Desiccation survival of parasitic nematodes

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SUMMARY

The ability of certain species of parasitic nematodes to survive desiccation for considerable periods is a fascinating example of adaptation to the demands of fluctuating environments that occasionally can become extreme and life threatening. Behavioural and morphological adaptations associated with desiccation survival serve primarily to reduce the rate of drying, either to prolong the time taken for the nematode's water content to reach lethal low levels or, in true anhydrobiotes, to enable the structural and biochemical changes required for long-term survival to take place. Examples of these adaptations are reviewed, together with information on the factors involved in rehydration that ensure successful exit from the dormant state. Information on desiccation survival is central to effective management and control options for parasitic nematodes. It is also required to assess the feasibility of enhancing the longevity of commercial formulations of entomopathogenic nematodes, both before and after application; current research and future prospects for enhancing survival of these bio-insecticides are discussed.

Key words: Anhydrobiosis, desiccation, nematodes, survival.

INTRODUCTION

Two types of adaptation, defined originally for survival of temperature stress (Precht, 1958; Cossins & Bowler, 1987), are capacity adaptation and resistance adaptation. Capacity adaptation enables an organism to grow and reproduce under harsh environmental conditions that differ markedly from conditions required by the majority of species for continuation of their life-cycles. Resistance adaptation enables an organism to suspend development and survive environmental extremes until favourable conditions return, when growth and development can re-commence. Among species of nematodes, capacity adaptation is demonstrated by certain free-living species living in extreme environments, such as deserts or the terrestrial Antarctic, whereas resistance adaptation is an attribute of some parasitic and free-living forms that ensures survival in a fluctuating environment. For many parasitic nematodes, the requirement to persist in the absence of a host also necessitates survival of unfavourable conditions. The associated behavioural, morphological and biochemical mechanisms to withstand environmental extremes, often features of specific stages in the nematode life-cycle, contribute to the survival strategy of each species.

One aspect of nematode survival, the ability to withstand desiccation for periods considerably in excess of the duration of the normal life-cycle, has generated much research interest. In part, this has been engendered by the historical fascination of the ability, rare in the animal kingdom, to recover from extreme body-water loss. However, the effectiveness of management and control options for parasitic nematodes is often conditional on an understanding of the temporal factors involved in survival; this has also underpinned research which aims to understand the mechanisms of survival with the long-term aim of assessing the feasibility of disrupting dormancy and improving nematode control, especially of plant-parasitic species. More recently, the use of entomopathogenic nematodes as an environmentally acceptable method of controlling economically important insect pests has resulted in research aimed at not only enhancing the longevity of the nematodes in commercial formulations but also increasing survival after application (Glazer *et al.* 1999).

TERMINOLOGY

Dormancy can occur at most stages of the nematode's life. Keilin (1959) separated dormancy, involving lowered metabolism, from cryptobiosis, where no metabolism could be detected. In practice, it is frequently difficult to separate quiescence from cryptobiosis and many authors have used the term cryptobiosis in an arbitrary sense to indicate long-term survival, usually for years, of adverse conditions. Evans & Perry (1976) considered that the fundamental criterion for separating categories within dormancy should be the cause of arrest in development, rather than metabolic state, and considered that cryptobiosis should be viewed as the same kind of phenomenon as quiescence. Subsequently, Evans (1987) distinguished between dormancy affecting ontogenetic development and that affecting somatic development.

Dormancy is usually subdivided into two categories, diapause and quiescence. Diapause is a state of arrested development whereby development

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does not occur until specific requirements have been satisfied, even if favourable conditions return. Quiescence is a spontaneous reversible response to unpredictable unfavourable environmental conditions and release from quiescence occurs when favourable conditions return. Adverse environmental conditions and the states they induce include cooling (cryobiosis), high temperatures (thermobiosis), lack of oxygen (anoxybiosis), osmotic stress (osmobiosis) and the subject of this review, dehydration (anhydrobiosis). These divisions are somewhat artificial. Nematodes are essentially aquatic organisms but many of the environmental stresses involve removal or immobilization of water. Desiccation concentrates body solutes and increases internal osmotic stress, which also may influence the rate of water loss. Exposure to hyperosmotic conditions causes partial dehydration of a nematode. Freezing may involve dehydration through sublimation of water from the solid phase (Wharton, this volume). Thus, it is unsurprising that the ability to survive one type of stress is frequently associated with increased resistance to others and nematodes exhibit similar behavioural, structural and physiological mechanisms to enhance their survival of different conditions.

OVERVIEW OF ANHYDROBIOSIS IN PARASITIC NEMATODES

Soil-dwelling nematodes are protected by the soil from extremes of moisture loss. Even when the soil water potential falls below $-1{\cdot}0$ MPa, the relative humidity in soil pores is still above 99% (Stirling, 1991). Species of plant ecto- and endoparasitic nematodes that attack roots growing in the upper and lower soil profiles may avoid a decrease in the availability of water in the upper layers by moving downwards in the soil. Other plant-parasitic species locate and invade roots of their recently germinated hosts near the soil surface, and some species climb plants when water films cover them. In general, soil-dwelling stages of animal-parasitic nematodes remain on the soil surface or associated vegetation until ingested by their hosts. Entomopathogenic nematodes move through the upper layers of the soil in search of prey or wait on the soil surface for the host to pass. Although some plant-parasitic nematodes, such as *Globodera rostochiensis*, that inhabit deep soil have mechanisms to endure desiccation, species, such as *Ditylenchus dipsaci*, inhabiting aerial parts of plants demonstrate the most spectacular intrinsic abilities to withstand severe desiccation. The ability to survive desiccation is often commensurate with a dispersal phase of the life-cycle and such dormant stages are frequently formed in response to food shortage.

