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Survival of *Ditylenchus destructor* in soil, hulls and seeds of groundnut (1)

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Summary – The survival of *Ditylenchus destructor* following storage of infected hulls and seeds of groundnut cv. Sellie and infested soil, and the capacity of *D. destructor* to undergo complete dehydration and enter in a state of anhydrobiosis was investigated. The results show that *D. destructor* can survive in the field in the absence of host plants and in hulls left in the field after harvest for at least 28 to 32 weeks. In contrast, nematode numbers in fragmented hulls and fragmented or whole seeds stored in paper bags at 10 °C (whole seeds) or 22 °C (fragmented hulls and seeds) decreased with increasing storage time, especially during the first 12 weeks. Very few nematodes survived in whole seeds stored in paper bags at 10 °C but the surviving nematodes were able to build up large populations and to cause damage. Finally, the results show that the freshness of the tissues played an important role in the efficiency of the soaking method : stored hulls and seeds must be soaked longer than 24 h to make a reliable estimate of the number of nematodes present.

Résumé – ***Survie de* Ditylenchus destructor *dans le sol et dans les enveloppes et graines d'arachide.*** – La survie de *Ditylenchus destructor* après stockage des enveloppes et des graines d'arachide cv. Sellie infestées et de sol infesté, ainsi que la capacité de *D. destructor* à entrer en anhydrobiose ont été étudiées. Les résultats montrent que *D. destructor* peut survivre pendant au moins 28 à 32 semaines en champ en l'absence de plantes hôtes dans les enveloppes restant sur place après récolte. Par contre, le nombre de nématodes dans les fragments d'enveloppes et de graines, ou dans les graines entières, stockés dans des sacs en papier à 10 °C (graines entières) ou à 22 °C (fragments d'enveloppes et de graines), diminue lorsque le temps de stockage augmente, surtout pendant les 12 premières semaines. Un faible nombre de nématodes survit dans les graines entières stockées à 10 °C dans des sacs en papier, mais ces nématodes sont capables de produire d'importantes populations et de causer des dégâts. Finalement, les résultats montrent que la fraîcheur des tissus joue un rôle important dans l'efficacité de la méthode de trempage : enveloppes et graines stockées doivent tremper plus de 24 heures pour que l'on puisse obtenir une estimation correcte du nombre des nématodes présents.

Key-words : *Ditylenchus destructor*, survival, groundnut.

Since its discovery on groundnut in South Africa (Jones & De Waele, 1988), *Ditylenchus destructor* Thorne, the potato rot nematode, has emerged as an important pest of groundnut (*Arachis hypogaea* L.) in this country. A nationwide survey revealed its presence in seeds of groundnut in all major groundnut-producing areas (De Waele *et al.*, 1989) while greenhouse experiments demonstrated that initial population densities of *D. destructor* as low as 50 nematodes per seedling caused severe damage to the appearance and yield of seeds of Sellie, the most widely grown groundnut cultivar in South Africa (Venter *et al.*, 1991). Outside South Africa, *D. destructor* has not been found on groundnut but is most important in the northern hemisphere as a pest of potato tubers and tubers of bulbous flowers (Hooper, 1973). The South African populations are considered to belong to a different ecotype (De Waele & Wilken, 1990) and race (De Waele *et al.*, 1991).

Since under field conditions, plants grown from nematode-free groundnut seeds are attacked by *D. destructor* following the dry winter season during which no crops are cultivated, *D. destructor* can survive in the field in the absence of a host plant. To overcome unfavourable environmental conditions and extend their survival, many nematodes have the capacity to depress their metabolic activity and arrest their development (Antoniou, 1989). Information on survival strategies of *D. destructor* as well in the field as in stored seeds of groundnut is lacking. This information, however is needed, firstly, to assist in the estimation of nematode numbers at the beginning of the growth season since the preplant damage threshold levels for *D. destructor* are near the reliable detection level (Venter *et al.*, 1991) and, secondly, to prevent the spread of *D. destructor* through infected seeds.

The objectives of this study were to study under lab-

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oratory and greenhouse conditions *i)* the survival capacity and virulence of *D. destructor* following storage of infected hulls and seeds of groundnut and infested soil and *ii)* the capacity of *D. destructor* to undergo complete dehydration and enter a state of anhydrobiosis.

