BURROWING-NEMATODE RESISTANCE OF BLACK SIGATOKA RESISTANT BANANA HYBRIDS

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ABSTRACT

Marin, D. H., T. B. Sutton, K. R. Barker, D. T. Kaplan, and C. H. Opperman. 1998. Burrowing-nematode resistance of black Sigatoka resistant banana hybrids. Nematropica 28:241-247.

Resistance of black Sigatoka resistant hybrids FHIA-01, FHIA-02, FHIA-03, and FHIA-21 to the burrowing nematode, *Radopholus similis*, was assessed under greenhouse conditions. Resistance was evaluated by inoculating the hybrid plants with 200 monoxenically-reared nematodes/plant. Banana plants produced by tissue culture were grown in 0.4-L Styrofoam cups, containing a 1:1 mix of a coarse and a fine sand, at ~27°C and 80% RH. Plants were allowed to acclimate and grow for 4 weeks prior to inoculation. Plant height, fresh shoot and root weights, nematode population levels, and root necrosis (0-100%) were determined 8 weeks after inoculation. Pisang Jari Buaya (accession III-106) and Grande Naine (*Musa* AAA, Cavendish subgroup) were used as resistant and susceptible controls, respectively. Final nematode population numbers and root-necrosis indices did not differ among FHIA-01, FHIA-03 and the susceptible control (Grande Naine). Although *R. similis* reproduced poorly in Pisang Jari Buaya, extensive root necrosis (~50%) was observed. Interactions of FHIA hybrids and nematode populations from Honduras and Costa Rica were also evaluated. The population from Costa Rica had a higher reproductive factor (4.0-7.8) than the population from Honduras (2.1-3.3). However, damage potential (root necrosis) did not differ greatly between the two populations.

Keywords: FHIA hybrids, *Musa* spp., *Radopholus similis*.

RESUMEN

Marin, D. H., T. B. Sutton, K. R. Barker, D. T. Kaplan y C. H. Opperman. 1998. Resistencia al nematodo barrenador de híbridos de banano resistentes a Sigatoka negra. Nematrópica 28:241-247.

La resistencia al nematodo barrenador, *Radopholus similis*, de los híbridos FHIA-01, FHIA-02, FHIA-03 y FHIA-21 fue evaluada bajo condiciones de invernadero. La resistencia se determinó inoculando las plantas con 200 nematodos reproducidos en cultivo monoxénico. Las plantas micropropagadas fueron aclimatadas en vasos de estereofón de 0,4 L, conteniendo una mezcla 1:1 de una arena gruesa y otra fina, a una temperatura de ~25°C y 80% de H.R. Las plantas fueron aclimatadas por 4 semanas antes de la inoculación. La altura de la planta, el peso fresco de la parte aérea y radicular, los niveles poblacionales de los nematodos, y la necrosis radicular (0-100%) fueron determinados 8 semanas después de la inoculación. Los cultivares Pisang Jari Buaya (introducción III-106) y Grande Naine (*Musa* AAA, subgrupo Cavendish) fueron utilizados como los testigos resistente y susceptible, respectivamente. La población final de nematodos y los índices de necrosis radicular no difirieron entre el FHIA-01, FHIA-03 y el testigo susceptible, Grande Naine. A pesar de que *R. similis* se reprodujo poco en Pisang Jari Buaya, la necrosis radicular fue relativamente alta (~50%). Se evaluó también la interacción entre poblaciones de *R. similis* de Honduras y Costa Rica con los híbridos de FHIA. La población de Costa Rica mostró una capacidad reproductiva mayor que la población de Honduras. Sin embargo, ambas poblaciones mostraron un daño potencial (necrosis radicular) similar.

Palabras clave: Híbridos de FHIA, *Musa* spp., *Radopholus similis*.

INTRODUCTION

Root and rhizome decay of bananas, caused primarily by the burrowing nematode, *Radopholus similis* (Cobb) Thorne, is the second most serious disease of Cavendish banana cultivars, following black Sigatoka (Gowen and Quénéhervé, 1990; Stover and Simmonds, 1987). *Radopholus* spp. are among the 10 most damaging nematode species worldwide. Crop losses in bananas to nematodes have been estimated to be 19.7% worldwide (Sasser and Freckman, 1987).

Nematode management practices in bananas include the application of granular nematicides in combination with agronomic practices, such as propping and guying (Gowen, 1995; Stover and Simmonds, 1987). Several problems have arisen with nematicides, including accelerated biodegradation of certain compounds, adverse effects on non-target organisms, contamination of water sources, and increased production costs (Jaramillo, 1988; Quénéhervé, 1993). Consequently, other options are needed to broaden and improve the management of nematodes in banana.

