Anhydrobiotic Coiling of Nematodes in Soil

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Abstract. Nematodes of three genera (*Acrobeloides* sp., *Aphelenchus avenae,* and *Scutellonema brachyurum*) were induced to coil and enter anhydrobiosis in drying soil of two types: sandy loam and loamy sand. Coiling was studied in relationship to soil moisture characteristics. Coiling and the physiological state of anhydrobiosis occurred before the water in sandy soils reached a water potential of −15 bars. Coiling was maximum at 3–6 bars, depending on the soil type and nematode species. It appeared that induction of coiling and anhydrobiosis were determined by the physical forces exerted by the water film surrounding the nematode, which, for these three species, was 6–9 monomolecular layers of water, rather than the % moisture and relative humidity of the soil *per se. Key Words:* anhydrobiosis, *Aphelenchus avenae, Acrobeloides, Scutellonema brachyurum,* soil moisture, survival, monomolecular layers of water.

Physiological and ultrastructural changes associated with anhydrobiosis and coiling in nematodes were demonstrated in 1974 with *Anguina tritici* larvae by Bird and Buttrose (1) and with *Aphelenchus avenae* larvae and adults by Crowe and Madin (5). Coiled nematodes in dry desert soils have been collected and observed by Demeure (7), Freckman, Kaplan and Van Gundy (9), and Freckman (10). Towson (16) observed coiled root-knot nematode larvae in desiccated soil under laboratory conditions. Although no coiled nematodes have been observed directly in dry soil, it has been postulated that they can survive long hot dry periods in the anhydrobiotic state (10).

Coiling, a morphological condition associated with anhydrobiosis, can be induced in *A. avenae* in the laboratory by slowly drying a minimum of 0.1 g wet weight mass of nematodes in chambers designed to maintain a relative humidity of 97% (6). The relative humidity of the soil pore spaces in field soil remains above 99% at the permanent wilting point (15 bars) (2). Our sampling of desert soils indicated that coiled nematodes were found in soils of less than 15 bars suction and suggested that moisture factors other than relative humidity were involved in initiating anhydrobiosis of single nematodes in soil. This research was done to induce coiling in drying soil, to study coiling in relation to the soil moisture characteristics of drying soil, and to observe anhydrobiotic nematodes directly in soil.

MATERIALS AND METHODS

Nematodes. Three nematode species were selected for study to represent the various nematode trophic groups commonly found in soil. The fungal feeder *Aphelenchus avenae* Bastian, 1865, was cultured in the laboratory on *Rhizoctonia solani* Kuhn, 1858 (8, 4). The bacterial feeder *Acrobeloides* sp. was collected from a Mojave desert soil near Rock Valley, Nevada, and cultured in a mixed bacterial culture on oatmeal and agar. The plant parasite

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Scutellonema brachyurum (Steiner, 1938) Andrassy, 1958, was collected before each experiment from moist soil around a banana plant on the Riverside campus of the University of California. All nematodes were extracted by the Baerman funnel technique for 24 h. Mixed larvae and adults were used in all experiments.

Soil moisture characteristics. The soil moisture of two soils, a loamy sand (92.4% sand, 3.9% silt, and 3.7% clay) and a sandy loam (72.8% sand, 21.2% silt, and 6.0% clay), was controlled on a pressure plate extractor (14) maintained at 20 C. Fig. 1 illustrates a cross-section of the extractor and soil sample. Pressure plates [5-bar pressure plate extractor (Cat. No. 1600) and 15-bar ceramic plate extractor (Cat. No. 1500); Soil Moisture Equipment Co., Santa Barbara, California] with two ranges of suction were used, 0–3 bars for preparing soils with suctions up to 3 bars, and 0–15 bars for preparing soils with suctions of 3–15 bars. For each suction, soil moisture content was determined by the weights of five replicates before and after samples were placed for 24 h in an oven at 105 C.