Materials and methods

EXPERIMENT 1

Soil (85 % sand, 8 % silt, 7 % clay) containing root fragments was collected immediately after harvest from a groundnut field infested with *D. destructor* and stored in an open 100 l container at 10 °C. Every 4 weeks, during 28 weeks, six 20-cm-diameter plastic pots were filled with this soil and planted with grain sorghum [*Sorghum bicolor* (L.) Moench] cv. DC 99, which is susceptible to *D. destructor* (Basson *et al.,* 1990). After 32 weeks, six 20-cm-diameter plastic pots were filled with the remaining soil and planted with nematode-free seeds of groundnut (*Arachis hypogaea* L.) cv. Sellie. Grain sorghum and groundnut seedlings were thinned to one per pot after emergence. The pots were regularly irrigated with a hydroponic nutrient (6.5 % N, 2.7 % P, 13 % K) dissolved in tap water and maintained in a greenhouse at 17-25 °C and a 13-hour photoperiod. Six weeks after planting, the grain sorghum plants were harvested and the fresh root weights determined. Nematodes were extracted from 5 g fresh roots using a centrifugal-flotation method (Coolen & D'Herde, 1972) and counted. Twenty-one weeks after planting, the groundnut plants were harvested. Nematodes were extracted from 5 g fresh hulls and 5 g fresh seeds by a soaking method (Bolton *et al.,* 1990) and counted.

EXPERIMENT 2

Fresh hulls and seeds of groundnut cv. Sellie heavily infected with *D. destructor* were fragmented and stored in paper bags at 22 °C. Every four weeks, during twenty weeks, 5 g hulls and 5 g seeds were soaked in tap water (Bolton *et al.,* 1990) for ten days at 22 °C. The nematodes which emerged from the tissues were counted daily.

EXPERIMENT 3

Fresh hulls and seeds of groundnut cv. Sellie were obtained from an infested field. Hulls and seeds were first rated for severity of disease symptoms (Venter *et al.,* 1991). Only hulls with a high rating and blemished seeds were selected. The hulls were fragmented into four quarters and mixed with steam sterilized sandy soil. The soil was then stored in an open container at 10 °C for 28 weeks to imitate hulls left in the soil in the field during the dry winter season. The whole seeds were stored in paper bags at 10 °C. Every 4 weeks, during 28 weeks, twenty-four 20-cm-diameter plastic pots containing a steam sterilized sandy soil (85 % sand, 8 % silt, 7 % clay) were planted as follows : eight pots with two infected seeds each; eight pots with nematode-free seeds of groundnut cv. Sellie plus a fragmented (four quarters) hull; eight pots with only nematode-free seeds of groundnut cv. Sellie. After emergence, the seedlings were thinned to one per pot. The pots were irrigated and maintained in a greenhouse as described for experiment 1. Twenty-four weeks after planting, the groundnut plants were harvested and the fresh root, peg, hull and seeds weights determined. The hulls and seeds were rated for severity of disease symptoms (Venter *et al.,* 1991). Nematodes were extracted from 5 g fresh roots and 1 g fresh pegs by a centrifugal-flotation method (Coolen & D'Herde, 1972) and from 5 g fresh hulls and 5 g fresh seeds by a soaking method (Bolton *et al.,* 1990).

To determine the number of *D. destructor* present in the stored hulls and seeds at each planting date, three Petri dishes each with two stored seeds and three Petri dishes each with a stored hull fragment, were soaked in tap water (Bolton *et al.,* 1990) on each planting date, and the nematodes which emerged from the tissues counted.

