Anhydrobiosis in the free-living antarctic nematode *Panagrolaimus davidi* (Nematoda : Rhabditida)

David A. WHARTON and Sharyn BARCLAY

Department of Zoology, University of Otago, PO Box 56,
Dunedin, New Zealand.

Summary – The antarctic nematode *Panagrolaimus davidi* Timm, 1971 can survive exposure to 99 % and 76 % relative humidity but will only survive anhydrobiotically at 0 % relative humidity if it is first dried at a higher relative humidity. Varying the water content of agar films showed an optimum rate of drying for survival but desiccation on agar films did not significantly enhance survival compared with drying from water at 99 % relative humidity. Slow rates of water loss allowed the nematodes to coil during desiccation. Increasing the severity of desiccation increased the lag phase before recovery upon rehydration. *P. davidi* is a slow-rate survivor, relying on slow rates of water loss from its moss habitat.

Résumé – *Anhydrobiose chez le nématode libre antarctique* **Panagrolaimus davidi** *(Nematoda : Rhabditidia)* – Le nématode antarctique *Panagrolaimus davidi* Timm, 1971 peut survivre à une exposition à des humidités relatives de 99 % et 76 %, mais ne peut survivre à l'humidité relative de 0 % qu'à l'état anhydrobiotique et après dessiccation à une humidité relative plus élevée. En faisant varier la teneur en eau de films d'agar, on met en évidence une valeur optimale de dessiccation pour la survie ultérieure, mais cette dessiccation sur film d'agar n'augmente pas significativement la survie en comparaison de la dessiccation en atmosphère aqueuse, à 99 % d'humidité relative. La perte en eau par le nématode étant lente, celui-ci s'enroule durant le processus de dessiccation. L'accroissement de la valeur de la dessiccation allonge la phase de repos précédant la récupération lors de la réhydratation. *P. davidi* est doué d'un pouvoir de survie assez bas, ceci lié aux faibles variations de teneur en eau des mousses qui constituent son habitat.

Key-words : *Panagrolaimus,* nematode, desiccation, anhydrobiosis.

Antarctic terrestrial invertebrates associated with algae, moss and lichens must be capable of withstanding the extreme environmental conditions to which they are exposed. Nematodes are important components of the terrestrial antarctic fauna (Maslen, 1979) and can tolerate exposure to low temperatures (Pickup, 1990*a, b, c;* Wharton & Brown, 1991). In addition to freezing stress, the terrestrial fauna may have to survive exposure to desiccation both during the summer, as a result of low precipitation and humidity, high winds and shallow soils; and during the winter, due to the freezing of available water (Pickup & Rothery, 1991). The survival of antarctic terrestrial invertebrates may be limited more by water availability than by low temperatures (Anon., 1981).

A variety of small invertebrates can lose all their body water and enter into a state of anhydrobiosis, in which their metabolism comes reversibly to a standstill. In nematodes, this ability is found amongst free-living species in sites exposed to desiccation stress (such as soil and moss) and in animal or plant parasitic nematodes which have free-living stages exposed to such stress (Evans & Perry, 1976; Evans & Womersley, 1980; Womersley, 1987). Although anhydrobiosis has been defined in terms of the cessation of metabolism this is difficult to demonstrate in practice (Barrett, 1982). A useful working definition for anhydrobiosis may be : the ability to

survive water loss and direct exposure to 0 % relative humidity. At such low levels of water availability metabolism, or at least normal metabolism, could not be sustained (Clegg, 1978). Upon rehydration anhydrobiotic nematodes do not recommence activity immediately but exhibit a " lag phase " during which hydration and metabolism rapidly return to normal levels but spontaneous activity does not occur for two to three hours (Barrett, 1991).

