J. exp. Biol. 143, 359–372 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

NATURAL DEHYDRATION REGIMES AS A PREREQUISITE FOR THE SUCCESSFUL INDUCTION OF ANHYDROBIOSIS IN THE NEMATODE *ROTYLENCHULUS RENIFORMIS*

BY CHRISTOPHER WOMERSLEY AND CHERIE CHING

Department of Zoology, University of Hawaii at Manoa, Honolulu, HI 96822, USA

Accepted 16 November 1988

Summary

Induction of anhydrobiosis in the nematode *Rotylenchulus reniformis* (Linford & Oliveira) was studied using direct exposure to elevated relative humidities and conditions resembling natural dehydration regimes. All larvae and preadults were unable to survive direct short-term exposure to 97 % relative humidity. However, dehydration of larvae on model substrates (0·5 % agar: 1·0 % agarose) that mimic the natural rate of soil moisture loss induced coiling and successful entry into anhydrobiosis. Coiling was maximized at 10–12 days and only coiled larvae survived dehydration, emerging as the preadult form. Larvae could withstand severe dehydration at 80 and 40 % relative humidity after the induction of coiling, but were unable to withstand direct exposure to 0 % relative humidity.

The levels of adaptation utilized by anhydrobiotes to ensure slow dehydration and the adaptive significance of this are discussed in relation to the different environments in which these organisms are found. The results suggest that *R. reniformis* is well-adapted to its environment and emphasize the importance of natural dehydration regimes for assessing anhydrobiotic potential.

Introduction

The ability of some organisms to survive extreme dehydration, a condition referred to as anhydrobiosis (Giard, 1894) has been known for nearly 300 years (Van Leeuwenhoek, 1702). Anhydrobiosis was thought to be rare, but in the last 50 years we have become aware that many animal species from quite diverse taxonomic groups seem to possess this remarkable ability. They are able to withstand removal of essentially all their intracellular water without suffering irreversible structural and functional damage (Crowe *et al.* 1987; Womersley, 1981*a*, 1987). Their adaptations include morphological changes and a reduction in metabolic rate, more commonly termed quiescence (Freckman & Womersley, 1983; Womersley, 1981*a*). As the desiccation period persists, some organisms are capable of lowering their metabolism to an undetectable level, entering a state of

Key words: dehydration regimes, anhydrobiosis, *Rotylenchulus reniformis*, tardigrades, rotifers, environment.

cryptobiosis (Womersley, 1981*a*,*b*, 1987). Although both quiescence and cryptobiosis induced by dehydration constitute anhydrobiotic survival, it is the latter state of dormancy – truly 'life without water' – that has evoked so much interest among biologists.

Most (if not all) anhydrobiotes are essentially aquatic. Some inhabit temporary bodies of water that, by virtue of their surrounding environment, are prone to rapid and prolonged periods of drought. Others rely on the levels of hydration found in the terrestrial environment, which can be highly variable and subject to rapid change. For these animals, adaptations to withstand dehydration stress are necessary for survival, their levels of adaptation reflecting the selection pressures operable within their natural habitat (Womersley, 1987).

The most successful anhydrobiotic animals belong to the phylum Nematoda. This is not surprising, considering the diversity of terrestrial habitats that are inhabited by nematodes and that are subject to the effects of partial or extreme dehydration. Womersley (1987) emphasized the importance of assessing the anhydrobiotic potential of any organism in relation to the dynamics of water availability in its natural environment. For example, the failure of some suspected nematode anhydrobiotes to survive experimental protocols appropriate for the induction of anhydrobiosis in organisms particularly well-adapted to rapid dehydration regimes has led to their being classified as having little or no anhydrobiotic potential (Womersley, 1981*b*).

Like many tardigrades and rotifers, these nematodes are soil-dwelling species and, although in some instances they are capable of controlling their rate of evaporative water loss through morphological and behavioural adaptations (Crowe & Madin, 1975; Demeure *et al.* 1979; Evans & Womersley, 1980; Freckman & Womersley, 1983), they cannot survive immediate exposure to extreme dehydration. Thus, as with other organisms inhabiting the upper soil profile (see Womersley, 1987, for review), anhydrobiotic survival is usually favoured by slow drying. But how slow?

Crowe & Madin (1975) first demonstrated the effects of controlled dehydration at elevated relative humidities (97% RH), by successfully inducing anhydrobiosis in mixed larval aggregates of the free-living mycophagous nematode *Aphelenchus avenae*. Adaptations to reduce rate of water loss in this case involved coiling of individual larvae to reduce surface area (Demeure *et al.* 1979; Freckman *et al.* 1977; Womersley, 1978) and mutual protection by clumping (Ellenby, 1969; Freckman & Womersley, 1983). However, even this level of dehydration would seem too stressful for some soil-dwelling nematodes that, as individuals, do exhibit coiling and reportedly can enter anhydrobiosis (Apt, 1976; Cairns, 1954; Womersley, 1981*b*). Presumably, these species (if capable of anhydrobiotic survival) must have adapted to natural rates of dehydration which, in the upper soil profile, are slow and dependent on the physical characteristics of the soil itself (Ekern, 1966; Simons, 1973). This relationship is not confined to nematodes. It can be applied to all organisms inhabiting the upper soil layers and may also be extended to those organisms inhabiting the bottom sediment of temporary ponds. Whether such

close relationship exists between anhydrobiotic survival in slow-dehydration strategists and the rate at which their surrounding substrate dries has yet to be demonstrated.

