# *In vitro* response of four species of nematodes to desiccation and discussion of this and related phenomena (1)

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ABSTRACT

Anhydrobiosis was induced in small quantities of nematodes ( ± 100) by slow desiccation using a modification of Simons' membrane filter technique and dehydration schedule. Tests for the degree of anhydrobiosis were percent coiling and survival after exposure at 0% r.h. for 24 h. Nematodes tested were *Aphelenchus avenae*, *Helicotylenchus dihystera*, *Scutellonema brachyurum* and *Acrobeloides* sp. The dehydration of all four individual nematodes induced 91 to 98% coiling. Survival to desiccation was not correlated with coiling but rather with duration of dehydration. Coiling appeared to be a short term physical response to dehydration while anhydrobiosis was a longer term physiological response to slow dehydration. The authors discuss the current concepts of cryptobiosis, anhydrobiosis, drought resistance and quiescence.

RÉSUMÉ

*Réponse* in vitro *de quatre espèces de nématodes à la dessiccation et discussion du phénomène et d'autres qui lui sont connexes*

Différents nématodes *(Aphelenchus avenae, Acrobeloides* sp., *Scutellonema brachyurum, Helicotylenchus dihystera)* ont été mis en anhydrobiose selon le procédé de Simons modifié par les auteurs. De petites quantités (≃ 100) de chacune des quatre espèces ont été déposées sur un filtre Millipore. Le filtre, humide, est ensuite placé dans une enceinte hermétiquement close où l'humidité relative a été équilibrée à 100% ; puis celle-ci est abaissée progressivement jusqu'à 97,7% par addition de glycérine.

Les auteurs ont noté le pourcentage de nématodes spiralés et le pourcentage d'individus qui survivent à une exposition de 24 heures à 0% d'humidité relative. Ces deux pourcentages sont d'autant plus élevés que la déshydratation de l'atmosphère de l'enceinte est plus progressive. La réaction de spiralisation du nématode apparaît être la réaction première à un environnement déficitaire en eau ; mais cette adaptation morphologique n'est pas suffisante pour permettre au nématode de survivre à une dessiccation prononcée (24 heures à 0% d'humidité relative). Aussi, la déshydratation du milieu environnant doit-elle déclencher au niveau du nématode des processus, autres que celui de la spiralisation, qui ne peuvent s'accomplir que si la déshydratation est lente et qui permettent aux nématodes de survivre au dessèchement. Les auteurs discutent les notions de cryptobiose, d'anhydrobiose, de résistance à la sécheresse et de quiescence chez les nématodes.

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An anhydrobiotic nematode forms a tightly-coiled spiral. This coiled shape has been described by Bird and Buttrose (1974) for *Anguina tritici* larvae and by Crowe and Madin (1974, 1975) for *Aphelenchus avenae* larvae and adults. These coiled anhydrobiotic nematodes were obtained from desiccated plant material and from large masses of nematodes (0.1 g wet weight), respectively. Simons (1973) used a membrane filter technique to study the desiccation of groups of 100 to 125 ectoparasite nematodes. This method approximates more closely the actual response of nematodes in the soil, since the nematodes probably respond as individuals rather than as a mass or cluster of nematodes. Ellenby's (1969) statement that only a few species of nematodes are able to withstand desiccation, was contradicted by Simons' (1973) work which showed that the drought-resistant species, *Tylenchorhynchus dubius*, and the more susceptible species, *Rotylenchus robustus*, could survive *in vitro* in humidities corresponding to pF 5.0 (93% rh) for one week. He suggested that tolerance to drought appeared to be a general phenomenon in plant nematodes. Cryptobiosis, however, may only occur in some nematodes (Van Gundy, 1965).

This paper reports the responses of four soil nematode species, two plant feeders, a bacterial feeder, and a fungivore to slow desiccation using a modification of Simons' membrane filter technique (1973) and discusses resistance to desiccation in nematodes.

## Materials and methods

### Nematodes

Four nematode species were selected for study representing the various trophic groups commonly found in soil. The fungal feeder, *Aphelenchus avenae* Bastian, 1865 was cultured in the laboratory on *Rhizoctonia solani* Kühn, 1858 (Cooper & Van Gundy, 1970 ; Evans, 1970). The bacterial feeder, *Acrobeloides* sp., was collected from a Mojave Desert soil in Rock Valley, Nevada, and cultured on a mixed bacterial culture on oatmeal agar. The plant parasites, *Scutellonema brachyurum* (Steiner, 1938) Andrássy, 1958 and *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961, were collected from a moist soil around banana plants from the University of California, Riverside campus, before each experiment. All nematodes were extracted by the Baermann funnel technique 24 h before each experiment. Mixed larvae and adults were used in all experiments.

