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STUDIES ON LIFE CYCLE HISTO PATHOLOGY AND POSSIBLE HOSTS OF THE NEMATODE SARISODERA AFRICANA.

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RAPPORT DE STAGE

par

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I - INTRODUCTION

Sarisodera africana Luc, Germani & Netscher 1973, has been found during a nematode survey on different varieties of Guinea grass, Panicum maximum (Jacq.) which are present in trial fields of ORSTOM, at Adiopodoumé (Ivory Coast). One variety from East Africa (var. T 29) proved to be attacked by a new species of Sarisodera. Only one species of this genus had been described before : Sarisodera hydrophila Wouts & Sher, 1971. The description given by Luc, Germani and Netscher concerned adult males, adult females, cysts, eggs and second stage larvae.

The description of the life cycle of Sarisodera africana has been made at the Laboratory of Nematology of O.R.S.T.O.M., Adiopodoumé, Ivory Coast.

Several experiments have been made in order to observe and describe the different stages of the life cycle and the time at which they appeared after inoculation.

II - BIOLOGICAL CYCLE

A - Experiment n° 1.

Material and methods.

The parasites were extracted from roots of Panicum maximum grown in pots and previously inoculated with second stage larvae of Sarisodera africana from the original population raised in a small plot on old plants of P. maximum, near the laboratory.

The roots of Panicum were extracted from inoculated pots. The roots were washed free from soil with a very strong stream of water on two sieves respectively 1 mm and 160 µm. The females and cysts were collected in the debris on the 160 µm sieve, under a dissecting microscope. They were put in Dropkin's solution for a week, to allow them to mature at the same time. Dropkin's solution was then replaced by water and the cysts were squashed on a nematode filter. After three days, the second stage larvae were counted and each pot, containing one plant of a local variety of Panicum, was inoculated with 1.300 larvae.

Every three days, the roots were extracted and stained in lactophenol-cotton blue, using de Guiran's method (1966).

Ten days after inoculation, the plants were up-rooted and replanted in sterile soil.

Results. (table 1).

TABLE 1

Time after inoculation (days)	Second stage larvae	third stage larvae	fourth stage larvae ♂	fourth stage larvae ♀
4	6	7		
7	6	11		
10	19	27		
13		1	9	3
16		7	9	1

After four days, three of the six second stage larvae observed were penetrating. While the genital primordium could not be observed in the inoculated second stage larvae, it was quite visible in the penetrating larvae (fig. 1 B).

The transplanting at the tenth day must have reduced the number of nematodes, probably by breaking parts of the young roots. The animals mounted in lactophenol could not be kept for a long time, because in white lactophenol the colour faded and the details could no longer be observed. Later on, we used, for mounting, lactophenol with 0,001 % cotton blue.

B - Experiment n° 2.

In order to avoid the mistakes of experiment n° 1 we started a whole new series.

Matérial and methods.

Very few females were to be found in the second series of inoculated pots. So, in this experiment, the inoculum was extracted directly from the small plot where the species is kept on old Panicum plants. Only white females were found. After three weeks in Dropkin's solution, few females were starting to turn brown. They were squashed, in water, on a nematode filter, kept at 27° C and, every day, the newly hatched larvae were collected and inoculated. An attempt was made, without success, to extract larvae from the roots with a mistifier (Seinhorst, 1950).

Pots were inoculated with 1.000 or 500 larvae and, twice a week, the roots were extracted and stained in the same way as in experiment n° 1. The mounting was made in lactophenol with 0,001 % cotton blue.

Results (table 2).

After 40 days, second stage larvae are observed again in the root. They belong to the next generation.

TABLE 2

Time after inoculation	nb of pots	l_2	l_3	third moult	l_4 ♂	l_4 ♀	♂	♀	eggs.
3	2	24	12						
7	1	53	22						
10	1	21	18	+					
14	2	33	6	+	8				
17	1							13	
31	2							16	+
42	1	13	10						

No female l4 and few male l4 have been found. We know, by experiment n° 1, that l4 of both sexes are found at 13 and 16 days. This stage must last only a short time, the fourth moult occuring rapidly after the third one. Females are observed 17 days after the inoculation and the eggs which will give rise to the next generation are present at the 31st day. The males, which are liberated from the roots immediately after the fourth moult, could not be observed.