EXPERIMENT 4

D. destructor of various life stages were obtained from monoxenic cultures on groundnut callus tissue (Van der Walt & De Waele, 1989). Thirty-five juveniles or 35 adults were handpicked and transferred onto 25 mm Millipore ® filter discs (0.65 µm pores). Perspex rings (24 mm in diameter, 3 mm high) contained the nematodes on each filter disc. Six discs with juveniles and six with adults were placed on watch glasses in humidity chambers (Simons, 1973; Demeure *et al.,* 1979) at 23 °C. The relative humidity (rh) within each chamber was regulated by glycerin-water solutions and by phosphorous pentoxide (0 % rh; Simons, 1973). Nematodes were dehydrated by successive exposure to 100 % rh for 24 h, 99.4 % rh for 24 h, 98.8 rh for 4 days and 0 % rh for 24 h. Then, the number of coiled nematodes was counted. Nematodes were rehydrated by successive 24 h exposure to 96, 97.7, 98.8, 99.4 and 100 % rh. They were then placed for 24 h in tap water and the number of active nematodes counted. To determine the virulence of the surviving nematodes, two seedlings grown from nematode-free seeds of groundnut cv. Sellie in 20-cm-diameter plastic pots containing a steam sterilized sandy soil (85 % sand; 8 % silt, 7 % clay) were each inoculated with a mixture of about 40 juveniles and adults. The pots were irrigated and maintained in a greenhouse as described for experiment 1. Twenty-one weeks after planting, the groundnut plants were harvested. The hulls and seeds were rated for severity of disease symptoms (Venter *et al.,* 1991). Nematodes were extracted from 5 g fresh hulls and 5 g fresh seeds by a soaking method (Bolton *et al.,* 1990) and counted.

In the experiments 1 and 3, storage time and nematode numbers recovered from roots, hulls or seeds were compared using regression analysis. In experiment 2,