It is difficult to compare the results of different workers on the survival attributes of various species of parasitic nematodes because the experimental conditions are rarely comparable. Only general conclusions can be drawn. In most examples of anhydrobiotic survival in nematodes, the individuals involved depend on mechanisms to reduce their rate of evaporative water loss. The majority of free-living stages of animal- and plant-parasitic nematodes show little intrinsic ability to control water loss and survive desiccation, being dependent on the environmental conditions of high relative humidity within soil pores or plant material to slow down or prevent water loss. Womersley (1987) grouped nematode anhydrobiotes into slow-dehydration and fast-dehydration strategists based on the water loss dynamics commensurate with survival. Slow-dehydration strategists depend on surrounding environmental conditions to control water loss and variations in the rate at which water is removed from a given nematode species and stage depend largely on the moisture loss characteristics of the soil in which it lives (Womersley, Wharton & Higa, 1998). Physical and behavioural adaptations to control the rate of water loss are frequently associated with fast-dehydration strategists such as the infective fourth stage larvae (L_4s) of *D. dipsaci* (Perry, 1977*a*, *b*).

Research into the mechanisms involved in anhydrobiotic survival has focused on three main areas: (1) behavioural and physical attributes that enhance survival, primarily associated with control of water loss from the nematode; (2) biochemical adaptations that maintain functional and structural integrity at the cellular level; and (3) the importance of rehydration in relation to metabolic and morphological changes required for successful revival. These aspects will be discussed using selected examples of animal- and plant-parasitic nematodes whose direct life-cycles involve survival outside the host.

BEHAVIOURAL RESPONSES THAT ENHANCE SURVIVAL

The two frequently quoted behavioural responses by some species of nematodes to removal of water are coiling and clumping. Coiling reduces the surface area of the nematode that is exposed to drying conditions and there is some experimental evidence to show that coiling reduces the rate of water loss of *D. myceliophagus* (Womersley, 1978). When exposed to desiccation, the infective larvae (L_3) of *Trichostrongylus colubriformis* form tight coils (Wharton, 1981). In the semi-endoparasitic nematode, *Rotylenchulus reniformis*, a direct relationship between coiling and anhydrobiotic survival has been shown (Womersley & Ching, 1989) but neither coiling nor the retention of moulted cuticles as sheaths (see below) enable this species to survive long-term dehydration. Womersley *et al.* (1998) considered that, although coiling may be an indicator of

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successful induction of anhydrobiosis, it cannot be used to distinguish between quiescent and cryptobiotic forms.

The classic image of anhydrobiotic nematodes is clumps of coiled, desiccated L_4s of *D. dipsaci*, yet only a very few species aggregate in this way. Although L_4s of *D. dipsaci* can survive extreme desiccation for long periods (see below), dry conditions rarely last long in the soil and the aggregations of dried L_4s, termed 'eelworm wool', are more usually associated with infected bulbs (Ellenby, 1969) or inside bean pods (Hooper, 1971). In infected narcissus bulbs, set out to dry at the end of the growing season, development is arrested at the L_4 stage and hundreds of L_4s issue from the basal plate and the lower end of the bulb scales and aggregate before drying. The death rate is greater on the outside of the aggregations and there is some evidence (Ellenby, 1969) that the outer fragments are drier. The death of the peripheral L_4s apparently provides a protective coat which aids survival of the L_4s in the centre of the 'wool' by slowing their rate of drying: the so-called 'eggshell' effect (Ellenby, 1968*a*). Thus, the outer layers perform the same function as physical structures discussed in the next section.

In other species of plant-parasitic nematodes, aggregations occur under natural conditions within modified plant tissue such as galls. *Anguina amsinckia* and *A. tritici* are examples of species that induce galls in the host inflorescence. Within the galls induced by *A. amsinckia* are hundreds of desiccated adults and larvae of all stages, many of which are coiled. However, not all nematodes need to coil to survive drying. The galls induced by *A. tritici* contain tightly packed aggregates of second stage larvae (L_2s) only, each of which remains uncoiled when dry. L_2s of *A. tritici* can survive severe desiccation as individuals (Ellenby, 1969), so the combination of this intrinsic ability and the behavioural adaptation of clumping, plus the protection of the gall tissue, enables L_2s to survive many years in the dry state.

Mass movement or swarming is found in mycophagous nematodes such as *D. myceliophagus* and *Aphelenchus avenae*, probably in response to lack of food or toxic products from decaying hosts. Swarming leads to aggregation and coiling during subsequent dehydration in the absence of a host but, in these examples, aggregation is not a behavioural response to desiccation *per se* (Womersley *et al.* 1998).

PHYSICAL ATTRIBUTES THAT ENHANCE SURVIVAL

Protection from environmental extremes is afforded by physical structures such as moulted cuticles, which are retained as protective sheaths, eggshells and resistant cuticles. One main function of such physical attributes is to control the rate of water loss. However, as will become clear, the ability to control the rate of water loss does not, by itself, ensure survival.

