

The role of the moulted cuticles in the desiccation survival of adults of *Rotylenchulus reniformis*

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SUMMARY

Rotylenchulus reniformis retains the moulted juvenile cuticles until the adult finally sheds them to become parasitic. The moulted cuticles have an important role in desiccation survival. The ensheathed adults are able to control their rate of water loss at 0, 60 and 80 % relative humidities for significantly longer periods than exsheathed adults. Successful shedding of the juvenile cuticles appears to be aided by frictional forces achieved by movement of the active nematodes against soil particles. When movement is inhibited, as in very dry soils, the adults are unable to shed these cuticles; the nematodes become quiescent and are able to control their water loss and thus survive desiccation.

RÉSUMÉ

Le rôle des cuticules exuviées dans la survie à la dessiccation des adultes de Rotylenchulus reniformis

Rotylenchulus reniformis conserve les cuticules juvéniles exuviées jusqu'à ce que, adulte, il les abandonne et devienne infestant. Ces cuticules jouent un rôle important dans la survie du nématode lors de la dessiccation. Les adultes pourvus de ces cuticules peuvent en effet contrôler leur niveau de perte en eau — à des humidités relatives de 0, 60 et 80 % — pendant des périodes beaucoup plus longues que les adultes qui en sont dépourvus. L'abandon des cuticules juvéniles est facilité par le frottement actif du nématode contre les particules de sol. Si les mouvements du nématode sont inhibés, comme dans les sols très secs, les adultes sont incapables de se débarrasser de ces cuticules; le nématode devient alors quiescent, peut contrôler ses pertes en eau et ainsi survivre à la dessiccation.

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, 1940, completes all its moults before the adult female becomes parasitic; adult males do not feed (Linford & Oliveira, 1940). It is a common root parasite of plants in tropical areas and is known to survive for long periods in dry soil. Survival of males and infective immature females in moist and in air-dried soil was noted by Birchfield (1967). Sivakumar and Seshadri (1976) found that the population surviving in dry soil consisted predominantly of fourth stage juveniles (J4) and young females and males, all ensheathed in moulted cuticles of the previous juvenile stages. Free-living stages of this species from dry soil were found to be coiled (Tsai & Apt, 1979) and Rodriguez-Fuentes (1980) recorded survival of J4 in dry soil for 25 months. Gaur and Haque (1986) subjected infested soil to slow air-drying after which young adult males and females in a coiled and shrunken anhydrobiotic state could be recovered after more than 27 months; on revival, they were found to have retained their infective and reproductive capacities.

Survival is related to the degree of moisture stress and the initial rate of moisture loss from the soil (Sehgal & Gaur, 1988, 1989; Womersley & Ching, 1989).

Much of the work on survival of *R. reniformis* does not define the rate of drying of the nematode or the humidity of the microenvironment between soil particles to which the nematode is directly exposed. In some species of nematodes, desiccation protection is afforded by a change in permeability of the cuticle which slows down the rate of water loss of internal structures (Ellenby, 1968a; Perry, 1977a). The retention of the juvenile cuticles by *R. reniformis* until the young adults become fully functional may be a significant factor in the desiccation survival of this species. Using interference microscopy (Ellenby, 1968b) to determine rate of water loss, the present study examines the role of the moulted cuticles in the ability of *R. reniformis* to survive desiccation.

The moulting juvenile stages of *R. reniformis* are relatively non-motile; only after the fourth and final

moult do young adults commence movement, still retaining the cuticular sheaths (Sivakumar & Seshadri, 1971). The possibility of abrasive or frictional forces between the moving nematode and the soil particles being involved in exsheathment was also investigated.

Materials and methods

Cultures of *R. reniformis* were maintained on tomato, cv. Pixie, in a glass-house at 22–30 °C. To obtain hatched J2, egg-sacs from females on roots of 30–44 day old plants were placed in glass distilled water (GDW) at 28 °C. Nematodes were allowed to moult up to young adults, still retaining the three moulted juvenile cuticles. Since the adults did not exsheath even after active movement for several days in water, some individuals were transferred to moist sand (150–400 µm particle size; FSA Laboratory Supplies) for 24 h to obtain exsheathed adults.

The ability of ensheathed and exsheathed adults of both sexes to survive desiccation was examined using the techniques of Perry (1977b). Individuals were transferred to artificial tap water (ATW) (Greenaway, 1970) on a glass slide and all superficial water was removed using micropipettes and filter paper slivers, preventing nematodes from coiling. The nematodes were dried for various periods in small 550 cm³ desiccation chambers containing glycerol solutions (Grover & Nicol, 1940) to give 60 and 80 % relative humidity (R.H.) or freshly activated silica-gel to give 0 % R.H. After desiccation, ATW was added and revival checked at intervals up to 3 days; the criterion for revival was movement of the nematode. A minimum of 100 nematodes of each sex were desiccated for each time interval at each humidity.

