EVALUATING HOST PLANT REACTION OF *MUSA* GERMPLASM TO *RADOPHOLUS SIMILIS* BY INOCULATION OF SINGLE PRIMARY ROOTS

B. De Schutter,[1] P. R. Speijer,[2†] C. Dochez,[2] A.Tenkouano,[1] and D. De Waele[3]

International Institute of Tropical Agriculture (IITA)—High Rainfall Station, Onne, Nigeria, c/o Lambourn & Co., Carolyn House, 26 Dingwall Road, Croydon CR9 3EE, England,[1] International Institute of Tropical Agriculture (IITA)—Eastern and Southern Africa Regional Center (ESARC), P.O. Box 7878, Kampala, Uganda,[2] and Laboratory of Tropical Crop Improvement, Catholic University Leuven (K.U. Leuven), Kasteelpark Arenberg 13, 3001 Leuven, Belgium.[3]

RESUMEN

De Schutter, B., P.R. Speijer, C. Dochez, A. Tenkouano y D. De Waele. 2001. Evaluación de la reacción hospedera de un germoplasma de Musa a *Radopholus similis* a través de la inoculación de raíces primarias individuales. Nematrópica 31:297-301.

Se evaluó un nuevo método para seleccionar fuentes de resistencia a *Radopholus similis* en un germoplasma de bananos y plátanos. Los experimentos se realizaron bajo condiciones experimentales similares en Nigeria y Uganda. En ambas localidades, *R. similis* fue capaz de penetrar, colonizar y reproducirse en segmentos de raíces primarias de 8 cm de largo de los cultivares susceptibles 'Agbagba' y 'Valery', aumentando respectivamente 40 y 50 veces la población de nematodos al cabo de 8 semanas después de la inoculación con 50 nematodos por segmento de raíz. El método permitió la detección de la resistencia de 'Yangambi km5', 'SH-3142', y la resistencia parcial de 'Gros Michel' a *R. similis*. Las tasas de reproducción de *R. similis* en segmentos de raíces primarias de 'Yangambi km 5' y 'SH-3142' fueron menores de 1 y 1,5 respectivamente, mientras que la tasa reproductiva en segmentos de raíces primarias de 'Gros Michel' fue 3.9 y 55.2 en 'Valery'. Estas diferencias fueron significativas de acuerdo a la pueba de contrastes ortogonales. Finalmente, el método permitió la identificación de varios híbridos, los cuales presentaron diferentes niveles de resistencia a *R. similis*.

Palabras claves: Germoplasma, metodología, *Musa*, nematodo barrenador, raíces primarias, *Radopholus similis*, resistencia, respuesta de hospedera, selección, susceptibilidad.

Of the plant-parasitic nematodes associated with banana and plantain in Africa, the burrowing nematode *Radopholus similis*, the root-lesion nematodes *Pratylenchus coffeae* and *Pratylenchus goodeyi*, the spiral nematode *Helicotylenchus multicinctus* and root-knot nematodes (*Meloidogyne* spp.) are the most common (Speijer and Fogain, 1999). With the exception of the sedentary root-knot nematodes, all these nematodes feed, migrate and multiply in the roots and corm, reducing the size of the root system by causing root necrosis.

The use of nematicides to control nematodes has adverse environmental effects and is too costly for subsistence farmers. A promising alternative is the use of nematode-resistant varieties. Such varieties can be obtained by selecting for the highest available resistance from among existing genotypes or by classical breeding. In both cases, the first step is the screening of germplasm for sources of resistance to nematodes. In *Musa*, resistance screening is usually undertaken either in pots or plastic bags placed in a greenhouse (see e.g., Fogain *et al.*, 1996; Stoffelen *et al.*, 2000) or in the field (see e.g., Price, 1994; Speijer *et al.*, 1999). Screening experiments in pots or plastic bags will only allow observations

[†]Paul R. Speijer died January 30, 2000.