Whether in soil or plant tissue, the majority of animal- and plant-parasitic nematodes experience dehydration stress as individuals and *in vitro* experiments aiming to examine survival attributes of species do not reflect the natural situation if percentage survival is assessed using clumps of nematodes, particularly as percentage survival is influenced by the size of the aggregation (Ellenby, 1969). However, care should be taken in extrapolating from the results of laboratory-based experiments, which examine the mechanisms involved in anhydrobiotic survival, to survival under field conditions. Frequently such experiments are interpreted as indicating considerably enhanced survival attributes, but the interpretations are not borne out by field data. In part, this may be due to the interaction of factors prevalent in natural environmental conditions. The development of *Ascaris* species occurs within the egg and the infective stage is protected by the eggshell until ingestion by the host. When exposed to desiccation, the eggs of several animal-parasitic nematodes lose water very slowly and the eggshell has been implicated in enabling the unhatched larvae to survive desiccation, the lipid layer providing the main permeability barrier to water loss (Wharton, 1980). However, the rate of water loss of unhatched larvae increases as an exponential function of increasing temperature (Wharton, 1979) and, although *Ascaris* eggs lose water very slowly relative to their surface-volume ratio (Wharton, 1979), they are sensitive to desiccation in the long term and egg mortality due to dehydration has been claimed to be responsible for the complete lack of transmission of *A. suum* under intensive indoor production systems (Roepstorff, 1997). On grass plots, high temperature in combination with severe dehydration in faecal samples may have contributed to the large mortality of *A. suum* (Larsen & Roepstorff, 1999).

Females of the plant-parasitic cyst nematodes become spherical (e.g. *Globodera* spp.) or lemon-shaped (*Heterodera* spp.) and, after death of the fertilized female, the cuticle becomes tanned to form a tough, brown cyst containing 100–500 eggs, each one containing a tightly coiled infective L_2. When exposed to desiccation, the permeability characteristics of the surface layers of the cyst wall of *G. rostochiensis* change as they dry faster than the rate at which water can be replaced from within the cyst, resulting in an effective barrier to further water loss (Ellenby, 1946). The eggshell also becomes differentially permeable as it dries resulting in a reduced rate of water loss of unhatched L_2s compared with free L_2s (Ellenby, 1968*a*). Ultimately, the unhatched L_2

becomes as dry as the hatched L_2 yet the former survives but the latter perishes; the rate of water loss is a decisive survival factor. The susceptibility of hatched L_2s of *G. rostochiensis* to environmental extremes is offset by a sophisticated host-parasite interaction whereby the L_2 does not hatch unless stimulated by host root diffusates, thus ensuring that a large population of infective L_2s are released close to susceptible roots. An initial phase in the hatching process is a change in the eggshell permeability induced directly by hatching factors in host diffusate (Jones, Tylka & Perry, 1998); alteration in eggshell permeability characteristics through the action of hatching factors results in an increased susceptibility of unhatched L_2s to dehydration stress (Perry, 1983).

Like the cyst nematodes, the root-knot nematodes (*Meloidogyne* spp.) are plant endoparasites but the female lays eggs into a gelatinous matrix consisting of an irregular meshwork of glycoprotein material. The gelatinous matrix shrinks and hardens when dried (Bird & Soeffky, 1972), thus exerting mechanical pressure on the eggs to inhibit hatching during drought conditions and ensuring that the infective L_2s are retained within the protection of the eggs and matrix. A third protective layer, which appears as an extracuticular subcrystalline layer in *M. charis* (Demeure & Freckman, 1981), also may function to slow the rate of water loss.

Although the cyst wall or gelatinous matrix and the eggshell enhance the survival of unhatched L_2s of cyst and root-knot nematodes, they do not result in unhatched L_2s of the different species being able to survive dehydration equally well. The ability to survive severe desiccation varies considerably between species and long-term anhydrobiosis seems to be associated primarily with those species, such as *G. rostochiensis*, that have a very restricted host range. For example, compared with *G. rostochiensis*, species such as *H. schachtii* hatch well in water without depending on host stimulation (Perry, Clarke & Hennessy, 1980) but withstand desiccation poorly (Ellenby, 1968*b*); the very wide host range of *H. schachtii* (some 218 plant species, including many weeds) ensures survival of populations until the main host crop becomes available.

Eggs of adult trichostrongyle nematodes that parasitize sheep and cattle are passed to the outside in faeces and also have to withstand environmental extremes. Waller & Donald (1970) considered that eggs of *Haemonchus contortus* and *Trichostrongylus colubriformis* will survive dehydration provided that development can proceed to the prehatch stage during drying, and before the embryo loses a critical amount of water. Under desiccating conditions, the eggshell of *H. contortus* is more permeable to water loss than that of *T. colubriformis*. The inner layer of the eggshell of *H. contortus* contains non-polar lipids of the hydrocarbon type, whereas the equivalent layer of *T. colubriformis* eggs contains either more polar unsaturated lipids or proteins (Waller, 1971); such physico-chemical differences between the eggshells of these and other species of nematodes may be correlated, in part, with differences in the ability of the eggshells to control water loss. The survival attributes of the infective larvae of *H. contortus* also have been examined experimentally. Under suitable environmental conditions, the first stage larva hatches from the egg and development proceeds to the L_2 and then to the infective third stage larva (L_3). The L_3 of *H. contortus* retains the cuticle of the previous stage as a sheath and development is arrested until exsheathment occurs in the rumen of the host. The ensheathed L_3 survives desiccation better than the exsheathed form: at 47% relative humidity ensheathed L_3 can survive for at least 4 weeks whereas the exsheathed L_3 perishes after 8 h (Ellenby, 1968*c*). On exposure to desiccating conditions, the sheath dries first and becomes increasingly impermeable, thus slowing down the rate of water loss of the enclosed L_3 and enabling it to survive (Ellenby, 1968*c*). Exsheathed L_3s of *T. colubriformis* will survive transfer to 0% relative humidity if they are first dried slowly at high humidity (Allan & Wharton, 1990).

