Journal of Nematology 22(3):321–326. 1990.

In Vitro Embryo Explant Cultures of Peanut to Evaluate Resistance to *Ditylenchus destructor*

D. De Waele, P. C. W. van der Walt, Ria Wilken, Selmaré Basson, and Elizabeth M. Jordaan[1]

Abstract: Population densities of *D. destructor* on embryo explants of 22 peanut genotypes grown in vitro were compared with those in roots and seeds of the same genotypes grown in the greenhouse. During the first 8 weeks after inoculation, the optimum incubation period was 6 weeks for maximum reproduction of *Ditylenchus destructor* on embryo explants of peanut (*Arachis hypogaea* L. cv. Sellie) inoculated with 250 nematodes at 25 C. Nematode numbers increased 17-fold. Deletion of $MnSO_4 \cdot H_2O$ and a higher KH_2PO_4 concentration in the medium resulted in higher nematode reproduction. Resistance or susceptibility to *D. destructor* was observed in seeds of several genotypes but was not matched by differences in host suitability in roots. The results indicate that the factor for resistance or susceptibility to *D. destructor* is synthesized in the seeds of peanut but is not translocated to the roots. Use of embryo explant cultures of peanut as a rapid method to evaluate resistance to *D. destructor* did not work under the conditions described.

Key words: Arachis hypogaea, culturing, *Ditylenchus destructor,* embryo explant, host nutrition, peanut, reproduction, resistance, screening, susceptibility.

Aseptic cultures of plant tissues and nematodes have been used for numerous purposes including the study of plant nematode interactions (14,20). Callus tissue and excised organ cultures are among the most widely used methods. Ectomigratory and endomigratory parasites are most successfully propagated on callus tissue; sedentary endoparasites do best on excised organ cultures (14,20). Because of their small size and controlled conditions, aseptic in vitro cultures have the potential to provide a less expensive and more reliable method for screening plants for resistance to nematodes than do field and greenhouse experiments. In callus tissue cultures, however, growth substances, which are major components of the culture medium, have been implicated in alterations in host–parasite interactions. Good reproduction of nematodes on callus tissues of plants that are resistant to, or are nonhosts of, those nematodes have been reported (22). Growth substances are not used in excised organ cultures, however, and screening of tomato for resistance to *Meloidogyne incognita acrita* Chitwood and *M. hapla* Chitwood (7,8), of soybean for resistance to *Heterodera glycines* Ichinohe (15), and of grape for resistance to *Pratylenchus vulnus* Allen & Jensen (19) using excised roots or shoot tips has been successful.

Since 1987, *Ditylenchus destructor* Thorne, the potato rot nematode, has emerged as an important pest of peanut in South Africa, causing severe damage to the appearance and yield of seeds (5,11). In infected plants, large numbers of this migratory endoparasite were found in hulls and seeds of mature pods; nematode population densities in roots and immature pods usually were low (12). Resistant cultivars could play an important role in any management program aimed at keeping *D. destructor* populations below damaging levels. Only three peanut cultivars, Sellie, Norden, and Harts, are grown in South Africa. Two more cultivars, Selmani and Misga, may be released in the near future. None of these cultivars are resistant to *D. destructor,* but field and greenhouse screening of the peanut germplasm collection available at the Grain Crops Research Institute (GCRI) has indicated that sources of resistance may be present (13). In the field and greenhouse, resistance of peanut to *D. destructor* can be determined only at pod maturity which occurs between 4 and 6 months after planting

Received for publication 5 September 1989.

[1] Grain Crops Research Institute, Private Bag X1251, Potchefstroom 2520, Republic of South Africa. Present address of first author: Plant Genetic Systems NV, Jozef Plateaustraat 22, 9000 Gent, Belgium.

The authors thank I. van den Berg, R. Swanepoel, R. Jantjies, U. Coen, and E. Setlhare for technical assistance.

and differs among genotypes. Although it is unknown if the factor for resistance or susceptibility to *D. destructor* is also present in the roots of peanut, a program was initiated at the GCRI to develop a rapid in vitro assay to evaluate sources of resistance. In vitro flowering and pod formation of peanut have been reported, but growth substances were necessary for their induction and development (18). In contrast, embryo explants of peanut can be grown aseptically without growth substances.