to be made for a relatively short period (2 to 3 months) of the crop cycle. During this period, the susceptibility of the genotypes can be determined by assessing the nematode reproduction ratio (final population divided by the initial population). Screening experiments in the field will allow observations to be made throughout the first crop cycle and subsequent ratoon crops. During this period, the susceptibility of the genotypes can be determined by assessing the nematode reproduction. If uninfected plants are included in the screening experiments, observations can also be made on the tolerance of the genotypes and effects on yield. Thus, field experiments provide the best possible evaluation of the response of genotypes to nematode parasitism. However, the number of genotypes that can be included in greenhouse and field experiments is limited, especially in field experiments. Also, for greenhouse experiments and for field experiments when a naturally infested field is not available, a large number of nematodes is necessary as inoculum (a minimum of 1 000 vermiform nematodes per 1-liter pot is required). Other problems in *Musa* are the differential growth rate of root systems, which vary between genotypes. This may complicate interpretation of the nematode population dynamics. Primary roots are produced in flushes and this may result in a root system that consists of a mixture of roots varying in age (Blomme, 2000). Differences in the reproduction (and root damage) of *R. similis* on 2-month-old sword sucker-derived plants and sword suckers of established mats were observed during early field screening of *Musa* genotypes in Uganda, suggesting a different host response to nematode infection of young root systems compared to old root systems (Speijer *et al.*, 1999).

The aim of our study was to develop a fast and reliable method for screening *Musa* germplasm for resistance to *R. similis,* a system that needs fewer plants and less space, a lower nematode inoculum than the screening methods currently in use, and that is not influenced by root growth rate or age. Throughout this communication the host response terminology proposed by Bos and Parlevliet (1995) is followed.

The experiments were carried out at the International Institute of Tropical Agriculture (IITA) High Rainfall Station at Onne in southern Nigeria (situated at sea level) and at the Sendusu farm of the Eastern and Southern Africa Regional Center of IITA at Namulonge in central Uganda (situated at 1000 m above sea level).

In Nigeria, the experiment was carried out under a roofed area with an average air temperature ranging from 25°C to 27°C and relative humidity ranging from 78% to 89%. The experiment included the cultivars 'Agbagba' (*Musa* AAB-group, plantain subgroup), which is a good host for *R. similis* (Price, 1994); 'Yangambi km 5' (*Musa* AAA-group), which is resistant to *R. similis* (Sarah *et al.*, 1992; Price, 1994; Fogain and Gowen, 1998); and the plantain hybrid 'TMPx 1658-4' with unknown host response to *R. similis.* In Uganda, the experiment was carried out in a screenhouse and included 'Yangambi km 5' and the diploid hybrid 'SH-3142', which is also resistant *to R. similis* (Pinochet and Rowe, 1979), 'Gros Michel' (*Musa* AAA, Gros Michel subgroup), which is partially resistant to *R. similis* (Mateille, 1992; Price, 1994), the susceptible cultivar 'Valery' (*Musa* AAA, Cavendish subgroup), the East African Highland banana-derived tetraploid hybrid 'TMHx 481K-1' and the diploid banana hybrids 'TMB2x 9128-3' and 'TMB2x 9722-1'. In both localities, suckers were used as planting material. The suckers were selected for absence of weevil damage, carefully pared to remove roots and corm tissue with symptoms of

nematode infection, and then hot water-treated (53°C to 55°C) for 20 minutes (Colbran, 1967) before planting. Three suckers of each genotype were planted in wooden boxes (0.5 m × 0.5 m × 0.5 m) containing sawdust. Four weeks after planting, three equally developed primary roots were selected from each sucker. At a distance of 5 cm from the corm, a segment of each selected root was carefully placed in a small plastic container (8 cm diameter, 5 cm high) two days before inoculation (Fig. 1). The roots were inoculated by pouring a 4 ml aqueous suspension containing 50 vermiform *R. similis* nematodes directly onto the 8-cm-long root segment, then covering it with heat-sterilized soil. In Nigeria, nematode inoculum was obtained by hand-picking *R. similis* from an aqueous suspension containing nematodes extracted from infected 'Valery' (*Musa* AAA-group) roots using a modified Baermann funnel technique (Speijer and De Waele, 1997). In Uganda, nematode inoculum was obtained from carrot disc cultures (Pinochet *et al.*, 1995). Inoculum was prepared by rinsing the Petri dishes containing the carrot discs with sterile distilled water and collecting the nematodes in a test tube. In both localities, the experiments were concluded 8 weeks after inoculation. The plastic containers with the root segments were carefully excavated. The root segments were removed, washed with tap water, chopped into 0.5 cm pieces, thoroughly mixed and macerated in a blender for two periods of 10 seconds separated by a 5-second interval. Nematodes were extracted from the root slurry overnight using a modified Baermann funnel technique (Speijer and De Waele, 1997). Per root segment, the nematodes in three 2 ml aliquots out of a 25 ml aqueous suspension were counted. Nematode population densities were log(x + 1) transformed prior to analysis (Gomez and Gomez, 1984). Orthogonal treatment contrasts were run to compare reproduction (SAS, 1997).