Some plant-parasitic and entomopathogenic nematodes also retain moulted cuticles to protect infective stages. L_2s of *Rotylenchulus reniformis* hatch in the soil and the post-hatch moulting from L_2 to adult is completed without feeding (Gaur & Perry, 1991*a*), resulting in a decrease in body volume from L_2s to adults (Bird, 1983). The young adults are enclosed in the three cuticular sheaths from the previous stages and remain inactive in dry soil until favourable conditions return allowing movement and exsheathment. Gaur & Perry (1991*b*) showed that the exsheathed adults survived poorly compared to ensheathed adults, and the sheaths aided desiccation survival by slowing the rate of drying of the enclosed adult. However, the reduced rate of water loss only assisted individuals to survive for periods over which water loss was controlled; they showed no ability for prolonged survival once their water content had been reduced to less than 10%. A similar situation appears to occur with the entomopathogenic nematode, *Heterorhabditis megidis*, where the sheath surrounding the infective larvae slows down the rate of drying of the enclosed larvae but does not result in them surviving for extended periods (Menti, Wright & Perry, 1997). Thus, whilst control of water loss enables some species to enter anhydrobiosis, *R. reniformis* and *H. megidis* are examples of nematodes that show little intrinsic ability for anhydrobiotic survival; control of water loss merely prolongs the time taken for the nematode's water content to reach lethal low levels.

O'Leary & Burnell (1997) isolated mutant lines of *H. megidis* with an increased tolerance to desiccation at low humidities. The surface of the sheaths of

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mutant lines is more negatively charged than that of the wild-type and removal of the outer layer, possibly the epicuticle, resulted in loss of the mutant phenotype (O'Leary, Burnell & Kusel, 1998). A strongly negative charge on the epicuticle has been found in larvae of *Strongyloides ratti* and related to desiccation tolerance (Murrell, Graham & McGreevy, 1983). O'Leary *et al.* (1998) suggested that the presence of a strongly ionized or polar coat on the surface of nematodes could facilitate the maintenance of a film of water over the cuticle.

The retention of moulted cuticles is found in other species of soil-dwelling nematodes but their presence does not necessarily indicate a rôle in desiccation survival; a sheath or sheaths also may afford protection against antagonistic organisms such as pathogenic fungi (Timper, Kaya & Jaffee, 1991). Species of *Steinernema*, another genus of entomopathogenic nematodes, have ensheathed infective soil-dwelling infective larvae but there is no evidence that the sheath aids desiccation survival (Campbell & Gaugler, 1991; Patel, Perry & Wright, 1997). The sheath of *Steinernema* spp. fits very loosely and is readily lost during movement through the soil whereas the sheath of *Heterorhabditis* spp. is closely associated with the nematode's body; the sheath of *Steinernema* may have no rôle in protection of the infective larva. Why such genera, occupying similar ecological niches, differ in this respect is not understood.

Nematodes parasitizing the aerial parts of plants provide some of the best examples of extended anhydrobiotic survival, and they also are able to tolerate very rapid dehydration regimes and repeated cycles of dehydration and rehydration. For example, L_4s of *D. dipsaci* have been revived after being stored in dry plant material for 23 years yet the total duration of the life-cycle ranges from only 19 to 23 days at 15 °C (Evans & Perry, 1976). In general, the survival of uncoiled, individual L_4, L_3 and L_2 in *in vitro* experiments can be expressed in weeks, days and minutes, respectively; in all cases survival increased with increase in humidity, especially in adults where survival was for hours at humidities under 50 % but for days at higher humidities (Perry, 1977*a*). L_3s lost water less slowly than L_4s but both lost water more slowly than L_2s and adults (Perry, 1977*b*). Thus, for this species, the slower dryers are the best survivors. The superior survival ability of L_4s appears to be linked to an intrinsic property of the cuticle to resist water loss. The cuticle of the L_4 dries more rapidly than deeper layers of the nematode and slows down the rate of water loss of internal, and perhaps more vital, structures (Ellenby, 1969; Perry, 1977*b*). The cuticular permeability barrier is heat labile and is destroyed by brief extraction with diethyl ether, indicating that an outer lipid layer, possibly the epicuticle, is involved (Wharton *et al.* 1988). The permeability barrier of the cuticle of *Anguina agrostis* was also considered to lie in the epicuticle and to be lipoprotein in nature (Preston & Bird, 1987; Bird & Zuckerman, 1989). Repeated cycles of desiccation and rehydration of L_4s of *D. dipsaci* resulted in a decrease in the percentage surviving each cycle; however, after the initial cycle the rate of drying of previously desiccated and revived individuals remained constant, irrespective of the number of cycles (Perry, 1977*c*). Thus, control of the rate of drying does not, of itself, guarantee survival, and death caused by repeated cycles of desiccation and rehydration is not associated with an altered ability to control water loss.

Although nematodes protected by cysts, eggshells, sheaths or impermeable cuticles may lose all their body water, the rate of water loss is much slower than that of unprotected individuals. However, only a few species are able to survive beyond the period during which water loss is controlled. With these species, additional biochemical adaptations are required for long-term survival of anhydrobiosis.

MORPHOLOGICAL CHANGES INDUCED BY DESICCATION

A slow rate of water loss appears to allow orderly packing and stabilization of structures to maintain functional integrity during desiccation. Experimental analysis of the water dynamics of individual L_4 of *D. dipsaci* exposed to 0 % and 50 % relative humidities demonstrated that water loss occurred in three distinct phases (Perry, 1977*b*). An initial rapid loss of water was followed by a period of very slow water loss before the third phase of rapid water loss to leave individuals with no detectable water content (Fig. 1). The first two phases are separated by a permeability slump during which the permeability of the cuticle, and hence the subsequent rate of water loss, is reduced (Perry, 1977*b*; Wharton, 1996). During the first phase, Wharton & Lemmon (1998), using freeze substitution techniques, observed rapid shrinkage of the cuticle, the lateral hypodermal cords and the muscle cells, followed by a slower rate of shrinkage during the second phase. The contractile region of the muscle cells appears to resist shrinkage until desiccation becomes severe during the third phase (Fig. 1). The mitochondria swell and then shrink during desiccation, which may indicate disruption of the permeability of the mitochondrial membrane (Wharton & Lemmon, 1998). A decrease in thickness of the hyaline layer, caused by shrinkage of its constituent muscle cells and epidermis, results in a decrease in diameter of L_4s of *D. dipsaci* that is of a much greater magnitude than the accompanying change in length, and which has not been observed in other nematodes (Wharton, 1996). For example, reduction in the rate of water loss of *Rotylenchus robustus* is achieved by controlled contraction of

