Biology of the plant-parasitic nematode *Scutellonema cavenessi* Sher, 1964 : reproduction, developement and life cycle

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SUMMARY

Life cycle, mode of reproduction, development inside egg and after hatching of *Scutellonema cavenessi* were studied using individuals recovered from dry soil kept at 25-28° for two years. After reactivation from anhydrobiosis *S. cavenessi* mated and laid eggs in the absence of plants. Newly hatching 2nd stage larvae were infective and invaded roots of hosts (sorghum and tomato), larvae left roots and returned to soil as 3rd or 4th stage larvae and developed to adults in soil, feeding on roots as ectoparasites. Data suggest that males are necessary for reproduction and that *S. cavenessi* is an amphimictic species.

RÉSUMÉ

Biologie du nématode phytoparasite Scutellonema cavenessi *Sher, 1964 : reproduction, développement et cycle biologique*

A partir d'échantillons de sols secs conservés depuis deux ans au laboratoire, les auteurs ont pu observer, après humidification, le cycle biologique complet de *Scutellonema cavenessi* en présence de deux plantes hôtes : le sorgho cv. 51-69 et la tomate cv. Rossol. Dans le sol sec, le nématode est en état d'anhydrobiose, état de résistance aux conditions de la saison sèche en zone sahélienne. Après humidification du sol la copulation intervient très rapidement et la première ponte apparaît après 9 jours : elle est suivie de l'éclosion de la larve du 2e stade 14 à 15 jours après la ponte ; dans l'eau distillée cette durée est légèrement inférieure (10 à 11 jours). Il apparaît que la reproduction est de type amphimictique avec méiose normale, et un nombre de chromosome n = 8. En présence de plantes hôtes, sorgho et tomate, les larves de 2e stade pénétrent dans le cortex des racines et s'y déplacent de façon inter- et intracellulaire. La 2e mue intervient dans le cortex de la racine 10 à 15 jours après inoculation. La 3e mue, entre le 20e et 27e jours, a été observée tout à la fois dans le cortex des racines et dans le sol. Les larves de 4e mue n'ont été extraites qu'à partir du sol, et ce entre le 34e et 41e jours après inoculation. A partir de ce moment les adultes ne sont retrouvés que dans le sol. Le cycle complet d'adulte anhydrobiotique à adulte est de 65 à 72 jours, à 28°.

Scutellonema cavenessi Sher, 1964, described from bush soil in Nigeria is one of the most common nematodes in the Senegalese Sahel where it has been found in soil around peanut, cowpea, lettuce, sweet pepper, tobacco, tomato, onion, papaya, cotton, millet and sorghum. This nematode has been reported to damage peanut production (Germani & Gautreau, 1977).

Little information is available regarding the biology of this parasite except that Demeure (1975, 1978*a* & *b*) showed the resistance of *S. cavenessi* to desiccation and to thermal stress. The present work, part of a study on the biology of *S. cavenessi* deals with the life cycle of this nematode and presents data on reproduction, on sorghum (*Sorghum bicolor* L.) cv. 51-69 and on tomato (*Lycopersicon esculentum* Mill.) cv. Rossol.

Materials and methods

The soil was dry when it was collected in April 1977, six months after the beginning of the dry season and was kept in polyethylene bags at 20-25° for two years. Moisture content of the soil was 0.5%, a value far inferior to that of soil at permanent wilting point (2.5%). All experiments described below were made with nematodes extracted from this soil. The anhydrobiotic condition of *S. cavenessi* was observed on nematodes extracted using the technique of Freckman, Kaplan and Van Gundy (1977).

For observations on the life cycle distilled water was added to bring soil to field capacity. The moistened soil was placed in a dark room at 28° for up to 24 days. *S. cavenessi* was collected from 100 cm³ samples at 24 hour intervals. Nematodes were extracted by centrifugation (Gooris & D'Herde, 1972).

Females extracted during the first nine days were observed for development of the ovary including fertilization and oogenesis.

In order to determine the role of males in oogenesis, adult nematodes (male and female) were extracted immediately after moistening infested soil. Under the dissecting microscope batches of either 50 females or 50 males were hand picked. In one case nematodes of both sexes were added to moist soil whereas in the other case only females were added. This experiment was repeated twice. The gonads were observed after 9 days. Temperature of the soils in all experiments concerning gonad development was 28°.

Observation on the development of ovaries was made using different techniques. Some observations were made on ovaries dissected and stained with orcein as described below. Fourth stage larvae and females were also stained *in toto* by killing nematodes in a drop of water by gentle heat. After keeping them for two hours or more in 20% acetic acid, nematodes were transferred to acetic orcein for 4 hours. Observations were made with the nematodes mounted in acetic orcein. Observations were also made on nematodes killed in hot FP4 : 1 (Netscher & Seinhorst, 1969), fixed in 4% formaldehyde and transferred to glycerin (Seinhorst, 1959).

Cytological observations were made by placing fertilized females in a small drop of distilled water on a microscope slide. After cutting each female at vulva level with an oculist scalpel, eggs and ovaries were expelled from the body. Slides were allowed to dry to let the ovaries and eggs adhere to the glass. Subsequently sides were immersed in 1N hydrochloric acid for 5 minutes after which they were fixed in acetic alcohol for 20 minutes (Triantaphyllou, 1962). After fixation, a drop of acetic orcein (2 g of orcein in 100 cm³ of 45% acetic acid) was placed on the nematode material and a cover slip applied. Observations were made immediatly, using high power magnification.

Gravid females were picked from a nematode suspension and kept in BPI watch glasses in phosphate buffer, pH 7.2, for studies on development inside. For photomicrographs and visual observations of egg laying and development of embryos at high magnification, hanging drop preparations of the newly laid eggs were made. Photomicrographs were taken with a Leitz "Orthomat" photomicroscope.

Development after hatching was studied by inoculating freshly hatched 2nd stage larvae on 5 day-old sorghum cv. 51-69 seedlings and 9 day-old tomato cv. Rossol seedlings grown in 1.1 l pots filled with sterile soil at 28° ± 3°. Pots were inoculated with ten second stage larvae each freshly hatched from an egg in

phosphate buffer pH 7.2. During 48 days three pots each of sorghum and tomato were examined at 5 or 7 day intervals. Roots were stained with acid fuchsin (Goodey, 1937), flattened between two microscope slides and observed under low power magnification. Nematodes were extracted from soil using the technique of Gooris and D'Herde (1972). The experiment with sorghum was repeated twice.

Observations made

Nematodes extracted from dry soil in 1.25 M sucrose were found to have a tightly coiled spiral shape, characteristic for anhydrobiotic nematodes (Demeure, Freckman & Van Gundy, 1979*a* & *b*). Under natural conditions anhydrobiotic coiling takes place when soil starts to desiccate, at the beginning of the dry season. Coiled nematodes constitute a resistant form when the environment is deficient in water (pF 4.2) (Demeure, 1978*b*). When optimal moisture content is restored (field capacity) nematodes lose their spiral shape and resume activity.

