Biology and host-parasite relations of a species of *Meloidoderita* (Nematoda : Criconematoidea)

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Summary

The life history of a population of *Meloidoderita* Poghossian, 1966, found in Israel, and its host-parasite relations on mint (*Mentha longifolia* (L) Huds.) were studied. The vermiform second stage female juvenile penetrated young roots of the host, coming to rest at or near the stele. Sedentary, swollen third and fourth stage juveniles were completely embedded within the root. The adult female ruptured the external root tissues and produced a gelatinous egg sac which covered its protruded body. Simultaneously, a thick-walled uterus expanded and filled the female body. Females deposited 85-90% of their eggs (average = 186 ; maximum observed = 393) in the egg sac. The remainder were retained within the uterus, which at the end of the female's life transformed into a brown cystoid structure with a sclerotic irregular wall. The body cuticle was shed. Males, present within about 2% of the egg masses, exhibited an underdeveloped stylet and oesophagus, and lacked a bursa. At optimal temperature of 25°, the life cycle was completed in 28 days, and at 18° in 68 days. No nematode development occurred at 33°.

As a result of nematode parasitism a feeding site was formed in the host root tissues comprising hypertrophied cells, usually in the pericycle, with enriched cytoplasm and organelle content, and enlarged, moderately-lobed nuclei. In cells closest to the nematode head, cell-wall breakdown occurred, and a syncytium was formed. No conspicuous galls were present on roots.

Host range studies revealed two additional mint species *(Mentha microphylla* and *M. spicata)* as hosts, while *M. piperita*, tomato, upland cotton, dwarf nettle and a local smartweed, were not infected by the nematode.

Résumé

Biologie d'une espèce de Meloidoderita *et relations avec la plante hôte (Nematoda : Criconematoidea)*

Le cycle d'une espèce de *Meloidoderita* Poghossian, découverte en 1966 en Israël, et ses relations avec la plante-hôte, la menthe (*Mentha longifolia* (L) Huds) ont été étudiés. Les juvéniles de deuxième stade pénètrent dans les jeunes racines de la plante-hôte et se localisent au voisinage du cylindre central. Les juvéniles des troisième et quatrième stades, renflés et sédentaires, sont entièrement inclus dans la racine. La femelle adulte rompt les tissus les plus externes de la racine et produit une masse d'œufs gélatineuse qui recouvre la totalité du corps. En même temps l'utérus, à parois épaisses grandit et remplit presque tout le volume du corps de la femelle. Les femelles pondent 85 à 90% des œufs (moyenne = 186 ; maximum observé = 393) dans la masse d'œufs, les autres étant retenus à l'intérieur de l'utérus qui se transforme en fin d'évolution en une structure cystoïde brune ayant une paroi dure, irrégulière, à ce moment la cuticule du corps a été éliminée. Les mâles, présents dans les masses d'œufs (2%) ont un stylet et un œsophage dégénérés et sont dépourvus de bursa. La durée du cycle complet, à la température optimale de 25°, est de 28 jours ; à 18° elle est de 68 jours, tandis qu'à 33° il n'y a plus de développement.

Le nématode produit une différenciation nutritionnelle dans les tissus de la racine, se traduisant par des cellules hypertrophiées, situées en général dans le péricycle, ayant un cytoplasme dense, de nombreux organites et des noyaux lobés. Dans les cellules les plus proches de l'extrémité antérieure du nématode, la paroi cellulaire disparaît et un syncytium est formé. Aucune formation nette de galles n'a été observée sur les racines.

Les études concernant la gamme d'hôtes ont montré que deux espèces supplémentaires de menthe *(Mentha microphylla* et *M. spicata)* sont sensibles tandis que *M. piperita*, la tomate, le cotonnier *(Gossypium hirsutum)*, l'ortie naine *(Urtica urens)* et *Polygonum equisetiforme* n'ont pu être infestés avec succès.

The genus *Meloidoderita* was established to accommodate a single, unusual, species, *M. kirjanovae* Poghossian, 1966, which was described from roots of *Mentha longifolia* (L) Huds. in Armenia, USSR. It was assigned to a newly-erected family — Meloidoderitidae — by Kirjanova and Poghossian (1973), who also reported its occurrence on *Mentha arvensis* L. and nettle (*Urtica dioica* L.). The male of *M. kirjanovae* was described by Poghossian (1975) from laboratory cultures of the nematode, also originating from Armenia, reared on *M. longifolia*. The species was also reported to be present on roots of *M. orientalis* and other plants in various sites in Uzbekistan, USSR (Narbaev, 1969). All these reports indicated that the nematode generally occurred in mixed populations with *Meloidogyne*, but, while inducing some root irregularities, did not form distinct galls.

The only references in the literature to *Meloidoderita* outside of these two Soviet Republics, come from Golden (1976) and Andrews, Krusberg and Golden (1977), who briefly reported having found a population in Maryland, USA, which reproduced on several smartweeds (*Polygonum* spp.), but failed to infect mint and nettle (1). Apparently, this population differs morphologically from *M. kirjanovae* and represents a separate species (A.M. Golden, *in litt*).

