

Desiccation stress and recovery in the anhydrobiotic nematode *Ditylenchus dipsaci* (Nematoda: Anguinidae)

DAVID A. WHARTON and OTTO AALDERS

Department of Zoology, University of Otago, P.O. Box 56, Dunedin, New Zealand; e-mail: david.wharton@stonebow.otago.ac.nz

Key words. Nematoda, *Ditylenchus dipsaci*, anhydrobiosis, cryptobiosis, lag phase, desiccation, recovery

Abstract. The plant-parasitic nematode *Ditylenchus dipsaci* shows a delay in recovery following a period of desiccation and reimmersion in water. This delay, called the “lag phase”, has been shown to be related to the severity of desiccation. It is the severity of the desiccation stress during dehydration, rather than the final relative humidity to which the animal is exposed which determines the length of the lag phase. A lag phase appears even after a brief exposure to desiccation. These results indicate that a period of repair, or the restoration of a normal physiological state, must be undertaken before activity can resume.

INTRODUCTION

Water is usually considered essential for life. However, some nematodes, and other small invertebrates, can lose all their body water and enter a state of anhydrobiosis, in which their metabolism comes reversibly to a standstill (Barrett, 1991; Crowe et al., 1992). Demonstrating that metabolism has ceased is, however, difficult and here we have assumed that survival at 0% relative humidity (RH) for at least 24 h indicates anhydrobiosis. Desiccation-induced quiescence is initiated by a mild desiccation stress (at a high RH) (Womersley et al., 1998). A slow rate of water loss is thought to be important for anhydrobiotic survival (Evans & Perry, 1976; Higa & Womersley, 1993). This allows the synthesis of trehalose which protects membranes during desiccation and rehydration by preventing membrane fusion and phase changes (Crowe et al., 1992). It also allows the orderly packing of body components, preventing structural damage (Bird & Buttrose, 1974; Crowe et al., 1978; Wharton & Barrett, 1985; Wharton & Lemmon, 1998).

Nematodes can be divided into fast-dehydration and slow-dehydration strategists, reflecting the desiccation stress and rates of water loss they face in their natural habitat (Womersley, 1987). *Ditylenchus dipsaci* is a plant parasitic nematode which infects the aerial parts of plants, where it is likely to be exposed to fast rates of water loss when the plant dies. It is a fast-dehydration strategist and can survive direct exposure to 0% RH (Perry, 1977; Wharton, 1996). During exposure to desiccation there is a decrease in cuticular permeability which decreases the rate of water loss (Wharton, 1996). The nematode is thus able to control its rate of water loss, whereas a slow-dehydration strategist relies on naturally slow rates of water loss from its soil, moss or similar habitat.

When anhydrobiotic nematodes are immersed in water they do not resume activity immediately but undergo a period of apparent inactivity referred to as the “lag period” (Barrett, 1982) or “lag phase” (Wharton et al., 1985). Metabolism, as indicated by heat output, oxygen uptake, or the production of carbon dioxide, begins im-

mediately upon immersion in water (Barrett, 1982). Water content also increases rapidly (Wharton et al., 1985). Why then does activity not start until after a lag phase of 2–3 h? During the lag phase there is an ordered series of morphological changes (Wharton & Barrett, 1985; Wharton et al., 1985). These observations suggest that some, as yet unknown, repair or restoration process occurs during the lag phase before activity can recommence.

The amount of repair to be undertaken might be expected to increase as the severity of desiccation increases. This would increase the time taken to complete the repair and hence the length of the lag phase. In this paper we test the hypothesis that the length of the lag phase increases with increasing severity of desiccation.

MATERIAL AND METHODS

Ditylenchus dipsaci (Kuhn) Filipjev was harvested from infected garlic, which had been stored dry at room temperature. The nematodes were separated from the plant material using a Baermann funnel (Hooper, 1986), washed three times in tap water and transferred to an artificial tap water (ATW: Greenaway, 1970). The nematodes were rehydrated for at least 12 h and the viability (as indicated by 100% moving) was checked before use for experiments. The nematodes from this material consist mainly of 4th-stage larvae (Wharton, 1996).