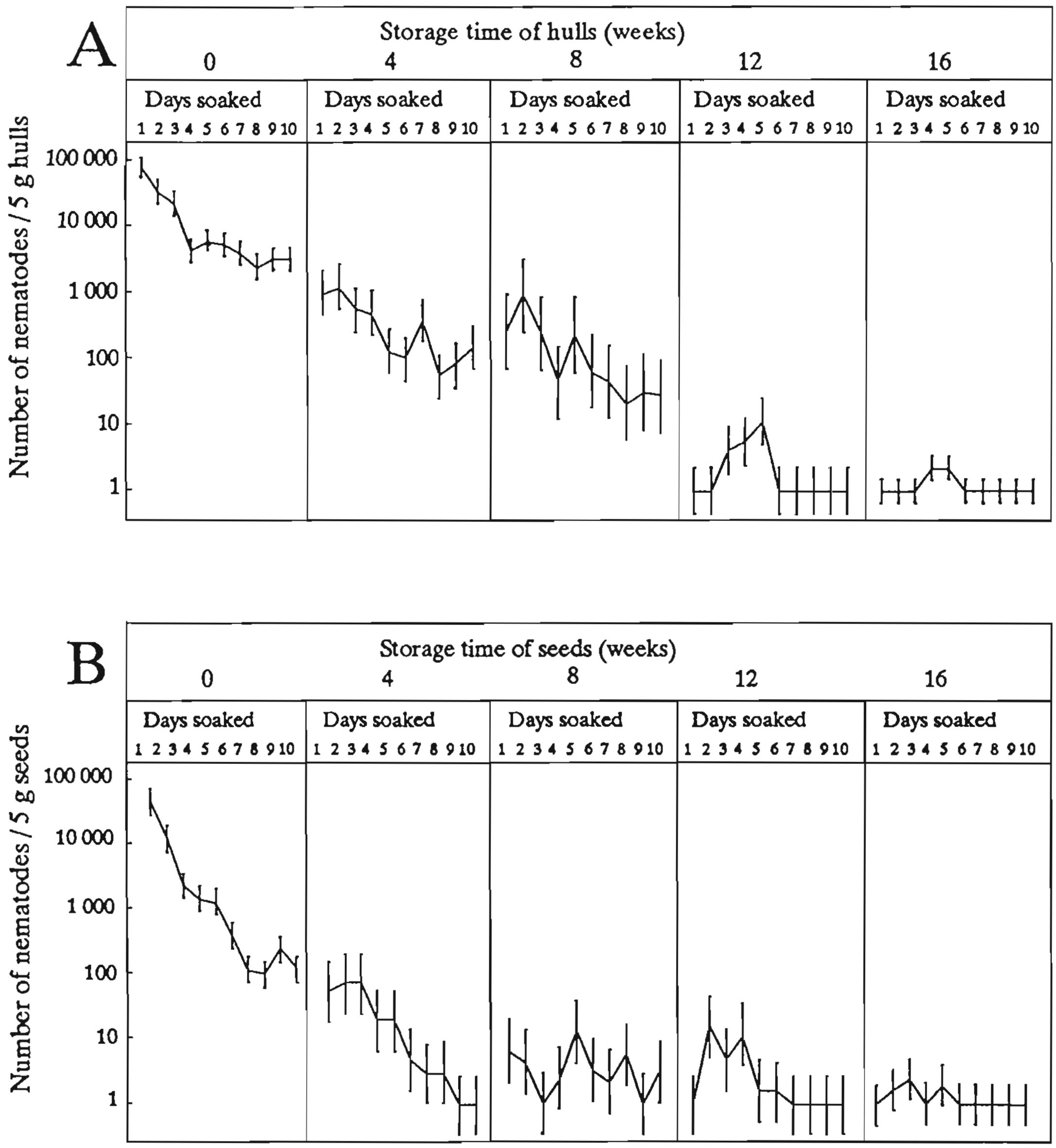

Fig. 2. Mean number (± 95 % confidence limits) of *Ditylenchus destructor* recovered from infected fragmented hulls (A) and fragmented seeds (B) of groundnut cv. Sellie stored in paper bags at 22 °C.

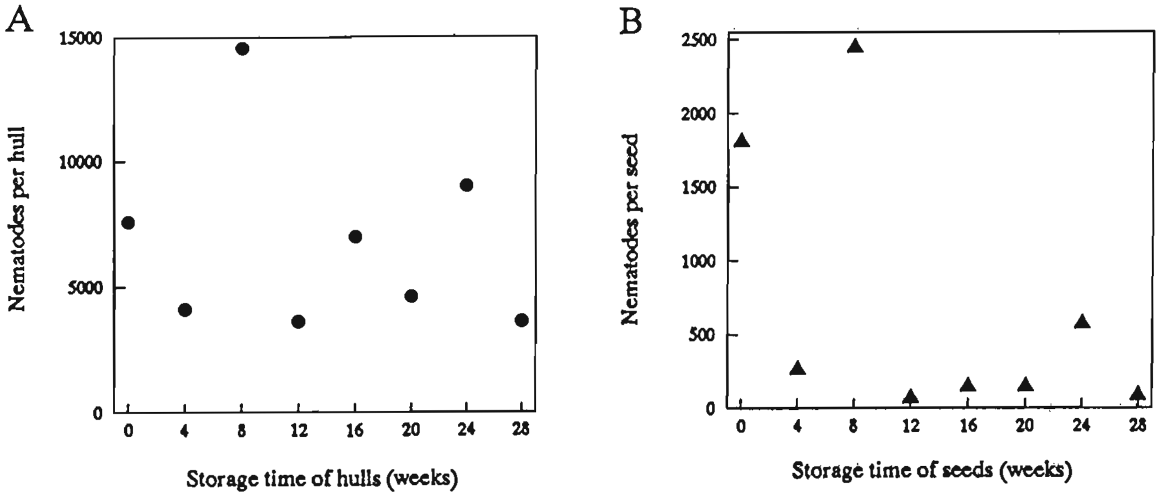

Fig. 3. Mean number of *Ditylenchus destructor* recovered from infected groundnut cv. Sellie fragmented hulls stored in soil at 10 °C (A) and whole seeds stored in paper bags at 10 °C (B).