During the summer of 1980, we encountered a population of *Meloidoderita*, occurring together with root-knot nematodes, *Meloidogyne* spp., on roots of natural *Mentha longifolia* [reported erroneously as *M. aquatica* in an earlier communication (Cohn & Mordechai, 1981)], growing along the slopes of the Golan Heights in the Upper Galilee district in northern Israel, close to a spring named Ein Notera. The nematode was subsequently reared and studied in the laboratory on its natural host. Females, males and juveniles of this population — sent to Dr. M.R. Siddiqi, C.I.H., St. Albans, England, for identification — generally fit the descriptions of the Soviet workers for *M. kirjanovae* with some minor differences : a somewhat shorter spear and absence of annules in the lip region of juveniles, and, perhaps more significantly, no visible bursa in the male. However, type material of *M. kirjanovae* was not available for comparative studies. Thus, while it is likely that our population is conspecific with *M. kirjanovae*, we prefer at this stage to withold specific determination, until its identity can be ascertained with a greater measure of confidence.

Since the general morphology of *M. kirjanovae* has been fairly well documented (Kirjanova & Poghossian, 1973) the emphasis in our studies was placed on the life history and host-parasite relations of the nematode. This is the subject of the present paper.

Materials and methods

Oval molting observations were carried out by placing living egg masses in tap water on glass slides and exerting gentle pressure on them with a glass cover slip.

For developmental studies the nematode was cultured on rooted *Mentha longifolia* plants, grown in a heat-disinfested sandy loam soil in 750 ml plastic pots. One week after planting, the plants were inoculated by placing five egg masses of *Meloidoderita* close to the roots, and covering with soil. The potted plants were maintained in controlled temperature water tanks, in groups, at 18, 23, 25, 27, 30 and 33°, and were watered via a glass tube inserted in each pot. At different intervals after inoculation, plants were removed, their roots were fixed and stained in boiling lactophenol-acid fuchsin solution for three minutes, then cleared and stored in lactophenol in Petri dishes. From this material, randomized samples were removed for examination and counts of nematode life stages.

For histological studies under the light microscope, roots were fixed, sectioned and stained as described by Cohn and Mordechai (1977) ; photographs of sectioned and unsectioned roots, as well as nematode life stages, were taken with a Wild Mka 4 photoautomat. For transmission electron microscopy, roots were fixed in glutaraldehyde and prepared by standard techniques. Sections were cut at 50-90 nm with a diamond knife on a Porter-Blum Ultramicrotome and micrographs were taken on a JEM-T-7 electron microscope at 80 KV.

Host range studies were carried out by inoculating eight seedlings or rooted cuttings of the plant varieties with five egg masses of *Meloidoderita*, and growing another eight replicates of each plant variety in a potted soil, naturally infested by the nematode. The plants were maintained in a growth chamber at 25-27°. Roots of all plants were examined for nematode infection fourteen weeks after inoculation and plants harbouring gravid females with egg masses were considered hosts.

(1) A full report of the work of Andrews *et al.*, (*Nematologica*, 27 : 146-159, 1981) appeared after submission of this manuscript.

