# Biology of the plant-parasitic nematode *Scutellonema cavenessi* Sher, 1964: anhydrobiosis

Yves Demeure

*Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal*

Summary

Female, male and larvae (3rd stage - 4th stage) of *Scutellonema cavenessi* are able to survive in dry soil for as long as nine months. Observations suggest that a migration from the higher dry depths to the lower more moist depths did not take place. Nematodes extracted from dry soil were found to have tightly coiled spiral shape characteristic for anhydrobiotic nematodes, Anhydrobiotic coiling in *S. cavenessi* has been induced *in vitro*, in a relative humidity chamber and 88% can be revived after one month in a desiccator at 0% relative humidity.

Résumé

*Biologie du nématode phytoparasite* Scutellonema cavenessi *Sher, 1964: anhydrobiose*

*Scutellonema cavenessi* survit dans un sol, sans plante hôte, à une période de sécheresse de 9 mois dans la proportion de 50%; chacun des stades de ce nématode (femelle, mâle et larves de 3$^{eme}$ et 4$^{eme}$ stade) survit dans de semblables proportions. La majorité des individus de la population de *S. cavenessi* est localisée au niveau 0-20 cm où les conditions environnantes (température, teneur en eau) sont extrêmement sévères. Aucun phénomène migratoire de l'horizon 0-20 cm vers l'horizon 20-40 cm n'a pu être enregistré pendant la durée de nos observations. En sol sec, femelles, mâles et larves de *S. cavenessi* adoptent un habitus spiralé ; cette conformation est à mettre en relation avec un état anhydrobiotique (= état résistant à l'environnement déficitaire en eau). Cette spiralisation anhydrobiotique a pu être induite *in vitro* chez 83,5% des individus d'une population de *S. cavenessi* et 88% de ces individus spiralés survivent à une exposition d'un mois à 0% d'humidité relative.

During the rainy season of the Senegalese Sahel (from june to september) just enough water (400-600 mm) is precipitated to allow the culture of one crop. Among the main crops grown, groundnut, millet and sorghum are often parasitized by the nematode *Scutellonema cavenessi* Sher, 1964.

During the nine months following the rainy season the soil is subjected to severe desiccation ; therefore, the nematode either escapes severe drought or possesses some means to resist desiccation. There are at least two means by which nematodes may survive, one is in perannual hosts adapted to this dry climate and the other is by migration of the nematodes to lower depths in the soil which are subjected to less desiccation. Previous studies (Demeure, 1975, 1978) have demonstrated the existence of anhydrobiotic mechanisms which might enable *S. cavenessi* to resist desiccation *in situ*.

Such a phenomenon either combined with migration or plant association, could explain the survival of this nematode under the stress of the dry climate conditions.

In this paper the results of field observations on the migration of *S. cavenessi* made at regular intervals during the dry season are given. Further the response of this nematode to slow desiccation *in vitro*, using a technique developed by Demeure, Freckman and Van Gundy (1979*b*) is reported.

## Material and methods

Soil samples were taken from a field at the Experimental Station of the ISRA (Institut Sénégalais de Recherches Agronomiques, Bambey, Senegal). During the previous rainy season this field was cropped with groundnut. Composition of the soil was : sand 85%, silt : 7% and clay : 8%.

Soil samples were taken at monthly intervals beginning on october 15th 1975, a date corresponding with the last rain of the year, and continued until july 15th 1976 when the first rainfall of the new crop season took place. Between these two dates rainfall was 1.1 mm (Tab. 1).

Table 1

Rainfall over the period of the experiment (CNRA, Bambey Experimental Station), mm per month

| | | |
|---|---|---|
| October | 1975 | 7 |
| November | » | 0 |
| December | » | 0 |
| January | 1976 | 0.8 |
| February | » | 0 |
| March | » | 0.3 |
| April | » | 0 |
| May | « | 0 |
| June | » | 1.8 |
| July | » | 101 |

Samples were taken from two soil depths, the first layer from 0-20 cm and the second 20-40 cm. To avoid mixing the soil of the two depths two sufficiently deep trenches, rectangular in section, one meter long and 50 cm wide were dug at places chosen at random in the field. Samples were taken from each of the four walls of each trench.