A restricted range of nematode species have been used as models for the study of anhydrobiosis (Evans & Womersley, 1980). The main species studied have been the infective stages of the plant parasitic nematodes *Ditylenchus dipsaci* and *Anguina tritici,* which can survive immediate exposure to low relative humidity, and the free-living fungivore *Aphelenchus avenae,* which must be dried at high relative humidity before it will survive exposure to more severe desiccation. The survival abilities of the infective juveniles of the animal parasite, *Trichostrongylus colubriformis* Giles, 1892, appear to be intermediate between these two groups (Allan & Wharton, 1990). Womersley (1987) has stressed the importance of exposing species to rates of water loss comparable to those found in their natural environment. The use of severe desiccation regimes in the laboratory has led to some nematodes being classed as desiccation intolerant although they may well have survived if allowed to dry

slowly. The phenomenon of anhydrobiosis in nematodes is clearly much more widespread than has been realised (Wharton, 1986).

Pickup and Rothery (1991) have recently described the desiccation survival abilities of the antarctic nematodes, *Teratocephalus tilbrooki* Maslen, 1979 associated with mosses and a *Ditylenchus* sp. associated with the more exposed branching thalli of lichens of the genus *Usnea*. Both these species are from Signy Island in the maritime antarctic. *Panagrolaimus davidi* Timm, 1971 has been isolated from the more extreme environment of the continental East Antarctic and can be easily cultured in the laboratory (Wharton & Brown, 1989). *P. davidi* may provide a useful comparison with species from the maritime antarctic and for the study of the mechanisms of anhydrobiotic survival in antarctic terrestrial nematodes.

Materials and methods

P. davidi was maintained in culture on agar plates (1 % agar, 0.1 % nutrient broth) at 15 °C and transferred to fresh plates at intervals (Wharton & Brown, 1989). 15 °C is the optimum temperature for culture growth (unpubl.). The nematodes fed on bacteria which grew from the original isolates, these have yet to be identified. Constant relative humidity (R.H.) chambers consisted of air tight, 700 cm³ plastic boxes containing saturated salt sludges (Winston & Bates, 1960). The R.Hs used were : 100 % R.H. (distilled water), 99 % R.H. (potassium sulphate), 76 % R.H. (sodium chloride) and 0 % R.H. (freshly-activated silica gel). The chemicals were spread as a layer at the bottom of the chamber, specimens were supported on wire mesh or plastic platforms and the chambers were maintained at 15 °C.

Exposure to constant relative humidity

Nematodes were dried in watchglasses containing 200 µl of nutrient agar to ensure a slow rate of water loss. Nematodes were washed off the surface of two-week old cultures and the suspension diluted to contain 50-100 per drop (47.5 µl) which were transferred to the watchglasses. Six replicates were kept at each of the R.H. conditions : 100 %, 99 %, 76 %, 0 % R.H. at 15 °C.

Each watchglass was weighed before the addition of agar, after the addition of agar, after the addition of nematodes and at hourly and/or daily intervals during desiccation until there was no further weight loss, and the rate of water loss from the nematodes' environment calculated. At 100 % R.H. no water was lost and these samples were observed when there was no further weight loss from the 99 % R.H. samples. When water loss was complete the number of coiled and uncoiled nematodes were counted. Specimens were considered to be coiled if all the muscles on one side of the body were contracted and the head or tail was in contact with the inside curve of the body.

When water loss was complete the specimens were retained at the test R.H. for a further 24 h. They were then transferred to artificial tap water (ATW : Greenaway, 1970). Recovery was assessed at intervals by counting the proportion of nematodes exhibiting movement after stimulating the specimens by swirling the contents of the watchglass.

Exposure to extreme desiccation at 0 % R.H.

Specimens were dried at 99 % and 76 % R.H., 15 °C and the rate of water loss and coiling determined as above. When water loss was complete the specimens were retained at the desiccating R.H. for a further 24 h and then transferred to 0 % R.H. at 15 °C for 24 h. Recovery after immersion in ATW was assessed as before.

