* sp. (oat). *M. naasi*: Wintok.

*Coffea* spp. (coffee). *M. exigua*: *C. arabica*—N39, Amfillo 1141-2, Dalle mixed 1150-2, Tafara Kela 1161-9 and Barbuk Sudan 1171-26. *C. canephora*—Robusta Collections 3 and 10, Laurenttii Coi. 10, Kawisari Cols. 6 and 8, and Bukobensis. *C. congensis*—Bangelan 5. *C. eugenoides*—one unnamed cultivar. Curie et al., 1970.

*Glycine max* (soybean). *M. incognita*: Hampton, Laredo, Delmar, Hutton, Peking, Hill, Dyer, Bragg, Jackson and Hardee. Moderately resistant: Blackhawk, Habard, Monroe, Illsoy, Bethel, Dare, York, Hood, Amredo, Laredo, Palmetto and Roanoke.

*M. javanica*: Hampton is moderately resistant.

*M. hapla*: Illsoy and Bragg are moderately resistant.

*M. arenaria*: Dyer and Bragg. Ibrahim, Ibrahim and Massoud, 1972; Hinson et al., 1973; Good, 1973.

*Gossypium hirsutum* and other *Gossypium* spp. (cotton). All cultivars and species are resistant to all *Meloidogyne* spp. except some races of *M. incognita*. Resistant to this race: Auburn 56, Clevewilt 6, Bayou, a wild selection of *Gossypium barbadense* and another wild selection from Mexico. Minton, 1962. (Note: Because of taxonomic transfers and varying resistance of cotton cultivars, there is considerable confusion about resistance of cotton cultivars. Local trials are advisable before making recommendations. Sasser, 1972b.)

*Lespedeza cuneata* (lespedeza). *M. hapla*: Selection 11,397 from Beltsville 23-864-8 and Alabama Inbred 503. Adamson et al., 1974.

*Medicago sativa* (alfalfa, lucerne). *M. incognita*: African, Hairy Peruvian, India, Sirsa and Sonora. Reynolds et al., .970. *M. javanica*: African, India and Moapa. Reynolds and O'Bannon, 1960.

*Nicotiana tabacum* and other *Nicotiana* spp. (tobacco). *M. incognita*: Coker 86, 254, 258 and 347; NC 79, 88, 95, 98 and 2512; Speight G-23, G-28, G-33 and G-41; Ga. 1469; and Virginia 080 and 770. Todd, 1976 b.

*M. javanica*: *N. repanda* and a cross, *N. repanda* x *N. sylvestris*. Calitz and Milne, 1962.

*Oryza sativa* (rice). *M. incognita* and *M. javanica*: International. Ibrahim, Ibrahim and Rezk, 1972.

*Prunus armeniaca* (apricot). *M. incognita* and *M. javanica*: Most cultivars are immune. Lownsbery et al., 1959.

*Prunus persica* (peach). *M. incognita*: Okinawa, Rancho Resistant, S-37, Shalil and Yunnan. Some selections of Bokhara and of Fort Valley 234-1 are resistant, others are susceptible.

*M. javanica*: Fort Valley 234-1 and Okinawa. Lownsbery et al., 1959. and Burdett et al., 1963.

*Prunus* spp. (plums). *M. incognita* and *M. javanica*: Marianna 2524 and 2623, Myrolaban 29, 29C, 29D and 29G. Lownsbery et al., 1959.

*Vicia* spp. (vetch). *M. incognita* and *M. javanica*: Alabama 1894, Warrior and 28 of 36 breeding lines of an interspecific cross, Alabama 1894 x P.I. 121275. Minton et al., 1966.

*Zea mays* (corn, maize). *M. arenaria, M. hapla, M. incognita* and *M. javanica*: 14 cultivars tested were immune to *M. hapla*. Reproduction of the other species was variable on all cultivars, and lowest on Pioneer 309B, Pioneer 511A and McNair 340. Baldwin and Barker, 1970b.

# Identification: Introduction and *Meloidogyne* Species of Cold Climates

## I. Introduction

Identification of *Meloidogyne* species is basic to research and to reporting of research results. If the identification is correct, the research information is automatically added to the existing knowledge of the species, and fits in its proper place. If the identification is erroneous, the research information may be added to information about another species where it does not belong, but causes confusion.

Identification of *Meloidogyne* species can be facilitated by dividing the 36 species into groups according to climate and host habits.

## A. Grouping of Species

Climate is determined partly by latitude; partly by altitude; and partly by proximity to large bodies of water, especially if warm or cold ocean currents flow nearby. These effects are apparent in climate maps with isotherms (Figs. 6.1 and 6.2).

As discussed in Chapter 6, I, the northern limit of *M. incognita* is at about the 30°F (-1.1°C) isotherm of average January temperatures, and the northern limit of *M. javanica* is near the 45°F (7.2°C) isotherm (Fig. 6.1). *M. hapla* occurs regularly north of these lines and also to the south as far as the 80°F (26.7°C) isotherm for average July temperature (Fig. 6.2). This suggests that *M. hapla* and 11 other species originally described from cool climates can be grouped as has been done in Table 8.1. *M. incognita*, *M. javanica*, *M. arenaria* and 21 other species described from warm climates are grouped in Table 9.1.

The species are further divided into groups by classes of preferred host plants. *Meloidogyne* species which do not have wide host ranges tend to have preferred hosts in one or more plant families or groups, such as grasses, woody plants, or species of one genus. These species have other host plants, but the probability that they will be found in the field on a host plant outside the group in which they are placed here is comparatively small.

In any case, the practical convenience of the grouping compensates for the possibility that it will lead to

Table 8.1. *Meloidogyne* species of cold climates grouped by host preferences with type hosts, type localities, and median larval lengths.

| Species and host preference | Type host | Type locality | Larval* length (mm) |
|---|---|---|---|
| Numerous hosts: | | | |
| M. hapla | Solanum tuberosum | NewYork, USA | 0.430 |
| Woody plants: | | | |
| M. ardenensis | Vinca minor | England | 0.412 |
| M. deconincki | Fraxinus excelsior | Belgium | 0.370 |
| M. litoralis | Ligustrum sp. | France | 0.390 |
| M. mali | Malus prunifolia | Japan | 0.420 |
| M. ovalis | Acer saccharum | Wisconsin, USA | 0.390 |
| Gramineae: | | | |
| M. microtyla | Festuca rubra | Ontario, Canada | 0.375 |
| M. naasi | Hordeum vulgare | England | 0.441 |
| M. ottersoni | Phalaris arundianacea | Wisconsin, USA | 0.465 |
| Cruciferae and Leguminosae: | | | |
| M. artiellia | Brassica oleracea capitata | England | 0.352 |
| Other hosts: | | | |
| M. kirjanovae | Lycopersicon esculentum | USSR | 0.396 |
| M. tadshikistanica | Pelargonium roseum | USSR | 0.392 |

*Median lengths. Most reported minima and maxima are about 12% more or less than the median.

temporary error. Identification should always be checked by a study of several distinct characters of the species. Reproductions of illustrations from the original or best available description are included in this book for that purpose. If the described characters are not found, it will be obvious that a mistake has been made or that the population is of a new species. If the population cannot be identified as any of the species illustrated in this book, material should be sent to Headquarters of the International *Meloidogyne* Project or to a professional taxonomist.

## II. Cold Climate Species

### A. *M. hapla*

The most abundant of the 12 cold climate species is *M. hapla*. It is widely distributed and has many host plants including economic crops and weeds. In the North Carolina Differential Host Test, *M. hapla* causes galls and reproduces on tobacco, pepper and peanut, but not on watermelon or cotton. Median length* of larvae is 0.430 mm (0.395-0.466 mm). Other characters are shown in Fig. 8.1. The perineal pattern is composed of smooth striae. Most patterns are nearly round (Figs. 8.1,J-M,and 8.2); some extend to one or both sides to form "wings" (Fig. 8.1,J,L,N). Often punctation is visible near the tail terminus (Fig. 8.2). Larvae may have blunt to bifid tails (Fig. 8.1,T, U). *M. hapla* galls often have one or more short lateral rootlets (Fig. 8.3).

### B. Species Infecting Woody Plants

The five species in this group, with type hosts, other hosts and type localities are :

*M. mali:* *Malus prunifolia* Borkh., other apple species, northern Japan.

*M. ovalis:* *Acer saccharum* Marshall, other maple species, Wisconsin, USA.

*M. litoralis:* *Ligustrum* sp., North Coast of France (Pas de Calais).

*M. ardenensis:* *Vinca minor* (periwinkle), *Ligustrum vulgare*, *Fraxinus excelsior* (ash), England.

*M. deconincki:* *Fraxinus excelsior* (ash), *Rosa* sp., Belgium.

Median larval lengths and ranges of these five species are similar and of no value for identification. Perineal patterns of *M. mali* (Fig. 8.4,I,J), *M. litoralis* (Fig. 8.5,D), and *M. ovalis* (Fig. 8.6,I-K) are round to oblong; *M. mali* has large phasmids, the other two do

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*The median is defined as the average of the highest and lowest measurements reported in the original description of the species.

not. *M. mali* males and larvae have lateral fields which are unusually wide (Fig. 8.4, D,E,O), about half the body width. *M. litoralis* females have the excretory pore about one-half stylet length from the anterior end (Fig. 8.5,A), *M. ovalis* (Fig. 8.6,H) about 1½ stylet lengths, and *M. mali* (Fig. 8.4,P) about 2 stylet lengths. These differences and study of the illustrations will separate the three species.

*M. ardenensis* and *M. deconincki* are much alike, and both have ash (*Fraxinus excelsior*) for a host plant. Females of both species have the excretory pore about one-half stylet length posterior to the lip region (Figs. 8.7,A,B and 8.8,A). Perineal patterns of both tend to have flattened arches. Apparently the two can best be separated by details of the perineal patterns (Figs. 8.7,D-F and 8.8,D-G).

### C. Species Parasitic on Gramineae

Three cold climate species of *Meloidogyne* are parasites of grasses. Their host plants and localities are:

*M. microtyla:* *Festuca rubra* cv Elco (fescue), Ontario (southern Canada).

*M. naasi:* *Hordeum vulgare* (barley), England.

*M. ottersoni:* *Phalaris arundinacea* (canary grass), Wisconsin (northern USA).

Other hosts of *M. microtyla* include oat (*Avena sativa*), barley (*Hordeum vulgare*), wheat (*Triticum vulgare*) and rye (*Secale cereale*). There was light galling and reproduction on bromegrass (*Bromus inermis*), orchardgrass (*Dactylis glomerata*) and timothy (*Phleum pratense*), but not on corn (*Zea mays*). There was heavy reproduction with light galling on white clover (*Trifolium repens*), and light reproduction with no galling on red clover (*T. pratense*). Sugarbeet (*Beta saccharifera*) was lightly galled with some reproduction (Mulvey et al, 1975).

Hosts of *M. naasi* include barley, wheat, ryegrass (*Lolium perenne* and *L. multiflorum*), couch grass (*Agropyron repens*), onion twitch (*Arrhenatherum elatius*), cocksfoot (*Dactylis glomerata*), fescue (*Festuca pratensis*), bluegrass (*Poa annua* and *P. trivialis*) and sugarbeet.

*M. microtyla* larvae have tails with a bluntly rounded end (Fig. 8.9,B); *M. naasi* larval tails taper to a narrowly rounded end which is sometimes forked (Fig. 8.10, G,J). Some females of *M. naasi* have the neck ventrally placed and a slight posterior protuberance (Fig. 8.11). The excretory pore is slightly anterior to the stylet knobs (Fig. 8.10,A); in *M. microtyla* it is 3 to 4 annules posterior to the base of the stylet. *M. naasi* perineal patterns have large phasmids and a fold of cuticle covering the anus; with
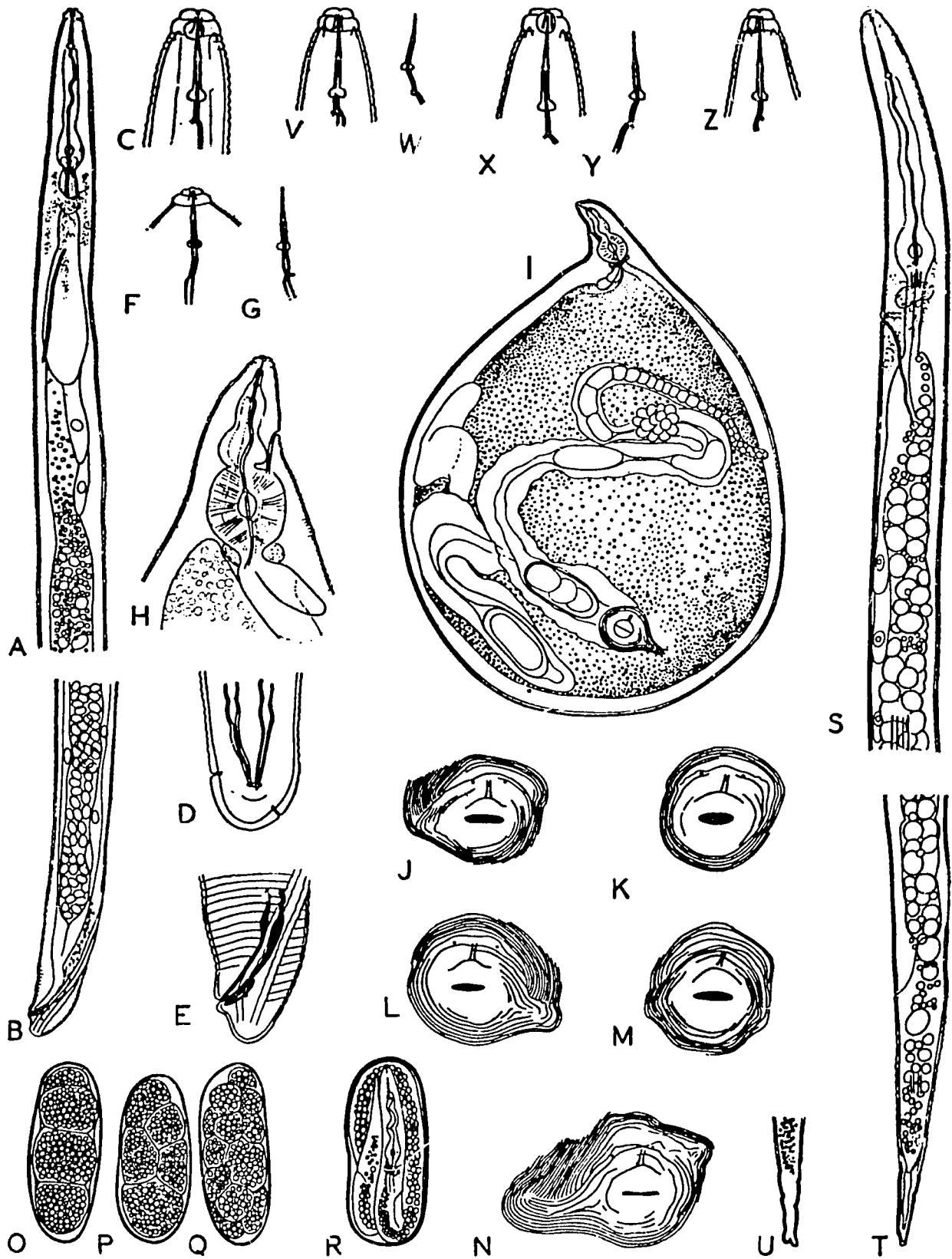
Fig. 8.1. *Meloidogyne hapla.* A-E, V, X, Z: Male. F,G: Female stylets. H-N, Female anterior, body (diagramatic) and five perineal patterns, of which J, L and N have wings. O-R: Eggs. S-U: Larvae. Chitwood, 1949.
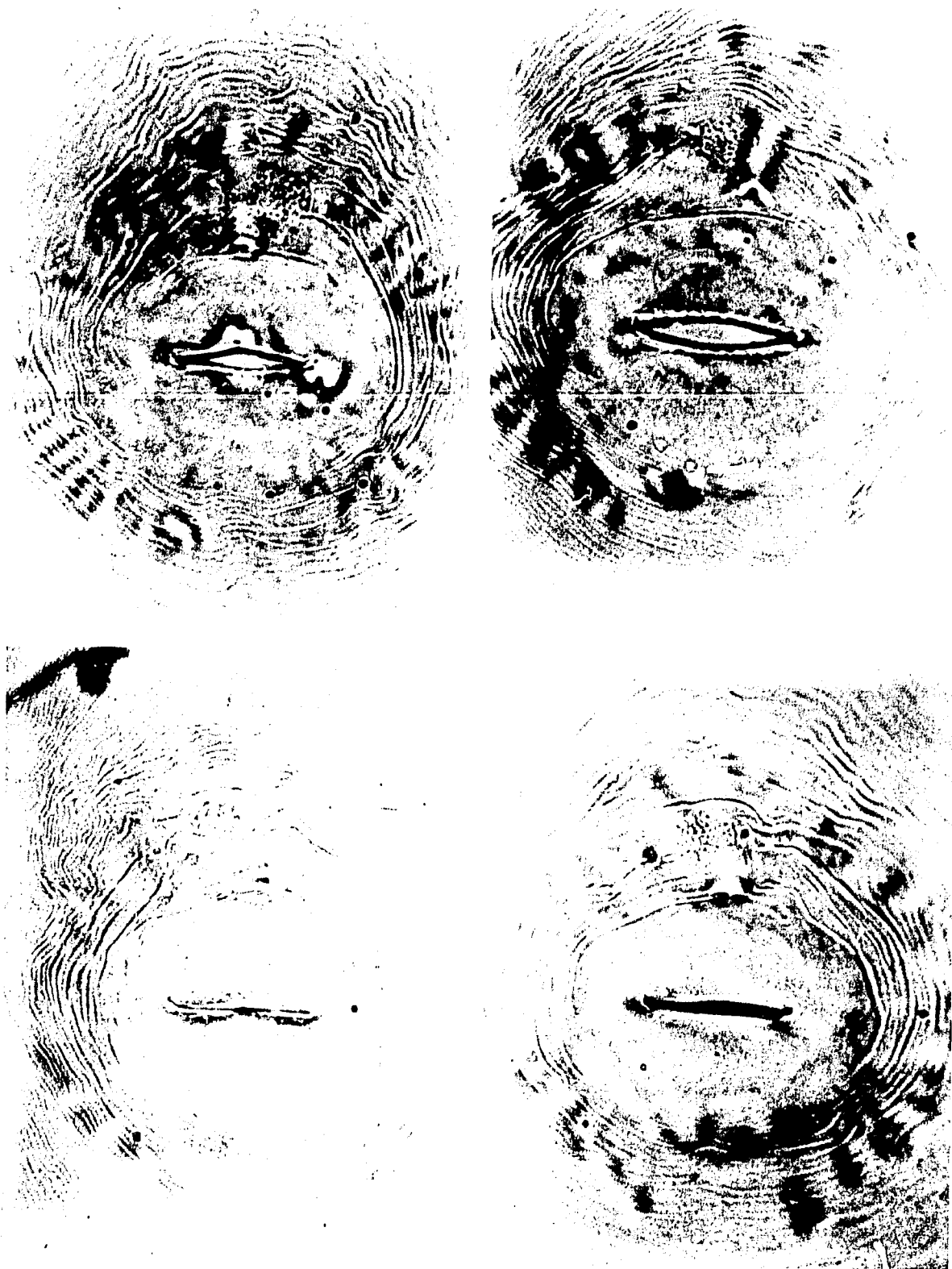
Fig. 8.2. *Meloidogyne hapla.* Photographs of perineal patterns. All four have stippling in a small area above the anus. This may or may not be visible, depending on fixation and mounting. Striae smooth to slightly wavy.
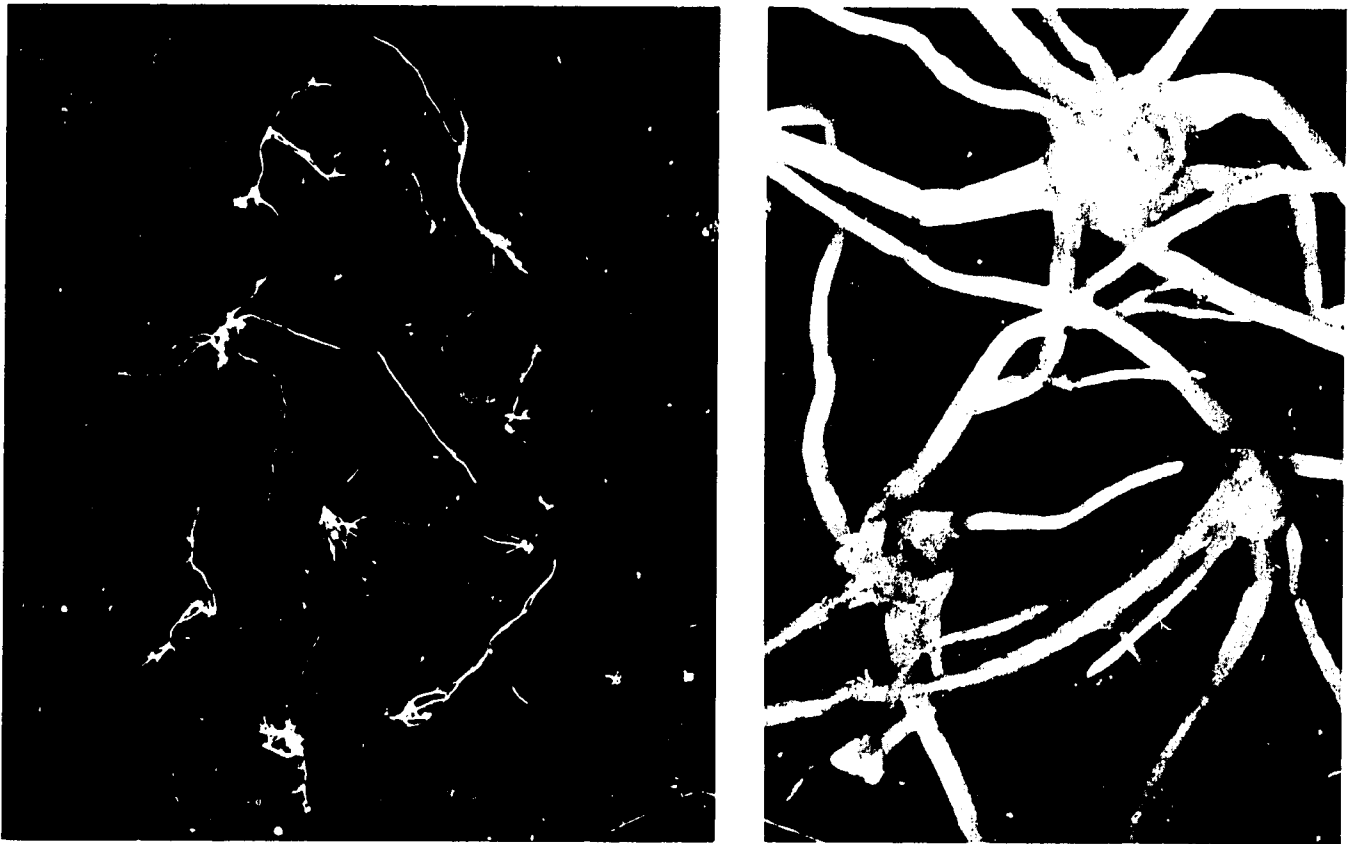
Fig. 8.3. Tomato roots (left) and peanut roots (right) with galls caused by *Meloidogyne hapla*. Lateral roots growing from the galls are characteristic of this species.

the phasmids for eyes and the vulva for mouth, the rounded patterns resemble monkey faces (Fig. 8.10,K-M). *M. microtyla* patterns are not round but have slight shoulders (Fig. 8.9,D,E).

*M. ottersoni* was originally described as *Hypsoperine ottersoni*. The female has a ventrally placed neck and distinct posterior protuberance (Fig. 8.12,L-Q). The larval tail is about 6 to 7 times as long as the anal body diameter (Fig. 8.12,B). Knobs of the female stylet are very small and the female excretory pore is just posterior to the stylet knobs (Fig. 8.12,I).

*M. artiellia* was described as a parasite of cabbage (*Brassica oleracea capitata*) in England. It also attacks kale (*B. oleracea v. acephala*), Brussels sprouts (*B. oleracea v. gemmifera*), swede (*B. napus v. napobrassica*), pea (*Pisum sativum*), bean (*Vicia faba*), clover (*Trifolium pratense*), and alfalfa (*Medicago sativa*). The species is easily identified by the distinctive perineal pattern and the short larval tail, length about 2 anal body diameters (Fig. 8.13,HI).

*M. tadshikistanica* is known to infect only two hosts, *Pelargonium roseum* (Geraniaceae) and spiderwort (*Tradescantia* sp.). According to Whitehead (1968) it differs from *M. incognita* in that the female excretory pore is opposite the median esophageal bulb (Fig. 8.14, A) compared to nearly opposite the stylet knobs in *M. incognita*.

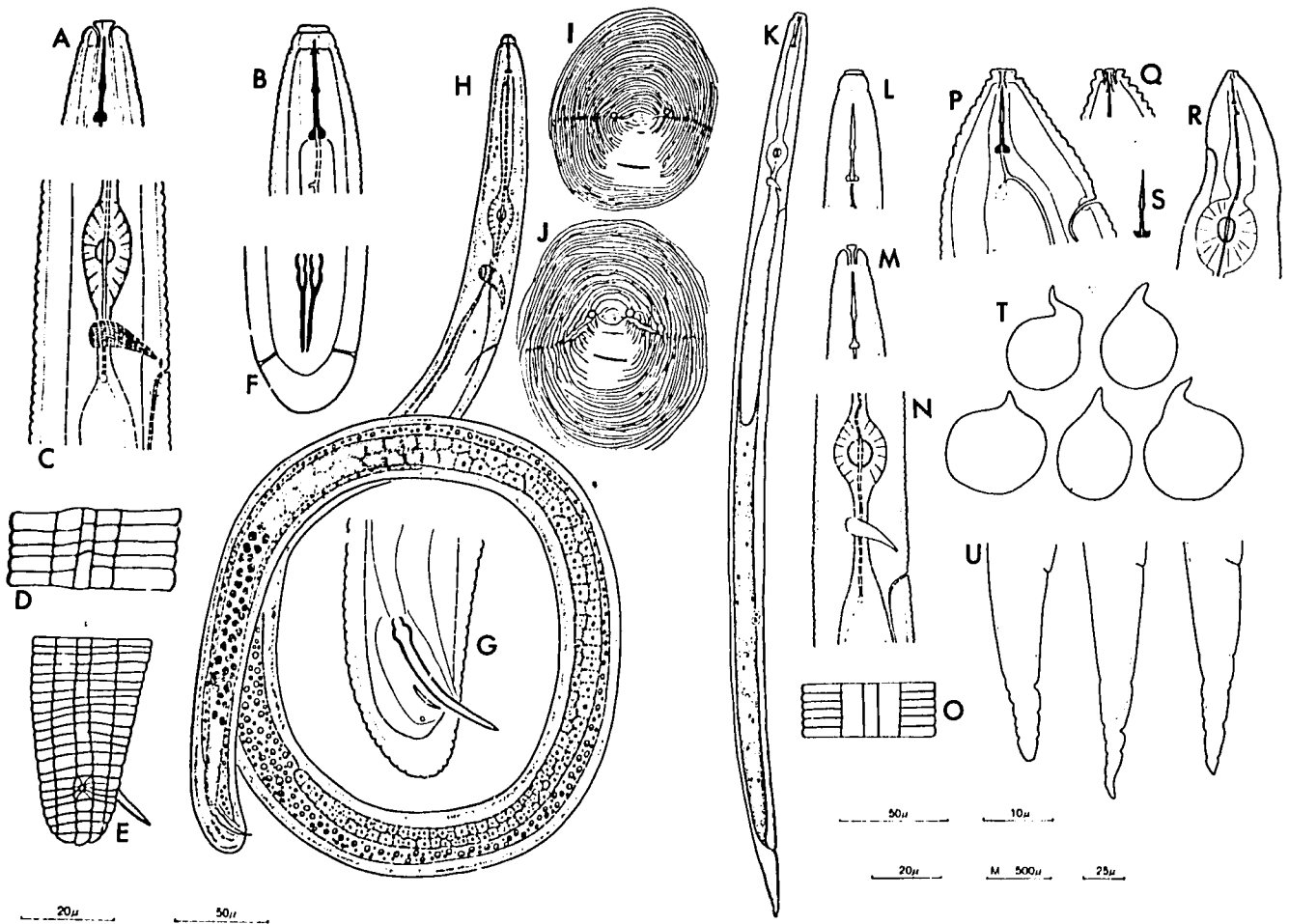*M. kirjanovae* was described as a parasite of tomato (*Lycopersicon esculentum*) in Russia (Fig. 8.15.).

