

## Desiccation survival of the rice stem nematode *Ditylenchus angustus*

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**Summary** – The desiccation survival of third (J3) and fourth (J4) stage juveniles and adults of *Ditylenchus angustus* was examined at different relative humidities on glass slides, on agar and agarose substrates and in infested rice stems and seeds. Individual nematodes show no intrinsic ability to control water loss and survive severe desiccation. Nematodes of all three stages are dependent on high humidities and/or protection by plant tissue for long-term survival. No single stage can be termed a “survival” stage; J3, J4 and adults showed similar survival attributes although J4 was consistently superior. J4 predominates in infested plant material and it is likely that J4 will be the principal stage involved in survival of the species after harvest. The presence of viable, dry juveniles and adults of *D. angustus* on freshly harvested rice seeds may be of importance for the dissemination of this species.

**Résumé – Survie du nématode des tiges du riz, *Ditylenchus angustus*, à la dessiccation** – La survie en état de dessiccation d’adultes et de juvéniles des 3<sup>e</sup> (J3) et 4<sup>e</sup> stades (J4) de *Ditylenchus angustus* a été étudiée à différentes humidités relatives, sur lames de verre, dans l’agar ou dans des substrats à base d’agar, ainsi que dans les tiges et les semences de riz. Le nématode ne fait montre d’aucune capacité à contrôler sa perte en eau et à survivre à une dessiccation très prononcée. Quel que soit leur stade, les nématodes sont dépendants d’une humidité élevée et/ou de la protection assurée par les tissus de la plante pour survivre de façon prolongée. Aucun stade ne peut être qualifié de « stade de survie » : J3, J4 et adultes montrent les mêmes caractéristiques de survie, encore que les J4 n’apparaissent plus performants. Les J4 prédominent dans le matériel végétal infesté et il est vraisemblable qu’ils constituent le stade principalement en cause pour la survie de l’espèce après la récolte. La présence d’adultes et de juvéniles desséchés, mais viables, dans les semences de riz peut jouer un rôle important dans la dissémination de l’espèce.

**Key-words** : Desiccation, survival, *Ditylenchus angustus*, dissemination.

The plant parasitic nematode, *Ditylenchus angustus*, is the cause of “Ufra” disease of rice and can cause devastating plant damage and crop loss. Mondal and Miah (1987) reported that 60-70 % of low lying areas in Bangladesh are now infested with *D. angustus*. In Thailand, Hashioka (1963) estimated the loss in grain yield due to this nematode was between 20-90 %. In Vietnam, Cuc and Kinh (1981) reported that 50 to 100 % loss occurred in deepwater, irrigated and lowland rice. Substantial crop losses of 90 % were observed in West Bengal (Pal, 1970) and in India yield loss of up to 30 % has been recorded (Rao *et al.*, 1986).

*Ditylenchus angustus* has been reported to survive for several months in dry plant material, mainly in the panicles and leaf sheaths (Butler, 1913; Cox & Rahman, 1979; Kinh, 1981). Controlling this nematode is difficult and chemical control methods are expensive and becoming unacceptable worldwide on environmental grounds. There is an urgent need to develop alternative control strategies, such as interference with nematode behaviour, as part of an integrated control programme. Such measures depend on a knowledge of the biology of *D. angustus* but there is little information about the relative survival ability of different stages and the accumulation and dissemination of nematodes.

As part of a programme of research on population

dynamics, development and survival of *D. angustus*, this paper presents results of investigations on the ability of third (J3) and fourth (J4) stage juveniles and adults of *D. angustus* to survive desiccation as individuals, on model substrates, in stems and on seeds and also the ability of these stages to control water loss. Preliminary studies showed that very few second stage juveniles (J2) were able to survive desiccation, even at the highest humidities and when protected by plant tissue, so this stage was not included in survival comparisons.

### Materials and methods

#### EXPERIMENTAL ANIMALS

Infected samples from rice crops, cv. Chet Som (local variety), showing symptoms of attack by *D. angustus* were received from Hau Giang province, Vietnam via Dr. R. Plowright, International Institute of Parasitology, St. Albans. Nematodes were extracted and identified before being inoculated on rice plants, cv. IR-36, kept in thermostatically controlled water tanks in a glasshouse at 28 °C and 87-90 % relative humidity (RH) to mimic lowland growing conditions. Nematodes were extracted from actively growing rice plants which were cut into small (1-2 cm) pieces and placed in artificial tap water (ATW; Greenaway, 1970); nematodes were used for

experimentation within 2 h of the start of extraction. Active nematodes were selected individually from nematode suspensions using total body length as the criterion for stage differentiation (Ibrahim & Perry, unpubl.).