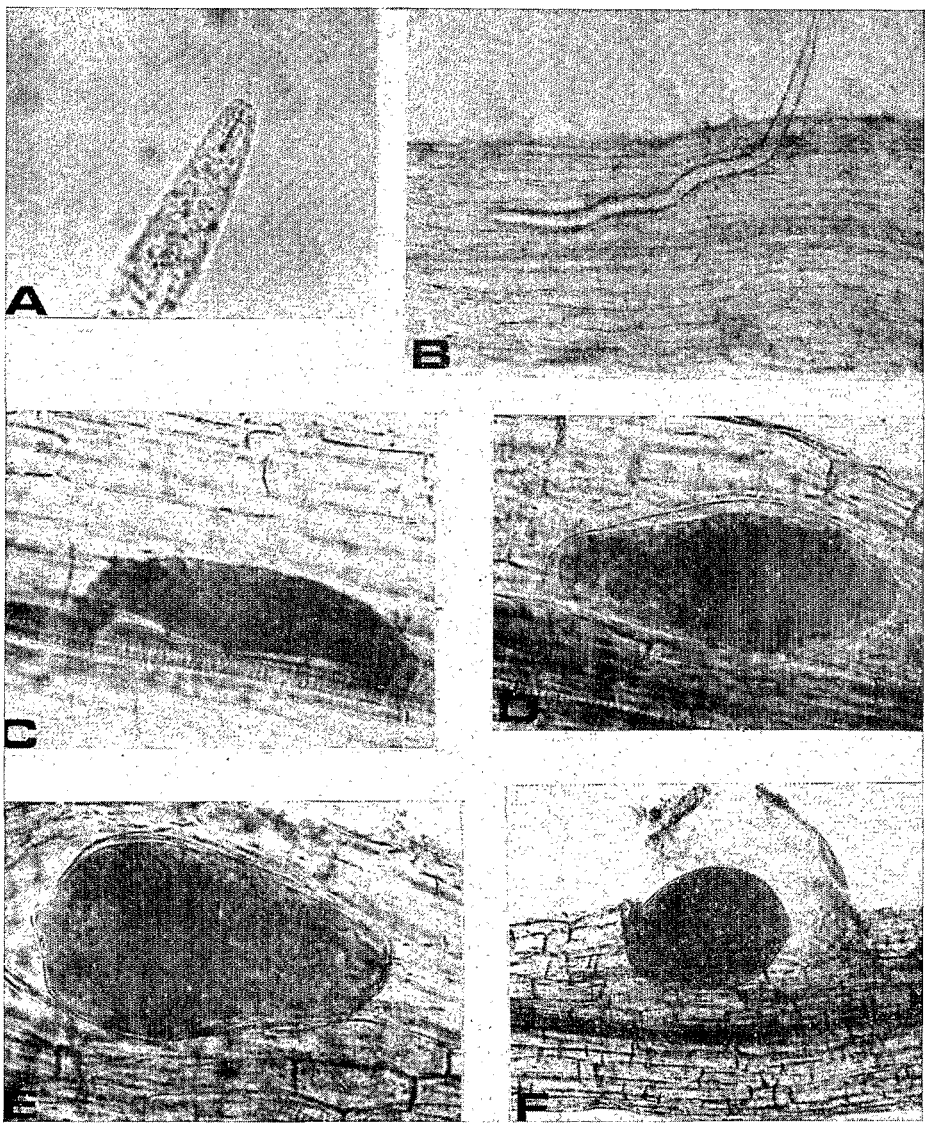

Fig. 1. Development of female juveniles of *Meloidoderita*. A (× 600) : Second-stage juvenile forced from egg, with molting cuticle attached ; B (× 165) : Second-stage juvenile penetrating root ; C (× 263), D (× 323) : third and fourth-stage juveniles embedded in root ; E (× 278) : Young female within root tissue ; F (× 143) : Female rupturing external tissue layers of root.

Results and discussion

Life history of *Meloidoderita*

Females: Hatching observations revealed that many juveniles emerged from eggs (under pressure) with molting cuticles attached to their bodies (Fig. 1 A) ; evidently, therefore, the juvenile undergoes its first molt within the egg, as is the case in most plant parasitic nematodes. The emerging second-stage female juvenile constitutes the infective stage of the nematode and when penetrating the young root of the host plant it became totally embedded within the tissue (Fig. 1, B, C). The juvenile came to rest with its head located at, or near the stele, and its body usually lying along the axis of the root.

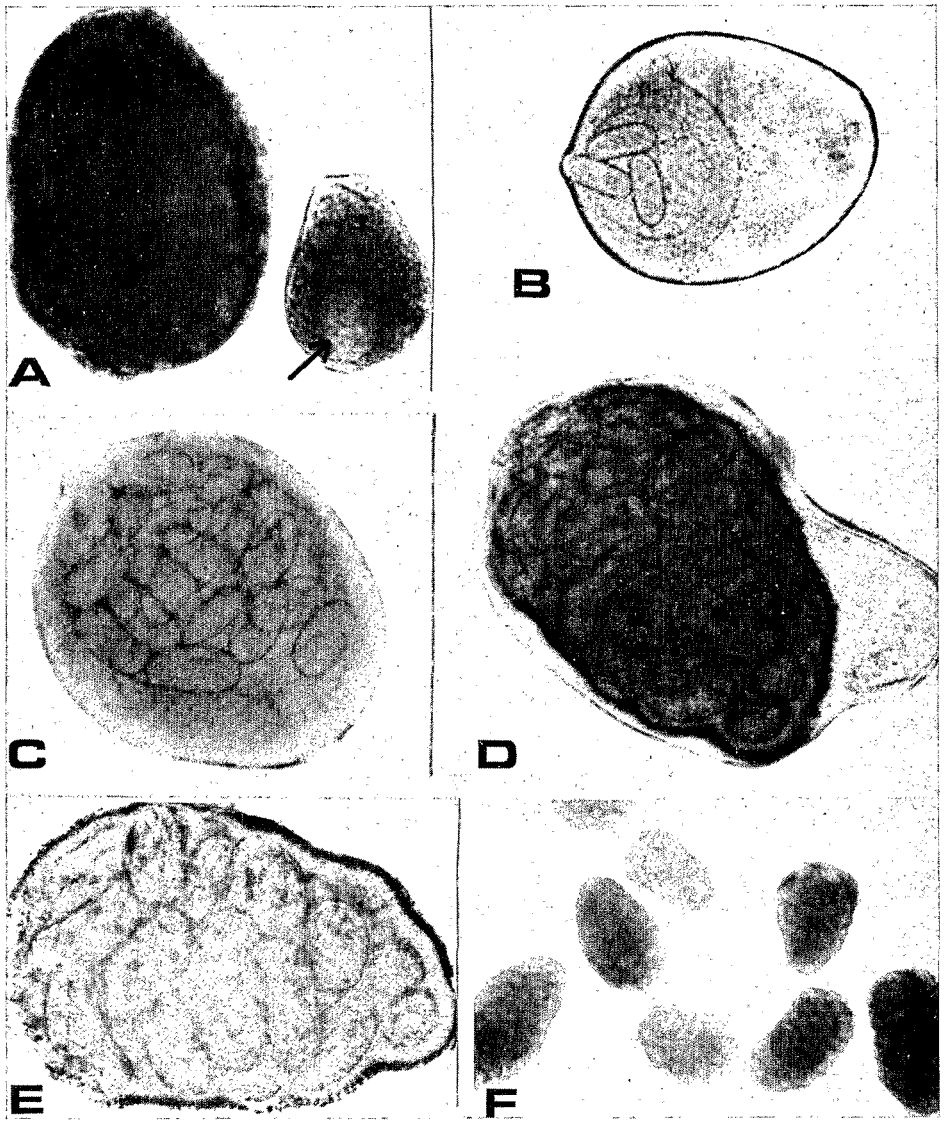

Fig. 2. Development of adult female of *Meloidoderita*. A (× 173) : Mature and young females (arrow indicates uterus) ; B, C (× 150) : Developing adult females, showing expansion of uterus and increased egg production ; D (× 188) : Mature female shedding cuticle and emerging as cyst ; E (× 150) : Single cyst ; F (× 38) : Group of cysts.