The surface area of each soil type was determined by the ethylene glycol monoethyl ether (EGME) adsorption method (3, 11) on dry soil. Soil was dried at 105 C for 24 h and then 1.1 g of dry soil was covered with 1 ml of EGME and placed in a desiccator over $CaCl_2$ for 72 h. The soil was weighed again to determine the amount of EGME adsorbed on the soil surface. The surface area, S, was calculated as follows:

$$S(m^2/g \text{ dry soil}) = \frac{\text{quantity of EGME adsorbed (g)}}{\text{quantity of dry soil (g)} \times k}$$

at 20–25 C, $k = 0.000286$ g/m^2

The average thickness of the water film around soil particles was calculated by measuring the surface area of the loamy sand (11.2 m^2/g) and sandy loam (15.2 m^2/g) and then estimating the soil moisture content when each particle was covered with one monomolecular layer of water (MLW). The formula and calculations are as follows:

$$\text{Soil moisture content (g water/g dry soil)} = \frac{\text{Soil surface area (m}^2\text{/g dry soil)} \times \text{molecular water weight (g)}}{\text{Molecular water surface (Å)} \times 6.023 \times 10^{23}}$$

Molecular water weight = 18^{-20} g
Molecular water surface = 10.8 Å

Soil moisture contents of the loamy sand and sandy loam were found to be respectively 3.1×10^{-3} g water/g dry soil and 4.3×10^{-3} g water/g dry soil (15).

$$\text{No. MLW} = \frac{\text{Soil moisture content (g water/g dry soil)}}{\text{Soil moisture content when each soil particle is covered with 1 MLW (g water/g dry soil)}}$$

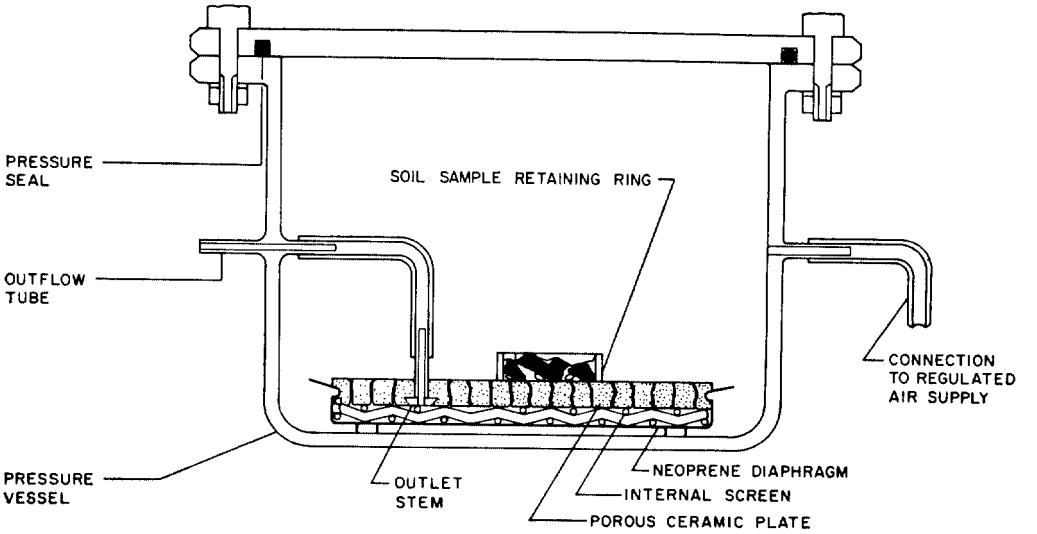

FIG. 1. Cross-section of ceramic pressure-plate cell showing soil sample in the extractor.

Treatments. Each experiment consisted of 30 polyethylene rings (2.5 cm in diameter and 1 cm high) placed on the pressure plate to represent 6 treatments each with 5 replicates. About 5 cc of dry soil was added to each ring. The samples were then moistened with water by capillary action to saturation. Nematodes were pipetted onto the surface of each wet soil sample. About 200 nematodes were used for *S. brachyurum,* and 1000 each of *A. avenae* and *Acrobeloides* sp.

Treatments consisted of 0, 0.1, 0.3, 0.5, 1, and 3 bars on the 0–3-bar plate and 0, 0.5, 1, 3, 6, and 9 bars on the 0–15-bar plate in those cases when nematodes did not reach maximum coiling on the 0–3-bar plate. Starting at saturation, suction was increased to the next treatment for 24 h, the container was opened, and the replicate rings and soil were removed from the plate with a spatula, placed in a plastic bag slightly larger than the ring, and heat-sealed. The container was closed, and the suction was increased to the next treatment for 24 h. All treatments were maintained in the plastic bags in a constant-temperature room until the end of the experiment. This ensured that all nematodes remained in the soil for the same length of time. The numbers of experiments were: two with *A. avenae,* three with *Acrobeloides,* and one with *S. bracyurum.* As a control, to determine whether pressure alone would initiate coiling and survival, nematodes were tested at 3.0 bars in moist soil not in contact with the porous pressure plate.