The objectives of our study were 1) to determine the effects of incubation period and culture medium composition on the reproduction of *D. destructor* on embryo explant cultures of peanut and 2) to test the usefulness of these cultures to evaluate the resistance of peanut genotypes to *D. destructor*. The results of the in vitro assay were compared with those of greenhouse experiments.

Materials and Methods

Laboratory experiments: Mature peanut (*Arachis hypogaea* L.) seeds were halved and the embryos excised from the cotyledons. Excised embryos were surface sterilized by immersion for 15 minutes in a 20% (v/v) commercial bleach solution (3.5% NaOCl) containing several drops of a wetting agent and rinsed four times in sterile water. One excised embryo was inserted in each culture tube (25 × 110 mm) containing 10 ml of a medium. In experiments 1, 2, and 4 the embryo explants were cultured on an orchid medium adjusted to pH 5.8 prepared as follows: to 500 ml water add 5 ml of stock solutions of $Ca(NO_3)\cdot 4H_2O$ (1 g/liter stock solution), KH_2PO_4 (0.25 g/liter), $MgSO_4\cdot 7H_2O$ (0.25 g/liter), $(NH_4)_2SO_4$ (0.5 g/liter), $FeSO_4\cdot 7H_2O$ (0.025 g/liter), and $MnSO_4\cdot H_2O$ (0.0075 g/liter) plus 25 g saccharose, and 7.5 g agar. In experiment 3, Gamborgs B5 medium (10) was used plus 0.5 g/liter casein-hydrolyzate, 10 g/liter sucrose, and 10 g/liter agar. The medium was adjusted to pH 5.8. To prevent contamination and dehydration of the medium and plant, each tube was sealed with parafilm. The embryo explant cultures were incubated at 26 C for 6–8 weeks under cool-white fluorescent tubes with 16-hour light and 8-hour dark periods.

Aseptic *D. destructor* mixed life stages were obtained from monoxenic cultures on callus tissue initiated from peanut leaves (21). Nematodes were pipetted in 0.25-ml aqueous suspensions on the medium. After incubation, the nematodes were extracted from the roots by cutting each root into pieces and soaking the pieces in tap water for 24 hours at room temperature. The callus was diluted with water and the number of nematodes was determined.

In experiment 1, 80 embryo explants (peanut cv. Sellie) on orchid medium were each inoculated with 250 *D. destructor*. From 1 until 8 weeks after inoculation, nematode numbers were counted weekly, 10 replications per week.

In experiment 2, embryo explants (peanut cv. Sellie) were cultured on 15 different variations of the orchid medium. Ten replications of each medium were inoculated with 125 *D. destructor*. Nematode numbers were counted 6 weeks after inoculation.

In experiment 3, embryo explants of five peanut cultivars (Norden, Selmani, Harts, Sellie, and Misga) and one breeding line (PC 137-K41) were cultured on Gamborgs B5 medium. Six replications of each genotype were inoculated with 90 *D. destructor*. Nematode numbers were counted 6 weeks after inoculation.

In experiment 4, embryo explants of 15 peanut genotypes were cultured on orchid medium. Ten replications of each genotype were inoculated with 100 *D. destructor*. Nematode numbers were counted 6 weeks after inoculation.

Greenhouse experiments: Nematode-free seeds of peanut were planted in 3-liter plastic pots (20 cm d) filled with steam-sterilized sandy soil (85% sand, 8% silt, 7% clay), and *Rhizobium* nitrogen-fixing bacteria were added to the soil. Seedlings were thinned to one per pot 2 weeks after planting, and *D. destructor* was added to the rhizosphere of each seedling 3 weeks after planting.