### Technique

A modification of Simons' technique (1973) is illustrated schematically in Figure 1. Approximately 100 nematodes were pipetted onto a Millipore filter (dia 13 mm ; aperature 0.6 µm) (Millipore Corporation, Bedford, Massachusetts, 01730, Catalog No. SSWP01300) over vacuum. The filter with nematodes was placed in a BPI watch glass and was suspended in a relative humidity chamber and exposed to relative humidities (rh) of 100%, 99.4%, 98.8%, and 97.7%. The percent of relative humidity of each chamber was regulated by using glycerin-water mixtures (Simons, 1973) adjusted to 0.998 and then 1.010, 1.020, 1.030 density. Glycerin was added to the chamber solution with stirring at daily intervals according to Table 1. The nematodes were exposed to each relative humidity for varying periods of time as shown in Table 2.

Table 1

Quantity of glycerin to add to adjust the glycerin-water mixture density at 1.010, 1.020 and 1.030

| *Density* | *Water-Glycerin Mixture* | *Calculated Relative Humidity* |
|---|---|---|
| 0.998 | 330 cc water, (1) | 100% |
| 1.010 | (1) + 12 cc glycerin, (2) | 99.4% |
| 1.020 | (2) + 13.3 cc glycerin, (3) | 98.8% |
| 1.030 | (3) + 13.3 cc glycerin | 97.7% |

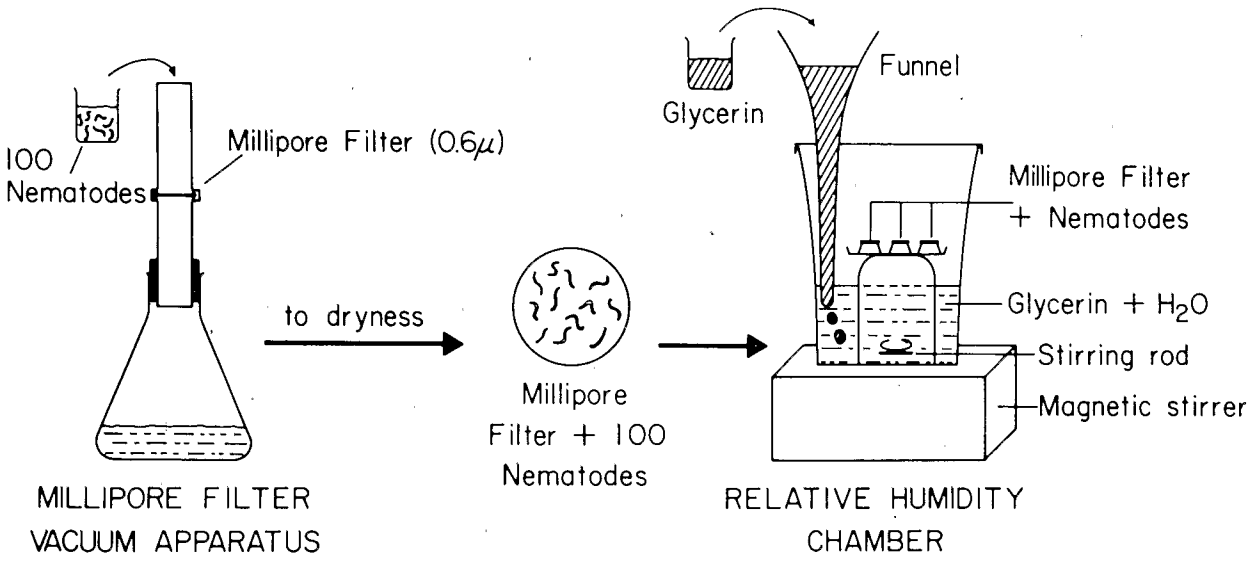



Fig. 1. Technique for preparing nematodes for placement on the Millipore filter and their suspension in the relative humidity chamber for dehydration.

