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Anhydrobiosis in Five Species of Plant Associated Nematodes[1]

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Abstract: Five species of nematodes—*Hemicriconemoides pseudobrachyurum, Hemicycliophora conida, Macroposthonia ornata, Aphelenchoides ritzemabosi,* and *Psilenchus hilarulus*—were desiccated to study their capacity to survive anhydrobiotically. Results indicate that the ability of the sheath to shrink quickly and its relatively loose attachment with the nematode body allow *H. conida* to survive longer than *H. pseudobrachyurum*; the survival of *M. ornata* was intermediate. *A. ritzemabosi* and *P. hilarulus* survived immersion in paraffin oil for 12 and 17 days, respectively. Both of these nematodes possess multiple contraction ability; i.e., coiling coupled with transverse and longitudinal folding of the cuticle. *P. hilarulus* is a new addition to the list of anhydrobiotic nematodes.

Key words: Aphelenchoides ritzemabosi, Hemicriconemoides pseudobrachyurum, Hemicycliophora conida, Macroposthonia ornata, Psilenchus hilarulus, cuticle, paraffin oil.

Although the first plant parasitic nematode to be discovered, *Anguina tritici* (Needham, 1743), was in the state of anhydrobiosis, this aspect of nematode behavior has been studied only recently. In addition to the early examples of *Tylenchus polyhypnus* (15), *Ditylenchus dipsaci* (5), and *Aphelenchoides ritzemabosi* (16), some of the records in recent years include *Helicotylenchus nannus* (10), *Globodera rostochiensis* (2), *Tylenchorhynchus dubius* (17), and *Scutellonema brachyurum* (1). Anhydrobiosis in nematodes has been reviewed by Simons (14), Evans and Perry (4), and, more recently, by Freckman (6). The list of nematodes exhibiting this phenomenon is still short. However, with the development of better techniques of extraction and/or inducing nematodes into anhydrobiosis, more species possessing properties to survive in this state are likely to be discovered.

Ellenby (2) reasoned that the cuticle of *Ditylenchus dipsaci* fourth-stage juveniles was important for the nematodes to withstand desiccation because upon drying it becomes increasingly impermeable to water loss. He also demonstrated that the exsheathed juveniles of *Haemonchus contortus* did not survive desiccation, whereas the sheathed ones did (3). The present study examined the desiccation survival of *Hemicriconemoides pseudobrachyurum* and *Hemicycliophora conida* (nematodes with extra cuticle or sheath), *Macroposthonia ornata* (belonging to the same group as the earlier two but without an extra cuticle), *Aphelenchoides ritzemabosi* (a known anhydrobiotic survivor), and *Psilenchus hilarulus* (frequently found in dry soil).

MATERIALS AND METHODS

Nematodes were maintained in a greenhouse on appropriate hosts and isolated as needed from infested soil by density flotation centrifugation. Isolated nematodes were subjected to three methods of desiccation and dehydrated to the point where they ceased movement and showed obvious signs of body shrinkage. The posture of dehydrated nematodes, their body length (as percentage of predehydrated length), the minimum time required to regain turgidity and resume body movements, and the survival time (the maximum period during which nematodes survived without water) were recorded.

Desiccated nematodes were observed and measured using a projection microscope

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and a light microscope. Surface changes of the nematodes during water loss were investigated with the scanning electron microscope (SEM). The method was a modification of that described by Roessner and Porstendoerfer (13) in that 15- × 15-mm cover slips holding dehydrated nematodes in paraffin oil (adhered to glass surface) were placed in erect position for 15 minutes in plastic boxes filled with 90% acetone (2–3 changes), followed by transfer to absolute acetone for 1 hour. In this way the nematodes were ready for observation in a relatively short time. At the same time paraffin oil was left in the boxes. Each experiment was repeated five times, and 10 nematodes of each species were taken every time.