#### DESICCATION OF NEMATODES ON GLASS SLIDES

Experimental techniques were similar to those of Perry (1979a). Nematodes were transferred individually to a drop of water on clean glass microscope slides. Before the slides were placed in constant humidity chambers, small pieces of filter paper were used to remove all superficial water from around the nematodes to prevent coiling and clumping (Perry, 1977a). Freshly activated silica gel was used as the desiccant for 0 % RH; glycerol/water solution (Grover & Nicol, 1940) were used for other humidities. To check humidity, paper hygrometers were placed inside the chambers. Desiccation was done at 23 °C for various periods at each humidity; 50 individuals of each stage were desiccated at each period.

After desiccation, slides were removed from the desiccation chambers and ATW was added immediately to the nematodes. Revival was checked at intervals until it reached a maximum; the criterion for revival was nematode movement. All slides were kept in covered Petri dishes at 23 °C and oxygenated ATW was added when necessary to replenish that lost by evaporation.

#### DESICCATION OF NEMATODES ON MODEL SUBSTRATES

In principle, experimental techniques were similar to those of Womersley and Ching (1989). Two model substrates, 0.5 % agar and 1 % agarose, were used to determine survival and morphological changes of *D. angustus*. Batches of approximately 50 nematodes of each stage were transferred to separate watchglasses, each containing 1 ml of either agar or agarose. Wherever possible, the stages were studied simultaneously, a series of watchglasses being left at 0, 40, 60, 80 and 97 % RH at 23 °C for various times. After each exposure time, the watchglasses were removed from the humidity chambers, ATW was added to the nematodes and revival checked at intervals until it reached a maximum. The procedure was repeated until, for each humidity, the time of exposure which 50 % of the nematodes of each stage would survive ( $S_{50}$  value) was determined.  $S_{50}$  values have been used by Ellenby (1968) and Perry (1977a), among others, to demonstrate marked differences in survival between stages and species of nematodes as a basis for studies on survival mechanisms.

In a separate series of experiments, nematodes of each stage in watchglasses, set up as above, were examined daily under a binocular microscope. During this procedure, a cover slide was placed on the top of the watchglass to minimise variations in the relative humidity.

Details of the coiling response of stages of *D. angustus* to

drying on agar and agarose were recorded by noting the number of individuals which were either loosely coiled or tightly coiled. Loose coiling occurred when the inactive nematode assumed a circular shape with anterior and posterior parts of the individual overlapping and touching; tight coiling was a progression from loose coiling when the nematode assumed the configuration of a watch spring, giving the maximum reduction in nematode surface area exposed to drying.

#### DESICCATION OF NEMATODES IN SEEDS AND STEMS

Mature rice plants, with well developed panicles, heavily infested with *D. angustus*, were cut at ground level. The above ground portions were placed into desiccation chambers at 40, 60, 80 and 97 % RH. At intervals of two months, a stem piece 5-7 cm long (mean weight  $0.403 \pm 0.05$  g;  $n = 12$ ) was cut into small sections and placed in ATW in a Petri dish at room temperature. At the same time, sections of panicles containing five to ten seeds (mean weight  $0.053 \pm 0.011$  g;  $n = 12$ ) were rehydrated in separate Petri dishes. At intervals during the subsequent 7 days, active nematodes were removed from the dishes, counted and their stage recorded. At the end of the revival period, the stem pieces or seeds were carefully teased apart to release any remaining nematodes; these were counted and their stage recorded. Where there were large numbers of nematodes present, counts were based on a 1 ml aliquot. For each stage, survival was represented by the number of actively moving nematodes expressed as a percentage of the total number of active and dead nematodes of the stage.

To determine the rate of moisture-loss of stems at each humidity level, stem pieces were weighed for two weeks at intervals (see Fig. 6).