C - Experiment n° 3.

In order to determine the time at which males start leaving the roots, some plants were transferred, after penetration of the larvae, into a liquid medium. So, males, after leaving the roots, dropped down to the bottom of the container, where they could be recovered.

Material and methods.

The inoculum had the same origin as in experiment n° 2. Two pots were inoculated with 1.000 larvae and nine with 500 larvae. After ten days, the plants were extracted and the roots carefuly washed free from soil. Then, they were put in opaque polythen tubes containing Kimura's nutrient solution (Yoshida et al. 1959), aerated with bubbling air. Aeration, in a preliminary test, had already proved to be necessary to keep the plants alive. Twice a week, the Kimura's solution was changed and the males were

counted. When all the males had left the roots, these were stained like in the previous experiments, in order to count the females.

Results. (table 3)

Between 13 and 16 days, males start leaving the roots. The numbers observed are too small for a statistical analysis. At 27 days, all the males were dead, except one. Nothing can be said about the numbers of females. They are low, compared with the numbers of males and it is quite possible that some of them have been lost during the manipulations.

TABLE 3

Temps (jours)	Inoc.= 1000 ♂			Inoc. = 500 ♀									
	1	2	Total	3	4	5	6	7	8	9	10	11	Total
13	0	0	0	0	0	0	0	0	0	0	0	0	0
16	5	0	5	2	3	3	1	0	0	3	9	1	22
20	2	146	148	16	22	6	9	8	2	8	31	25	127
23	6	0	6	4	0	3	0	2	2	0	2	7	20
27	0	6	6	1	0	0	2	0	0	0	0	3	6
Total ♂	13	152	165	23	25	12	12	10	4	11	42	36	175
♀ (28 jours)	0	2	2	5	12	30	8	5	2	2	1	7	72

In the pots inoculated with 500 l_2, the observed sex ratio is : 2,43.

D - Experiment n° 4.

Material and methods.

The purpose of this experiment was to test other methods of extraction of nematodes from the roots.

We used nematodes from the same suspension as in experiment n° 2. Pots were inoculated with different numbers of nematodes : 5.000, 1.000 or 500 and several extraction methods were tried.

I. In order to extract females, the domestic waring blendor was used. The roots were stained with acid fuchsin (Goodey, 1937) and mixed several times for respectively 5, 10, 20 and 40 seconds in water. The liquid was, then, poured over two sieves, respectively 2 mm and 35 µm and the females were collected among the debris on the 35 µm sieve. This procedure is commonly followed to recover the females of Heterodera oryzae from rice roots.

II. In order to collect and count males present in soil, free living nematodes are extracted with Seinhorst's elutriator, 20, 26, 28, 62, 64 and 67 days after inoculation.

III. Because many females could have been lost during the manipulations of the roots, an attempt was made to recover females in the soil. In a first series, the roots were washed with all the soil contained in a pot, on four sieves, respectively 4 mm, 1 mm, 280 µm and 160 µm. But, by this method, mistakes were made too easily and, in a second series, the roots were extracted from the soil with a good quantity of soil surounding them and washed over two sieves, respectively 1 mm and 160 µm. The debris collected on the 160 µm sieve were put in a sugar solution with a density of 1,15 (460 g of sugar in one liter of water). After decantation, the females were counted in the supernatant.

Results.

I - After using the waring blendor, no female was found.

II - The results obtained in extracting males from soil with Seinhorst's elutriator are shown in table 4.