Paraffin oil desiccation: When a nematode is lifted from water suspension with a hair tip, a small quantity of water is carried along with its body. This water becomes visible if the nematode is immediately immersed in a drop of paraffin oil on a glass slide and observed under a binocular microscope (Fig. 1). Nematodes remain active in this water until it disappears. Then they assume morphological adaptations. To study desiccation response in this system, female nematodes were immeresed in paraffin oil (Merck 7161) in depression slides and moved about to remove the water surrounding their bodies. Selected specimens were transferred into a small drop of fresh paraffin oil on a microscope slide and covered with a coverglass supported by stainless steel wires slightly larger in diameter than the nematodes. The slides were left under room conditions (22 C ± 4, 26% relative humidity) until the point of dehydration. The paraffin oil was then blotted from the dehydrated nematodes which were washed three times with tap water. The dehydrated nematodes were rehydrated by placing them in tap water in depression slides.

Humid chamber desiccation: Observation chambers similar to those described by Roessner (11) were used, but instead of soil, moist filter papers were placed inside. The nematodes were suspended in a drop of distilled water in the chamber. The water was allowed to slowly evaporate until the point of dehydration was reached. Nematodes were rehydrated by water vapors after irrigating the chamber.

Instant dehydration: To study the immediate anhydroprotective response of the sheath, nematodes were subjected to rapid drying. Mature females were picked from the water suspension on the tip of a hair lifter and held at room conditions for various lengths of time. Dehydrated nematodes were placed in tap water in depression slides, and their morphological state was immediately noted under a binocular microscope.

RESULTS

Paraffin oil desiccation: The body length of all the species was reduced during desiccation (Table 1). Upon rehydration, *Hemicriconemoides pseudobrachyurum* survived only 40 minutes. The body of the nematode contracts more rapidly than the enclosing sheath; the contraction starts before the disappearance of free water. Eventually the sheath also contracts and again tightly encloses the nematode. Finally the nematode assumes a semicircular or coiled form (Fig. 2).

In contrast, the sheath of *Hemicycliophora conida* contracts more rapidly than the nematode and does not remain as a loose structure as in turgid nematodes. However, after a time the body of the nematode shrinks at the same rate as the sheath. When dehydrated the sheath assumes a "telescope" or "skirt" form at the vulval region (Fig. 3).

The desiccation responses of *Macroposthonia ornata* differed from *Hemicricone-*

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FIGS. 1–6. Effects of anhydrobiosis on nematodes. 1) *Hemicycliophora conida.* Intended to show the amount of water that could be carried when a nematode is lifted from water suspension and transferred to paraffin oil. 2) *H. pseudobrachyurum.* Paraffin-dried nematode in coiled posture. 3) *H. conida.* SEM photograph showing "skirting" of the sheath at the vulval region. 4) *Macroposthonia ornata.* SEM photograph showing difference in the interannular spaces; less at anterior end (top), more towards the middle of the body. 5) *Aphelenchoides ritzemabosi.* SEM view of a part of spiral. Note the transverse and longitudinal folding of the cuticle. 6) *Psilenchus hilarulus.* SEM view of part of spiral. Note the transverse and longitudinal folding of the cuticle.