Table 2

Four sequence treatments (1, 2, 3, and 4) used for nematode dehydration from 100% to 97.7% relative humidity in chamber.

| *Density* | 0.998 | 1.010 | 1.020 | 1.030 | *Total Dehydration Time from 100% to 97.7% rh* |
|---|---|---|---|---|---|
| Treatment 1 | 1 day | 1 day | 1 day | 1 day | 4 days |
| Treatment 2 | 2 days | 2 | 2 | 2 | 8 |
| Treatment 3 | 3 days | 3 | 3 | 3 | 12 |
| Treatment 4 | 4 days | 4 | 4 | 4 | 16 |

After the nematodes had been exposed to at least 24 h of 97.7% rh, four replicates were tested for nematode survival by returning to water and four replicates were exposed to 0% rh over $P_2O_5$ for 24 h before returning to water. Crowe and Madin (1975) have suggested that nematodes in the state of anhydrobiosis are capable of being dried over $P_2O_5$. The experimental temperature was 23° and each experiment was repeated twice.

NEMATODE OBSERVATIONS

Nematodes were counted on the filter and in the dish for percentage coiling under a microscope and then placed in a film of water for 48 h and observed for motility as an indication of survival. Millipore filters with nematodes from 97.7% rh were attached with double face cellophane tape on a scanning electron microscope (SEM) plug, treated with gold and observed at

800 and 2 000 magnifications on a Joelco SEM (Model No. JSM-U3). Percentage of coiled and active nematodes were analyzed after angular transformation (angle = arcsin $\sqrt{\text{percentage}}$).

## Results

The percentage of coiled nematodes (Tab. 3, Fig. 2) increased significantly (P = 0.05) with all nematode species when the dehydration time in the relative humidity chamber was lengthened from four days (Treatment 1) to sixteen days (Treatment 4). More than 90% of *H. dihystera* and *S. brachyurum* were coiled when the

Table 3

Percentage of coiled *Aphelenchus avenae*, *Helicotylenchus dihystera*, *Acrobeloides* sp. and *Scutellonema brachyurum* from Treatment 1, 2, 3, or 4

| *Treatment* * | *1* | *2* | *3* | *4* |
|---|---|---|---|---|
| *Aphelenchus avenae* | 53.7 | 69.4 | 72.2 | 91.2 |
| *Helicotylenchus dihystera* | 86.6 | 90.9 | 91.6 | 98.7 |
| *Acrobeloides* sp. | 2.1 | 25.4 | 40.8 | 90.9 |
| *Scutellonema brachyurum* | 76.8 | 91.7 | 97.5 | 96.2 |

* See Table 2.

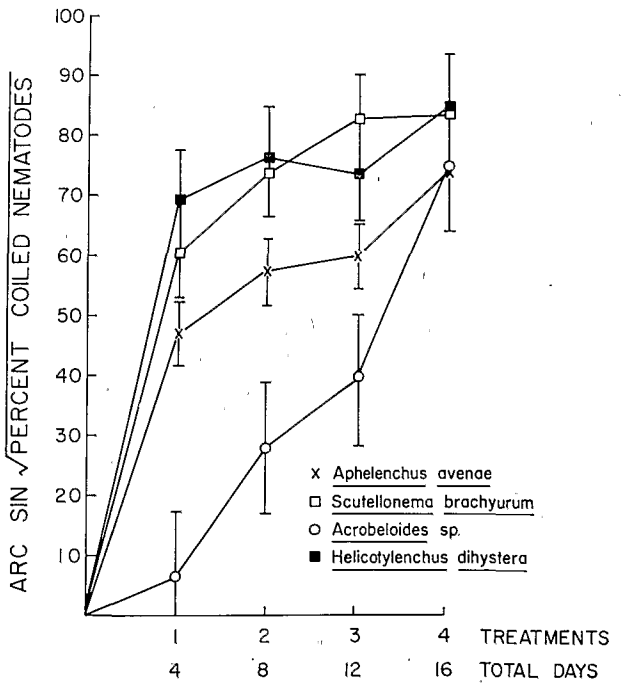



Fig. 2. Arcsin $\sqrt{\text{percent coiled}}$ *Aphelenchus avenae*, *Helicotylenchus dihystera*, *Acrobeloides* sp. and *Scutellonema brachyurum* from Treatments 1, 2, 3, or 4. Significance of differences are indicated at the 0.05 level of probability. (LSD test.)