Ditylenchus destructor was obtained as described for laboratory experiments and consisted of mixed life stages. Nematodes were pipetted in 10-ml aqueous suspensions into holes in the soil around the roots of the seedlings. Plants were maintained at 20–25 C with a 13-hour photoperiod and fertilized weekly by irrigation with tap water containing a nutrient solution (6.5% N, 2.7% P, 18% K). At harvest, fresh root and seed weights were determined. Nematodes were extracted from a 5-g subsample of fresh roots per plant using the centrifugal-flotation method (3) and from a 1-g subsample of fresh seeds per plant by soaking the tissues in shallow water in petri dishes for 24 hours at room temperature (1).

In experiment 5, peanut cultivars Sellie, Norden, Harts, Selmani, and Misga and the breeding line PC 137-K41 were planted. Eight replications of each genotype were inoculated with 3,500 *D. destructor*. Harts was harvested 15 weeks after planting, Misga at 18 weeks, Sellie and PC 137-K41 at 21 weeks, and Norden and Selmani at 24 weeks.

In experiment 6, 15 peanut genotypes were planted. Each genotype was replicated five times and each plant was inoculated with 5,000 *D. destructor*. All genotypes were harvested 20 weeks after planting.

Statistics: In all experiments, tubes or pots were arranged in a completely randomized design. All data, except those of experiment 1, were subjected to analysis of variance. Means were separated using the Student-Newman-Keuls range test.

Results and Discussion

During the first 8 weeks after inoculation, the maximum number of *D. destructor* was produced on embryo explants of Sellie at 6 weeks when a 17-fold increase in number of nematodes was observed (Table 1). This is the first report of the successful culturing of *D. destructor* on excised organ cultures. On excised clover and tomato roots, *D. destructor* either did not develop large populations (4) or was unable to penetrate the roots and failed to reproduce (9). Either the cell walls were too thick for the nematodes to penetrate, or the nematodes did not have sufficient leverage or traction in the medium (9). Peanut callus tissue cultures inoculated with 50 *D. destructor* yielded 600 times as many nematodes 5 weeks later (21). In general, with the exception of members of the family Heterodoridae, nematodes reproduce more rapidly on callus tissues of a plant than on excised root cultures or intact seedlings (14).

Table 1. *Ditylenchus destructor* juveniles and adults that developed in vitro on embryo explants of Sellie peanut.

Weeks after inoculation	Mean no. nematodes†		Total no. nematodes‡	
	Roots	Agar	Mean	Range
1	126	4	130	56–197
2	1,146	15	1,161	487–1,947
3	2,254	41	2,295	633–4,068
4	1,584	348	1,932	810–4,695
5	756	316	1,072	382–1,645
6	3,948	223	4,171	2,092–8,322
7	1,185	1,529	2,714	257–3,379
8	904	856	1,760	600–3,643

† Mean of 10 replications; inoculum density was 250 nematodes per replication.

‡ Total number of nematodes extracted from the roots and agar.

Inoculation of embryo explants grown on the standard medium with 125 *D. destructor* resulted in a 20-fold increase in nematode numbers 6 weeks after inoculation (Table 2). Deleting the $MnSO_4 \cdot H_2O$ and doubling the KH_2PO_4 concentration resulted in significantly ($P = 0.05$) higher nematode numbers than in the standard medium. Without $MnSO_4 \cdot H_2O$ the increase in numbers of *D. destructor* was 58-fold, and with the increased level of KH_2PO_4 the increase was 66-fold. Deleting or increasing the concentration of all other elements and saccharose had no significant effect on reproduction of *D. destructor*. Few studies have dealt with the effects of host nutrition on nematode reproduction in excised organ cultures; however, direct effects of the medium on juveniles and on root penetration, development, and sex ratio of *Meloidogyne* spp. on excised tomato

TABLE 2. *Ditylenchus destructor* juveniles and adults that developed in vitro on embryo explants of Sellie peanut grown at different fertility levels, 6 weeks after inoculation.