Fig. 1. Root placed in cup about 5 cm from the corm.

Radopholus similis was able to penetrate, colonize and reproduce in the susceptible cultivars. From the root segments of 'Agbagba' and 'Valery', 2 023 and 2 761 nematodes, respectively, were recovered representing a 40- and 55-fold population increase (Table 1). In both Nigeria and Uganda, the reproduction ratio's of 'Yangambi km 5' and 'SH-3142' were less than 1 and 1.5, respectively. In Uganda, the reproduction ratio of 'Gros Michel' was only 3.9 compared with 55.2 for 'Valery'. The reproduction ratio's of 'Yangambi km 5', 'SH-3142' and 'Gros Michel' were all lower ($P < 0.001$) compared to the reproduction ratio's of either 'Agbagba' or 'Valery' as shown by the orthogonal contrasts (Table 2).

In Nigeria, 'TMPx 1658-4' was less susceptible to *R. similis*, with a reproduction ratio that was not different from that of

Table 1. Reproduction ratio (Rr) of *Radopholus similis* in 8-cm-long primary root segments of *Musa* genotypes measured 8 weeks after inoculation with a suspension containing 50 vermiform nematodes (Pi) per primary root segment.

Genotype	n	Pf[y]	Rr[z]
		Nigeria	
Yangambi km 5	9	36	0.7
Agbagba	9	2 023	40.5
TMPx 1658-4	9	191	5.7
		Uganda	
Yangambi km 5	9	25	0.5
Valery	9	2 761	55.2
Gros Michel	9	196	3.9
TMHx 418K-1	9	937	18.7
TMB2x 9128-3	9	27	0.7
TMB2x 9722-1	9	1 083	21.7
SH-3142	9	77	1.5

[y]Pf = Final nematode population (including all vermiform developmental stages).
[z]Rr = Pf/Pi.

'Yangambi km 5' ($P > 0.05$), but was lower ($P < 0.001$) compared to 'Agbagba' (Table 2). In Uganda, the reproduction ratio's of all three hybrids included in the experiment were lower ($P < 0.05$) than that of 'Valery' (Table 2). The reproduction ratio of 'TMB2x 9128-3' was less than 1 and not different from that of 'Yangambi km 5' ($P > 0.05$).

The results of our study show that inoculating single primary roots of *Musa* with a low inoculum of *R. similis* allows the identification of resistance at an early stage of plant growth. Similar results were obtained for the host response of the *R. similis*-resistant genotype 'Yangambi km 5' in Nigeria and Uganda. The screening method used needs fewer plants and space than the screening methods currently in use and a low nematode inoculum. By using single roots, the evaluation of the host response to nematode attack is not influenced by differences in root growth rates among *Musa* genotypes. Moreover, primary roots of the same age can be selected for inoculation, avoiding bias caused by differences in host response to *R. similis* related to root age.

Table 2. Orthogonal contrasts between the reproduction of *Radopholus similis* on single primary root segments of *Musa* genotypes.

	Nigeria	
Genotype	Contrast with 'Yangambi km 5'	Contrast with 'Agbagba'
Yangambi km 5		***
Agbagba	***	
TMPx 1658-4	NS	***
	Uganda	
Genotype	Contrast with 'Yangambi km 5'	Contrast with 'Valery'
Yangambi km 5		***
Valery	***	
Gros Michel	NS	***
TMHx 418K-1	NS	***
TMB2x 9128-3	NS	***
TMB2x 9722-1	**	**
SH-3142	NS	***

***Contrast significant ($P < 0.001$).
NS: Contrast not significant ($P > 0.05$).

ACKNOWLEDGMENTS

Financial support by the Flemish Agency for Development Co-operation and Technical Assistance (VVOB) and the Directorate General for International Co-operation (DGIC, Belgium) is gratefully acknowledged. The authors wish to thank Mr. Idowu Rotifa and Mrs. Pamela Mpirirwe for helping with data collection. This is IITA manuscript number IITA/00/JA/76.

