Journal of Nematology 33(2–3):147–151. 2001.

Aseptic Culture Systems of *Radopholus similis* for In Vitro Assays on *Musa* spp. and *Arabidopsis thaliana*[1]

A. Elsen,[2] K. Lens,[2] D. T. M. Nguyet,[3] S. Broos,[2] R. Stoffelen,[2] and D. De Waele[2]

Abstract: Radopholus similis is one of the most damaging nematodes in bananas. Chemical control is currently the most-used method, but nematode control through genetic improvement is widely encouraged. The objective of this study was to establish an aseptic culture system for *R. similis* and determine whether *R. similis* can infect and reproduce on in vitro banana plantlets and in vitro *Arabidopsis thaliana*. In the study's first part, a suitable aseptic culture system was developed using alfalfa callus. *Radopholus similis* could penetrate and reproduce in the callus. Six weeks after inoculation with 25 females, the reproduction ratio was 26.3 and all vermiform stages were present. The reproduction ratio increased to 223.2 after 12 weeks. Results of a greenhouse test showed that *R. similis* did not lose its pathogenicity after culturing on alfalfa callus. In the study's second part, the infection and reproduction of the nematodes cultured on the callus were studied on both in vitro banana plantlets and *A. thaliana*. *Radopholus similis* infected and reproduced on both banana and *A. thaliana*. Furthermore, nematode damage was observed in the root systems of both hosts. These successful infections open new perspectives for rapid in vitro screening for resistance in banana cultivars and anti-nematode proteins expressed in *A. thaliana*.

Key words: alfalfa callus, *Arabidopsis thaliana,* burrowing nematode, *Medicago sativa,* method, Musa (AAA), nematode, *Radopholus similis*.

Among nematodes parasitizing bananas throughout the world, *Radopholus similis* (Cobb) Thorne, the burrowing nematode, is one of the most damaging (Sarah et al., 1996), causing severe yield losses in commercial and in local consumption cultivars (Sarah, 1989; Davide, 1996). Chemical control is currently the most-used method to manage the nematodes although nematicides are dangerous, toxic, and expensive. Therefore, nematode control through genetic improvement of banana is widely encouraged. Many *Musa* cultivars have been screened to find resistance against these root pathogens (Pinochet, 1996). Screening research is time consuming because it must be carried out under both field and greenhouse conditions (Pinochet, 1988; Price, 1994). In vitro screening could facilitate and hasten incorporation of genetic nematode control into bananas. Wang et al. (1997) successfully developed an in vitro screening method for a related burrowing nematode, *R. citrophilus,* in commercial *Anthurium* hybrids, and the method holds promise for use with *Musa* (INIBAP, 1998). However, a useful in vitro screening method requires aseptic nematode cultures. The most commonly used in vitro technique for culturing *R. similis* is carrot discs (O'Bannon and Taylor, 1968). Although reproduction is very high, this culturing technique makes in vitro studies difficult because the nematodes are easily contaminated. Excised organ cultures, like excised soybean roots (Huettel, 1989), and callus cultures have been investigated for monoxenic culturing of *R. similis*. Reproduction on callus of citrus leaves (Inserra and O'Bannon, 1975), banana fruit (Brown and Vessey, 1985), and carrot (Reise et al., 1987) was poor, whereas *R. similis* reproduced successfully on alfalfa callus (Castro and Ferraz, 1990; Ko et al., 1996; Myers et al., 1965).

In addition to screening existing *Musa* germplasm and improved hybrids from the breeding programs, an in vitro screening method would aid in evaluation of anti-nematode proteins for their effectiveness against the burrowing nematode. *Arabidopsis thaliana* Lineaus is used as a model system for genetic studies. Anti-nematode proteins are easily expressed in transgenic lines. Sijmons et al. (1991) reported that *A. thaliana* was a good host for migratory endoparasitic and sedentary nematodes. Therefore, *A. thaliana* was suggested as a new model system for plant-parasitic nematodes.

The objective of this study was to establish an aseptic culture system for *R. similis* and determine whether *R. similis,* reared on the callus, could infect and reproduce on in vitro banana plantlets and in vitro *A. thaliana*.