This study was undertaken to define the conditions necessary for the successful induction of anhydrobiosis in a suspected nematode anhydrobiote inhabiting Hawaiian soils. *Rotylenchulus reniformis* is unique in that development from egg to preadult can proceed without feeding and involves a fully hydrated but quiescent developmental phase between the second-stage larva and the preadult form (Bird, 1984). Only the adult female is parasitic on plant root systems. Both second-stage larvae and preadults have been extracted from dry soil in low numbers (Apt, 1976) and in the coiled form (Tsai & Apt, 1979), although coiling was not thought to be an adaptation towards survival.

In this paper we will show that dehydration of *R. reniformis* at elevated humidity levels does not afford survival. However, the use of model substrates whose drying characteristics mimic those of Hawaiian soil does induce coiling and anhydrobiosis in this nematode, suggesting that the relationship between substrate drying rates and dehydration survival may be the most important factor for anhydrobiotes that have evolved as slow-dehydration strategists.

Materials and methods

Experimental animals

Mixed larval and adult samples of *R. reniformis* were obtained from pineapple field soil by Baermann funnel extraction (Hooper, 1970). Individuals were cleaned by active migration through wetted tissue and collected for experimentation using a glass micropipette. To obtain pure second-stage larvae, nematode cultures were first established on susceptible tomato plants potted into infected soil in clear plastic cups. After 6–8 weeks, infected plant root systems were removed, washed carefully and the egg masses hand-picked with the aid of a dissecting microscope. Egg masses were placed in soil water at room temperature (25 °C) and the second-stage larvae allowed to hatch. Soil water was obtained by flooding potted field soil samples, collecting the percolate overnight, and filtering before use. These larvae were cleaned as before and used in experiments within 24 h of hatching.

Dehydration chambers and experimental protocol

For both direct dehydration of larvae and preadults and dehydration of second-stage larvae in soil or model substrates, glass desiccators were used. All experiments were conducted at room temperature (25 °C). Specific relative humidity (RH) levels were maintained using glycerol/water (Grover & Nicol, 1940) or sulphuric acid/water solutions (Solomon, 1951). Phosphorous pentoxide was used as desiccant for the 0 % RH chambers. For nematodes to be directly dehydrated approximately 50 larvae and preadults were transferred to clean glass slides, excess water was removed (Womersley, 1978), and the slides were placed at

the desired RH for various times. Rehydration was by the immediate addition of soil water to the slides or by allowing the nematodes to rehydrate at 100 % RH for 24 h before adding soil water. Survival was determined by counting the number of actively moving nematodes and the number of inactive nematodes present at hourly intervals during a minimum rehydration period of 7 days. In addition, the vital stain Trypan Blue was added to determine the morphological and physiological viability of active and inactive nematodes. Biological integrity of active nematodes was also assessed by determining their ability to produce viable egg masses after inoculation onto tomato seedling roots. For nematodes desiccated in soil, a known number of second-stage larvae were added to 0·5–1·0 g soil samples contained in glass tubes (0·5 cm i.d. × 3 cm) and sealed at one end with dental wax. Following desiccation, the soil was extracted as before or allowed to rehydrate for 24 h at 100 % RH before extraction. Soil samples were also extracted using the method of Freckman *et al.* (1977) to determine if any coiled individuals were present.

Soil and model substrate drying curves

Because of problems encountered in successfully extracting all nematodes from dry soil samples, it was impossible accurately to quantify the number of individuals entering into anhydrobiosis. To overcome this we decided to define a model substrate or substrates to which nematodes could be added and observed through the dehydration process and that would allow a rate of water loss similar to that of a Hawaiian red clay soil (low humic latosol). To simulate natural drying of the upper layer of Hawaiian soil, 0·5–1·0 g soil samples were placed in tared glass boats, weighed and dried at 0, 69 and 97 % RH. Samples were removed periodically, weighed, and oven-dried at 105 °C for 24 h to give a dry mass comparison for calculation of actual water content. Two differently prepared latosols were analysed. 'Wet' soil represented flooded soil allowed to drain overnight. 'Dry' soil represented oven-dried soil (105 °C/24 h) to which a known quantity of water (approx. 38 % moisture = maximum field capacity) was added before desiccation.

