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Ecological and Physiological Survival Strategies of Plant-Parasitic Nematodes

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Introduction

'Survival strategies' are usually taken to mean the responses of organisms to the particular biological and physical challenges that may result in their death. Nematodes face a number of such challenges i.e. biological challenges and physical challenges. Biological challenges include: running out of food, predation, pathogens and competition. Parasitic species face the additional problems of the immune, and other, responses of their hosts and the problems of infecting new hosts. Physical challenges include: temperature extremes, desiccation, high pressure, acid or alkaline conditions, osmotic and ionic stress, anoxia and exposure to various types of radiation. The responses of nematodes to a physical challenge, however, also involve morphological, physiological, biochemical and molecular mechanisms. There may also be an interaction between the physical and biological challenges faced by nematodes.

There are four responses

1. **Migratory response:** The nematode might move away from the extreme conditions to a less stressful environment that is more favourable to its growth and reproduction.
2. **Capacity adaptation:** The nematode may adapt to the challenge and so be able to grow and reproduce under extreme conditions.
3. **Timing mechanism:** The life cycle of plant parasitic nematodes (PPNs) may include timing mechanisms that enable the parasite to avoid seasonally stressful conditions and to synchronize its life cycle with that of its host.
4. **Resistance adaptation:** The nematode may survive extremes in a dormant state until conditions suitable for growth and reproduction return.

Adaptation strategies in plant-parasitic nematodes

Different PPNs also follow several survival strategies against environmental extremes and host response (Perry, 2011). Two types of dormancy, 'quiescence' and 'diapause' often lengthen survivability of unhatched larvae in soil living nematodes including root-knot nematode (Perry, 1989). Cryobiosis, thermobiosis, anoxybiosis, osmobiosis and desiccation are different survival mechanisms that are also observed in nematodes (Wright & Perry, 2006). First discovered PPNs, *Anguina tritici* have also special adaptation aptitude against desiccation (Needham, 1743). Commonly, life cycle of PPNs is divided into different distinct stages such as egg, four juvenile stages (J1, J2, J3 and J4) and adults and different major PPN also exhibits different adaptation mechanism at their different life stages.

Eggs of root-knot nematode (RKN) are laid in mucoid protein mass that provides safety to eggs water loss and predators (Eisenback, 1985). Furthermore, extreme temperature makes this glycoprotein shriveled and hard that promotes mechanical pressure and hinders the hatching of J2 larva that is prone to drought environment (Wright & Perry, 2006). Additionally, gelatinous matrix defends the eggs from the attack of some soil microorganism as was observed in *M. javanica* (Orion & Kritzman, 2001). During dry conditions, a dried eggshell can also decrease water loss rate by changing its permeability (Ellenby, 1968). Additional research on eggshell permeability was done by Wharton (1980) and he observed that the lipid layer of the eggshell played a major role for lowering the water loss during desiccation. Not all PPN have survival capability. Mostly, the species with limited hosts such as potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) gained well adaption features for undesirable circumstances (Perry, 2002). The unhatched larvae of this nematode can survive many years in cyst without host (Turner & Rowe, 2006). Ellenby (1946) observed that the permeability of cyst wall exterior and eggshell of *G. rostochiensis* changed at desiccated conditions which helped cyst wall and eggshell to restrict their water losing rate compared to favourable conditions. This helps the unhatched susceptible J2 to survive in the egg and hatching factors (host root exudates) are obligatory for this nematode to hatch their J2 (Wright & Perry, 2006). J2 of soybean cyst nematode *Heterodera glycines* hatched in water at optimum conditions but at the end of the plant growing season when environment is becoming complex, most of the J2 remains in the cyst and hatching is solely dependent on hatching factors, irrespective to the origin of hatching factors; i.e. natural (Ishibashi *et al.*, 1973) or artificial (Thompson & Tylka, 1997). Other cyst nematodes, such as *H. carotae* (Greco, 1981), *H. goettingiana* (Greco *et al.*, 1986), *H. sacchari* (Ibrahim *et al.*, 1993) contain the same characteristics. Even, RKN, *M. chitwoodi* and *M. triticoryzae* (Gaur *et al.*, 2000; Wesemael *et al.*, 2006) also showed the same features for hatching of J2.