In this position, the nematode underwent two additional molts, its body swelling in size, but still remaining entirely embedded within the root (Fig. 1C, E). Only in the adult female stage, were the outer root tissues ruptured and the nematode assumed a position with its posterior end protruding from the root (Fig. 1 F). This semiendoparasitic condition is therefore, secondary, as is the case in species of *Heteroderà* (Williams, 1978), and unlike *Tylenchulus semipenetrans* (Van Gundy, 1958) and *Rotylenchulus* spp. (Birchfield, 1962), which only partly penetrate the root in the juvenile stage, with parts of their bodies protruding beyond the plant surface throughout their entire life cycle.

Female maturation involved a significant increase in body size. Already in the young female, before the onset of egg production, an unusually thick-walled uterus was visible, and body growth bet-

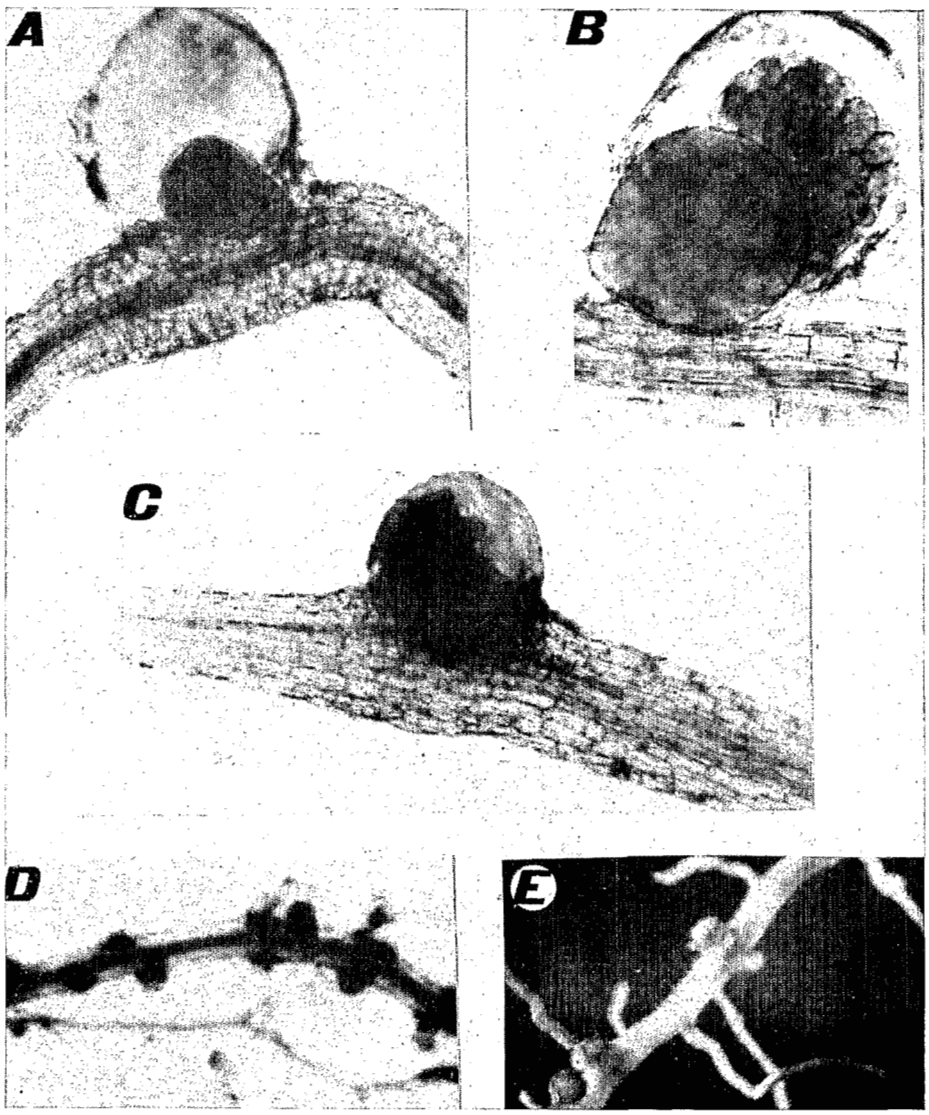

Fig. 3. Adult female of *Meloidoderita* on roots of mint. A (× 66) : Protruding female producing gelatinous matrix ; B (× 75), C (× 45) : Females depositing eggs in matrix ; D (× 6) : Root of *Mentha longifolia* infected with Israeli population of *Meloidoderita* ; E (× 8) : Root of *Mentha longifolia*, infected with *M. kirjanovae* (material by courtesy of Dr. E. Krall).

ween this stage and the mature female was observed to be as much as three-fold (Fig. 2 A). As described in detail by Kirjanova and Poghossian (1973), the development of the adult female is accompanied by a progressive expansion of the uterus which eventually fills the entire volume of the female body (Fig. 2 B, C). After rupturing the root tissues, the female secretes a gelatinous matrix which finally covers its whole body, and into which she lays most of her eggs (Fig. 3 A-C). The dynamics of the egg-laying process is illustrated in Fig. 4 ; from this graph it is evident that egg production began some 15 days after inoculation, most eggs being rapidly deposited in the matrix, and a small number being retained in the uterus. After reaching a peak about 30 days following inoculation, the number of intact eggs in the matrix began to drop due to hatching of juveniles. Retention of newly-layed uterine eggs, however,

continued at a slow rate until about 46 days after inoculation, when large scale cyst formation could be observed and further egg production probably ceased.