Different substrates, including fine glass beads (80–400, 300–700 μm in diameter) and solutions (0·25–10·0 % w/v) of agar, agarose and Sephadex (50–100, 80–120, 100–300 and 200–400 μm), were analysed for rates of evaporative water loss at 97 % RH and compared to the moisture-loss characteristics observed for soil. 1 ml of each solution was added to preweighed deep well or cavity glass slides. For the glass beads, 0·5 g was added to the wells or cavities and enough water added to fill all interstitial spaces. All slides plus substrate were reweighed and placed at 97 % RH. Replicate slides were weighed at 5-min intervals for the first 6 h and dry masses determined as before. Remaining slides were weighed either hourly for a further 6 h or at 2-, 6-, 8- and 14-day intervals before determination of moisture content. Initial rates of evaporative water loss from soil and model substrates were compared statistically by analysis of covariance.

Desiccation of second-stage larvae on model substrates

Two model substrates, 0·5 % agar and 1·0 % agarose, were chosen as most closely simulating soil in terms of initial rates of evaporative water loss. Approximately 50 freshly hatched active second-stage larvae were transferred to cavity glass slides prepared with these substrates. Surplus water was removed and the slides placed at 97 % RH. Slides were examined daily and nematode morphology, i.e. whether active, C-shaped or coiled, recorded. Once coiling had been maximized, slides were either rehydrated, to determine the level of survival, or placed at lower RH levels for 1 week before being rehydrated. In all experiments, desiccated nematodes were allowed to revive for a minimum period of 7 days. During this period, successful revival was assessed daily as previously described.

Results

Direct dehydration experiments

Mixed nematode samples (larvae and preadults) extracted from infected soil were unable to survive short-term exposure to 97 % RH. Preadults showed little ability to survive, and only a few second-stage larvae revived after 2 h. Subsequent observations on extracted nematodes showed high morphological variability, many larvae and preadults appearing heavily vacuolated. This suggested a depletion of food reserves, which are a necessity for successful induction of slow-dehydration strategists into anhydrobiosis (Demeure *et al.* 1978). This variability was eliminated through the use of second-stage larvae synchronously hatched from egg sacs. Similarly, these results (Fig. 1) demonstrated that unstarved larvae could not survive direct exposure to reduced RH levels. At 90 and 95 % RH decreases in survival were evident after 10 min (as determined by the uptake of the vital stain

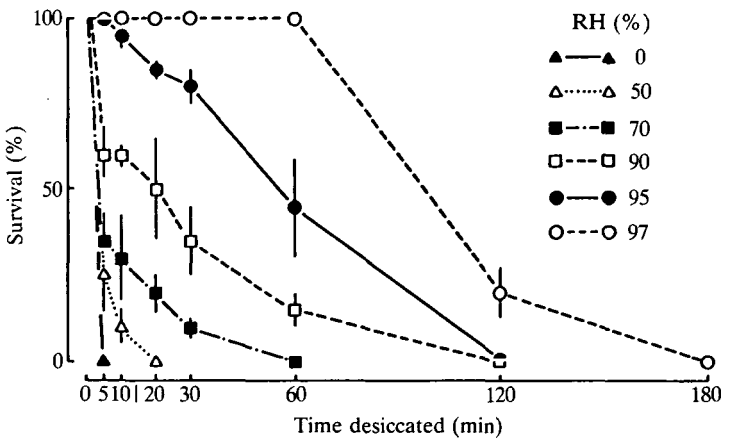

Fig. 1. Percentage survival of second-stage *Rotylenchulus reniformis* larvae following direct desiccation at various relative humidities. ($N = 3$, bars indicate ±S.D.; approximately 20 nematodes per sample.)

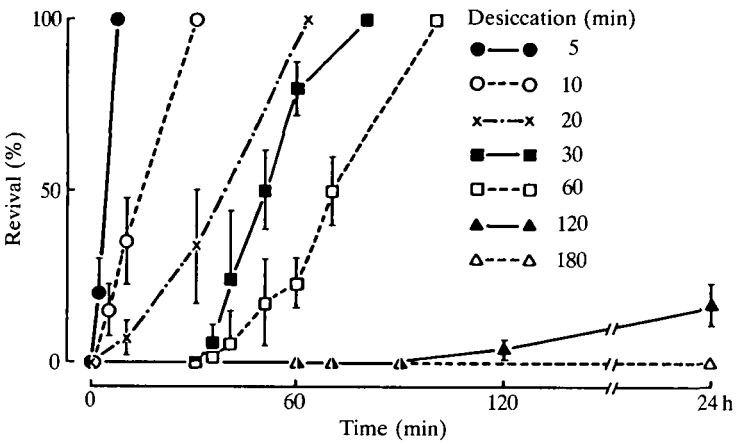

Fig. 2. Time taken to achieve maximum revival of second-stage *Rotylenchulus reniformis* larvae following various intervals of short-term desiccation at 97 % RH. ($N = 3$, bars indicate ± S.D; approximately 20 nematodes per sample.)

Trypan Blue) and all larvae were dead after 3 h of desiccation (Fig. 1). Viability of larvae desiccated at 97 % RH was maintained at 100 % for 1 h, but dropped rapidly after 2 h and no revival was recorded after 3 h. In all short-term dehydration experiments the time needed to achieve maximum revival increased with time desiccated at 97 % RH (Fig. 2). This probably reflects the time taken to replace water lost during desiccation and suggests that, at 97 % RH, the nematodes have no control over this loss. In all short-term experiments, revival rates were the same for larvae exposed to immediate rehydration as for those prehydrated at 100 % RH before the addition of bulk water. Morphologically, none of the second-stage or preadult nematodes subjected to humidities of 97 % or less exhibited any coiling behaviour.