Under drying condition, species of *Anguina* and *Ditylenchus* showed coiling and clumping like features in their life cycle that helps to avoid water loss by reducing their surface (Crowe & Madin, 1975). *Ditylenchus myceliophagus* also lessen their water loss becoming coiled (Womersley, 1978). Furthermore, at the last stage of the crop growing season, *D. dipsaci*, stops their development at J4 stage due to shortage of food and make large aggregations and start coiling. This attribute known as 'eelworm wool' that helps them to remain alive for many years at desiccated condition by the sacrifice of peripheral J4s of this aggregation (Ellenby, 1969). Rice grain nematodes, *Aphelenchoides besseyi* also stops their multiplication at ripening stage of rice and adults become coiled and clumped which helps them surviving for 2-3 years with dry grains (Perry & Moens, 2011). On the other hand, J2 of 2 gall producing nematode *Anguina tritici* and *A. pacificae* become anhydrobiotic but remain uncoiled and survived many years (McClure *et al.*, 2008). So, coiling is not obligatory for survival during desiccation. Nevertheless, *D. dipsaci* and *A. tritici*, can overcome desiccated environment and survive many years in above ground plant parts (Moens & Perry, 2009). Sheath retaining is also a survival mechanism against desiccated condition.

Young adults of *Rotylenchulus reniformis* remains confined with all 3 moulted cuticle of their larval stage which facilitates them of being dormant in adverse condition until situation becomes amiable for their survival (Gaur & Perry, 1991). Dauer phenomenon is a special characteristic of nematodes that helps them to survive at stress condition. Dauer is very common in *C. elegans* where dauer larvae restrict its biological activity and survive periods at desiccated situation (Kenyon, 1997). J2, J3, or J4 stages are performed as dauer in *A. tritici*, *C. elegans* and *D. dipsaci* respectively (Bird & Bird, 1991). Pine wood nematode *Bursaphelenchus xylophilus*, also contains a dauer stage that facilitates the transportation to pine trees with the help of insect vector *Monochamus* (Mota & Vieira, 2008).

Behavioural responses

Various types of aggregation, swarming and synchronized movements occur in nematodes. Some of these behaviours are associated with responses to environmental stress, including desiccation. Croll (1970) suggested that aggregation was associated with quiescence (reduced metabolism) and with survival responses to adverse environmental conditions, while swarming was involved in migration and dispersal. However, aggregation can occur in the absence of an environmental stress and swarming may be associated with such a stress. Aggregation as the clumping of nematodes either spontaneously in culture or in water or when they are drawn together by the evaporation of water to form a clump and Swarming as a coordinated movement by a mass of nematodes from one location to another. Swarming may be followed by aggregation. The best known example of aggregation is the accumulation of large numbers of *D. dipsaci* on the surface of stored flower bulbs (Christie 1959). Croll (1970) mentions synchronized movements as another category and gives the movement of *Panagrellus redivivus* on to the walls of a culture vessel as an example.

Factors in survival of nematodes

Hypobiosis" is the form of survival covering all the biological and metabolical states of an organism that are associated with inactive life and lowered metabolism.

"Dormacy" is the state of inactivity associated with a low but measurable metabolism, as contrasted with latent life or "cryptobiosis" which is associated with a nondetectable metabolism.

Dormancy (Hypometabolism)

It is a period in an organism's life cycle when growth, development, and (in animals) physical activity are temporarily stopped. This minimizes metabolic activity and therefore helps an organism to conserve energy. Dormancy tends to be closely associated with environmental conditions.

1. **Predictive dormancy** occurs when an organism enters a dormant phase before the onset of adverse conditions.
2. **Consequential dormancy** occurs when organisms enter a dormant phase after adverse conditions have arisen.It is considered to be a physiological state of dormancy with very specific initiating and inhibiting conditions.