TABLE 4

Inoculum	Days after inoculation	Days after begenning of the 2d generation	nb of pots	nb of
5.000	20		1	185
5.000	26		1	3
1.000	28		2	2
5.000	62	20	1	21
500	64	22	4	0
500	67	25	4	0

III - The numbers of females extracted by washing roots and soil over sieves are shown in table 5. In experiment n° 2, where females were counted directly on roots previously washed free from soil before staining, we normally observed 3 or 4 females per pot inoculated with 1.000 larvae. It is obvious that many females were lost by washing the roots and fourth stage female larvae may have been lost in the same way.

TABLE 5

Days after inoculation	number of pots	Inoculum	number of ♀
35	1	5.000	27
62	1	5.000	57
64	4	500	72
67	4	500	67

It is obvious that females are easily separated from the roots when the plant is uprooted and the roots are washed. The roots must be removed gently and females must be looked for in the soil surrounding them.

After 28 days males are no more found in soil until the next generation appears. Their life must be relatively short.

E - Discussion.

The numbers of animals observed in the roots at the first generation are low. This may be due to different causes. One of them is that the conditions in a pot are not the same as in a field. In order to obtain larger numbers of animals, it would be better to wait until the parasite has completed a second generation. On the local variety of *P. maximum* which we used in the previously described experiments, we observed, at the second generation, in a pot inoculated with 5000 l_2, 57 females and 21 males and, in two pots inoculated with 500 l_2, 61 females.

Another reason for the fact that we observed relatively small populations can be that the variety was not the most suitable host for *S. africana*. We inoculated, with 1000 l_2, a pot containing the variety T29 on which the species has been discovered. After 12 days, we found 1 l_2, 45 l_3, 30 male l_4 and 15 female l_4. It is obvious that T29 is a better host than the variety used in the previously described experiments. Unfortunately, very few cuttings of T29 were available.

III - DESCRIPTION OF LIFE STAGES

The descriptions of adults, second stage larvae and eggs have been given by Luc, Germani and Netscher (1973).

Eggs : In the females starting to turn brown, we already found eggs in all stages of development (fig. 1 A).

To repeat shortly the characteristics of eggs : They are 105 µm long and 40,5 µm wide. Their shape is cylindrical with two hemispheres at the ends. The development begins with divisions in two, four, eight sixteen cells and so on. Then, the embryos appear , which show one flexure. At the end of its development, the embryo has three flexures.

As already noted by the authors of the species, we did not observe any gelatinous matrix containing eggs.

Second stage larva.

The second stage larva leaves the egg and penetrates in the roots of the host plant. It remains in the cortex and puts its head firmly into the central cylinder. It generally assumes a position parallel to the longitudinal axis of the root.

The genital primordium, observed in penetrating larvae (fig. 1 B) is divided in two cells with visible nuclei.

We do not know how long the second stage larva can stay in the egg, in a dormant stage, but, presumably it can stay there a very long time, may be several years like in Heterodera species.

The presence of second stage larvae belonging to the next generation has been observed 42 days after inoculation.

Second moult. (fig. 1 C)

Immediately after having reached its final position, the second stage larva undergoes its second moult. This moult occurs inside the skin of the second stage larva which has the dimensions of about 0,300 mm in length and 0,038 mm in width. Three days after inoculation, there was already evidence of third stage larvae being present.

Third stage larva (fig. 1 D, E, F, G and 3 B)

This stage begins shortly after the penetration of second stage larvae into the root and seems to last a long time for fourth stage larvae were not found before 12 days after inoculation. The third stage larva assumes the same

position parrallel to the longitudinal axis of the root, as the second stage larva (fig. 1 D).

It is wider and a little shorter than the second stage larva. In one case, the measures were 0,262 mm for the length and 43 µm for the width in a newly formed third stage larva.

The genital primordium is 30 µm in length and oval (Fig. 1 E). Further on, during this stage, the sexes can be distinguished as the genital primordium starts growing. In males, it elongates at the anterior end (Fig. 1 F) which becomes reflexed (Fig. 3 B) and then, the posterior end starts elongating. In females, the genital primordium elongates in two branches at the anterior end.