Soil dehydration experiments

Attempts to simulate natural rates of dehydration by adding nematodes to soil of known moisture content and desiccating at 97 % RH were not quantifiable. At best, only 50 % of added individuals were subsequently extracted from the soil, but most of these were live. Formaldehyde extraction of dried soil samples (Freckman *et al.* 1977) to retrieve coiled individuals was moderately successful, but because of the nature of the extraction procedure we were unable to assess their viability. However, these results were encouraging and indicated that to induce coiling and successful entry into anhydrobiosis the rate of drying required by *R. reniformis* had to be far slower than that afforded by direct exposure to 97 % RH.

Moisture release curves

The moisture release curves for both Hawaiian low humic latosol soils (wet an

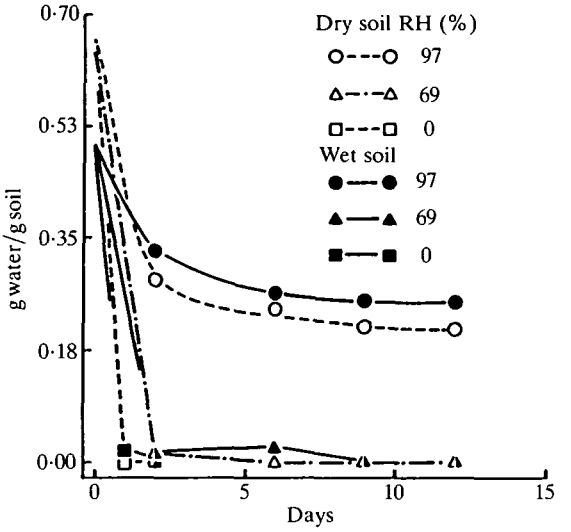

Fig. 3. Moisture characteristic curves for a Hawaiian low humic latosol soil desiccated at various relative humidities (see Materials and methods for definition of wet and dry soil).

dry) show a two-component system (Fig. 3). Initially, pore water evaporated from the interstitial spaces (component 1). Between the fifth and sixth day, moisture contents stabilized at about 15%, the remaining moisture representing water locked into compound particles or aggregates (component 2). These aggregates constitute the soil macrofabric for low humic latosols (Sharma & Uehara, 1968). Differences between the moisture release curves for the two soil models suggested that drying soil before the addition of water caused a partial breakdown of component 2, increasing the overall effect of component 1 on evaporative water loss (Fig. 3). At 0% and 69% RH, water evaporated rapidly from both components of the wet and dry soil models. Because only water in component 1 of the soil is available to the nematode, we concentrated on simulating this rate of water loss in model substrates at 97% RH.

Of the model substrates tested, results with Sephadex were highly variable and water loss from glass bead/water mixtures was rapid. Statistical analysis of initial drying rates (6 h at 97% RH) for wet soil and substrates composed of 0·25–10·0% agar or agarose showed that the slopes of the regression lines for 0·5% agar and 1·0% agarose were the most similar to that of wet soil (Table 1). There was no significant difference in the rates of moisture loss between wet soil and 0·5% agar (Table 1; $P > 0{\cdot}05$). Although 1·0% agarose was significantly different, its water loss characteristics were similar to those of soil and 0·5% agar; both model substrates were therefore used in further studies.

Extended moisture release curves of 0·5% agar and 1·0% agarose compared favourably with that of wet soil (Fig. 4) for the first 48 h. However, unlike in either

Table 1. *Linear regression analyses of and results of* F-*test* (P *value) for comparison of initial rates of moisture loss from soil with that from model substrates at 97% RH*

Substrate	Line of best fit	*F*	r^2	*P* value for *F*-test
Soil	y = 100·26–0·068x	992·46	0·969	
0·5% agar	y = 100·38–0·073x	953·93	0·968	0·0764
1·0% agarose	y = 99·60–0·075x	955·63	0·968	0·0203

y = percentage of moisture left in substrate, x = time.

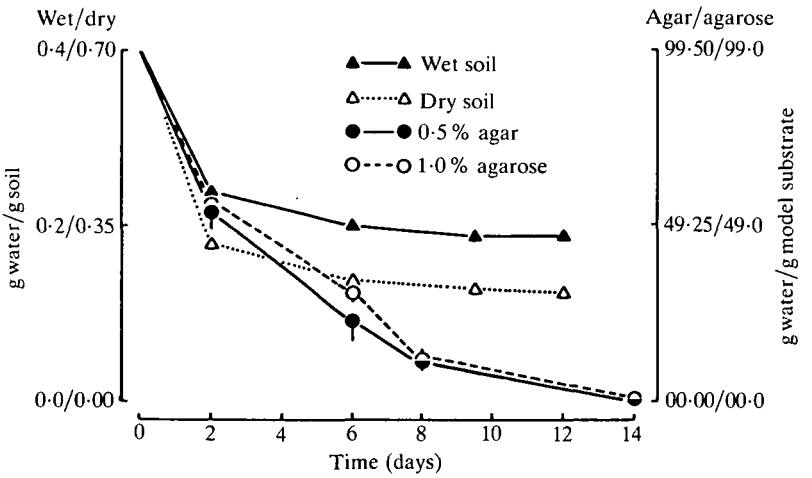

Fig. 4. Comparison of moisture characteristic curves for wet and dry low humic latosols and model substrates.

soil model, moisture loss did not stabilize. Although the loss was apparently constant for 48 h, this slowed and eventually approached 0% after 14 days.