Treatment	Ml of component/500 ml total solution†						g/500 ml		No. nematodes per embryo explant‡
	$Ca(NO_3)\cdot 4H_2O$ (1 g/l)	KH_2PO_4 (0.25 g/l)	$MgSO_4\cdot 7H_2O$ (0.25 g/l)	$(NH_4)_2SO_4$ (0.5 g/l)	$FeSO_4\cdot 7H_2O$ (0.025 g/l)	$MnSO_4\cdot H_2O$ (0.0075 g/l)	Saccharose	Agar	
K	5	5	5	5	5	5	25	7.5	2,489 abc
1	0	5	5	5	5	5	25	7.5	2,047 ab
2	10	5	5	5	5	5	25	7.5	3,711 abcd
3	5	0	5	5	5	5	25	7.5	521 a
4	5	10	5	5	5	5	25	7.5	8,188 de
5	5	5	0	5	5	5	25	7.5	2,378 abc
6	5	5	10	5	5	5	25	7.5	1,464 ab
7	5	5	5	0	5	5	25	7.5	739 a
8	5	5	5	10	5	5	25	7.5	6,942 cde
9	5	5	5	5	0	5	25	7.5	6,433 cde
10	5	5	5	5	10	5	25	7.5	3,364 abcd
11	5	5	5	5	5	0	25	7.5	10,732 e
12	5	5	5	5	5	10	25	7.5	4,331 abcd
13	5	5	5	5	5	5	0	7.5	7,910 cde
14	5	5	5	5	5	5	50	7.5	6,084 bcde

† Stock solutions.

‡ Mean of 10 replications; inoculum density was 125 nematodes per replication. Column means followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls range test.

and cucumber roots have been reported (6,16,17).

Among the six embryo explant cultures, in vitro reproduction of *D. destructor* per explant 6 weeks after inoculation was significantly ($P = 0.05$) higher in roots of PC 137-K41, Misga, and Sellie than in roots of Selmani and Norden (Table 3). In PC 137-K41, the increase in number of nematodes was 68-fold; Norden, at six-fold, had the lowest ($P = 0.05$) increase. In the greenhouse, numbers of *D. destructor* per gram roots 6 weeks after inoculation were comparable among all genotypes (Table 3). At harvest, significantly ($P = 0.05$) more nematodes were extracted from roots of Sellie and PC 137-K41 than from the other genotypes. Population density of *D. destructor* per gram seeds at harvest was significantly ($P = 0.05$) higher in Harts than in all other genotypes. Misga supported the lowest ($P = 0.05$) number of nematodes. Six weeks after inoculation, numbers of *D. destructor* on embryo explants were much higher than in roots of plants grown in the greenhouse (Table 3).

Among the 16 genotypes, in vitro reproduction of *D. destructor* per embryo explant, measured 6 weeks after inoculation, was significantly ($P = 0.05$) higher on embryo explants of EP 3 and Selection 5 than on embryo explants of all other genotypes except Val Red (Table 4). The increase in

TABLE 3. *Ditylenchus destructor* juveniles and adults that developed in vitro in embryo explants and in the greenhouse in roots and seeds of six peanut genotypes.

Genotype	No./embryo explant†	No./g roots‡		No./g seeds† at harvest
		6 weeks after inoculation	Harvest§	
Norden	563 a	69 a	18 a	3,245 b
Selmani	2,087 b	65 a	30 a	1,530 b
Harts	2,322 bc	99 a	16 a	4,727 c
Sellie	3,313 c	82 a	92 b	1,914 b
Misga	4,142 c	30 a	28 a	485 a
PC 137-K41	6,147 c	63 a	92 b	1,903 b

Column means followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls range test.

† Mean of six replications after 6 weeks incubation. Initial inoculum density was 90 nematodes per replication.

‡ Mean of eight replications; initial inoculum density was 3,500 nematodes per replication.

§ Harts was harvested 15 weeks after planting, Misga at 18 weeks, Sellie and PC 137-K41 at 21 weeks, Norden and Selmani at 24 weeks.

TABLE 4. *Ditylenchus destructor* juveniles and adults that developed in vitro on embryo explants and in the greenhouse in seeds of 16 peanut genotypes, 6 and 18 weeks after inoculation.