The spear is less robust than in the second stage larva (respectively 7-14 µm and 18-20 µm). The basal swellings are not so distinct as the basal knobs of the preceding stage.

The tail is about 10 µm long and bears a mucro. The anus is dorsal.

As already observed in H. schachtii (Raski, 1950) and in H. oryzae (Berdon & Merny, 1964) the median bulb in female third stage larvae is larger than in male third stage larvae (23-25 µm against 18-20 µm).

The cuticle is marked by irregular longitudinal ornementations (Fig. 1 G) except for the head region which has narrow transverse annulations.

The fully developped third stage male larva is 0,284-0,380 mm in length and 0,059-0,093 in width while the fully developped third stage female larva is about 0,300 mm long and 0,095-0,108 mm wide.

Third moult (fig. 1 H and I)

The third moult of both sexes occurs inside the skin of the third stage larva. Evidence of the third moult in males is 10 days after inoculation. It was 10 to 13 days for the female larvae.

At this stage, male larvae are coiled twice (fig. 3A) and the single-branched genital primordium is well elongated (fig. 1 I). In female larvae, the two branches of the genital primordium are almost as long as the body itself (fig. 1 H).

Fourth stage larva. (fig. 2 A and B)

The fourth stage male larva grows completely inside the skin of the third stage larva. It elongates in anterior and posterior directions forming two coils (fig. 1 I) At the end of the development of this stage, the body of the developping male is coiled three times and a weakly developped spear can be distinguished.

In this stage, wheras males grow in length, females grow in width (fig. 2 A). Their dimensions vary from 0,365 to 0,390 mm in length and from 0,170 to 0,195 mm in width. The length of the spear is 79 µm. The anus is subterminal. The cuticle is marked by irregular longitudinal lines (fig.2 B), except for the head region which bears narrow annulations.

Fourth moult. (fig. 2 C and D).

In males, evidence of the fourth moult is between 13 and 16 days after inoculation. It is completely inside the skin of the third and fourth stage larvae. At this time, the spicules and the spear can be distinguished (fig. 2 C) ;

During the fourth moult of the female larva the dimensions were 0,450-0,530 mm in length and 0,202-0,210 mm in width. At this stage, the vulva is beginning to open (fig.2 D).

Male.

Males start leaving the roots between 14 and 16 days after inoculation. Most of them are liberated at about the 20th day and, at the 28th day almost all have left the roots That last day, free moving nematodes have been extracted from the soil and only a few males were left. Their morphological characteristics have been fully described by Luc et al. (1973).

Female.

The first adult females were observed 17 days after inoculation. In the original description, the young female has not been fully described because the anterior end of the extracted females was always damaged. In one case, we could obtain a young female with complete head and oesophagus. The head and the neck are very small compared with the size of the entire animal. The cuticle is marked by irregular transverse annelations, the spear is 33 µm long and the median bulb has a diameter of about 23 to 25 µm.

An attempt has been made to extract entire females from the roots without injuring their necks. Parts of roots, wherein the presence of females had been observed, were put in a solution of cellulase (Merck 2329) at a concentration of 30-50 mg in a buffer at pH 5,5 (86 cm^3 of a solution of 2,101 g of citric acid in 100 cm^3 of water and 114 cm^3 of a solution of 10,752 cm^3 of $Na_2 HPO_4$). The root pieces remained in the buffer 16-20 hours at 30°C. The females could then be extracted without damaging their head but much debris remained around the head and had to be removed (fig. 2 E). Unfortunately, only rather old females could be collected that way but the method can be tried to extract younger females.

IV - HISTOLOGICAL STUDIES

Material and methods. P. maximum cuttings have been planted in frames with glass at one side. The frames were put in sand, leaning with the glass side under. S. africana was inoculated, using the same larvae suspension as in experiment n° 2. The roots were periodically observed through the glass and parts of roots bearing females were cut off. They were killed with F.P. 4-1, fixed in Bouin's fluid and embedded in paraffin. Sections 15 µm thick were made with a microtome and stained, using Gason's fluid (orange G, anilin blue, phosphotungstic acid and acid fuchsin).