Dehydration of nematodes on model substrates

For second-stage larvae desiccated at 97% RH on 0·5% agar or 1·0% agarose, coiling was maximized after 10–12 days (Fig. 5). Coiling occurred more rapidly in nematodes on 1·0% agarose and was initiated after the third day of desiccation when the larvae migrated to the edge of the substrate or onto the glass slide surface to coil. Nematodes remaining on the substrate rarely coiled, and usually became C-shaped, indicating entry into the hydrated quiescent phase. Conversely, coiling of nematodes desiccated on 0·5% agar occurred mainly on or in the substrate. We are unable to explain these behavioural differences. Subsequent revival of the nematodes after 17 days at 97% RH showed that survival was higher in the agar model (Fig. 6). Results are expressed as percentage survival of coiled nematodes, since in all model substrate experiments only coiled individuals survived. Uncoiled

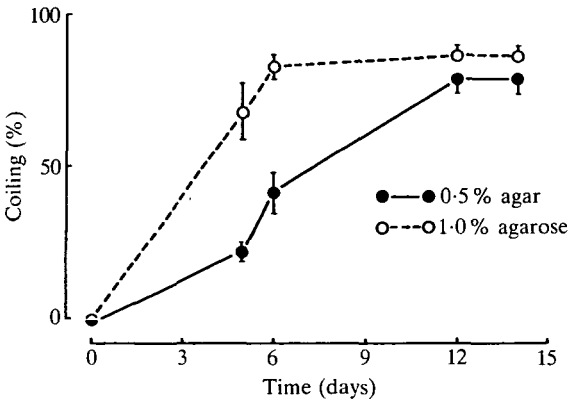

Fig. 5. Time taken to achieve maximum coiling of second-stage *Rotylenchulus reniformis* larvae during desiccation at 97% RH on model substrates. ($N = 3$, bars indicate ±S.D.; approximately 50 nematodes per sample.)

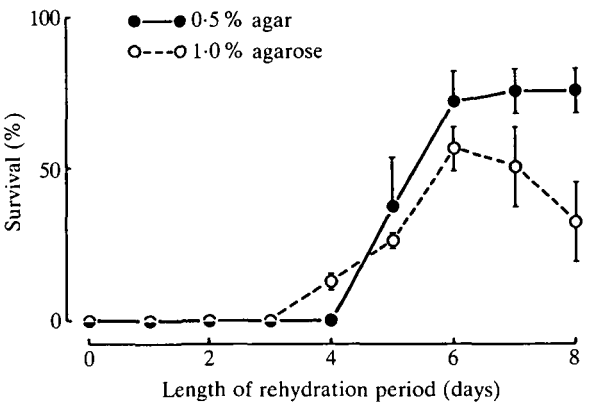

Fig. 6. Percentage survival and length of time necessary to achieve maximum revival of second-stage *Rotylenchulus reniformis* larvae after 17 days at 97% RH on model substrates (results are expressed as percentage survival of coiled nematodes). ($N = 3$, bars indicate ±S.D.; approximately 50 nematodes per sample.)

and C-shaped nematodes readily incorporated Trypan Blue on rehydration, indicating a total loss of viability. Conversely, coiled nematodes did not take up the stain and, once active, were able to develop to the adult stage and successfully produce viable egg masses when reinoculated back onto tomato seedling roots. The time necessary for maximum revival was long, 6 days, and over 80% of the larvae revived were in the preadult form. These results indicated that most nematodes entered the quiescent developmental phase after coiling (all experiments used freshly hatched second-stage larvae). The time required to revive nematodes successfully (determined by active movement) was presumably due to nematodes continuing development in the quiescent phase after rehydration. The

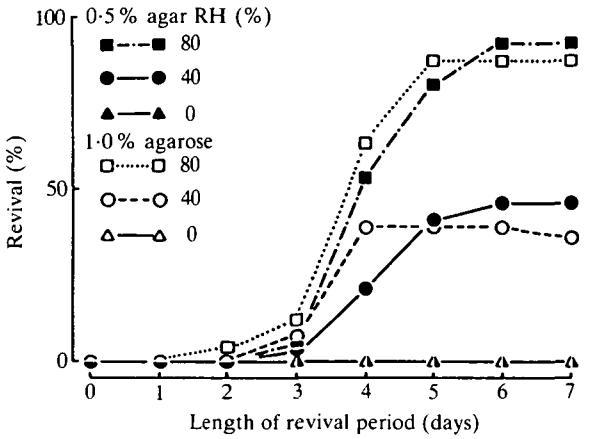

Fig. 7. Percentage survival of second-stage *Rotylenchulus reniformis* larvae after 7 days at 0, 40 or 80 % RH following 14 days at 97 % RH on model substrates. ($N = 1$; approximately 50 nematodes per sample point; results are expressed as percentage survival of coiled nematodes.)