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Fig. 8.4. *Meloidogyne mali.* A-H: Male. I,J: Perineal patterns. K-O, U: Larvae. P-T: Female. The wide lateral fields of males and larvae and the location of the female excretory pore are useful identification characters. Itoh, Ohshima and Ichinohe, 1969.
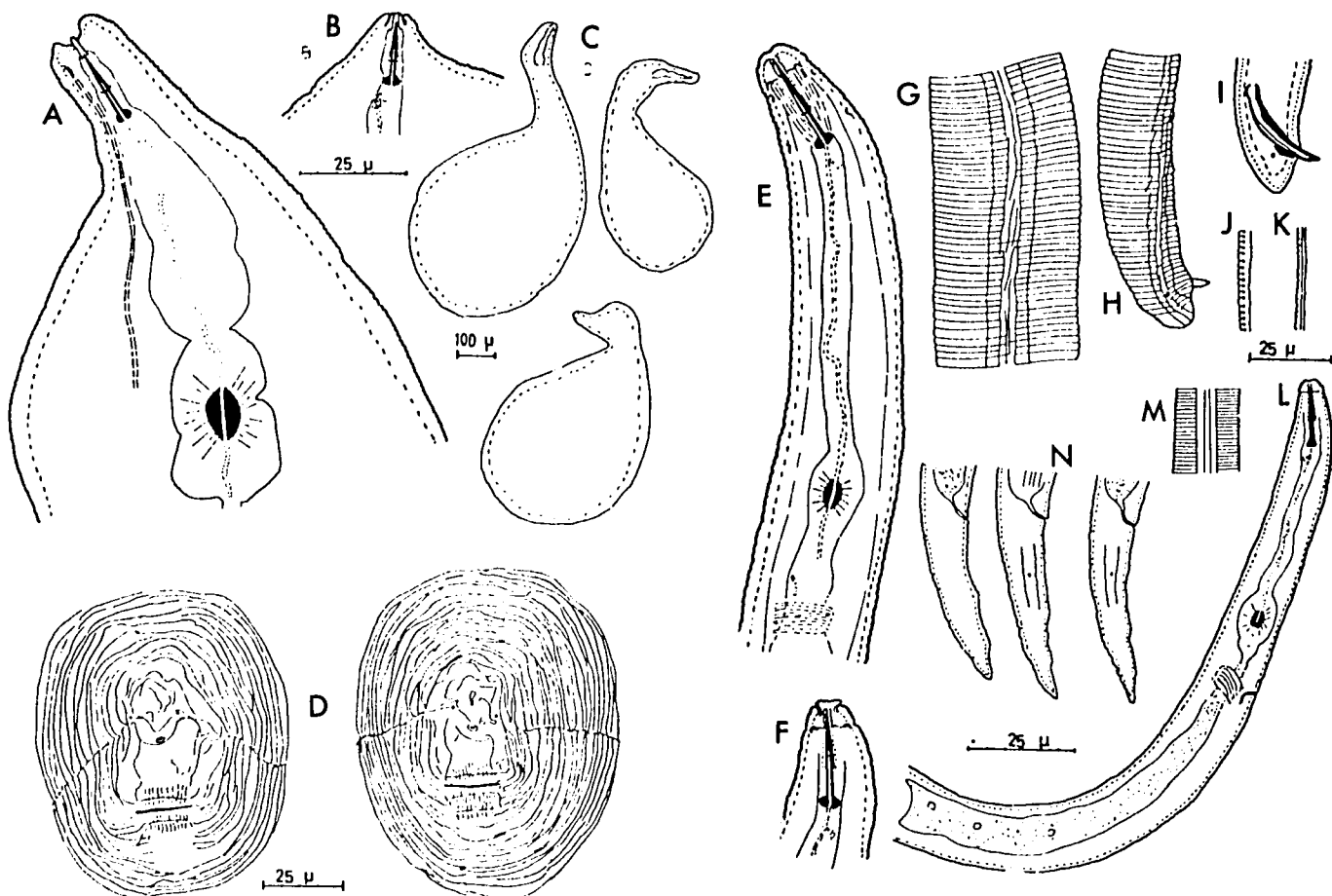
Fig. 8.5. *Meloidogyne litoralis.* A-D: Female. E-K: Male. L-N: Larvae. For separation from *M. mali* and *M. ovalis*, the location of the female excretory pore opposite the stylet is distinctive. Elmiligy, 1968.
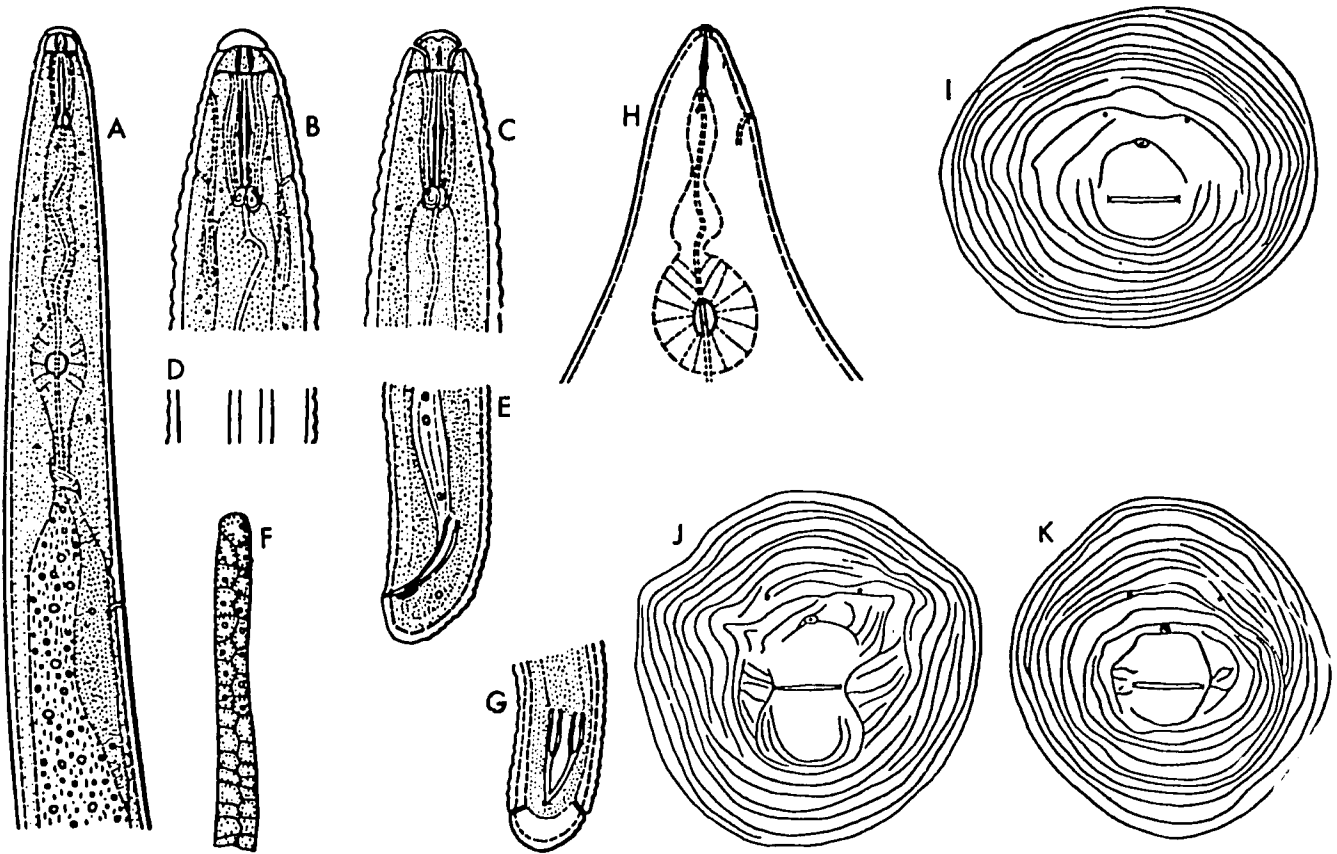
Fig. 8.6. *Meloidogyne ovalis*. A-G: Male. H-K: Female. The rounded female pattern is distinctive for separation from other cool climate species infecting woody plants. Riffle, 1963.
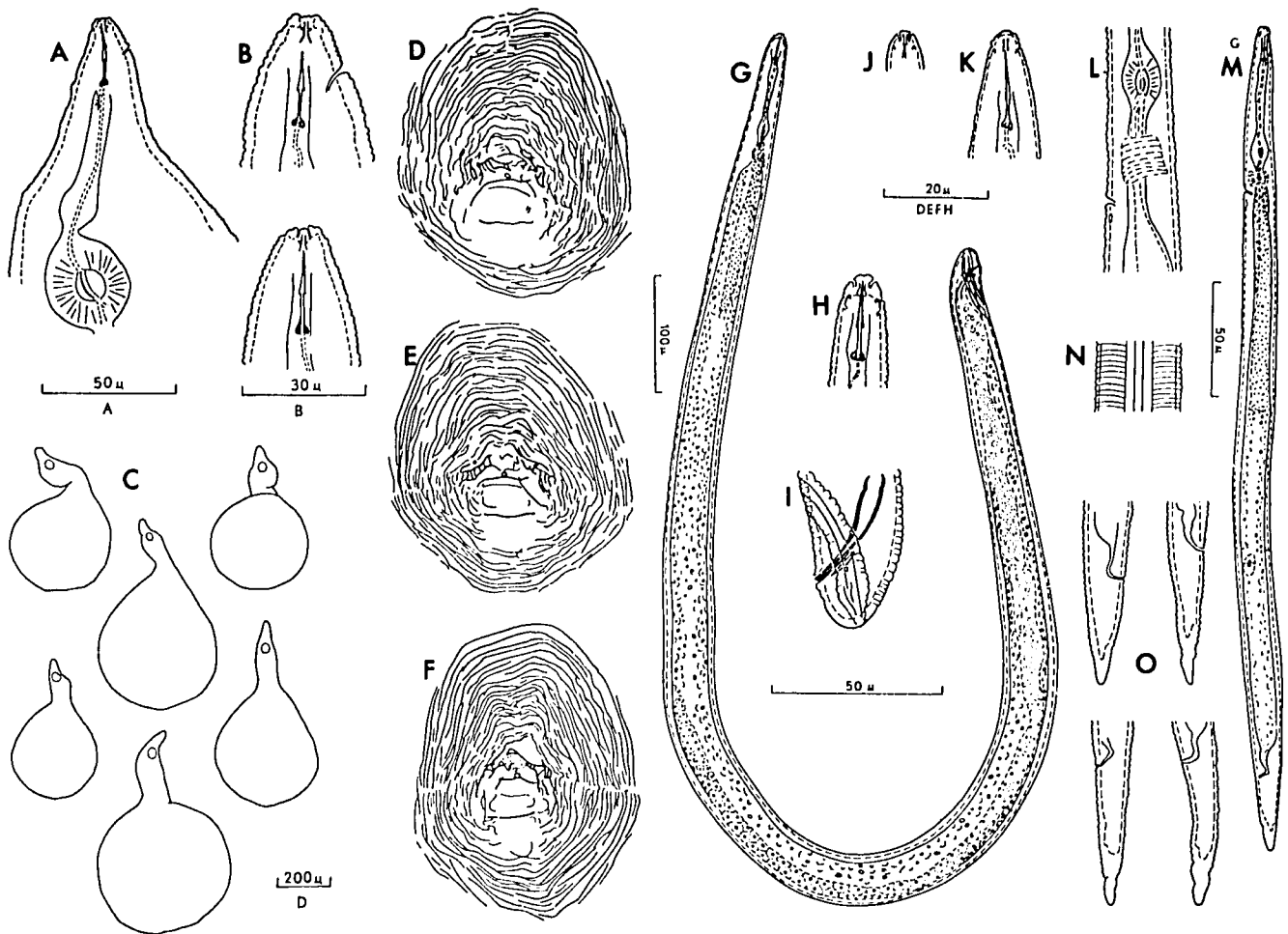
Fig. 8.7. *Meloidogyne ardenensis.* A-F: Female. G-I: Male. K-O: Larva. Compare perineal pattern with Fig. 8.8. Santos, 1967.
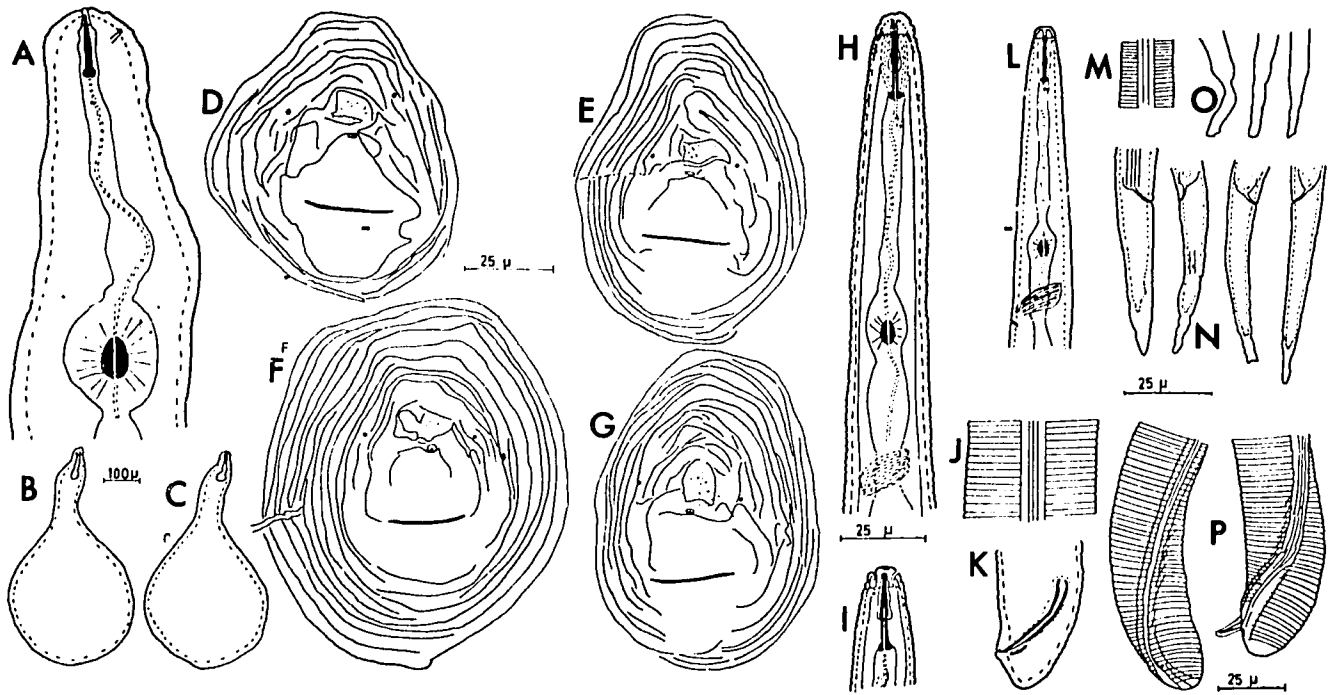
Fig. 8.8. *Meloidogyne deconincki*. A-G: Female. H-L, P: Male. M-O: Larvae.
Compare perineal pattern with Fig. 8.7. Elmiligy, 1968.
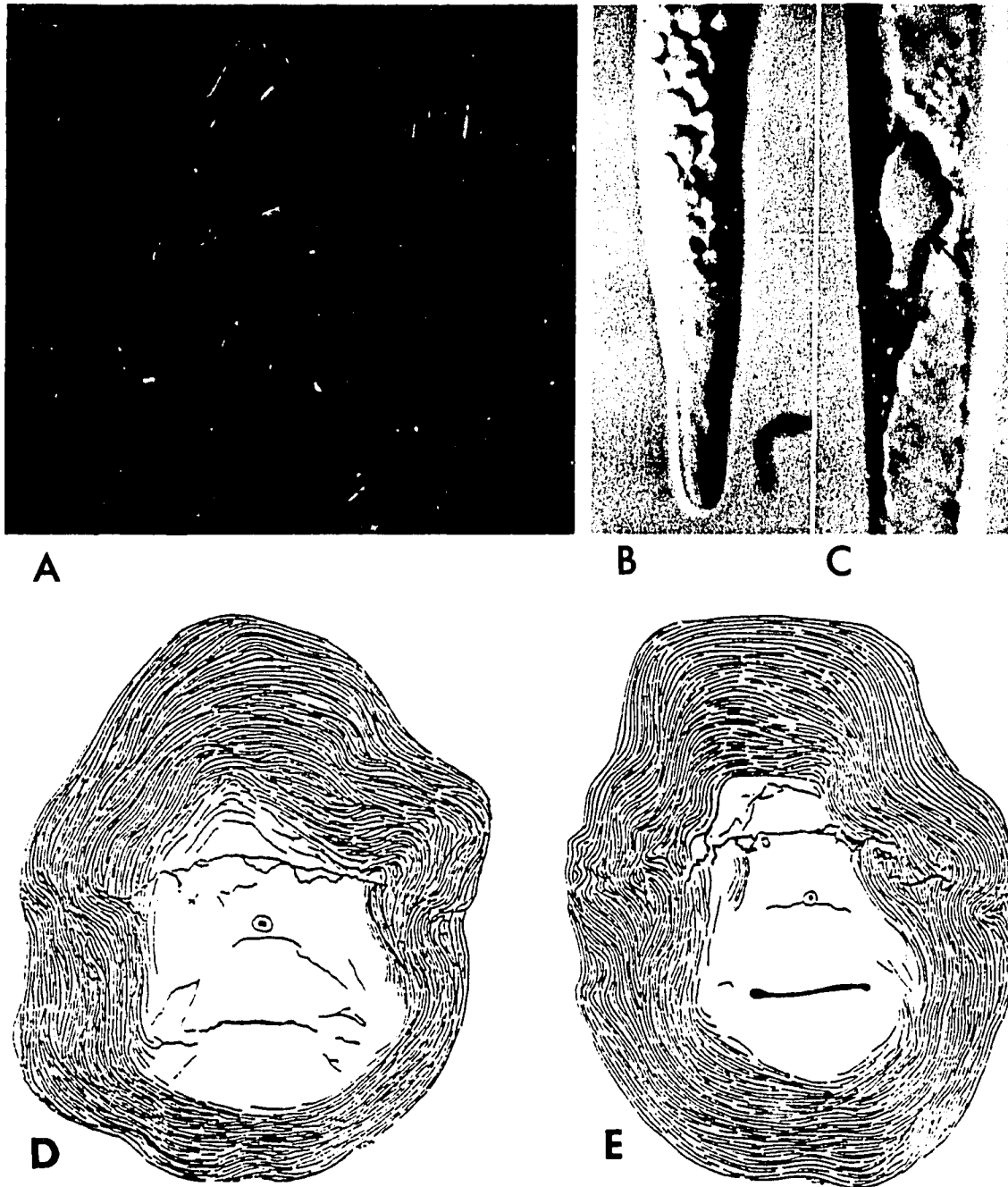
Fig. 8.9. *Meloidogyne microtyla.* A, D, E: Perineal patterns. B: Bluntly round-
ed larval tail is useful identification chara ter. C: Inflated rectum of larva. A, B
and C from Mulvey et al., 1975. D and E original drawings by Karen McKee,
IMP.
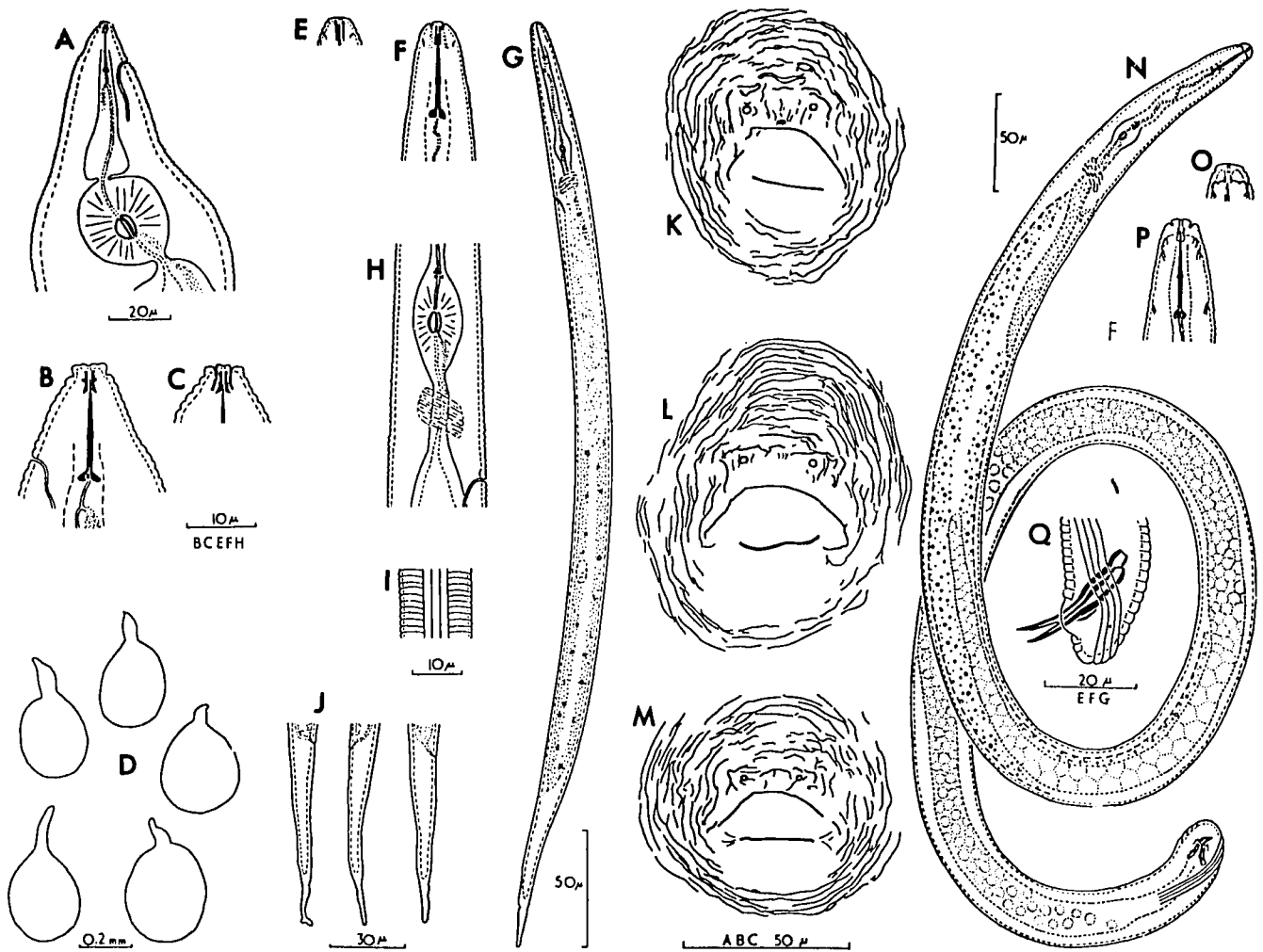
Fig. 8.10. *Meloidogyne naasi*. A-D, Female. E-J: Larvae. K-M: Perineal patterns. N-Q: Male. The large phasmids are eyes in a "monkey face" perineal pattern. Franklin, 1965.
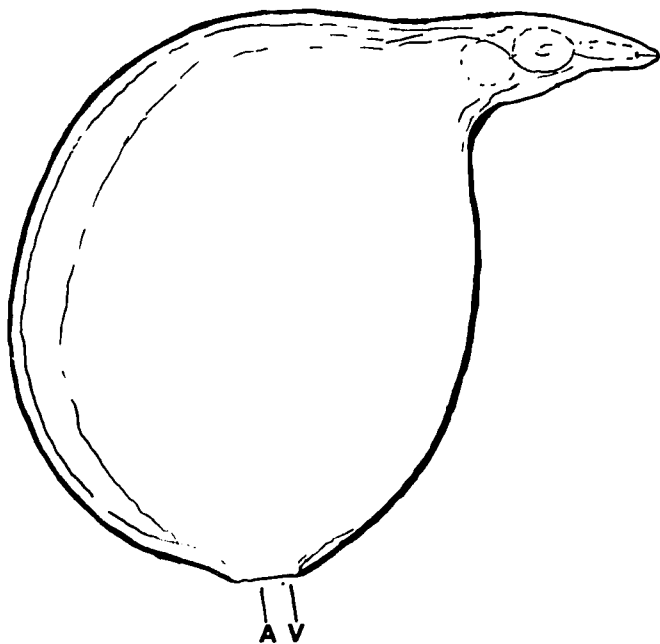


Fig. 8.11. Mature female of *Meloidogyne naasi* in lateral view. Traced from a photograph to show oval body and ventrally placed neck. Siddiqui and Taylor, 1970.
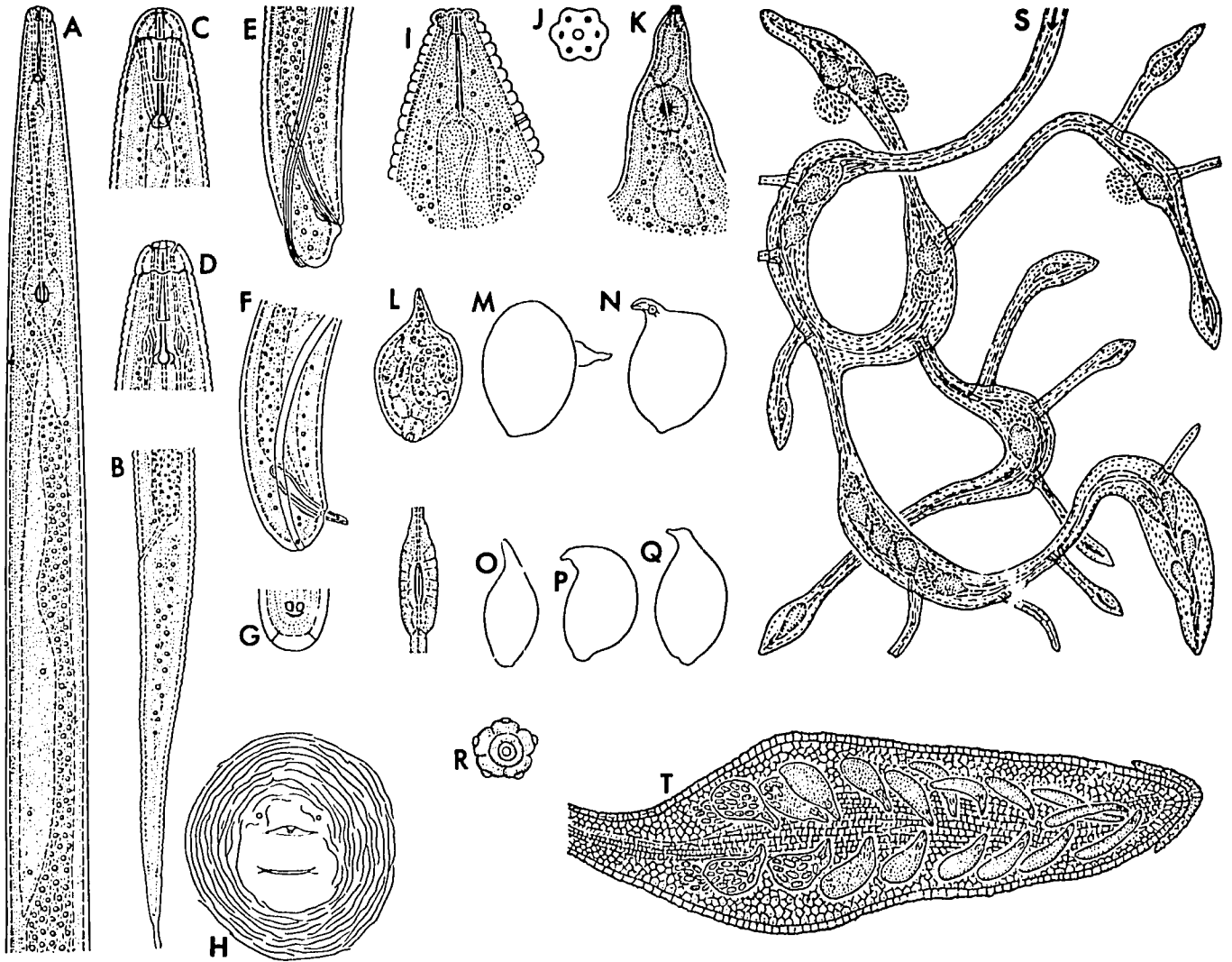
Fig. 8.12. *Meloidogyne ottersoni*. A, B: Larva. C-G: Male. H-Q: Female. R: *Duboscqia* parasites.S,T: infected grass roots. Note that nematodes in grass roots have their heads toward the root tips. This species was originally described as *Hypsoperine ottersoni*, and females in lateral view have ventrally located necks and posterior protuberances. The female stylet knobs are very small. The larval tail is 6 to 7 times the anal body diameter. Thorne, 1969.
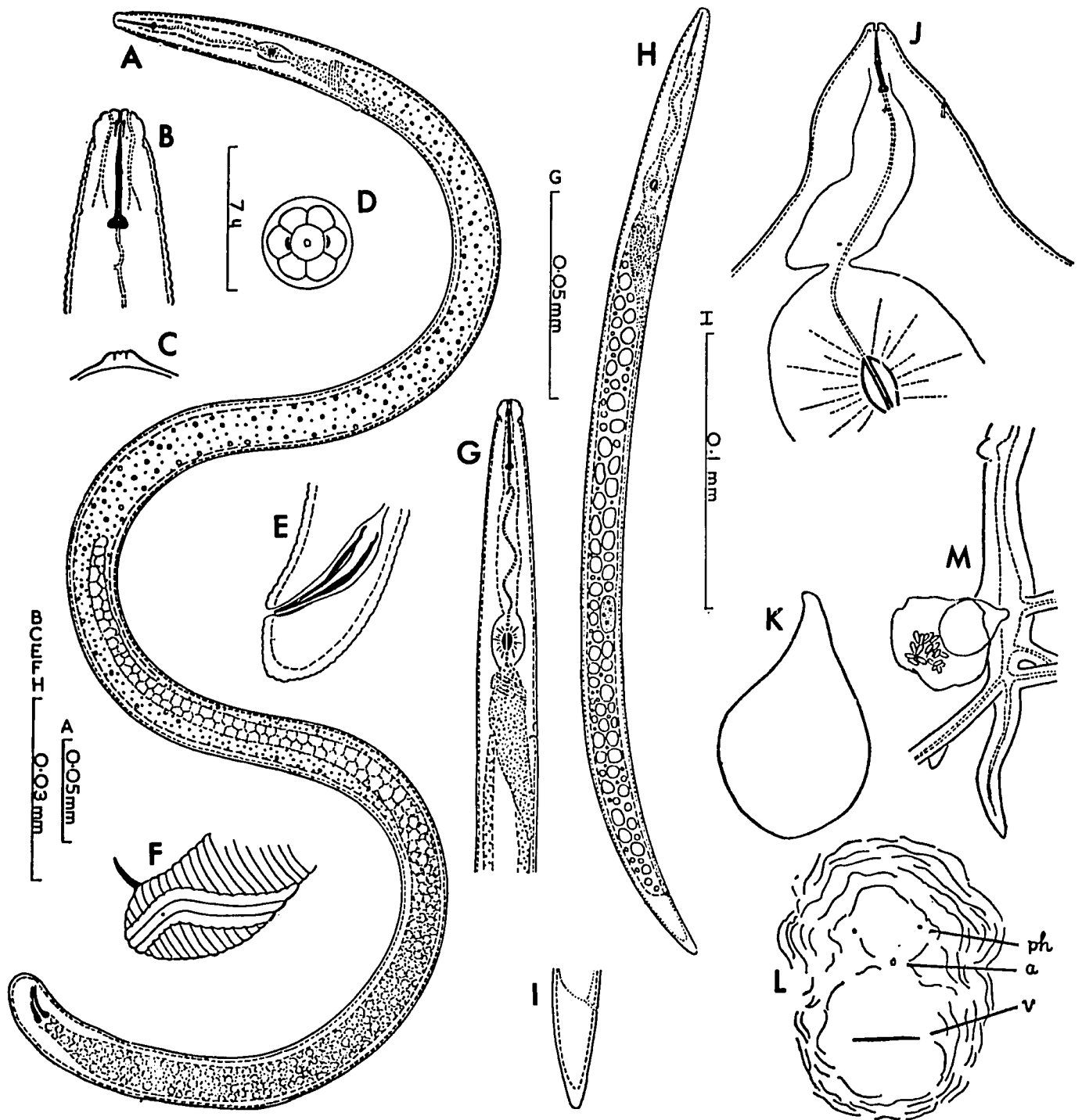
Fig. 8.13. *Meloidogyne artiellia*. A-F: Male. G-I: Larva. J-L: Female M: Infected cabbage root. The short larval tail with rounded tip is distinctive. Franklin, 1961.
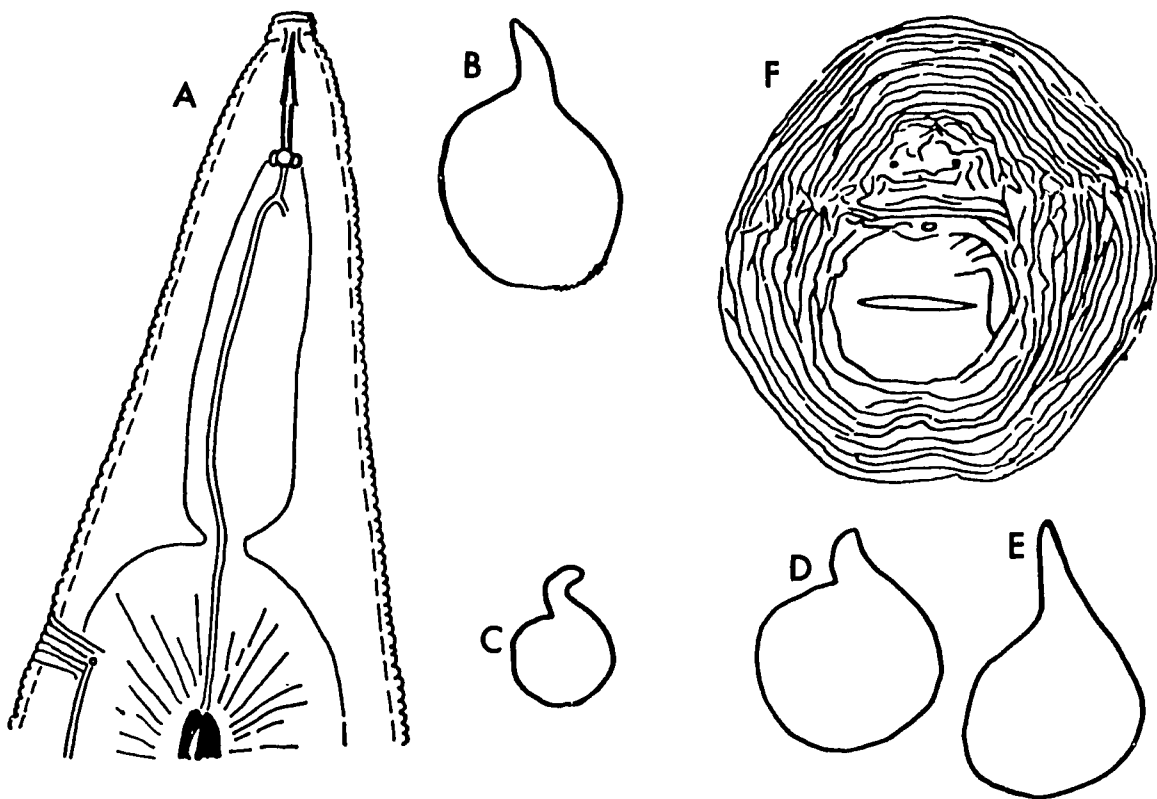
Fig. 8.14. *Meloidogyne tadshikistanica.* A: Female, anterior. B-E: Female body shapes. F: Perineal pattern. This species is much like *M. incognita*, but differs in the location of the female excretory pore (4½ stylet lengths posterior to the head apex compared to one stylet length for *M. incognita*). Kirjanova and Ivanova, 1965.
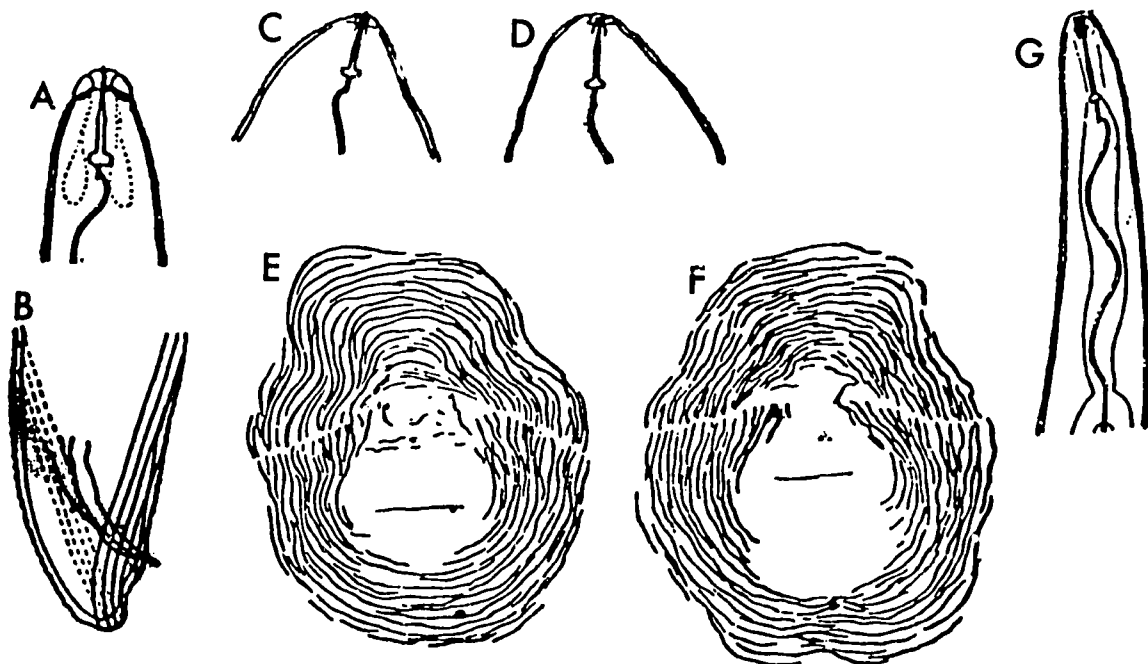


Fig. 8.15. *Meloidogyne kirjanovae.* A-C: Male. D-G: Female. H: Larva. This species can be separated from *M. tadshikistanica* by the perineal pattern. Terenteva, 1965.