Reproduction

Development of female gonads was followed from 4th stage larvae to egg laying females.

Prior to the last moult ovaries consisted of a zone of undifferentiated cells (Fig. 1). In females extracted from infested soil, one day

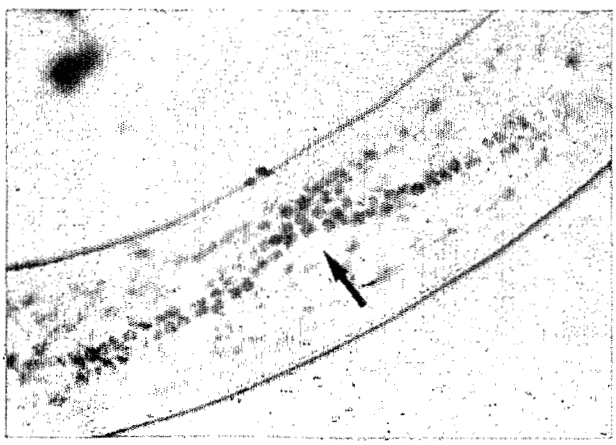

Fig. 1. Reproductive system of fourth-stage larva (female) (arrow).

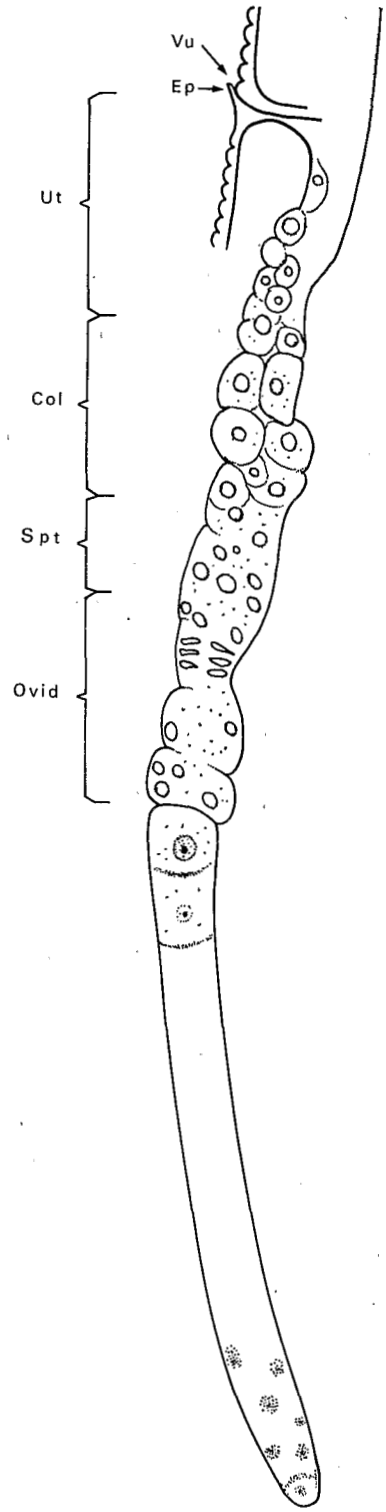

Fig. 2. Diagram of reproductive system of female freshly extracted from dry soil. Vu : vulva ; Ep : Epiptygma ; Ut : uterus ; Col : columella ; Spt : spermatheca ; Ovid : oviduct.

after moistening, cells constituting the uterus, quadri-columella, spermatheca and oviduct were small, making it difficult to distinguish between these structures (Fig. 2). At this stage the germinal and multiplication zone occupy approximately half the length of the ovary. Four days after moistening the soil, pairs of males and females in the process of copulation were extracted (Fig. 3 & 4) with regularity. It should be noted that copulating males and females were extracted between the 4th and 12th days after moistening of the soil. After this period, no female coupled to a male was extracted and 80 to 90% of the females showed indications of fertilization.

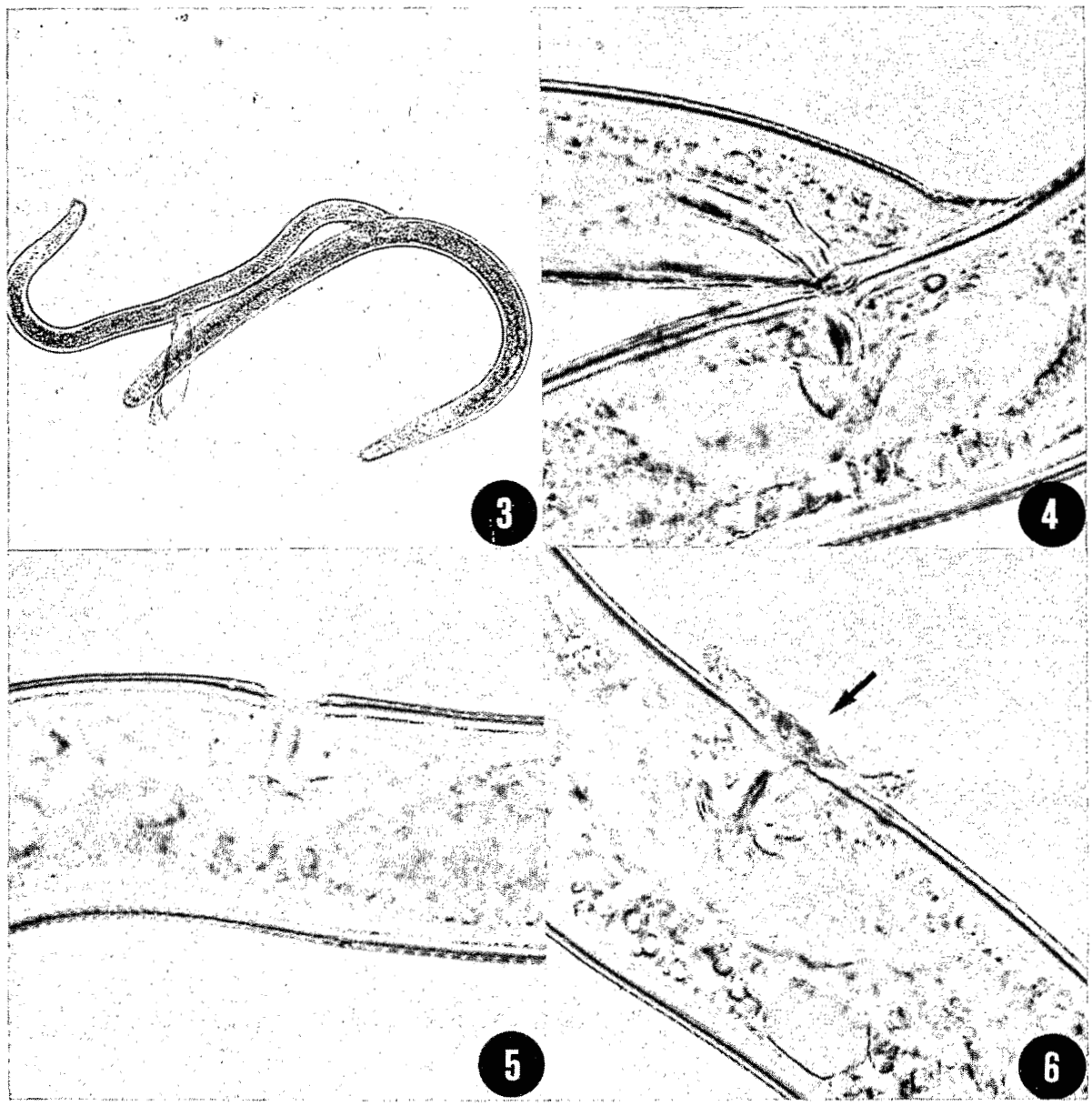

Fig. 3 to 6 ; 3 : Copulation ; 4 : Position of spicules during copulation ; 5 : Vulva and uterus before copulation ; 6 : Vulva and uterus after copulation : note the plug on the vulva (arrow) and the increase in volume of the uterus.