Counts carried out on fifteen females revealed an average total egg production of 186 per female (maximum : 393), of which an average of 169 (85-90%) were deposited in the external egg sac, and an average number of 19 (10-15%) were retained within the body. Maximum number of eggs in a single sac was 337 and a maximum of 63 uterine eggs within a single female, was recorded.

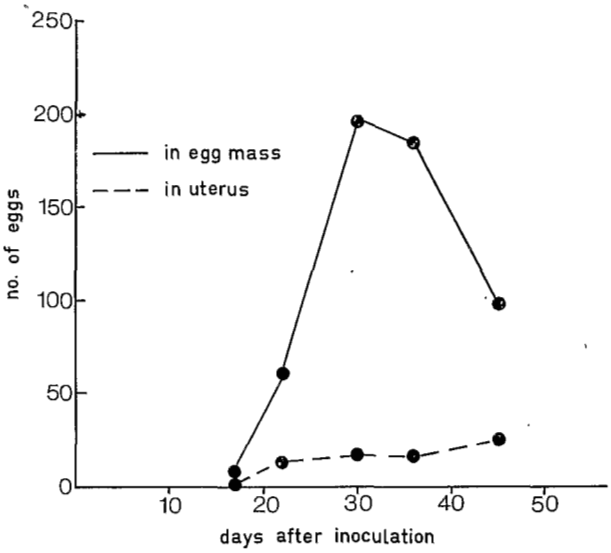

Fig. 4. Egg production by adult female of *Meloidoderita* (counts are means of five replicates).

Initial cyst formation coincided approximately with the beginning of hatching of juveniles within the external egg sacs. Earliest cysts were observed at 30 days after inoculation (at the favourable temperature levels), and by the 45th day, about 26% of the females could be designated as « cysts. » As described in detail by Kirjanova and Poghossian (1973), the egg-filled uterus, which by this time filled almost the entire body volume, transformed into a brown cystoid structure, its wall becoming sclerotic and irregular in texture (Fig. 2 D-F), and the body cuticle was shed (Fig. 2 D). Within the next two weeks the cysts darkened, eventually turning to a dark brown colour.

These structures differ considerably — both in appearance and probably in function — from genuine cysts as we know them in the genus *Heterodera*. Their walls are elastic and not as rigid as in *Heterodera* cysts ; furthermore, being irrigularly wavy, they do not always provide a continuous cover for their egg content, and sometimes do not fully enclose all the eggs. The eggs themselves are very firmly attached to the internal uterine substance, from which they do not separate. *Meloidoderita* eggs are apparently not preserved for long periods of time whithin such cysts ; indeed, after several weeks, most cysts, still attached to roots, contained empty eggs with broken shells, and few juveniles were often seen emerging from the cyst « mass ». Evidently, therefore, these structures, while apparently representing a primitive form of cyst, do not serve as an effective or persistent protectant for eggs, as do the *Heterodera* cysts.

Males: Within about 2% of the egg masses, one to four males were encountered. Several of these had molting cuticles still attached to them (Fig. 7 C), and thus could obviously be identified as offspring of the particular female beneath the egg-mass, rather than having migrated from outside. Furthermore, often more than one molting cuticle was present at the same time, indicating that development of juveniles into adult males was a relatively short process and apparently did not necessitate feeding on the host tissues. The males generally fit the description of the male of *M. kirjanovae* (Poghossian, 1975), bearing an indistinct, fragmentary stylet and an under-developed oesophagus. As was visible from the early cuticles, the stylet was fairly well developed in the juvenile stages, but degenerated with the successive molts (Fig. 7 C). No bursa was visible in our specimens (Fig. 7 D) ; Poghossian (1975) reported a very narrow bursa in *M. kirjanovae*, which « is extremely difficult to discern, even in living specimens. » This question, therefore, requires further study. Biologically, the males of *Meloidoderita* are clearly similar to those of the allied genera, *Tylenchulus* (Van Gundy, 1958) and *Sphaeronema* (Thorne, 1961), in bearing a degenerate digestive system. Lack of a bursa in the male, would further confirm the already recognized affinity of the family Meloidoderitidae to the families Tylenchulidae and Sphaeronematidae within the suborder Criconematina (Siddiqi, 1980). Regarding the functionality of the male, actual copulation was not observed, but active males were sometimes seen in the vulval region of females, beneath the gelatinous matrix prior to egg production.

Temperature requirements

Periodic observations on nematodes reared on *M. longifolia* at different temperature levels, indicated a favourable range of 23-30°, with optimum development at 25° (Fig. 5) ; at this temperature, completion

of the life cycle was most rapid (28 days), and average egg production was maximum (199 per female). For the purpose of these comparative studies, the life cycle was considered completed when juveniles were first observed emerging from eggs. Since inoculation was performed with egg masses, and not juveniles, the actual times for life cycle completion would therefore be slightly less than the values presented here.

At 18°, rate of nematode development was markedly slowed down, and at 33°, no development whatsoever was observed.