# 9
# Identification of *Meloidogyne* Species of Warm Climates

## I. Introduction

The 24 Meloidogyne species listed in Table 9.1 have been described from warm climates. The most widespread and common are *M. incognita*, *M. javanica* and *M. arenaria*, all of which have numerous hosts including economic crops grown in many parts of the world. A considerable part of the work of the International *Meloidogyne* Project will be research on these three species.

*M. incognita*, *M. javanica* and *M. arenaria* can be identified by study of the morphological characters listed in Table 9.2 and the illustrations Figs. 9.1 to 9.9. It is also important to identify the four races of *M. incognita* and the two races of *M. arenaria* by use of the North Carolina Differential Host Test as described in Appendix 1.

## II. Descriptions of the Species
## A. Species with Many Host Plants

### 1. *M. incognita*

As discussed in Chapter 5, the name *M. incognita* as it is now used designates a group containing four

Table 9.1. *Meloidogyne* species of warm climates grouped by host preferences, with type hosts, type localities, and median larval lengths.

| Species and host preference | Type host | Type locality | Larval* length (mm) |
|---|---|---|---|
| Numerous hosts: | | | |
| *M. arenaria* | *Arachis hypogaea* | Florida, USA | 0.470 |
| *M. incognita* | *Daucus carota* | Texas, USA | 0.376 |
| *M. javanica* | *Saccharum officinarum* | Java | 0.370 |
| *Coffea* species: | | | |
| *M. africana* | *Coffea arabica* | Kenya | 0.425 |
| *M. coffeicola* | *Coffea arabica* | Brazil | 0.380 |
| *M. decalineata* | *Coffea arabica* | Tanganyika | 0.522 |
| *M. exigua* | *Coffea* sp. | Brazil | 0.346 |
| *M. megadora* | *Coffea canephora* | Angola | 0.480 |
| *M. oteifae* | *Pueraria javanica* | Congo | 0.360 |
| Other woody plants: | | | |
| *M. brevicauda* | *Camellia sinensis* | Ceylon | 0.525 |
| *M. indica* | *Citrus aurantifolia* | India | 0.414 |
| Gramineae: | | | |
| *M. acronea* | *Sorghum vulgare* | Republic of South Africa | 0.450 |
| *M. graminicola* | *Echinochloa colonum* | Louisiana, USA | 0.449 |
| *M. graminis* | *Stenotaphrum secundatum* | Florida, USA | 0.465 |
| *M. kikuyensis* | *Pennisetum clandestinum* | Kenya | 0.325 |
| *M. spartinae* | *Spartina alterniflora* | South Carolina, USA | 0.762 |
| Soybeans: | | | |
| *M. bauruensis* | *Glycine max* | Brazil | 0.348 |
| *M. inornata* | *Glycine max* | Brazil | 0.397 |
| Other hosts: | | | |
| *M. ethiopica* | *Lycopersicon esculentum* | Tanganyika | 0.407 |
| *M. lordelloi* | *Cereus macrogonus* | Brazil | 0.360 |
| *M. lucknowica* | *Luffa cylindrica* | India | 0.492 |
| *M. megriensis* | *Mentha longifolia* | Armenia, SSR | 0.412 |
| *M. thamesi* | *Boehmeria utilis* | Florida, USA | 0.443 |

*Median lengths. Most reported minima and maxima are about 12% more or less than the median.

Table 9.2. Identification characters of the most common *Meloidogyne* species of warm climates.

| Species and larval length* (mm) | Identification features of perineal pattern and other characters useful for identification |
|---|---|
| *M. incognita* 0.376 (0.360-0.393) | Perineal pattern as shown in Figs. 9.1,B,F,G,M,R,S and 9.2. Female excretory pore opposite stylet knobs (Fig. 9.1,D). |
| *M. javanica* 0.370 (0.340.0-0.400) | Perineal pattern with distinct lateral lines; few or no striae cross lateral lines from dorsal to ventral sector (Figs. 9.3,C,D,G,N,O.Z,AA,BB,CC, 9.4 and 9.5). Female excretory pore 2½ stylet lengths posterior to apex of head (Fig. 9.3,A). |
| *M. arenaria* 0.470 (0.450-0.490) | Perineal pattern with rounded or slightly flattened arch, indented near lateral lines, with short striae and some forked striae along lateral lines (Figs. 9.6,F and 9.7). Longest larvae of any species in this group. Female excretory pore 2 stylet lengths posterior to apex of head (Fig. 9.6,D). |

*The first figure is the median length which is close to, but not identical with the average. The figures in parentheses are the reported range of larval length and are generally about 12% more or less than the median.

races. Experience with the North Carolina Differential Host Test for 20 years, and tests of numerous populations of *M. incognita* from many parts of the world have shown that the most common, Race 1, does not reproduce on root-knot-resistant tobacco cultivar NC 95, on Deltapine 16 cotton or Florrunner peanuts. It causes galls and reproduces on cv California Wonder pepper (*Capsicum frutescens*), on cv Charleston Grey watermelon and cv Rutgers tomato. The other three races occur in various parts of the world but are much less common than Race 1. Race 2 reproduces on cv NC 95 tobacco. Race 3 reproduces on cv Deltapine 16 cotton. Race 4 reproduces on both the cotton and tobacco cultivars.

Perineal patterns are all of the type shown in Figs. 9.1,B,F,G,M,R,S and 9.2; and other morphological characters, so far as now known , are as given in the other illustrations of Fig. 9.1. It is planned that the work of the International *Meloidogyne* Project will include a thorough study of the morphology of all stages of the four races. In the meantime, cooperators are requested to bring any morphological differences in populations identified by the Differential Host Test to the attention of the Principal Investigator at Project Headquarters.

Median larval length of *M. incognita* is 0.376 mm (0.360-0.393 mm). The female excretory pore is opposite the stylet knobs (Fig. 9.1,D).

### 2. *M. javanica*

*M. javanica* is widespread in the tropics and the warmer regions of the Temperate Zone. In the Differential Host Test, populations from all over the world have infected tobacco, watermelon and tomato, but not cotton, pepper and peanut. Populations of *M.*

*javanica* which infect strawberry have been reported only three times (Taylor and Netscher, 1975). *M. javanica* has a distinctive perineal pattern with definite incisures on the lateral lines separating the striae of the dorsal and ventral sectors (Fig. 9.4 and 9.5). These lines can be traced on the female body from the perineal region to the neck. Few or no striae cross the lateral lines of the perineal pattern. Median larval length of *M. javanica* is 0.370 mm (0.340-0.400 mm). *M. javanica* and *M. incognita* can be separated by their perineal patterns, but not by larval length. The excretory pore of *M. incognita* females is one stylet length from the apex of the head compared to 2½ stylet lengths for *M. javanica* females. Other characters of *M. javanica* are shown in Fig. 9.3.

### 3. *M. arenaria*

The majority of *M. arenaria* populations which have been tested by differential hosts in North Carolina reproduce on tobacco, pepper, watermelon, peanut and tomato (Race 1). Some populations (Race 2) do not reproduce on peanut and reproduce poorly if at all on pepper. Median larval length of *M. arenaria* is 0.470 mm (0.450-0.4.0 mm). This median is nearly 0.100 mm more than the median larval lengths of *M. incognita* and *M. javanica*, and there is no overlapping of ranges. Perineal patterns of *M. arenaria* may be difficult to identify since they vary from patterns resembling *M. hapla* to patterns resembling *M. incognita*. Some patterns have "wings" (Fig. 9.6,F). Other characters of *M. arenaria* and *M. hapla* are similar, and their distribution overlaps in the USA at the southern limit of *M. hapla* and the northern limit of *M. arenaria*. The differential plant in the host test is watermelon, which is always infected by *M.*
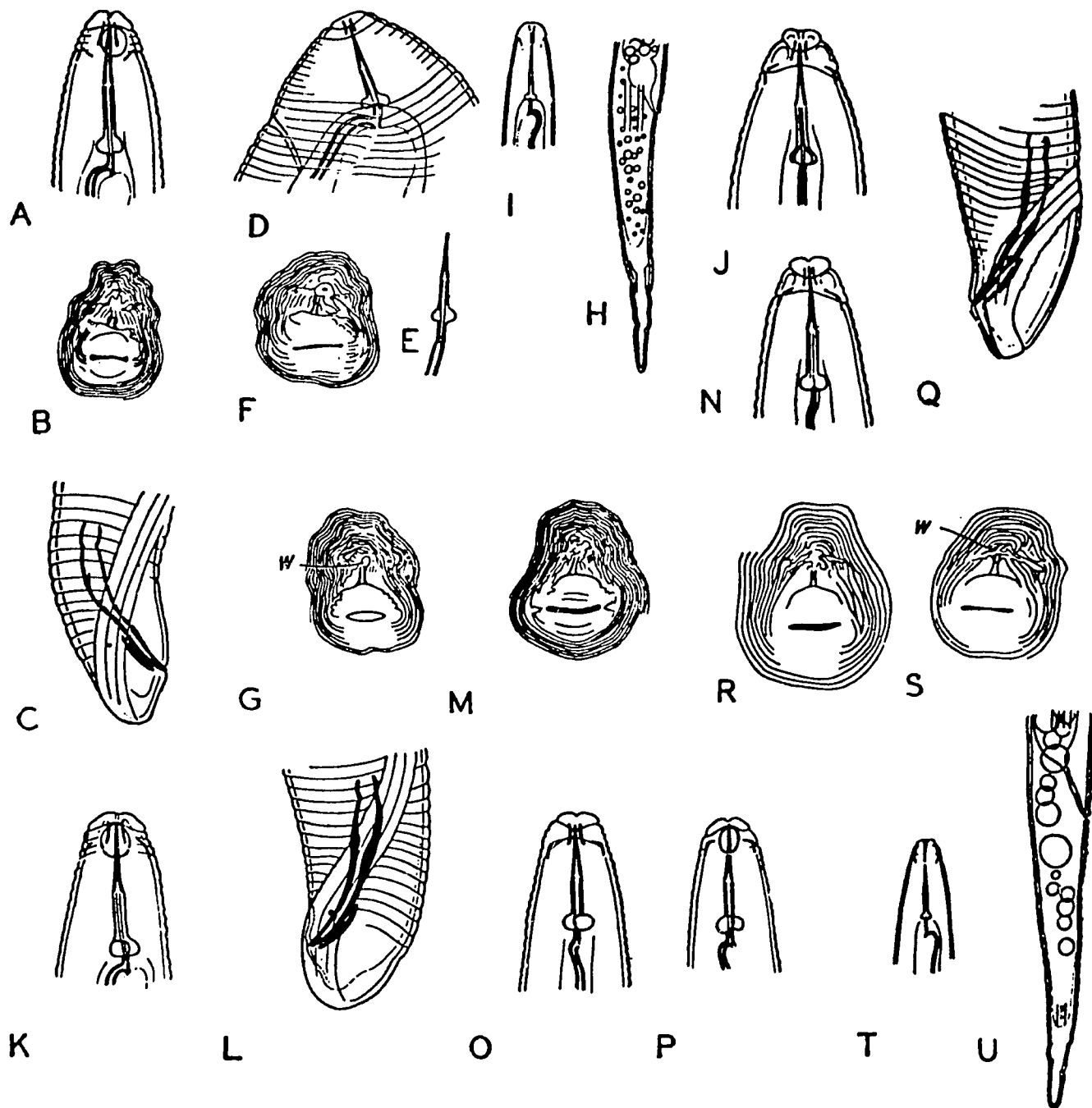
Fig. 9.1. *Meloidogyne incognita.* A, J, K, N, O, P: Male heads, C, L, Q: Males, posterior. D, E: Female, anterior and stylet. B, F, G, M, R, S: Perineal patterns. H, I, T, U: Larvae. Female excretory pore is about opposite stylet knobs. Chitwood, 1949.
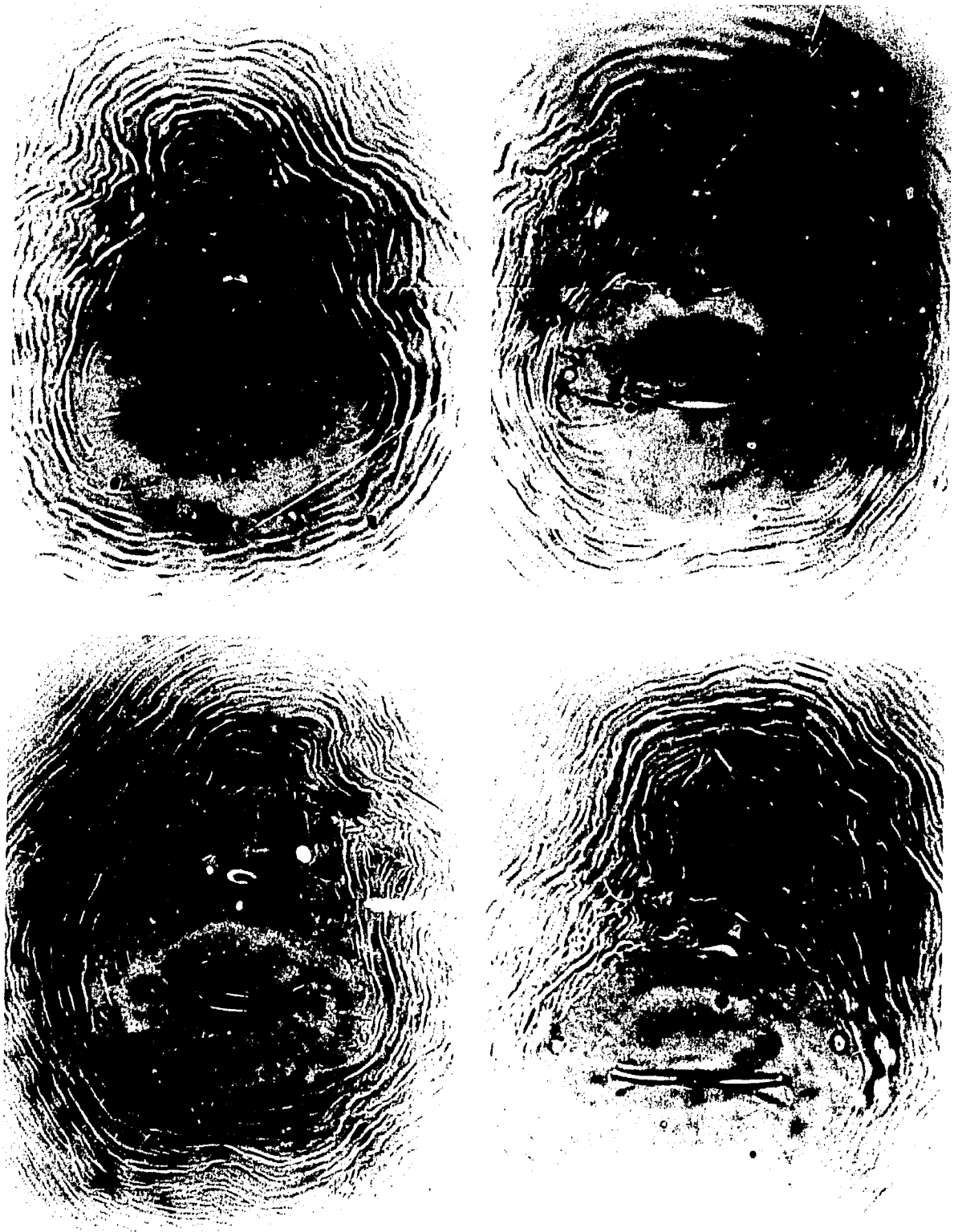
Fig. 9.2. *Meloidogyne incognita.* Photographs of perineal patterns. Elongated with more or less flattened dorsal arch. Striae smooth to wavy with some forking at lateral lines.
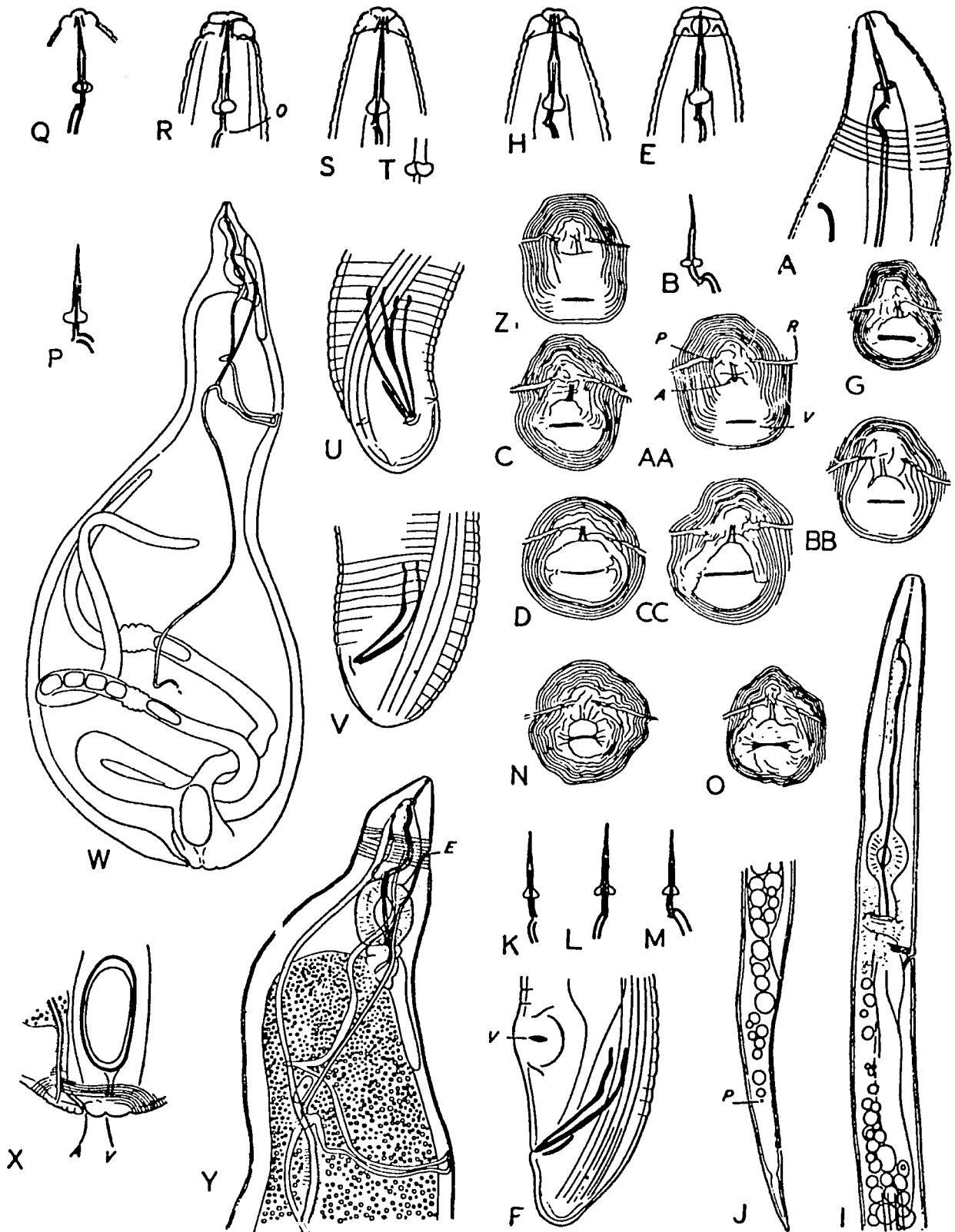
Fig 9.3. *Meloidogyne javanica.* A: Female, anterior. B, K, L, M: Female stylets. C, D, G, N, O, Z, AA, BB, CC: Perineal patterns. E, H, R, S: Male heads. F: Male (intersex), posterior with rudimentary vulva. I, J: Larva. P, Q: Female stylets. U, V: Male, posterior. W, Y: Female body (diagrammatic) and female, anterior. X: Female, posterior. Female excretory pore is 2½ stylet lengths posterior to head apex. Chitwood, 1949.
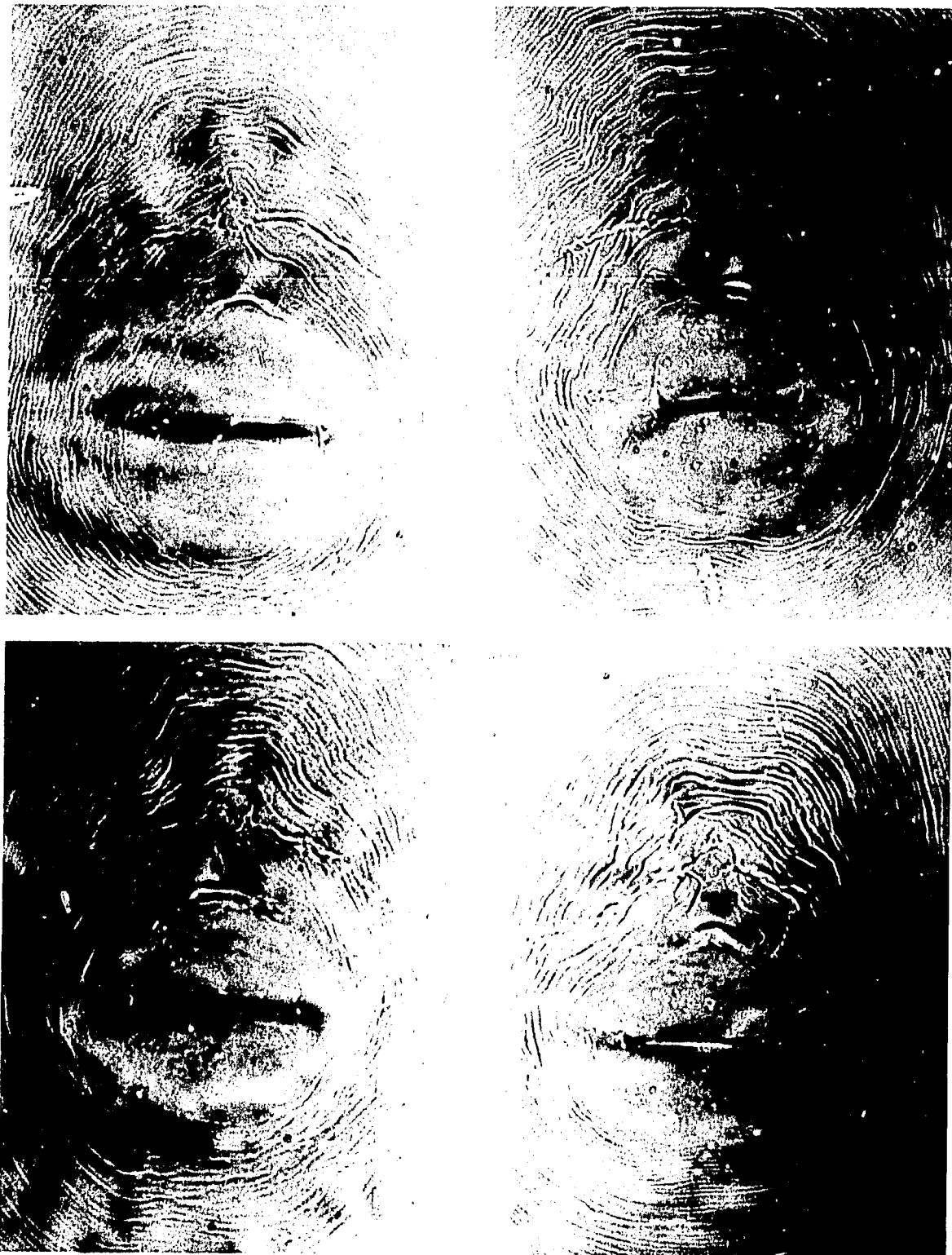
Fig. 9.4. *Meloidogyne javanica.* Photographs of perineal patterns. Dorsal arch rounded to flattened. Lateral incisures distinct. Few striae extending unbroken from dorsal to ventral sector.
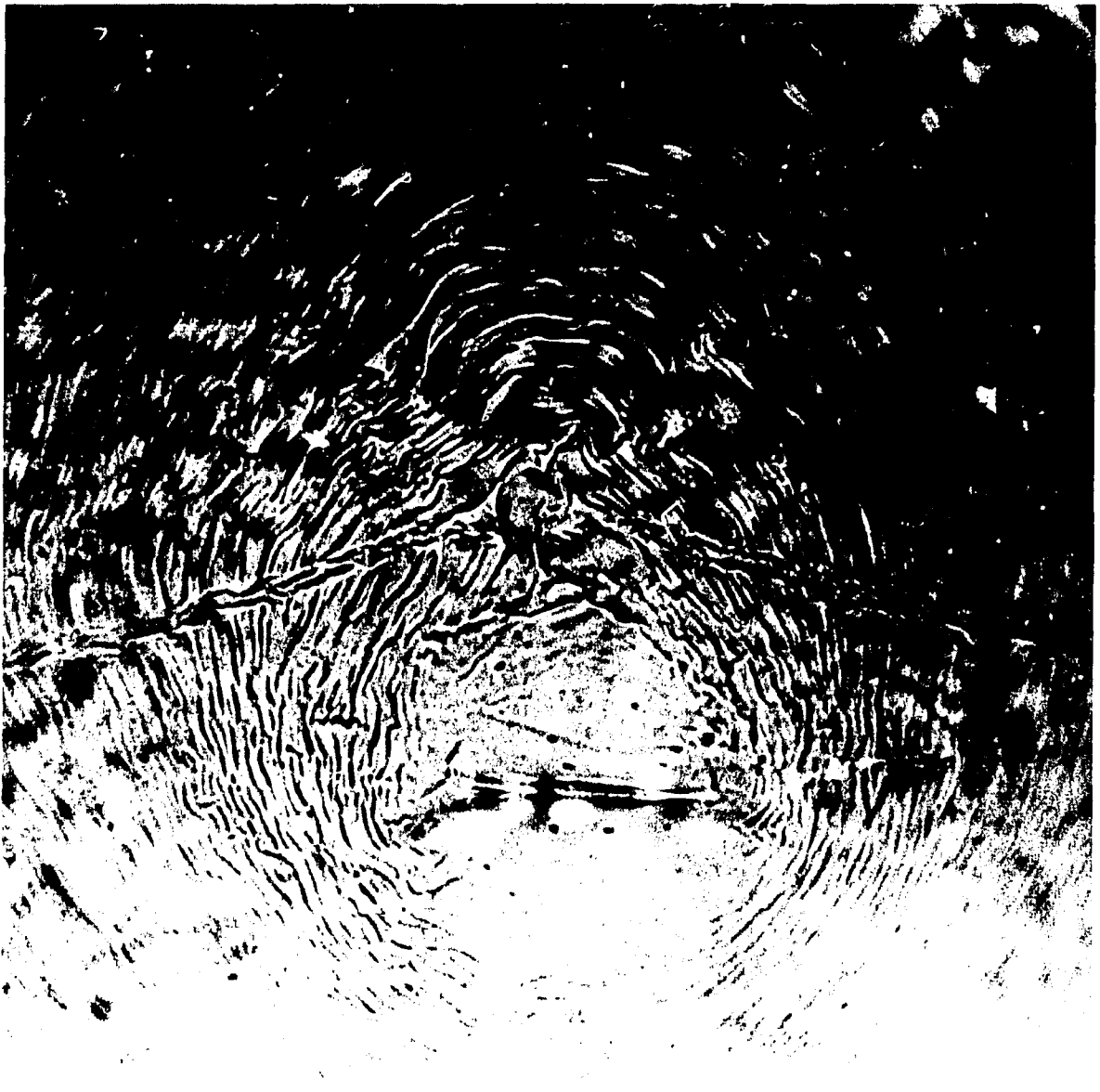
Fig. 9.5. Photograph of perineal pattern of *M. javanica*. The lateral lines have definite borders. They lead to the tail terminus. Note that the lateral lines are in focus in this picture taken with an oil immersion objective. Compare with out of focus folds along lateral lines in Fig. A-1.1, C, D.

*arenaria* and never by *M. hapla*. Other characters of *M. arenaria* are shown in Fig. 9.6.

As shown in Fig. 9.9, *M. arenaria* invades shells and stems of peanuts, causing knots and other abnormalities. Other organisms cause rotting of the invaded tissue. Root systems galled by *M. arenaria* may have m..ny small lateral roots, but these rarely grow directly from the gall (Fig. 9.8) as lateral roots grow from galls caused by *M. hapla* (Fig. 8.3).