Nematodes were extracted using elutriation techniques developed by Seinhorst (1956, 1962). To observe anhydrobiotic *S. cavenessi*, nematodes were extracted from soil by a sugar flotation technique, using 1.25 M sucrose (Freckman, Kaplan & Van Gundy, 1977). The total number of *S. cavenessi* extracted was counted using a dissecting microscope.

The soil moisture content of soil from both depths sampled was calculated from the loss of weight of soil samples placed in an oven at 105° for 24 hours.

Soil temperatures at 10 cm and 50 cm depths were obtained from the meteorology section of the ISRA Bambey.

Resistance to drought was also studied by submiting *S. cavenessi* to air maintained at low relative humidity. Eight lots, each consisting of 100 hand picked nematodes, placed in water were concentrated on a Millipore ® filter each and placed in a humidity chamber where they were exposed to slow desiccation using a modification of Simons' dehydration schedule (Demeure, Freckman & Van Gundy, 1979). Nematodes were exposed for two days at each of the following relative humidities : 100%, 99.4%, 98.8% and 97.7%. After the nematodes had been exposed for two days to 97.7% relative humidity, four of the batches were returned to optimal moisture by placing the Millipore filter in a dish with a film of water ; the other four replicates were exposed to 0% relative humidity by placing the filter in a desiccator filled with $(SiO_2)_x$ for 24 hours before placing them in a film of water. In an other experiment the same procedure was followed but this time the nematodes were kept at 0% relative humidity during 30 days. Temperature for these experiments was maintained at 28°. All experiments were repeated twice. Populations of *S. cavenessi* in all experiments were composed of larvae ($L_3$ and $L_4$) and of males and females. Survival of nematodes was determined by counting individuals actively moving after having been placed in water for 48 hours. The percentage of coiling was determined by counting the number of coiled nematodes on the filter directly under the microscope.