Materials and Methods

Alfalfa callus: Alfalfa seeds (*Medicago sativa* Lineaus) were sterilized with a 15-minute soak in concentrated H_2SO_4 followed by four rinses with sterile, distilled water; a 15-minute soak in $HgCl_2$ (1,000 ppm in 30% ethanol); and four rinses with sterile, distilled water (Riedel and Foster, 1970). Sterile 4-day-old alfalfa seedlings, produced from these seeds on plates of check agar (10 g sucrose, 2 g yeast agar, 10 g agar, 1,000 ml water), were placed on slants. These slants were prepared from 14-ml aliquots of White's medium (White, 1963), modified by adding 0.2 ppm α-NAA and 2 ppm 2,4-D. Seven to 10 days later, allowing the callus to develop, the calli were transferred to petri dishes containing the same medium.

Received for publication 23 February 2000.

[1] Supported in part by a grant from the European Community (INCO DC, ERB IC18 CT97-0208), ABOS-IITA, the Catholic University Leuven, and the Flemish Association for Development Cooperation and Technical Assistance (VVOB). This research was carried out in the framework of PROMUSA.

[2] Laboratory of Tropical Crop Improvement, Catholic University Leuven, K. Mercierlaan 92, 3001 Leuven, Belgium.

[3] Vietnam Agricultural Science Institute (VASI), Van Diem, Than Tri, Hanoi, Vietnam.

The authors thank J. Reynders for technical assistance, Nathalie Barthels (R. U. G.) for supplying material and G. Gheysen (R. U. G.) and E. Van Damme (K. U. Leuven) for critically reviewing the manuscript.

E-mail: annemie.elsen@agr.kuleuven.ac.be

This paper was edited by B. C. Hyman.

Initiation of the nematode culture on alfalfa callus: An *R. similis* isolate from Uganda, previously cultured on carrot discs, was surface-sterilized for 2 minutes in 0.01% $HgCl_2$, followed by two rinses of sterile, distilled water. With a sterile micropipet, 25 females were inoculated on each alfalfa callus. The petri dishes were incubated at 28 ± 0.5 °C in the dark, allowing the nematodes to feed and reproduce on the callus. After 5 weeks the nematodes began moving from the callus. To maintain stock cultures of the sterile *R. similis* isolate, fresh alfalfa calli were infected with a small piece of infected callus containing an undetermined number of *R. similis*. After two subcultures, the nematodes were placed on PDA (potato dextrose agar) and NA (nutrient agar) to test for bacterial and fungal contamination.

Nematode inoculum: To extract the nematodes, the callus was chopped and put on a sterile 70-µm-pore sieve. The sieve was placed on a sterile watch glass containing sterilized water. Within 48 hours the living nematodes migrated through the sieve into the water. Prior to inoculation, the nematodes were collected from the bottom of the watch glass. The extraction process was carried out under sterile conditions at room temperature.

Evaluation of the alfalfa callus compared to carrot discs as tissue to culture nematodes: Alfalfa calli were established in petri dishes containing 20 ml modified White's medium. Each callus was inoculated with 25 female *R. similis* extracted from callus stock cultures. Fresh carrot discs were prepared and inoculated with 25 female *R. similis* from the same Ugandan isolate but maintained on carrot discs (Moody et al., 1973). Each treatment was replicated 10 times. The alfalfa calli and the carrot discs were incubated at 28 ± 0.5 °C in the dark. After 5 weeks the nematodes were extracted using a Baermann funnel for the calli and the maceration-sieving technique for the carrot discs (Baermann, 1917; Speijer and De Waele, 1997). Numbers of nematodes were determined by counting one 6-ml aliquot of a homogenized 150-ml suspension.

Reproduction of R. similis on alfalfa callus as a function of time: Alfalfa calli were established in petri dishes containing 20 ml modified White's medium. Each callus was inoculated with 25 female *R. similis* extracted from callus stock cultures. The calli were incubated at 28 ± 0.5 °C in the dark. Six, 9, and 12 weeks after inoculation, eight petri dishes were analyzed by extracting nematodes from the callus and the medium separately, using the maceration-sieving technique. Numbers of juveniles, females, and males were determined by counting three 2-ml aliquots taken from a 50-ml homogenized suspension.