Nematode extraction. Nematodes were extracted from the soil samples in 1.25 M sucrose (9). The percentage of coiled nematodes in the 1.25 M sucrose solution was calculated, after which they were returned to water for 24 h and the percentage of active nematodes was determined as an indication of survival.

SEM examination. Some soil from the 9-bar treatments was glued with silver conductive paint on a scanning electron microscope (SEM) plug, treated with gold, and observed at 800 to 1,400 magnifications on a Joelco SEM (Model No. JSM-U3).

RESULTS

Figs. 2 and 3 graph the relationship of soil suction, soil moisture, and average water film thickness to coiling and survival of adults and larvae of *A. avenae, S. brachyurum,* and *Acrobeloides* sp. in the loamy and sandy loam soil, as detailed in Table 1. In loamy sand soil, respective coiling began at soil moisture contents of 2.8, 2.8, and 2%, i.e., when the water films around soil particles were respectively 9, 9, and 6 MLW thick. Coiling in sandy loam soil began

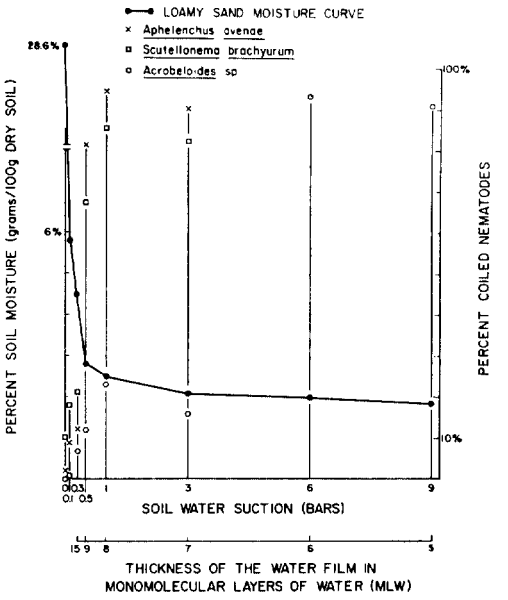

FIG. 2. Soil moisture characteristic of a loamy sand (92.4% sand, 3.9% silt, and 3.7% clay) and percentage of coiling of *A. avenae* (x), *S. brachyurum* (□), and *Acrobeloides* sp. (o).

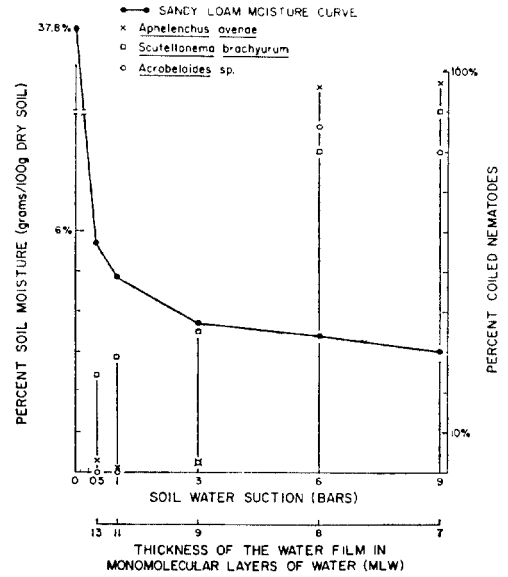

FIG. 3. Soil moisture characteristic of a sandy loam (72.8% sand, 21.2% silt, and 6% clay) and percentage of coiling of *A. avenae* (x), *S. brachyurum* (□) and *Acrobeloides* sp. (o).

TABLE 1. Relationship of soil suction, soil moisture, and water film thickness in number of monomolecular layers of water (MLW) to coiling and survival of adults and larvae of *A. avenae, S. brachyurum,* and *Acrobeloides* sp. in two soil types.