## B. Species Attacking Coffee

### 1. South American Species

*M. exigua*, type species of the genus *Meloidogyne*, was first described as a cause of root galling of coffee trees in Brazil (Goeldi, 1887). It was redescribed by Lordello and Zamith (1958). *M. exigua* is a very common root-knot nematode of coffee in South and Central American countries.
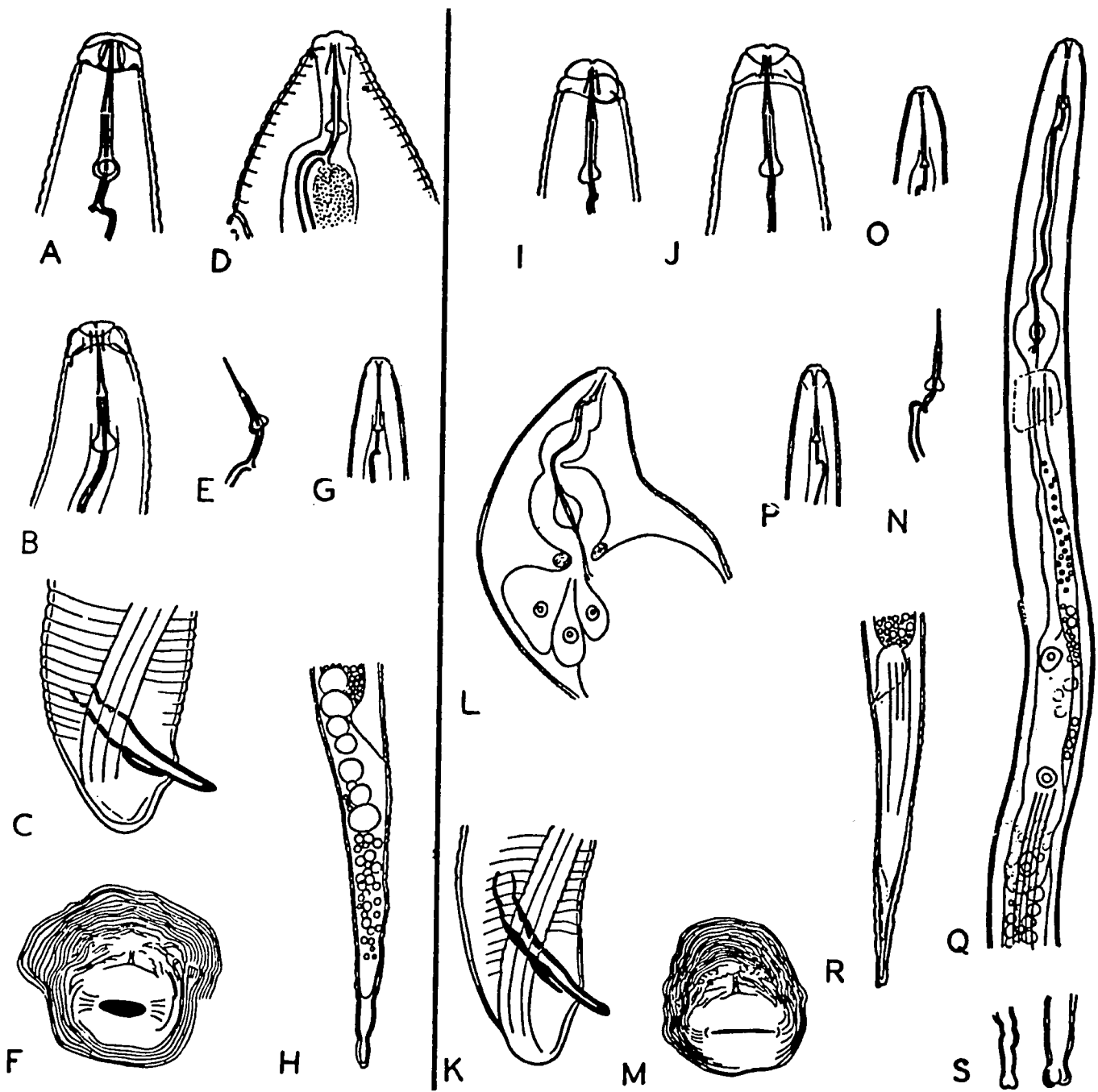
Fig. 9.6. *Meloidogyne arenaria*. A-C: Male. D, E: Female, anterior and stylet. G, H: Larva. *Meloidogyne thamesi*. I, J: Male, anterior, K: Male, posterior. L-N: Female, anterior, perineal pattern and stylet, O-S: Larvae. Chitwood, 1949.

Other species having *Coffea* as type host are:

M. *Coffeicola:* *Coffea arabica*, Brazil. It was reported that coffee trees infected by M. *coffeicola* commonly die, and that more than 15,-000 trees on a single plantation had died (Lordello and Zamith, 1960).

M. *decalineata:* C. *arabica*, Tanganyika (Tanzania).

M. *megadora:* *Coffea canephora*, C. *arabica*, C. *congensis* and C. *eugenoides*, Angola.

M. *africana:* C. *arabica*, Kenya.

M. *oteifae:* *Pueraria javanica* (kudzu) and also
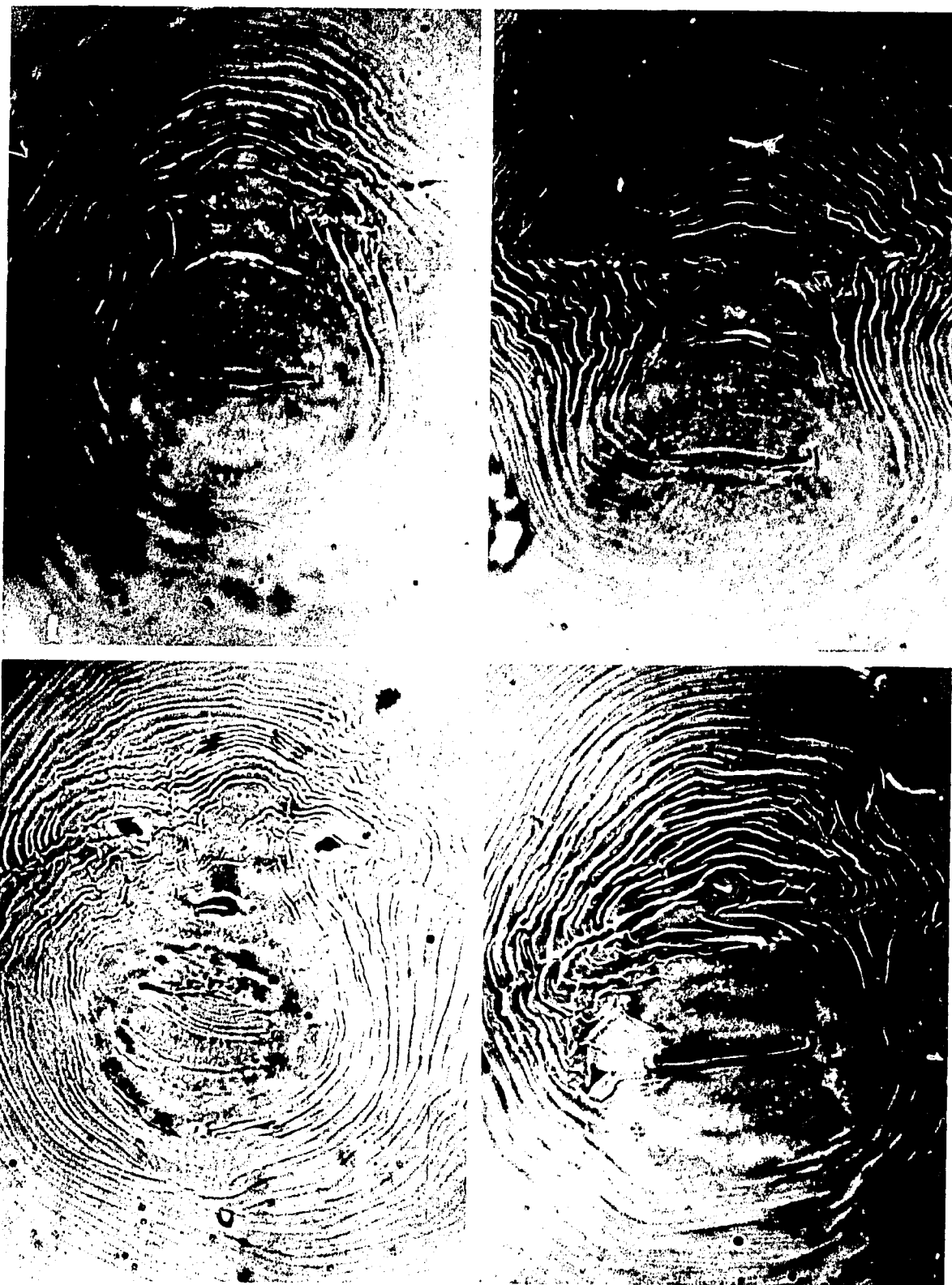
Fig. 9.7. *Meloidogyne arenaria.* Photographs of perineal patterns. Arch rounded to flattened. Striae smooth to wavy, slightly ..idented at laternal lines. Forking, and short irregular striae near lateral lines.
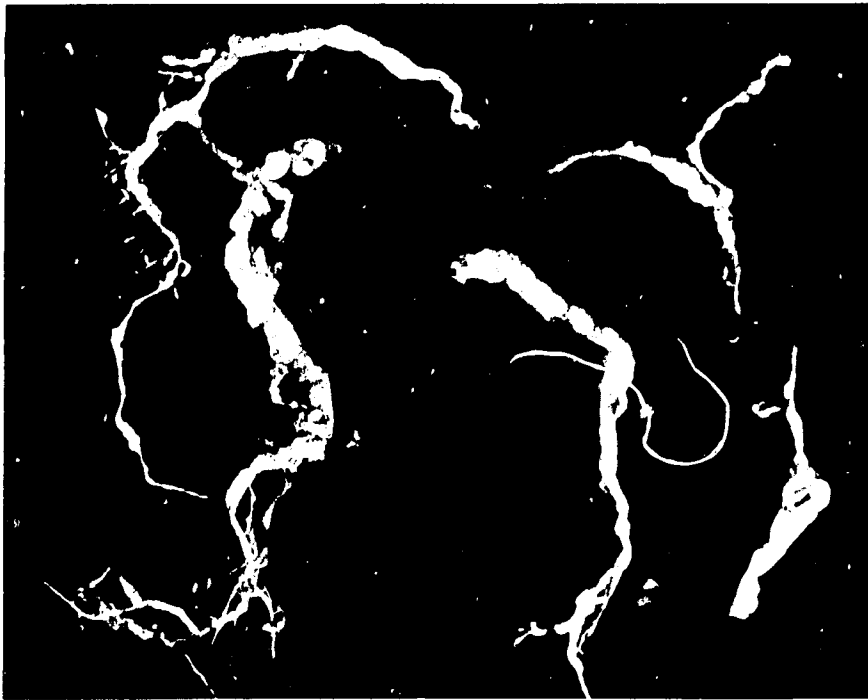
Fig. 9.8. Roots with galls caused by *Meloidogyne arenaria*. The short lateral roots do not grow from the galls.
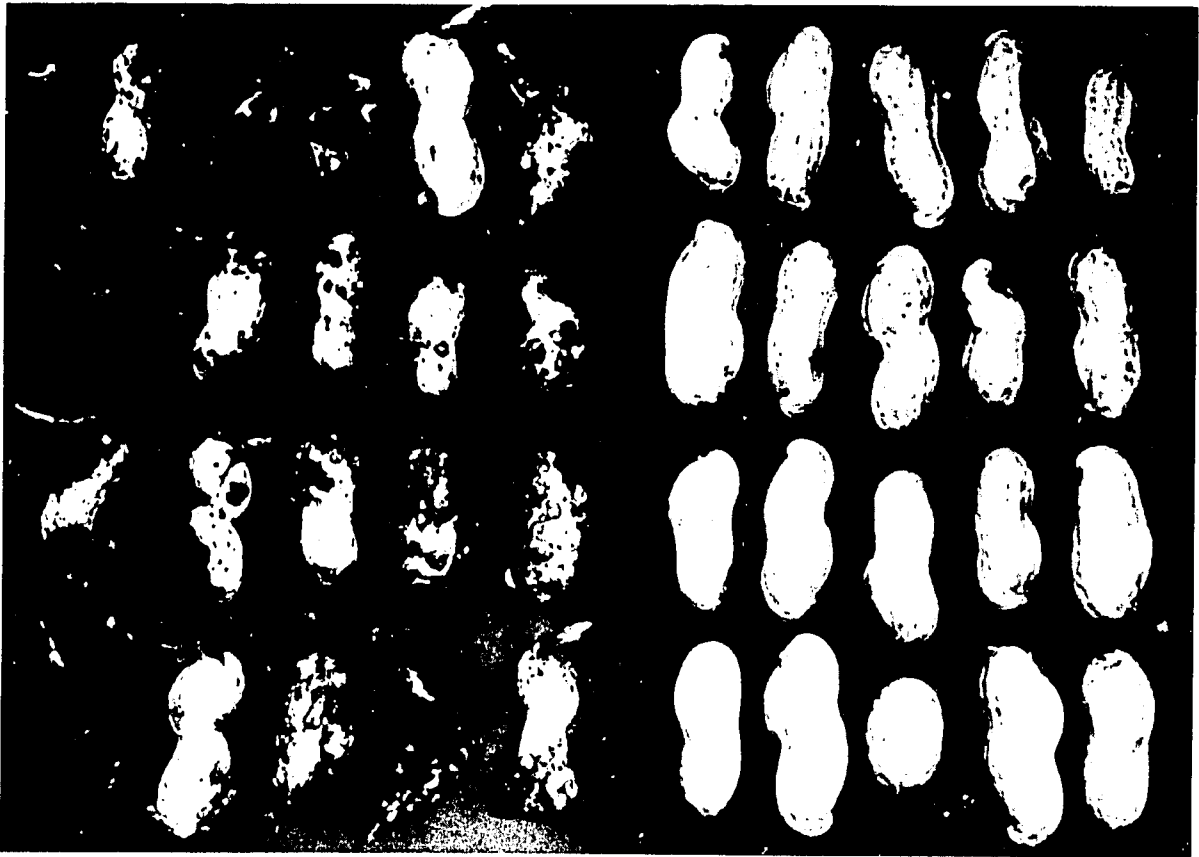
Fig. 9.9. *Meloidogyne arenaria* galls on peanut shells (left) compared with slightly damaged shells (right). Below: Heavily infected peanut shells and stems.
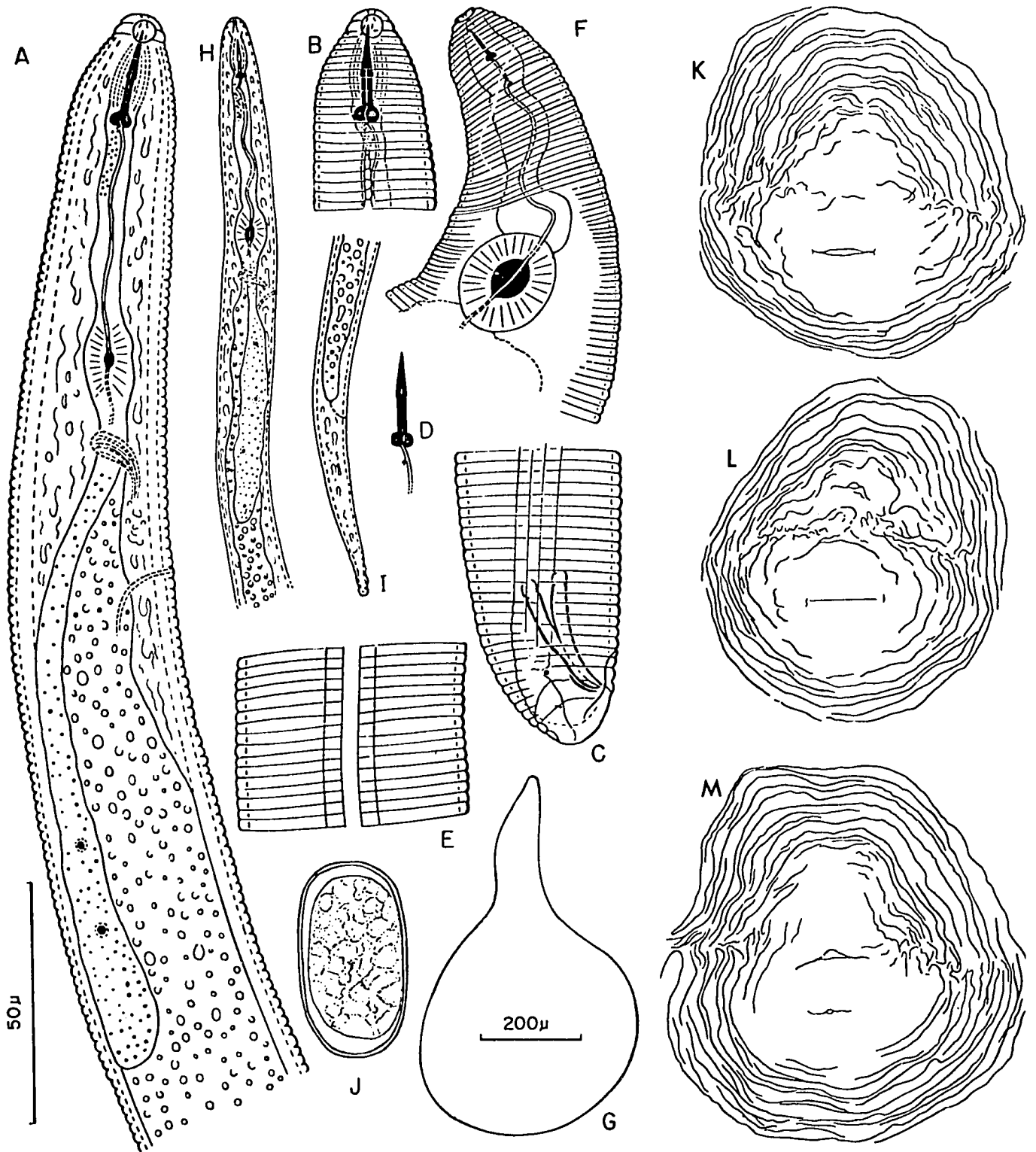
Fig. 9.10. *Meloidogyne exigua.* A-E: Male anterior, head, posterior, stylet and lateral field, respectively. F, G: Female anterior and body shape. H, I: Larva anterior and posterior. J: Egg. K-M: Perineal patterns. Lordello and Zamith, 1958.
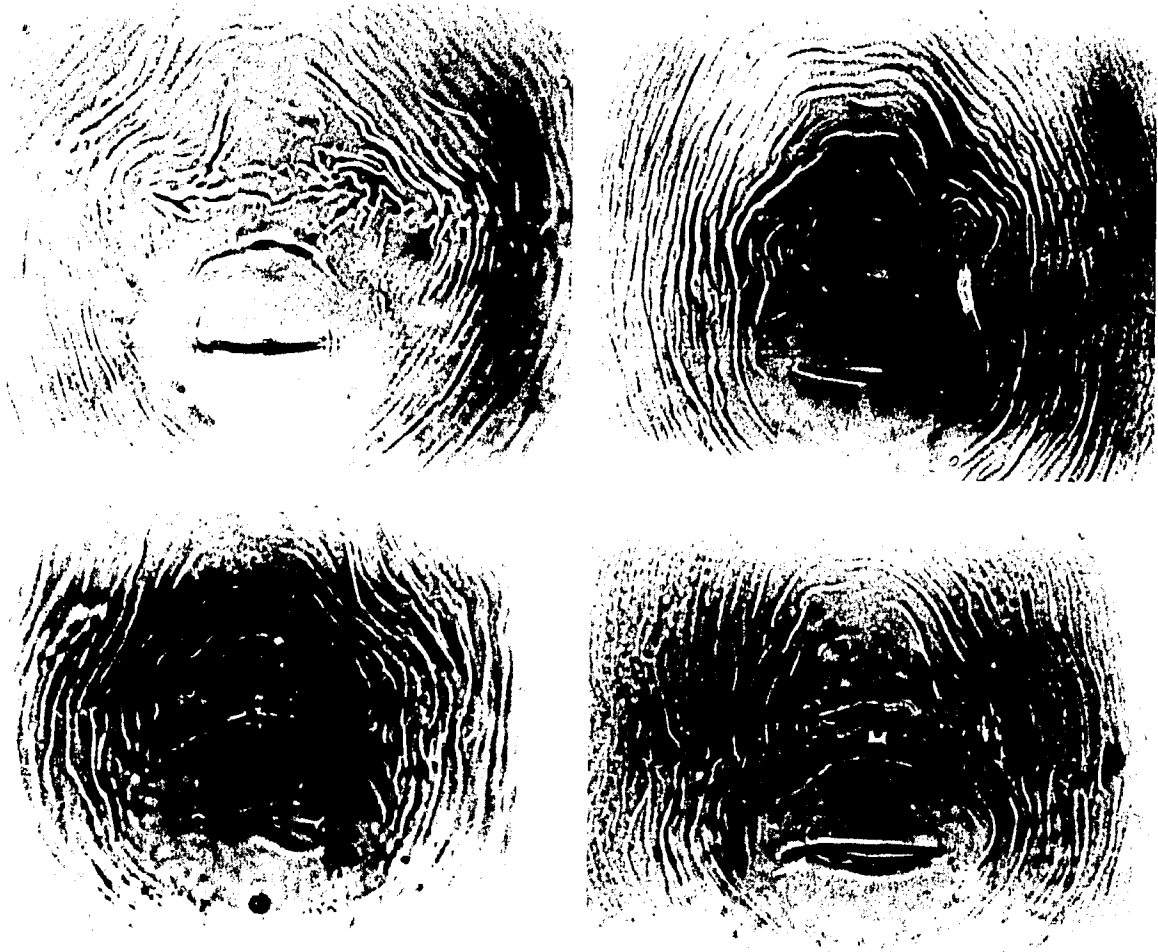
Fig. 9.11. *Meloidogyne exigua.* Photographs of perineal patterns. Arch more or less flattened and indented laterally. Striae widely spaced. Broken and folded striae ventral to inconspicuous lateral lines.

found on *C. canephora (C. robusta)* in Yangambi, Congo.

The South American species, *M. exigua* and *M. coffeicola,* can be easily separated by perineal patterns, and by shape of the female bodies (globular with a short neck (Fig. 9.10,G) and elongated with a long neck (Fig. 9.13,F), respectively. The larval tail of *M. exigua* is about 5 anal body widths long compared to only 3 anal body widths for *M. coffeicola* (Figs. 9.10,I and 9.13, I).

Roots of a *Coffea* sp. grown by Dr. Paulo de Souza in a glasshouse at North Carolina State University and infected by *M. exigua* had numerous terminal root galls (Fig. 9.12). Lordello 1972, p. 273) illustrates similar galls. He also illustrates a root from a coffee tree attacked by *M. coffeicola* (Ibid. p. 277). The root is "thickened and heavily cracked" and the cortex is rough because of cracking and detachment of cortical tissues.

2.  African Species

Two of the African species, *M. decalineata* and *M. megadora,* have distinctive perineal patterns (Figs. 9.14,B and 9.15,C). Median larval lengths are 0.522 mm (0.471-0.573 mm) and 0.480 mm (0.413-0.548 mm), respectively. Perineal patterns of *M. africana* (Fig. 9.16,B) and *M. oteifae* (Fig. 9.17,H,I) are very similar to each other, but different from those of *M. decalineata* and *M. megadora.* The larvae of *M. oteifae* have tails with narrow rounded ends (Fig. 9.17,K); larval tails of *M. africana* have wider rounded ends (Fig. 9.16,A).

Whitehead (1969) reports that in Africa *C. arabica* is occasionally attacked by *M. javanica and M. incognita;* and that *C. robusta* is occasionally attacked by *M. incognita. M. arenaria* and *M. hapla.* Lordello (1972) lists *M. incognita* and *M. inornata* as parasites of *C. arabica* in Guatemala. Flores and Yepez (1969)
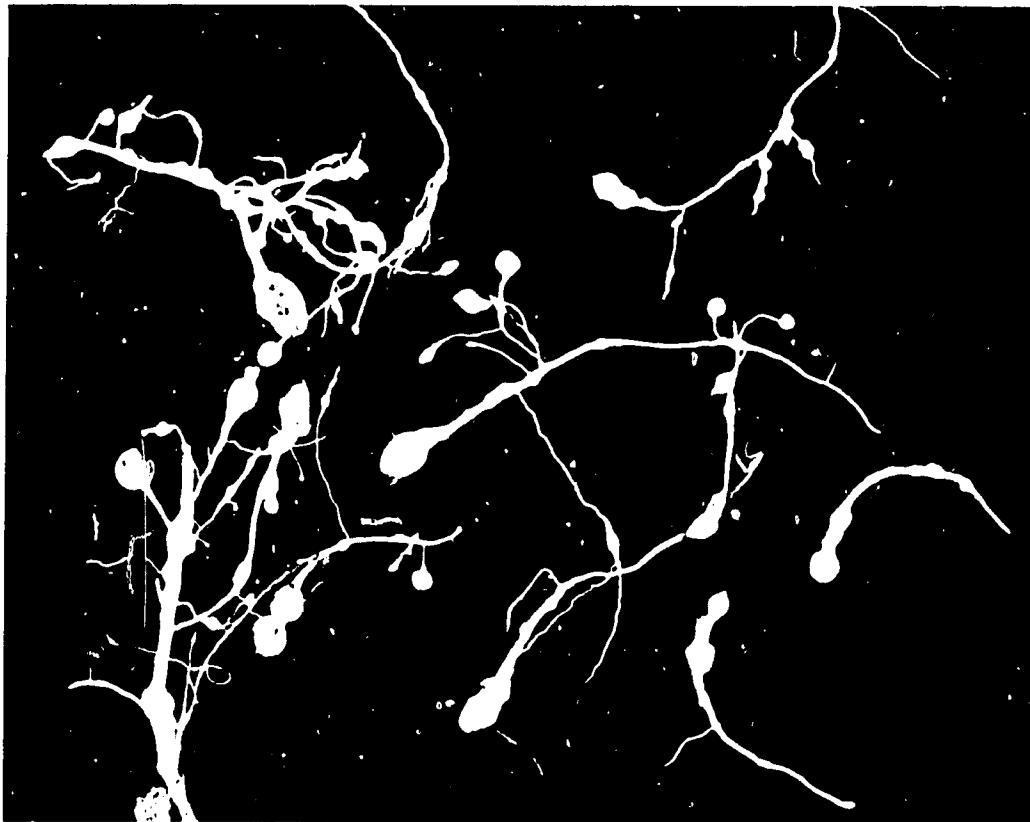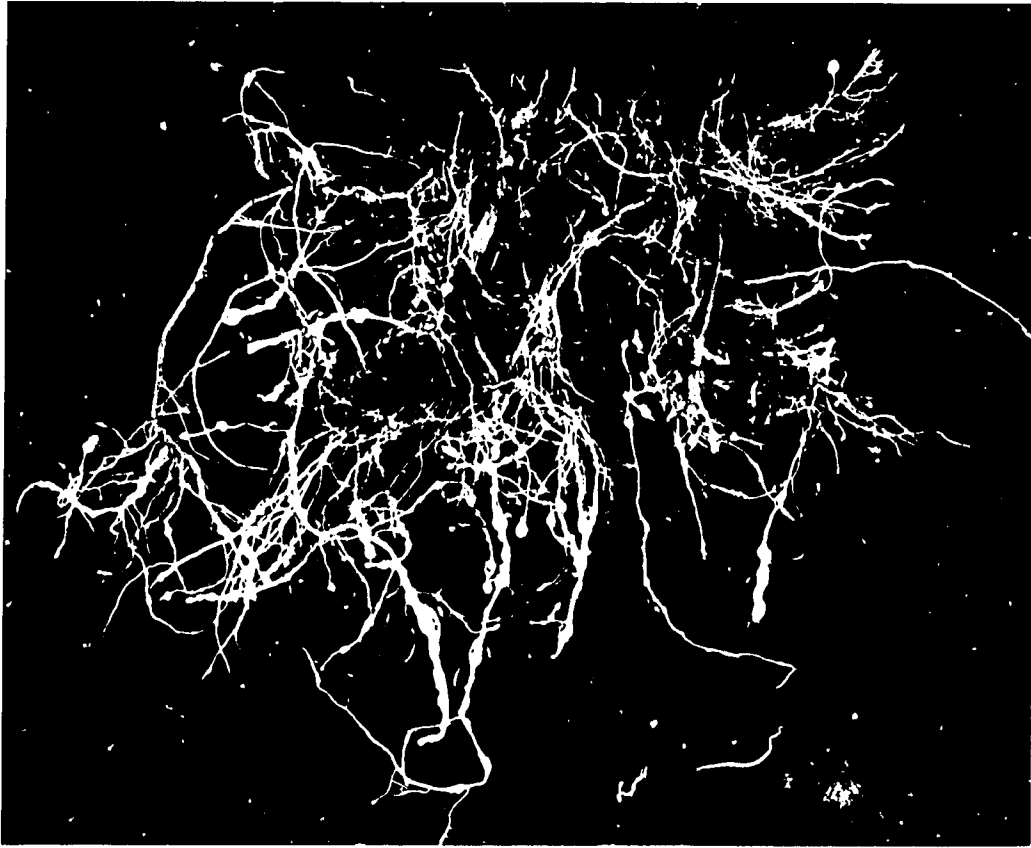
Fig. 9.12.   *Meloidogyne exigua* galls on *Coffea* sp. Many galls are terminal. The largest galls are about 4 mm in diameter. Specimens by courtesy of Dr. Paulo de Souza.
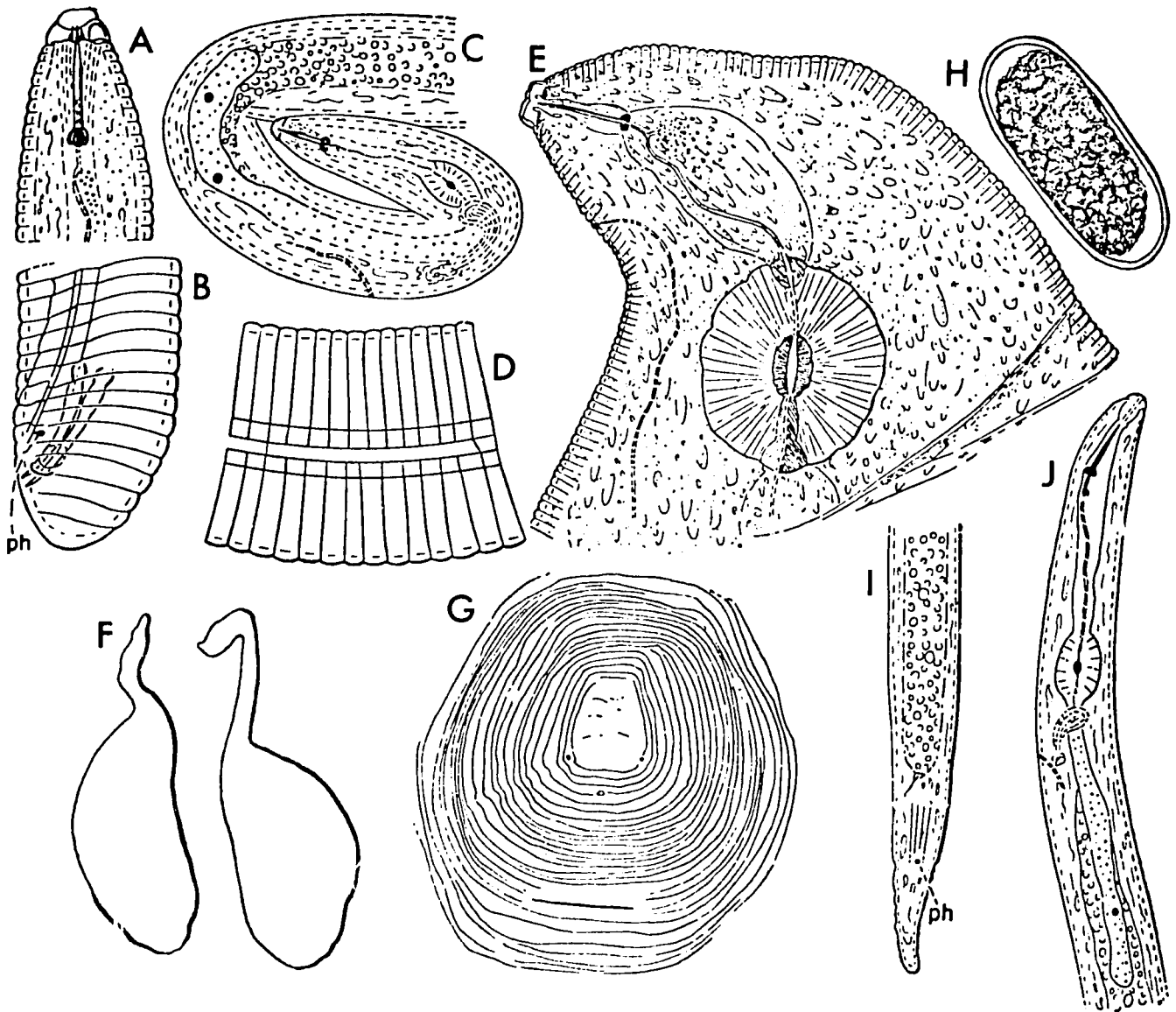
Fig. 9.13. *Meloidogyne coffeicola*. A-D: Male. E: Female anterior. F: Female body shapes. G: Perineal pattern. H: Egg. I, J: Larva. Female body shapes are distinctive and larval tail is 3 anal body widths long. Distinguished from *M. exigua* by the perineal pattern with striae between anus and vulva. Also by female body shapes and larval tails. Lordello and Zamith, 1960.

state that "Although *M. incognita* was also observed attacking coffee in the Eastern Region (of Venezuela), *M. exigua* is responsible for most of the infestations throughout the country."

## C. Species Infecting Other Woody Plants

### 1. *M. brevicauda*

*M. brevicauda* is a parasite of tea (*Camellia sinensis*) in Sri Lanka (Ceylon). The females are described as being globular with short necks if feeding on roots with soft tissue. In roots with corky cortical tissue and woody central cylinders, they have elongated bodies with narrow necks (Fig. 9.18,D) and are encased in tubular cavities with smooth slimy linings. The female excretory pore is opposite the stylet knobs, the neck is distinctly annulated, and the elongated perineal pattern is distinctive (Fig. 9.18, A-C). Median larval length of 0.525 mm (0.460-590 mm). The larval tail is about twice the anal body diameter, and the tip is broadly rounded. Larvae are coarsely annulated (Fig. 9.18, E-I).