Test for pathogenicity: In vitro propagated 'Grande Naine' (*Musa* AAA, Cavendish group) tissue culture plants were used. After propagation, regeneration, and rooting (Banerjee and De Langhe, 1985), the in vitro propagated plantlets were transplanted into 1-liter plastic pots filled with a 2:1 mixture of autoclaved peat and quartz sand. The pots were maintained in a greenhouse at an ambient temperature of 20–27 °C and a 12-hour photoperiod. To adapt to the greenhouse conditions, the plants were kept under a plastic cover for 2 weeks, which was gradually opened during the subsequent 2 weeks. The pots were irrigated as needed and fertilized every 3 weeks. Eight weeks after planting, each plant was inoculated with 1,000 vermiform nematodes. Eight plants were inoculated with *R. similis* cultured on carrot discs, and another eight were inoculated with alfalfa callus cultured nematodes. The plants were harvested 8 weeks after inoculation. The percentage of necrotic root tissue was assessed for each plant. In addition, nematode population density per root system and per-gram fresh root weight was determined. The maceration-sieving technique was used to extract nematodes For the extraction, a subsample of 15 g of fresh roots was taken and nematodes in a 6-ml aliquot taken from a homogenized 150-ml suspension were counted.

Reproduction of R. similis, extracted from alfalfa callus, on the Musa cultivar Grande Naine (AAA) grown in vitro: Aseptic shoots of the *Musa* cultivar Grande Naine (AAA, Cavendish group) were transferred to jars containing MS (Murashige and Skoog) rooting medium (Banerjee and De Langhe, 1985). The jars, containing two plantlets each, were incubated at 28 ± 0.5 °C with a 16-hour-light/8-hour-dark cycle. Three weeks later, 10 jars were inoculated with 50 female *R. similis* extracted from alfalfa callus. Mature females were collected individually with a sterile micropipet and placed in a drop of sterile water on the MS medium near the shoots. Eight weeks after inoculation, the nematodes were extracted from the roots and the medium separately, using the maceration-sieving technique. Numbers of juveniles, females, and males were counted in one 6-ml aliquot taken from a 70-ml homogenized suspension.

Reproduction of R. similis, extracted from alfalfa callus, on A. thaliana grown in vitro: Seeds of *A. thaliana* (Colombia 0 ecotype) were surface-sterilized with a 2-minute soak in 95% ethanol, followed by a 12-minute soak in sterile water with 5% NaOCl and 0.1% Tween, and three rinses with sterile, distilled water. The sterilized seeds were sown on germination medium (Valvekens et al., 1988). Ten-day-old seedlings were transferred to and arranged on a thin layer of Knop medium (Sijmons et al., 1991). Petri dishes, each containing three seedlings, were placed slightly tilted to promote unidirectional root growth. After growth for 10 more days at 22 ± 0.5 °C (16-hour-light/8-hour-dark cycle), roots were inoculated with 20 female *R. similis* per root system (60 females per petri dish). The petri dishes were incubated at 28 ± 0.5 °C with a 16-hour-light/8-hour-dark cycle. Ten weeks after inoculation, the nematodes were extracted using a Baermann funnel. Numbers of juveniles, females, and males were counted in three 2-ml aliquots from a homogenous 25-ml suspension.

Different extraction techniques were used in the dif-

ferent experiments, depending on the type of tissue. If the tissue was very soft or fine, like the alfalfa callus in the first experiment or the *A. thaliana* roots, the Baermann funnel was used. By using this technique, only living vermiforms could be extracted, while the maceration-sieving technique allowed extraction of dead as well as living vermiforms.

Results

Evaluation of the alfalfa callus compared to carrot discs as tissue to culture R. similis: Radopholus similis reproduced well on both alfalfa callus and carrot disc, with a 137 and 379-fold increase of the initial population, respectively (Table 1). Taking into account that the amount of tissue available to the nematodes was similar in both treatments, carrot disc was a significantly better host tissue ($P \leq 0.05$) than alfalfa callus. The *R. similis* population cultured on alfalfa callus tested negative for bacterial and fungal contamination on PDA and NA.

