

Some Factors Affecting Survival of Desiccation by Infective Juveniles of *Orrina phyllobia*¹

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Abstract: The survival of desiccation by J4 *Orrina phyllobia* was examined at controlled relative humidities. When nematodes were transferred from water to air at 10% relative humidity (rh), 80% died within 30 minutes. When nematodes were transferred from water to air with rh at 70% or greater for ca. 15 minutes prior to being transferred to 10% rh, more than 90% of them survived desiccation. This phenomenon is referred to as preconditioning and occurred at much faster rates (2-30 minutes) than has been observed for other nematode species (24 hours). Differences in preconditioning rates may be due to technique-dependent variations in boundary layer resistance around nematodes during desiccation.

Key words: anhydrobiosis, relative humidity.

Anhydrobiosis is a usual part of the life cycle of *Orrina phyllobia* (Thorne) Brzeski. Infective juveniles leave the old host through the abscission of dried nematode-infected leaves. Large numbers of viable dried juveniles are frequently found on plant surfaces and in the upper few centimeters of soil (11). Occasionally, large numbers of dead *O. phyllobia* have been recovered from soil. The objective of the following research was to define optimum conditions for desiccation survival by *O. phyllobia* infective juveniles.

MATERIALS AND METHODS

Orrina phyllobia infective juveniles were collected, stored, extracted from plant tis-

sue, and isolated as previously described (12). The techniques used to control drying differed substantially from techniques that have been used conventionally for more than two decades to study nematode anhydrobiosis. Because of the markedly different results we obtained, our techniques will be described in detail.

Orrina phyllobia juveniles were dried in a constant airflow at controlled relative humidity (rh) as follows. Nematodes were placed on a 19-mm-d circular coverslip in the absence of liquid water by placing one drop (0.2 ml) of nematode suspension (ca. 300 nematodes) on one half of the coverslip. A bisected 47-mm membrane filter was laid across the opposite half of the coverslip with its cut edge 1-2 mm away from the nematode suspension drop and was stuck to the coverslip by wetting the cut edge with a drop of distilled water. A scalpel was used to connect the nematode suspension drop to the edge of the filter. Water in the nematode suspension gradually moved into the filter, leaving nematodes on the glass against the edge of the filter. By gently removing the filter, most of the nematodes were kept on the coverslip with

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essentially all free water removed. The coverslip was placed within a 25-mm-d in-line filter holder with the nematode side exposed to a 3-m second⁻¹ airflow. The absorption of water by the filter and the placement of the coverslip within the holder were coordinated so that the coverslip was held directly in the airflow at the initial experimental rh while the last trace of visible water disappeared. The filter was removed within the airflow only after water was no longer observable.

After the subjection of nematodes to experimental rh's, the in-line filter holder was opened with air still flowing at the final experimental rh, and the coverslip was flooded with distilled water directly under the air current. Flooded coverslips were kept in petri dishes for 20–28 hours at ca. 23 C, then photographed for survival index (SI) measurements (13). A visual estimate of SI was also made for each coverslip to confirm data obtained photographically.

Relative humidity was controlled by passing air through one or more of ten glycerol solutions (0–96% by weight). To permit sequencing versatility, the solutions were contained in polyvinyl chloride cylinders. The cylinders were plugged with rubber stoppers, through which were passed polyethylene tubes for air exchange and were interconnected by vinyl tubing and stopcock valves; this approach allowed conversion from any rh in the 10–100% range to any other within 5 seconds. Total airflow was controlled and monitored with a regulator/flowmeter on a compressed-air tank providing dried air (6% rh). Relative humidity was monitored by directing all the exhaust air from the coverslip holder into the cuvette of a Licor LI-1600® porometer. Porometer readings for airflow temperature and rh were checked against a wet bulb thermometer made by wrapping a wetted membrane filter around a thermistor inserted within the airflow.

Two series of exposures were conducted. In the first series, nematodes were transferred from 100% rh to 10, 30, 50, 70, and 90% rh for 2, 4, 8, 16, 32, and 60 minutes. After each exposure, airflow rh was changed to 100% for at least 1 minute prior to opening the coverslip holder. Two controls were included in each exposure. The first control consisted of placing one drop of nematode suspension on a cover-

slip, then immediately flooding the coverslip with water. The second control consisted of inserting a prepared coverslip into the airflow at 100% rh for 1 minute, then removing it, flooding it with water and immediately examining nematodes under the stereomicroscope to confirm that a high level of activity was achieved within 1 or 2 seconds.

The second series of exposures tested the hypothesis that preexposure to high rh is necessary for nematodes to survive drying at low rh. Juveniles were maintained for various periods of time (0.5–60 minutes) at 50, 60, 70, 80, 90, 95, and 97% rh before changing to 10% rh, and then were held at 10% rh for an additional 15 minutes. Preexposures to 80% and higher rh's were repeated with a second nematode population.