Effect of agar volume on recovery

The volume of agar used for desiccation experiments determines the amount of water to be lost and hence the rate of drying of the specimen. Slow rates of drying may enhance survival but a long drying time (up to 30 days using 200 µl of agar at 99 % R.H.) may adversely affect survival if the nematodes deplete the available nutrients. The amount of water to be lost was varied by modifying the amount of agar and the volume of water added with the nematodes (Table 1). In each case 50-100 nematodes were added to the sample.

Table 1. Conditions for determining the effect of agar volume on recovery.

Agar (µl)	Water + nematodes (µl)	Total water (µl)
200	47.5	245.5
90	11.9	100
40	10.9	50
5	5.1	10
0	10	10

Six replicates of each condition were prepared, transferred to 99 % R.H., 15 °C and weighed at intervals until no further weight loss was recorded. Coiling and drying rates were determined as before. The samples were kept at 99 % R.H. for a further 24 h and then transferred to 0 % R.H., 15 °C for 24 h. Recovery after rehydration in ATW was assessed as before.

Statistical analysis

All percentages were transformed to normalise the data. Arcsin transformation was used before significance testing using *t* tests or ANOVA and square root transformation before regression analyses.

Results

EXPOSURE TO CONSTANT R.H.

The coiling, rate of drying and recovery of *P. davidi* after exposure to various constant R.Hs. is summarised in Table 2. At 100 % R.H. no drying of the agar occured, the nematodes remained motile and there was no coiling. After exposure to 0 % R.H. there was no recovery after immersion in ATW. Coiling and recovery were significantly higher at 99 % R.H. than at 76 % R.H. (t tests : $p < 0.05$).

The rate of recovery after exposure to 99 % R.H. and 76 % R.H. is shown in Fig. 1. There was some recovery in the first hour with an indication of a plateau being reached after 4 h in the 99 % R.H. samples and after 24 h in the 76 % R.H. samples. Immediately after the addition of water the nematodes became transparent and swelled. Some coiled individuals began to straighten. This was presumably a physical effect since these speciments showed no further movement for more than 1 h before normal locomotion commenced.

It appears that the slower the rate of drying, the greater is the extent of coiling and the higher is the recovery from desiccation (Table 2). This was investigated by regression analysis of the data for 99 % R.H. and 76 % R.H. The relationship between coiling and recovery was significant (Fig. 2A : $t = 7.47$, $r^2 = 84.8$, $df = 10$), as was the relationship between log drying and recovery (Fig. 2B : $t = -4.63$, $r^2 = 68.2$, $df = 10$). The graph of coiling against rate of drying suggests a log/log relationship (Fig. 2C : $t = -5.4$, $r^2 = 76.4$, $df = 9$).

RECOVERY FROM EXTREME DESICCATION

The coiling, rate of drying and recovery of *P. davidi* after exposure to extreme desiccation at 0 % R.H., after drying at two constant R.Hs., are summarised in Table 2. During rehydration after exposure to 99/0 % R.H.
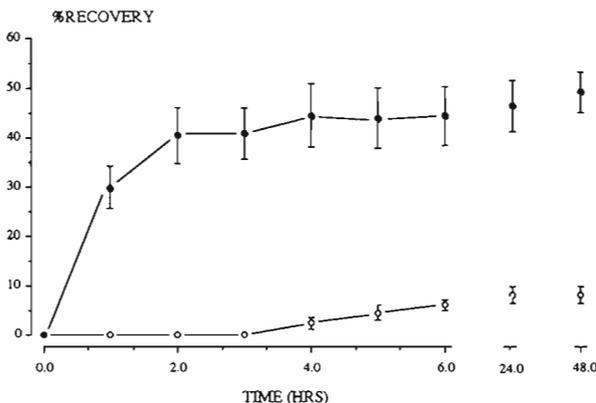
Table 2. The effect of various desiccation regimes on coiling, recovery and rate of drying of *Panagrolaimus davidi*.