The rate of water loss of ensheathed and exsheathed adult males and females was determined after drying individuals for various periods at 0, 60 and 80 % R.H. After desiccation, liquid paraffin was added to the slide to cover the specimens and a cover-slip placed in position. The water content of a minimum of ten individuals for each time period at each humidity was measured using interference microscopy (Ellenby, 1968b). Refractive index data, rather than the water content values derived from them, were used for the statistical analysis for reasons given by Ellenby and Perry (1976).

The difficulty in obtaining exsheathed adults when nematodes were kept in water indicated that frictional forces as the nematodes move through soil may aid in the final shedding of the juvenile cuticles. To examine this aspect, 3.5 cm diameter clay pots with fibre wicks at the base were set up containing moist sand (150–400 µm particle size) and approximately 500 moulting juveniles were transferred to each pot. The pots were placed in similar sand subjected to moisture tensions of pFs "0" (no tension applied), 1.24, 1.54 and 1.85 maintained manometrically; a control batch was kept in ATW. The

nematodes were removed from individual pots after 3, 6, 9 or 14 days and the proportion of juvenile stages and ensheathed and exsheathed adults (males and females together) was determined using a stereomicroscope at 100 × magnification. The experiment was replicated three times.

Results

Survival of adult *R. reniformis* after desiccation as uncoiled individuals at all three humidities was for minutes only. Although there is a slight suggestion from the results (Fig. 1) that the females survived marginally

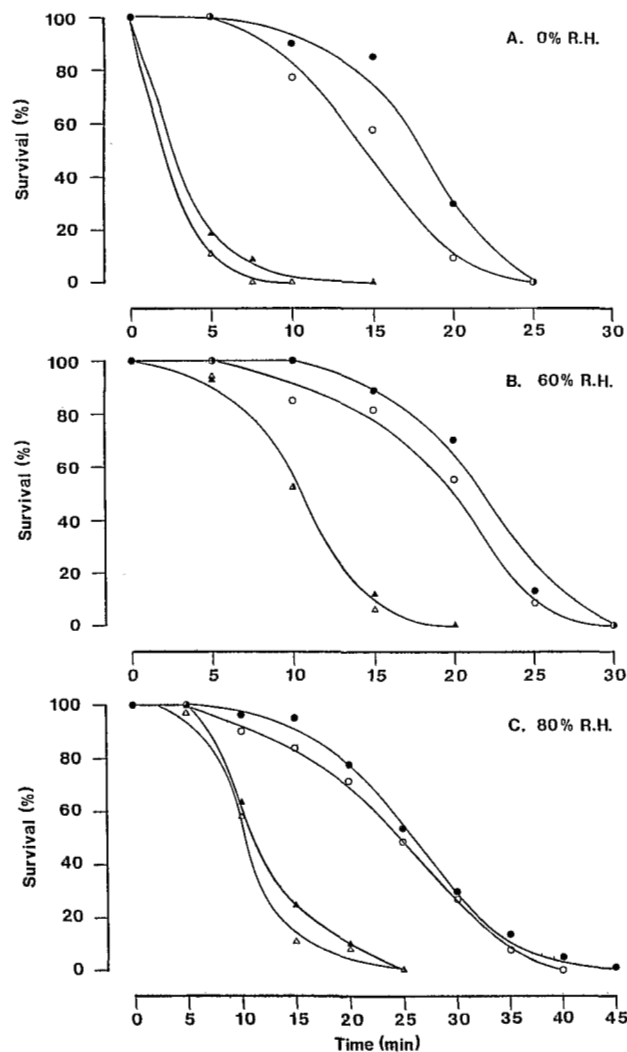


Fig. 1. Survival of ensheathed male (○) and female (●) adults and exsheathed male (△) and female (▲) adults of *Rotylenchulus reniformis* after desiccation for various periods at, A : 0 %; B : 60 %; C : 80 % relative humidity.

better than males, the most marked difference is in the desiccation survival of the ensheathed and exsheathed forms. Exsheathed forms survived poorly, the S_{50} (survival of 50%) times being about 3, 10 and 11 min at 0, 60 and 80% R.H. respectively. By contrast the S_{50} values for ensheathed forms are 21 and 25 min at 60 and 80% R.H. and even under the very severe desiccation conditions of 0% R.H., the S_{50} was between 15 to 18 min (Fig. 1 A).