RESULTS

When nematodes were dried at constant rh, they died only at rh ≤ 50% (Fig. 1). The number of nematodes dying per minute and the final percentage killed were inversely related to airflow rh. Water potentials of air at 50, 30, and 10% rh (20 C) are -930, -1,600, and -3,100 × 10⁵ Pa, respectively. Forty to fifty percent of the nematodes held at 50% rh were killed after 1 hour (data not shown). In three separate experiments, ≥ 80% of nematodes held at 10% rh were killed in < 10 minutes.

Nematode survival at 10% rh was increased from 20 to 100% by preexposure to 3-m second⁻¹ airflow at ≥ 70% rh (-480 × 10⁵ Pa). This phenomenon will be referred to as preconditioning (Figs. 2, 3). The period of time that nematodes had to be exposed to high rh to achieve 100% preconditioning was very short (1–30 minutes) and, at rh ≥ 80% (-300 × 10⁵ Pa), the time required was directly related to preconditioning water potential. This result suggested that the attainment by nematodes of a critical water content was instrumental to preparation for rapid drying. Below 80% rh, preconditioning rate did not increase as rh was decreased. At 70% rh, 100% preconditioning required ca. 6 minutes compared with 1–4 minutes required at 80% rh. At 50% rh, preconditioning did not begin until sometime between 30 and 60 minutes; during this period

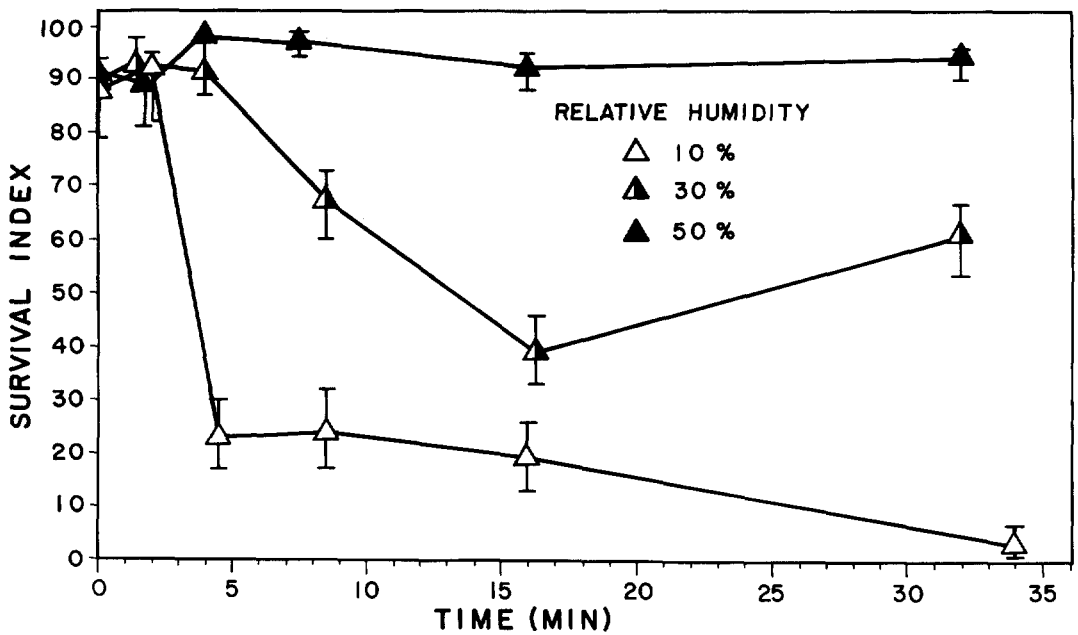


FIG. 1. The survival index (photographically measured percent moving) of *Orrina phyllobia* infective juveniles after 20–28 hours in distilled water following exposure to 3-m second⁻¹ airflow at 10, 30, and 50% relative humidity for various time intervals. Brackets are binomial confidence intervals at $P = 0.05$.

ca. 50% of the nematodes died and 50% preconditioned.

The second nematode population required about twice as long as the first population to precondition at each rh examined. The second population had been kept in distilled water about 24 hours longer prior to desiccation.

Prior to and during rehydration, nematodes that had been fully preconditioned at high rh where characterized by coiling and ca. 50% length reduction. Nematodes that were rapidly dried without preconditioning treatment did not coil and were collapsed laterally with little length reduction. Nematodes which, according to SI measurements, were partially preconditioned were intermediate in appearance when desiccated and during rehydration contained nonrefractive vacuolar regions along the length of the body.