Genotype	Source	No./embryo explant	No./g seed
Bolivia 163/66	Bolivia	99 a	1 a
Umtali 28	Zimbabwe	162 ab	2,054 bc
Golden 1495	Israel	174 ab	2,446 cd
P.I. 1365553	U.S.A.	177 ab	401 ab
Swallow	South Africa	197 ab	119 ab
P.I. 295233	U.S.A.	226 ab	1 a
69-101	Senegal	277 abc	310 ab
PC 113	South Africa	283 abc	529 ab
66 G 293	South Africa	294 abc	11 a
Chalimbana	Malawi	347 c	4,191 d
P.I. 268729	U.S.A.	357 c	1,355 bc
Spanish Valencia	U.S.A.	491 cd	981 abc
Natal Common	South Africa	515 cd	516 ab
Val Red	South Africa	707 de	412 ab
Selection 5	South Africa	818 e	2,933 cd
EP 3	South Africa	874 e	4 a

Mean of 10 explant or 5 seed replications; inoculum density was 100 (in vitro) and 5,000 (greenhouse) nematodes per replication. Column means followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls range test.

number of nematodes was nine-fold in EP 3 but in Bolivia 163/66, which had the fewest nematodes, there was no increase in nematode numbers.

At 18 weeks after inoculation in a greenhouse, population densities of *D. destructor* per gram seeds were significantly ($P = 0.05$) higher in Chalimbana than in any other genotype except Golden 1495 and Selection 5 (Table 4). Fewer than 20 nematodes were extracted per gram seeds of Bolivia 163/66, P.I. 295233, EP 3, and 66 G 293.

Greenhouse tests demonstrated differences in *D. destructor* host suitability among the genotypes and indicated the presence of factor(s) for resistance to *D. destructor* in Bolivia 163/66, P.I. 295233, EP 3, and 66 G 293. Although the exact nature of the resistance factor(s) is unknown, a comparison between the number of nematodes extracted from the roots at harvest and the seeds of greenhouse-grown Norden, Selmani, Harts, Sellie, Misga, and PC 137-K41 shows that nematode reproduction in the roots did not parallel reproduction in the seeds. Apparently the factor(s) for resistance or susceptibility to *D. destructor* is (are) restricted to the seeds. In contrast, Chambers and Epps (2) suggested that the factor for resistance or susceptibility to *H. glycines* in soybean is not synthesized in one part of the plant and translocated to other parts, but it is genetically inherent in tops, stems, and roots of resistant plants. However, *H. glycines* attacks the roots of soybean, whereas *D. destructor* attacks the seeds of peanut. Also, the pods of peanuts develop in a unique way. After fertilization, the ovary situated at the base of the flower elongates into a positively geotropic stalk-like peg. After penetration of the soil, the tip of the peg where the fertilized egg is situated forms the pod and the seeds develop inside the pod. Obviously roots and pods have numerous morphological and physiological differences. Because differences in host suitability were observed in vitro, other factors for resistance or susceptibility to *D. destructor* may be present in the roots. Host nutrition and excision of the embryo may have affected the genotypes in different ways.

The factor(s) for resistance or susceptibility to *D. destructor* in peanut is (are) apparently synthesized in the pods but not translocated to the roots. Therefore, a comparison between the number of nematodes extracted from greenhouse-grown seeds at harvest and from embryo explants 6 weeks after inoculation were not com-

parable. Consequently, embryo explants of peanut cultured as in these studies can not be used to evaluate the resistance of peanut genotypes to *D. destructor*. In contrast, screening soybean genotypes for resistance to *H. glycines* was successful using excised root cultures (15).

Literature cited

1. Bolton, C., D. De Waele, and S. Basson. 1990. Comparison of two methods for extracting *Ditylenchus destructor* from hulls and seeds of groundnuts. Revue de Nématologie, in press.

2. Chambers, A. Y., and J. M. Epps. 1967. Nature of soybean resistance to *Heterodera glycines*. Plant Disease Reporter 51:771–774.