Table 4

Percentage of nematode revival after exposure at 0% rh for 24 h with nematodes from Treatment 1, 2, 3, and 4.

| | *Treatments* * | | | | |
|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | |
| *Aphelenchus avenae* | 18.8 | 25.2 | 17.9 | 20.6 | No significant difference |
| *Helicotylenchus dihystera* | 0.4 | 21.3 | 23.9 | 21.0 | |
| Angle | 1.5 | 26.5 | 28.9 | 27.0 | LSD (P = 0.05) = 6.5 |
| *Acrobeloides* sp. | 0 | 9.2 | 5.6 | 20.5 | |
| Angle | 0 | 11.1 | 11.2 | 26.4 | LSD (P = 0.05) = 8.6 |
| *Scutellonema brachyurum* | 0 | 1.1 | 1.2 | 0.9 | No significant difference |

* See Table 2.

duration of dehydration between 100% and 97.7% rh was eight days (Treatment 2) whereas the dehydration time required for 90% of *A. avenae* and *Acrobeloides* sp. to coil was sixteen days (Treatment 4). The percentage of nematode survival after 97.7% rh for Treatments 1-4 was 95-96%, 93-94%, 93-94%, and 92-93% for *A. avenae*, *H. dihystera*, *Acrobeloides* sp. and *S. brachyurum*, respectively.

Nematode survival after a 24 h exposure to 0% rh, maintained by using $P_2O_5$, is presented in Table 4. The length of dehydration time did not appear to affect the survival of *A. avenae*, since between 17-25% of the nematodes survived in Treatments 1-4. However, the survival of *H. dihystera* increased significantly ($P = 0.05$) when the dehydration time was lengthened from Treatment 1 to either Treatment 2, 3 or 4. *Acrobeloides* sp. survival also increased significantly between Treatments 1, 2 and 4, with no significant difference between Treatments 2 and 3. Only 1% of the *S. brachyurum* survived 0% rh.

### Nematode observations

Examination of the Millipore filters under the SEM revealed individually, as opposed to mass, coiled anhydrobiotic nematodes. The typical morphology of *A. avenae*, *H. dihystera*, *Acrobeloides* sp. and *S. brachyurum* after drying to 97% rh on Millipore filters is illustrated in Fig. 3.

## Discussion

It is pertinent to this work to discuss the current concepts of cryptobiosis, anhydrobiosis, drought resistance and quiescence. There are three theories which distinguish the various types of survival in nematodes. Van Gundy (1965) and Cooper and Van Gundy (1971) broadened the Keilin (1959) concept of latent life in animals [this has been reviewed more recently by Evans and Perry (1976)]. Their theory is based on the lack of metabolism of an organism, i.e., no metabolism being the most resistant state, cryptobiosis, and a lowered but detectable metabolism being defined as dormancy, quiescence. Anhydrobiosis, cryobiosis, anoxybiosis and osmobiosis are descriptive terms of the environmental pathway by which nematodes proceed from the active to the cryptobiotic state (Tab. 5). This view recognized that there are no clear-cut stages of anhydrobiosis or lowered metabolism until cryptobiosis is reached. Thus it is possible for nematodes to be drought-resistant or slightly quiescent in the anhydrobiotic state and not cryptobiotic. Eventually, with the development of more sensitive equipment to detect metabolism, the gradation between these states of dormancy and cryptobiosis may become clearer.

Table 5

Relationship of the various states of metabolic activity of inactive organisms as proposed by Keilin (1959)

*Active life* = normal metabolism

- *Hypobiosis*
  - *Dormancy* (hypometabolism)
    - *hibernation*
    - *aestivation*
    - *diapause*
    - *quiescence*
  - *Latent life* (ametabolism)
    - *cryptobiosis*
    - *anabiosis*
    - *abiosis*

The other two theories of nematode survival disregard presence or absence of metabolism as being a criterion to distinguish cryptobiosis. Crowe and Cooper (1971) define cryptobiosis on the basis of the structural integrity of the cryptobiote. If the structural integrity of an organism remains intact, the organism is capable of resuming the active state. They note that the structural integrity of a cryptobiote will remain organized and intact in spite of exposure to extremes of heat, cold, ionizing radiation and even chemicals. There are four types of cryptobiosis : anhydrobiosis, which is brought on by extensive water loss