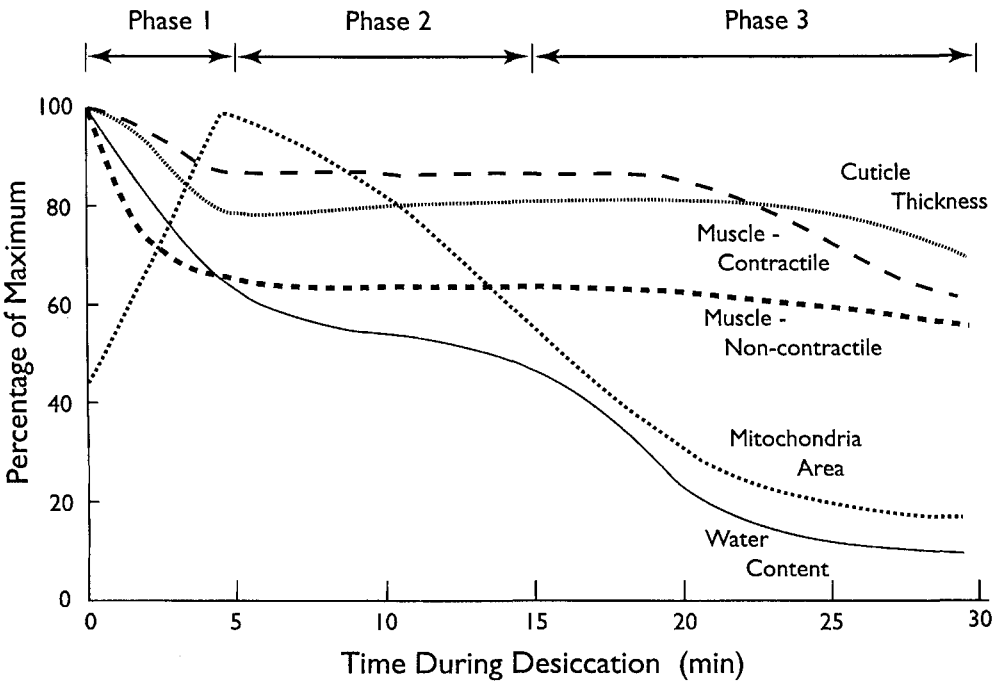

Fig. 1. Changes accompanying desiccation of L_4 of *Ditylenchus dipsaci* following placement of hydrated individuals in 50% relative humidity at time zero. Nematode water content data were calculated from Perry (1977*b*). Data for cuticle thickness, muscle region thickness and mitochondrial profile area were calculated from Wharton & Lemmon (1998). The three phases reflect differences in the rate of water loss (Perry, 1977*b*).

cuticular annuli resulting in decreased length, but not diameter, of the nematode (Rössner, 1973; Rössner & Perry, 1975). The large lipid reserves found in some nematodes, such as *D. dipsaci* and *A. tritici*, may prevent structural damage. Intestinal cells of *D. dipsaci* changed little during desiccation, possibly because the lipid droplets they contain resist shrinkage (Wharton & Lemmon, 1998).

Lee (1972) considered that the infective L_3 of *Nippostrongylus brasiliensis* became coated with a thin monolayer of lipid, obtained from the skin and hairs of the prospective host, that may reduce the rate of water loss from the nematode. Bird & Buttrose (1974) found differences between hydrated and desiccated L_2s of *A. tritici* in the structure of the external cortical layer of the cuticle. In desiccated L_2s, the outermost osmiophilic layer is doubled in thickness, indicating an increase in lipids. An outer layer is present in desiccated L_2s of *A. amsinkia* (Womersley *et al.* 1998) and in aggregations of *D. myceliophagus* (Perry, unpublished). However, the biochemical nature of these layers has not been investigated and it is not known whether they derive from the nematode or the host.

BIOCHEMICAL AND MOLECULAR CORRELATES OF ANHYDROBIOSIS

The biochemical adaptations associated with desiccation survival have been reviewed by Barrett (1991) and Womersley *et al.* (1998). At water contents below about 20%, there is no free water in the cells. This 20%, usually referred to as 'bound water', is involved in the structural integrity of macromolecules and macromolecular structures, such as membranes. The water content of desiccated, anhydrobiotic nematodes is estimated to be about 1–5%, so it is probable that the bound water has been lost although there is no experimental evidence that nematodes can survive the complete loss of structural water. Research on biochemical attributes of organisms that may be associated with anhydrobiosis has centred on molecules that might replace bound water and preserve structural integrity. Crowe & Crowe (1999) have recently presented a summary and supporting evidence for this 'water replacement' hypothesis in relation, primarily, to retention of membrane stability during desiccation. In nematodes, there is only limited information on biochemical mechanisms of desiccation survival. Most research has concentrated on the free-living mycophagous nematode, *Aphelenchus avenae*, but it is not certain that this is a useful 'model' for plant- and animal-parasitic nematodes capable of surviving anhydrobiotically.