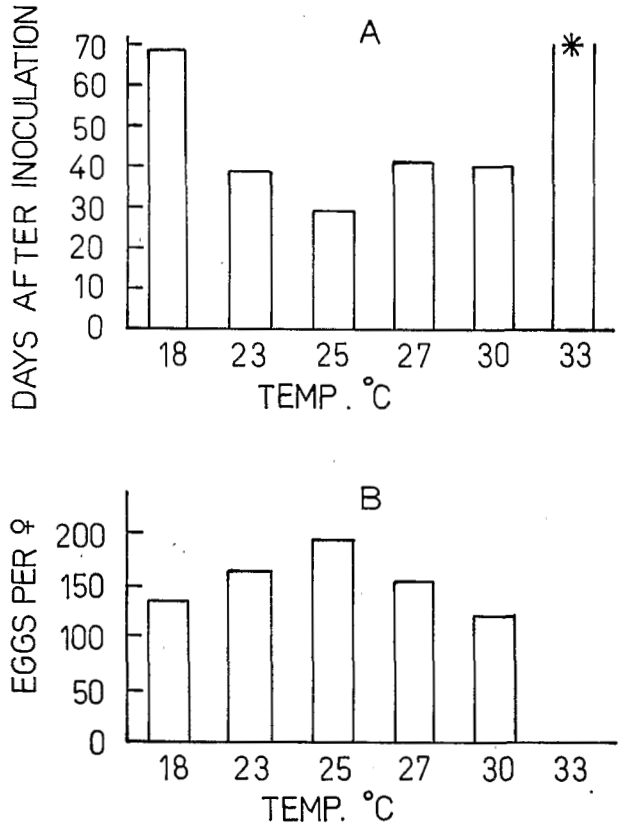

Fig. 5. Effect of temperature on rate of development of *Meloidoderita*, reared on *Mentha longifolia*. A : Time required for completion of life cycle (* no nematodes seen on roots) ; B : Total egg production (in uterus + egg mass).

Host range

The plant species tested as possible hosts were : three additional species of *Mentha* (*M. microphylla* C. Kock, *M. spicata* L. and *M. piperita* L.) one smartweed (*Polygonum equiseliforme* S. *et* S.), dwarf nettle (*Urtica urens* L.), tomato (*Lycopersicon esculentum* Mill.), and upland cotton (*Gossypium hirsutum* L.). Of these only *M. microphylla* and *M. spicata* were designated as hosts, in addition to *M. longifolia*. All other plant species not only failed to support nematode reproduction, but were not found to harbour any life stage of the nematode in their roots. *M. kirjanovae* has been reported to infect *Urtica dioica* L. in the Soviet Union, (Kirjanova & Poghossian, 1973), and four smartweeds have been found to serve as hosts for the American *Meloidoderita* population (Andrews, Krusberg & Golden, 1977). Evidently our population shows a distinct preference for *Mentha* species, and in this respect, too, is similar to the Soviet populations of *M. kirjanovae*.

From the information available at present, *Meloidoderita* is obviously a highly specific parasite with a very selective host range. This would partially account for the very limited distribution of the genus throughout the world.

Histopathology

The nematode parasitized the young roots of the plant. It did not form distinct galls, but the location of the female was often marked by a slight swelling of the root tissue around it (Fig. 3), especially on very thin roots. This pattern of parasitism is in general agreement with that described for *M. kirjanovae* by Kirjanova and Poghossian (1973).

Upon coming to rest after penetrating the root, the nematode created a typical feeding site within the stele tissues. In most cases, the nematode head was situated in, or close, to the endodermis, and feeding induced a mild hypertrophic reaction in the pericycle tissue (Fig. 6 A, B). This reaction included an enlargement of the cell volume, a marked enrichment of the cell protoplasm, an increased number of organelles within the cytoplasm, and an enlargement and some lobing of the nucleus (Fig. 7 A, B). Expansion and protoplasmic density were greatest in cells in closest proximity to the nematode head — so that in cross section the feeding zone appeared as a group of enlarged pericycle cells extending around the root to either side of the head, decreasing gradually in size with increased distance from the head. As feeding continued, there was partial cell wall breakdown in the affected cells closest to the nematode head, and thus a syncytium was formed in this area (Fig. 6 C). Cell wall dissolution was particularly clearly observed under the electron microscope (Fig. 7 A, B). Some parenchyma cells in the stele close to the pericycle layer also sometimes contained a dense protoplasm, but generally no change in