71
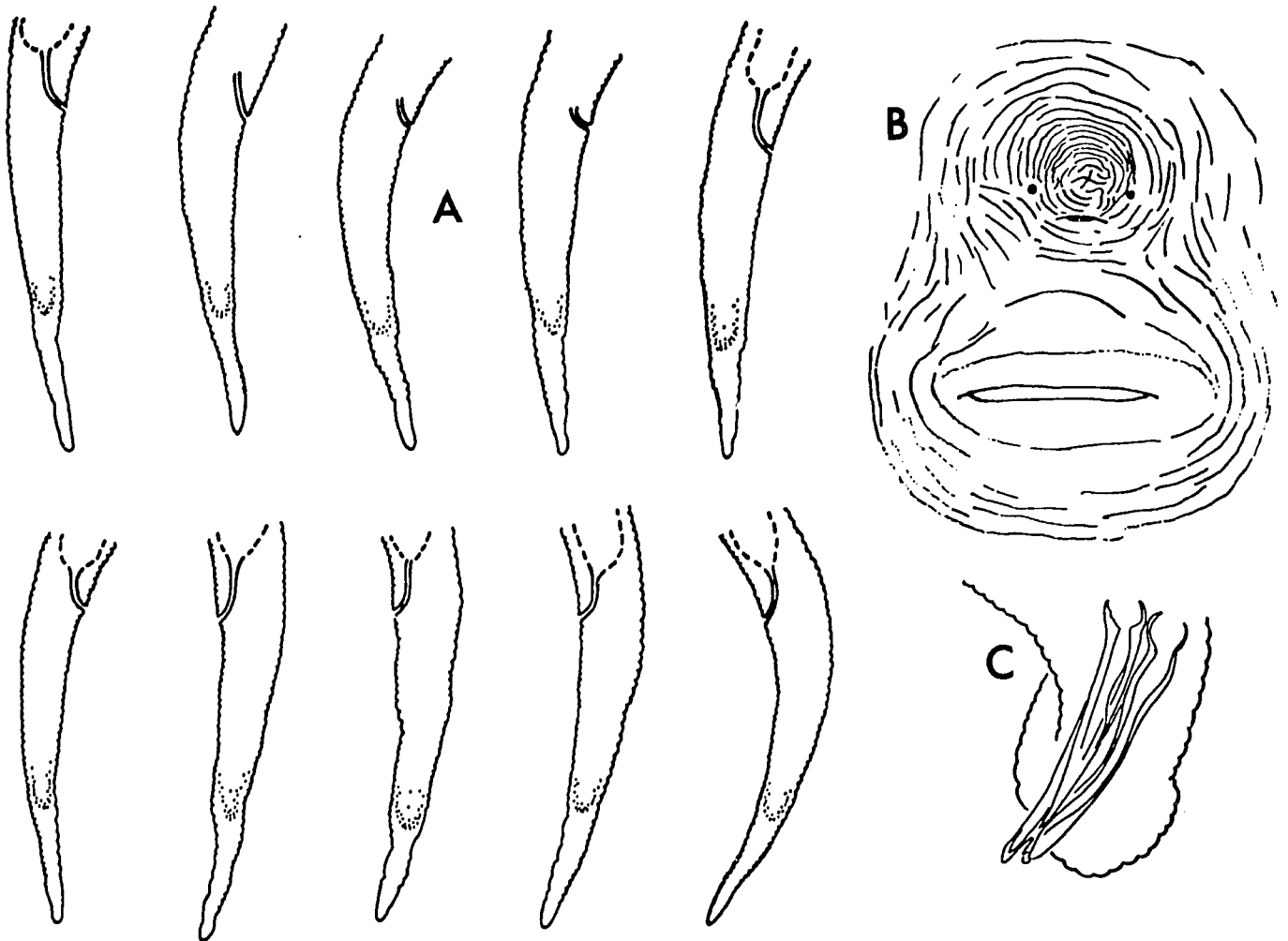
Fig. 9.14. *Meloidogyne decalineata.* A: Larval tails. B: Perineal pattern. C: Male posterior. Whitehead, 1968.

## 2. *M. indica*

*M. indica* is the only named *Meloidogyne* species described as reproducing on *Citrus* spp. The female body is described as saccate with a short neck. The perineal pattern is faint and composed of smooth striae, many of which are concentric around the tail terminus, others cross the area between anus and vulva (Fig. 9.19, F). The female stylet knobs are wide, either concave anteriorly or sloping posteriorly (Fig. 9.19,D,E). Larval tails are short, about 1½ to 2 times the anal body diameter (Fig. 9.19,A). The only known hosts are *Citrus* species.

## D. Species Attacking Gramineae

In warm climates, five species of *Meloidogyne* have been described from Gramineae. With their type hosts and localities, they are:

*M. spartinae: Spartina alterniflora* (smooth cordgrass), South Carolina (southern USA).

*M. acronea: Sorghum vulgare* (sorghum), South Africa.

*M. graminis: Stenotaphrum secundatum* (St. Augustine grass), Florida (southern USA).

*M. graminicola: Echinochloa colonum* (barnyard grass), Louisiana (southern USA).

*M. kikuyensis: Pennisetum clandestinum* (millet), Kenya.

The first three were formerly placed in the genus *Hypsoperine*, and the females have the ventrally located neck and perineal protuberance of that genus. *M. spartinae* has the longest larvae yet described for any *Meloidogyne* species; median length is 0.762 mm (0.612-0.912 mm). Larval lengths are 0.450 mm (0.440-0.460 mm) for *M. acronea* and 0.465 mm (0.420-0.510 mm) for *M. graminis*. The larvae of *M. spar-*
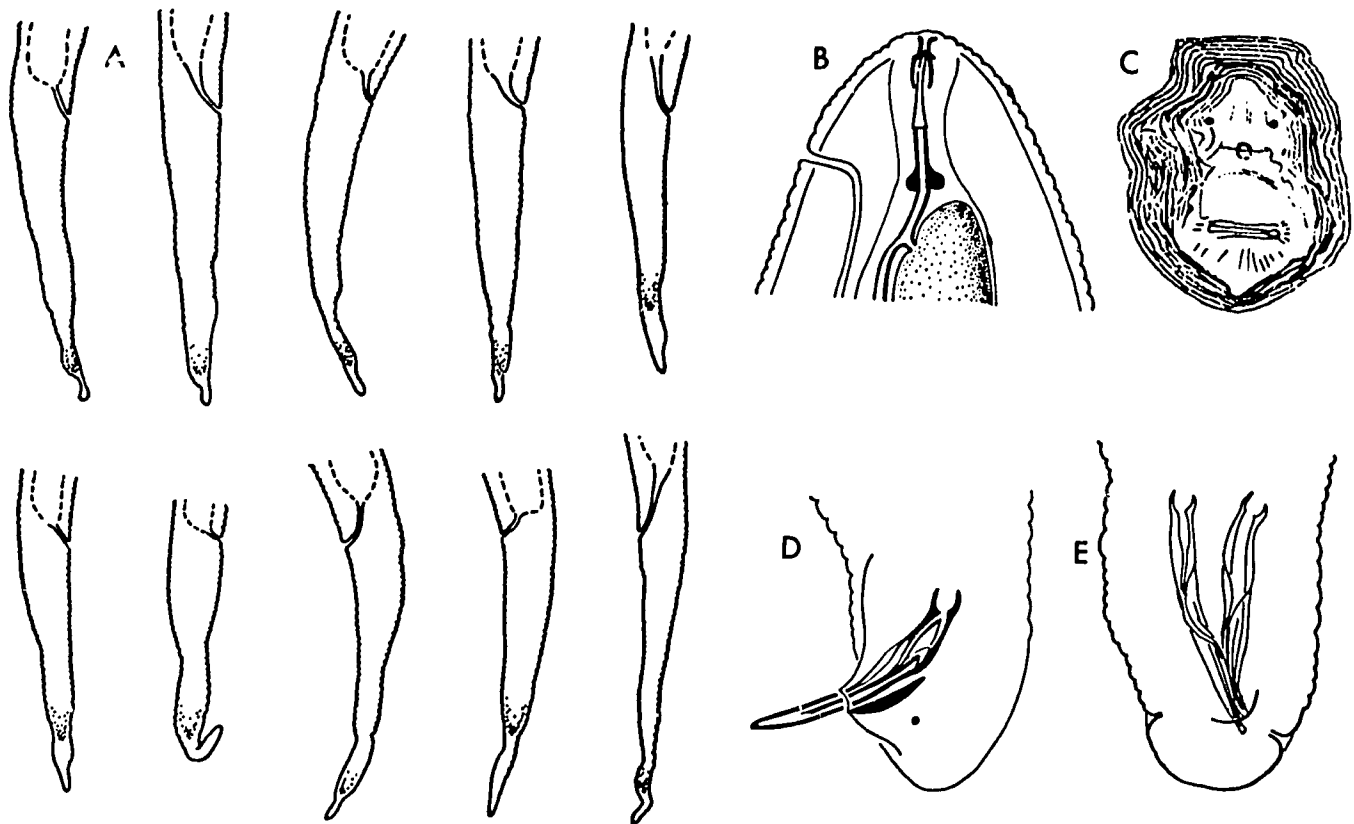
Fig. 9.15. *Meloidogyne megadora*. A: Larval tails. B, Female anterior. C: Perineal pattern, to be compared with Fig. 9.14, B. D, E: Male posterior. Whitehead, 1968.

*tinae* have long tails ending in an oval bulb and mucro (Fig. 9.20,G). The type host of this species is smooth cordgrass, which grows in tidal marshes where the sodium chloride content of the water is about 2%.

*M. acronea* and *M. graminis* are separated by the shapes and proportions of the larval tails; *M. acronea* tails (Fig. 9.21,E) have broadly rounded ends with a very much shorter hyaline portion than the tapering larval tails of *M. graminis* (Fig. 9.22,D). Tails of *M. acronea* larvae are 5½ times as long as the anal body diameter; the tails of *M. graminis* are 7½ anal body diameters long (Fig. 9.22,D). The female excretory pore of *M. acronea* is about three stylet lengths posterior to the apex of the head, compared to about one for *M. graminis* (Fig. 9.21,B and 9.22,B).

*M. kikuyensis* and *M. graminicola* can be separated by their respective larval lengths, 0.325 mm (0.290-0.360 mm) and 0.449 mm (0.415-0.484 mm). The larval tail of *M. kikuyensis* (Fig. 9.23,E,Q) is about 2½ times as long as the anal body diameter and tapers to a rounded point. The larval tail of *M. graminicola* is nearly 6 times as long as the anal body diameter (Fig. 9.24,E). The perineal pattern of *M. kikuyensis* has

distinctive cheek-like structures at both ends of the vulva. These are not present in *M. graminicola* (Figs. 9.23,T and 9.24,G).

*M. graminicola* is a common parasite of rice in India, Thailand and Laos; and 31 rice cultivars were infected in experiments in Louisiana (USA) (Golden and Birchfield, 1968). In Thailand it is found in rice seedbeds, which are usually kept wet but not flooded. Galls are often terminal on roots of rice seedlings, and seedlings are stunted. When transplanted in flooded paddies, infection does not spread to new roots (Taylor, 1968).

## E. Species Parasitic on Soybeans

*M. inornata* and *M. bauruensis* were described as parasites of soybeans in Brazil. *M. inornata* was found in the Campinas region of Brazil where soybean cultivars were tested for resistance. The species attracted attention when one of these, cv La-41-1219, which was highly resistant to *M. inornata* at Campinas was not resistant in the Bauru region where *M. incognita* occurs.
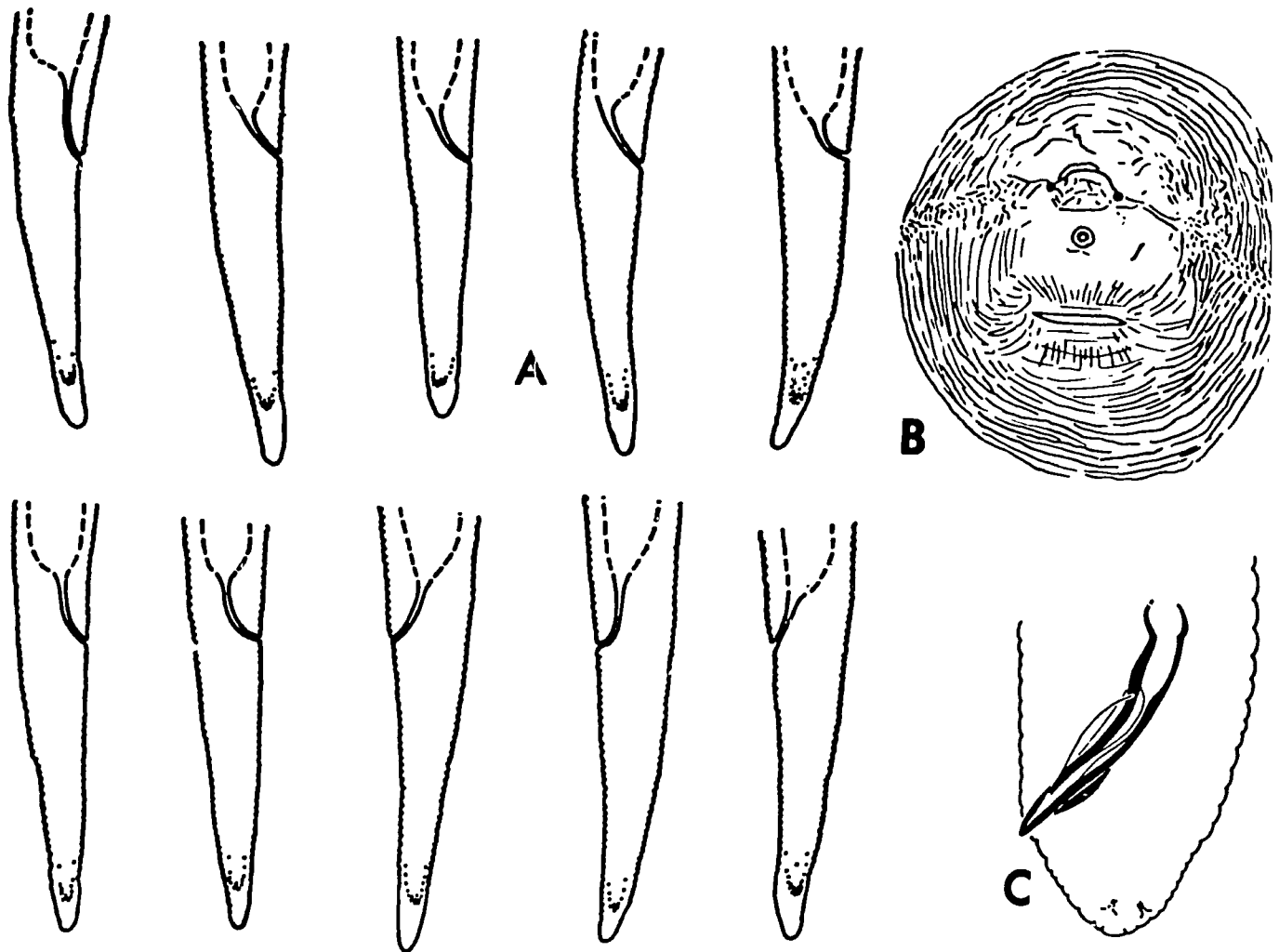
Fig. 9.16. *Meloidogyne africana.* A: Larval tails. B: Perineal pattern. C: Male posterior. Whitehead, 1968.

*M. inornata* is described as closely related to *M. incognita*, the most definite difference being the female excretory pore which is one stylet length posterior to the apex of the head in *M. incognita* (Fig. 9.1,D) and 2½ stylet lengths in *M. inornata* (Fig. 9.25,D).

*M. bauruensis,* in other soybean cultivar trials, attacked only cultivar Abura and was originally described as a subspecies, *M. javanica bauruensis.* The most distinctive characters are the female excretory pore one-half stylet length posterior to the apex of the head (Fig. 9.26,E) compared with 2½ stylet lengths for *M. javanica* (Fig. 9.3,A). Perineal patterns of *M. bauruensis* (Fig. 9.26,G) have lateral lines which are less evident than those of *M. javanica,* with striae often extending unbroken from the dorsal to the ventral sectors.

## F. Species Reported from Other Host Plants

### 1. *M. thamesi*

When Chitwood (1949) redescribed *M. arenaria,* he also described "a very similar nematode collected by W. H. Thames from ramie (*Boehmeria utilis*) in Florida (USA)." He illustrated this as "*M. arenaria* from *Boehmeria*" (Fig. 9.6,I-S). Later he made it a subspecies, *M. arenaria thamesi,* referring to "a vertical series of transverse marking" in the perineal pattern and the blunt tail of the larvae which tends to be bifid or trifid (Fig. 9.6,R,S) (Chitwood, Specht and Havis, 1952). This species has been identified in California (USA) on various hosts. Whitehead (1968) obtained a population from the type host and locality. He remarks that the patterns fitted Chitwood's
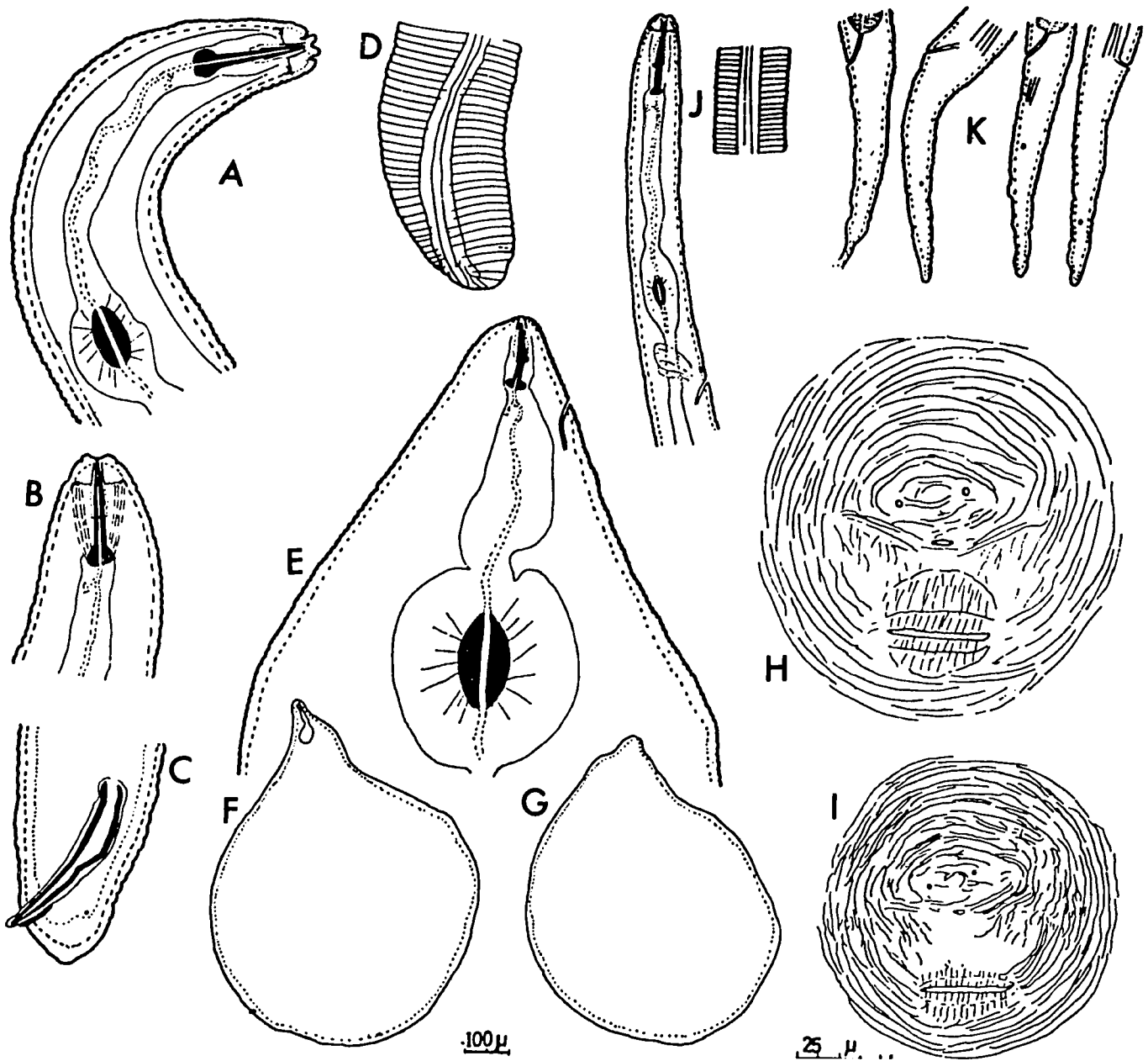
Fig. 9.17.  *Meloidogyne oteifae.* A-D: Male anterior, stylet, spicule and posterior. E-G: Female anterior and body shapes. H,I: Perineal patterns. J, K: Larvae. Elmiligy, 1968.

description and remained fairly constant during the period of culture. Di Muro (1971) reported that *M. thamesi* was next in importance to *M. incognita* in tobacco fields in Italy. Otherwise it has seldom been reported.