Four types of dormancy in eggs of *Heterodera schachtii* is recognized (Zheng and Ferris 1991). Some eggs hatched quickly in water, some required host-root diffusate for rapid hatch, while others hatched slowly in water or in host-root diffusate. Stimulus for hatching and ending of dormancy in various species include such factors as temperature (Van Gundy 1985) or the presence of host plant or root leachate (Huang and Pereira 1994; Sikora and Noel 1996). The quality of the latter depended on crop cultivar, phenology, and other factors (Sikora and Noel 1996). Interpretation of dormancy and diapause in nematode eggs is further complicated in that the induction of dormancy in cyst nematodes varies seasonally, and may be dependent on temperature or host phenology (Yen *et al.* 1995; Sikora and Noel 1996). Diapause and developmental dormancy seem to apply mostly to the egg stage and to juvenile stages within eggs, although instances of diapause in later juvenile stages or adults are known, mainly in a few animal-parasitic nematodes (Evans and Perry 1976).

Diapause is a mechanism used as a means to survive predictable, unfavorable environmental conditions, such as temperature extremes, drought, or reduced food availability. Diapause as a developmental arrest which is temporarily irreversible, so development will not resume, even under favourable conditions, until some intrinsic changes have been completed.

Resting stages in the life cycle

Infective stages: The life cycles of most parasitic nematodes include an infective stage in which development is arrested until infection occurs. The resting stage is usually the infective juvenile and this is often the stage of the life cycle that is most resistant to environmental stress.

Dauer juvenile formation: *Caenorhabditis elegans* produces a special survival stage called a dauer juvenile (dauer larva) in response to deteriorating environmental conditions. This is an alternative developmental pathway that leads to the formation of dauer juveniles, rather than to normal J3 and reproductive development. E.g. infective juvenile (J2) of some anguinid plant-parasitic nematodes have also been referred to as dauer juveniles (Womersley *et al.,* 1998).

Diapause: It is defined as a period of developmental arrest that is either programmed into the life cycle **(**obligatory diapause) or is triggered by environmental conditions (facultative diapause). Diapause and other delays in developmental stages that are common in insects (Chapman 1971; Romoser & Stoffolano 1998) occur in some nematodes also (Evans & Perry 1976; Wharton 1986). Although diapause is not necessarily due to adverse environmental conditions nor ended by presence of favorable conditions (Chapman 1971; Evans & Perry 1976; Wharton 1986), it is nonetheless a critical survival mechanism during cold seasons and in the absence of a host. The increase of egg hatching in *Meloidogyne naasi* by chilling is a well-known example of diapause in a plant-parasitic nematodes (Van Gundy 1985). In some species of root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* spp., *Globodera* spp.) nematodes, a portion of the eggs hatch rapidly while others hatch gradually over time (DeGuiran 1979; Zheng & Ferris 1991; Huang and Pereira 1994).

Egg diapause:- Hatching of the eggs of the plant-parasitic nematodes *Heterodera avenae* and *Meloidogyne naasi is* greater after chilling. The response to chilling varies among isolates of these nematodes and may allow overwintering or the survival of a dry season.

Quiescence: "Quiescence" most accurately describes dormancy in nematodes. The arrested development or movement of the nematode during quiescence or cryptobiosis is usually caused by some unfavorable environmental conditions. It may be associated with a particular stage of development but is not necessarily associated with some type of morphogenesis. Quiescence is contrasted with diapause i.e., development will not resume unless or until certain physiological processes have been completed, even though the environment is favorable.

Cryptobiosis: Cryptobiosis is an ametabolic state of life entered by an organism in response to adverse environmental conditions such as desiccation, freezing, and oxygen deficieny. In the cryptobiotic state, all measurable metabolic processes stop, preventing reproduction, development, and repair. When environmental conditions return to being hospitable, the organism will return to its metabolic state of life as it was prior to the cryptobiosis. E.g. nematodes stored under dry conditions survive for long periods of time. *Tylenchus polyhypnus* was revived after 39 years, *Anguina tritici, Ditylenchus dipsaci* and *Tylenchus balsamophilus* after 20 years storage in plant tissue (Fielding 1951).

Chemobiosis is the cryptobiotic response to high levels of environmental toxins. It has been observed in tardigrades.

Cryobiosis is a form of cryptobiosis that takes place in reaction to decreased temperature. Cryobiosis initiates when the water surrounding the organism's cells has been frozen, stopping molecule mobility and allowing the organism to endure the freezing temperatures until more hospitable conditions return. The nematodes which are freezing-susceptible are those which escape freezing injury by super-cooling at temperatures just below 0° C, but which freeze and die at some temperature. The freezing-resistant nematodes are not injured by freezing. The group of nematodes considered susceptible to chilling injury may be further segregated into sensitive and tolerant.