% r.h.	% coiled (mean ± S.E.)	Rate of drying %/hour (mean ± S.E.)	Maximum % recovery (mean ± S.E.)	T_{50} (hours)
100	0 ± 0	0 ± 0	100 ± 0	0
99	65.3 ± 5.9	0.3 ± 0.1	89.4 ± 2.7	0.6
76	3.7 ± 1.4	4.3 ± 1.0	65.1 ± 2.6	3.5
0	2.3 ± 1.2	15.8 ± 0.8	0 ± 0	–
99/0	62.3 ± 3.6	0.16 ± 0.01	49.0 ± 3.2	0.75
76/0	5.2 ± 0.8	2.2 ± 0.1	8.3 ± 1.7	4.7

there was some recovery during the first hour after immersion in ATW, rising to a plateau after 4 h. After exposure to 76/0 % R.H. no recovery was observed until 3 h after immersion in ATW, rising to a plateau after 6 h (Fig. 3). Recovery was higher after exposure to 99/0 % R.H. than after 76/0 % R.H. (t test : $p < 0.05$). Recovery was lower after 99/0 % R.H. than 99 % R.H. and after 76/0 % R.H. than 76 % R.H. (t tests : $p < 0.05$). The nematodes did show some recovery after exposure to 0 % R.H. after drying at a higher R.H. whereas drying at 0 % R.H. resulted in no recovery (Table 2).

EFFECT OF AGAR VOLUME ON RECOVERY

Reducing the amount of agar in the sample increased the rate of drying (Table 3). The amount of agar in the sample, and hence the amount of water to be lost and the rate of drying, had a significant effect on the recovery after 24 h rehydration (ANOVA : $p < 0.05$). For specimens in agar, there was an optimum rate of drying (Fig. 4). Specimens dried from water with no agar had the highest rate of drying but did not have the lowest rate of revival (Table 3). There were, however, no significant differences in revival between specimens dried from water and those dried from the various concentrations of agar (Fisher PLSD : $p > 0.05$), indicating that

Fig. 1. Recovery with time of *Panagrolaimus davidi* during rehydration after desiccation on agar films at 99 % R.H. (●) and 76 % R.H. (○).

Table 3. The effect of sample agar volume on coiling, recovery and rate of drying of *Panagrolaimus davidi*.

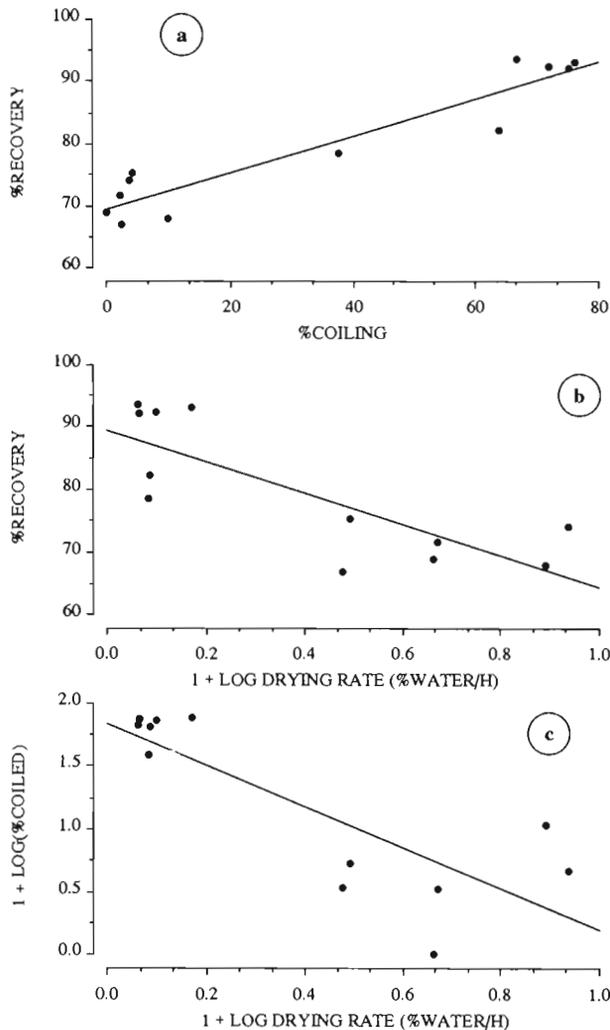| Agar μl	% coiled (mean ± S.E.)	Rate of drying %/hour (mean ± S.E.)	% recovery at 24 h (mean ± S.E.)
200	62.3 ± 3.6	0.16 ± 0.01	46.2 ± 5.2
90	53.7 ± 4.3	0.22 ± 0.01	54.4 ± 3.9
40	40.4 ± 3.6	0.33 ± 0.01	63.8 ± 6.2
5	41.1 ± 3.3	0.61 ± 0.02	43.3 ± 2.9
0	50.3 ± 2.7	1.1 ± 0.01	54.4 ± 2.6