Reproduction of R. similis on alfalfa callus as a function of time: The number of nematodes extracted from the alfalfa callus and the modified White's medium increased over time (Table 2). Within 6 weeks, the life cycle was completed as indicated by the recovery of 220 females per petri dish and the presence of males. At that time, the reproduction ratio was 26.3. The population continued increasing 119.2 and 223.3-fold 9 and 12 weeks after inoculation, respectively, which indicates that alfalfa callus cultured on White's medium is a good host for *R. similis.* The proportion of females and males to all vermiform stages, counted in the callus and the medium, was 33% and 2% at 6 weeks, 38% and 8% at 9 weeks, and 42% and 7% at 12 weeks after inoculation, respectively. All vermiform developmental stages were extracted from the callus and the medium. Toward the end of the experiment more nematodes were moving into the medium, and many nematode clusters were observed on the modified White's medium. The percentage of nematodes recovered from the medium, compared to the total population counted in the petri dishes, increased from 15% at 6 weeks after inoculation to 68% at 12 weeks after inoculation.

Test for pathogenicity: On the 'Grande Naine' plantlets, the *R. similis* population cultured on alfalfa callus proved to be as pathogenic as the population cultured on carrot discs. No differences ($P \leq 0.05$) were observed between the two populations concerning damage or reproduction (Table 3).

Reproduction of R. similis, extracted from alfalfa callus, on the Musa cultivar Grande Naine (AAA) grown in vitro: The in vitro 'Grande Naine' plantlets were a good host for *R. similis:* the nematode population increased 126-fold in 8 weeks, reaching on average 6,282 juveniles, females, and males (Table 4). The nematodes were able to penetrate and reproduce in the roots, and 81% of the total population was recovered from the root system. All vermiform developmental stages (juveniles, females, and males) were extracted from the roots as well as from the medium. The proportion of females and males counted in the roots and the medium was 4% and 3% in the roots and 12% and 4% in the medium, respectively.

Reproduction of R. similis, extracted from alfalfa callus, on A. thaliana grown in vitro: Radopholus similis could successfully penetrate and develop in *A. thaliana* under monoxenic conditions. Ten weeks after inoculation, the population reached 975 individuals (Table 4). The recovery of 367 females and the presence of males indicated that the life cycle was completed. All vermiform developmental stages were observed in the medium prior to extraction. Lesions on *A. thaliana* were observed as localized yellowing of the cortex tissue.

Table 1. Reproduction of *Radopholus similis* (Ugandan isolate) on alfalfa callus and carrot disc 5 weeks after inoculation with 25 females, incubated at 28 ± 0.5 °C in the dark.

Tissue	Total final population	R r[b]
Alfalfa callus	3,433 a[a]	137 a
Carrot disc	10,893 b	379 b

[a] Data are means of 10 replicates. Means in the same column followed by the same letter are not different ($P \leq 0.05$) according to the Tukey-test. Data were log(x + 1) transformed prior to statistical analysis.
[b] R r = Reproduction ratio.

Discussion

Alfalfa callus has proved to be a good host tissue for aseptic culturing of different migratory plant-parasitic nematodes, including *R. similis* (Myers et al., 1965; Castro and Ferraz, 1990; Ko et al., 1996). Our results confirmed the good-host capacity, although a different growth medium was used instead of the Krusberg' medium. For the production of *Pratylenchus penetrans,* the modified White's medium (containing 0.2 ppm α-NAA and 2 ppm 2,4-D) used in our experiments proved to be as good as Krusberg's medium (Riedel and Foster, 1970).