To expose nematodes to desiccation, a drop of suspension containing approximately 100 nematodes was transferred to a coverslip and the surface water removed using a fine pipette and filter paper. Four such coverslips were prepared for each exposure tested. The coverslips were immediately transferred to a relative humidity (RH) chamber at 20°C. RH chambers consisted of airtight, 700 ml plastic boxes, with the specimens supported above the surface of a saturated salt sludge, or other material, which regulated the RH. The following RHs and salts were used: 98% RH – K₂SO₄, 76% RH – NaCl, 50% RH – an 81.77% (w/w) solution of glycerol in distilled water, 33% RH – MgCl₂·6H₂O, 0% RH – freshly-activated silica gel (Grover & Nicol, 1940; Winston & Bates, 1960).

To determine recovery and length of the lag phase after exposure to desiccation, the coverslip was inverted onto the surface of ATW in a watchglass at room temperature. After a few minutes the nematodes detached from the surface of the coverslip, which was then removed. Recovery was assessed at intervals by

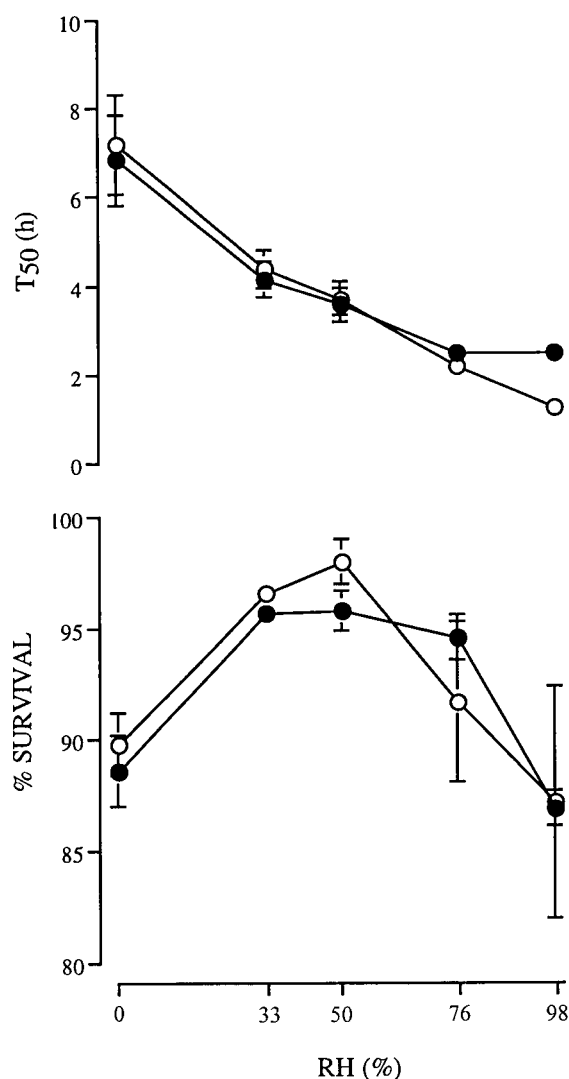


Fig. 1. The effect of relative humidity on the lag phase (top, T_{50} : $N = 32$) and the survival (bottom, $N = 4$) of *D. dipsaci* after exposure for one day (○) or six days (●). Vertical lines represent standard errors.

counting the proportion of nematodes moving, after a mechanical stimulus; which consisted of expelling the nematode suspension from a pipette. The length of the lag phase was determined by calculating the time at which 50% of the nematodes became active (T_{50}), and its standard error, using the methods of probit analysis (Finney, 1952). Where recovery was slow and/or not all nematodes recovered, the specimens were left overnight and the recovery counted after 24 h of rehydration. The use of the 24 h data, however, tended to overestimate the T_{50} . We therefore used time intervals up to and including the time at which the sample reached 90% of its maximum recovery for the T_{50} calculations. This resulted in 8–9 counts of 4 replicate samples, yielding a total count of 32–36 for the T_{50} calculation.

Does the severity of desiccation affect the lag phase and recovery?

Nematodes were exposed to 98%, 76%, 50%, 33% or 0% RH for one or six days. They were then rehydrated in ATW and recovery and the T_{50} determined.