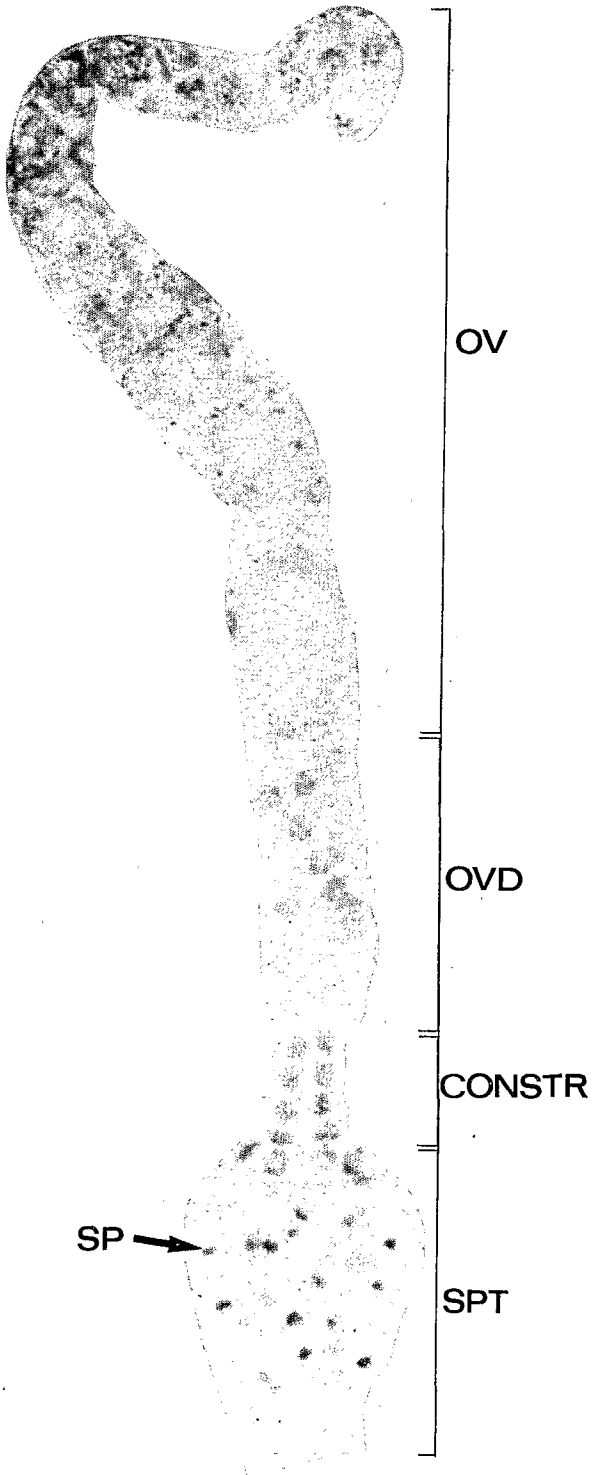

Fig. 7. Composite photomicrograph of reproductive system after copulation. Ov : ovary ; Ovd : oviduct ; Constr : constriction ; Spt : spermatheca ; Sp : spermatozoids (stained with acetic orcein).

Following copulation there was an appearance of a "mucuslike" plug in the vulva (Fig. 6), and an increase in volume of the uterus (Fig. 6) and the spermatheca which could easily be distinghuished from the oviduct and quadricollumella contained spermatozoa (Fig. 7). Oogenesis was initiated after copulation and the

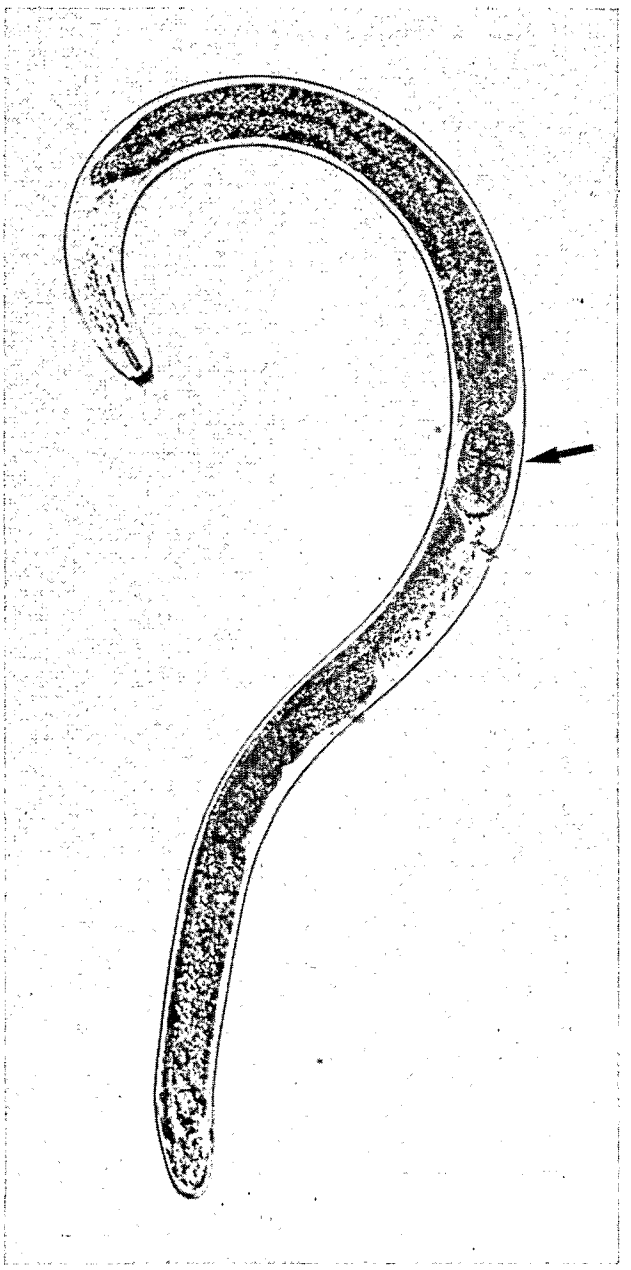

Fig. 8 : Gravid female with egg (arrow).

first eggs appeared eight days after moistening the soil (Fig. 8).