Results. In only one case has a female be observed undisturbed with the head in the central cylinder. Other females had been removed, probably by the blade of the microtome (fig. 2 F). Host reactions like giant cells or thylles, as found in Heterodera oryzae (Berdon & Merny, 1964), have never been observed. Not uncommonly, the female remains in the cortex without damaging it. This is remarkable because Heterodera females leave the root except for their head region. When females of Sarisodera do not break through the cortex, hardly any difference can be observed with the rest of the root, except for a swelling of the root, caused by the female itself. In this case, the host tissues around the female display a pink to violet reaction. This coloration of the host tissues could not be explained.

V - OTHER POSSIBLE HOST PLANTS

The only known host plant of S. africana is Panicum maximum. The host range of the species would be of great interest. The three other species of Heteroderidae known in Africa and all belonging to the genus Heterodera have been found as parasites of Graminae and their host ranges, which are under study, are quite different from one another.

As a first step in the study of the host range of S. africana, second stage larvae have been inoculated to three graminae : maize, rice and sorghum, and to soya.

Maize. Nine pots with three seven days old maize plants were inoculated, each with 2.000 larvae. After 12 days, the roots of two pots were extracted and stained, one with cotton blue and the other with acid fuchsin ; five second stage larvae and 119 third stage larvae were observed in the first pot and nine second stage larvae and 19 third stage larvae in the second one.

Fourteen days after inoculation, the plants of five pots were put in aerated nutrient solution (Kimura) ; 17 days and 20 days after inoculation the solution was changed and, the last day, the roots were stained with acid fuchsin. They were treated in the blendor in order to count eventual females. At the same day, the roots of the two last pots were equally stained and treated in the blendor. Just one male has been observed in the nutrient solution and no female was found after treatment with the waring blendor.

As already stated, the blendor does not seem to be a good mean for extracting S. africana females, for it probably destroys them. However, as only one male has been found, it seems likely that no female has developped, the development of the parasite in maize being stopped, mostly at the third stage larva.

Rice. Three pots, each containing four rice plants (var. Moroberekan), were inoculated with 2.000 larvae ; 21 days after inoculation, the roots of two pots were extracted and stained. The roots of the third pot were stained 28 days after inoculation. In one of the two first pots, eleven third stage larvae were observed, some of which, all males, were undergoing the fourth moult. In the other pot, two third stage larvae were observed. In the roots stained 28 days after inoculation, nothing was found. The fact that nothing was observed on old roots means that none of the previously observed third stage larvae had developped into females.

Rice is not a suitable host for S. africana.

Sorghum. Two pots, each containing three twelve day old sorghum plants were inoculated with respectively 1.000 and 1.300 larvae. In the roots of one pot, extracted seven days after inoculation, nothing was observed and only three second stage larvae in the roots of the second pots, extracted twelve days after inoculation.

Sorghum is not a host of S. africana.

Soya. Two pots, each containing two soya plants were inoculated with respectively 650 and 700 larvae. No penetration was observed in the roots stained eight days after inoculation.

Conclusion. The host range of S. africana is far from being known, but this first attempt to determine it indicates that it must be very different of these of the three African Heterodera species : it is a parasite neither of rice, which is a host of Heterodera oryzae and H. sacchari, nor of sorghum, which is a host of H. gambiensis, nor of maize which is, in a lower extent, parasitized by H. oryzae.