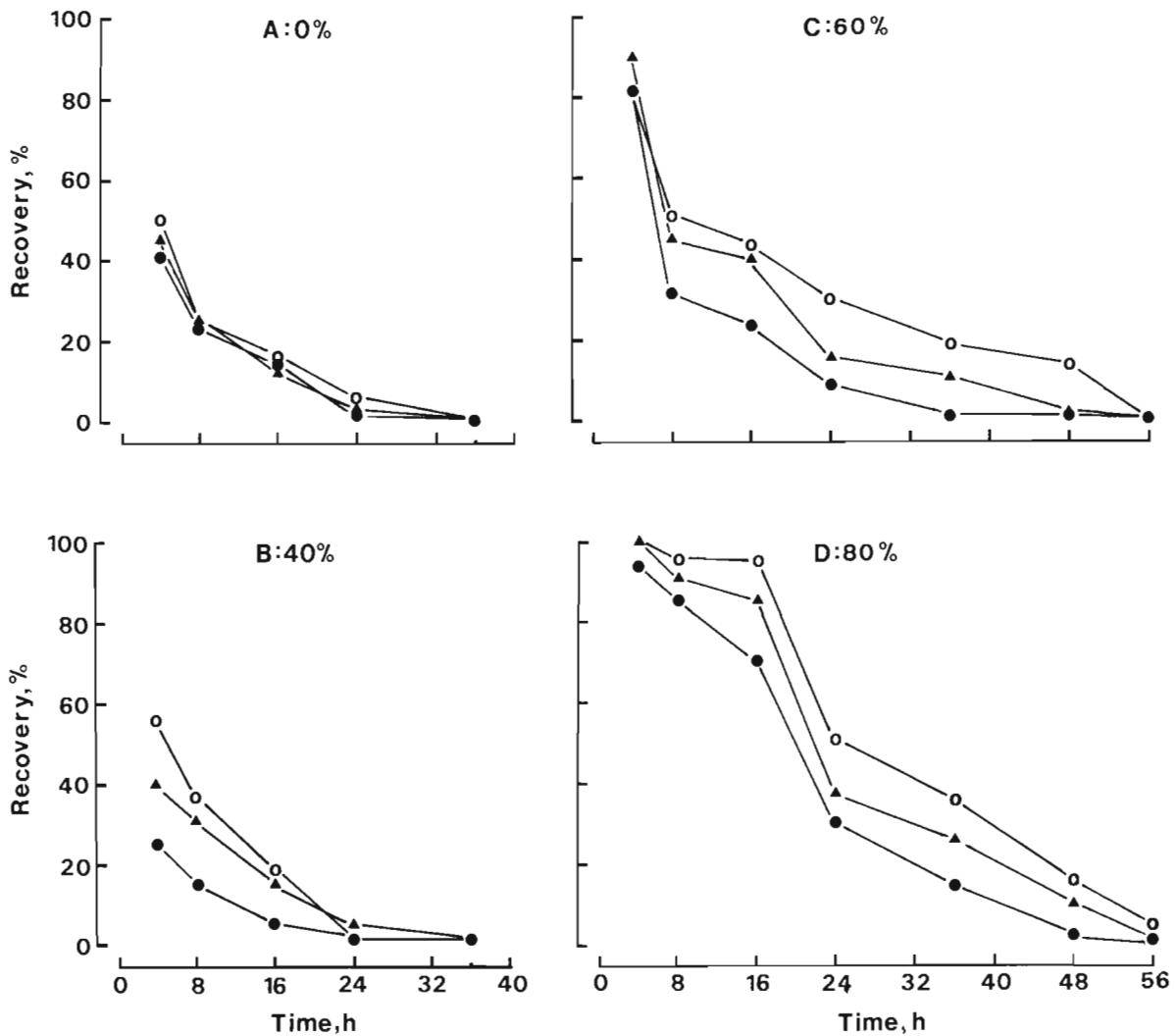
#### MEASUREMENT OF WATER LOSS

J3, J4 and adults of *D. angustus* were desiccated on glass slides for various times at 0, 60 and 80 % RH using techniques described by Perry (1977b). After the desiccation period, liquid paraffin was added to cover the specimens and a cover-slip placed in position. The water content of individual nematodes was determined by quantitative interference microscopy (Ellenby, 1968). Data were analysed by two way analysis of variance using refractive index determinations, rather than the water content values derived from them, for reasons given by Ellenby and Perry (1976).

## Results

#### DESICCATION OF NEMATODES ON GLASS SLIDES

Survival of J3, J4 and adults increased with increase in RH and, at each humidity, decreased with increase in period of desiccation (Fig. 1). Between 40-50 % of all three stages survived 4 h at 0 % RH but survival decreased to less than 10 % after 24 h exposure. Under the least severe desiccation conditions of 80 % RH, survival



**Fig. 1.** The survival of third (●) and fourth (○) stage juveniles and adults (▲) of *Ditylenchus angustus* after desiccation as individuals on glass slides at 0, 40, 60 and 80 % RH for various periods.

was only reduced to below 50 % after 24 h exposure. Although the survival of the three stages was similar at each of the four humidities tested, J4 were always marginally better than J3 or adults. Revival after exposure to 0 % RH started after 2 h rehydration whereas, at higher humidities, revival was more rapid, often starting within 30 min. of immersion in ATW.