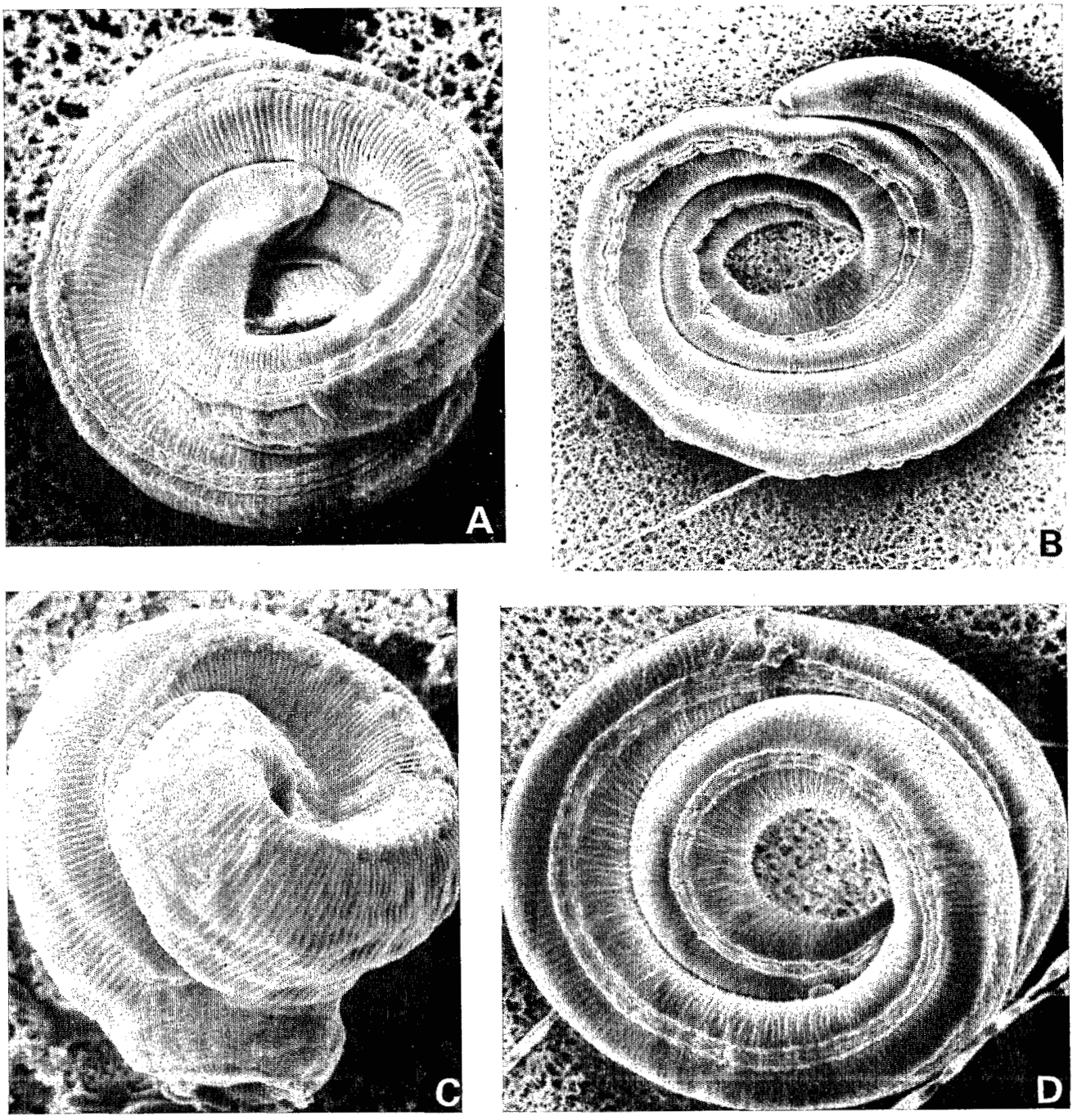



Fig. 3. Scanning electron microscope photographs of A) *Aphelenchus avenae* ( × 2 000) ; B) *Scutellonema brachyurum* ( × 800) ; C) *Acrobeloides* sp. ( × 2 000) and D) *Helicotylenchus dihystera* ( × 1 000) taken on Millipore filter after dehydration to 97.7% relative humidity.

through evaporation ; osmobiosis, which is induced by removal of water from organnisms by an external solution that has high osmotic pressure ; cryobiosis, which occurs when the organism is frozen, and anoxybiosis, which is induced when the external oxygen concentration falls below the level required to support oxidative metabolism. Osmobiosis and cryobiosis are defined as being similar to anhydrobiosis since both are induced by the unavailability of water which is needed for normal metabolic reactions.