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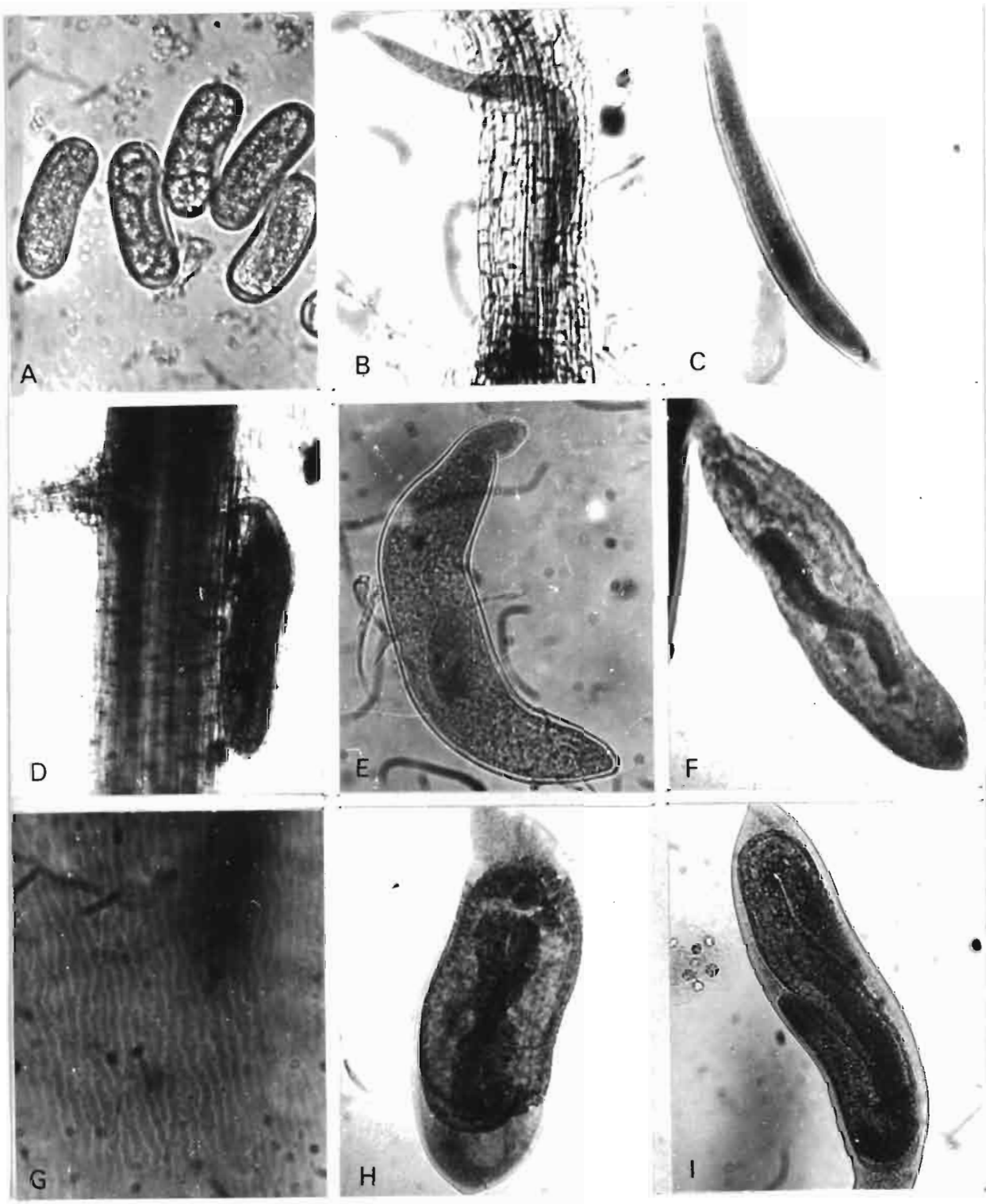

Fig. 1 - Sarisodera africana - A : eggs - B : penetrating second stage larva - C : second moult - D : third stage larva in a root - E : newly formed third stage larva : genital primordium undifferenciated. F : male third stage larva - G : cuticle ornementations of a third stage larva - H : third moult of a female larva - I : third moult of a male larva.