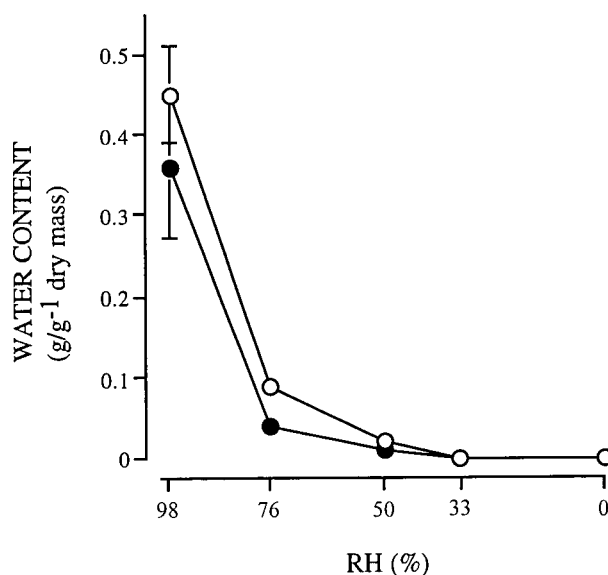


Fig. 2. Water content of *D. dipsaci* after exposure to various relative humidities for one day (○) or six days (●). Vertical lines represent standard errors ($N = 4$).

Water content determinations

The water contents of nematodes exposed to 98%, 76%, 50%, 33% or 0% RH for up to six days was determined gravimetrically as described by (Wharton, 1996).

What is the minimum period of desiccation that induces a lag phase?

Nematodes were exposed to 98% RH for various periods up to 7 days or to 50% RH for short periods of time (2–30 min). They were then rehydrated in ATW and recovery and the T_{50} determined.

Does desiccation at a higher RH affect the lag phase and recovery after exposure to 0% RH?

Nematodes were exposed to 98% RH for one to seven days, followed by one day at 0% RH. Nematodes were also exposed to 0% RH for one to eight days, to give the same total desiccation periods. They were then rehydrated in ATW and recovery and the T_{50} determined.

Nematodes were also exposed to 76%, 50%, 33% and 0% RH for one day, followed by one day at 0% RH. They were then rehydrated in ATW and recovery and the T_{50} determined.

RESULTS

Does the severity of desiccation affect the lag phase and recovery?

The length of the lag phase increased with decreasing RH and hence increasing severity of desiccation for nematodes exposed to desiccation for one or for six days (Fig. 1). The increase in T_{50} with decreasing RH was significant for both one day's (regression analysis: $t = -11.6$, $p < 0.05$) and six days' exposure ($t = -4.88$, $p < 0.05$). There were no significant differences between the T_{50} after one and six days' exposure with the exception of at 98% RH ($t = 3.97$, $p < 0.05$).

Survival after exposure to desiccation was high (>85%) after exposure to all RHs tested and after both one day's and six days' exposure (Fig. 1). Survival was significantly higher at intermediate RHs (factorial ANOVA af-

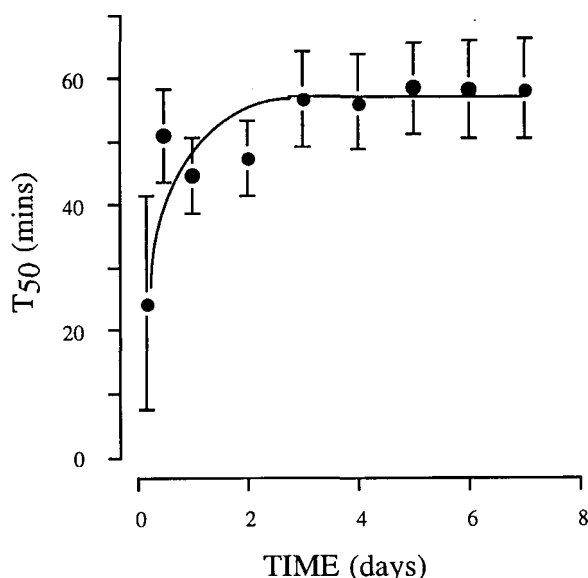


Fig. 3. The effect of period of exposure to 98% RH on the lag phase (T_{50} : $N = 36$) of *D. dipsaci* upon reimmersion in water. Vertical lines represent standard errors.

ter arcsin transformation: $F = 5.41$, $p < 0.05$) but the duration of exposure had no significant effect ($F = 0.99$, $p < 0.05$).