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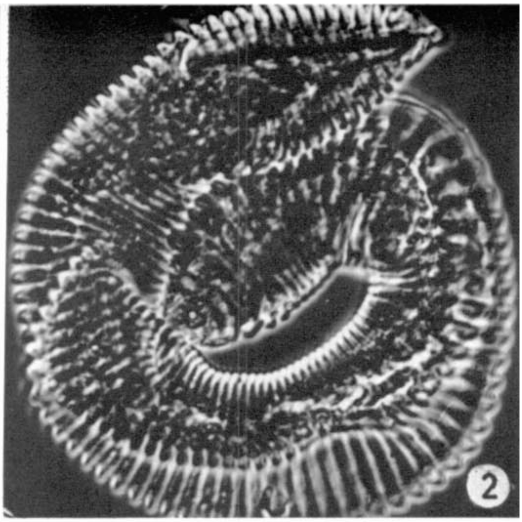
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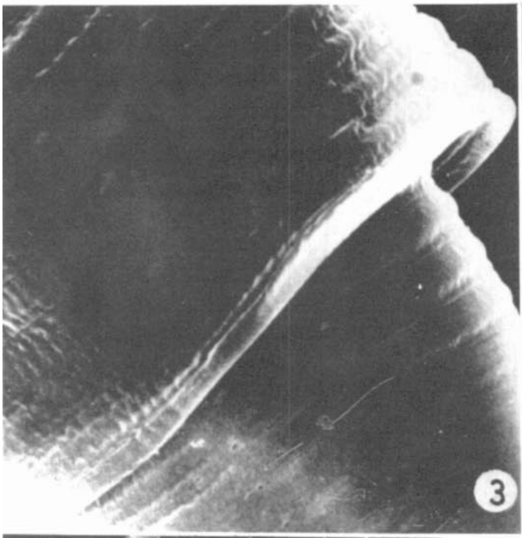
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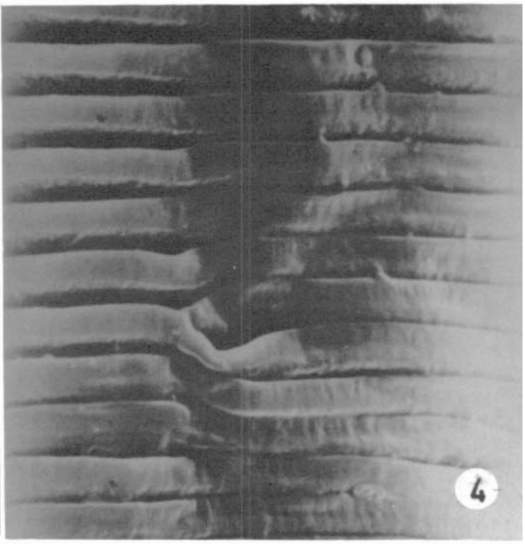
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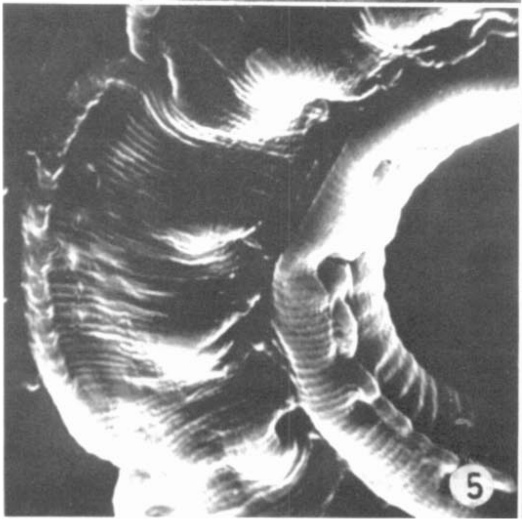
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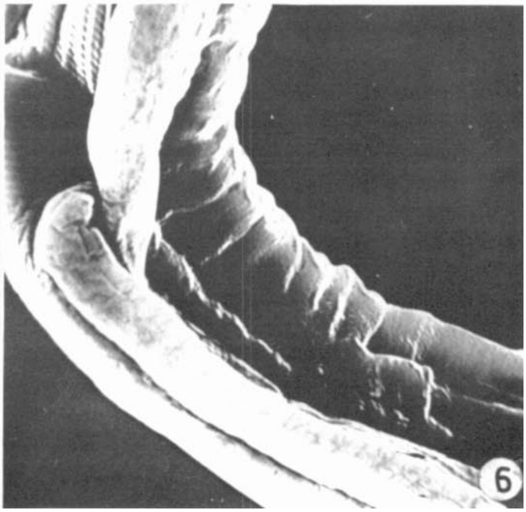
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SCALE: 2 25 µm, 3,4 13 µm, 5 10 µm, 6 15 µm

TABLE 1. Minimum times required for nematodes to regain turgidity and resume activity, survival times, and final body length (as percentage of original length) after dehydration in paraffin oil.