#### DESICCATION OF NEMATODES ON MODEL SUBSTRATES

The  $S_{50}$  values for J3, J4 and adults desiccated on 0.5 % agar and 1 % agarose at different RHs (Fig. 2) show clearly that nematodes survived desiccation on agarose better than on agar. For all stages, the  $S_{50}$  value

at humidities up to and including 60 % RH did not exceed 10 days. However, at higher humidities survival was considerably enhanced. For example, at 80 % RH the  $S_{50}$  values for J3, J4 and adults on agarose were 20.5, 40.0 and 27.5 days, respectively, while the equivalent values on agar were 12.0, 17.0 and 15.0 days (Fig. 2).

As with the data for survival on slides, results from tests on model substrates show that J4 are able to survive longer than J3 or adults. The maximum  $S_{50}$  value was 58.0 days for J4 on agarose at 97 % RH; under these conditions the values for J3 and adults were 34.5 and 45.0 days, respectively (Fig. 2).

The percentages of nematodes assuming loosely or

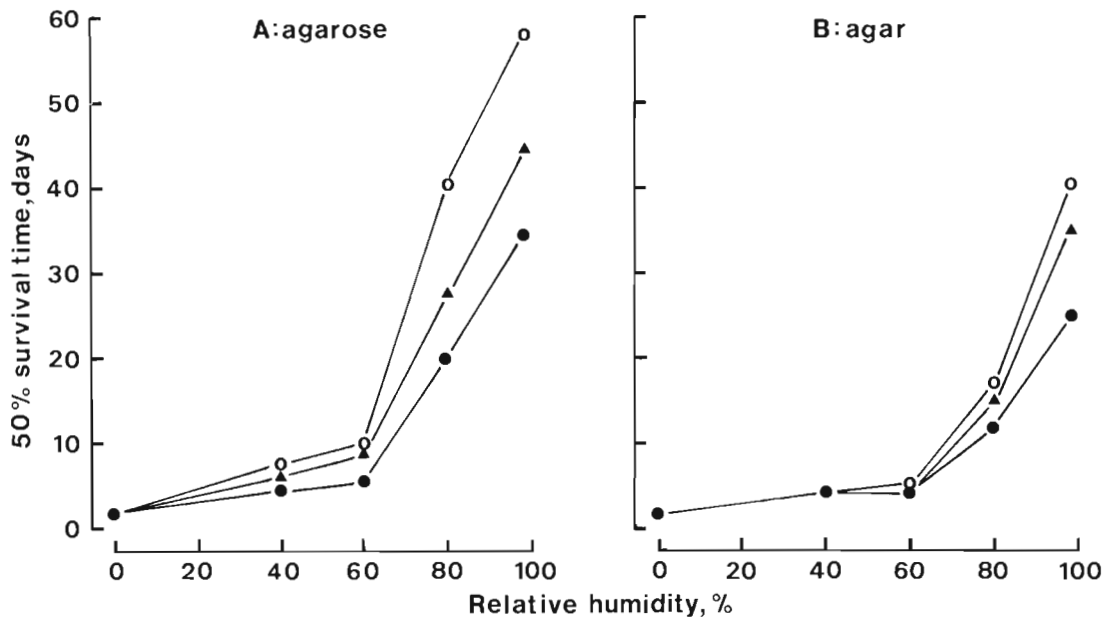


Fig. 2. The 50% survival time ( $S_{50}$ ) of third (●) and fourth (○) stage juveniles and adults (▲) of *Ditylenchus angustus* after desiccation at 0, 40, 60, 80 and 97% RH on agarose and agar.