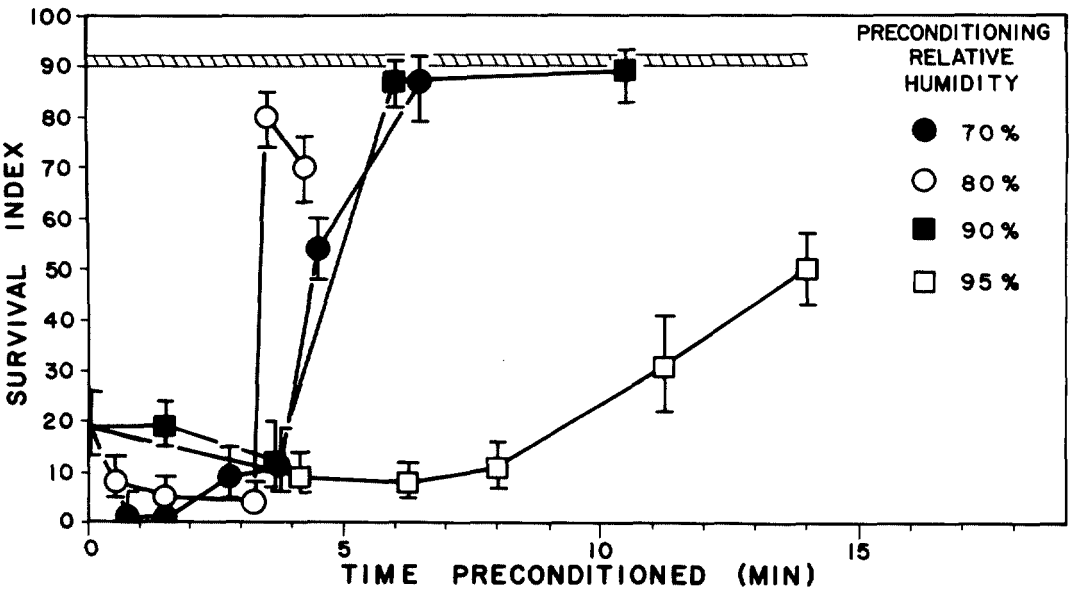
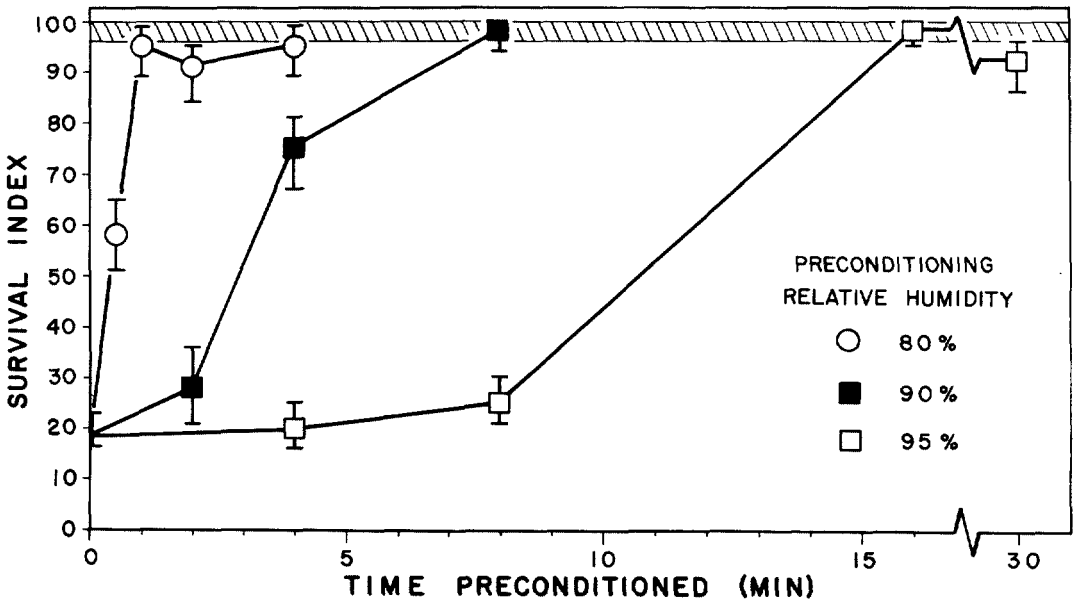
DISCUSSION

The short time periods required for desiccation preconditioning by *O. phyllobia* (1–30 minutes) are in agreement with the ability of large numbers of *O. phyllobia* to survive desiccation on aerial plant surfaces following intermittent wetting. The inability

of *O. phyllobia* juveniles to survive instantaneous transfer from water to air at low relative humidities agrees with numerous observations on other nematode species (3,7). Postural and length differences between slowly and rapidly dried *O. phyllobia* were similar to those reported for *Aphelenchus avenae* Bastian by Crowe et al. (1).