Due to the limited availability of females with well developed eggs and the rapidity of oogenesis, meiosis could only be partially followed. Observations suggest that oocytes undergo a normal meiosis. During anaphase and telophase I, sperm could be observed at the anterior pole of the egg (Fig. 9 A & B). Although no prometaphase or metaphase of meiosis I could be observed, telophase plates in polar bodies permitted eight chromosomes to be distinguished, each consisting of a pair of chromatids (Fig. 9 C & D). In one egg a metaphase II was observed (Fig. 9 E).

Hand picked females placed in moist sand in the absence of males had poorly developed ovaries ; spermatozoa were not observed and oogenesis had not taken place. In one case an oocyte was found in which no meiotic activity could be demonstrated, the egg consisted of an egg shell containing no material that could be stained with orcein. In females which had been exposed to males, fertilization and oogenesis were observed.

Egg laying

When the egg passed into the uterus the nematode undulated intensely and the pseudocoelomic fluid circulated rapidly around the uterus and the vagina. The egg was then rapidly and forcefully ejected. It took a few seconds for the egg to be laid (Fig. 10 A-C). First eggs were laid nine days after moistening the soil.

Females of *Scutellonema cavenessi* extracted from dry soil, stored for 30 months, were able

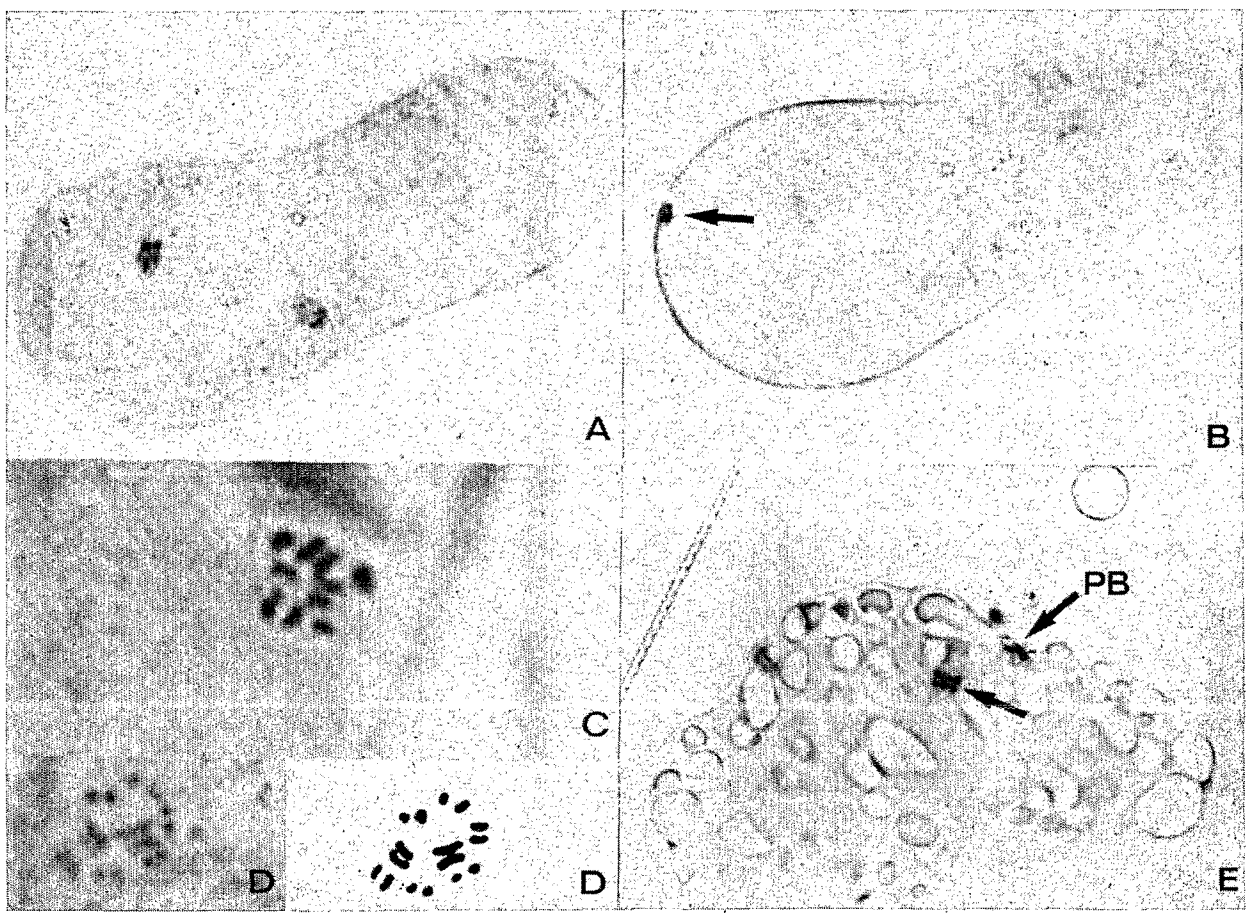

Fig. 9. A : Meiosis (telophase I). B : Same ovocyte as in A, spermatozoid (arrow) ; C, D : Telophase plate of polar bodies (n = 8) ; E : Metaphase II (arrow) ; pb : polar body.

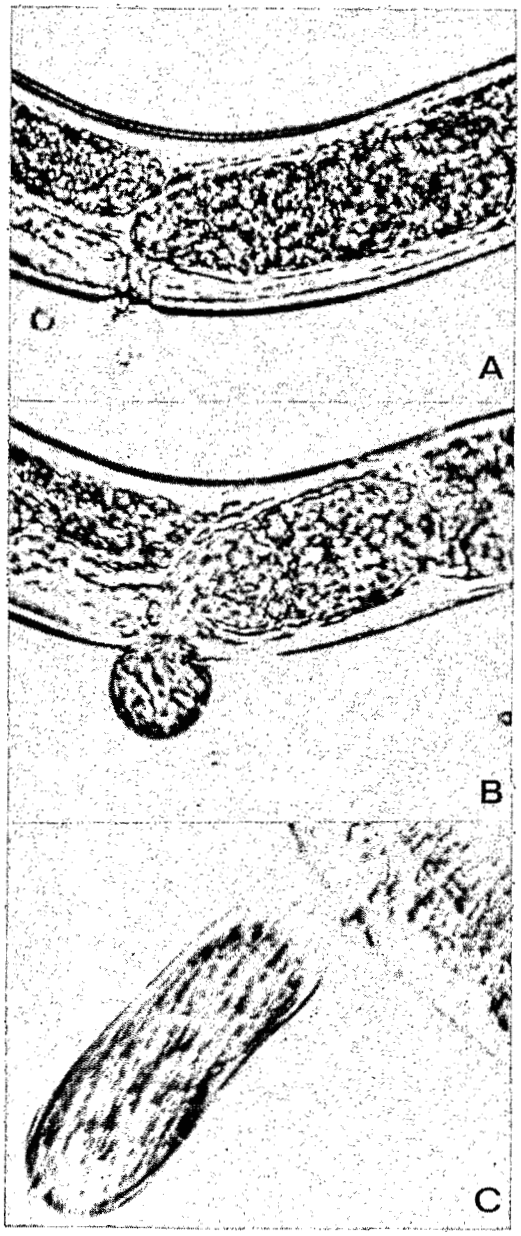

Fig. 10. A-C : Successive stages in egg expulsion.

to lay at least two eggs. Following the production of these eggs, laying stopped but resumed if nematodes had the opportunity to feed on roots of sorghum plants grown on agar. Although it was difficult to count the number of eggs laid by a single female because some eggs laid on agar in a Petri dish culture of sorghum escape observation, an average production of five to ten eggs per female was estimated in ten days.