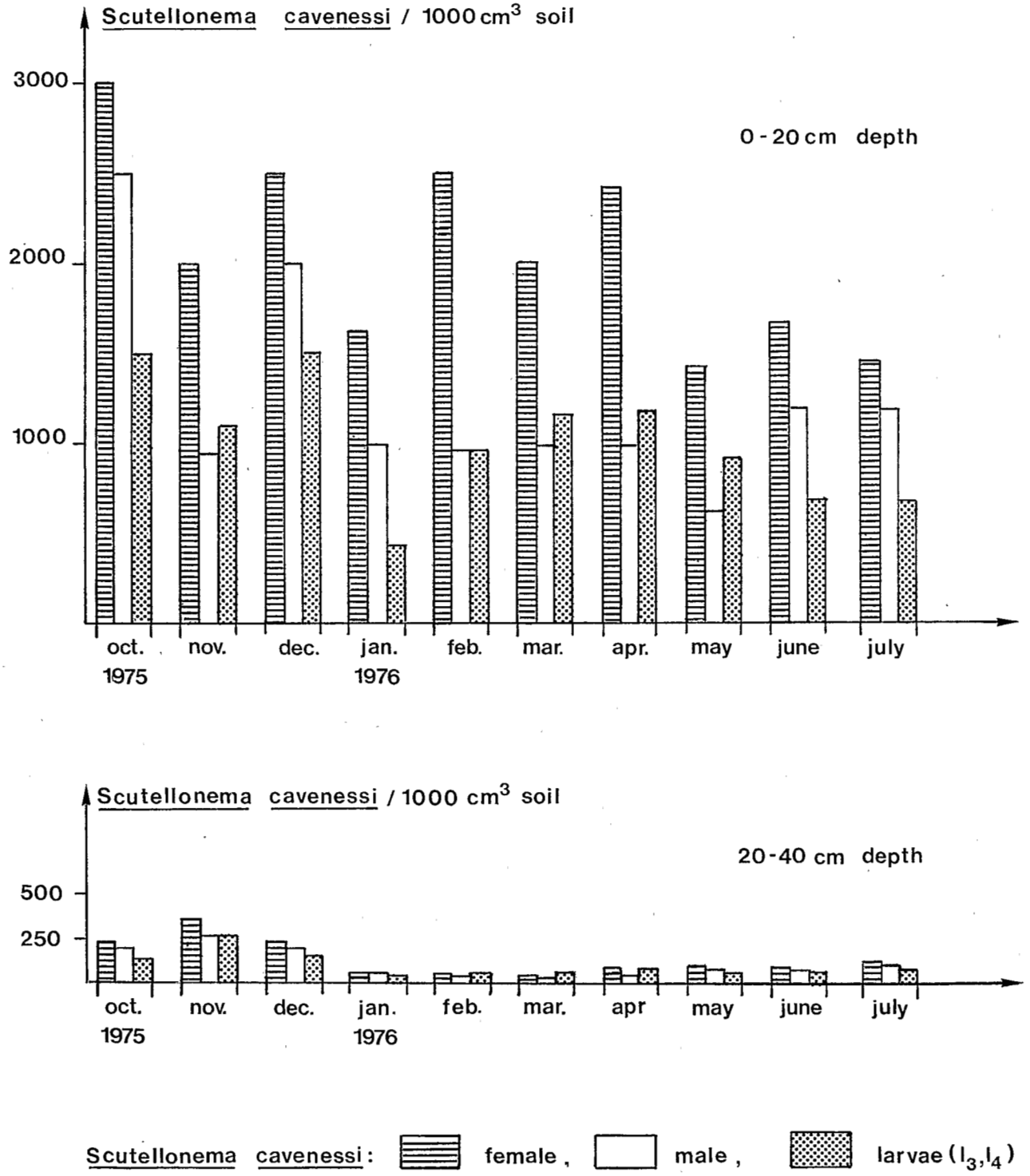


Fig. 1. Size of the population of *Scutellonema cavenessi* (female, male, larvae) during the dry season (October 1975-July 1976) at 0-20 cm depth and 20-40 cm depth.

Nematodes extracted by the sugar flotation technique were photographed in a drop of sugar 1.25 M. Photographs of nematodes taken from Millipore filter were placed in 5% formaldehyde (Freckman, Kaplan & Van Gundy, 1977). All photographs were taken with a Leitz "Orthomat" photomicroscope.

## Results

During the dry season the number of individuals of all stages decreases progressively to about 50% of the number of nematodes in July compared to that found in October. This observation was made in both depths studied (Fig. 1). Comparing the number of individuals of the different stages observed in both depths it is evident that at any time the number of nematodes in the lower depth is lower than that in the upper depth (5 to 10% of the number of individuals in the upper depth). Second stage larvae and eggs were never extracted from soil during the dry season. During the dry period of the year 90%-95% of *S. cavenessi* (females, males and larvae) extracted were coiled (Fig. 3 A-C).

Soil moisture content in the upper 20 cm of soil decreases rapidly during the first month after the last rain and remains stable for the rest of the dry season (0.3%) (Fig. 2). Soil moisture content in the depth 20-40 cm decreases gradually to reach a constant level for the rest of the dry season in february (Fig. 2). With the exception of a rapid rise in moisture content in the upper depth during the month of july (period corresponding with the first rain) moisture content of the lower depth is always higher than that of the upper 20 cm.

Moisture content of this soil at permanent wilting point is 2.3%, a value far superior to any moisture content measured in both depths, during the entire dry season.

In the experiments where drought was induced artificially 83.5% of the nematodes were tightly coiled after treatment until 97.7% relative humidity (Fig. 4 A-C) ; 97% of these nematodes revived after 48 hours in a film of water.