The accumulation of the disaccharide, trehalose, the only naturally occurring non-reducing disaccharide of glucose, during water loss of anhydrobiotic nematodes has been reported frequently. In *A. avenae*, glycogen and lipid reserves are converted to trehalose and glycerol, respectively (Madin & Crowe, 1975). L_4 of *D. dipsaci* and L_2 of *Anguina tritici* also sequester trehalose, but not at the expense of lipid reserves; in these stages, other carbohydrates, such as myo-inositol and ribitol may be involved (Womersley, 1987). Lipid reserves are maintained at

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high levels in many anhydrobiotic nematodes and provide a food source for the nematode after revival and before they are able to feed on a host. The reported accumulation of glycerol in *Aphelenchus avenae* during desiccation (Madin & Crowe, 1975) was considered by Higa & Womersley (1993) to be an artefact due to the anaerobic conditions produced in large aggregates and not an adaptation to anhydrobiosis. There is no evidence that glycerol is preferentially synthesized during desiccation of *Anguina tritici* or *D. dipsaci* (Womersley & Smith, 1981; Womersley, Thompson & Smith, 1982). Womersley *et al.* (1998) considered that this is consistent with the fact that glycerol is highly fusogenic in dry membrane systems and, thus, would have an adverse effect on membrane stability during desiccation. Some nematodes, such as *Ditylenchus* (= *Orrina*) *phyllobius* accumulate no extra polyols during water loss yet can survive very rapid drying (Robinson, Orr & Heintz, 1984). Barrett (1991) considered it possible that such species may normally have large amounts of tissue polyols when active.

Several rôles for the involvement of trehalose in desiccation protection have been advanced and are detailed by Barrett (1991) and Crowe & Crowe (1999); only a brief summary is given here. Trehalose may replace bound water by attaching to polar side groups on proteins and phospholipids, thus maintaining the balance between hydrophilic and hydrophobic forces acting on the molecules and preventing their collapse. Preventing cross-linkage of molecules and fusion of membranes as bulk water is removed also preserves membrane stability. Stabilizing the membranes allows them to remain in a liquid crystalline phase and prevents a phase change to a gel state which would cause loss of the contents of cells and membrane vesicles during rehydration. Stabilization of molecules in the dry state also requires vitrification, which keeps membranes in a glass-like state to prevent a variety of deterioration processes (Levine & Slade, 1992; Crowe, Carpenter & Crowe, 1998). Trehalose also may prevent protein denaturation. Glucose reacts with the amino-acid side chains of proteins to form brown pigments called melanoidins. By contrast, trehalose does not react with proteins in this way and also appears to suppress this adverse reaction of other sugars with proteins (Loomis, O'Dell & Crowe, 1979). Trehalose can act as a free-radical scavenging agent to reduce random chemical damage (Barrett, 1991).

Synthesizing trehalose during dehydration may indicate preliminary preparation for a period in the dry state, but it does not necessarily mean that preservation of biological integrity and thus survival during subsequent severe desiccation is assured. Research on *Aphelenchus avenae* and *D. myceliophagus* illustrates this point. During desiccation preconditioning at 97% relative humidity, large aggregates (> 115 mg wet wt.) of *A. avenae* maximised their trehalose content after 72 h (Crowe & Madin, 1975), whereas small aggregates (< 10 mg wet wt.) achieved similar concentrations within the first 24 h (Higa & Womersley, 1993). However, only large aggregates survived direct transfer to extreme conditions such as 0% relative humidity; small aggregates needed further slow drying, by sequential transfer to successively lower humidities, to enable the nematodes to survive severe desiccation (Higa & Womersley, 1993). The rate of drying of large aggregates was probably reduced by the 'eggshell' effect mentioned previously in connection with survival of *D. dipsaci* in 'eelworm wool'. The research by Higa & Womersley (1993) contradicts the view that, once trehalose synthesis is complete, nematodes can survive further desiccation irrespective of the subsequent rate of water loss. It appears that, following trehalose synthesis, other, at present unknown, adaptations are required at the cellular and subcellular levels for nematode survival, and rate of drying still has to be controlled (Higa & Womersley, 1993). In contrast to *D. dipsaci*, individuals of *D. myceliophagus* survive desiccation poorly as individuals, even when dried at high humidities, and show no intrinsic ability to control water loss (Perry, 1977*a*, *b*). When raised on different food sources and exposed to various desiccation regimes, aggregates of *D. myceliophagus* contained different amounts of trehalose (*ca.* 3–16% dry wt.), depending on treatment, yet the nematodes are unable to survive direct exposure to low relative humidity (Womersley & Higa, 1998). The survival of aggregates of *D. myceliophagus* was unrelated to their trehalose content, and elevated levels of trehalose did not enhance anhydrobiotic survival of this species.

In general, biochemical changes during drying of infective larvae of the entomopathogenic nematode, *S. carpocapsae*, parallel those observed in *A. avenae* and *D. myceliophagus*. When infective larvae were dried slowly at 97% relative humidity, glycogen and lipid reserves declined while the trehalose content increased from *ca.* 0·2% dry wt. in fully hydrated nematodes to a maximum of *ca.* 7%; however, the larvae were only able to survive at high relative humidities (Womersley, 1990). More research is needed to examine whether trehalose is implicated in the desiccation survival of other species or strains of entomopathogenic nematodes. Although steinernematids are not considered to be anhydrobiotes (Womersley, 1990), desiccation-tolerant strains have been isolated from a semi-arid region in Israel (Glazer *et al.* 1996; Solomon, Paperna & Glazer, 1999) but the adaptations enabling these strains to survive have not yet been investigated. Future work may focus on genetic transformation of entomopathogenic nematodes to improve their environmental tolerance (reviewed by Burnell & Dowds, 1996) and thus enhance survival of commercial formulations during storage and after foliar