Osmobiosis is the least studied of all types of cryptobiosis. Osmobiosis occurs in response to increased solute concentration in the solution the organism lives in. E.g. most nematodes are capable of tolerating and surviving at osmotic pressures up to 15-20 atm there is considerable variability in their tolerance to single ions. When the nematodes *T. Christiei*, *T. claytoni*, *H. erythrinae* (adults), *M. Javanica* and *T. semipenetrans* (larvae) were bathed in solutions of copper ions, they were still active, respectively, in the following solutions: 0 to 0.25, 0 to 4, 4 to 8, 16 to 32, >32 ppm for 24 hr.

Anhydrobiosis: Some nematodes can withstand moderate desiccation better than others, probably because the water distribution in the cells and the nature of the protein constituents are such that withdrawal of water does not cause the disruption of their intimate structure. *Ditylenchus dipsaci* larvae and *Trichostrongylus colubriformis* larvae have survived desiccation at 50 per cent relative humidity and 24° C for at least 32 days.

Survival responses to desiccation: For most nematodes, activity ceases once the surrounding water film evaporates. The loss of activity is due to the adherence of nematodes to the substrate in the absence of water or to the loss of turgor pressure, without which movement cannot occur. Nematodes may survive partial desiccation with a reduced rate of metabolism (desiccation-induced quiescence) or, after complete desiccation, metabolism may cease altogether (anhydrobiosis). A slow rate of water loss is important for anhydrobiotic survival. *Anguina tritici*, *D. dipsaci* and *Tylenchus* have been showed potentiality of being enliven from desiccation after 20 year (Perry & Moens, 2011), 28 years (Fielding, 1951) and 39 years (Steiner & Albin, 1946) respectively. Cuticle lipid content of desiccated J4 of *D. dipsaci* and J2 of *A. tritici* have been increased compared to hydrated juveniles of those nematodes (Bird & Buttrose, 1974; Preston & Bird, 1987).

Anhydrobiotic nematodes can be divided into two groups: depending upon the rates of water loss from their environment that they will survive. Slow-dehydration strategists depend upon their surroundings, such as soil or moss, drying slowly; fast-dehydration strategists can survive rapid loss of water from their surroundings but themselves have mechanisms for ensuring the slow loss of water from their bodies. Adaptations for reducing the rate of water loss include both behavioural and physiological mechanisms (Table 1).

Table 1. Examples of nematode survival after lengthy time in anhydrobiosis

Nematode	Normal active habits	Anhydrobiosis conditions	Time in anhydrobiosis	Reference
Anguina agrostis	Foliar plant parasite	Dried plant material	4 yr	Fielding, 1951
A. tritici	Foliar plant parasite	Dried plant material	9-30 yr	Fielding, 1951
Ditylenchus dipsaci	Foliar plant parasite	Dried plant material	16- 23 yr	Fielding, 1951
Ditylenchus dipsaci	Foliar plant parasite	-80°C	5 yr	Cooper *et al.*, 1971
Filenchus polyhypnus	Foliar in moss	Dried plant material	39 yr	Steiner and Albin, 1946
Helicotylenchus dihystera	Plant parasite in soil	Dry soil	250 d	Aroian *et al.*, 1993
Pratylenchus penetrans	Plant parasite in soil, roots	Dry soil	770 d	Townshend, 1984

Anoxybiosis: *Bursaphelenchus xylophilus* can survive more than 14 days under anoxic conditions (Kitazume *et al.*, 2018). Many of the soil-inhabiting and intestine-inhabiting nematodes live in habitats that can at times be abnormally low in oxygen content. The nematodes most susceptible to low oxygen levels were those in the process of molting. Physiologically, this is a very active stage in the life cycle of the nematode, during which there is apparently a high demand for oxygen. Oxygen consumption of the surviving infective larvae in the egg was uniform and low. Anaerobic conditions also had pronounced effects on the growth of the developing nematode, egg production, hatching and movement.

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