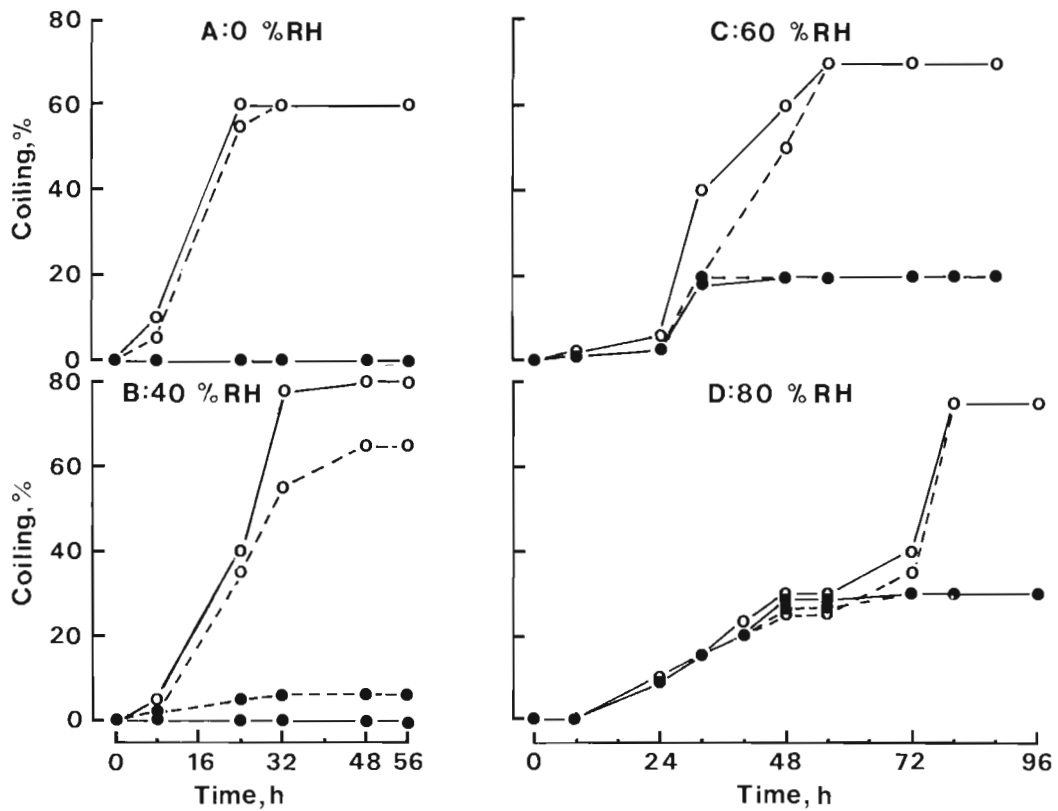


Fig. 3. The percentage of *Ditylenchus angustus* showing a loosely (○) or tightly (●) coiled response to desiccation at 0, 40, 60 and 80% RH on agarose (—) and agar (---).

tightly coiled postures after desiccation on agar or agarose at different RHs for various period of times are given in Fig. 3. Tight coiling was restricted to J3 and J4. Drying at 0 and 40 % RH was too rapid to allow the nematodes to coil tightly on either substrate; only a few (3 %) coiled tightly on agar (Fig. 3). At 60 and 80 % RH, the percentage of tightly coiled individuals increased to 20 % and 30 % respectively.

The maximum percentage of loose coiling was reached very rapidly on both substrates at low humidities but the response was slower with more gradual desiccation at higher humidities; for example, at 0 % RH a maximum of 60 % of the nematodes were loosely coiled within 32 h exposure whereas at 80 % RH the maximum value of 75 % was not achieved until 85 h exposure (Fig. 3). In general, loose coiling of nematodes desiccated on agarose occurred sooner than on agar. At 97 % RH, nematodes of all stages remained active.

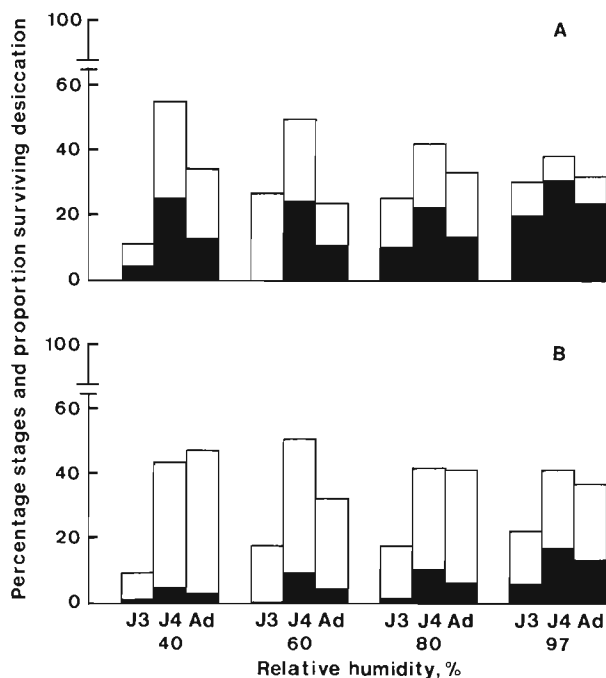
#### DESICCATION OF NEMATODES IN SEEDS AND STEMS

Seeds and plant stems were examined after 2, 4 and 6 months storage at various RHs; the number of J3, J4 and adults present as a percentage of the total number of active and dead nematodes extracted and the proportion of each of these stages which survived are presented in Fig. 4 for experiments with seeds and Fig. 5 for experiments with stems. Data for the 6 month desiccation pe-