Water content determinations

The nematodes retained water after exposure to 98% and 76% RH but exposure to lower relative humidities resulted in the loss of measurable body water (Fig. 2).

What is the minimum period of desiccation that induces a lag phase?

The lag phase increased with the time of exposure to 98% RH up to a period of three days' exposure (Fig. 3). There was no effect on survival which was high ($> 95\%$) after all periods of exposure.

Nematodes exposed to 50% RH for two or five minutes recovered activity immediately upon immersion in water. A delay in recovery (a lag phase) appeared after 10 min exposure and the length of the lag phase increased with increasing time of exposure to 50% RH (Fig. 4).

Does desiccation at a higher RH affect the lag phase and recovery after exposure to 0% RH?

Desiccation at 98% RH before exposure to 0% RH substantially reduced the lag phase upon recovery compared to nematodes which were exposed to 0% RH directly (Fig. 5). For example the T_{50} of nematodes exposed to 98% RH for one day followed by 0% RH for one day was 1.07 ± 0.11 h, whereas that of nematodes exposed to 0% RH for one day directly was 7.18 ± 1.11 h. The length of the lag phase increased significantly with the time of exposure directly to 0% RH (comparing one and eight days' exposure: $t = -2.29$, $df = 70$, $p < 0.05$), whereas there was no significant increase in the lag phase with increased time of exposure to 98% RH before 1 day at 0% RH (comparing one and seven days at 98% RH: $t = -1.68$, $df = 46$, $p > 0.05$).

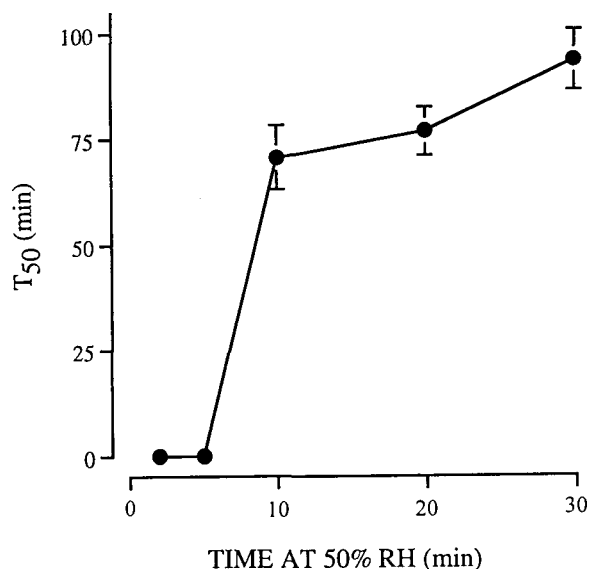


Fig. 4. The effect of period of exposure to 50% RH on the lag phase (T_{50} : $N = 36$) of *D. dipsaci* upon reimmersion in water. Vertical lines represent standard errors.

Survival after exposure to 98% RH followed by 1 day at 0% RH was consistently higher than when exposed to 0% RH directly (Fig. 5). There was an indication that survival declined with the time of exposure to 0% RH but the results were not consistent.

The lag phase increased with a decrease in the relative humidity during the initial exposure to desiccation, before exposure to 0% RH, but there was no clear effect on survival (Fig. 6).

CONCLUSIONS AND DISCUSSION

The length of the lag phase increased as the severity of desiccation increased (i.e. decreased RH). Not all water was lost from nematodes exposed to 98% and 76% RH even after 6 days. This may explain their more rapid recovery compared with those exposed to lower RHs. Nematodes exposed to 50%, 33% or 0% RH, however, had little or no detectable water and yet the length of the lag phase still increased with decreasing RH. Increased length of exposure to desiccation (one or six days) increased the length of the lag phase at 98% RH but there was no significant effect at lower RHs. At 98% RH the length of the lag phase increased to a maximum after 3 days' exposure. Survival was high ($> 85\%$) in all these experiments, although there was some indication that the nematodes survived best at intermediate RHs.

At high RH the desiccation stress would be insufficient to induce a state of anhydrobiosis and the nematodes would be in a state of desiccation-induced quiescence (Womersley et al., 1998). However, a lag phase appears even after a desiccation stress which does not involve the loss of almost all body water. This suggests that even a mild desiccation stress is likely to invoke some of the adaptations involved in anhydrobiotic survival.