Suction (bars)	Soil moisture content (%)	Water-film thickness (MLW)	% coiled nematodes			% activity after treatment		
			Aphelenchus avenae	*Scutellonema brachyurum*	*Acrobeloides* sp.	*Aphelenchus avenae*	*Scutellonema brachyurum*	*Acrobeloides* sp.
Sandy loam (72.8-21.2-6.0)								
0.0	37.8	89	0	0	0	89	98	97
0.1	16.0	38	0	11	0	88	98	84
0.3	9.0	21	1	18	0	93	99	97
0.5	5.7	13	3	24	0	93	93	100
1.0	4.8	11	1	28	0	95	94	95
3.0	3.7	9	2	35	2	94	96	78
6.0	3.4	8	96	80	86	95	94	87
9.0	3.0	7	97	90	80	95	97	83
Loamy sand (92.4-3.9-3.7)								
0.0	28.6	92	2	10	0	91	97	96
0.1	5.8	19	9	18	1	95	96	97
0.3	4.5	15	12	21	7	96	98	97
0.5	2.8	9	81	67	12	97	98	95
1.0	2.5	8	94	85	23	96	98	98
3.0	2.1	7	90	82	16	98	97	98
6.0	2.0	6	—	—	93	—	—	92
9.0	1.8	6	—	—	91	—	—	92

— no data taken.

when the soil moisture content reached 3.4% or when the water film was 8 MLW thick. Generally, there was an inverse relation between soil moisture content and coiling, i.e., as the soil pore spaces began to empty, the nematodes began coiling. Coiling was maximum when the water film around the soil particles reached 8–6 monomolecular layers of water, regardless of soil type. Nematode survival after each treatment was 78–100%. Pressure controls up to 3.0 bars did not increase coiling (8% of *A. avenae* were coiled at 3.0 bars pressure) nor did it affect survival in moist soil (94% of *A. avenae* were revived from 3.0 bars).

Examination of dry soil with the SEM provided *in situ* observation of single coiled anhydrobiotic nematodes in soil pore spaces. Fig. 4-A,B,C illustrates the typical shapes in soil treated at 9 bars of suction. The nematodes often appeared to be adhering to some soil particle surface or to each other (Fig. 4-D).

DISCUSSION

These results clearly indicate that the coiling of nematodes in drying soil and the physiological state of anhydrobiosis started long before the water in the sandy soils reached the water potential of −15 bars. Although the three nematodes reacted differently to drying, they all achieved maximum coiling at 6 bars of suction in the sandy loam. Madin and Crowe (13), from laboratory studies on anhydrobiosis, indicated that at least 4 days of slow drying at 97% rh were needed for nematodes to shift their metabolic processes from a lipid-glycogen to a glycerol-trehalose storage. Our nematodes were subjected to 6 days of drying at different pressures to ensure maximum coiling and inducement of anhydrobiosis at each pressure. This is substantiated by the high level of nematode activity after each treatment (Table 1). The low levels of coiling (<10%) at 0 to 0.3 bar in loamy sand and 0 to 1 bar in sandy loam may be attributed to some drying at the soil surfaces during storage in plastic bags before extraction.

The general inverse relation between soil moisture content and coiling varied between the two soil types. About 80% of all three nematodes were coiled when moisture content was between 2.0% and 2.8% in the loamy sand and at 3.4% in the sandy loam. Freckman (10) suggested that nematodes went in and out of anhydrobiosis in desert soils at a moisture content of about 2.5%. On the basis of calculated water film thickness around soil particles in monomolecular layer of water (MLW) in both soils, it appeared that there was a closer relationship between number of MLW and coiling than between moisture content and coiling. The relative humidity in the soil pore spaces was between 100 and 99% at these moisture levels (2).

Wallace (17) studied the movement of nematodes in water film and demonstrated that there was an optimum film thickness for nematode movement on flat surfaces and that nematode movement in soil was optimum when soil pores were about half drained of free water. Figs. 2 and 3 suggest that coiling takes place when the soil pores have been completely drained of free water and the water film thickness surrounding the soil particles is between 6 and 9 MLW. These data support the work of Wallace (17), who proposed that the physical forces of the water films surrounding nematodes in the soil pore spaces (Fig. 5) are an important factor affecting the behavior of nematodes. As a further extension of Wallace's theory, it is the physical forces of the soil particles on the monomolecular layers of water molecules that induce nematodes to coil and enter the state of anhydrobiosis. This is further supported by Low (12) who suggested that water films of different thicknesses have different viscosities.

