Note brève

COMPARISON OF TWO METHODS FOR EXTRACTING *DITYLENCHUS DESTRUCTOR* FROM HULLS AND SEEDS OF GROUNDNUT

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Nematodes were not considered major pests of groundnut (*Arachis hypogaea* L.) in South Africa until *Ditylenchus destructor* Thorne, the potato rot nematode, was found infecting pods and seeds, causing severe damage (Jones & De Waele, 1988; De Waele *et al.*, 1989).

Nematodes are usually extracted from hulls (broken pods) and seeds of groundnut by soaking the macerated tissues in water in Petri dishes (Bridge *et al.*, 1977) or by placing the macerated tissues on sieves in a mist chamber (Minton, Hammons & Parham, 1970; Smith, Boswell & Thames, 1978; Starr, 1984). The same methods are also used for the extraction of nematodes from seeds maturing above ground (Babatola, 1984; Hooper, 1984; Panchbhai, Varma & Reddy, 1986). The centrifugal-flotation method, often used to extract nematodes from root tissues, is seldom used for the extraction of nematodes from seeds.

For large numbers of samples, the soaking method is more convenient than the mistifier method because of the limited space within most mist chambers. Preliminary observations showed that after one week specimens of *D. destructor* still emerged from groundnut seeds soaked in water. Soaked tissues release products, such as starch, causing the water to become cloudy, and often become contaminated with fungi after a few days. Therefore it is preferable to collect the extracted nematodes as soon as possible.

The objective of our study was to compare the soaking and centrifugal-flotation methods for extracting *D. destructor* from hulls and seeds of groundnut. The mistifier method was not included in this study since preliminary observations showed that fungi developed more rapid and abundant in the warm, humid mist chamber than in Petri dishes.

Materials and methods

Nematode-free groundnut seedlings (*Arachis hypogaea* L. cv. Sellie) were raised in 20-cm-diam. plastic pots filled with 3 dm^3 steam sterilized sandy soil and thinned to one per pot after emergence. Mixed life stages of *D. destructor*, obtained from hulls and seeds of infected Sellie groundnut pods from a field in the northern Transvaal and soaked in shallow water in Petri dishes for 24 hours at room temperature, were pipetted in 10-ml aqueous suspensions into holes in the soil around the roots in each pot at three weeks after planting. To obtain pods and seeds with different final nematode population densities, seedlings were inoculated with five different initial populations : 500, 1000, 2000, 4000 and 8000 nematodes per pot. The pots were randomized and the treatments replicated eight times. Eighteen weeks after inoculation, the pods were collected, broken open and the seeds separated from the hulls. The hulls were chopped in smaller parts with a scalpel and the seeds broken in two. To obtain homogenous subsamples, hulls and seeds of each treatment were combined.

Method 1 : Soaking

Per treatment, six subsamples each of 5 g hulls and 5 g seeds were soaked in tap water in Petri dishes at 22 °C. The influence of additional reproduction during soaking was reduced by decanting the nematodes which had moved out of the tissues after 24 hours and, from then onwards, every three or four days.

Method 2 : Centrifugal-flotation

Per treatment, six subsamples each of 5 g hulls and 5 g seeds were macerated in 50 cm^3 of tap water in a domestic blender at high speed for 2 min to release the nematodes from the tissues. The suspension of nematodes and tissue fragments was then washed through a 750-µm-aperture sieve placed on top of a 45-µm-aperture sieve. The residue on the 45-µm-aperture sieve was collected and poured into two 50 cm^3 centrifuge tubes. About 1 cm^3 of kaolin was added to each tube, the mixture thoroughly stirred and centrifuged at 1750 *g* for 5 min. The supernatant was then decanted and a sucrose solution (density 1.13 g/cm^3) added to the tubes. The mixture was thoroughly stirred and centrifuged at 1750 *g* for 1 min. The supernatant was washed through a 45-µm-aperture sieve, the residue collected and the nematodes counted.

The total number of nematodes recovered by the soaking method after fourteen days was used as the standard against which the extraction methods were compared.