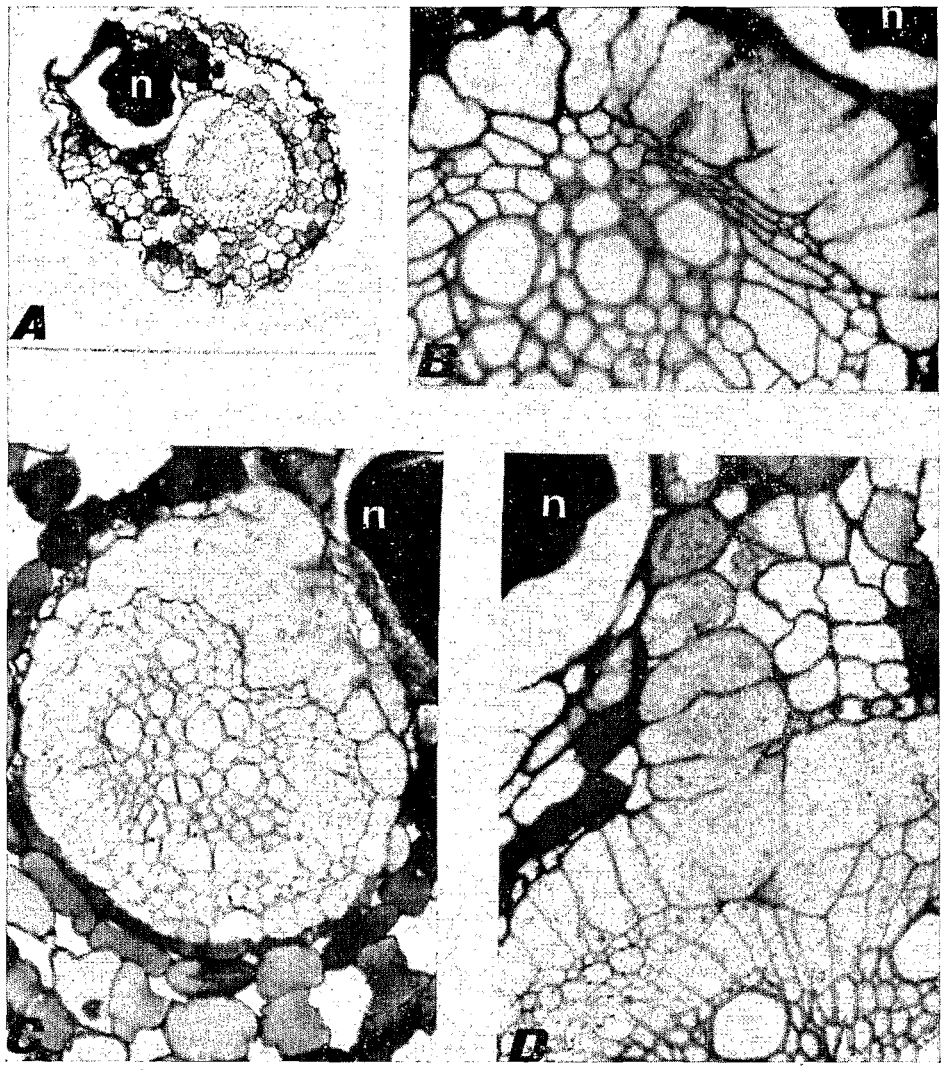

Fig. 6. Histopathology of *Meloidoderita* in roots of *Mentha longifolia*. A (× 113), B (× 540), C (× 323) : Reaction of pericycle and adjacent cells to nematode feeding, and formation of syncytium near nematode head (n = nematode head) ; D (× 480) : Hypertrophied parenchyma cells "leading" to syncytium in pericycle tissue from head of nematode situated within the cortex.

their size was observed. Occasionally, the nematode came to rest within the cortex ; in such cases a number of cortical parenchyma cells, differentiated from neighbouring normal cells by a slightly larger size and dense protoplasm, could be seen « connecting » the nematode head to the typical feeding site in the pericycle tissue (Fig. 6 C).

Fixed roots of *M. longifolia*, parasitized by *M. kirjanovae* females originating from Armenia, were kindly supplied to the senior author by Dr. E. Krall, Estonian SSR, in 1974. Although detailed histopathological study of this material was precluded for technical reasons, in cross sections carried out on these roots, it was possible to discern the location of a stained zone, consisting of slightly enlarged, dense cells, in the pericycle tissue, as was observed in the Israeli material. This is an additional indication of similarity between the Israeli and the Soviet *Meloidoderita* populations.

The plant reaction induced by *Meloidoderita* described above is, on the whole, fairly similar to the host response observed to feeding by *Roty-*