A high percentage (89%) of *S. cavenessi* revived after exposure at 0% rh for one day ; after exposure during 30 days at 0% rh, 88% of the nematodes survived (Tab. 2).

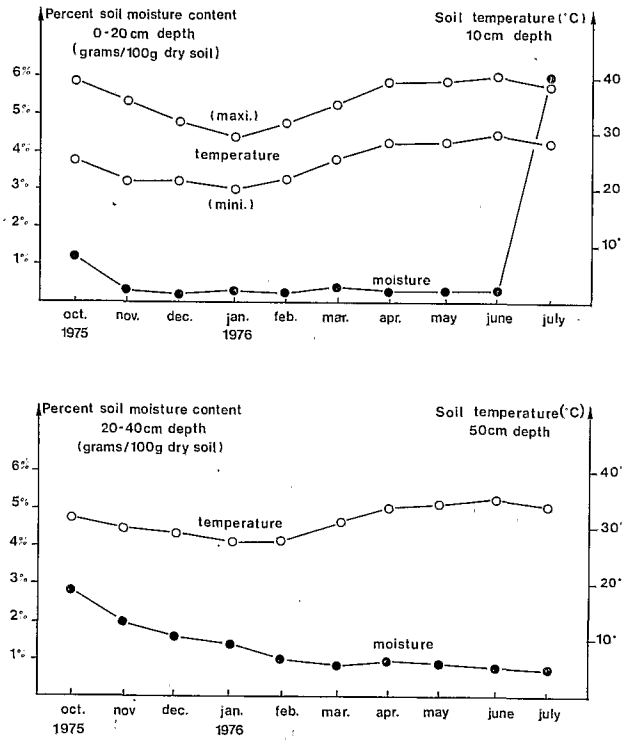


Fig. 2. Soil moisture (0-20 cm depth and 20-40 cm depth) and temperature (10 cm depth and 50 cm depth) during the dry season (October 1975-July 1976) at ISRA Experimental Station (Bambey).

## Discussion

Observations made during the dry season suggest that there was little if any migration from the surface 20 cm to the more moist and colder (Fig. 2) lower depths. In fact we did not observe a significant increase in size of the *S. cavenessi* population in the depth 20-40 cm in relation to a significant decrease in size of the population in the depth 0-20 cm.

The fact that no larvae and eggs ever were extracted during the dry season makes it most unlikely that reproduction of *S. cavenessi* enabled this nematode to keep its population level during this period. Almost all individuals of all stages of *S. cavenessi* which survive the dry season are coiled. It has been demonstrated that a coiled habitus often is related to an anhydrobiotic state of the nematode (Crowe & Madin, 1974 ; Freckman, Kaplan & Van Gundy, 1977 ; Freckman, 1978 ; Demeure, Freckman