Albeit a poorer host than carrot disc, alfalfa callus has important advantages. First, the nematodes feed on sterile tissue and appear to be free of bacteria and fungi (after testing on PDA and NA). Second, this culturing system needs to be subcultured less frequently and the nematodes are more easily available. And third, the maintenance of the nematode culture is less labor-intensive because a piece of infected callus or medium is transferred to a fresh callus (Myers et al., 1965). In alfalfa callus, the population can reach the same level as in carrot disc (Stoffelen et al. 1999a), although a longer incubation time is needed. The different extraction techniques used in the first experiment may have influenced the results. The higher population recovered from carrot discs could be explained by a proportion of dead vermiform nematodes.

TABLE 2. Reproduction of *Radopholus similis* (Ugandan isolate) on alfalfa callus 6, 9, and 12 weeks after inoculation with 25 females, incubated at 28 ± 0.5 °C in the dark.

Time (weeks)	Nematodes in callus				Nematodes in medium					
	Juveniles	Females	Males	Total	Juveniles	Females	Males	Total	P f[a]	R r[b]
6	383	160	13	556	38	60	3	101	657 a[c]	26.3 a
9	1,246	654	123	2,023	363	491	104	958	2,981 b	119.2 b
12	908	746	143	1,797	1,971	1,591	223	3,784	5,581 c	223.2 c

[a] P f = Final population including juveniles, females, and males extracted from callus and medium.
[b] R r = Reproduction ratio = Pf/Pi = Final population/Initial population.
[c] Data are means of eight replicates. Means in the same column followed by the same letter are not different ($P \leq 0.05$) according to the Tukey-test. Data were log(x + 1) transformed prior to statistical analysis.

Although alfalfa callus was used by others to culture *R. similis,* the pathogenicity after culturing on alfalfa callus was never tested. The pathogenicity of other nematodes has been reported to be unaltered after culturing under aseptic conditions. This was reported for *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa propagated on alfalfa callus (Högger, 1969), and *Heterodera schachtii* propagated on transformed beet roots (Paul et al., 1987). This study confirmed it for the first time for *R. similis* after culturing on alfalfa callus, using susceptible banana plants in the greenhouse.

Based on the good reproduction of *R. similis* on in vitro 'Grande Naine' plantlets and the necrosis observed in the root systems, this in vitro system could be further developed to reveal variations in resistance within the *Musa* spp. at a very early stage. Preliminary results indicated that resistance to *R. similis* could be identified in plantlets growing in rooting medium under in vitro conditions (INIBAP, 1998). To obtain a reproducible method, it is important to include a highly susceptible cultivar, like 'Grande Naine', as a reference. In the past, 'Grande Naine' was used in greenhouse screening experiments as a susceptible reference cultivar (Stoffelen et al., 1999b). According to Mateille (1990), in vitro systems are unsuitable for screening banana cultivars for resistance to *R. similis* because the in vitro plantlets are too sensitive to reveal variation in resistance. However, if the period prior to analysis is not too long and the container is not too small, thus allowing extensive root growth, the nematodes won't be too detrimental to the plantlets and variations in resistance might be revealed.

Sijmons et al. (1991) established *A. thaliana* as a model for plant-parasitic nematodes. It was suggested that expressing gene products detrimental to nematodes in this plant might rapidly lead to engineering new varieties with increased resistance to nematodes. For *R. similis,* the most important nematode parasitizing bananas, no information was available on whether *A. thaliana* could be used as a model host for *R. similis.* In this study, penetration and reproduction were confirmed on bananas for the first time and an in vitro evaluation system with *A. thaliana* was further optimized for this tropical endoparasitic nematode. Two important modifications were made to the in vitro evaluation system: (i) after transfer from germination medium to Knop medium, a 10-day period allowed the plants to develop a branched root system; and (ii) after the inoculation, the petri dishes were incubated at 28 °C. This temperature is more optimal for reproduction of *R. similis* (Fallas and Sarah, 1995) and does not restrict further growth of the *A. thaliana* plantlets.

In conclusion, in vitro alfalfa callus is an aseptic culturing system that supplies sufficient sterile *R. similis* on a continuous basis. The nematodes cultured on the callus can infect and reproduce in in vitro banana plantlets and in vitro *A. thaliana.* Callus culture does not affect pathogenicity as compared to an *R. similis* population cultured on carrot disc. This opens new perspectives for rapid in vitro screening and rapid testing of interesting anti-nematode proteins expressed in transgenic lines of *A. thaliana.* Still, the use of these systems for screening for resistance to *R. similis* must be confirmed.