## 2.  *M. ethiopica*

*M. ethiopica* was described as a parasite of tomato in Tanganyika (Tanzania) and is reported to occur also in Rhodesia and South Africa. It is close to *M. arenaria* with perineal patterns (Fig. 9.27,F) very similar to that species, but the male head (Fig. 9.27,C,E) is more tapering and has two annules of equal length posterior to the lip region on the sublateral head sectors, compared to one wide and two small annules for *M. arenaria* (Fig. 9.5,A,B). "The spicules are thicker walled with strongly ridged shafts" (Fig. 9.27,D) Whitehead (1968).
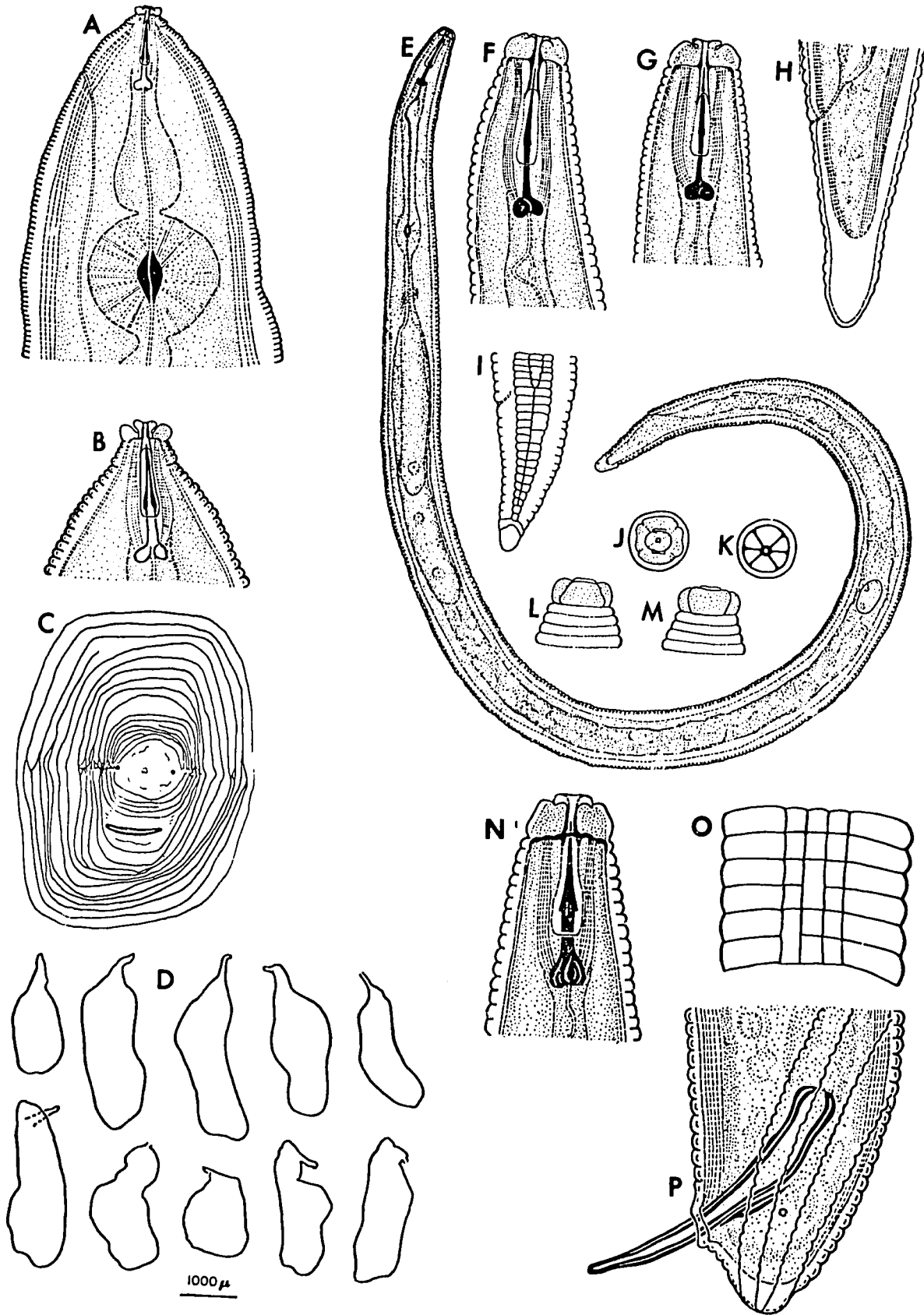
Fig. 9.18. *Meloidogyne brevicauda.* A, B: Female anterior. C: Perineal pattern. D: Female body shapes. E-M: Larvae. N-P: Male. The perineal pattern and short larval tail are distinctive. Loos, 1953.
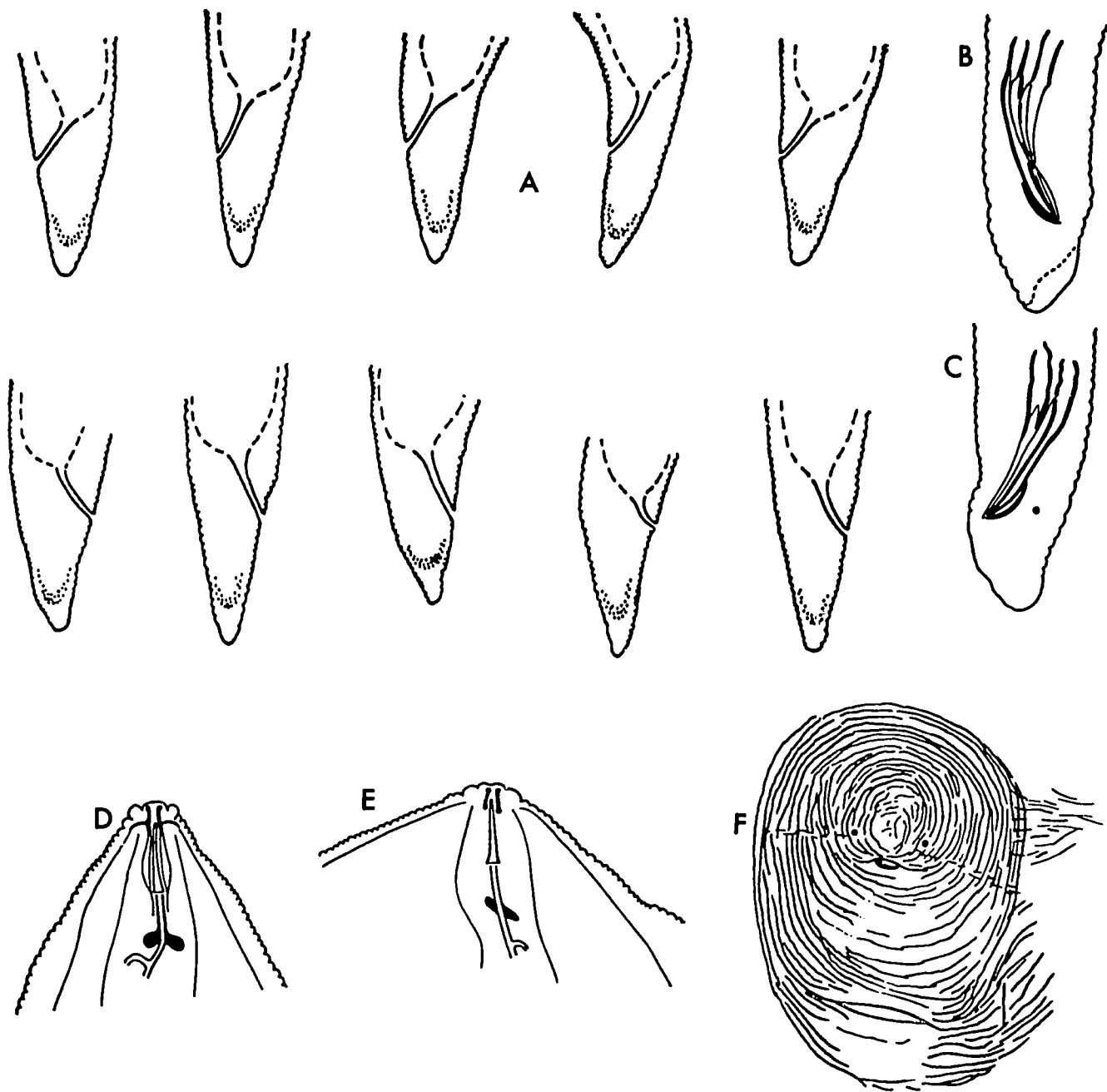
Fig. 9.19. *Meloidogyne indica.* A: Larval tails. B, C: Male posterior. D, E: Female anterior. F: Perineal pattern. Whitehead, 1968.
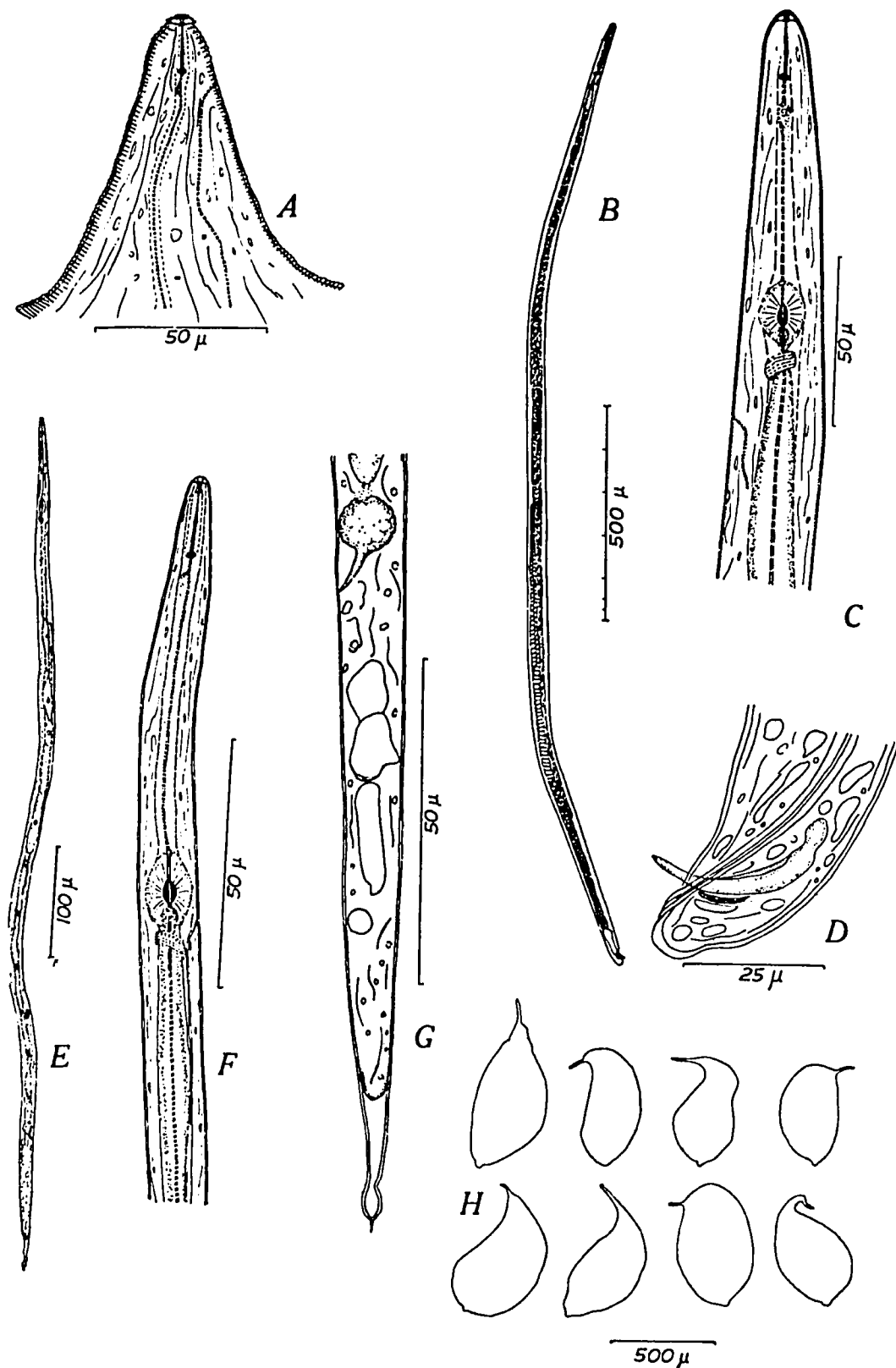
Fig. 9.20. *Meloidogyne spartinae.* A: Female anterior. B-D: Male. E-G: Larvae. H: Female body shapes. The larval tail is distinctive for length (about 9 times anal body diameter) and for the bulb and mucro at the end. Female body has ventrally located neck and posterior protuberance. Rau and Fassuliotis, 1965.
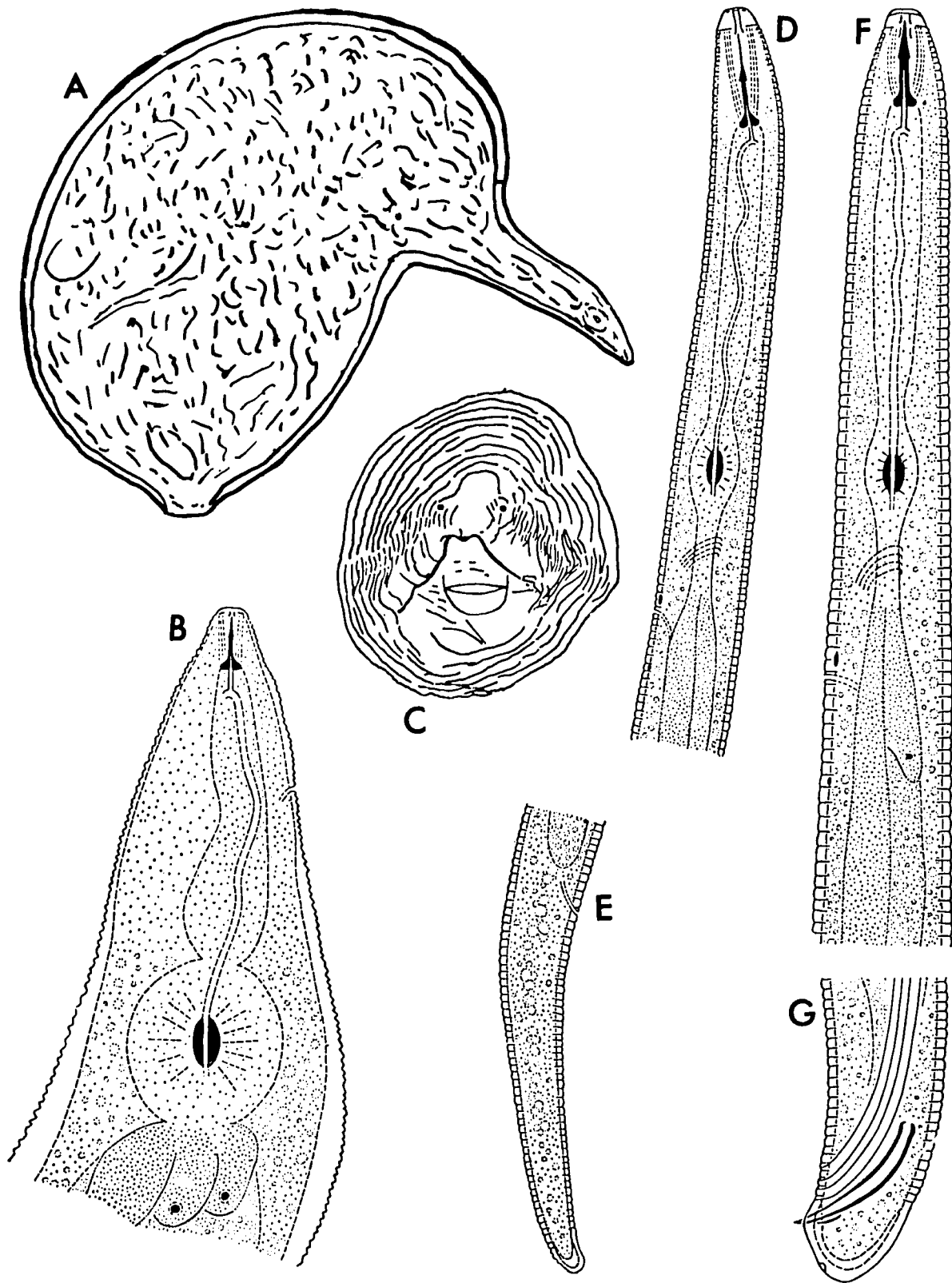
Fig. 9.21. *Meloidogyne acronea.* A: Female body shape (outline traced from photo). B: Female anterior. C: Perineal pattern. D, E: Larva. F, G: Male. Female has ventrally placed neck. Larval tail has broadly rounded end with very short hyaline portion. Coetzee and Botha, 1975. Perineal pattern from Whitehead, 1968.
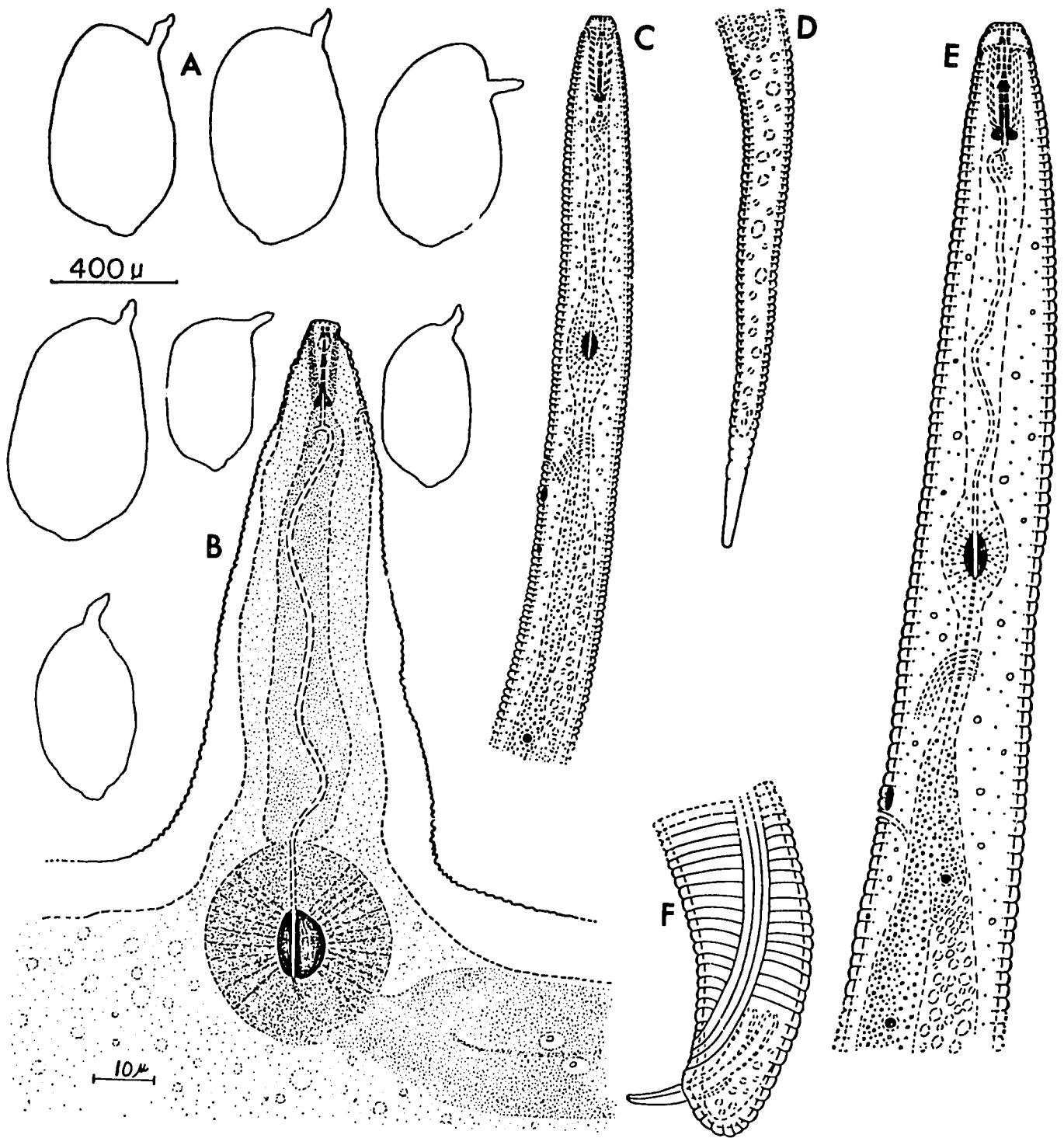
Fig. 9.22.   *Meloidogyne graminis*. A: Seven body shapes. Body is oval with a posterior protuberance. Position of neck is ventral. B: Female anterior. Note ventral position of esophageal glands. C, D: Larva. E,F: Male. Larval tail is 7½ times anal body diameter, has a long hyaline portion and narrow rounded tip. Sledge and Golden, 1964.
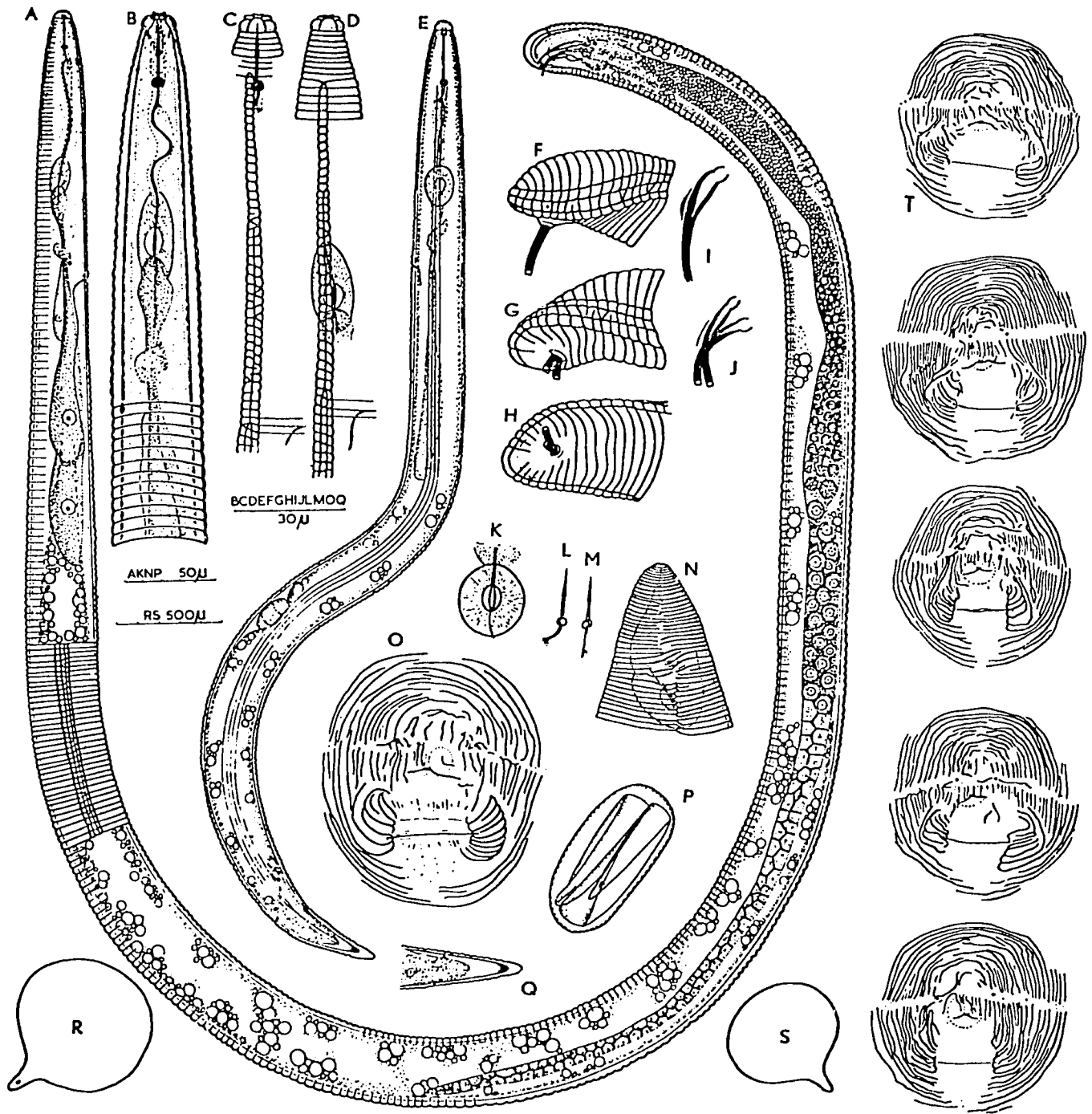
Fig. 9.23. *Meloidogyne kikuyensis*. A-D: Male anterior. E, Q: Larva. F-J: Male posterior and spicules. K-N: Female. O, T: Perineal patterns. P: Egg with larva. R, S: Female body shapes. The larval tail is about 2½ anal body diameters long. De Grisse, 1960.
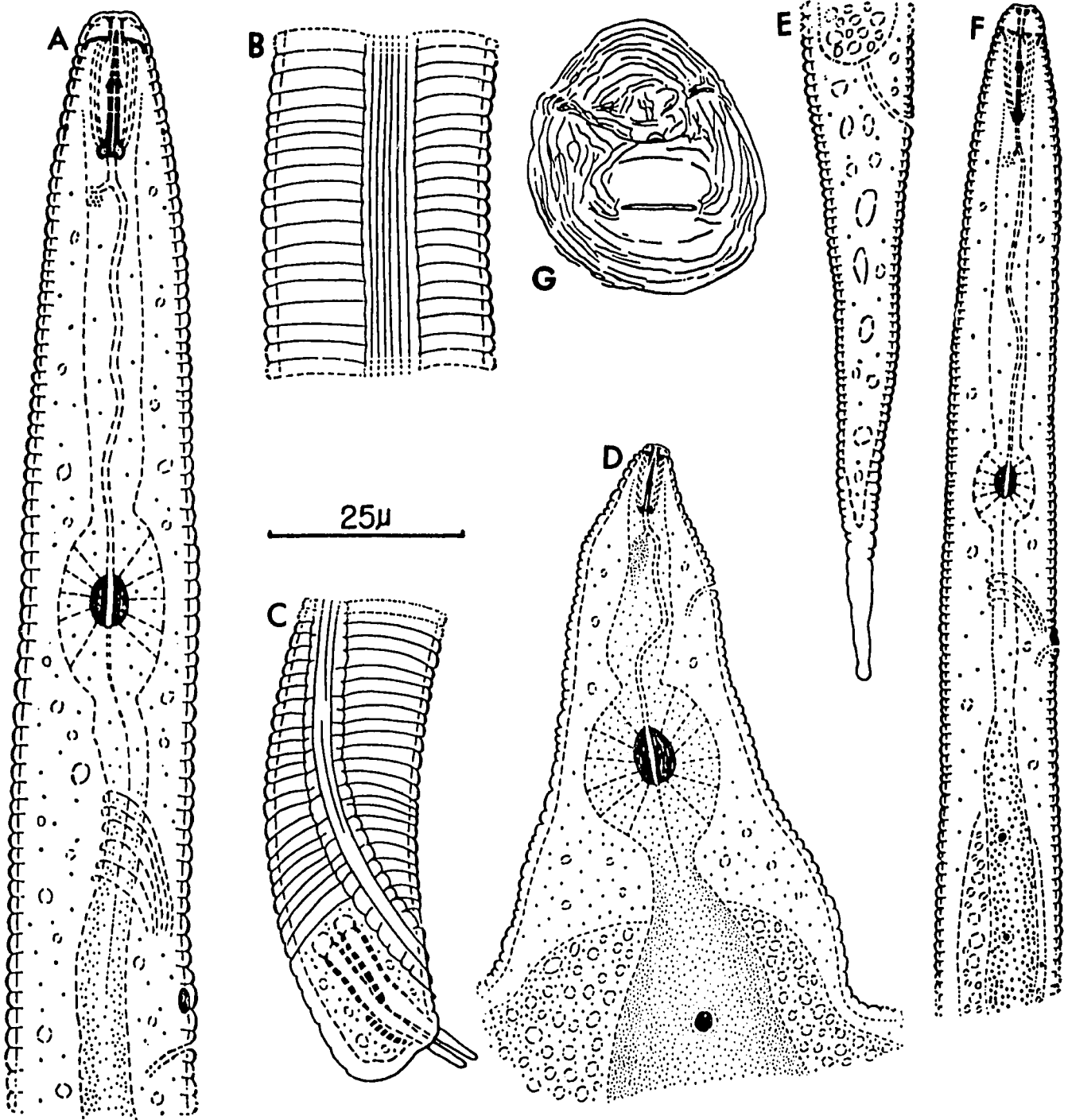
Fig. 9.24. *Meloidogyne graminicola*. A-C: Male anterior, lateral field and posterior. D: Female anterior. E, F: Larva G: Perineal pattern :Whitehead, 1968). Golden and Birchfield, 1965.
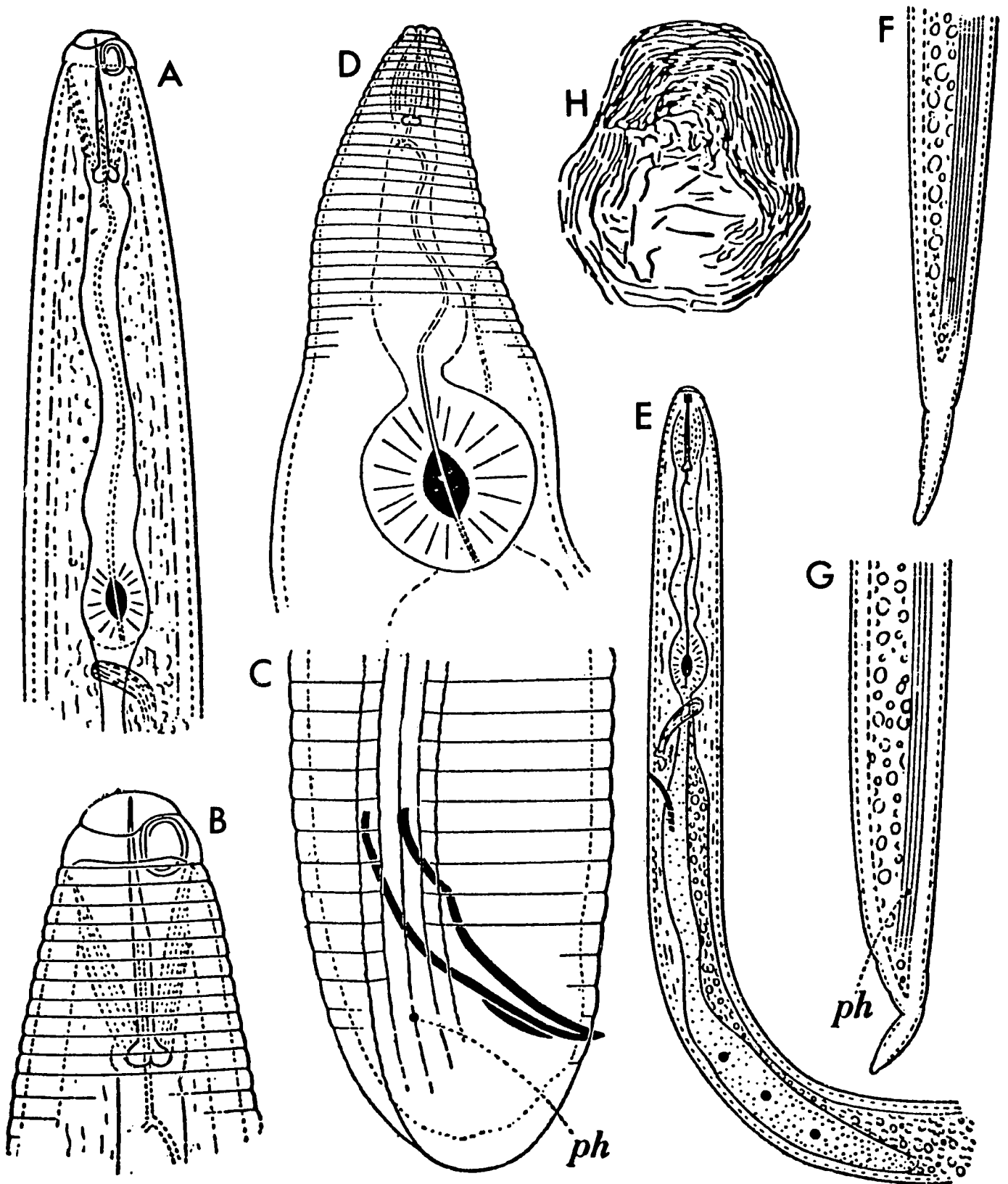
Fig. 9.25. *Meloidogyne inornata*. A-C: Male anterior, stylet and posterior. D: Female anterior. E-G: Larvae. H: Perineal pattern. Excretory pore of female is 2½ stylet lengths posterior to apex of head. Lordello, 1956a.
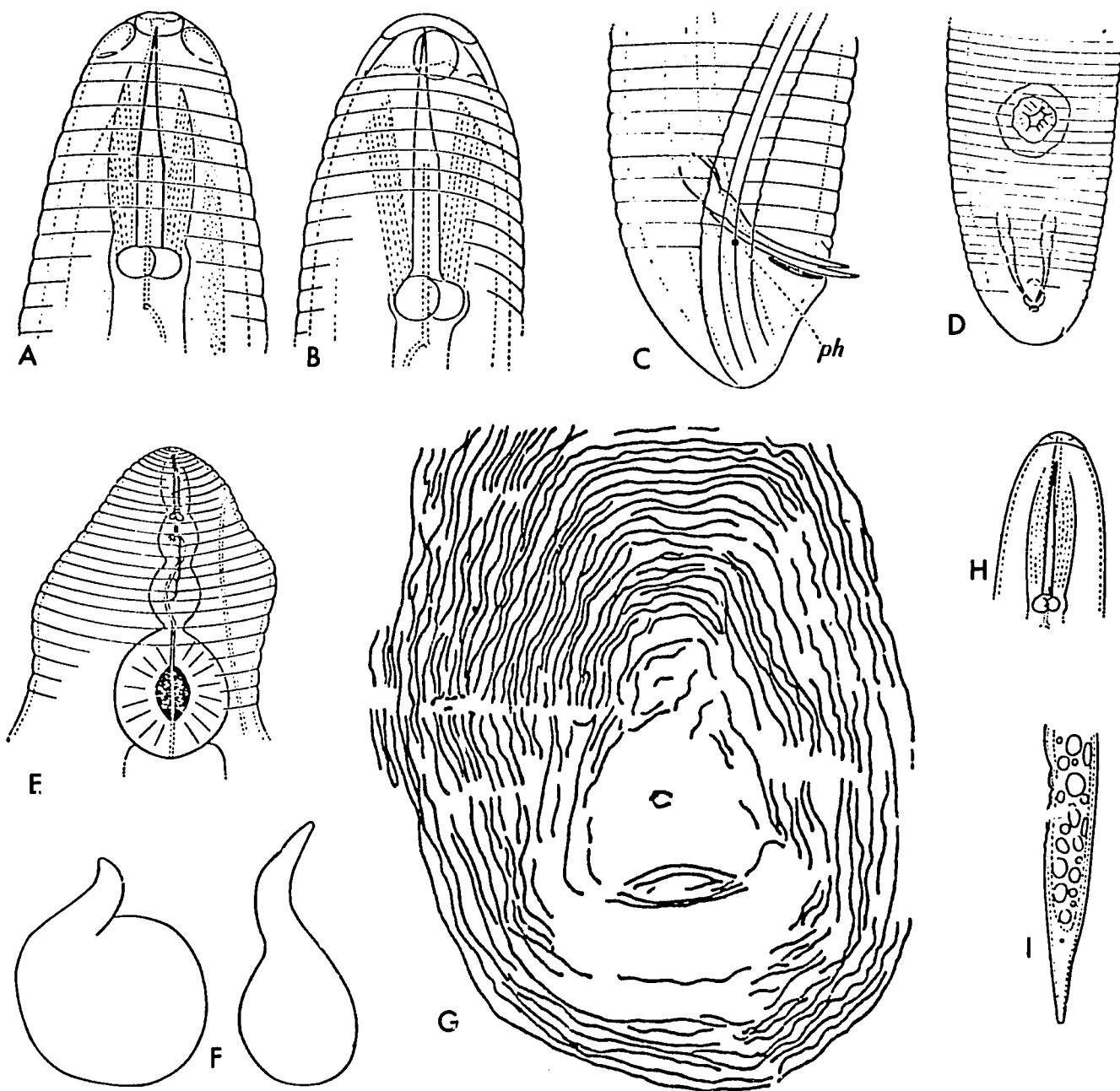
Fig. 9.26. *Meloidogyne bauruensis*. A-D: Male anterior and posterior. E: Female anterior. F: Female body shapes. G: Perineal pattern. H, I: Larva anterior and posterior. Lordello, 1956b.
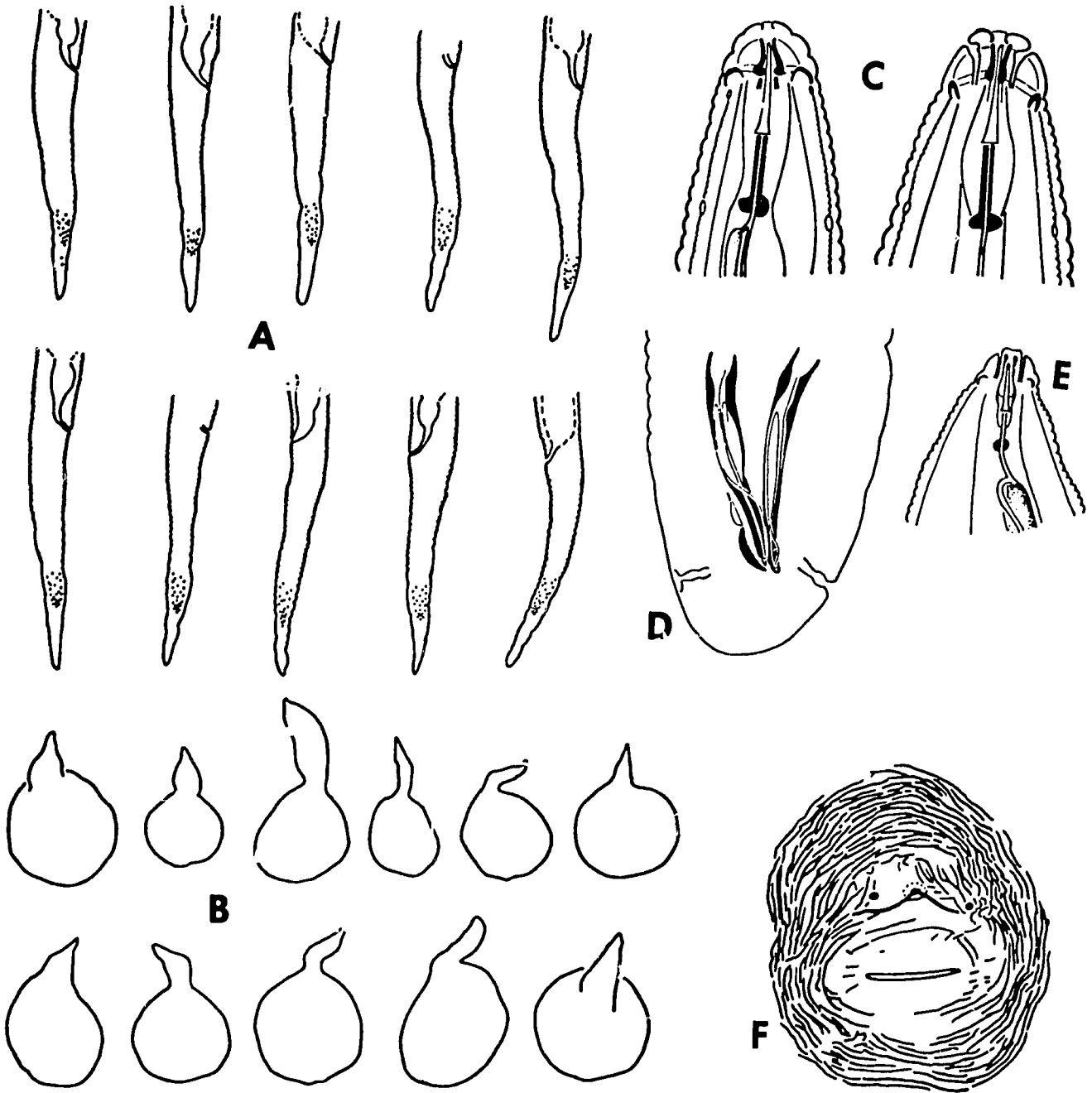
Fig. 9.27. *Meloidogyne ethiopica*. A: Larval tails. B: Female body shapes. C: Male anterior. D: Male posterior. E: Female anterior. F: Perineal pattern. Whitehead, 1968.
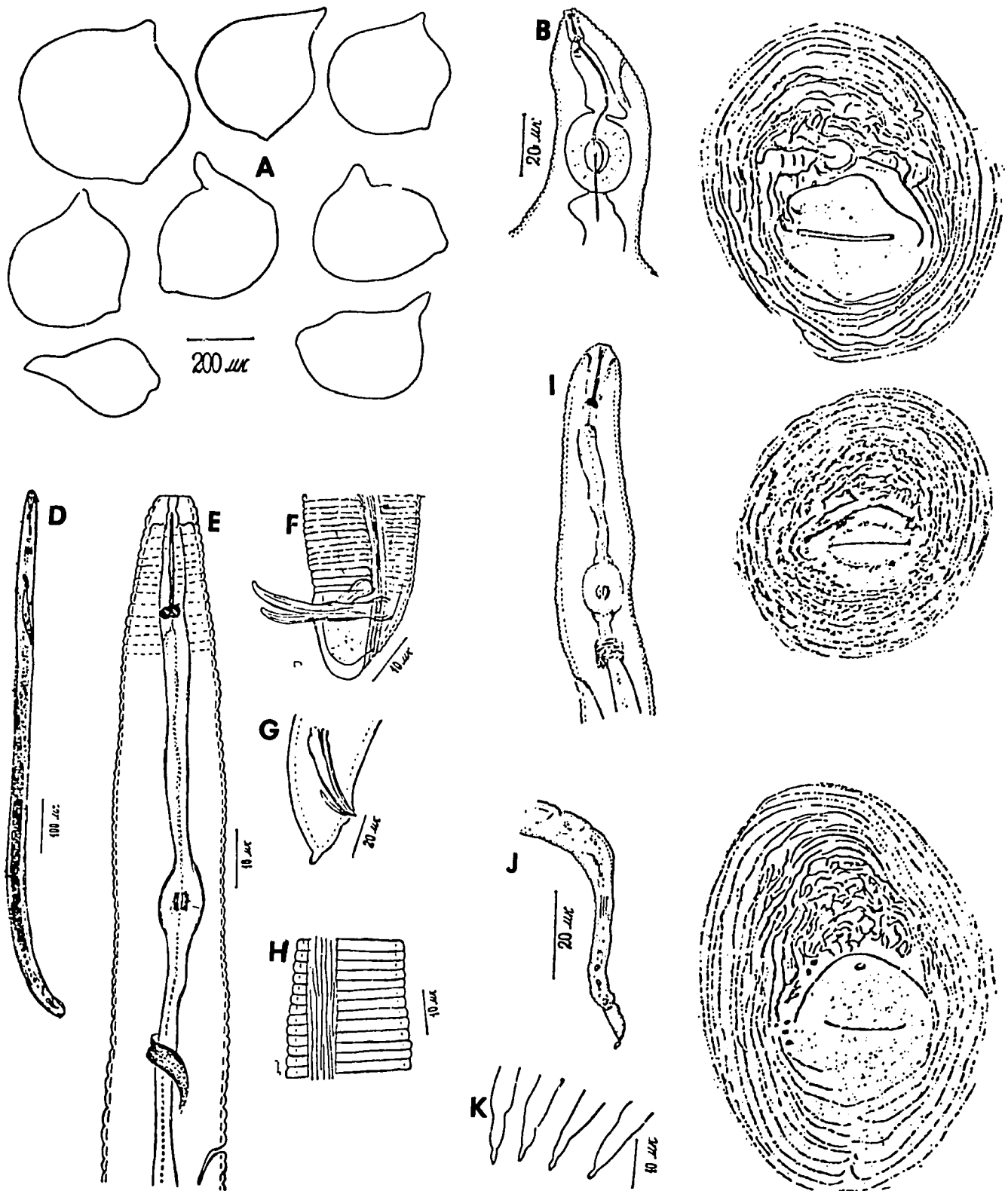
Fig. 9.28. *Meloidogyne megriensis*. A: Female body shapes. Seven of the eight have a short nec.· at an angle to the axis of the body, and a posterior protuberance. B: Female anterior. C: Perineal patterns. D-H: Male, full length, anterior, two posterior ends with different shapes, and lateral field. I-K: Larvae. This species was originally described in the genus *Hypsoperine*. Pogosyan, 1971.
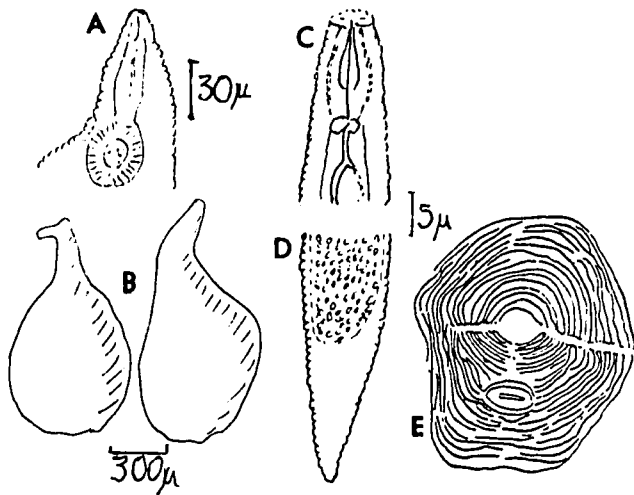
Fig. 9.29. *Meloidogyne lordelloi.* A, B: Female. C, D: Larva. E: Perineal pattern. Perineal pattern differs from *M. javanica* in having striae between anus and vulva. Da Ponte, 1969.