Fig. 2. *Panagrolaimus davidi* after desiccation on agar films at 99 % or 76 % R.H.; showing the relation between coiling and recovery (A) and between the rate of drying of the agar and recovery (B) and coiling (C).

potential starvation effects during long periods of drying did not affect survival.

THE EFFECT OF DESICCATION ON THE LAG PHASE

This was determined by estimating, from graphs, the time at which 50 % of the nematodes which were going to recover, recovered during rehydration (T_{50}). Increasing the severity of the desiccation regime, increased the lag phase and decreased recovery (Table 2).

Discussion

Desiccation-tolerant nematodes can be divided into those which survive a moderate degree of desiccation stress and enter into a state of desiccation-induced quiescence, and those which survive extreme desiccation,
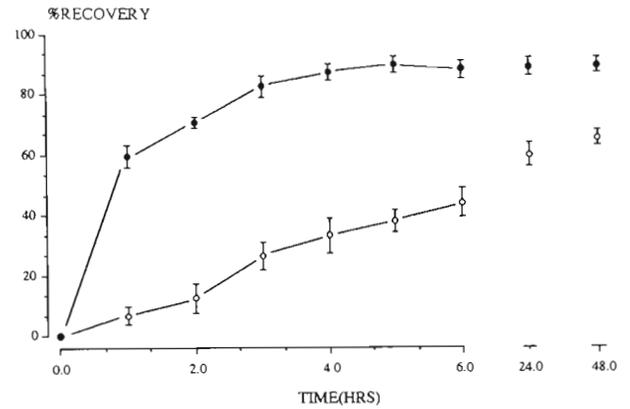

Fig. 3. Recovery with time of *Panagrolaimus davidi* during rehydration after exposure to 0 % R.H. following desiccation on agar films at 99 % R.H. (●) and 76 % R.H. (○).

that is exposure to 0 % R.H., and enter into a state of anhydrobiosis. By definition, anhydrobiosis (" life without water ") could not be applied to nematodes such as *Rotylenchulus reniformis* which do not survive exposure to 0 % R.H. (Womersley & Ching, 1989) and can not therefore survive the loss of all free water. Pellets of *A. avenae* exposed to 80 % R.H. contain 28-34 % water (Crowe & Madin, 1975).

Anhydrobiotic nematodes fall into two groups : those that survive direct exposure to low relative humidity (mainly plant parasites) and those that survive low relative humidity only after drying at a high relative humidity or after drying at slow rates of water loss in their natural habitat (such as moss or soil), or in artificial substrates which have similar drying characteristics
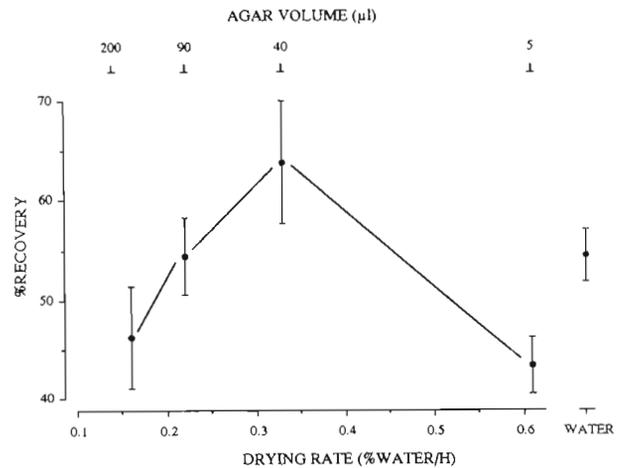

Fig. 4. The effect of drying rate on recovery of *Panagrolaimus davidi* after 24 h rehydration following desiccation from water or in various concentrations of agar, and exposure to 0 % R.H.