The ability of *A. avenae* to survive rapid drying was closely correlated with the onset of polyol accumulation, specifically, the synthesis of trehalose and glycerol from lipid reserves (2,9). Evans and Womersley (8) reported a similar increase in the trehalose content of *Ditylenchus dipsaci* (Kuhn) Filipjev during desiccation. It might be construed, based on the time required for rapid polyol synthesis to initiate in *A. avenae* (24 hours at 97% rh), that polyol accumulation in response to water stress necessarily takes a long time in nematodes. However, if polyol accumulation is the mechanism responsible for desiccation preconditioning in *O. phyllobia*, it can occur within 30 minutes.

The faster preconditioning response we obtained for *O. phyllobia* may be the result of technique-related differences in conditions immediately surrounding organisms.



FIGS. 2, 3. Effect of preconditioning by preexposure to high relative humidity on the desiccation survival of two populations of *Orrina phyllobia* infective juveniles. 2) Nematodes were preexposed to high relative humidity and subsequently desiccated for 15 minutes at 10% relative humidity within a 3-m second⁻¹ airflow. Survival index (photographic percent moving) was measured after 20–28 hours reacclimation in distilled water. Striped zone indicates confidence interval for controls. 3) Results similar to those illustrated in Fig. 2 that were obtained with a second experimental population of *Orrina phyllobia* juveniles.

Net unidirectional flux of water across any interface is a function of the hydraulic conductance of that interface and the water potential drop across it. Specifically, $J_v = L_p(\Delta\Psi)$, where J_v is the volumetric flux of

water (m second⁻¹), L_p is the hydraulic conductance (m second⁻¹ bar⁻¹, 1 bar = 10⁵ Pa), and $\Delta\Psi$ is the water potential drop (bars) across the interface (10). A similar expression describes isothermal flux of

water evaporating from a wet surface. Specifically, $J_v = KL(V/\lambda)^{1/2}\Delta\Psi$, where K is a constant, L is the hydraulic conductivity ($\text{m}^2\text{second}^{-1}\text{bar}^{-1}$) of air within the boundary layer above the surface, V is the velocity of air moving across the surface, and λ is the distance the air moves across the surface. For still air, where small air convection currents occur, V is, by convention, approximated by 0.1 m second^{-1} (10). Clearly, V can have a very big effect on water evaporation. Since J_v is linearly related to the square root of V , hydraulic flux across vapor boundary layers is most sensitive to air velocity variation at low air speeds. Where flux is nonisothermal, -82 bars is added to $\Delta\Psi$ for each 1 C increase from water to air. Small thermal gradients thus can be quite important.

When water leaves a drying nematode, two serial conductances are involved: that of the nematode cuticle and that of the vapor boundary layer. Therefore, the drop in water potential to which the nematode is directly exposed, i.e., the drop across the cuticle, may be expressed as $\Delta\Psi_c = \Delta\Psi_t - \Delta\Psi_a$, where $\Delta\Psi_t$ is the total water potential drop from inside the nematode to air above the vapor boundary layer, and $\Delta\Psi_a$ is the water potential drop across the boundary layer; $\Delta\Psi_c$ is organism-dependent, whereas $\Delta\Psi_a$ is external condition-dependent. If $\Delta\Psi_a$ is of the same order of magnitude as $\Delta\Psi_c$ or greater, the condition to which a nematode is directly exposed may differ substantially from that predicted by rh measurement.

Nematode cuticle permeability to water has been measured for several species by placing nematodes in deuterated or tritiated water and assaying radioactivity of washed animals through time. The quantity that is calculated with this method is the permeability coefficient, which may be expressed as $P_w = J_v(c_o - c_i)^{-1}$, where c_o and c_i are the concentrations of labeled water outside and inside the nematode. The permeability coefficient has units of m second^{-1} and does not directly consider $\Delta\Psi$. It is theoretically related to hydraulic conductance according to $L_p = (7.5 \times 10^{-4}\text{ bar}^{-1})P_w$ (4). Accordingly, the permeability coefficients calculated by Wright and Newall (15) for various nematode species (0.8 to $6 \times 10^{-8}\text{ m second}^{-1}$) predict hydraulic conductances of 0.6×10^{-11} to 0.5×10^{-10}

$\text{m second}^{-1}\text{bar}^{-1}$. Utilizing values of K and L from Milburn (10), we calculate similar boundary layer conductances (10^{-11} to $10^{-10}\text{ m second}^{-1}\text{bar}^{-1}$) for conditions under which nematodes have been air dried experimentally. These estimates suggest that boundary layer conductance is as important as cuticle permeability during nematode desiccation.

In previous studies investigating nematode desiccation, nematodes were dried against solutions with known water potentials (usually H_2SO_4 or glycerol) within desiccators containing relatively still air (2,5,6,14). In view of the markedly different results that were obtained for *O. phyllobia*, it may be useful to vary boundary layer conductance during nematode desiccation via controlled airflow and controlled thermal gradients to better understand effects caused by vapor-induced water potential differentials across the nematode cuticle.

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