### 3. *M. megriensis*

*M. megriensis* was described as *Hypsoperine megriensis* on *Mentha longifol.* (mint) in Megri, Armenia SSR. Mature females have globular bodies with short ventrally placed necks and distinct perineal protuberances (Fig. 9.28,A). Perineal patterns are round to oval, and some have punctations surrounding the vulva and anus or rather thick broken striations on the dorsal arch (Fig. 9.28,C). One drawing of the male tail shows a distinct projection at the terminus, another does not (Fig. 9.28,F,G). Larval tails tend to have a bulbous end (Fig. 9.28,-J,K). Median length of larvae is 0.412 mm (0.358-0.467 mm), and the one illustrated has a tail about 7 anal body diameters long (Fig. 9.28, J).

### 4. *M. lordelloi*

*M. lordelloi* was described from Brazil as a parasite of cactus (*Cereus macrogon .*). Measurements of the species are similar to those of *M. javanica.* The perineal pattern is also much like that of *M. javanica*, but differs in having striae between the anus and vulva, and wide lateral lines which often do not reach the edges of the pattern (Fig. 9.29,E).

### 5. *M. lucknowica*

*M. lucknowica* was described as a parasite of the sponge gourd (*Luffa cylindrica*) at Lucknow, India. The female bodies illustrated vary greatly in shape (Fig. 9.30,B), and some have a slight protuberance of the perineal region. Perineal patterns have distinct lateral lines between the dorsal and ventral sectors,

with few or no striae extending unbroken from one sector to the other (Fig. 9.30,C). The incisures continue to the levels of the stylet. In these respects, the females resemble *M. javanica.* Larval length is 0.492 mm (0.410-0.575 mm), larval tails are of various shapes (Fig. 9.30,I), and larval stylet length (from point to posterior surface of knobs) is 0.014 mm (Fig. 9.30,F). The most definitive characters are six lateral lines in the middle of the male body (Fig. 9.30,E); the gubernaculum, which is heart-shaped in ventral view (Fig. 9.30,D); and the unequal lengths of the male spicules (Fig. 9.30,D,G).

## III. Summary

### A. Approach to Identification

Identification of *Meloidogyne* species is facilitated by consideration of everything known about a population, including location, climate, botanical relationships of the host plant, and what has been found previously under similar conditions and on related plants. This mental approach brings to mind a few probabilities which can be quickly checked by reference to original or other available descriptions.

Identification procedure: An efficient procedure for the less experienced nematologist, or one starting a survey in an unexplored region, is as follows: 1) Collect widely from the crop plants of the region, taking care to get good representative samples from each field. 2) Identify the root-knot nematodes by the North Carolina Differential Host Test as outlined in Appendix 1. This will provide identification of *M. arenaria, M. hapla, M. incognita* and *M. javanica*, the species most likely to be present It will also detect races and mixed infestations. The result will be identification of the principal species of the region and their host plants.

The Differential Host Test should be supplemented with careful study of the females, males and larvae by microscope. With practice in identification by microscope, the nematologist will soon be able to identify the species of most importance in his region. Populations which cannot be identified by the Differential Host Test c: n be compared with other species in the various groups of Tables 8.1 and 9.1.

### B. Identification Technique

To obtain "typical" perineal patterns or average larval lengths, it is necessary to examine a representative sample of a population. This sample should consist of nematodes from various parts of the field, and it is most important that at least 10 to 20 females or larvae be examined.
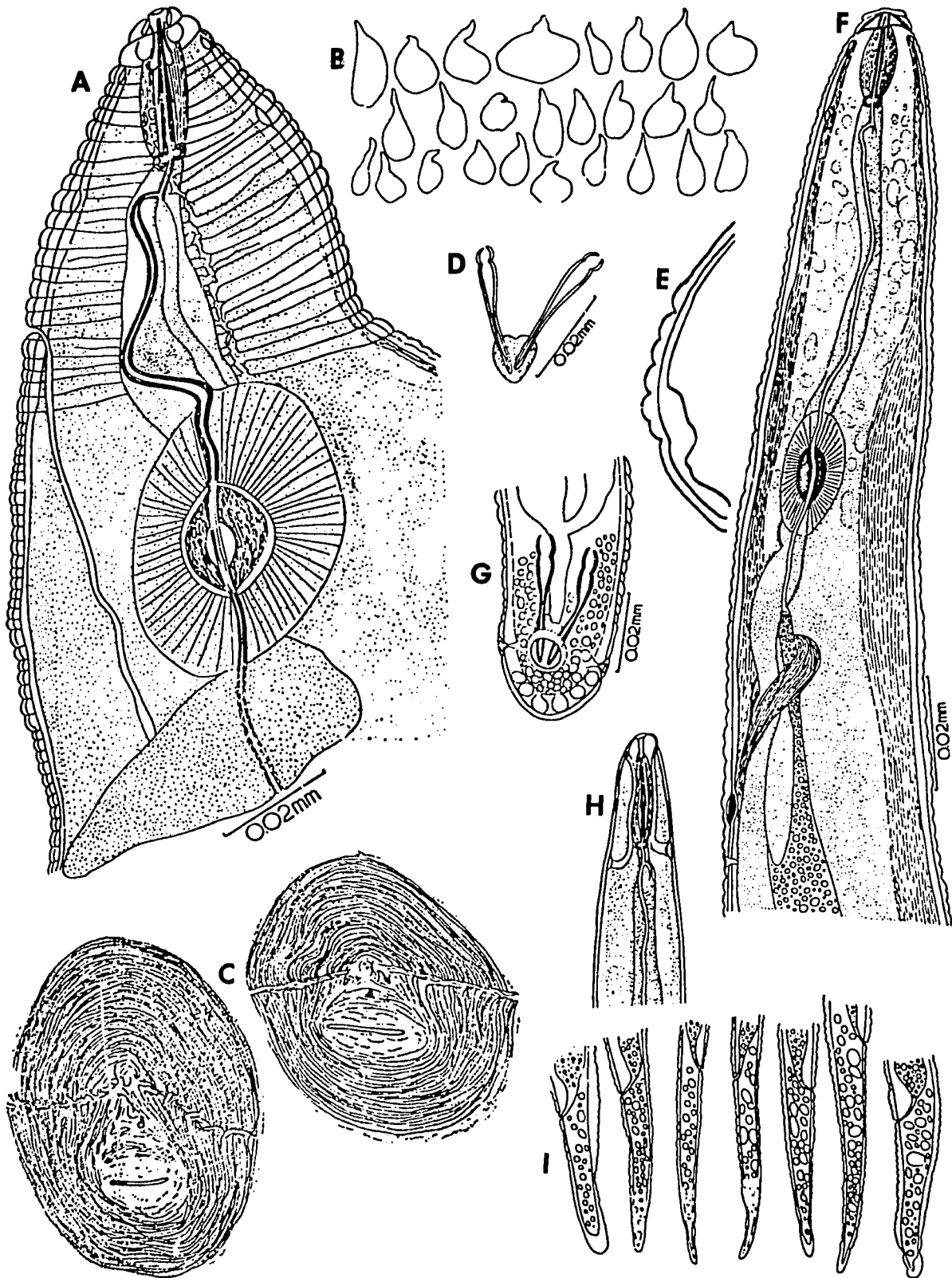
Fig. 9.30. *Meloidogyne lucknowica.* A: Female anterior. B: Female body shapes. C: Perineal patterns. D-G: Male, spicules and heart-shaped gubernaculum, cross section of lateral field showing 6 lateral lines, anterior and posterior, showing spicules of uneven length. H, I: Larva anterior and larval tail shapes. Singh, 1969.

# 10
# Integrated Control of *Meloidogyne* Species

## I. Introduction

The basic objective in control of *Meloidogyne* species is economic, to increase quantity and quality of yield of farm produce. The procedures always involve reduction of a nematode population, or making it much less infective than it would be otherwise. Usually, the nematode population is in the soil, but it may be in planting stock (transplants, tubers, corms) or in the roots of growing plants.

Since the objective is economic, care and calculation are needed to be sure that expenses, including money, labor, and loss of income due to changes in customary farm practices, do not exceed the value of probable benefits. Because of farming risks, such as unfavorable weather, diseases, pests, or a poor market, the expected benefits should exceed the expenses by a ratio of at least three to one, and preferably more.

## II. General Principles for Control of *Meloidogyne* Species

### A. Soil

Before planting a crop susceptible to a *Meloidogyne* species, the soil population of eggs and infective larvae of that species should be reduced as much as is economically feasible. Infectivity of soil populations can be reduced by use of nematicides as discussed in Chapter 11; by crop rotations, and sometimes by special methods, such as flooding, or drying the soil by repeated cultivation during dry seasons.

### 1. Crop Rotation

*Meloidogyne* species are obligate and specialized parasites. Mobility is limited to short distances; only a small proportion move as far as 50 centimeters from the place where they are hatched. Movement of larvae through the soil is difficult, and movements are random until within a few centimeters of a root. Larvae are hatched with a limited supply of energy food and cannot infect plants when this is exhausted in their search for a root in which they can feed and reproduce (Wallace, 1973). A *Meloidogyne* population in a field which contains no host plants will become non-infective and die sooner or later of starvation.

In crop rotation for control of *Meloidogyne* species, susceptible crops are rotated with immune or resistant crops. Usually the susceptible crop is the most

profitable and the rotation crops less profitable.

For example, tomatoes are a profitable crop but susceptible to all the common species of *Meloidogyne*. After a tomato crop is harvested, the root-knot nematode population of the soil is high. A second crop of tomatoes would be severely damaged. If the nematode species present is not *M. hapla* or Race 1 of *M. arenaria*, a tomato crop can be followed by peanuts without risk of damage to the peanuts. While the peanuts are growing, the nematodes cannot reproduce. Instead, many of the larvae in the soil die or become non-infective because of starvation and the attacks of predators, fungi and diseases. If the population is reduced sufficiently, tomatoes can be grown again after peanuts without serious injury.

In a field infested with Races 1 or 2 of *M. incognita*, *M. arenaria*, *M. javanica* or *M. hapla*, cotton can be used as a nematode-resistant rotation crop.

Many other combinations of crops may also be used. The first and most important requirement is that the rotation crops be immune or highly resistant to the species and race of *Meloidogyne* present.

The second requirement is weed control. *Meloidogyne* species can reproduce on many weeds, and their presence in the field can prevent success of a rotation.

A rotation for control of *Meloidogyne* species should be planned so that the nematode population is at its lowest level when the principal, or most profitable and most susceptible, crop is planted. This crop grows well because it is not heavily attacked early in the growing season; but at the end of the season, the nematode population will have increased through several generations. The population is again reduced by growth of an immune or highly resistant crop. With good choice of resistant rotation crops and good weed control, the reduction is enough so that the profitable susceptible crop can be grown the next season.

Immune or highly resistant rotation crops may be naturally immune. Peanuts, for example, are immune to all known races of *M. incognita*, to *M. javanica*, and to Race 2 of *M. arenaria*, but not to *M. hapla*. Cotton is highly resistant to all of the common root-knot nematodes except Races 3 and 4 of *M. incognita*.

Cultivars of crop plants may also be immune or resistant, selected or developed for resistance by plant breeders. Lists of cultivars reported to be resis-

tant are given in Tables 7.1 and 7.2. These lists should be used with caution. Identification of races of *M. incognita* and *M. arenaria* (Chapter 5) introduces a different concept of resistance. Cultivars reported resistant to these species may not be resistant to all races. On the other hand, cultivars not reported resistant may in fact be resistant to one or more of the races.

Selection of immune or resistant plants for use in nematode rotations involves consideration not only of their effect on the nematode population, but also of their agronomic and economic advantages and disadvantages. Many of the factors to be considered may be unknown for the regions in which cooperators of the International *Meloidogyne* Project are working. Cooperators can make important contributions by experiments with crop rotations. There are many variations and questions that can be answered only by field trials with crops which are saleable on local markets. The data from such trials should include estimates of *Meloidogyne* populations, yield, and economic data.

In the great majority of rotations for control of *Meloidogyne* species, growth of the most profitable crop is possible in alternate years, or every third year, with no advantage for longer rotations.

## 2. Effects of Crop Rotation on Crop Yields

Experiments in Florida (USA) resulted in reduction of populations of *M. incognita*, *M. javanica* and of sting nematode (*Belonolaimus longicaudatus*) after growing hairy indigo (*Indigofera hirsuta*). Snap beans grown in the plots had average yields of 8923 kg/ha compared to 3387 kg/ha following sorghum (*sorghum bicolor*), and 5592 kg/ha following sesbania (*Sesbania macrocarpa*). In another experiment, yield of cucumbers averaged 27,187 kg/ha after hairy indigo compared with 1585 kg/ha after sorghum, and 1220 kg/ha after sesbania (Rhoades, 1976).

On soil in Florida infested with both *M. incognita* and the soybean cyst nematode, *Heterodera glycines*, rotation with corn (*Zea mays*) and soybean cultivar Hampton (resistant to *M. incognita*) did not increase yields of soybean cultivar Pickett beyond an average of about 53% of yields on nearby experimental plots. This was due to the presence of *H. glycines* and illustrates the difficulty of control of two species of nematodes by rotation.

## B. Special Methods for Control of *Meloidogyne* Species

### 1. Flooding

Where water is abundant and fields are level it is sometimes possible to control *Meloidogyne* species by flooding the land to a depth of 10 cm or more for several months. Flooding does not necessarily kill the eggs and larvae of root-knot nematodes by drowning. It does inhibit infection and reproduction on any plants which grow while the field is flooded. Flooding experiments are best evaluated by measuring yields of a subsequent crop, not by survival of larvae. Larvae may survive flooding but not be infective.

### 2. Desiccation

In some climates, the *Meloidogyne* population of fields can be reduced by plowing at intervals of two to four weeks during the dry season. This exposes eggs and larvae to desiccation, and many in the upper layers of soil are killed. This may be sufficient to increase a subsequent susceptible crop significantly.

### 3. Antagonistic Plants

As discussed previously, larvae of *Meloidogyne* species which enter the roots of certain immune plants die in a few days. This suggests that use of such antagonistic plants in rotations would be more effective than plants which do not kill larvae. Among the plants which have been tested are *Tagetes* spp. (marigold), *Chrysanthemum* spp., and *Ricinus communis* (castor bean).

These antagonistic plants were compared with fallow in glasshouse tests for control of *M. incognita*. Root-knot indexes (0 to 5 scale) of bioassay plants after 90 days were: marigold 1.0, castor bean 1.9, chrysanthemum 0.5 and fallow 0.4. When tomatoes were grown with the antagonistic plants, average root-knot indexes increased to 3.4. All of the plants used have been shown to contain toxins which can kill nematodes. In this experiment, all were resistant to *M. incognita* but not more effective than fallow in reducing the population in 90 days.

No evidence that marigold, chrysanthemum or castor bean kill nematodes in soil was found; populations in tomato plants grown with antagonistic plants were not different from populations in tomato grown alone (Hackney and Dickerson, 1975)

Belcher and Hussey (1977) in their review of previous literature point out that the effect of *Tagetes* (marigold) species on populations of *Meloidogyne* species is highly variable, depending on the combination of species and possibly cultivar of *Tagetes* and the species and probably race of *Meloidogyne*. Their own experiments indicated that reduction of populations of *M. incognita* by *Tagetes patula* was primarily due to an antagonistic or trap-crop effect. Second-stage larvae entered roots but

there was no giant cell formation and a hypersensitive necrotic reaction. In 12 weeks, populations in flats in a glasshouse were reduced 97% by marigold, but only 70% by peanut (*Arachis hypogaea*) which is also a non-host plant.

## C.  Planting Material

*Meloidogyne* species are not found in true seed; they may be found in "seed potatoes" or other vegetative material used for planting, such as corms, bulbs or roots. It is possible to kill the nematodes in some planting material by use of chemicals (Table 10.1), or by hot-water treatment (Table 10.2). Both methods must be used with care since too much heat or exposure to chemicals can damage growth. Often, it is better and safer to discard infected material than to use heat or chemicals.

For transplanting, only seedlings grown in nematode-free seedbeds should be used. Any of the nematicides listed in Table 11.1 can be used in seedbeds with good results, but there are some advantages in using fumigants containing methyl bromide (MBr). When applied to seedbeds at rates of about one pound (453 grams) to 10 square yards (8.4 square meters), methyl bromide kills plant-parasitic nematodes, soil insects, bacteria, fungi and most weed seeds. The treatment is more expensive than use of other nematicides but produces clean, healthy seedlings and eliminates hand weeding of seedbeds.

If seedlings for transplanting are obtained from commercial growers, they should be examined for root-knot symptoms before purchase. When nursery stock of perennials is obtained, it should be examined even more carefully for evidence of nematode damage.

## III.  Integrated Programs for Control of Nematodes

### A.  Annual Crops

The principal crops of North Carolina are tobacco, corn (maize), peanuts, soybeans and cotton. Tobacco has the highest value, averaging more than $6,000 per hectare. The Research and Extension Services of North Carolina State University have developed a very advanced integrated program for control of plant-parasitic nematodes, diseases, and pests of tobacco.

The principles and procedures used in development of this program are useful in *Meloidogyne* control elsewhere, and for other annual crops:

1) The first step was a survey to determine the distribution of *Meloidogyne* species in the state. It was found that *M. incognita* was in practically every tobacco field, and that *M. hapla, M. arenaria* and *M. javanica* were much less common.

2) Field trials of cultivars of the principal crops of the state were conducted to determine resistance to the above four *Meloidogyne* species. An important part was rotation experiments to determine effects of resistant cultivars on *Meloidogyne* populations.

3) A plant breeding program for development of

Table 10.1.   Control of *Meloidogyne* species by dipping planting material in nematicides.

| Plant | *Meloidogyne* Species | Nematicide Common and Trade Names | Concentration in parts per million and percent of active ingredient | Time of Submersion | Reference |
|---|---|---|---|---|---|
| *Actinidia chinensis* (Kiwi fruit, Chinese gooseberry) | *M. hapla* | ethoprop (MOCAP) | 1000 ppm 0.1% | 60 min | Dale and van der Mespel 1972 |
| *Cornus florida* (dogwood seedlings) | *M. incognita* | fensulfothion (DASANIT) | 1000 ppm 0.1% | 15 min | Johnson, et al., 1970 |
| *Gladiolus* sp. (gladiolus corms) | *M. incognita* | fensulfothion (DASANIT) or ethoprop (MOCAP) | 600 ppm 0.06% 900 ppm 0.09% | 15 min 15 min | Overman, 1969 |
| *Prunus persica* (peach roots) | *M. incognita* | fensulfothion (DASANIT) | 1000 ppm 0.1% | 30 min | Ponchillia, 1973 |
| *Rosa* sp. (rose roots) | *M. hapla* | ethoprop (MOCAP) or fenamiphos (NEMACUR) | 1000 ppm 0.1% 1000 ppm 0.1% | 30 min 30 min | Dale, 1973 |

Table 10.2. Hot-water treatments for control of root-knot nematodes[1] in planting material.[2] Suggested temperatures and times of submersion.[3]

| Planting material | Temp. °C | Time min. | Reference |
|---|---|---|---|
| *Begonia* tubers | 48 | 30 | Gillard, 1961 |
| | 45 | 60 | Ibid. |
| *Caladium* tubers | 50 | 30 | Rhoades, 1964, 1970 |
| *Dioscorea* spp. (yam tubers) | 51 | 30 | Hawley, 1956 |
| *Fragaria chiloensis* (strawberry roots) | 52.8 | 5 | Goheen and McGrew, 1954 |
| *Humulus lupulus* (hop rhizomes) | 51.7 | 5 | Maggenti, 1962 Maggenti and Hart, 1963 |
| *Ipomoea batatas* (sweet potato) | 46.8 | 65 | Anon., 1968 |
| | 50 | 3-5 | Martin, 1970 |
| *Prunus avium* (cherry rootstocks) | 50-51.1 | 5-10 | Nyland, 1955 |
| *Prunus persica* (peach rootstocks) | 50-51.1 | 5-10 | Nyland, 1955 |
| *Rosa* sp. (rose roots) | 45.5 | 60 | Martin, 1968 |
| *Solanum tuberosum* (potato tubers) | 46-47.5 | 120 | Martin, 1968 |
| *Vitis vinifera* (grape rootstocks) | 51.7 | 5 | Meagher, 1960 (preferred) |
| | 57.2 | 2 | Ibid. |
| | 47.8 | 30 | Lear and Lider, 1959 |
| | 50 | 10 | Ibid. |
| | 51.7 | 5 | Ibid. |
| | 52.8 | 3 | Ibid. |
| | 52 | 5 | Moller and Fisher, 1961 |
| *Zingiber officinale* (ginger rhizomes) | 45-55 | 10-50 | Colbran and Davis, 1969 |

1. Any species of the genus *Meloidogyne*.
2. In any hot-water treatment, the combination of temperature and time which controls the nematodes may also damage the planting stock. To avoid damage, careful control of temperature is needed. Dormant planting stock is less likely to be injured.
3. Before using any treatment on large quantities of valuable material, it should be tested on a small scale with careful observation of treated material for damage. Differences in heat tolerance of cultivars are always possible.

tobacco cultivars resistant to Race 1 of *M. incognita* and to the diseases black shank, Fusarium wilt, Granville wilt, and mosaic was started. This program is still in progress.

4) Tobacco seedbed procedures to reduce occurrence of root knot and other soil-borne diseases, such as tobacco mosaic, were developed.

5) Crop schedules to include tobacco as often as feasible were devised.

6) Extensive trials were made of all available nematicides in combination with rotations, resistant cultivars, and sanitary practices to obtain maximum profits.

7) A service for identifying *Meloidogyne* and other plant-parasitic nematodes in soil samples from farms was organized.

8) Throughout the whole program, the Extension Service arranged the maximum possible publicity, always stressing advantages and profits. Field trials were conducted in cooperation with farmers in various parts of the State, and farmers in the vicinity were invited to "field days" to see the results.

9) This integrated program has been of immense value to the state of North Carolina. On problem fields where the average yearly income without control is $1,047 per hectare: 1) sanitary procedures, such as plowing up tobacco roots at the end of the season, add $711; 2) rotation with resistant crops adds $1,104; 3) use of resistant tobacco varieties adds $939; and 4) use of soil chemicals (particularly combined nematicides and insecticides) another $1,378. The total increase is $4,132 per hectare, and the total value is $5,179 or almost five times as much as without nematode and disease control.

The following aspects of the North Carolina program for control of nematodes on tobacco should

be emphasized:

1) The principal nematode species and races present must be identified.

2) All available cultivars of crops grown locally should be tested to measure reproduction of the species and races during the growing season in the field.

3) A program for breeding root-knot-resistant cultivars of the principal and most profitable crop should be initiated if possible. Resistant cultivars should be obtained from other countries.

4) If any of the crops are transplanted, experiments with nematicides in seedbeds are necessary.

5) Using information and experience obtained by field experiments, an integrated program of nematode control should be developed. This may involve rotations, nematicides, resistant cultivars, soil management practices and marketing procedures, and should be directed toward maximum profits for the farmers.

6) The program requires participation of research workers, extension agents, farmers, marketing specialists and administrators. A very important part of the program is development of the interest of those who can contribute.

## B. Perennial Crops

### 1. Soil

Control of *Meloidogyne* species in soil for planting of perennial plants in orchards, vineyards, plantations (banana, coffee, tea, sugarcane) is more difficult than control for annual crops. The principal difference is depth of treatment with nematicides, especially when replanting where the same crop has grown before. More nematicide and different application methods are used. (Taylor and Sasser, 1978).

### 2. Planting Stock

Transplants of perennials are usually obtained from commercial nurseries. Before purchase, the root systems should be carefully examined for knots caused by *Meloidogyne* species and for lesions caused by *Pratylenchus* species. If either are found, the stock should not be purchased.

If knots or lesions are found after purchase, the roots should be treated with nematicides (Table 10.1) or with hot water (Table 10.2). Root-knot-resistant or immune rootstocks should always be used if available.

At present, there is no chemical control for *Meloidogyne* species on roots of perennials which have been planted and have become established. Eventually the problem may be solved by use of systemic nematicides.

Extra care, such as frequent light irrigation and applications of fertilizer, may help maintain growth and delay decline of root-knot-infected perennials.

Immune cover crops will delay decline. Cover crops susceptible to the same *Meloidogyne* species infecting the perennials will cause increased damage.

## IV. Biological Control

### A. Reviews

Sayre (1971) and Webster (1972) have reviewed the literature on biological control of plant-parasitic nematodes. Both authors discuss various soil organisms antagonistic to nematodes.

Predators include fungi, nematodes, turbellarians, enchytraeids, insects and mites. Parasites include viruses, protozoa, bacteria and fungi.

### B. Predacious and Endozoic Fungi

Two types of fungi kill nematodes, nematode-trapping and endozoic parasitic. The trapping fungi capture nematodes by means of adhesive networks, adhesive knobs attached to the hyphal network by short lateral branches, and hyphal rings, some of which constrict to capture nematodes which try to pass through. Among the fungus genera, some of the best known are *Arthrobotrys*, which has constricting rings and adhesive networks, and *Dactylella*, with adhesive knobs and loops. The nematode-trapping fungi apparently produce a toxin which kills the nematode. The fungi then invade its body.

The endozoic parasitic fungi which infect *Meloidogyne* species and other plant-parasitic nematodes have spores which adhere to nematode cuticle and germinate, forming tubes which penetrate into the body. A common example is *Catenaria anguillulae*.

Nematode-trapping and nematode-parasitic fungi are common and perhaps plentiful in many agricultural soils. Their influence on populations of plant-parasitic nematodes under natural conditions is very difficult to measure. Usually the only indication is a small percentage of individuals recently killed or not yet dead in soil samples processed by centrifugal flotation techniques. Those seen are only a small proportion of those affected; the nematodes killed only a day or two previously have already disintegrated.

Numerous attempts have been made to use fungi for biological control of plant-parasitic nematodes. Such attempts seldom succeed if only a fungus culture is added to soil. If very large amounts (10 to 150 metric tons per hectare) of organic matter are also added, results are better. The organic matter

may change the soil environment in ways which stimulate the fungi, the decomposing organic material may produce substances directly toxic to the nematodes, or the abundance of organic matter may cause a large increase in the population of free-living nematodes feeding on bacteria, followed by an increase in enemies of nematodes which attack both free-living and parasitic species.

Since the fungi are often present in soils, it is logical to add the organic matter without the fungus culture. This is economical on small and valuable plots. On a field scale, other control methods are less expensive than obtaining the large amounts of organic matter and distributing it over the soil (Webster, 1972).

An important factor which is seldom considered in biological control is the enormous reproduction potential of *Meloidogyne* species. In a glasshouse experiment, larval populations of *M. arenaria* on red clover increased from 100 per pot to 180,000 in 15 weeks. This figure is nearly consistent with a 12X increase for three generations. *Meloidogyne* females seldom produce less than 500 eggs; a 12X increase can occur if only about 2.5% of the larvae live to reproduce (Chapman, 1963).

## C. Predacious Nematodes

Predatory nematodes attacking other nematodes have been reported in the genera *Mononchus, Mononchoides, Butlerius, Anatonchus, Diplogaster, Tripyla, Seinura, Dorylaimus* and *Discolaimus*. Those which have been observed in the laboratory have killed many nematodes each day.

## D. Predacious Arthropods

Tardigrades, collembola and mites have been observed feeding on nematodes in the laboratory.

## E Predacious Worms

A turbellarion (small flatworm) feeds on nematodes and other microscopic animals. Enchytraeids have also been reported as feeding on nematodes, but there is little evidence that they feed on plant parasites.

## F. Protozoan Parasites

The best known protozoan parasite of nematodes is *Duboscqia* penetrans Thorne, 1940 (Fig. 10.1). It was

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*Incorrectly spelled "Duboscquia" in nematological literature, including Thorne's "Principles of Nematology" (1961). Spelling "Duboscqia" as in the original description of the species (Thorne, 1940) is correct and must be used in
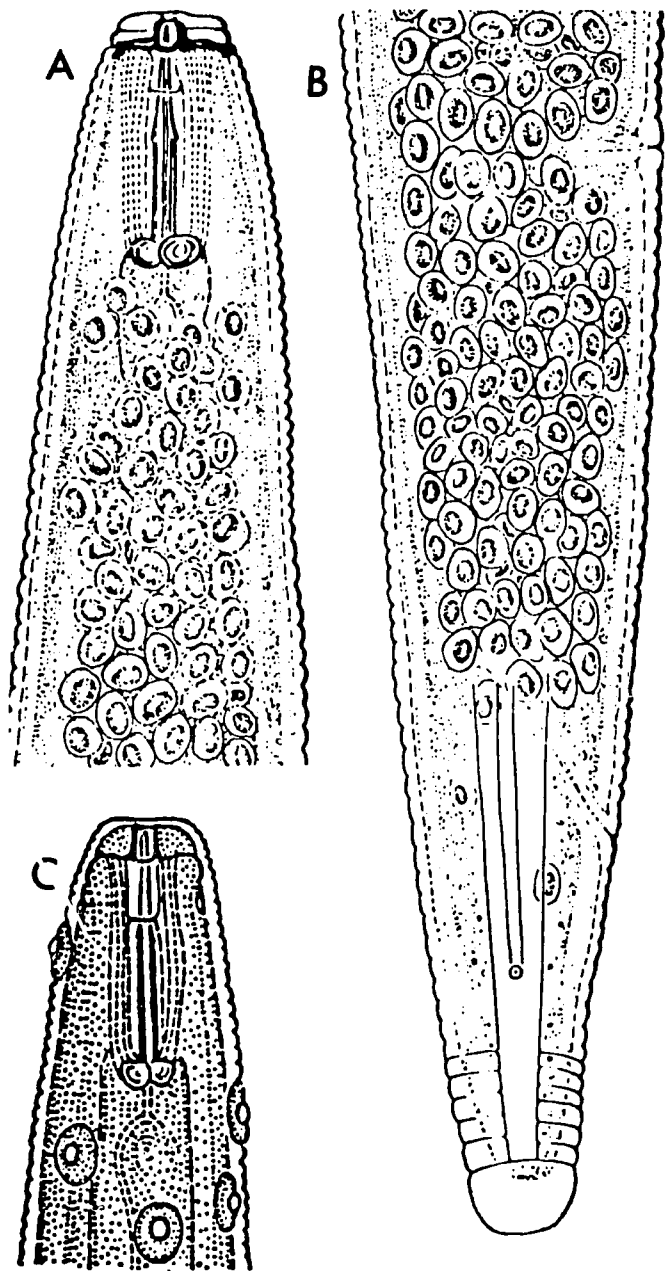


Fig. 10.1. *Duboscqia penetrans.* A, B: In *Pratylenchus pratensis.* Thorne, 1940, C: On the cuticle of *Radopholus gracilis.* Thorne, 1961.

believed to be a protozoan, but was redescribed as *Bacillus penetrans* by Mankau (1975). The life cycle was described by Mankau and Imbriani (1975). The dome-shaped spores are often seen on *Meloidogyne* larvae, and females containing large numbers are sometimes found. Another somewhat similar parasite is shown in Fig. 8.12,R.

accordance with Article 32(a) of the International Code of Zoological Nomenclature. The word is derived from a personal name Duboscq to which is added the suffix -ia as recommended by Appendix D, Section VI, 37 of the Code.