Some slow-dehydration strategists show a similar response. The lag phase increases as the severity of desiccation increases in *Panagrolaimus davidi*, a free-living

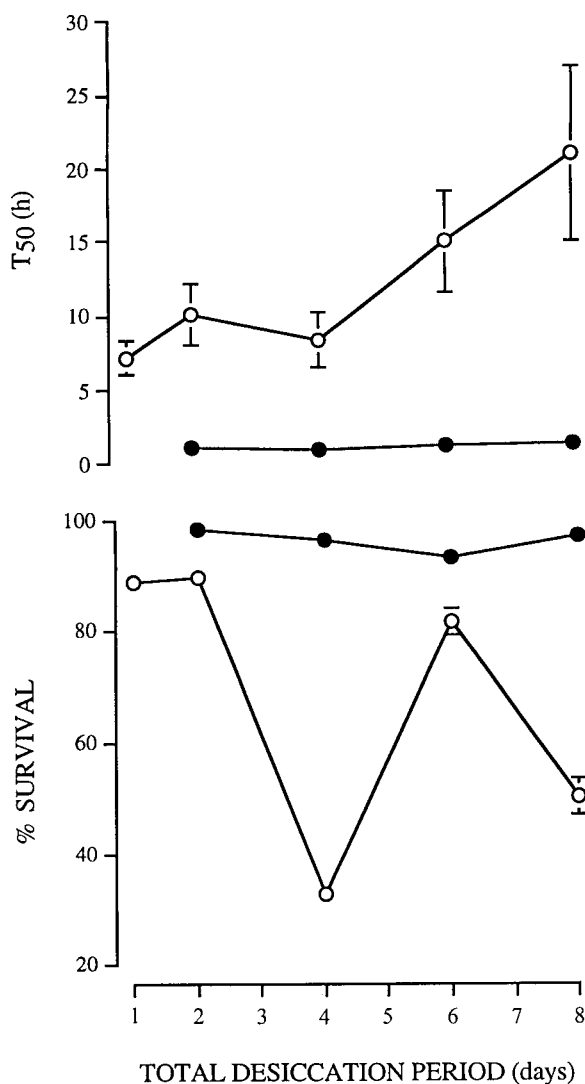


Fig. 5. The effect of period of exposure to 98% RH, followed by 0% RH for one day (●) and of direct exposure to 0% RH for various periods (○) on the lag phase (top, T_{50} : $N = 36$) and survival (bottom, $N = 4$) of *D. dipsaci* upon reimmersion in water. Vertical lines represent standard errors.

nematode (Wharton & Barclay, 1993), and in *Trichostongylus colubriformis*, a free-living infective larva of an animal parasitic nematode (Allan & Wharton, 1990). These slow-dehydration strategists require desiccation at a high RH before they will survive exposure to low RH. Although *D. dipsaci*, a fast-dehydration strategist, will survive immediate exposure to low RH the severity of desiccation influences the time taken to recover upon rehydration.

In *D. dipsaci* the length of the lag phase was substantially reduced if the nematodes were desiccated at a high RH before exposure to 0% RH. It therefore appears that it is the RH during the period of water loss which determines the length of the lag phase, rather than the lowest RH to which the nematode is exposed. This effect occurs after 1 day's exposure to 98% RH and is not affected by further exposure to 98% RH.

Our initial hypothesis, that the length of the lag phase will increase as the severity of desiccation increases (de-