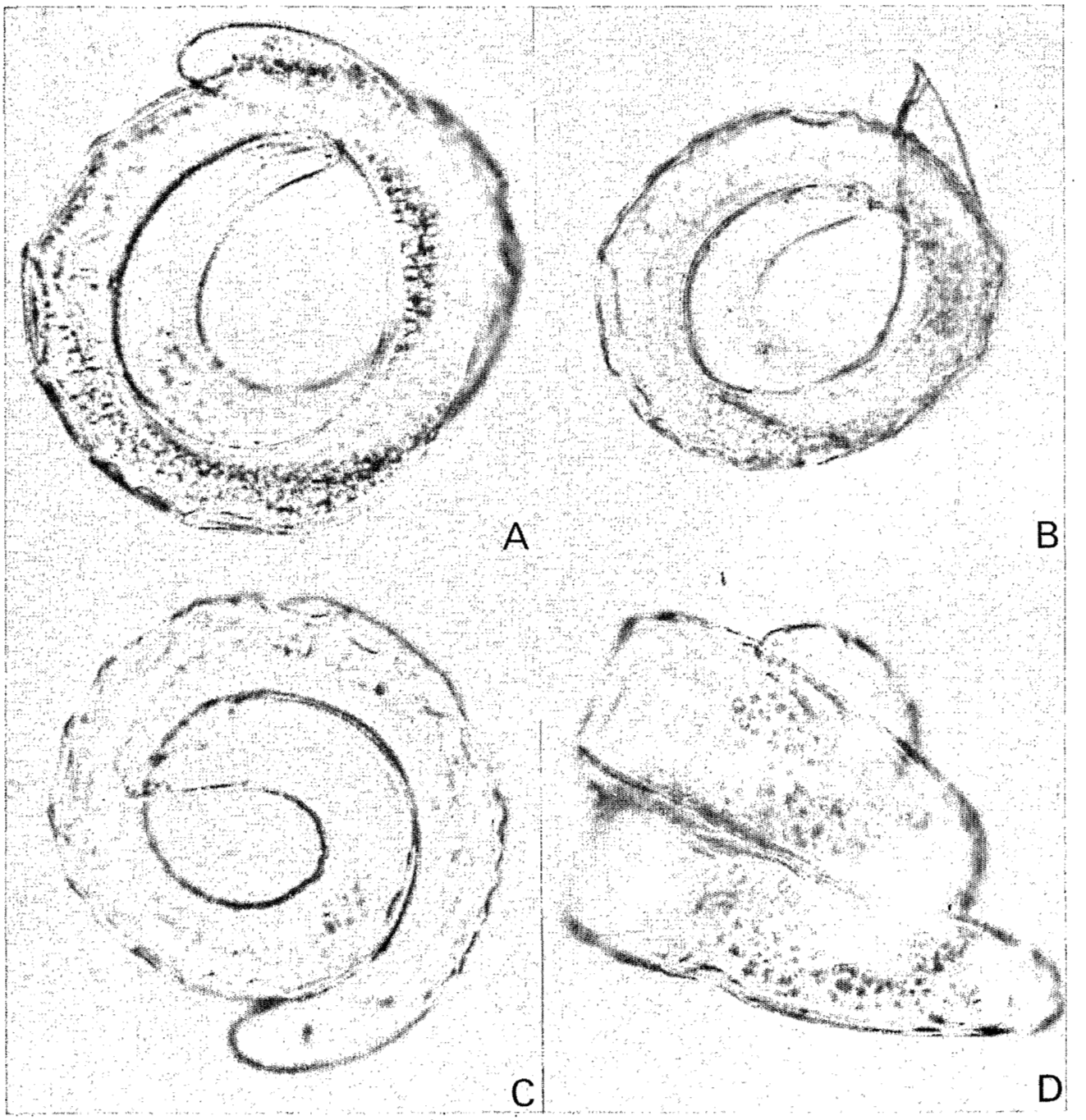


Fig. 3. Morphological aspect of *Scutellonema cavenessi* female (A) male (B) and larvae (C-D) extracted from dry soil in 1.25 M sucrose as according to Freckman, Kaplan and Van Gundy (1977) technique.

Table 2

Percentage of coiled *Scutellonema cavenessi* and percentage of nematode revival after treatment until 97.7% relative humidity (rh), after exposure at 0% rh for one day or thirty days

| | |
|---|---|
| Coiled *Scutellonema cavenessi* (%) | 83,5% |
| % nematode revival after treatment until 97.7% rh | 97% |
| % nematode revival after exposure at 0% rh for one day | 89% |
| % nematode revival after exposure at 0% rh for one month | 88% |