TABLE 3. Test for pathogenicity of *Radopholus similis* cultured on alfalfa callus and carrot disc, on 'Grande Naine' in the greenhouse (analysis 8 weeks after inoculation with 1,000 vermiforms).

	Root weight (g)	Root necrosis (%)	*R. similis* in total root system[b]	*R. similis* per gram fresh root[b]
'Grande Naine' inoculated with *R. similis* cultured on alfalfa callus	113 a[a]	15 a	10,929 a	90 a
'Grande Naine' inoculated with *R. similis* cultured on carrot disc	129 a	9 a	5,726 a	55 a

[a] Data are means of eight replicates. Means in the same column followed by the same letter are not different ($P \leq 0.05$) according to the Tukey-test.
[b] Data were log(x + 1) transformed prior to statistical analysis.

TABLE 4. Reproduction of *Radopholus similis* (Ugandan isolate) on in vitro plants of 'Grande Naine' (*Musa* AAA) and *Arabidopsis thaliana* 8 and 10 weeks, respectively, after inoculation with 50 and 60 females, respectively, incubated at 28 ± 0.5 °C (16/8 hours light regime).

Host	Juveniles	Females	Males	Total	P f[a]	R r[b]
Grande Naine nematodes						
in roots	4,798	187	128	5,113	6,282	125.6
in medium	975	146	48	1,169		
A. thaliana	441	367	167	975	975	16.3

[a] P f = Final population including juveniles, females, and males extracted from callus and medium.
[b] R r = Reproduction ratio = Pf/Pi = Final population/Initial population.
All data are means of eight replicates.

LITERATURE CITED

Baermann, G. 1917. Eine einfache Methode zur Auffindung von *Ankylostonum* (Nematoden) Larven in Erdproben. Geneeskundig tijdschrift Nederland-Indië 57:131–137.

Banerjee, N., and E. De Langhe. 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). Plant Cell Reports 4:351–354.

Brown, S. M., and J. C. Vessey. 1985. Rearing of *Radopholus similis* on banana fruit callus. Revue de Nématologie 8:179–190.

Castro, M. E. A., and S. Ferraz. 1990. Multiplication of *Pratylenchus brachyurus, P. zeae, Radopholus similis,* and *Tylenchorhynchus* sp. in monoxenic culture on alfalfa callus. Nematologica Brasileira 14:103–120.

Davide, R. G. 1996. Overview of nematodes as limiting factor in *Musa* production. Pp. 27–31 *in* E. Frison, J. P. Horry, and D. De Waele, eds. Proceedings of the workshop on new frontiers in resistance breeding for nematode, fusarium, and sigatoka, 2–5 October 1995, Kuala Lumpur, Malaysia, INIBAP, Montpellier.

Fallas, G., and J. L. Sarah. 1995. Effect of temperature on the in vitro multiplication of seven *Radopholus similis* isolates from different banana-producing zones of the world. Fundamental and Applied Nematology 18:445–449.

Högger, C. H. 1969. Comparison of penetration of potato roots by *Pratylenchus penetrans* grown in tissue cultures and from field populations. Journal of Nematology 1:10.

Huettel, R. N. 1989. In vitro screening of soybean lines to *Radopholus similis* and *R. citrophilus*. Nematropica 19:143–149.

INIBAP. 1998. Networking banana and plantain: INIBAP Annual report 1997. International Network for the Improvement of Banana and Plantain, Montpellier. Pp. 29–30.

Inserra, R. N., and J. H. O'Bannon. 1975. Rearing migratory nematodes in citrus callus and roots produced from citrus leaves. Journal of Nematology 7:261–263.

Ko, M. P., D. P. Schmitt, and B. S. Sipes. 1996. Axenizing and culturing endomigratory plant-parasitic nematodes using Pluronic F127, including its effects on population dynamics of *Pratylenchus penetrans*. Journal of Nematology 28:115–123.