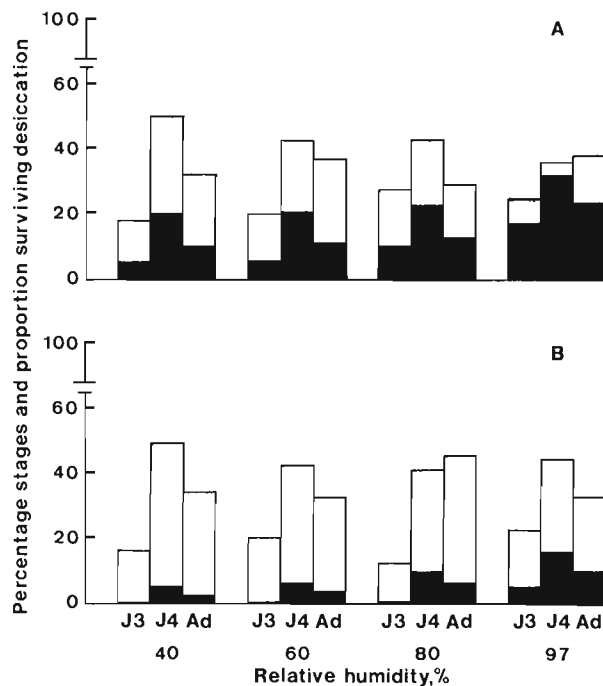
riod are not included because no nematodes survived at 60 % RH and the maximum number surviving at other humidities was 14 (of which 10 were J4) in seeds stored at 97 % RH; thus, survival in both seeds and plant tissue for 6 months was negligible.

There were  $405.1 \pm 56.6$  nematodes present in each stem piece (equivalent to 1005 nematodes per g of tissue) and  $527.8 \pm 123.7$  nematodes in each batch of five to ten seeds (equivalent to 9940 nematodes per g of seeds). Overall, the percentage of J3, J4 and adults was 21.1 %, 43.3 % and 35.6 %, respectively, in stems and 22.2 %, 44.4 % and 33.4 %, respectively, in seeds. Results for each humidity at 2 and 4 months storage conform to this pattern; all stages were present with J4 and adults predominating.

The survival of *D. angustus* in seeds (Fig. 4) and stems (Fig. 5) was similar and the data confirm and extend results from experiments on slides and model substrates: although J4 survived desiccation better than other stages tested, J3 and, especially, adults were also able to survive dehydration for periods of 2 months or more. J3 was consistently the poorest at surviving desiccation with less than 10 % surviving 4 months at 40, 60 and 80 % RH; at 97 % RH 25.3 % survived in seeds and 22.1 % in stems. J4 survived better than adults at all humidities (Figs 4, 5). For example, at 40, 60, 80 and 97 % RH, 45.1, 48.1, 52.4 and 79.4 %, respectively, of



**Fig. 4.** The percentage of third and fourth stage juveniles and adults of *Ditylenchus angustus* present (□) and the proportion of each stage which survived (■) in rice seeds kept for A) 2 months and B) 4 months at 40, 60, 80 and 97 % RH.



**Fig. 5.** The percentage of third and fourth stage juveniles and adults of *Ditylenchus angustus* present (□) and the proportion of each stage which survived (■) in stems of rice plants kept for A) 2 months and B) 4 months at 40, 60, 80 and 97 % RH.

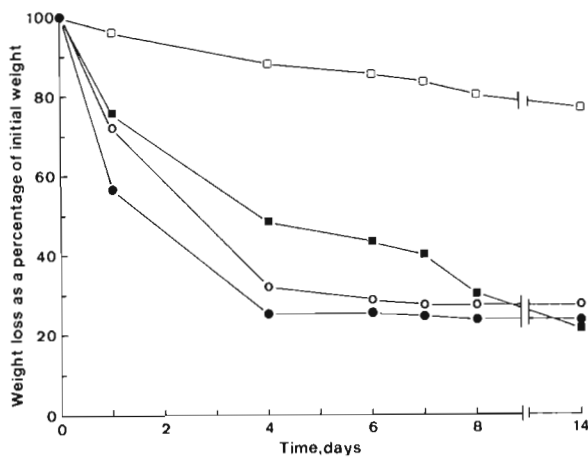


Fig. 6. The rate of weight loss of stems of rice plants after drying for various periods at 40 (●), 60 (○), 80 (■) and 97 % RH (□).

J4 survived in seeds for 2 months; the equivalent values for survival of adults were 37.1, 44.7, 39.3 and 75.0 %. Even after 4 months storage at 60 % RH, approximately 25 % of J4 survived in either seeds or stems. At 97 % RH fungal contamination was noted on the plant tissue, especially after long periods of storage, which may have adversely affected nematode survival.

The weight loss of stems, as a percentage of the original weight, was less than 25 % after 14 days at 97 % RH (Fig. 6). Water loss from stems dried at 40 and 60 % RH occurred during the first four days exposure and subsequently stabilised; the rate of drying of stems at 80 % RH slowed after the first day (Fig. 6). When seeds were stored at these humidities, the maximum loss in weight was 17.4 % after 14 days at 40 % RH.