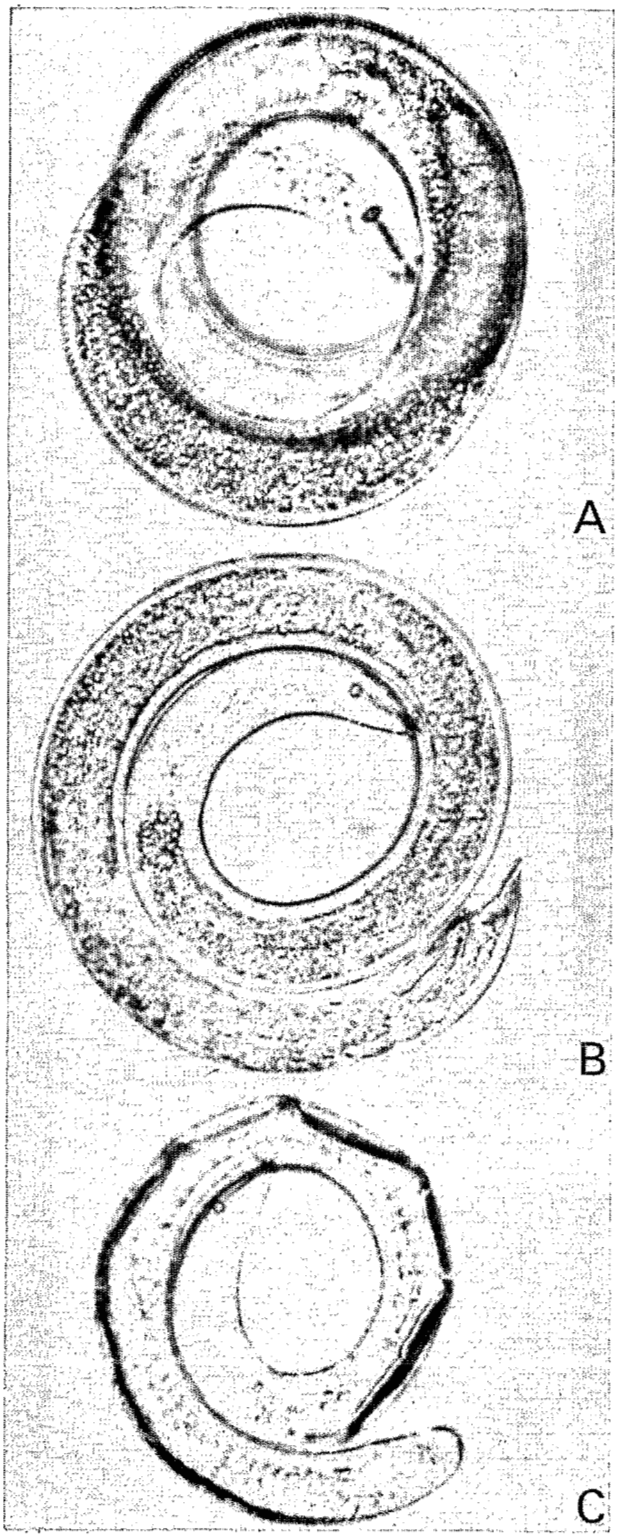


Fig. 4. Morphological aspect of *Scutellonema cavenessi* female (A) male (B) and larva (C) dehydrated on Millipore filter as according to Demeure, Freckman and Van Gundy (1977) technique.

& Van Gundy, 1979*a*, *b*). Accepting this point of view, *S. cavenessi* survives during the dry season in an anhydrobiotic state.

One may oppose the preceding statement by pointing to the fact that all nematodes extracted from soil in our experiments were exposed to a hypertonic solution (1.25 M sugar solution). This treatment might cause the nematode to coil. In order to approach the atmospheric conditions existing in dry soil, nematodes were exposed to 97.7% relative humidity. Nematodes responded to this treatment by coiling. Therefore it seems very likely that the coiling of nematodes extracted from soil was due to drought and not to the exposure to sugar.