There was no significant difference ($P > 0.05$) between the water content of ensheathed and exsheathed females (73.2% and 71.9%, respectively) or between

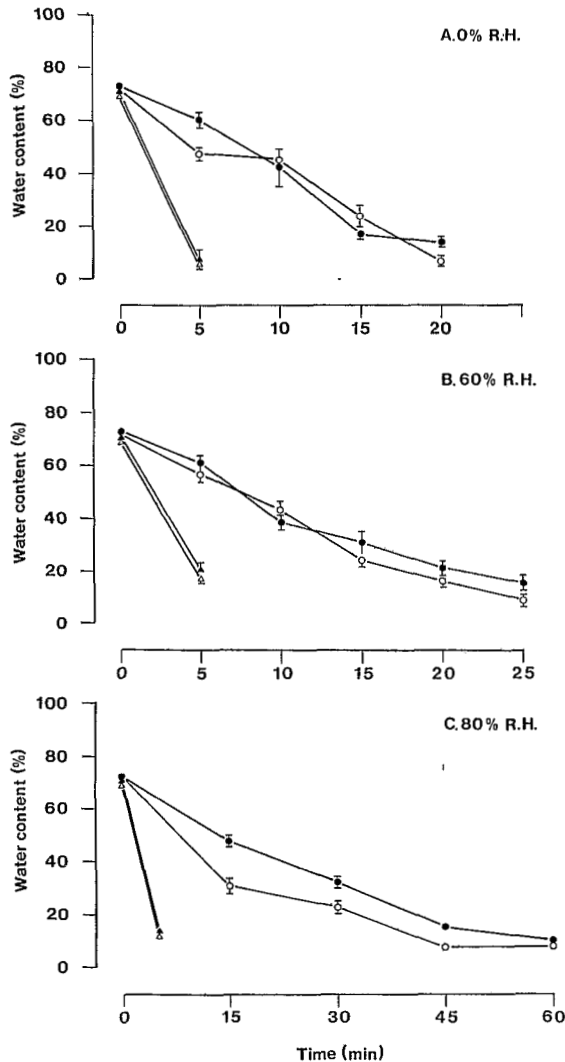


Fig. 2. The water content of ensheathed male (O) and female (●) adults and exsheathed male (Δ) and female (▲) adults of *Rotylenchulus reniformis* after desiccation for various periods at, A : 0% ; B : 60% ; C : 80% relative humidity.

ensheathed and exsheathed males (71.6% and 69.8%, respectively) before exposure to desiccation.

Results from studies on the water content of ensheathed and exsheathed adult males and females after desiccation at 0, 60 and 80% R.H. show that the retained moulted cuticles have a marked effect on the rate of water loss (Fig. 2). At all three humidities the exsheathed forms dry very rapidly and even at 80% R.H. the water content of both sexes was reduced to less than 15% after only 5 min drying. By contrast, the ensheathed forms dry slowly. For example, at 60% R.H. the water content of individuals after 5 min drying is over 55% and is not reduced to 15% until 25 min exposure (Fig. 2 B); even severe desiccation at 0% R.H. (Fig. 2 A) reduces the water content of individuals to less than 15% only after 20 min drying. Increase in humidity slows the rate of drying of ensheathed forms but the difference is more marked between 60 and 80% R.H. than between 0 and 60% R.H.

At each humidity for ensheathed and exsheathed forms, the rate of water loss of males and females was examined separately. Two way analysis of variance of the data at each humidity revealed no significant difference ($P > 0.05$) between the rate of water loss of males and females at 0% R.H. and 60% R.H. However, there is a difference in the rate of water loss of ensheathed forms at 80% R.H., where females lose water significantly ($P < 0.01$) more slowly than males.

The three replicates of the pot tests to study development of ensheathed forms at different suction pressures all showed a similar pattern; results of the first trial are given in Figure 3. By 3 days after suction had been applied, the majority of the populations at each pF and in the water control had moulted to the J3 stage, with the exception of nematodes at pF 1.85 where 28% were still J2 and 25% had moulted to J4 stage (Fig. 3 A). However, by 6 days the greatest proportion of the nematodes from each pF and controls were at J4 stage (Fig. 3 B). By 9 and 14 days differences between treatments had become more marked. At the lowest moisture levels of pF 1.54 and 1.84, where the sand was dry, the majority of the nematodes had moulted to ensheathed adults (Fig. 3 C) but only a few had exsheathed even by 14 days (Fig. 3 D). In the water control none had exsheathed by 9 days, most remaining either as J4 or ensheathed adults (Fig. 3 D); in the saturated sand at pF "0", 18.8% of the nematodes had exsheathed by 9 days rising to 56.6% by 14 days. The most rapid development to exsheathed adults and also the greatest proportion of exsheathed individuals occurred at pF 1.24 where 60.7% were exsheathed adults by 9 days rising to 71.3% by 14 days.