Nematode	Turgidity (min)	Activity (min)	Survival time (maximum)	Final length (%)
Hemicriconemoides pseudobrachyurum	29	55	40 min	66
Hemicycliophora conida	120	150	6 hr	60
Macroposthonia ornata	26	39	90 min	53
Aphelenchoides ritzemabosi	3	12	12 days	56
Psilenchus hilarulus	3	17	17 days	50

moides pseudobrachyurum in two major respects. In *M. ornata,* the narrowing of interannular spaces was more pronounced at the anterior and posterior ends than at the middle of the body (Fig. 4) and the nematode contracted in length proportionally more; it was only 53% of the original length, as compared to 66% in *H. pseudobrachyurum* (Table 1).

Coiling coupled with transverse and longitudinal folding of cuticle were the main morphological adaptations of *Aphelenchoides ritzemabosi* (Fig. 5). However, 30% of the nematodes assumed other postures—straight, slightly curved, or irregularly twisted.

Psilenchus hilarulus frequently made coils, but this was not the only adaptative measure. As compared with *A. ritzemabosi,* other postures—helix, C-shaped, and straight—were more frequent (40%). SEM revealed strong indications of transverse and longitudinal folding of cuticle (Fig. 6).

The rate of water uptake was slowest (2 hours) in *H. conida.* After prolonged moistening, the sheath resumed turgidity more rapidly than the nematode inside. Compared to this nematode, *H. pseudobrachyurum* and *M. ornata* took up water quickly—in 29 and 26 minutes, respectively (Table 1). The rate of water uptake in *A. ritzemabosi* and *P. hilarulus* was essentially similar. Both species, although surviving a considerably longer period of desiccation, resumed turgidity rapidly (Table 1).

Resumption of activity by the different species correlated with their rate of water uptake (Table 1). *A. ritzemabosi* revived a few minutes faster than *P. hilarulus,* although both took up water at the same rate.

Humid chamber dehydration: Only minor differences occurred in the responses of nematodes to drying in a chamber compared with paraffin oil desiccation. Contraction of the body length was the principal response of the three criconematids, whereas *A. ritzemabosi* and *P. hilarulus* frequently made coils. It was difficult to judge the rate of water uptake by the desiccated nematodes as they received moisture slowly through the raised humidity of the atmosphere inside the chamber. Moisture may have come in contact with the nematode's lower side; i.e., the side facing water in the bottom of the chamber which escaped observation. However, starting from the moment the first water droplet became visible touching the nematode body, the times of water uptake and consequent assumption of activity were recorded; these differed little from those recorded in the paraffin oil method.

Instant dehydration: Only in *M. ornata* were anterior-posterior body contractions noted. *H. pseudobrachyurum* maintained its form and did not shrink when given 3 minutes of exposure on a hair tip. Measurement of the body length of other species immediately upon transfer to water was not possible due to the irregular shrinkage and twisted state of the body. Results of this experiment are summarized in Table 2.

Summing up the results based upon these observations, the species tested could be categorized as follows, depending upon their ability to survive desiccation.

- Desiccation susceptible:
  - *Hemicriconemoides pseudobrachyurum*
  - *Macroposthonia ornata*
- Desiccation tolerant:
  - *Hemicycliophora conida*
- Anhydrobiotic survivors:
  - *Psilenchus hilarulus*
  - *Aphelenchoides ritzemabosi*

TABLE 2. Responses of five nematode species to instant dehydration.

Nematode	Maximum exposure withstood (min)	State upon releasing to water	Time taken in resuming turgidity (min)	Time taken in resuming activity (min)
Hemicriconemoides pseudobrachyurum	3	Not shrunken		Immediately
Hemicycliophora conida	3	Shrunken irregularly	2–3	2–3
Macroposthonia ornata	2	Collapsed at middle of body, contracted in length	1–2	1–2
Aphelenchoides ritzemabosi	4	Severely shrunken, irregularly coiled	ca. 1	1–2
Psilenchus hilarulus	4	Same as *A. ritzemabosi*	ca. 1	1–2

While making the preceding gradations, it has been recognized that several days of slow dehydration are required for nematodes to enter into true anhydrobiosis; i.e., shifting their metabolic processes from one to the other type (8).