(such as agar : Womersley & Ching, 1989). Although it is difficult to assess the conditions of desiccation in an agar film, such substrates may produce slower rates of drying or less abrupt exposure to desiccation and thus more closely mimic drying conditions in the nematode's natural environment than does direct exposure to lowered relative humidity.

Womersley (1987) has suggested that the abilities of nematodes to withstand desiccation stress is related to the stresses normally experienced in their environment. Fast-rate survivors are thus adapted to survive high rates of water loss from their environment in the aerial parts of plants; such as lichen fruiting bodies, drying plant stems and foliage. Slow-rate survivors rely on slow rates of water loss from their natural environment.

P. davidi can not survive direct exposure to 0 % R.H. but will survive after drying at a high relative humidity. Drying on agar films did not significantly enhance survival. There was no obvious indication that a particular stage in the life cycle was especially resistant but this was not investigated in detail. *T. tilbrooki* shows little ability to control its rate of water loss (Pickup & Rothery, 1991) and may also require desiccation at a high relative humidity before it can survive anhydrobiotically. A *Ditylenchus* sp., however, can control its rate of water loss and survive direct exposure to 0 % R.H.; although survival is enhanced if it is desiccated at a higher relative humidity (Pickup & Rothery, 1991). *P. davidi* and *T. tilbrooki* are thus slow rate survivors relying on slow rates of water loss from their moss habitats. *Ditylenchus* sp. is a fast rate survivor and is adapted to survive the high rates of water loss likely to be experienced in its exposed lichen habitat.

The role of coiling in nematode desiccation survival is unclear. In *P. davidi* coiling is related to a slow rate of drying and higher survival. In *Rotylenchulus reniformis* only coiled nematodes survive desiccation (Womersley & Ching, 1989). Coiling is usually considered to reduce the rate of water loss by reducing the surface area exposed to desiccation. Coiling on its own, however, does not appear to have a significant effect on the rate of water loss (Womersley, 1978).

The time taken for *P. davidi* to recover from desiccation stress upon rehydration (" the lag phase ") increases with the severity of desiccation. A series of morphological and physiological changes occur during the lag phase following the rehydration of anhydrobiotic *Ditylenchus dipsaci* (Wharton *et al.*, 1985, 1988; Wharton & Barrett, 1985). Changes in the dimensions of the muscle cells and the permeability of the cuticle indicate the repair and recovery of normal physiological function in anhydrobiotic nematodes. The amount of repair to be undertaken, and hence the length of the lag phase, might be expected to be related to the degree of stress and damage which occurs during desiccation.

P. davidi can survive both low temperatures (Wharton & Brown, 1991) and desiccation. It is thus well adapted to survive the extreme environmental conditions of its terrestrial antarctic environment.

References

ALLAN, G. S. & WHARTON, D. A. (1990). Anhydrobiosis in the infective juveniles of *Trichostrongylus colubriformis* (Nematoda : Trichostrongylidae). *Int. J. Parasit.*, 20 : 183-192.

ANON. (1981). Terrestrial biology. *British Antarctic Survey Annual Report, 1980-1981 :* 48-59.

BARRETT, J. (1982). Metabolic responses to anabiosis in the fourth stage juveniles of *Ditylenchus dipsaci* (Nematoda). *Proc. R. Soc. London,* B 216 : 159-177.