decrease in survival that occurred after the sixth day in nematodes desiccated on 0·1 % agarose (Fig. 6) was due to surviving second-stage larvae either dying or entering into the hydrated quiescent phase. However, none of these larvae successfully developed to the preadult form. Survival of second-stage larvae was not apparent in the agar model. Again, no differences were observed in revival rates between nematodes prehydrated before the addition of bulk water and those subjected to immediate rehydration.

Results of subjecting coiled larvae to reduced humidity levels for 7 days after induction into anhydrobiosis for 14 days at 97 % RH are presented in Fig. 7 and expressed as percentage survival of coiled nematodes. After 7 days at 80 % RH, approximately 90 % of the coiled nematodes revived on both model substrates, indicating successful entry into anhydrobiosis. However, this success rate was reduced to 39 and 46 %, respectively, on 1·0 % agarose and 0·5 % agar after 7 days at 40 % RH (Fig. 7). No coiled larvae survived exposure to 0 % RH for 7 days after induction at 97 % RH.

Discussion

Like most other anhydrobiotes studied, slow dehydration is a prerequisite for the successful induction of anhydrobiosis in *R. reniformis*. However, the required rate of evaporative water loss is much slower than that reported for other anhydrobiotic organisms (Crowe, 1972; Crowe & Madin, 1975; Perry, 1977; Womersley, 1978). The present results show that the larvae and preadults of this nematode are unable to survive even short-term direct exposure to 97 % RH. Conversely, these animals are able to survive in high numbers if subjected to

drying conditions similar to those that occur in native soils. Further, the use of model substrates to simulate natural dehydration regimes demonstrates a distinct relationship between rate of substrate moisture loss and anhydrobiotic survival in this nematode.

The use of model substrates to demonstrate the above relationship has proved very successful in the present work, allowing not only the induction of anhydrobiosis, but also direct observation and measurement of morphological/behavioural adaptations. Even so, it is difficult to assess just how closely our model substrates simulate drying in natural soils. The moisture characteristic curves for wet soil and model substrates in essence represent drying of a bare soil in the absence of a water table. In the natural habitat a water table would be present and, as atmospheric evaporation removes water from the upper soil layers, this would allow a steady flow (through capillary rise) from the ground water source below to the evaporative sink above. Presumably, this would help maintain a high relative humidity (> 97 % RH) in the interstitial spaces of the drying soil. This suggests that, although we were able to induce anhydrobiosis in *R. reniformis*, the actual rates of evaporative water loss experienced by the nematodes may have been higher than those afforded by the natural soil environment.

The relationship between substrate drying rates and anhydrobiotic survival does not apply to all anhydrobiotes. Some live in more exposed environments where slow evaporative water loss can only be achieved using specific physical, behavioural and morphological adaptations (Womersley, 1987). It is these adaptations, or lack of them, that dictate how fast an exposed animal can be dried, the levels of adaptation tending to reflect the different rates of dehydration stress to which the organisms are normally exposed. We can demonstrate the relationship between level of adaptation and rate of evaporative water loss if we consider the anhydrobiotic organisms studied so far with respect to their natural environments.

The upper soil profile is the most stable environment in which anhydrobiotes occur. Organisms inhabiting the middle layers of this region are most often subjected to extremely slow rates of dehydration stress and seem to have developed little resistance to evaporative water loss. They dehydrate individually and appear to be adapted to the moisture-loss characteristics of their surrounding substrate. This is apparently the case for *R. reniformis*. The present work indicated no morphological or behavioural adaptations other than coiling, and this could only be achieved over extended periods, i.e. 9–12 days. The coiling response has been suggested to indicate successful induction into anhydrobiosis (Crowe & Madin, 1975; Demeure *et al.* 1979). Our present results substantiate this view in that only coiled nematodes were able to survive dehydration. This is contrary to the work of Tsai & Apt (1979), who concluded that coiling did not contribute to the anhydrobiotic survival of *R. reniformis*. Even so, the overall effects of coiling alone on rate of evaporative water loss are minimal (Womersley, 1978); thus, it is not surprising that, when removed from their natural environment, individual nematodes are unable to survive direct exposure to 97 % RH and below.