Development inside eggs

Average dimensions of eggs were 70 × 20 µm ; eggs usually were laid unsegmented (Fig. 11 A). Sometimes, however, cleavage divisions occurred inside the body and eggs with two, three or four blastomeres were observed. The first division was transverse and stage II blastomeres were visible 2 to 4 hours after egg deposition (Fig. 11 B). The second, third and fourth divisions (Fig. 11 C, E) were also transverse, resulting in the formation of five blastomeres in tandem in 20 to 24 hours. Then rapid cell divisions led to the formation of many celled stages (Fig. 11 F, G). The gastrula stage was reached 6 days after oviposition (Fig. 11 H). Cells differentiated into two zones of dissimilar densities ; the light zone became the oesophagus and the dark zone intestine.

Cell differentiation leading to the formation of the first stage larva (Fig. 11 I, L) took place from the 7th to the 9th days after egg laying. The second stage larvae (Fig. 11 N) hatched within 1-2 days after the first moult. The time required from egg laying to hatching in distilled water was 10-11 days. Second stage larvae were recovered from soil, starting 24 days after moistening. Thus, development inside eggs in soil took place in fourteen to fifteen days.

Development after hatching

Development after hatching of *Scutellonema cavenessi* consisted of three larval stages and adults.

Second-stage larvae

Just after hatching (Fig. 12) second stage larvae were about 200 µm long and 15 µm wide. They were characterized by a pointed tail ; in all other stages the tail is blunt. Five days after inoculation second stage larvae were observed in the cortex of the roots of the host plants i.e. sorghum and tomato (Fig. 14 & 19). Larvae moved intracellulary and intercellulary, perforating cell walls on their way (Fig. 15). In only one case, a larva was observed feeding on the central cylinder of a tomato root (Fig. 20). Sometimes larvae were coiled inside cells of the cortex of tomato roots (Fig. 21).

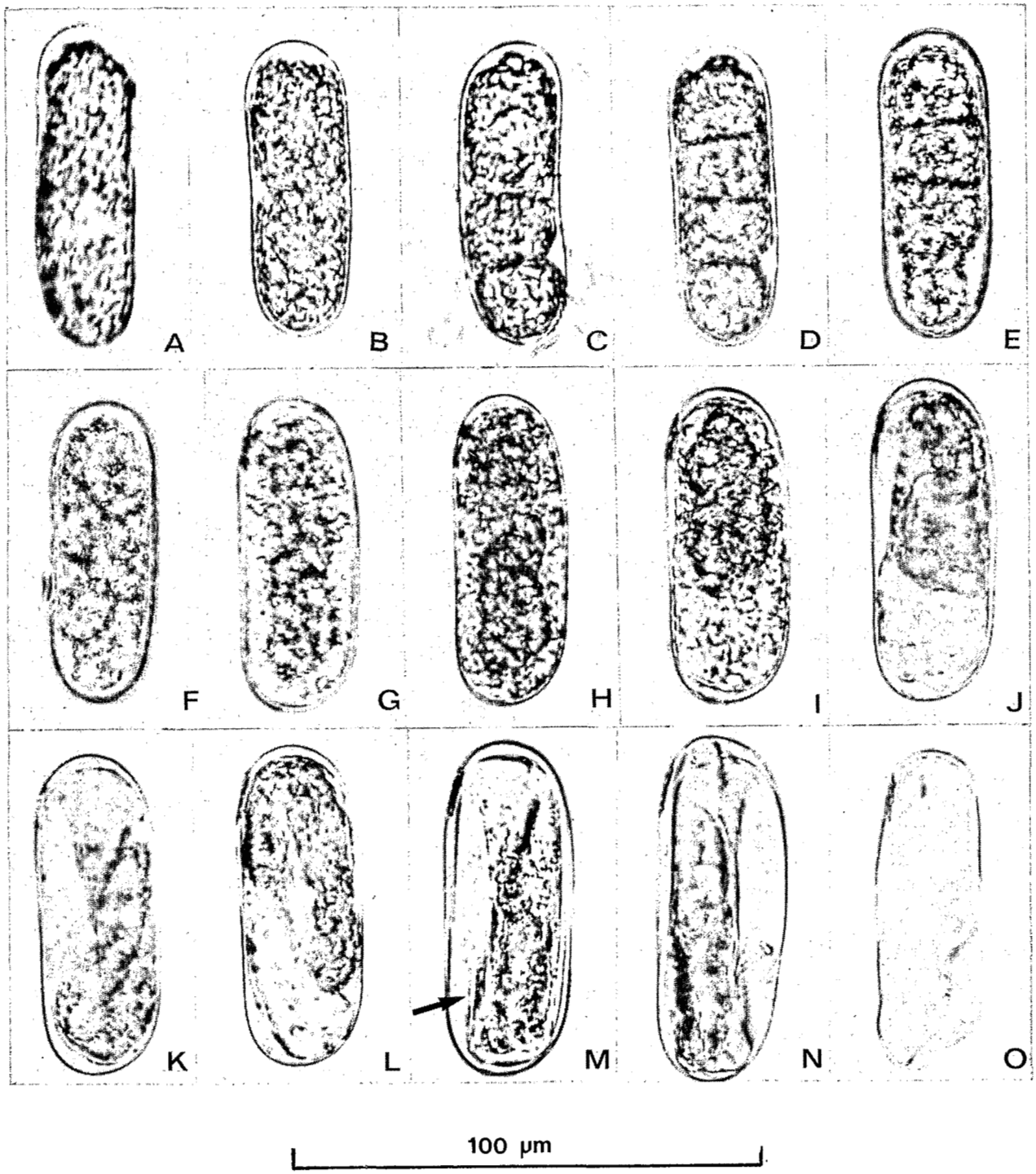

Fig. 11. A-N. Egg of *Scutellonema cavenessi* in various stages of development. A : One celled stage ; B : Two celled stage ; C : Three celled stage ; D : Four celled stage ; E : Five celled stage ; F. G : Multiple celled stage ; H : Gastrula ; I : "Tadpole" stage ; J. K, L : First stage larvae ; M : Second stage larvae, note the stylet (arrow) ; N : Second stage larvae prior to hatching ; O : Egg shell after hatching.