It has been demonstrated for *Aphelenchus avenae*, *Acrobeloides* sp., *Scutellonema brachyurum*, that coiling is not caused by the relative humidity of the air in the soil pores but by the physical forces exerted on the nematode by the film of water around the soil particles (Demeure, Freckman & Van Gundy, 1979*a*). The effect of coiling would be the reduction of the surface of the tegument exposed to an environment deficient in water (Bird & Buttrose, 1974). It seems probable that the same holds for *S. cavenessi* in drying soil.

Anhydrobiosis in *S. cavenessi* is a quiescence, because it depends on external factors; it is caused by unfavorable conditions such as the begining of the dry season. As soon as conditions become favourable (rainy season) the opposite takes place : reactivation.

As a consequence of the anhydrobiotic state of the nematode during which its metabolic activity is slowed down (Keilin, 1959) its food reserves are not exhausted (Cooper & Van Gundy, 1979). This may be the reason that females of *S. cavenessi* stored during 30 months in dry soil are capable of laying eggs without feeding (Demeure, Netscher & Quénéhervé, 1980).

The experiments *in vitro* demonstrate that it is possible to produce anhydrobiotic *S. cavenessi* on Millipore filters which are capable of surviving at least thirty days at 0% relative humidity. It may be possible to use the Millipore technique to induce anhydrobiosis on populations of *S. cavenessi* and maybe other nematodes for the purposes of storing alive nematodes in the laboratory for long periods of time.

ACKNOWLEDGMENTS

The author thanks M. Luc for helpful comments, C. Netscher for translating the text into English and B. Souchaud and M. Chpiliotoff for technical assistance.

REFERENCES

BIRD, A. F. & BUTTROSE, M. S. (1974). Ultrastructural changes in the nematode *Anguina tritici* associated with anhydrobiosis. *J. Ultrastruct. Res.*, 48 : 177-189.

COOPER, A. F. & VAN GUNDY, S. D. (1971). Senescence, quiescence and cryptobiosis. In : Zuckerman, B. M., Mai, W. F. & Rhode, R. A. (Eds) *Plant parasitic nematodes*, Vol. 2. London & New York, Academic Press, 347 p.

CROWE, J. H. & MADIN, K. A. (1974). Anhydrobiosis in nematodes : Evaporation water loss and survival. *J. exp. Zool.*, 193 : 323-333.

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). Influence des températures élevées sur les états actifs et anhydrobiotiques du nématodes *Scutellonema cavenessi*. *Revue Nématol.*, 1 : 13-19.

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

*Accepté pour publication le 21 avril 1980.*

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.

DEMEURE, Y., NETSCHER, C. & QUÉNÉHERVÉ, P. (1980). Biology of the plant parasitic nematode *Scutellonema cavenessi* Sher, 1964 : reproduction, development and life cycle. *Revue Nématol.*, 3 : 213-225.

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

FRECKMAN, D. W. (1978). Ecology of anhydrobiotic soil nematodes. In : Crowe, J. H. & Clegg, J. S. (Eds) *Dried Biological Systems (Ann. Meet. Amer. Inst. Biol. Sci. E. Lansing, Michigan, 21-26 August 1977)*. London & New York, Acad. Press : 345-357.

FRECKMAN, D. W., DEMEURE, Y., MUNNECKE, D. & VAN GUNDY, S. D. (1980). Resistance of anhydrobiotic *Aphelenchus avenae* to methyl bromide fumigation. *J. Nematol.*, 12 : 19-22.

KEILIN, D. (1959). The problem of anabiosis or latent life : history and current concepts. *Proc. R. Soc.*, B 150 : 149-191.

PIERRE, F. (1958). Ecologie et peuplement entomologique des sables vifs du Sahara nord occidental. *CNRS, Publ. Centre Rech. Sahar., Sér. Biol.*, 1 : 333 p.

SEINHORST, J. W. (1956). The quantitative extraction of nematodes from soil. *Nematologica*, 1 : 249-267.

SEINHORST, J. W. (1962). Modifications of the elutriation method for extracting nematodes from soil. *Nematologica*, 8 : 117-128.