application. A transgenic approach would utilise the considerable information available from the *Caenorhabditis elegans* genome sequencing project (www.sanger.ac.uk/Projects/C_elegans/wormpep/) and has been used already by Gaugler, Wilson & Shearer (1997) to introduce a heat-shock protein gene, *hsp70*A, from *C. elegans* into *H. bacteriophora* to enhance thermotolerance. If trehalose is implicated in the survival of species and/or strains of entomopathogenic nematodes, then the use of genes for enzymes involved in the synthesis of trehalose, such as *tps* 1 coding for trehalose-6-phosphate synthase, may cause trehalose overproduction and enhanced survival (Vellai *et al.* 1999). Information on trehalose metabolism and its rôle in life-cycle physiology of animal- and plant-parasitic nematodes is also essential to evaluate possible novel control strategies. For example, if trehalose is important for the survival of animal-parasitic nematodes, enzymes of trehalose metabolism may offer molecular control targets as trehalose metabolism appears not to be important in mammals (Behm, 1997).

There is a shortage of information on other metabolic adaptations or changes involved in anhydrobiosis. The most detailed studies have been on L_4s of *D. dipsaci*. Barrett (1982) found that desiccation of these nematodes did not result in any appreciable denaturation of metabolic enzymes. There was no increase in the frequency of breaks in DNA obtained from desiccated L_4s compared with hydrated L_4s but as this also was observed in the desiccation-intolerant free-living nematode *Panagrellus redivivus*, it appears that DNA stability is a general feature of biological material and is not associated specifically with organisms able to enter anhydrobiosis (Barrett & Butterworth, 1985).

Completion of the sequencing of the 100 million base pair *C. elegans* genome provides a useful resource for the examination of the genetic induction of the survival forms in parasitic nematodes. The survival form of *C. elegans*, termed the 'dauer larva', represents a developmental arrest (Riddle & Albert, 1997) essentially similar to that found in some animal-parasitic nematodes, such as *Strongyloides ratti* which can switch between free-living and parasitic life-cycles in response to environmental cues (Viney, 1996). Dauer larvae are specialised L_3s enclosed by a dauer-specific cuticle and exhibit several characteristics including reduced metabolism, elevated levels of several heat shock proteins and an enhanced resistance to desiccation (Kenyon, 1997). They are formed, not in response to adverse environmental conditions acting on the L_3s but in response to specific factors acting on the L_1s and early L_2s. The factors initiating dauer formation are food availability, temperature and levels of a *C. elegans*-specific pheromone; details of the interaction of these factors and the *daf* genes (*daf* = dauer formation) involved have been reviewed by Riddle & Albert (1997). The information from this research may be relevant to other nematode groups as, in broad terms, diapause in plant- and animal-parasitic nematodes (Evans & Perry, 1976; Perry, 1989) and the formation of the infective larvae of entomopathogenic nematodes (Womersley, 1990) encompass developmental adaptations similar to dauer formation in *C. elegans*. Future research to investigate whether there are homologues of *daf* genes in parasitic nematodes will be an instructive first step.

REHYDRATION

Successful survival of desiccation requires not only completion of the induction into anhydrobiosis but also that changes during rehydration are ordered and controlled. Essentially, these changes reverse those that occur during drying but morphological and metabolic readjustments do not all occur at similar times (Fig. 2). The rate of rehydration by desiccated individuals when placed in water is very rapid and seems not to relate to the length of time they had been desiccated. L_2s of *G. rostochiensis* and L_4s of *D. dipsaci* took up water at the same rate (Ellenby, 1968*a*) and comparisons of all stages of *D. dipsaci* showed that, irrespective of the fact that they had been desiccated for different periods at 0% relative humidity, there were no differences in the rate of water uptake (Perry, 1977*b*). In all cases, the initial rate of rehydration was rapid with 50% water content being achieved in only a few minutes. The water content of L_4s of *D. dipsaci* increased logarithmically for up to 2·4 h of rehydration (Wharton, Barrett & Perry, 1985) whereas during rehydration of L_2s of *A. agrostis* cuticle permeability initially increased slightly followed by a sharp decrease in permeability between 1 and 8 h, after which there were two successive slower declines in permeability up to 24 h (Preston & Bird, 1987). These species are able to revive from the desiccated state on immediate transfer to water. With *Aphelenchus avenae*, successful revival depends on slow rehydration in saturated atmospheres (*ca.* 100% relative humidity) (Crowe, Hoekstra & Crowe, 1992). It appears that the fast-dehydration strategists are also fast-rehydration strategists and the slow-dehydration strategists are slow-rehydration strategists. Thus it is probable that the water dynamics of dehydration and rehydration are linked. The definitions of the two groups given by Womersley (1987) may be extended to characterize one group that can withstand fast water loss and gain and the second group that requires slow, controlled water loss and gain.

Although L_4s of *D. dipsaci* rehydrate very rapidly, there is a delay of several hours before the onset of locomotory activity (Fig. 2). Barrett (1982) termed this delay the 'lag phase' and considered that it may be necessary to restore membrane function. The