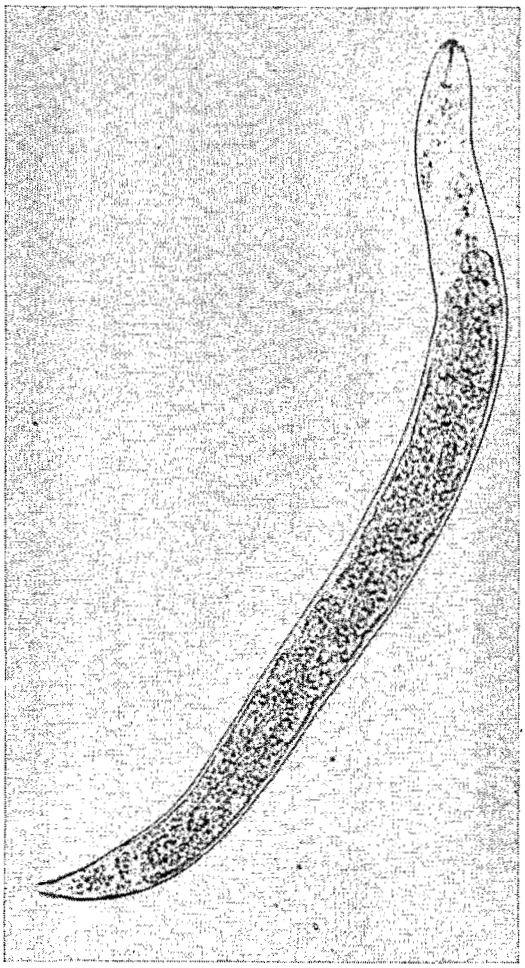

Fig. 12 : *Scutellonema cavenessi*, Second-stage larva after hatching.

Tomato roots, infected by second-stage larvae of *S. cavenessi* exhibited a slight browning of cells in the vicinity of the nematodes especially near the head of the nematode (Fig. 19). The browning was accompanied by a slight thickening of the cell walls (Fig. 19). These observations were not confirmed in thin histological sections of tomato roots infected by second-stage larvae (Fig. 22). Five days after penetration the average length of the nematodes observed in sorghum roots was 219 µm.

Second moult

The second moult took place inside the roots ten to fifteen days after inoculation. With only one exception larvae undergoing this moult were not extracted from soil around inoculated roots. The moult could be identified by the clear regions in the anterior and posterior end of the nematode (Fig. 16).

Third stage larvae

Fifteen days after inoculation the majority of larvae were in the third stage, average length of larvae in sorghum roots was 330 µm. Twenty days after inoculation to sorghum, all nematodes were still found embedded in the roots (Fig. 17). Of those inoculated to tomato, 23% of the third stage larvae counted were recovered from soil the others were observed inside the roots. Average length of third stage larvae observed on sorghum (20 days after inoculation) was 412 µm.

Third moult

Nematodes inoculated onto both sorghum and tomato undergo a third moult between 20 and 27 days after inoculation. These observations could be made in nematodes extracted from soil as well as in nematodes embedded in roots (Fig. 18 & 23), independant of the host plant used. Larvae present in soil ceased all activity at the time of moulting and assumed a "C" shape. Twenty seven days after inoculation 41% of the larvae recovered from sorghum pots 9% were found in the roots and 91% in the soil. Average length of these larvae from sorghum pots was 478 µm.

Fourth stage larvae

Thirty four days after inoculation 9% of the larvae were recovered from roots and 91% from soil for both sorghum and tomato. Average length of nematodes inoculated onto sorghum was 581 µm. Thirty four days after inoculation onto tomato, a browning of cells around the fourth-stage larvae was observed probably as a result of very slight wounds made by intracellular wandering larvae. This was not observed in uninfected roots.

Fourth moult

The fourth moult took place between 34 and 41 days after inoculation of second-stage larvae.