In all instances where direct exposure to 97% RH and below has resulted in successful survival, the organisms concerned have been associated with far more stressful environments than the upper soil profile (i.e. surface layers of the upper soil profile, soil–air interface, plant–air interface, etc.) and have shown a natural propensity for clumping, have been used in clumps, or have possessed specific morphological adaptations which enable them, as individuals, to control their rate of water loss. For example, Crowe (1972) demonstrated that dehydrating tardigrades at high humidity allowed them to undergo an anterior–posterior contraction called a 'tun', which effectively removed areas of high permeability in the cuticle from direct contact with the air. Similar adaptations have been observed for rotifers (Hickernell, 1917). Both these organisms are most often found in the surface layers of the upper soil profile or shallow sediment layers of temporary ponds. There is also evidence to suggest that clumping of rotifers at the sediment–air interface can result in survival when populations are high at the time of drying (Dickson & Mercer, 1967). Our own observations on the chironomid *Polypedilum vanderplanki* indicate that without tube formation in the pond sediment and correct instar folding during dehydration, anhydrobiotic survival is not possible.

Swarming and clumping for mutual protection, like coiling, are natural phenomena amongst free-living nematodes that occupy the upper soil surface layers or soil–air interface and those that are associated with the most extreme anhydrobiotic environments, i.e. within the aerial parts of plants or at the plant–air interface (Ellenby, 1969). In the latter cases, some species enhance slow drying by staying within plant tissue or in parts of the plant modified by the nematodes themselves (Evans & Womersley, 1980; Womersley, 1987). Others coil and clump at the plant–air interface and use specific morphological adaptations that ensure slow drying of individual larvae. For example, Perry (1977) showed that individuals of the stem nematode *Ditylenchus dipsaci* could slow their rate of drying through differential permeability of the cuticle. Not all nematodes in this environment require slow drying. Some die if dried slowly (Hendriksen, 1984; Robinson *et al.* 1984) and tend to require a more rapid rate of dehydration. However this is not the norm.

Crowe & Madin (1975) concluded that slow dehydration allowed the initiation of biochemical adaptations that appear to be a prerequisite for survival in most anhydrobiotes (Evans & Womersley, 1980; Madin & Crowe, 1975; Womersley & Smith, 1981). The preferential storage of specific metabolites has been linked to the maintenance of the structural and functional integrity of biomembranes and biomacromolecules as structural water is removed (Crowe & Crowe, 1982; Crowe *et al.* 1987). The poor survival of *R. reniformis* at 40% RH and lack of survival at 0% RH after induction into anhydrobiosis suggests that it may be incapable or only partially capable of such metabolic adjustments and that it is not as well adapted to anhydrobiotic survival as organisms found in more stressful environments. However, considering the stability of the upper soil profile in terms of rate and extent of dehydration stress, metabolic adaptations may be unnecessary; it is

highly unlikely that *R. reniformis* would ever be subjected to such rigorous dehydration regimes.

Overall, the present work suggests that soil-dwelling anhydrobiotes are poorly adapted to controlling evaporative water loss. For them, the rate at which the surrounding substrate normally dries would appear to dictate how slowly water must be removed to induce anhydrobiosis. Under these conditions individual *R. reniformis* larvae show a level of anhydrobiotic survival that is commensurate with the level of dehydration stress to which they would normally be exposed, suggesting that they are well adapted to this mode of survival within the confines of their natural habitat. However, without dehydration regimes that mimic substrate moisture loss, anhydrobiosis cannot be successfully induced, underlining the importance of this relationship for assessing the anhydrobiotic potential of soil-dwelling organisms under laboratory conditions.

The authors wish to thank Dr Walter Apt and Donna Meyer of the Department of Plant Pathology, UH Manoa, for supplying infected soil and helping with extraction procedures. We would also like to thank Janet Chinen for her taxonomic expertise and help in identification of larval and preadult stages of the nematode. This research was supported by USDA-406, grant no. 85-SR-2-2654 and BRSG 2507RR07026-21.

References

Apt, W. (1976). Survival of reniform nematodes in desiccated soils. *J. Nematology* **8**, 278.

Bird, A. F. (1984). Growth and moulting in nematodes: moulting and development of the hatched larva of *Rotylenchulus reniformis*. *Parasitology* **89**, 107–119.

Cairns, E. J. (1954). Relationship of the environmental moisture conditions of the mushroom spawn nematode, *Ditylenchus* sp., to its control by heat. *Mush. Sci.* **2**, 164–166.

Crowe, J. H. (1972). Evaporative water loss by tardigrades under controlled relative humidities. *Biol. Bull. mar. biol. Lab., Woods Hole* **142**, 407–416.

Crowe, J. H. & Crowe, L. M. (1982). Induction of anhydrobiosis: Membrane changes during drying. *Cryobiology* **19**, 317–328.

Crowe, J. H., Crowe, L. M., Carpenter, J. F. & Wistrom, C. A. (1987). Stabilization of dry phospholipid bilayers and proteins by sugars. *Biochem. J.* **242**, 1–10.

Crowe, J. H. & Madin, K. A. C. (1975). Anhydrobiosis in tardigrades and nematodes: Evaporative water loss and survival. *J. exp. Zool.* **193**, 323–334.

Demeure, Y., Freckman, D. W. & Van Gundy, S. D. (1979). Anhydrobiotic coiling of nematodes in soil. *J. Nematol.* **11**, 189–195.