Evans and Perry (1976) follow Laudien's (1973) concept of grouping all resting stages under dormancy. Their criterion for separating resting stages is the cause of arrested development. Cryptobiosis is simply defined as a form of facultative quiescence, as is anhydrobiosis, anoxybiosis, cryobiosis and osmobiosis. These conditions are all caused by unfavorable environmental factors and are reversed by favorable environmental conditions. The other categories are obligate quiescence, facultative diapause and obligate diapause.

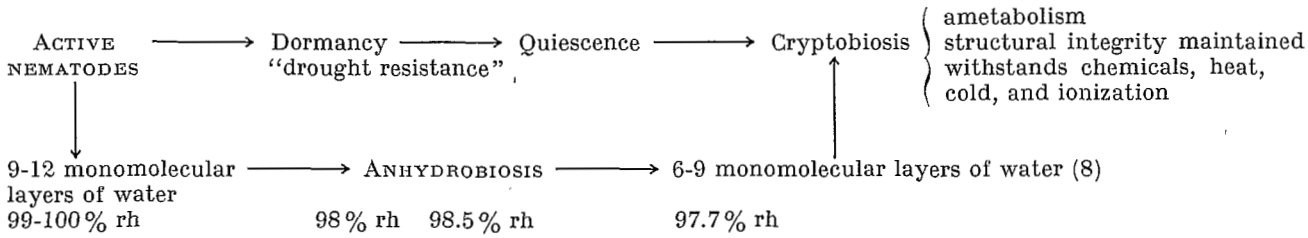
Because we consider that the nematodes experiencing a diapause are the exception rather than the rule, and that the ability to enter the latent state at any stage of the life cycle due to unfavorable conditions is the more widespread phenomenon in soil nematodes, we prefer a combination of the first two theories (Tab. 6). Simons' (1973) suggestion that drought resistance is a general phenomenon in plant nematodes can now be extended to other soil nematodes. Drought resistance would be dormancy or quiescence as induced by anhydrobiosis. The nematodes would not have reached the more resistant state of anhydrobiosis and cryptobiosis, as is seen with stages of *Ditylenchus dipsaci* (Perry, 1977).

Our results with single nematodes of different species and representing different trophic groups suggest that there are differences in the physical integrity of coiling in single nematodes (Fig.3) versus clumps of nematodes, in addition to various levels of reduced metabolism, when nematodes enter the various stages of anhydrobiosis (dormancy, quiescence, cryptobiosis). These results have been confirmed further by *in vivo* studies (Demeure, 1975 ; Demeure, Freckman & Van Gundy, 1979 ; Freckman, Kaplan & Van Gundy, 1977).

As noted by Simons (1973) and others (Ellenby, 1968 ; Endo, 1962 ; Perry, 1977), the rate of dehydration appears to be the controlling factor of nematode survival in environments having less than 100% rh. We also observed two separate responses to slow desiccation or anhydrobiosis : 1) a physical response or coiling, and 2) a physiological response. Coiling was a short-term response and was proportional to increased dehydration time. Madin and Crowe (1975) found that spiral formation of pellets of *A. avenae* was completed hours before the nematodes could be exposed to dry air. The cryptobiotic stage of anhydrobiosis was a longer term response to a slow rate of drying, enabling the nematode to survive at relative humidities such as 0% for 24 h. Twenty to 25% of *A. avenae*, *H. dihystera* and *Acrobeloides* sp. survived 0% rh for 24 h, whereas only 1% of *S. brachyurum* survived. Of these species, the desert nematode, *Acrobeloides* sp. appeared to be more resistant to the decreasing relative humidity and coiled later than the other three species. It is not clear

Table 6

A classification of the various types of nematode survival in dry soils.



why a mass of anhydrobiotic nematodes clump together and survive chemicals such as $P_2O_5$, thus defining them as in the cryptobiotic state, whereas individual nematodes of the same species will not.

In conclusion, it appears that anhydrobiosis and the cryptobiotic stage of anhydrobiosis are general responses that occur in many soil and plant nematodes and are not limited to a few species. However, nematode survival may be dependent on the rate of response to desiccation, which will vary with species (Demeure, 1975 ; Demeure, Freckman & Van Gundy, 1979), life stages (Perry, 1977 ; Simons, 1973) and ecological habitat (Demeure, Freckman & Van Gundy, 1979 ; Freckman, Kaplan & Van Gundy, 1977).

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