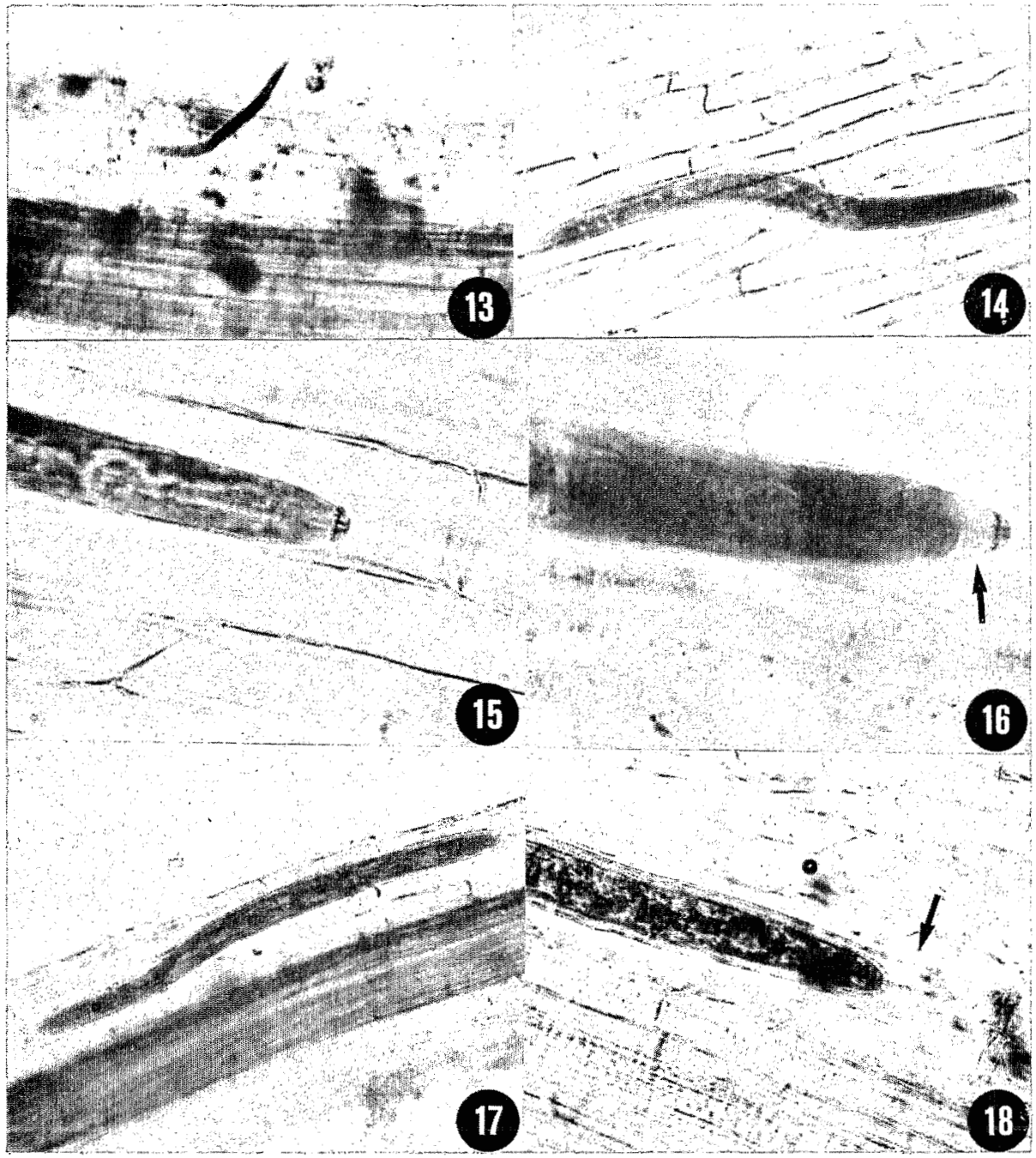

Fig. 13 to 18 ; 13 : Second-stage larva penetrating sorghum root ; 14 : Second-stage larva in sorghum root five days after inoculation ; note pointed tail ; 15 : Second-stage larva in cell of sorghum root ; 16 : Second-moult in sorghum root 15 days after inoculation ; note clear region in the anterior part of the body (arrow) ; 17 : Third-stage larva in sorghum root 20 days after inoculation ; 18 : Third moult in sorghum root ; note clear region in the posterior part (arrow).

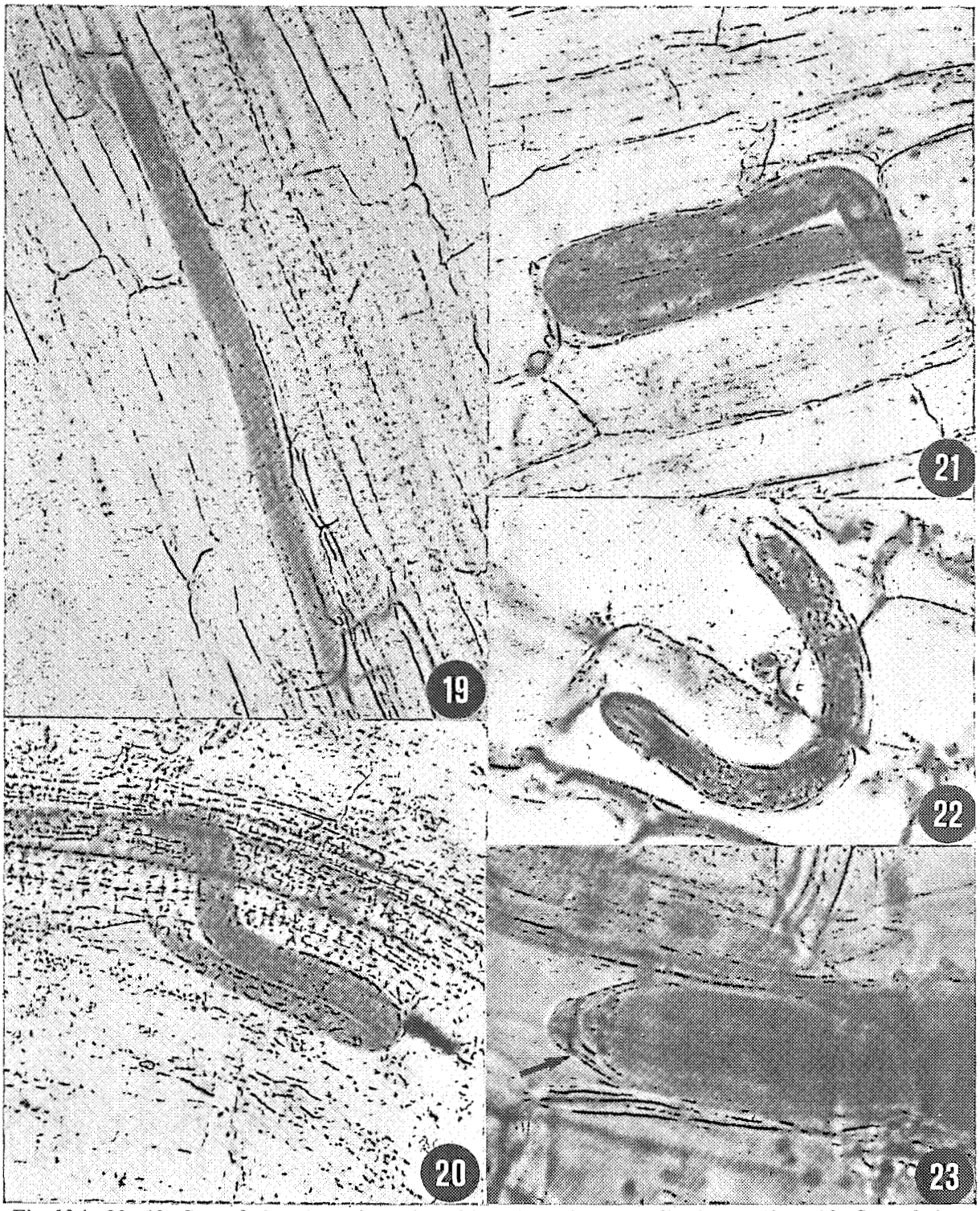

Fig. 19 to 23 ; 19 : Second-stage larva in cortex of tomato root 5 days after inoculation ; 20 : Second-stage larva feeding on the central cylinder of a tomato root five days after inoculation ; 21 : Second-stage larva coiled in tomato root 5 days after inoculation ; 22 : Histological section of infected root of tomato with second stage larva (stained with safranin and fast green) ; 23 : Third moult in tomato root (arrow).

Moulting fourth-stage larvae were only recovered from soil and were never observed in roots. As observed in previous moults, larvae were immobile and assumed a "C" shape. Forty one days after inoculation on sorghum, mean length of nematodes was 637 μm.

Adults

The first males and females appeared between 41 and 48 days after inoculation. Adults were extracted from soil only.

Discussion

Scutellonema cavenessi when stored in soil for a period of 30 months under anhydrobiotic conditions were capable of laying a few eggs without feeding when soil moisture conditions became favourable. Four days after moistening the soil, copulation took place and eggs were laid five days later. Subsequent egg production, the larval development inside eggs produced second-stage larvae in fourteen days in the absence of host plants.