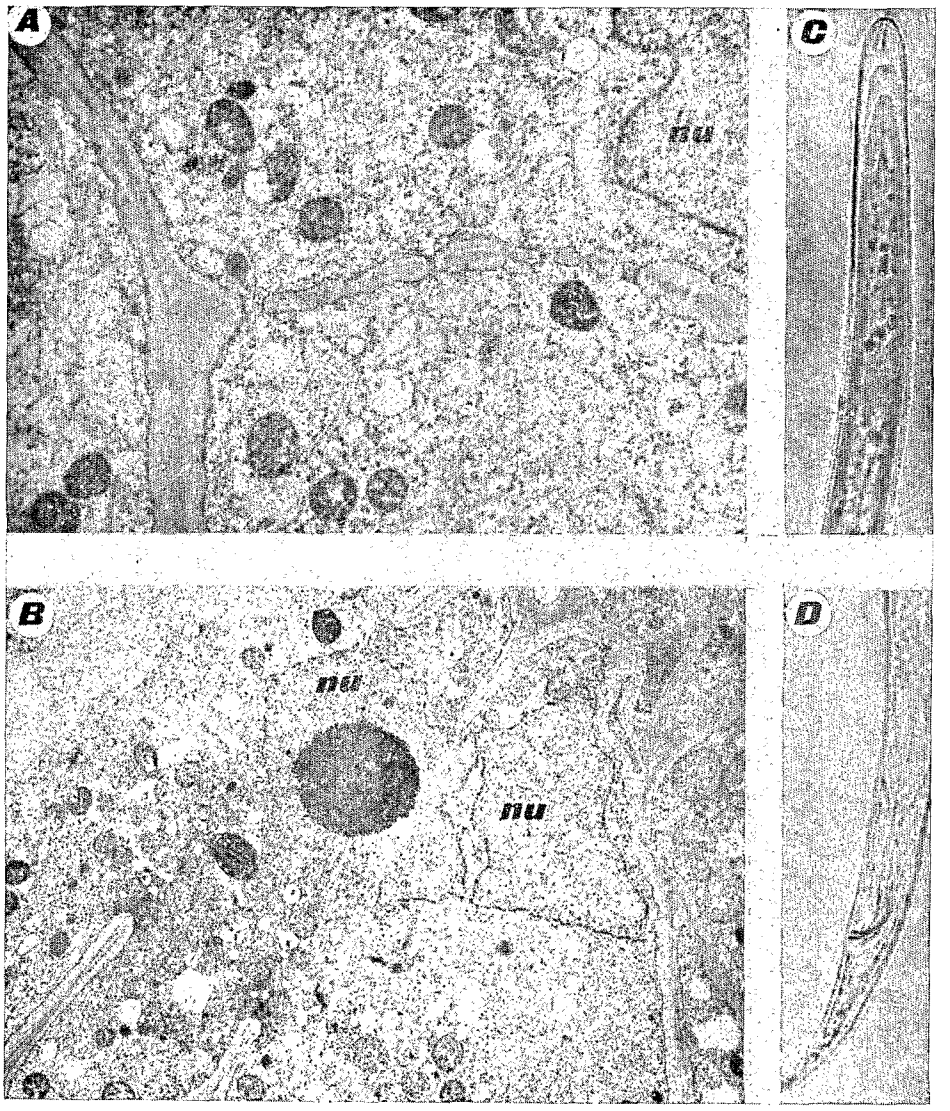

Fig. 7. A (× 8 100), B (× 4 763) : Electronmicrographs of syncytium in *M. longifolia* roots : note cell wall breakdown, movement of slightly enlarged and moderately lobed nuclei (nu), dense cytoplasm and organelle proliferation ; C (× 750) : Anterior end of adult *Meloidoderita* male, with molting cuticles attached : note progressive degeneration of stylet ; D (× 750) : Posterior end of male, showing lack of bursa.

lenchulus reniformis, where hypertophy of pericycle cells (Cohn, 1973) and cell wall breakdown in an area close to the nematode head have also been observed (Rebois, Madden & Eldridge, 1975). However, the actual syncytial region induced by *Meloidoderita* seems to be somewhat larger and contains more nuclei, apparently being created by an amalgamation of more cells. In comparison with its most closely-related genera, the plant reaction to *Meloidoderita* is certainly more specialized, since *Tylenchulus semipenetrans*, a cortical parasite, induces a more primitive, less differentiated cell response, lacking a syncytium (Himmelhoch *et al.*, 1979). The histopathology of *Sphaeronema* — another closely-related nematode genus — has not hitherto been investigated.

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REFERENCES

ANDREWS, S.W., KRUSBERG, L.R. & GOLDEN, A.M. (1977). The host range of a *Meloidoderita* species from Maryland. *J. Nematol.*, 9 : 261-262.

BIRCHFIELD, W. (1962). Host parasite relations of *Rotylenchulus reniformis* on *Gossypium hirsutum*. *Phytopathology*, 52 : 862-865.

COHN, E. (1973). Histology of the feeding site of *Rotylenchulus reniformis*. *Nematologica*, 19 : 455-458.

COHN, E. & MORDECHAI, M. (1977). Uninucleate giant cell induced in soybean by the nematode *Rotylenchulus macrodoratus*. *Phytoparasitica*, 5 : 85-93.

COHN, E. & MORDECHAI, M. (1981). Biology of a species of *Meloidoderita* found in Israel. *Summaries, 13th Ann. Meeting, OTAN, Guayaquil, Ecuador, June, 22-26, 1981*, unpaginated.

GOLDEN, A.M. (1976). First occurrence and morphology of a *Meloidoderita* species in the United States. *J. Nematol.*, 8 : 286.

HIMMELHOCH, S., COHN, E., MORDECHAI, M. & ZUCKERMAN, B.M. (1979). Changes in fine structure of citrus root cells induced by *Tylenchulus semipenetrans*. *Nematologica*, 25 : 333-335.

KIRJANOVA, E.S. & POGHOSSIAN, E.E. (1973). [Redescription of *Meloidoderita kirjanovae* Poghossian, 1966 (Nematoda : *Meloidoderitidae*) n. fam.] *Parazitologiya*, 7 : 280-285.

NARBAEV, Z.N. (1969). [Members of *Heterodera* and *Meloidoderita* in Uzbekistan]. *Matern. nauch. Konf. vses. Obsheh. Gel'mint Part* 1 : 195-200.

POGHOSSIAN, E.E. (1966). [A new nematode genus and species of the family *Heteroderidae* (Nematoda) in the Armenian SSR.] *Dokl. Akad. Nauk Arm. SSR.*, 42 : 117-123.

POGHOSSIAN, E.E. (1975). [Description of the male of *Meloidoderita kirjanovae* Poghossian, 1966 (Nematode, Meloidoderitidae).] *Dokl. Akad. Nauk Arm. SSR.*, 60 : 252-255.

REBOIS, R.V., MADDEN, P.A. & ELDRIDGE, B.J. (1975). Some ultrastructural changes induced in resistant and susceptible soybean roots following infection by *Rotylenchulus reniformis*. *J. Nematol.*, 7 : 122-129.

SIDDIQI, M.R. (1980). Taxonomy of the plant nematode superfamily *Hemicycliophoroidea*, with a proposal for *Criconematina*, new suborder. *Revue Nématol.*, 3 : 179-199.

THORNE, G. (1961). *Principles of Nematology*. New York, Mc Graw-Hill Book Co. Inc., 553 p.

VAN GUNDY, S.D. (1958). The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. *Nematologica*, 3 : 283-294.

WILLIAMS, T.D. (1978). Cyst nematodes : biology of *Heterodera* and *Globodera*. In : Southey, J.F. (Ed.) *Plant Nematology*, London, H.M.S.O. : 156-171.

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