Demeure, Y., Reversat, G., Van Gundy, S. D. & Freckman, D. N. (1978). The relationship between nematode reserves and their survival to desiccation. *Nematrop.* **8**, 7–8.

Dickson, M. R. & Mercer, E. H. (1967). Fine structural changes accompanying desiccation in *Philodina roseola* (Rotifera). *J. Microscopie* **6**, 331–348.

Ekern, P. C. (1966). Evaporation from bare low humic latosol in Hawaii. *J. appl. Meteorol.* **5**, 431–435.

Ellenby, C. (1969). Dormancy and survival in nematodes. *Symp. Soc. exp. Biol.* **23**, 572–576.

Evans, A. A. F. & Womersley, C. (1980). Longevity and survival in nematodes: Models and mechanisms. In *Nematodes as Biological Models*, vol. 2 (ed. B. M. Zuckerman), pp. 193–211. New York: Academic Press.

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. & WOMERSLEY, C. (1983). Physiological adaptation of nematodes in Chihuahuan desert soils. In *New Trends in Soil Biology* (ed. Ph. Lebrun, H. M. André, A. De Medts, C. Grégoire-Wibo & G. Wauthy), pp. 395–403. Proc. 8th Int. Colloq. Soil Zool. Ottignies-Louvain-la-Neuve: Imprimeur, Dieu-Brichart.

GIARD, A. (1894). L'anhydrobiose en ralentissement des phénomènes vitaux. *Cr. Soc. Biol., Paris* **46**, 497.

GROVER, D. W. & NICOL, J. M. (1940). The vapour pressure of glycerin solutions at 20°. *J. Soc. chem. Ind.* **59**, 175–177.

HENDRIKSEN, N. B. (1984). Anhydrobiosis in nematodes: Studies on *Plectus* sp. In *New Trends in Soil Biology* (ed. Ph. Lebrun, H. M. André, A. De Medts, C. Grégoire-Wibo & G. Wauthy), pp. 387–394. Proc. 8th Int. Colloq. Soil Zool. Ottignies-Louvain-la-Neuve: Imprimeur, Dieu-Brichart.

HICKERNELL, L. M. (1917). A study of dessication in the rotifer *Philodina roseola* with special reference to cytological changes accompanying desiccation. *Biol. Bull. mar. biol. Lab., Woods Hole* **32**, 343–407.

HOOPER, D. J. (1970). Extraction and handling of plant and soil nematodes. In *Laboratory Methods for Work with Plant and Soil Nematodes*, Tech. Bull. II (ed. J. Southey), M.A.F.F., 5th edn. London: HMSO.

MADIN, K. A. C. & CROWE, J. H. (1975). Anhydrobiosis in nematodes: Carbohydrate and lipid metabolism during rehydration. *J. exp. Zool.* **193**, 335–342.

PERRY, R. N. (1977). The water dynamics of stages of *Ditylenchulus dipsaci* and *F. myceliophagus* during desiccation and rehydration. *Parasitology* **75**, 45–70.

ROBINSON, A. F., ORR, C. C. & HEINTZ, C. E. (1984). Some factors affecting the survival of desiccation by infective juveniles of *Orrina phyllobia*. *J. Nematol.* **16**, 86–91.

SHARMA, M. L. & UEHARA, G. (1968). Influence of soil structure on water relations in low humic latosols: water retention. *Proc. Soil. Sci. Soc. Am.* **32**, 765–770.

SIMONS, W. R. (1973). Nematode survival in relation to soil moisture. *Meded. LandbHoogesch. Wageningen* **73**, 1–85.

SOLOMON, M. E. (1951). Control of humidity with potassium hydroxide, sulphuric acid, or other solutions. *Bull. ent. Res.* **42**, 543–554.

TSAI, B. Y. & APT, W. (1979). Anhydrobiosis in the reniform nematode: survival and coiling. *J. Nematol.* **11**, 316.

VAN LEEUWENHOEK, A. (1702). On certain animalcules found in the sediment in gutters of the roofs of houses. In *The Selected Works of Anton Van Leeuwenhoek* (translated by S. Hoole) **2**, 207–213.

WOMERSLEY, C. (1978). A comparison of the rate of drying of four nematode species using a liquid paraffin technique. *Ann. appl. Biol.* **90**, 401–405.

WOMERSLEY, C. (1981*a*). Biochemical and physiological aspects of anhydrobiosis. *Comp. Biochem. Physiol.* **68**A, 249–252.

WOMERSLEY, C. (1981*b*). The effect of myo-inositol on the ability of *Ditylenchus dipsaci*, *D. myceliophagus* and *Anguina tritici* to survive desiccation. *Comp. Biochem. Physiol.* **68**A, 249–252.

WOMERSLEY, C. (1987). A reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. In *Vistas on Nematology* (ed. J. Veech & D. W. Dickson), pp. 165–173. MD: Soc. Nematol. Publ.

WOMERSLEY, C. & SMITH, L. (1981). Anhydrobiosis in nematodes. I. The role of glycerol, myo-inositol and trehalose during desiccation. *Comp. Biochem. Physiol.* **70**B, 579–586.