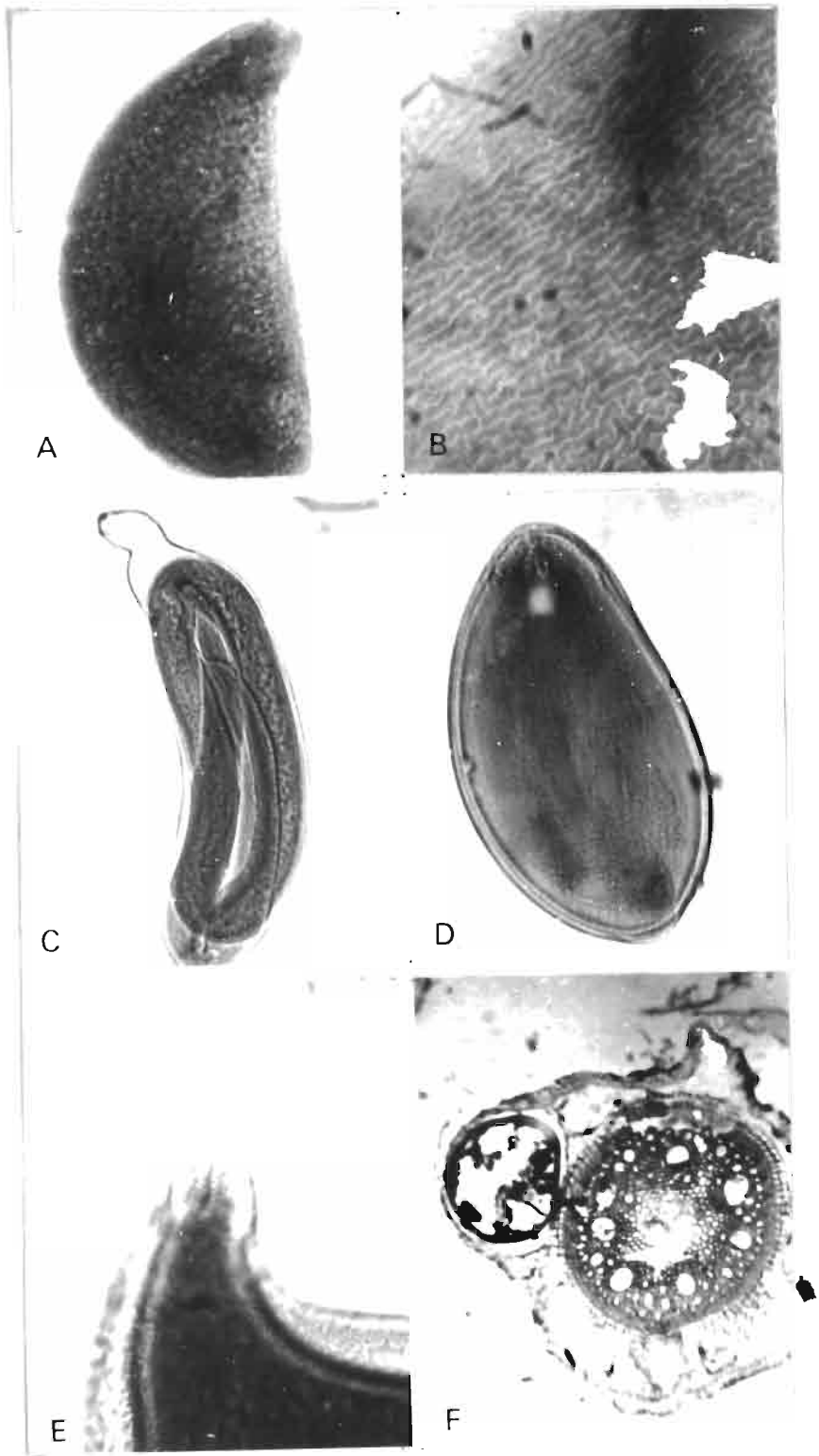

Fig. 2 - Sarisodera africana - A : male fourth stage larva - B : Cuticle ornementations of a fourth stage female larva - C : fourth moult of a male larva - D : fourth moult of a female larva - E : head of a female - F : section through a root at the feeding point of a female.