LITERATURE CITED

BLOMME, G. 2000. The interdependence of root and shoot development in banana (*Musa* spp.) under field conditions and the influence of different biophysical factors on this relationship. Ph.D. Thesis. Katholieke Universiteit Leuven, Belgium.

BOS, L., and J. E. PARLEVLIET. 1995. Concepts and terminology on plant/pest relationships: toward consensus in plant pathology and crop protection. Annual Review of Phytopathology 33:69-102.

COLBRAN, R. C. 1967. Hot water tank for treatment of banana planting material. Advisory Leaflet no 924: 4. Queensland Department of Primary Industries, Division of Plant Industry, Brisbane, Australia.

FOGAIN, R., and S. R. GOWEN. 1998. 'Yangambi km5' (*Musa* AAA, Ibota subgroup): a possible source of resistance to *Radopholus similis* and *Pratylenchus goodeyi*. Fundamental and Applied Nematology 21:75-80.

FOGAIN, R., S. R. GOWEN, and F. MEKEMBA. 1996. Screening for susceptibility to *Radopholus similis*: evaluation of plantains AAB and diploid AA, AB and BB. Tropical Agriculture (Trinidad) 73:281-285.

GOMEZ, K. A., and A. A. GOMEZ. 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons, New York, NY, U.S.A.

MATEILLE, T. 1992. Comparative development of three banana-parasitic nematodes on *Musa acuminata* (AAA group) cvs Poyo and Gros Michel *vitro*-plants. Nematologica 38:203-216.

PINOCHET, J., C. FERNANDEZ, and J.-L. SARAH. 1995. Influence of temperature on the *in vitro* reproduction of *Pratylenchus coffeae*, *P. goodeyi* and *Radopholus similis*. Fundamental and Applied Nematology 18:391-392.

PINOCHET, J., and P. R. ROWE. 1979. Progress in breeding for resistance to *Radopholus similis* on bananas. Nematropica 9:76-78.

PRICE, N. S. 1994. Field trial evaluation of nematode susceptibility within *Musa*. Fundamental and Applied Nematology. 17:391-396.

SARAH, J. L., F. BLAVIGNAC, F. SABATINI, and M. BOISSEAU. 1992. Une méthode de laboratoire pour le criblage variétal des bananiers vis-à-vis de la résistance aux nématodes. Fruits 47: 559-564.

SAS. 1997. SAS Guide for Personal Computers. 6th edition. Cary, NC, U.S.A.

SPEIJER, P. R., E. BOONEN, D. VUYLSTEKE, R. L. SWENNEN, and D. DE WAELE. 1999. Nematode reproduction and damage to *Musa* sword suckers and sword sucker derived plants. Nematropica 29:193-203.

SPEIJER, P. R., and D. DE WAELE. 1997. Screening of *Musa* germplasm for resistance and tolerance to nematodes. INIBAP Technical Guidelines 1. INIBAP, Montpellier, France.

SPEIJER, P. R., and R. FOGAIN. 1999. *Musa* and *Ensete* nematode pest status in selected African countries. Pp. 99-108 *in* E. A. Frison, C. S. Gold, E. B. Karamura and R. A. Sikora, eds. Mobilizing IPM for Sustainable Banana Production in Africa. Proceedings of a Workshop held in Nelspruit, South Africa, 23-28 November 1998. INIBAP, Montpellier, France.

STOFFELEN, R., R. VERLINDEN, N. T. XUYEN, R. SWENNEN, and D. DE WAELE. 2000. Host plant response of *Eumusa* and *Australimusa* bananas (*Musa* spp.) to migratory endoparasitic and root-knot nematodes. Nematology 2:907-916.

Received: *1.II.2001*

Recibido:

Accepted for publication: *5.VII.2001*

Aceptado para publicación:

BLANK PAGE USED IN PAGE COUNT