WATER LOSS STUDIES

There was no single stage with a marked ability to control its rate of water loss (Fig. 7). The rate of drying at 0 % RH was rapid for all stages, with water contents being reduced to below 20 % within 5 min. Increase in humidity reduces the rate of drying; for example, at 0 % RH the water content was below 10 % within 10 min, whereas after 10 min. at 80 % RH the water content of adults and J3 was between 30 and 40 % and was over 45 % for J4 (Fig. 7). At 0 and 60 % RH, there was no significant difference ( $P > 0.05$ ) in the rate of water loss of J3, J4 and adults, whereas, at 80 % RH, the rate of water loss of J4 during 20 min. exposure was significantly slower ( $P < 0.001$ ) than that of the other two stages. At this latter humidity, Figure 7 indicates that the drying of *D. angustus* can be divided into at least three phases. The first phase is a rapid loss of water during the first 10 min. of drying; the second phase is an apparent degree of stability of water content of individuals between 10 min of drying; the second phase is an apparent de- after 15 min. Subsequent water loss (possibly a fourth

phase of drying) is slow, probably due to the prior removal of almost all available water.

Discussion

The genus *Ditylenchus* includes species showing differing abilities and strategies to survive desiccation. With *D. dipsaci* it is the J4 which accumulates during host plant senescence and which has remarkable abilities to survive desiccation (Ellenby, 1968; Perry, 1977a); by contrast, *D. myceliophagus* is poor at surviving desiccation and all stages accumulate when food is exhausted (Perry, 1977a). *D. angustus* is a serious problem in rice

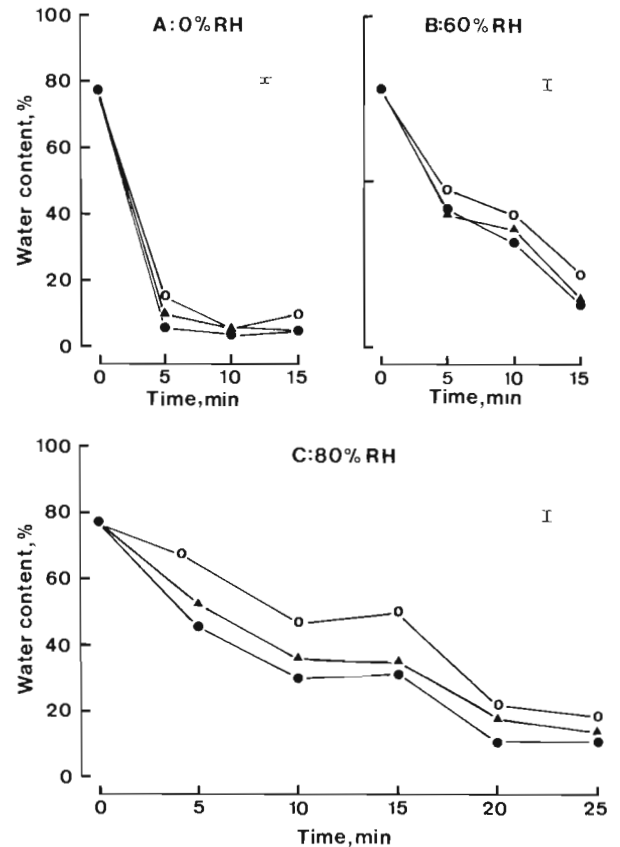


Fig. 7. The change in water content of third (●) and fourth (○) stage juveniles and adults (▲) of *Ditylenchus angustus* after desiccation as individuals at 0, 60 and 80 % RH. Vertical bars are the standard error of the difference at each humidity.

growing regions (Bridge *et al.*, 1990) and it is known to survive and to be disseminated in dry crop residues, mainly panicles enclosed or partially enclosed in leaf sheaths (Cox & Rahman, 1979; Kinh, 1981). The population dynamics and accumulation of stages of *D. angustus* during host senescence is the subject of a separate study (Ibrahim & Perry, unpubl.) but it is clear from the

present work that in infested old rice plants accumulation of one specific life cycle stage does not occur; J3, J4 and adults were all present in stems and seeds, although J4 generally predominated.

The survival studies demonstrated that no single stage of *D. angustus* can be termed a "survival" stage; J3, J4 and adults showed similar survival attributes although J4 was consistently superior. Perry (1977a) argued that, although the ability of J4 of *D. dipsaci* to survive desiccation was markedly greater than other stages, the designation as "survival" stage was inaccurate as J3 and adults showed considerable survival attributes. However, *D. dipsaci* is an endoparasite and the J4 is the infective stage which accumulates at the end of the host's growing season, so survival of the species between seasons depends on the J4. *D. angustus* is an ectoparasite, with J3, J4 and adults all capable of invading a host (Ibrahim & Perry, unpubl.), and survival of the species is thus not so dependent on a specific stage; in this respect *D. angustus* is similar to *D. myceliophagus* (Perry, 1977a). Of the three species, the survival ability of *D. angustus* is greater than *D. myceliophagus* but markedly inferior to *D. dipsaci* (Perry, 1977a). Although there is no specific "survival" stage of *D. angustus*, the preponderance of J4 in senescing plants, together with the superior survival attributes of this stage means that it is probable that the J4 will be the principal stage that survives drying and subsequently invades new hosts.