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## V.  Other Control Methods

### A.  Quarantine

Large-scale methods of controlling *Meloidogyne* species by quarantine to prevent entry of infected planting material are effective only if there is an efficient organization for inspection and identification. Quarantines which regulate growth of highly susceptible crops on infested fields are more effective, but only if the species has not yet spread to all of its potential distribution area. Eradication campaigns are expensive and often fail (Oostenbrink, 1972).

### B.  Electricity

Various attempts have been made to kill *Meloidogyne* larvae in soil by electricity. Following apparent success, a series of experiments in Rhodesia made it clear that power required to control nematodes in the field would be far beyond the capabilities of farm equipment (Stokes and Martin, 1954).

Ultrahigh frequency (UHF) electromagnetic energy killed *Rotylenchulus reniformis* in the upper 10 cm of soil but had no effect at 15 cm. The killing was apparently due to heating of the soil (Heald and Wayland, 1975).

# 11
# Nematicides

## I. Introduction

Nematicides* are chemicals used for control of plant-parasitic nematodes. Widespread use started about 1950 and has increased to a business estimated at nearly $100,000,000 per year. Table 11.1 includes nematicides which are widely available.

Farmers use nematicides to increase crop values per hectare, that is, to increase profits. Increase in crop value may be due to a larger yield; usually there is also an increase in quality and a larger percentage of the produce can be sold (Fig. 11.1). Nematodes are killed in the process, though not always immediately. The effect on the crop is similar if they are only made non-infective.

The principal use of nematicides is for control of nematode populations in soil before planting annual crops. Application of nematicides to soil is called "treating the soil" or "soil treatment," and "treated" soil is often compared with "untreated" soil. Some nematicides are also used to kill nematodes infecting planting material.

### A. Economics of Nematicides

Nematicides are relatively expensive, and their application requires labor and specialized equipment.

*Also spelled nematocide. Nemacide is a synonym which is seldom used.

Very early in the development of nematicide markets, it became evident that it was not possible to kill all the nematodes in farm soil and that attempts to do so by increasing the amount (dosage) per hectare was not an efficient use of nematicides or the farmer's money. Recommended application rates are now calculated to produce the largest number of dollars increase in crop value (quantity and quality) for each dollar invested in nematicides and their application. When growers use nematicides, they expect an increase in crop value of at least three or four times the investment.

### B. Soil Fumigants

The older nematicides are liquids which are injected beneath the soil surface. They evaporate to produce fumes which kill nematodes and are called "soil fumigants." Fumes from soil fumigants diffuse through the soil, are dissolved in soil water, and enter nematode bodies through the cuticle.

### C. Non-fumigants and Systemics

Newer nematicides are water soluble and are called "non-fumigant" nematicides. Non-fumigant nematicides are distributed through the soil by percolation of water and also enter nematode bodies through the cuticle.

### Table 11.1 Nematicides available on world markets*

| Common Name | Registered Trade Name Manufacturer | Chemical name | Formulation and Classification |
|---|---|---|---|
| Aldicarb | (TEMIK) Union Carbide Corp. | 2-methyl-2-(methylthio) propionaldehyde 0-(methylcarbamoyl) oxime | Granular nematicide/insecticide |
| Carbofuran | (FURADAN) Niagara Chem. Div., FMC Corp. | 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methycarbamate | Granular and flowable nematicide/insecticide |
| Chloropicrin | Great Lakes Chem. Corp. | trichloronitromethane | Liquid fumigant nematicide/insecticide |
| DBCP* | (FUMAZONE) Dow Chem. Co. (NEMAGON) Shell Dev. Co. | 1,2-dibromo-3-chloropropane | Emulsifiable and non-emulsifiable liquid nematicide |
| 1,3-D | (TELONE) Dow Chem. Co. | 1,3-dichloropropene and related hydrocarbons | Liquid fumigant nematicide |
| DD Mixture | (DD) Shell Dev. Co. (VIDDEN-D) Dow Chem. Co. | 1,3-dichloropropene and 1,2-dichloropropane and related hydrocarbons | Liquid fumigant nematicide |
| EDB | (DOWFUME W-85) Dow Chem. Co. | ethylene dibromide | Liquid fumigant nematicide |
| Ethoprop | (MOCAP) Mobile Chem. Co. | 0-ethyl S,S-dipropyl phosphorodithioate | Granular or emulsifiable liquid nematicide/insecticide |
| Fenamiphos | (NEMACUR) Chemagro Agri. Div., MOBAY Chem. Co. | ethyl 4-(methylthio)-m-tolyl isoprophyl-phosphoramidate | Granular or emulsifiable liquid nematicide |
| Fensulfothion | (DASANIT) Chemagro Agri. Div., MOBAY Chem. Co. | 0,0-diethyl 0-[p-(methylsulfinyl) phenyl] phosphorothioate | Granular nematicide |
| MBr | (DOWFUME MC-2) Dow Chem. Co. | methyl bromide | Gas fumigant nematicide |
| Oxamyl | (VYDATE) E. I. duPont de Nemours and Co. | methyl-N',N'-dimethyl-N-[(methylcarbamoyloxy]-1-thioxamidate | Granular or emulsifiable liquid nematicide/insecticide |

*DBCP has recently (1977) been removed from the market and manufacture discontinued

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Fig. 11.1.  The carrot on the left is marketable, the two on the right are deformed and not marketable because they were infected by root-knot nematodes early in the growing season.

The newest types of nematicides are systemic. They can be taken up by plants through the roots after application to the soil; or through foliage after spray application, then translocated to roots to kill nematodes feeding on the plants. Presumably they are taken up by nematodes in feeding, but might also enter nematode bodies through nematode cuticle in contact with plant tissue.

## D.  Phytotoxicity and Specificity

Some nematicides are phytotoxic when applied to the soil, and decompose into non-toxic compounds in a few days or weeks. These are applied before planting (preplanting). Others are not toxic and can be applied at planting time or after planting (postplanting). A few are especially phytotoxic to certain crop plants, and many are recommended for use on only a limited number of crops.

Nearly all nematicides will control most species of plant-parasitic nematodes in soil, but some are reported to be more effective against cyst nematodes of the genus *Heterodera*. *Meloidogyne* species are killed readily by all nematicides.

## E.  Effect of Nematode Populations

In farm fields, application of nematicides is followed by a decrease of infectivity of nematodes in the soil. Since the decrease is not necessarily correlated with the number of living *Meloidogyne* larvae or other nematodes in the soil, it is best measured by comparative infection of indicator plants from treated and untreated soil 3 to 6 weeks after planting.

Depending on soil temperature, the *Meloidogyne* larval population will start to increase when the first eggs hatch 20 to 40 days after planting; from then on, the increase will be rapid for about 60 days (2 or 3 generations), and will continue until the roots die af-

## Table 11.2 Addresses of manufacturers or suppliers of nematicides.

| Manufacturers or Suppliers of Nematicides | Addresses |
|---|---|
| Chemagro Agric. Div. | MOBAY Chemical Corp., P.O. Box 4913, Kansas City, MO 64120, USA |
| Dow Chemical Co. | Agricultural Dept., P.O. Box 1706, Midland, MI 48604, USA |
| Dow Chemical (Australia), Ltd. | P.O. Box 384, North Sydney, N.S.W. 2060, Australia |
| E. I. duPont de Nemours & Co. | Biochemicals Dept. New Prod. Development, 1007 Market St., Wilmington, DE 19898, USA |
| E. I. duPont de Nemours & Co. (Australia), Ltd. | P.O. Box 930, North Sydney, N.S.W. 2060, Australia |
| DuPont do Brazil S.A. | Rua da Consolacao, 57-60 andar, Sao Paulo, Brazil |
| DuPont of Canada Ltd. | Montreal, Quebec, Canada |
| DuPont de Colombia, S.A. | P.O. Box 15024, Bogota, Colombia |
| Dupont Philippines | P.O. Box 1718 MCC, Makati, Rizal, Philippines 31117 |
| Great Lakes Chemical Corp. | Agr. Chem. Res. and Dev., P.O. Box 2200, West Lafayette, IN 47906, USA |
| Mobil Chemical Co. | Industrial Chemicals Div., 401E. Main St., Richmond, VA 23208, USA |
| Niagara Chemical Div. | FMC Corp., 100 Niagara St., Middleport, NY 14105, USA |
| Shell Development Co. | Biological Sciences Center, P.O. Box 4248, Modesto, CA 95352, USA |
| Shell Chemical (Australia) Pty. Ltd. | P.O. Box 1713P, Melbourne, Victoria 3000, Australia |
| Shell Chemicals U.K. Ltd. | 39-41 St. Mary's Street, Ely, Cambridgeshire, U.K. |
| Shell Oil (New Zealand) | P.O. Box 2091, Wellington, New Zealand |
| Union Carbide Corp. | Agricultural Products & Services, 4708 Kirkwood Highway, Wilmington, DE 19808, USA |
| Union Carbide Australia, Ltd. | G.P.O. Box 5322, Sydney, N.S.W. 2001, Australia |

ter harvest. *Meloidogyne* species increase rapidly on the healthy root systems of plants in soil treated with nematicides and less rapidly on damaged roots in untreated soil. If the infestation is heavy, roots in untreated soil will have severe damage early in the season, be infected by bacteria and fungi, and have a large amount of decay before harvest.

## F. Residues

Most nematicides decompose in the soil, leaving residues which are not toxic to plants nor taken up by the plants in sufficient quantity to be objectionable. An exception is bromine which is a considerable part of dibromochloropropane (DBCP) and ethylene dibromide (EDB). Bromine is toxic to onions, garlic, and a few other plant species. It is readily taken up by other plants and is detectable in milk from cows fed on peanut hay from fields where DBCP or EDB has been used.

## II. Application of Nematicides to Farm Fields*

### A. Overall Treatment

Nematicides may be applied to the whole area of a field with the intention of controlling a large part of

*This subject was reviewed by J. M. Good (1969).

the soil population. This is "overall" or "solid" application. For this purpose, soil fumigants are usually injected into the soil in parallel lines 30 cm apart and at a depth of about 20 cm. Fumes diffuse from these lines, and control is uniform throughout the top 40 cm of soil.

Liquid non-fumigant nematicides may be diluted and sprayed uniformly over the surface.

Granular non-fumigant nematicides are distributed uniformly over the soil surface. Efficiency of non-fumigant nematicides is increased if they are mixed with the top 10 to 20 cm of soil.

### B. Row Treatment

If a crop is to be planted in rows spaced 60 cm or more apart, "row" treatment may be used. Usually one or two lines of fumigant nematicide or a band of non-fumigant nematicide about 25 to 30 cm wide is applied for each row.

The area between rows is left untreated. Only a small proportion of larvae in untreated areas migrate to infect germinating seedlings or transplants in the early stages of growth when they are most vulnerable. By the time the roots have grown out of the treated area, the plants are large enough to escape serious damage. Many vegetable and field crops are treated by the "row" method. It requires one-fourth to one-

half the amount of nematicide needed for a hectare, reduces labor of application, and is often the most efficient way of using nematicides.

For planting fruit or nut trees and vineyards, a strip of soil one to three meters wide is treated for each row.

## C. Spot Treatment

If the crop is widely spaced in a row, a very large saving of nematicide is possible by use of "spot" treatments. A single injection of fumigant nematicide produces a spot of treated soil about 30 to 40 cm in diameter in which one plant can grow. This method is not always the most economical; the nematicide must be applied by hand applicators, which requires more labor than row treatment by tractor applicators.

"Site" treatment is a variation of spot treatment and is used for planting perennials. Treated sites vary in size from circles one meter to three meters in diameter according to the size of the tree or vine to be planted.

# Appendix 1

# Identification of *Meloidogyne* Species

## I. North Carolina Differential Host Test

### A. Introduction

The North Carolina Differential Host Test is designed to identify the most widely distributed *Meloidogyne* species and races, namely, *M. incognita* (4 races), *M. arenaria* (2 races) *M. javanica* and *M. hapla*. It can be used for surveys with a test for each new population found, and is especially useful for identification of populations from new locations or new hosts in the territory under survey.

### B. Procedure

1. Collect soil samples from a field infested with *Meloidogyne*.

   a) If no plants are growing, the samples should come from the rows where plants were grown the previous season.

   b) If plants are growing, take combined soil and root samples with as many knotted roots as possible.

   c) A sample can consist of knotted roots only. The sample should be composite, at least 1000 cubic centimeters of soil or 500 grams of roots, from at least five locations in the field.

2. Divide the sample and use it to inoculate 8 or more pots. Pots should hold 1000 cc of sandy soil or soil mixed with sand. (Note: Substitute plastic bags or tin cans if pots are not available. Punch holes for drainage.) Transplant nematode-free tomato seedlings in the pots and keep them in a glasshouse if available. Or in warm countries, place outdoors in shade, on boards supported 20-30 cm above ground level. (Pots placed on the ground or on a plastic sheet on the ground will often become contaminated by soil nematodes.) Water the pots daily and fertilize lightly every two weeks with a complete fertilizer. Keep populations separated on the glasshouse bench, or on a separate board if outdoors. Cross contamination between populations can confuse data if different species of *Meloidogyne* are involved. After a minimum of 45 days, your inoculum will be ready to use.

3. For a host test, you will need inoculum for 24 pots. The best potting soil is sandy loam mixed with an equal amount of coarse sand. This mixture must be free of plant-parasitic nematodes, particularly *Meloidogyne* species. Soil sterilized with methyl bromide or soil sterilized by steam can be used if available. An easy way to kill *Meloidogyne* species is to spread the soil in a layer 2-cm thick on a concrete floor or on a plastic sheet and expose it to the sun until dry.

4. Empty 6 of the inoculum pots, cut the roots into short pieces and mix roots and soil. Divide this mixture into 24 parts and use one part to inoculate each of 24 pots of nematode-free soil. Probably the best procedure is to fill the pots three-fourths full of sterilized soil, add the inoculum and finish filling with a layer of sterile soil about 2-cm thick. Fill 4 pots with sterilized soil only.

   Keep the other two pots in case you need to start over.

5. Transplant 1 or 2 seedlings of the following cultivars in each of 4 inoculated pots: 1) NC 95 tobacco, 2) Deltapine 16 cotton, 3) California Wonder pepper, 4) Charleston Grey watermelon, 5) Florrunner peanut, and 6) Rutgers tomato. The uninoculated pots are planted with Rutgers tomato. (Note: Seed of these cultivars are available from the International *Meloidogyne* Project Headquarters, P. O. Box 5397, Raleigh, North Carolina 27607, USA.)

6. Taking care to prevent contamination as outlined in section 2 above, water, fertilize, and allow the plants to grow for 50 days if the temperature range is 24° to 30°C. If the temperature is higher, grow for 45 days; if lower, for 55 days.

7. Remove plants from pots, wash root systems gently, and examine with a dissecting microscope or magnifying glass. Look for galls and mature (light brown) egg masses. Rate each root as follows: no galls or egg masses = 0; 1-2 galls or egg masses = 1; 3-10 = 2; 11-30 = 3; 31-100 = 4; more than 100 = 5. Make two separate tables, one for galls and the other for egg masses. For each plant, calculate an average root-knot index for galls and a separate one for egg masses. Usually the indexes for galls and egg masses will be alike; but in some cases, the egg mass index will be smaller. If the egg mass index is smaller, use it for making the final decision. Susceptibility is measured by reproduction, not by galling.

## C. Identification

Identification is by Table A-1.1. If all goes well, *Meloidogyne* species or races and plant combinations with plus (+) ratings in Table A-1.1 will have average ratings of 4.0 or more, and those with minus (-) ratings will have average ratings of zero, 1.0 or 2.0.

Tomato is susceptible to all species and races and is the indicator plant. If the tomato plants from inoculated pots are heavily infected, other positive ratings will be high. If they are low, the probability is that the inoculum was inadequate and other ratings will be abnormally low. Ratings should be judged partly in relation to the tomato ratings. Infection of tomatoes in the uninoculated pots indicates that the potting mixture was infested, and that the experiment should be repeated.

If a population with two or more *Meloidogyne* species is tested, there will be too many plus (+) ratings. In this case, the experiment should be repeated, using egg masses from differential host test plants, that is, plants which have different signs in the table. In a mixture of *M. incognita* and *M. javanica*, for instance, pepper is the differential host plant.

To gain experience in identification by morphological characters, species identified by the differential hosts should be verified through microscopic examination using the technique of the following section III of this appendix. The illustrations and information of Chapters 8 and 9 are for this purpose.

## D. Limitations of the Differential Host Test

The main purposes of the North Carolina Differential Host Test are to identify the four species and six races shown in Table A-1.1, and to detect pathogenic variation within populations of species.

Results cannot be interpreted as establishing the host range of these species and races. As discussed in Chapter 7, root-knot resistance of crop cultivars varies widely. Testing of all cultivars of any one crop would be practically impossible. But until this is done, no one can say that a nematode species never reproduces on a given plant species. Also, as discussed in Chapter 5,V, there is evidence of individual variation in *Meloidogyne* populations.

As Sasser (1954) stated early in the development of the Differential Host Test, "The relatively small number of plants tested in these studies did not reveal any general pattern which would enable the prediction of resistance or susceptibility among plant species." Later work with many more *Meloidogyne* populations and additional plant cultivars has not changed this basic statement. The most that can be said of the test in its present form is that *M. incognita* populations which reproduce on peanuts, *M. hapla* populations which reproduce on watermelon, or *M. arenaria*, *M. javanica* and *M. hapla* populations which attack cotton, have not been found.

When an apparently new host of a species is found, there is a possibility that the cultivar is susceptible only to the particular population tested and not

Table A-1.1. Differential Host Test Identification Table

| *Meloidogyne* species and race | Differential Host Plant Cultivars* | | | | | |
|---|---|---|---|---|---|---|
| | Tobacco NC 95 | Cotton Delta-pine 16 | Pepper California Wonder | Watermelon Charleston Grey | Peanut Flor-runner | Tomato Rutgers |
| *M. incognita* | | | | | | |
| Race 1 | − | − | + | + | − | + |
| Race 2 | + | − | + | + | .. | + |
| Race 3 | − | + | + | + | − | + |
| Race 4 | + | + | + | + | − | + |
| *M. arenaria* | | | | | | |
| Race 1 | + | − | + | + | + | + |
| Race 2 | + | − | − | + | − | + |
| janica | + | − | − | + | − | + |
| *M. hapla* | + | − | + | − | + | + |

* Tobacco *(Nicotiana tabacum)* cv NC 95; cotton *(Gossypium hirsutum)* cv Deltapine 16; pepper *(Capsicum frutescens)* cv California Wonder; watermelon *(Citrullus vulgaris)* cv Charleston Grey; peanut *(Arachis hypogaea)* cv Florrunner; tomato *(Lycopersicon esculentum)* cv Rutgers.

susceptible to other populations.

Variation in plant cultivars and in individuals of *Meloidogyne* populations indicates that infection and reproduction with any combination of *Meloidogyne* species and crop species is possible. See the section on designation of races (Chapter 5,I).

## II. Standardized Host Test

The North Carolina Differential Host Test can be standardized by using *Meloidogyne* eggs as inoculum. The procedure is as follows:

1) Wash soil from galled roots of a plant grown in inoculated sterile soil and harvested when the egg masses are light brown, usually at least 45-55 days after inoculation.

2) Cut roots into pieces about 2-cm long and place in a container of 500-ml capacity with 200 ml of 0.5% sodium hypochlorite (NaOCl) solution. Shake vigorously by hand for 3 minutes. This dissolves the gelatinous matrix of the egg mass. (Do not expose the roots to the NaOCl solution for longer than 4 minutes.)

3) Pour the liquid suspension of eggs through two sieves, the first of 100 mesh to 200 mesh (openings 0.149 to 0.074 mm), the second 500 mesh (openings 0.028mm). Eggs separated from the egg masses pass through the first sieve and are retained by the second one. Eggs on the 500 mesh sieve are washed free of NaOCl solution by a slow stream of cold tap water, then washed into a container previously marked to contain one liter. The roots in the original container can be washed twice more with water to obtain additional eggs.

4) Concentration of eggs per milliliter in the container is determined by filling the container to the one-liter line, and counting numbers in three samples of one milliliter each.

5) Use 10,000 eggs per pot for inoculum.

Experiments with this method indicated that eggs are surface-sterilized and are more uniform inoculum than larvae of varying ages. Egg masses were also good inoculum but cannot be standardized.

For assessing reproduction, eggs can be counted by the same procedure with substitution of 1.0% NaOCl solution, which frees more eggs than the 0.5% solution (Hussey and Barker, 1973).

## III. Identification by Microscope

Because of variation, individual specimens of *Meloidogyne* species cannot be positively identified by study of morphological characters. Populations of *Meloidogyne* species can be identified if an adequate sample of the population is studied. An adequate

sample is never less than 10 females and their egg masses from 10 different parts of the field. Identification is facilitated if all available information about the population is utilized. This subject is discussed more fully in Chapter 8 and 9.

### A. Females

Females of *Meloidogyne* species are prepared for identification by fixing in approximately 2% formaldehyde over night or longer. (Note: Commercial formalin is about 37% formaldehyde by volume or 40% by weight. A formula for approximately 2% formaldehyde is one part of commercial formalin and 19 parts water.) Roots containing *Meloidogyne* species can be fixed and stored in this solution. Females are obtained by dissecting roots under a binocular dissecting microscope. If mounted on microscope slides in 2% formalin, specimens are usable for a few days. If mounted in lactophenol and sealed, they will last indefinitely.

One way of mounting females for identification is to cut the body as near the posterior end as possible, turning the cut piece so that the pattern is on the upper side. The cover glass is placed over both pieces. This system keeps body and perineal pattern together, and one or two females can be mounted on each slide.

Identification characters of females are the relation of the axis of the neck to the axis of the body, the position of the excretory pore, and the perineal pattern.

The relation of the neck axis and body axis can be studied under the dissecting microscope. Specimens turned to a lateral view will show neck displacement if it is present.

The excretory pore is located by following the excretory tube which is easier to see than the pore. On specimens lying in the ventral position, both excretory pore and tube may be difficult to see, and it will be necessary to look at other specimens.

### B. Larvae

The most useful identification character of larvae of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* is average larval length, obtained by measurement of at least 20 larvae, preferably from at least 4 egg masses. It is the average of all lengths measured, and should be very near the median larval length given in Tables 8.1 and 9.1. For exact measurements, larval length is measured along the center line of the body by use of a camera lucida. Many fixed larvae lie in a curve which approximates one-sixth of a circle. True length of such larvae is approximately the

straight-line distance from head to tail tip plus 5%. The straight-line can be measured by use of an ocular micrometer, and is sufficiently accurate to separate larvae of *M. arenaria* (median length 0.470 mm) from larvae of *M. incognita* (0.376 mm) and *M. javanica* (0.370mm). Lar···e of *M. hapla* (0.430 mm) can be separated from tn; longer larvae of *M. arenaria*, and from the shorter larvae of *M. incognita* and *M. javanica*.

For some species, larval tail lengths and larval tail
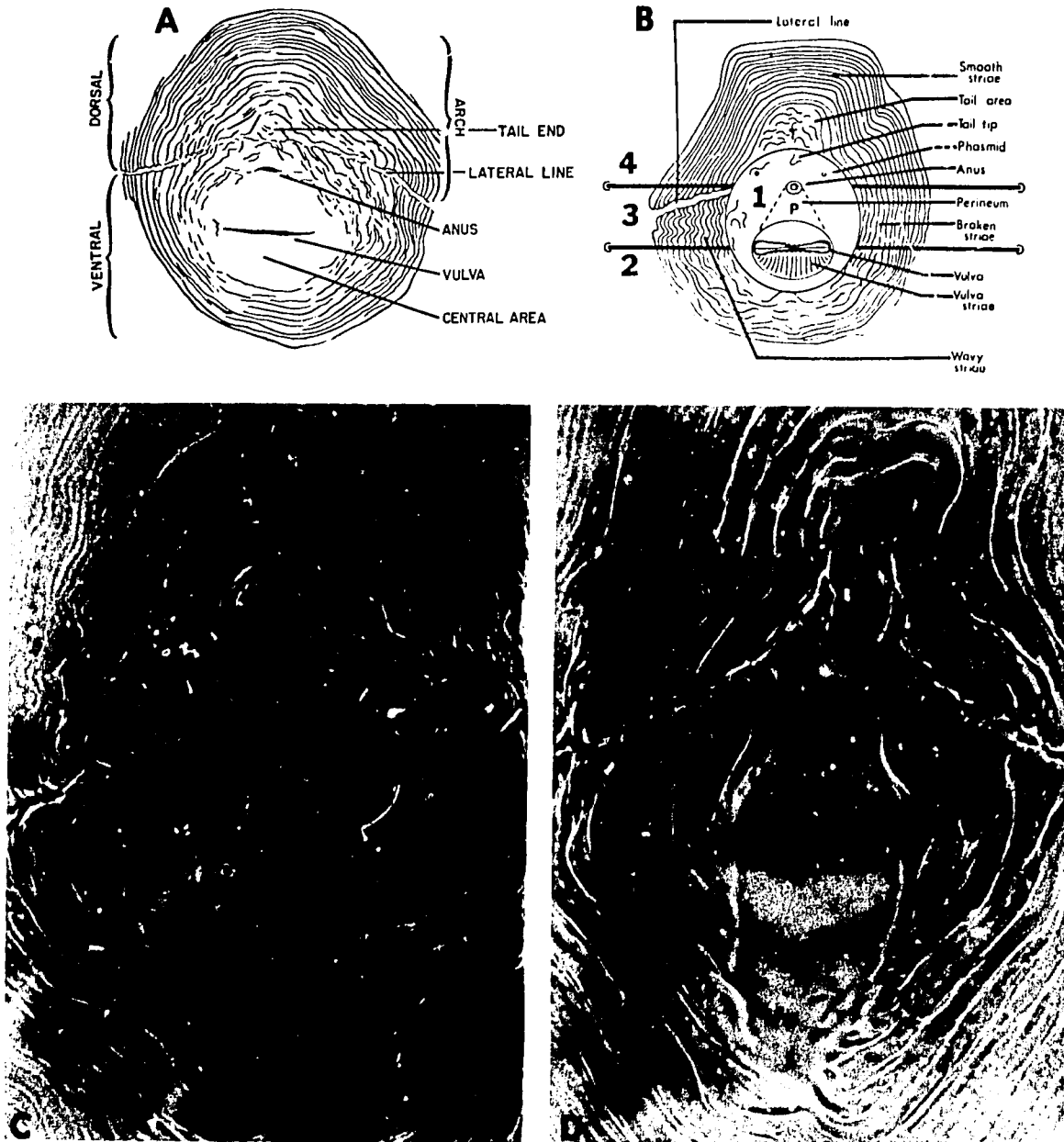


Fig. A-1.1. Perineal pattern nomenclature, two systems: A: Drawing of perineal pattern of *M. javanica* (Taylor, Dropkin and Martin, 1955). B: Perineal pattern divided into sectors to facilitate description. Sector 1 is the area around the vulva and anus. Sector 2 is the area beneath the vulva. Sector 3 is defined by horizontal lines passing through vulva and anus, respectively. Sector 4 is the part above the anus. Striae in the various sectors can be described as not present, sr. ooth, wavy, broken, unbroken, regular or irregular (Esser, Perry and Taylor, 1976). C: Perineal pattern with fold along lateral line at left, but not along lateral line at right. D: Perineal pattern with folds along both lateral lines.

shapes are useful identification characters.

## C. Perineal patterns

After identification of *Meloidogyne* populations by the Differential Host Test, checking the location of the female excretory pore and the median larval length, study of the perineal pattern will provide additional confirmation of identification of the common *Meloidogyne* species.

Many workers mount 4 to 10 perineal patterns on a microscope slide, using the method described by Taylor and Netscher (1974).

1) The infected roots are placed in 0.9% sodium chloride solution and the females are dissected out from the root under the dissecting microscope.

2) The females are transferred to 45% lactic acid in a plastic petri dish or to a piece of plastic in a glass petri dish and the posterior end cut off with an eye knife (a very small scalpel used by ophthalmologists) or a sharpened needle.

3) Body tissues are removed by lightly brushing with a flexible bristle or fibre.

4) The perineal pattern is trimmed and transferred to glycerine on a microscope slide. A cover slip is applied and the mount sealed with wax or other cover glass sealer.

Perineal patterns are formed by expansion and alteration of the larval body and retain the lateral lines, the tail tip, and the phasmids. The lateral lines point to the tail tip (end) as shown in Fig. A-1.1,A, which is a pattern of *M. javanica* and has the distinct lateral lines of that species. Lateral lines are more or less visible in other species as a series of breaks or irregularities in the striae.

Figure A-1.1,A and B shows two ways of naming parts of perineal patterns. But word descriptions of patterns are of little value for identification. There is no substitute for comparing drawings or preferably photographs of patterns with specimens, looking for similarities, not for differences. Differences are always present, but there are more similarities, and typical patterns can always be found. Look for patterns like the ones in Chapters 8 and 9 of this book and try to find typical patterns.

Caution: Perineal patterns often become folded along the lateral lines, some species more than others. Figure A-1.1, C and D shows folds: the first along lateral line at left, but not along lateral line at right; the second along both lateral lines. These can and often have been mistaken for definite incisures. Folds can be easily recognized as such by focusing slowly up and down.

## D. Males

Males of *Meloidogyne* species are of little value for routine identifications.

# Appendix 2

# Obtaining Large Quantities of Eggs and Larvae

## I. For Host Tests, Breeding Programs, Biochemical Tests, and Experiments

Large quantities of *Meloidogyne* eggs and larvae are needed. Some useful methods for isolating eggs or larvae are as follows.

### A. From Roots with Few Exposed Egg Masses

Infected roots are washed and cut into pieces about 5-mm long. A 5-gram portion is placed in a mixer (homogenizer, Waring Blender) with 500 ml of water. The mixer is run at slow speed for 15 seconds. The resulting suspension is poured through a coarse sieve (1.000 mm openings) and a fine sieve (0.010-0.030 mm openings), then washed thoroughly. The residue on the fine sieve is washed into a centrifuge tube to which one cubic cm of kaolin powder is added. After thorough mixing, it is centrifuged for 5 minute and the supernatant poured off. A sugar (sucrose) solu-tion of 1.15 specific gravity is added, the mixture again stirred, and again centrifuged for 4 minutes. This brings the eggs to the top and they are poured in a fine sieve (Coolen and D'Herde, 1972).

### B. From Roots with Many Exposed Egg Masses

Agitate roots with exposed egg masses in water, and stroke them with a brush to dislodge the egg masses. Collect on a 60-mesh sieve (0.420 mm open-ings), and process in a mixer (homogenizer, Waring Blender) with 500 ml of 1% sodium hypochlorite (NaOCl) solution for 40 seconds. This releases eggs from the egg masses. They are separated from large debris by passage through 100-mesh (0.149 mm open-ings) and 400-mesh (0.037mm) sieves. Centrifuging with water, then with sucrose solution (454 grams of sucrose in 1,000 ml of water), and washing, removes small debris and leaves the eggs clean (McClure et al., 1973).

# Appendix 3

# Testing Plants for Resistance

## I. Preliminary Tests

Tests for resistance are best made with seedlings germinated in sterile soil and transplanted in soil heavily inoculated with eggs, larvae, or egg masses. The soil temperature is maintained at an average of 25°C, and plants examined at intervals for galling and egg production. When moderate to heavy egg production is found, a breeding line may be discar-ded. Plants with light galling and low egg production are kept for 50 to 60 days before final ratings are given. Lines with the lowest egg production are kept and retested.

## II. Field Tests

In the later stages of development of cultivars, breeding lines are tested on a larger scale, with replications in different parts of the field. Lines with low egg production are retested in as many different fields as possible until it is certain that their resistance is true. Final selections are made by trials of yield potential in fields infested with *Meloidogyne* species and in similar soil with nematicide treatment (Kinloch and Hinson, 1972).

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