Discussion

Moulting in nematodes may involve enzymatic degradation of the old cuticle and the involvement of

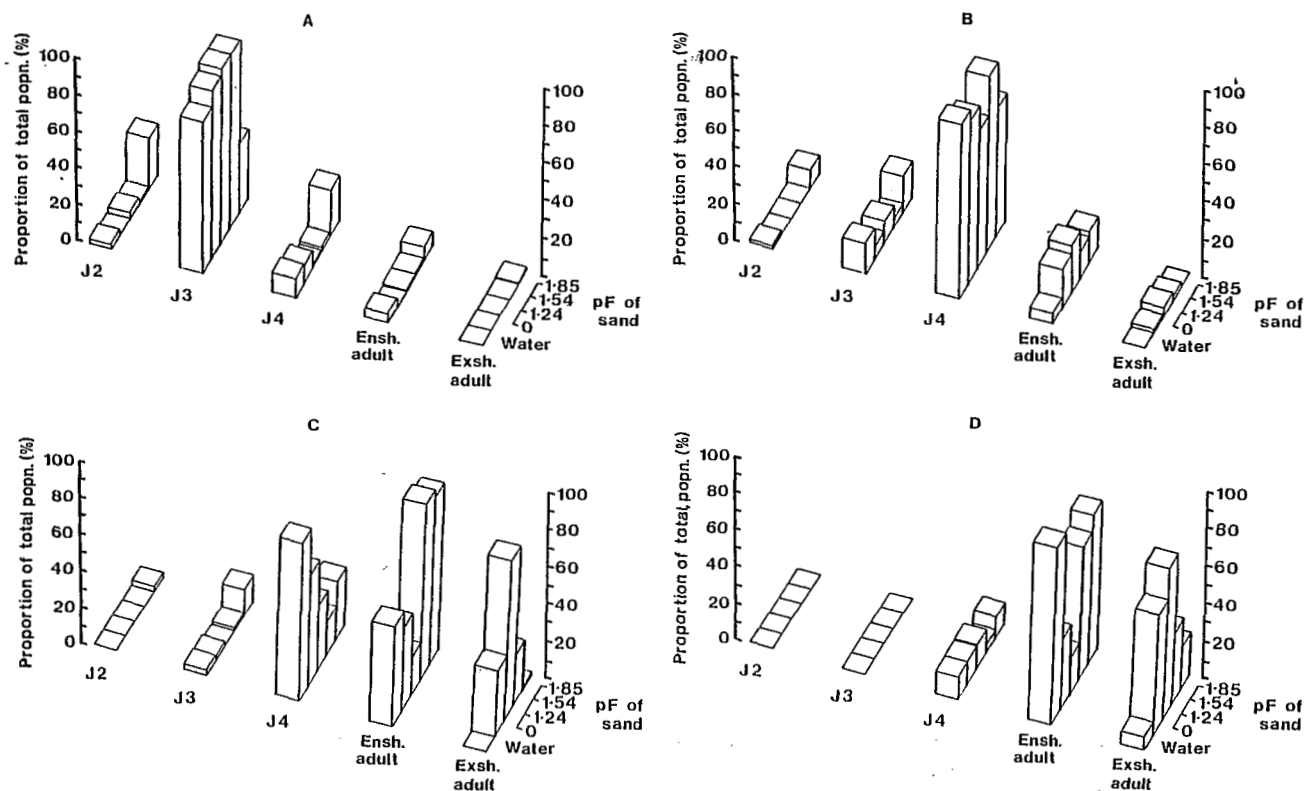


Fig. 3. The development and exsheathment of *Rotylenchulus reniformis* after, A : 3 days; B : 6 days; C : 9 days; and D : 14 days in water controls or sand at four different suctions.

enzymes in the moulting of *R. reniformis* has been suggested by Bird (1984) in his detailed examination of growth and moulting in this nematode. However, it is clear that with some species, including *R. reniformis*, abrasion against substrate particles is also important for successful shedding of the moulted cuticle. Wright and Perry (1991) found that adult *Aphelenchoides hamatus* in microscope slide preparations frequently became trapped in the old cuticle and they suggested that, under natural conditions, mechanical interactions with the environment would aid in rupturing the cuticle. In *R. reniformis*, this dependence on movement and abrasion for exsheathment is an advantage in dry soils. The small pot experiments show that, in very dry conditions where movement is inhibited (Wallace, 1958), ensheathed adults accumulate. The ensheathed forms are more resistant to desiccation and thus survival of the population is enhanced.