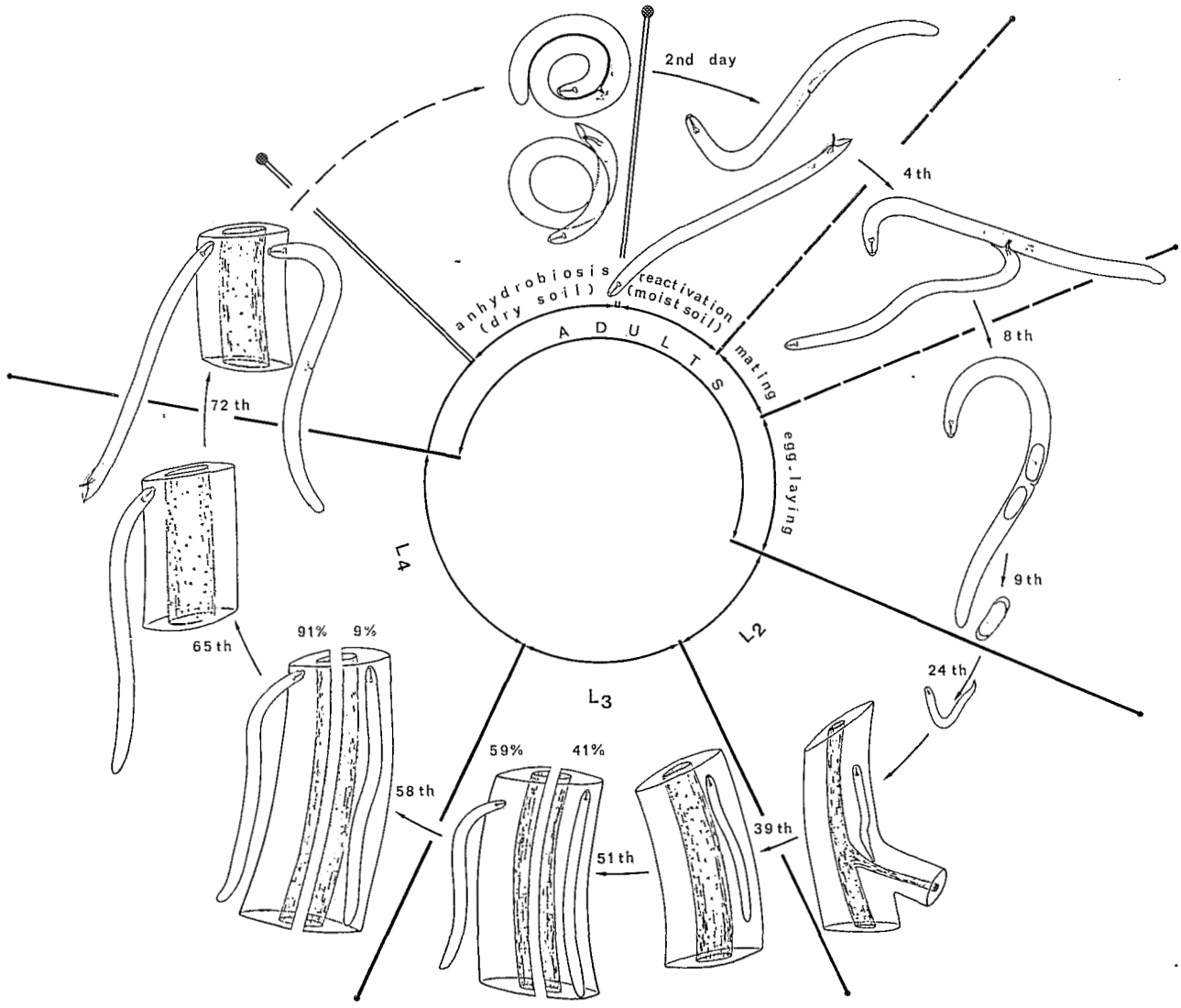

Fig. 24 : Diagramatic life cycle of *S. cavenessi* under experimental conditions.

In the case of *S. cavenessi* egg production in the presence of a host was increased when compared with egg production without hosts.

Cytological evidence obtained in this study provides strong indications that *S. cavenessi* is an amphimictic organism because egg production does not take place without fertilization ; since the development of the spermatheca and the presence of spermatozoa in females of *S. cavenessi* depends on elapsed time between moistening of the dry soil and extraction of nematodes, the use of these characters should be avoided in differentiation of species of this genus as proposed by Sher (1964).

The three moults observed during the development from second-stage larvae to adult, occured in a period of 41 to 48 days. Thus, the total time necessary for the production of another generation of adults starting from adults present as anhydrobiotic nematodes is between 65 to 72 days at 28°.

Under experimental conditions the second-stage larva was the infective stage of the parasite. This and the third stage in the development of *S. cavenessi* constituted the endoparasitic phase of the nematode life cycle. In some cases endoparasitic fourth stage larvae were observed but almost all of them feed ectoparasitically. During its life cycle *Scutellonema cavenessi* is first endoparasitic and then becomes progressively ectoparasitic (Fig. 24).

At present nothing is known about the longevity of the nematodes which gave rise to the new generation. Neither is it known what happens to newly formed adults. The fact that ovaries of females surviving the dry season were not clearly differentiated suggests that they belonged to a new generation and that females giving rise to this generation did not survive. This aspect of the *S. cavenessi* biology is being investigated in a study of the periodicity of egg laying of this nematode.

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REFERENCES

DEMEURE, Y. (1975). Résistance à la sécheresse en zone sahélienne du nématode phytoparasite *Scutellonema cavenessi* Sher, 1963. *Cah. ORSTOM, Sér. Biol.*, 10 : 283-292.

DEMEURE, Y. (1978*a*). Influence des températures élevées sur les états actifs et anhydrobiotiques du nématode *Scutellonema cavenessi*. *Revue Nématol.*, 1 : 13-19.

DEMEURE, Y. (1978*b*). *Les causes de la survie de certains nématodes phytoparasites* (Scutellonema cavenessi *et* Meloidogyne *spp.*) *pendant la saison sèche dans le Sahel sénégalais.* Thèse 3e cycle, Biologie appliquée, Univ. Lyon, 72 p.

DEMEURE, Y., FRECKMAN, D. W. & VAN GUNDY, S. D. (1979*a*). Anhydrobiotic coiling of nematodes in soil. *J. Nematol.*, 11 : 189-195.

DEMEURE, Y., FRECKMAN, D. W. & VAN GUNDY, S. D. (1979*b*). *In vitro* response of four species of nematodes to desiccation and discussion of this and related phenomena. *Revue Nématol.*, 2 : 203-210.

FRECKMAN, D. W., KAPLAN, D. T. & VAN GUNDY, S. D. (1977). A comparaison of techniques for extraction and study of anhydrobiotic nematodes from dry soil. *J. Nematol.*, 9 : 176-181.

GERMANI, G. & GAUTREAU, J. (1977). Résultats agronomiques obtenus par des traitements nématicides sur arachide au Sénégal. *Cah. ORSTOM, Sér. Biol.*, (1976) 11 : 193-202.

GOODEY, T. (1937). Two methods for staining nematodes in plant tissues. *J. Helminth.*, 15 : 137-144.

GOORIS, J. & D'HERDE, C. J. (1972). A method for the quantitative extraction of eggs and second stage juveniles of *Meloidogyne* spp. from soil. *Stat. Nematol. Entomol. Res. Stat. Ghent*, 35 p.

NETSCHER, C. & SEINHORST, J. W. (1969). Propionic acid better than acetic acid for killing nematodes. *Nematologica*, 15 : 286.

SEINHORST, J. W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4 : 67-69.

SHER, S. A. (1964). Revision of the Hoplolaiminae (Nematoda). III. *Scutellonema* Andrássy, 1958. *Nematologica*, 9 : 421-443.

TRIANTAPHYLLOU, A. C. (1962). Oogenesis in the root knot nematode *Meloidogyne javanica*. *Nematologica*, 7 : 105-113.