All three nematodes responded differently to moisture losses in soil. The microbial feeder *Acrobeloides* sp. seemed to coil more slowly than the other two species. This nematode had been previously isolated from the desert soil, and its behavior in this experiment suggested that it was adapted for maximum activity during brief periods of moisture in normally dry desert soils. The fungal feeder *A. avenae* has been a model system for studying the nature of anhydrobiosis in nematodes. The plant parasite *S. brachyurum* was isolated from a moist semi-tropical ornamental planting in Riverside. It appeared to be somewhat less resistant to drying, and some individuals began coiling as soon as the soil started to dry (0.1 bar) and progressively continued to

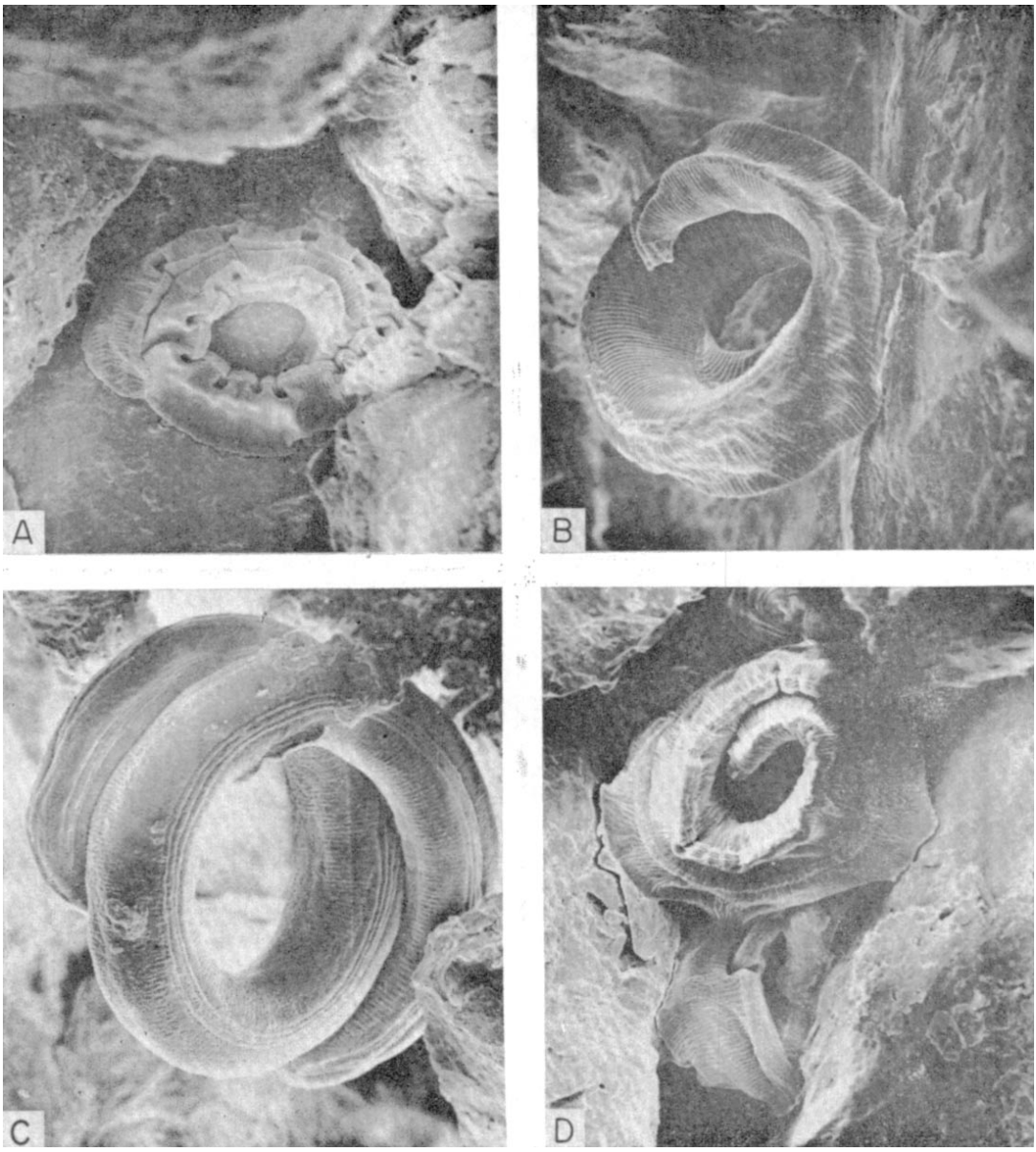

FIG. 4. Scanning electron-microscope photographs of: A) *A. avenae* (×1000), B) *Acrobeloides* sp. (×1400), C) *S. brachyurum* (×800), and D) *A. avenae* (×1000) taken *in situ* in soil treated at 9 bars suction.

coil until the maximum was reached. Coiling of *S. brachyurum* is a natural behavioral response to extraction from soil and may have contributed to a higher coiling response at the low suctions (0–0.3 bar). It is doubtful that the coiled nematodes were in the state of anhydrobiosis at those low suctions. The length and width of 15 preserved