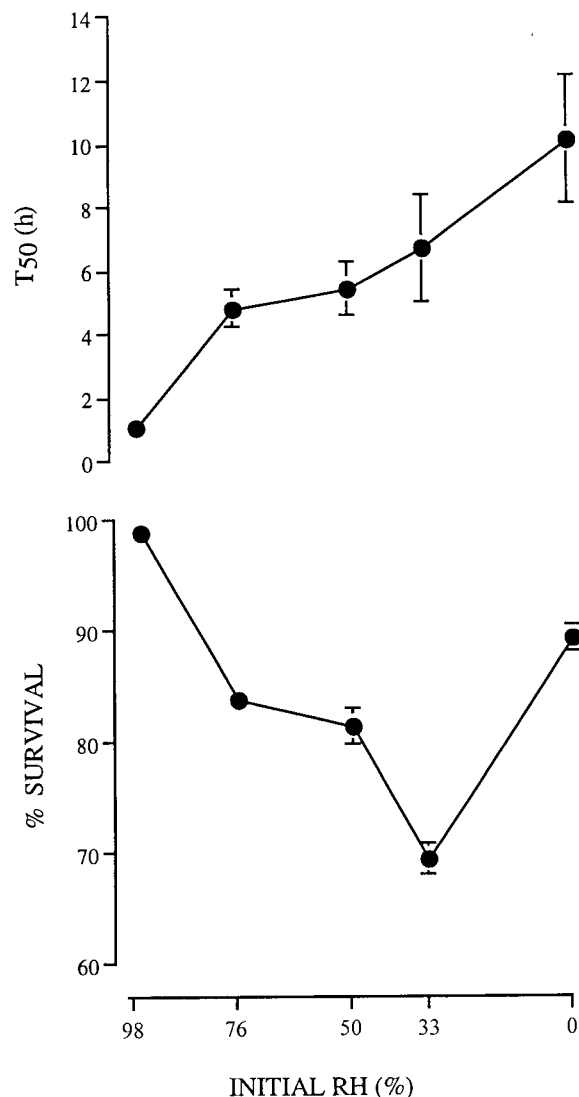


Fig. 6. The effect of the initial RH nematodes were exposed to for one day before exposure to 0% RH for one day, on the lag phase (top, T_{50} : $N = 32$) and survival (bottom, $N = 4$) of *D. dipsaci* upon reimmersion in water. Vertical lines represent standard errors.

creased RH), is confirmed. We have also shown that it is the RH to which the nematodes are exposed while they are losing water, rather than the final RH, which is important in determining the length of the lag phase. This would be related to the rate of water loss and emphasises the importance of a slow rate of water loss in anhydrobiosis (Evans & Perry, 1976; Higa & Womersley, 1993), even for a fast-dehydration strategist.

This provides further evidence that the lag phase represents a period during which damage or disruption sustained during desiccation is repaired and the normal physiological state restored. The amount of damage or disruption would be expected to increase as the rate of water loss during desiccation increased, and hence the time taken to restore the physiological condition of the nematode would also increase. There may also be an accumulation of damage over a long period when anhydrobiotic nematodes are stored dry, due perhaps to oxidation (Barrett, 1982). This would also be expected to be re-

flected in the length of the lag phase, although damage may accumulate to the point where the nematode would not recover.

There is also direct evidence for the operation of repair mechanisms during the lag phase. The cuticle of *D. dipsaci* and *Anguina agrostis* becomes more permeable after desiccation and the cuticular permeability barrier is restored upon rehydration (Preston & Bird, 1987; Wharton, et al., 1988). The restoration of the permeability barrier is sensitive to metabolic inhibitors which block enzyme activity and post-transcriptional protein synthesis (Wharton, et al., 1988), suggesting an active repair mechanism. Nematodes can, however, recover activity in the presence of inhibitors of protein and RNA synthesis (Barrett, 1982). Inorganic ions and primary amines leak from anhydrobiotic specimens of *Aphelenchus avenae* upon rehydration, indicating a loss of membrane integrity or an increase in permeability (Crowe et al., 1979). This leakage ceases during the lag phase, suggesting membrane repair or restoration of permeability barriers. Loss of ions (K^+ , Na^+ , Mg^{++} , Ca^{++}) during rehydration has also been reported from *Anguina tritici* (Womersley, 1981). The termination of leakage could indicate the repair of damaged membranes or a physical change as they take up water which restores their ability to act as a permeability barrier.

A delay in recovery appeared after only 10 min exposure to 50% RH; a period of repair is necessary even after this brief period of desiccation. The nature of the repair mechanisms involved are unknown. Possibilities include membrane repair and the restoration of the ionic gradients which are essential for nerve and muscle function.

ACKNOWLEDGEMENTS. We would like to thank K. Knight for the supply of infected garlic for our initial infections, K. Judge for technical assistance and the Division of Sciences, University of Otago for a Summer Research Bursary.

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Received September 29, 1998; accepted February 19, 1999