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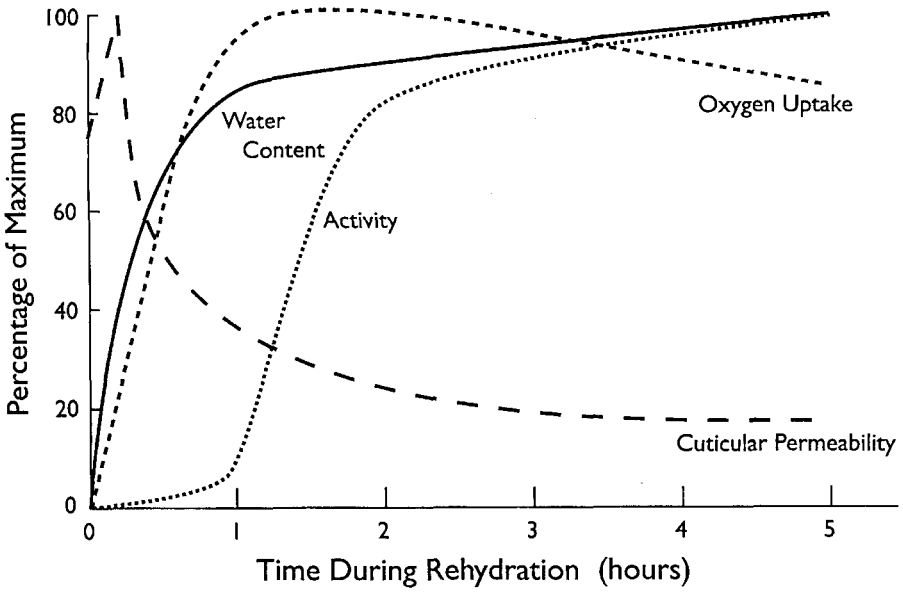

Fig. 2. Changes accompanying rehydration of L_4 of *Ditylenchus dipsaci* following placement of desiccated individuals in water at time zero. Nematode water content data were calculated from Perry (1977*b*). Cuticular permeability data were calculated from Wharton *et al.* (1988). Oxygen uptake and activity data were calculated from Barrett (1982). Activity is defined as the percentage of L_4s showing movement. The time difference between water uptake and activity is the 'lag phase' (Barrett, 1982). (Redrawn from Barrett, 1991.)

water permeability characteristics of the cuticles of *D. dipsaci* and *A. agrostis* are altered during desiccation and the permeability barrier associated with these species is restored during the lag phase (Wharton *et al.* 1985; Preston & Bird, 1987). The length of the lag phase in *D. dipsaci* increased as the severity of the desiccation stress during dehydration increased (Wharton & Aalders, 1999). A similar relationship between the length of the lag phase and the severity of desiccation has been demonstrated in *T. colubriformis* (Allan & Wharton, 1990). The restoration of the cuticular permeability barrier during rehydration can be prevented by inhibitors that block enzyme activity and post-transcriptional protein synthesis (Wharton *et al.* 1988), indicating an active repair mechanism. Leakage of inorganic ions during rehydration has been demonstrated in *Aphelenchus avenae* (Crowe, O'Dell & Armstrong, 1979) and in *Anguina tritici* (Womersley, 1981). The leakage ceases during the lag phase, indicating the repair of damaged membranes or the restoration of the permeability barrier due to a physical change associated with rehydration.

Morphological changes occur gradually throughout the lag phase. Muscle cells of L_4s of *D. dipsaci* increase in thickness and, in *D. dipsaci* and *A. agrostis*, small lipid droplets coalesce within the intestine to form large droplets (Wharton & Barrett, 1985; Wharton *et al.* 1985; Preston & Bird, 1987). There is a decrease in body length of *D. dipsaci* (Wharton *et al.* 1985) and *T. colubriformis* (Allan & Wharton, 1990) during the lag phase. This may indicate a contraction of the muscle cells as they recover and, in *T. colubriformis*, there is evidence of a change in the arrangements of muscle filaments in the contractile region of the muscle cells (Allan & Wharton, 1990).

Analyses of metabolic changes during rehydration have been confined almost entirely to L_4s of *D. dipsaci*. Barrett (1982) found that metabolism of L_4s, as measured by heat output, oxygen uptake or $^{14}CO_2$ production from labelled substrates, begins immediately after hydration. The metabolite profiles recover quickly during hydration with noticeable changes after 10 min and completion by 1 h. However, the ATP content does not recover as rapidly as those of the other metabolites; after 10 min there is little change and even after 1 h it is still low (Barrett, 1982). The slow trehalose depletion (up to 48 h to return to pre-desiccation levels) may be associated with the slow recovery of ATP levels. Mitochondria swell during rehydration before adopting a normal morphology (Wharton & Barrett, 1985); immediately after hydration, the mitochondria are essentially uncoupled and there is no oxidative phosphorylation (Barrett, 1982). Barrett (1982) suggested that during the dehydration-rehydration cycle, membrane function is disrupted and the lag phase reflects the time required to restore metabolic and ionic gradients.

There seems to be negligible protein synthesis during the first 2 h of rehydration and L_4s of *D. dipsaci* revive successfully in the presence of inhibitors of protein and RNA synthesis (Barrett, 1982). However, there is an increase in the activity of certain enzymes involved in prevention of cellular aging though free-radical scavenging reactions and negation of lipid peroxidation. For example, during

rehydration of *A. avenae* an increase in superoxide dismutase activity occurs (Womersley, 1987) and catalase activity essentially triples during the first 4 h (Gresham & Womersley, 1991).

CONCLUSIONS

The urgent need for environmentally-acceptable methods to control pests has provided the impetus for research on the use of entomopathogenic nematodes as bioinsecticides and has renewed interest in studies on aspects of the survival of parasitic nematodes outside their hosts. In turn, this has generated further research on the morphological and biochemical adaptations associated with anhydrobiosis. It is clear, from this review, that only a limited number of nematode species have been used as the basis for detailed research and there is still much to be understood about the biochemical changes during desiccation and successful revival and the genetic control associated with induction of the dormant state. There is no 'model nematode' that can serve to provide the information about dormancy as species and stages of nematodes exhibit a variety of adaptations, and different combinations of these adaptations are associated with different nematodes to ensure anhydrobiotic survival.

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