The number of nematodes extracted by the soaking method from hulls and seeds after 24 hours and after fourteen days were subjected to regression analysis.

Results and discussion

The means of the total numbers of *D. destructor* recovered from the hulls and seeds by the different extraction methods are presented in Table 1. The different initial nematode populations resulted in different final nematode populations : 73 080 to 142 178 nematodes in 5 g of hulls and 93 856 to 186 024 nematodes in 5 g of seeds.

The efficiency of the soaking method (24 hours) was significantly higher and more consistent (as expressed by the coefficient of variation) than the efficiency of the centrifugal-flotation method (Table 2). The soaking method is also an inexpensive and rapid method involving few steps. The absence of any sieving during the soaking method may have reduced the loss of larvae compared with the centrifugal-flotation method. The recovery of immobile adults by the soaking method indicates that the nematodes not only actively moved out of the tissues but that they were also passively released by swelling and bursting of the tissues.

In the soaking method, few nematodes were recovered after ten days. Most adults were recovered after 24 hours. Few adults were observed after that time. Consequently, additional reproduction through egg laying was low. Hatching of eggs within the tissues could however not be eliminated during the incubation period. Therefore, the total number of nematodes recovered

Table 1

Numbers of *Ditylenchus destructor* extracted from 5 g samples of groundnut hulls and seeds by the soaking and centrifugal-flotation methods, eighteen weeks after inoculation

	Hulls				*Seeds*			
*Pi**	*Soaking* 24 h	*Soaking* 7 days	*Soaking* 14 days	*Centrifugal-flotation*	*Soaking* 24 h	*Soaking* 7 days	*Soaking* 14 days	*Centrifugal-flotation*
500	91 464	116 420	142 178	34 232	60 020	93 683	136 355	21 534
1 000	49 956	69 528	94 023	32 035	64 149	118 277	156 876	10 852
2 000	40 687	70 345	87 779	30 166	38 410	80 046	109 381	19 592
4 000	35 291	61 048	73 080	16 310	48 753	77 182	93 856	13 397
8 000	44 833	70 706	91 386	37 719	99 695	162 359	186 024	17 004

Numbers are the means of six replicates.
* Initial nematode population density per pot.

Table 2

Efficiency* of the soaking and centrifugal-flotation methods for extracting *Ditylenchus destructor* from 5 g samples of groundnut hulls and seeds

	Hulls			*Seeds*		
*Pi***	*Soaking* 24 h (%)	*Soaking* 7 days (%)	*Centrifugal-flotation* (%)	*Soaking* 24 h (%)	*Soaking* 7 days (%)	*Centrifugal-flotation* (%)
500	64	80	24	44	69	16
1 000	53	74	34	41	76	7
2 000	46	80	34	35	73	18
4 000	48	83	22	52	82	13
8 000	49	78	41	54	88	9
Overall mean	53.4	80.8	31.2	45.8	77.3	12.8
S.D.	10.5	7.8	11.1	10.4	11.3	6.6
C.V.	19.7	9.7	35.6	22.7	14.6	51.6

* The number of nematodes extracted after fourteen days by the soaking method was used as a standard against which the extraction methods were compared.
** Initial population density per pot.

after fourteen days is an estimation of the total number of all life stages, including eggs, present inside the tissues at the beginning of the incubation period. Soaking the tissues for only 24 hours gives a reliable estimation of this number. The relationship between the number of nematodes extracted after 24 hours (x) and fourteen days (y) was : $y = 37415 + 1.132x$ ($r = 0.911$, $P = 0.05$) for hulls and $y = 48663 + 1.411x$ ($r = 0.827$, $P = 0.05$) for seeds.

Preliminary observations have shown that the freshness of the tissues may play an important role in the soaking method : few nematodes were released after 24 hours from infected seeds, stored for several weeks at room temperature. After 48 hours soaking, however, the number of nematodes extracted from stored seeds was high. In stored seeds, the nematodes may be in an anhydrobiotic state and therefore their recovery was probably better after 48 hours soaking. The extraction of *D. destructor* from stored seeds needs further investigation. During the present study only fresh seeds were used.

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