BARRETT, J. (1991). Anhydrobiotic nematodes. *Agric. Zool. Rev.*, 4 : 161-176.

CLEGG, J. S. (1978). Hydration-dependent metabolic transitions and the state of cellular water in *Artemia* cysts. *In :* Crowe, J. H. & Clegg, J. S. (Eds). *Dry biological systems.* New York, Academic Press : 117-153.

CROWE, J. H. & MADIN, K. A. C. (1975). Anhydrobiosis in nematodes : evaporative water loss and survival. *J. exp. Zool.*, 193 : 323-334.

EVANS, A. A. F. & PERRY, R. N. (1976). Survival strategies in nematodes. *In :* Croll, N. A. (Ed.). *The organisation of nematodes.* New York & San Francisco, Academic Press : 383-424.

EVANS, A. A. F. & WOMERSLEY, C. (1980). Longevity and survival in nematodes : models and mechanisms. *In :* Zuckerman, B. M. (Ed.). *Nematodes as biological models. Vol. 2.* New York & London, Academic Press : 193-211.

GREENAWAY, P. (1970). Sodium regulation in the freshwater mollusc *Limnea stagnalis* (L) (Gastropoda, Pulmonata). *J. exp. Biol.*, 53 : 147-163.

MASLEN, N. R. (1979). Additions to the nematode fauna of the antarctic region with keys to taxa. *Br. Antarctic Surv. Bull.*, 49 : 207-229.

PICKUP, J. (1990a). Seasonal variation in the cold-hardiness of a free-living predatory nematode, *Coomansus gerlachei* (Mononchidae). *Polar Biol.*, 10 : 307-315.

PICKUP, J. (1990b). Seasonal variation in the cold hardiness of three species of free-living antarctic nematodes. *Funct. Ecol.*, 4 : 257-264.

PICKUP, J. (1990c). Strategies of cold-hardiness in three species of antarctic dorylaimid nematodes. *J. comp. Physiol.*, B 160 : 167-173.

PICKUP, J. & ROTHERY, P. (1991). Water-loss and anhydrobiotic survival in nematodes of Antarctic fellfields. *Oikos*, 61 : 379-388.

WHARTON, D. A. (1986). *A functional biology of nematodes.* London & Sydney, Croom Helm, v + 192 p.

WHARTON, D. A. & BARRETT, J. (1985). Ultrastructural changes during recovery from anabiosis in the plant parasitic nematode, *Ditylenchus. Tissue & Cell,* 17 : 79-96.

WHARTON, D. A. & BARRETT, J. & PERRY, R. N. (1985). Water uptake and morphological changes during recovery from anabiosis in the plant parasitic nematode, *Ditylenchus dipsaci. J. Zool., London,* 206 : 391-402.

WHARTON, D. A. & BROWN, I. M. (1989). A survey of terrestrial nematodes from the McMurdo Sound region, Antarctica. *N. Z. J. Zool.,* 16 : 467-470.

WHARTON, D. A. & BROWN, I. M. (1991). Cold tolerance mechanisms of the antarctic nematode *Panagrolaimus davidi. J. exp. Biol.,* 155 : 629-641.

WHARTON, D. A., PRESTON, C. M., BARRETT, J. & PERRY, R. N. (1988). Changes in cuticular permeability associated with recovery from anhydrobiosis in the plant parasitic nematode, *Ditylenchus dipsaci. Parasitology,* 97 : 317-330.

WINSTON, P. W. & BATES, D. H. (1960). Saturated solutions for the control of humidity in biological research. *Ecology,* 41 : 232-237.

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. (1987). A reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. *In :* Veech, J. A. & Dickson, D. W. (Eds). *Vistas on Nematology.* Hyattsville, MD, USA, Society of Nematologists Inc. : 165-173.

WOMERSLEY C. & CHING, C. (1989). Natural dehydration regimes as a prerequisite for the successful induction of anhydrobiosis in the nematode *Rotylenchulus reniformis. J. exp. Biol.,* 143 : 359-372.