DISCUSSION

The sheath of *H. pseudobrachyurum* and *H. conida* was expected to be of anhydroprotective value, as in *Haemonchus contortus* (3). This was, however, not the case with *H. pseudobrachyurum.* The sheath of this nematode is attached to the body at the mouth, excretory pore, and vulva. Being a thicker and stronger structure, the sheath does not shrink at the same rate as the enclosed nematode. Thus the nematode is not allowed to contract freely to prevent water loss. The noncontracted sheath appears to remain permeable, allowing loss of water from the nematode body beyond tolerable limits. In this respect, the sheath of this nematode appears to be disadvantageous, instead of being of any advantage, as far as the nematode's desiccation tolerance is concerned.

The disadvantage of close contact between the two cuticles of *H. pseudobrachyurum* is also shown by the relatively longer survival of *M. ornata.* This species, which is a close relative of *H. pseudobrachyurum,* differs from it in lacking a sheath. It can contract freely, and thus tolerance to desiccation is enhanced. However, when the nematodes were dried rapidly, the sheath of *H. pseudobrachyurum* could protect the nematode from collapsing. This could be due to the thickness of this structure, coupled perhaps with the presence of water between the two cuticles, which for a short time has survival value.

The sheath of *H. conida,* however, behaved differently and to the advantage of the nematode. Though its attachment to the main body is also at the head and vulva, it allows more freedom to the nematode inside. This is because the distance between the two cuticles throughout the body length is greater than in *H. pseudobrachyurum,* and the points of attachment are not located at the extreme ends as in the other nematode; thus a proportionally greater area of the body can adapt according to the need. Further, the sheath of this nematode shrinks more rapidly than that of *H. pseudobrachyurum* and thus is capable of effectively checking loss of water. As observed by Ellenby (2) in the case of *Ditylenchus dipsaci* larvae, rapid shrinking of the outer structure protects the inner and more vital ones from drying.

The peculiar contraction of *M. ornata,* more at the two ends than at the middle of the body, may be due to the capability of this nematode to compartmentalize its body with the help of the deep annulations, thereby closing the body openings situated at the two ends, in order to check loss of water. However, it is not clear if its better survival (in comparison to *H. pseudobrachyurum*) depends on this capability (i.e., body compartmentalization) or is due to the lack of a stiff sheath.

Psilenchus hilarulus is a new addition to the list of nematodes exhibiting anhydrobiosis. Like *Aphelenchoides ritzemabosi,* this nematode has multiple contraction capability; i.e., coiling coupled with transverse and horizontal folding of the cuticle and thereby reducing the exposed surface area to a great extent. *P. hilarulus* is a close ally of *Tylenchus polyhypnus* which has been reported by Steiner and Albin (15) to have survived a record period of time in the anhydrobiotic state. It remains to be determined if this shared property of the two nematodes is also indicative of their phylogenetic relationship.

Behavior of nematodes on a glass surface has been found useful in understanding the dehydration of nematodes (2,3,9,12). The method of immersion in paraffin oil has some additional advantages: (a) lipid or other coatings on the nematode body are not lost; (b) nematodes remain protected from external attack by micro-organisms, especially when they are left for periods of days or weeks; and (c) micro-environmental parameters affecting the nematode do not change so greatly as in other methods because the animal is surrounded by paraffin oil. Further work in this direction may lead to a control over the micro-environment influencing the nematode, such as developing a technique whereby an optimum thickness of monomolecular layers of water could be maintained around the animals for the time required to induce them into anhydrobiosis (1). However, extra care must be taken in handling nematodes in such studies because animals in cryptobiosis are reported to have little or no capability to recover from physical damage (7).

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