3. Coolen, W. A., and C. J. D'Herde. 1972. A method for the quantitative extraction of nematodes from plant tissues. Merelbeke, Belgium: State Nematology and Entomology Research Station.

4. Darling, H. M., L. R. Faulkner, and P. Wallendal. 1957. Culturing the potato rot nematode. Phytopathology 47:7 (Abstr.).

5. De Waele, D., B. L. Jones, C. Bolton, and E. van den Berg. 1989. *Ditylenchus destructor* in hulls and seeds of peanut. Journal of Nematology 21:10–15.

6. Dropkin, V. H., and W. R. Boone. 1966. Analysis of host–parasite relationships of root-knot nematodes by single-larva inoculations of excised tomato roots. Nematologica 12:225–236.

7. Dropkin, V. H., and R. E. Webb. 1967. Resistance of axenic tomato seedlings to *Meloidogyne incognita acrita* and to *M. hapla*. Phytopathology 57:584–587.

8. Dropkin, V. H., D. W. Davis, and R. E. Webb. 1967. Resistance of tomato to *Meloidogyne incognita acrita* and to *M. hapla* (root knot nematodes) as determined by a new technique. Journal of the American Society for Horticultural Science 90:316–323.

9. Faulkner, L. R., and H. M. Darling. 1961. Pathological histology, hosts, and culture of the potato rot nematode. Phytopathology 51:778–786.

10. Gamborg, O. L., R. A. Miller, and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research 50: 151–158.

11. Jones, B. L., and D. De Waele. 1988. First report of *Ditylenchus destructor* in pods and seeds of peanut. Plant Disease 72:453.

12. Jones, B. L., and D. De Waele. 1990. Histopathology of *Ditylenchus destructor* on peanut. Journal of Nematology, in press.

13. Jordaan, E. M., D. De Waele, and P. J. A. van der Merwe. An evaluation of groundnut germplasm for resistance to *Ditylenchus destructor* (Nematoda). Phytophylactica, in press (Abstr.).

14. Krusberg, L. R., and D. E. Babineau. 1977. Application of plant tissue culture to plant nematology. Pp. 401–419 *in* W. R. Sharp, P. O. Larsen, E. F. Paddock, and V. Raghavan, eds. Plant cell and tissue culture. Columbus: Ohio State University Press.

15. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1988. Screening soybean for resistance to *Heterodera glycines* Ichinohe using monoxenic cultures. Journal of Nematology 14:593–594.

16. McClure, M. A., and D. R. Viglierchio. 1966. Penetration of *Meloidogyne incognita* in relation to growth and nutrition of sterile, excised cucumber roots. Nematologica 12:237–247.

17. McClure, M. A., and D. R. Viglierchio. 1966. The influence of host nutrition and intensity of infection on the sex ratio and development of *Meloidogyne incognita* in sterile agar cultures of excised cucumber roots. Nematologica 12:248–258.

18. Narasimhulu, S. B., and G. M. Reddy. 1984. In vitro flowering and pod formation from cotyledons of groundnut. Theoretical and Applied Genetics 69: 87–91.

19. Palys, J. M., and C. P. Meredith. 1984. Dual cultures of grape (*Vitis* spp.) and the lesion nematode (*Pratylenchus vulnus*) for studies of nematode tolerance. Plant Disease 68:1058–1060.

20. Riedel, R. M., M. Alves de Lima, and M. Martin. 1983. Establishment of nematode germplasm banks. Pp. 880–903 *in* D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada, eds. Handbook of plant cell culture, vol. 1. Techniques for propagation and breeding. New York: Macmillan Publishing Company.

21. Van der Walt, P. C. W., and D. De Waele. 1989. Mass culture of the potato rot nematode *Ditylenchus destructor* on groundnut callus tissue. Phytophylactica 21:79–80.

22. Webster, J. M., and D. Lowe. 1966. The effect of the synthetic plant-growth substance, 2,4-dichlorophenoxyacetic acid, on the host–parasite relationships of some plant-parasitic nematodes in monoxenic callus culture. Parasitology 56:313–322.