At 0 and 85 % RH, J4 of *D. dipsaci* had  $S_{50}$  values of 3.5 days and more than 4 weeks, respectively (Perry, 1977a). The remarkable ability of individual J4 of this species to survive severe desiccation depends partly on a decrease in permeability of the drying cuticle which slows the rate of drying of inner, and perhaps more vital, layers of the nematode (Ellenby, 1968a; Perry, 1977b). The drying curve of J4 can be divided into three phases: an initial rapid loss of water, followed by a degree of stability in the nematode's water content with a final phase of rapid drying (Perry, 1977b). By contrast, when dried as individuals, J3, J4 and adults of *D. angustus* were unable to withstand prolonged desiccation at low humidities; their survival was for hours only. There was also no indication from the water content studies that individuals had any intrinsic ability to control their rate of water loss and the slowing down of water loss, manifest by a degree of stability in the water content, was only apparent in *D. angustus* at 80 % RH.

The survival and water loss studies indicate that *D. angustus* is dependent on environmental conditions to survive dehydration. A controlled rate of drying successfully induces anhydrobiosis; protection within plant tissue and high humidities, resulting in an induced slow rate of drying, enables several species of nematodes to survive long periods of desiccation (Evans & Perry, 1976). Survival of the reniform nematode, *Rotylenchulus reniformis*, is related to the degree of moisture stress and the initial rate of water loss from the soil (Sehgal & Gaur,

1988, 1989; Womersley & Ching, 1989). In addition, morphological adaptations, such as the retention of the moulted cuticles (Gaur & Perry, 1991), and behavioural adaptations, such as coiling (Womersley & Ching, 1989), enhance survival of this species. Agar and agarose, used as model substrates by Womersley and Ching (1989) to mimic the natural rate of soil moisture loss experienced by *R. reniformis* in drying soils, were used in the present work to examine the coiling response of *D. angustus* to slow drying conditions. Compared to experiments using glass slides, survival was increased on both substrates from hours to weeks but the percentage survival was greater on agarose than agar. On agar the nematodes migrated into the substrate and remained there, especially at low RHs, but some nematodes coiled on or at the edge of the substrate. However, on agarose some nematodes migrated into the substrate but they did not remain there; they moved to the edge of substrate where they coiled. The slow rate of water loss from model substrates at high humidities (Womersley & Ching, 1989) enabled some individuals to coil, although formation of tight coils was never noted with adults. Coiling, as a physical adaptation by some species of nematodes to enhance desiccation survival, has been reported by several workers (for example, Mankau and Mankau, 1964); the reduction of the exposed surface area afforded by coiling is also likely to slow the nematode's rate of drying (Evans & Perry, 1976). On model substrates, *D. angustus* were also seen to aggregate and this clumping was noted in dry stems and, especially, in infested seeds. The aggregation of nematodes, such as occurs with J4 of *D. dipsaci* to form "eelworm wool", is a further behavioural response to facilitate survival during desiccation (Evans & Perry, 1976).

This study confirms the finding of Butler (1913) that *D. angustus* can survive for several months in plant material. It is also interesting that the nematodes can survive in seeds. Seth (1939; quoted by Seshadri and Dasgupta, 1975) observed *D. angustus* on rice grains but Butler (1913) did not record this and the chance of transmission on seed was thought to be minimal (Seshadri & Dasgupta, 1975). However, the present work demonstrates the presence and survival of *D. angustus* in freshly harvested seeds. The nematodes were found mainly between the glumes and the caryopsis, and sterile seeds from heavily infested plants frequently contained masses of coiled, dry *D. angustus* between the glumes; in one seed 2400 nematodes were recorded. Although the "empty" sterile seeds will probably be lost during threshing (Plowright, pers. comm.) other seeds containing nematodes will remain. Clearly, the presence of nematodes in seeds is of importance for dissemination and may enable the species to cross quarantine barriers. However, as rice grains have to be dried to a moisture content of 14 % or below before they can be stored (De Datto, 1981), this will probably be sufficient to eliminate viable *D. angustus* from seeds. The efficacy of seed treat-

ment to ensure *D. angustus* free material is the subject of further study.

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