Variations in soil moisture content only affected the final shedding of the cuticles. Moulting by the juvenile stages progressed through to ensheathed adults at all soil moisture tensions and by 6 days the majority of nematodes in all treatments were J4, similar to the development times recorded by Sivakumar and Seshadri (1971)

and Bird (1984). Although there is an indication of a delay in the final moult from J4 to adult in water controls, it appears that abrasion against soil particles is not required for moulting to the adult and this is consistent with the observations that juvenile stages are relatively non-mobile (Sivakumar & Seshadri, 1971).

There is an indication from the results that the females survive desiccation and control their rate of water loss more effectively at 80 % R.H. than males. The significance of this under natural conditions is unclear but it is interesting that Linford and Oliveira (1940) considered that only the females feed on host plants and there have been reports of parthenogenetic populations from Japan (Nakasono, 1983). Perhaps survival of the females rather than males is relatively more important to the persistence of populations of *R. reniformis*.

The retained moulted cuticles of *R. reniformis* aid in desiccation survival by slowing the rate of drying of the enclosed adult. In the animal parasitic nematode, *Haemonchus contortus*, the J2 cuticle is retained by the J3 infective juvenile and the ensheathed form survives desiccation better than the exsheathed form. Ellenby (1968c) showed that when ensheathed individuals were exposed to desiccation at 47 % R.H., the sheath dried

first and slowed the rate of drying of the enclosed juvenile. However, even under these severe conditions of desiccation, the ensheathed individuals of *H. contortus* can survive for up to 4 weeks (Ellenby, 1968c), whereas ensheathed *R. reniformis* can survive for minutes only even at 80 % R.H.

Control of the rate of drying is an important factor in desiccation survival and nematodes able to enter the anhydrobiotic state often have mechanisms to control the rate of water loss (Evans & Perry, 1976). Individual *Ditylenchus dipsaci* J4 can survive considerable periods of severe desiccation (Perry, 1977b). The cuticle of this species is instrumental in controlling rate of water loss (Ellenby, 1968a; Perry, 1977a) and there is evidence that membrane repair (Wharton, Barrett & Perry, 1985) and the reestablishment of the cuticular permeability barrier (Wharton *et al.*, 1988) are central to successful emergence from anhydrobiosis during rehydration. However, whereas individual *D. dipsaci* and ensheathed *H. contortus* control their rate of water loss down to low levels over periods of minutes and hours respectively, they can survive severe desiccation for periods of days and weeks. By contrast the reduced rate of water loss by ensheathed *R. reniformis* only enables individuals to survive for the period that water loss is controlled; they show no ability for prolonged survival once their water content has been reduced to less than 10 %. Thus, while control of water loss enables some species successfully to enter anhydrobiosis, *R. reniformis* shows 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.

Clearly factors other than control of the rate of drying are involved in desiccation survival and probably include morphological, physiological and biochemical mechanisms. The severe drying conditions of the survival and water loss studies in the present work were designed to examine whether the moulted juvenile cuticles aided the survival of individual adult nematodes. Such conditions are unlikely to relate to those experienced by *R. reniformis* in its natural environment where the humidity in soil pores is likely to be very high. In addition to the importance of the moulted cuticles, an environmentally induced slow rate of drying may allow behavioural adaptations that further enhance survival. For example, dehydration of *R. reniformis* on model substrates that mimicked the natural rate of soil moisture loss induced coiling and enabled individuals to survive extended periods of desiccation (Womersley & Ching, 1989). Thus, structural and behavioural adaptations considerably enhance the chances of survival in dry soils and this species seems well adapted to meet the survival requirements under natural conditions.

In naturally slow drying soils *R. reniformis* can survive in the absence of host crops (Sehgal & Gaur, 1988, 1989) although alternate wetting and drying of infested soils resulted in considerable reduction in populations (Gaur

& Sehgal, 1989). In *D. dipsaci*, repeated cycles of desiccation and rehydration progressively reduced the percentage of viable J4 but this was not associated with any decrease in the ability to control drying (Perry, 1977c). In both species a combination of factors, including energy depletion, probably contributed to mortality. However, it would be interesting to determine if the activation and subsequent exsheathment of adults of *R. reniformis*, thus making them vulnerable to drying, was a major factor in the population decline observed by Gaur and Sehgal (1989).

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