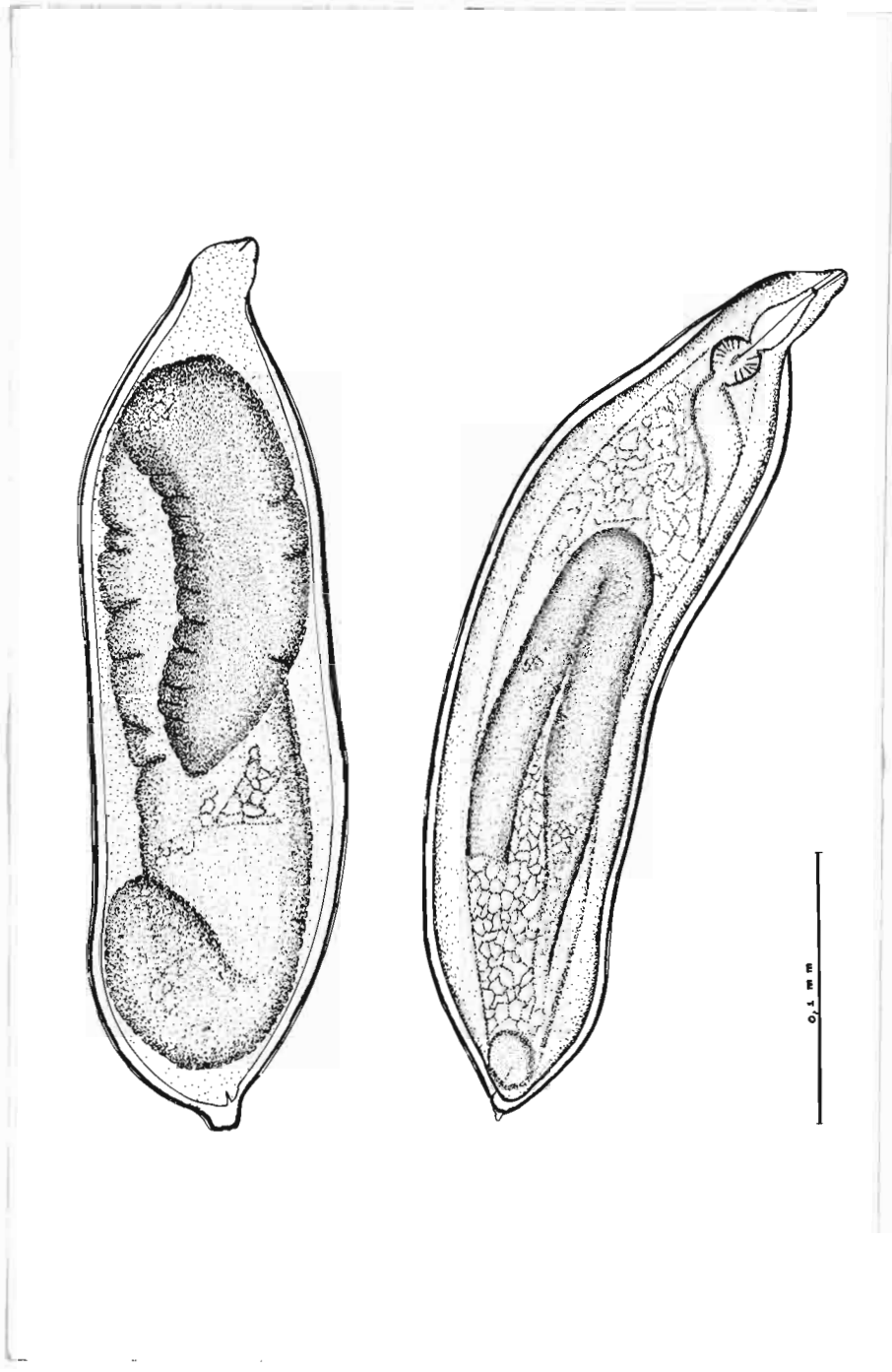

Fig. 3 - A : third moult of a male larva - B : male third stage larva with reflexed genital primordium.