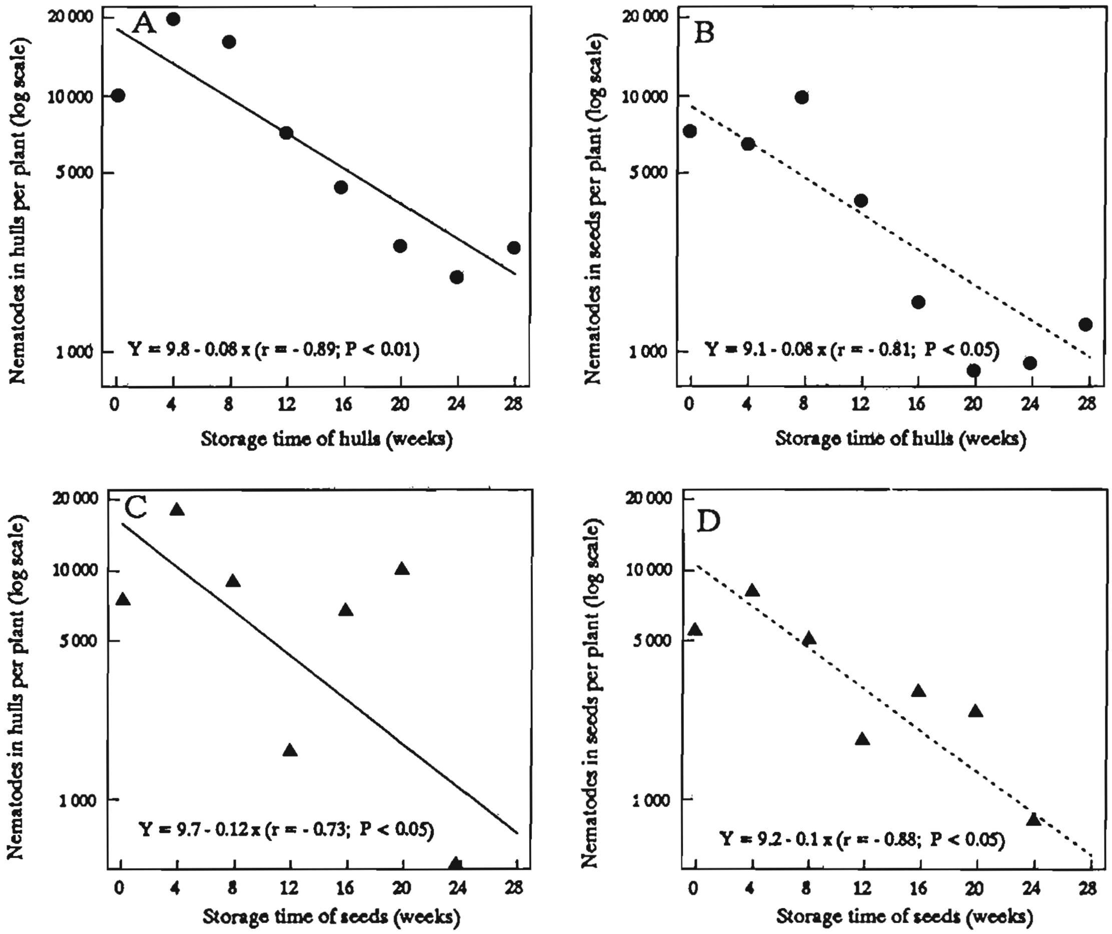

Fig. 4. Correlations between mean number of *Ditylenchus destructor* recovered from hulls (A, C) and seeds (B, D) of groundnut cv. Sellie grown during 24 weeks in soil together with infected fragmented hulls stored in soil at 10 °C (B, D) and whole seeds stored in paper bags at 10 °C (A, C).

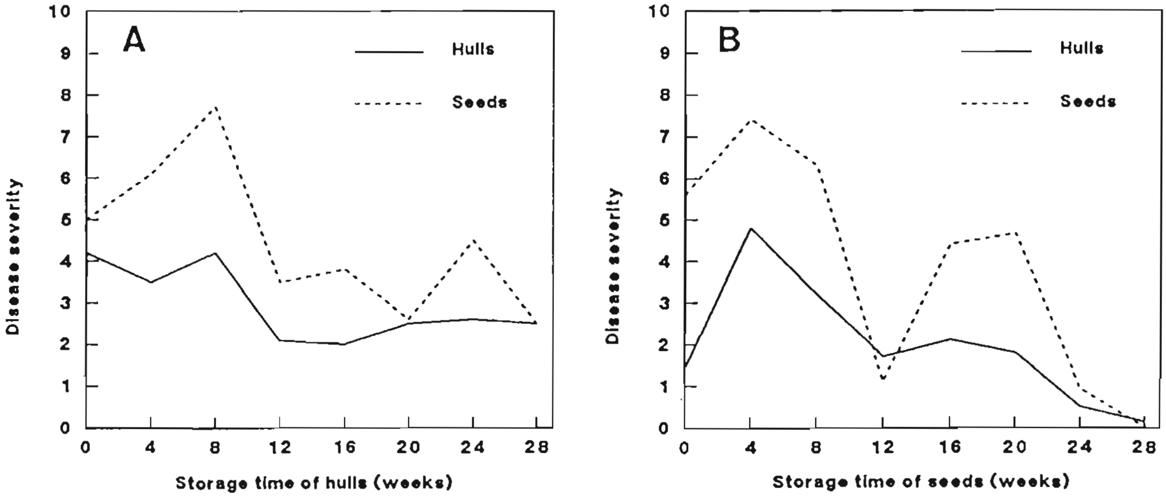

Fig. 5. Disease severity of hulls and seeds of groundnut cv. Sellie grown during 24 weeks in soil together with *Ditylenchus destructor* infected fragmented hulls stored in soil at 10 °C (A) and whole seeds stored in paper bags at 10 °C (B).