Mateille, T. 1990. Monoxenic culture of banana-parasitic nematodes on *Musa acuminata* cv. Poyo shoots. Journal of Nematology 22:608–611.

Moody, E. H., B. F. Lownsbery, and J. M. Ahmed. 1973. Culture of the root-lesion nematode *Pratylenchus vulnus* on carrot discs. Journal of Nematology 5:225–226.

Myers, R. F., W. A. Feder, and P. C. Hutchins. 1965. The rearing of *Radopholus similis* (Cobb) Thorne on grapefruit, okra, and alfalfa root callus. Proceedings of the Helminthological Society of Washington 32:94–95.

O'Bannon, J. H., and A. L. Taylor. 1968. Migratory endoparasitic nematodes reared on carrot discs. Phytopathology 58:385.

Paul, H., C. Zijlstra, J. E. Leeuwangh, F. A. Krens, and H. J. Huizing. 1987. Reproduction of the beet cyst nematode *Heterodera schachtii* Schm. on transformed root cultures of *Beta vulgaris* L. Plant Cell Reports 6:379–381.

Pinochet, J. 1988. A method for screening bananas and plantains to lesion-forming nematodes. Nematodes and the borer weevil in banana: Present status of research and outlook. Proceedings of a workshop held in Bujumbura; 7–11 December 1987; Bujumbura. INIBAP, Montpellier. Pp. 62–65.

Pinochet, J. 1996. Review of past research on *Musa* germplasm and nematode interactions. Pp. 32–44 *in* E. Frison, J. P. Horry, and D. De Waele, eds. Proceedings of the workshop on new frontiers in resistance breeding for nematode, fusarium, and sigatoka, 2–5 October 1995, Kuala Lumpur, Malaysia. INIBAP, Montpellier.

Price, N. S. 1994. Field trial evaluation of *Musa* varieties and other crops as hosts of *Pratylenchus goodeyi* in Cameroon. Afro-Asian Journal of Nematology 4:11–16.

Reise, R. W., R. N. Huettel, and R. M. Sayre. 1987. Carrot callus culture of endoparasitic nematodes. Journal of Nematology 19:387–389.

Riedel, R. M., and J. G. Foster. 1970. Monoxenic culture of *Ditylenchus dipsaci* and *Pratylenchus penetrans* with modified Krusberg's and White's medium. Plant Disease Reporter 54:251–254.

Sarah, J. L. 1989. Banana nematodes and their control in Africa. Nematropica 19:199–216.

Sarah, J. L., J. Pinochet, and J. Stanton. 1996. The burrowing nematode of bananas, *Radopholus similis* Cobb, 1913. *Musa* pest fact sheet no. 1. INIBAP, Montpellier.

Sijmons, P. C., F. M. W. Grundler, N. von Mende, and P. R. Burrows. 1991. *Arabidopsis thaliana* as a new model host for plant-parasitic nematodes. Plant Journal 1:245–254.

Speijer, P. R., and D. De Waele. 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines 1.

Stoffelen, R., M. I. Jimenez, C. Dierckxsens, V. T. T. Tam, R. Swennen, and D. De Waele. 1999a. Effect of time and inoculum density on the reproductive fitness of *Pratylenchus coffeae* and *Radopholus similis* populations on carrot discs. Nematology 1:243–250.

Stoffelen, R., R. Verlinden, N. T. Xuyen, R. Swennen, and D. De Waele. 1999b. Screening of Papua New Guinea bananas to root-lesion nematodes and root-knot nematodes. InfoMusa 8:12–15.

Valvekens, D., M. Van Montagu, and M. Van Lijsebettens. 1988. *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection. Proceedings of the National Academy for Science (USA) 85:5536–5540.

Wang, K., A. R. Kuehnle, and B. S. Sipes. 1997. In vitro screening for burrowing nematode, *Radopholus citrophilus,* tolerance and resistance in commercial *Anthurium* hybrids. In Vitro Cellular Developmental Biology-Plant 33:205–208.

White, P. R. 1963. The cultivation of animal and plant cells. 2nd edition. New York: Ronald Press.