nematodes were measured, and neither the differences between the diameters of females of *A. avenae* (24 μm), *S. brachyurum* (35 μm), and *Acrobeloides* sp. (37 μm) nor the difference between the surface areas of these nematodes (respectively 71.10^3, 86.10^3, and 69.10^3 μm^2) can explain the different responses of *Scutellonema* and *Acrobeloides* from *Aphelenchus* to moisture losses in soil. The *in situ* photographs of all three nematodes (Fig. 4) suggest that cuticle and bodies of *A. avenae* and the *Acrobeloides* sp. collapse and shrink more than *S. brachyurum*.

In conclusion, the three nematodes tested can be induced to coil and enter

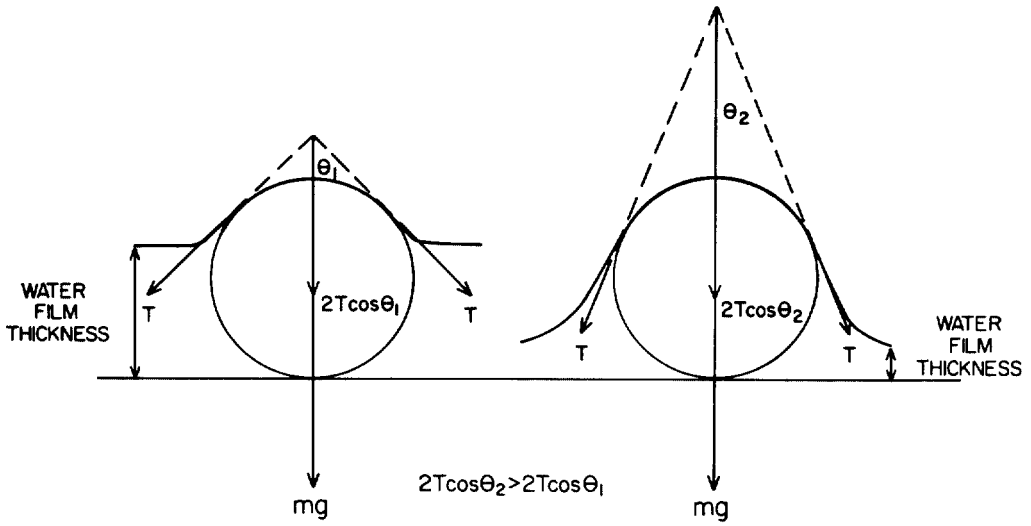

FIG. 5. A comparison of the external physical forces acting on a nematode at rest in a water film [after Wallace (1959)].

anhydrobiosis by slowly drying moist soil. Depending upon soil types, the nematodes may begin coiling at about 0.5 bar of suction and reach maximum coiling and anhydrobiosis at suctions of 6 bars. The trigger mechanism for inducing coiling and anhydrobiosis appears to be physical forces brought to bear on the nematode surface by the thickness of the water film surrounding the nematode. The optimum thickness to induce anhydrobiosis was 6 to 9 monomolecular layers of water molecules.

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