determination of preplant soil population densities since special methods are necessary to extract anhydrobiotic nematodes from soil samples (Freckman *et al.*, 1977). Venter *et al.* (1991) reported that under greenhouse conditions an initial population density of 50 *D. destructor* in the rhizosphere of a groundnut seedling was sufficient to cause economic losses. Since probably many of the nematodes remain in a state of anhydrobiosis until planting, or overwinter in the soil as eggs as has been suggested by Thorne (1961), soil extraction methods will not be reliable for preplant soil population density determination. Fall sampling of fields to estimate the preplant soil population densities for the following growth season or the use of indicator plants, such as grain sorghum cv. DC 99, may be a more reliable alternative although the latter method is elaborate and time-consuming.

The results confirm earlier suggestions that *D. destructor* is able to undergo complete dehydration and enter in a state of anhydrobiosis. A *D. destructor* in coiled position on the surface of a cotyledon of a mature seed was shown by Jones and De Waele (1990). The results further demonstrate that rehydrated *D. destructor* are able to invade groundnut hulls and seeds and to cause damage. De Waele *et al.*, (1990) recovered large populations of *D. destructor* from roots, pegs, hulls and seeds of groundnut grown in infested soil but from which no nematodes were extracted before planting using a modified decanting and sieving method followed by centrifugal-flotation.

In contrast with nematodes in soil and hulls left in soil, nematode numbers in fragmented hulls and fragmented or whole seeds stored in paper bags at 10 °C (whole seeds) or 22 °C (fragmented hulls and seeds) decreased with increasing storage time, especially during the first 12 weeks. This may indicate that these conditions are less suitable for *D. destructor* to undergo complete dehydration and enter in a state of anhydrobiosis. Crowe and Madin (1975) showed that too rapidly dehydrated *Aphelenchus avenae* did not form coils and were killed. *A. avenae* which were dehydrated slowly at 97 % rh for 72 h formed coils and survived.

Although relatively few nematodes survived in whole seeds stored in paper bags at 10 °C, the surviving nematode populations were high enough to build up large populations and to cause damage. Whole seeds stored for up to 24 weeks and then planted in nematode-free soil yielded seeds with a disease severity of 1 or higher. According to the official grading regulations in South Africa, seeds with a disease severity lower than 1 are graded as choice edible seed; those with a disease severity equal to or higher than 1 but lower than 2 are down-graded as standard edible seed while those with a disease severity higher than 2 are further down-graded as crushing seed. The producer prices for the 1991-1992 season are US $ 602, US $ 532 and US $ 247/metric ton for choice edible, standard edible and crushing seed, respectively (Venter *et al.*, in press). In fact, it is not so important if *D. destructor* survives in large or small numbers because the results confirm previous reports (Venter *et al.*, 1991) that a small initial population density of this nematode present in the field at the beginning of the growth season may build up a large population causing damage. One of the main factors contributing to this high damage potential is the high reproductive potential of *D. destructor*. At 28 °C, the length of the life cycle of *D. destructor* was 6-7 days (De Waele & Wilken, 1990). Under greenhouse conditions, an initial population density of 50 nematodes per seedling resulted in a 341-fold population increase at harvest (Venter *et al.*, 1991).

The significant negative correlations between storage time of infected hulls in soil and of infected seeds in paper bags at 10 °C and infection of new tissues suggest that storage time had an influence on the potential of *D. destructor* to invade or colonize groundnut hulls and seeds. This decline in virulence, however, is compensated by the high reproductive potential.

Finally, the freshness of the tissues played an important role in the efficiency of the soaking method as already suggested by Bolton *et al.* (1990). Stored hulls and seeds must be soaked longer than 24 h to make a reliable estimate of the number of nematodes present in the tissues. Soaking for 5 days should be sufficient. Longer soaking at room temperatures is often impossible because of the growth of bacteria and fungi which may influence the efficiency of the extraction method. Apparently rehydration of the nematodes in the stored seeds takes longer than 24 h.

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