The incorporation of resistance through plant breeding offers an important advantage over other forms of nematode control. Development of useful nematode resistance in bananas and plantains has not been easy as these plant species are genetically complex. Additionally, important features, such as bunch size and shelf life, are likely to be altered or lost during the breeding process (Pinochet, 1988a). Resistance to *R. similis* has been identified in the Pisang Jari Buaya group (Wehunt *et al.*, 1978) and one clone, SH-3142, derived from a Pisang Jari Buaya parent (accession II-115) and SH-173, has been used extensively by banana breeders to develop burrowing-nematode resistant clones (Rowe, 1984; Pinochet, 1992; Gowen, 1994). One or more dominant alleles confer resistance to *R. similis* (Pinochet, 1988a, 1992, 1996; Pinochet and Rowe, 1979). Thus, it is possible to incorporate resistance from Pisang Jari Buaya into tetraploid or diploid hybrids (Ortíz, 1995; Pinochet, 1992, 1996).

In 1993, FHIA (Fundación Hondureña de Investigación Agrícola) released the first black Sigatoka resistant hybrid that could be planted as a replacement of varieties belonging to the Musa AAA Cavendish subgroup. FHIA-01 (Goldfinger) is a tetraploid derived from crossing SH-3142 onto a Dwarf Prata triploid (FHIA, 1993). SH-3142, derived from a Pisang Jari Buaya parent and SH-1734, is highly resistant to *R. similis* and is the most promising diploid tested for nematode resistance (Pinochet and Rowe, 1979; Pinochet, 1992). Other selections, in addition to FHIA-01, have been released in the last few years, mostly for screening evaluations in the International *Musa* Testing Program (IMTP, an INIBAP-IPGRI sponsored program) and/or for national programs in collaboration with FHIA.

There is evidence that *R. similis* populations from bananas from Latin America, the Caribbean Islands, Africa and Asia vary in their aggressiveness (reproductive fitness and damage potential) (Fallas *et al.*, 1995; Hahn *et al.*, 1995,1996; Marin, 1997; Pinochet, 1988b, 1992; Sarah *et al.*, 1993; Tarte *et al.*, 1981). Thus, a screening program for nematode resistance should utilize different populations of *R. similis* because of these differences. The objective of this research was to determine the resistance of newly released FHIA hybrids to two populations of *R. similis*, using a greenhouse screening technique (Marin, 1997).

MATERIALS AND METHODS

Two experiments were conducted in the greenhouse to evaluate the resistance of FHIA hybrids (Table 1) to *R. similis*. The

Table 1. FHIA hybrids selected to test burrowing-nematode resistance under greenhouse conditions.

Hybrid	Breeder's code[y]	Genome	Cross (parents)
FHIA-01	SH-3481	AAAB	Prata Ana × SH-3142
FHIA-02	SH-3486	AAAB	Prata Ana × SH-3393[z]
FHIA-03	SH-3565	AABB	SH-3386 × SH-3320
FHIA-21	SH-3460	AAAB	AVP-67 × SH-3142

[y]The prefix SH corresponds to "Selection Honduras", designated by the breeding program at FHIA.
[z]SH-3393 is a diploid (2N) product of the cross SH-3142 × SH-3217.

first experiment evaluated resistance against a nematode population from Costa Rica (CR1), in FHIA-01, FHIA-03, Pisang Jari Buaya (Musa AA accession III-106) and Grande Naine (*Musa* AAA, Cavendish subgroup). Pisang Jari Buaya and Grande Naine were the resistant and susceptible controls, respectively. In the second experiment resistance of FHIA-01, FHIA-02, FHIA-03 and FHIA-21 was evaluated against *R. similis* populations from Honduras (H1) and Costa Rica (CR5). These populations were selected because of their aggressiveness, moderate and high respectively (Marin, 1997). Both experiments included non-inoculated controls.

Plants of each genotype from tissue culture were grown in 0.4-L Styrofoam cups using a resistance-screening procedure that proved to be reliable in earlier related experiments (Marin, 1997). A 1:1 mixture of steamed coarse river sand and 212 µm white quartz sand (Whitehead Brothers Co., Florham Park, NJ) was used as substrate. A complete nutrient solution (Chem-Gro, Hydro-Gardens Inc., Colorado Springs, Co.), based on 100 mg L^{-1} N, was added twice a week, and deionized water was added as needed. The same amounts of fertilizer and water were added to all treatments. Plants were allowed to acclimate and grow for 4 weeks at ~27°C and 80% RH in the greenhouse before inoculation.

Monoxenic carrot-disk cultures (O'Bannon and Taylor, 1968) of *R. similis* were established from infected banana roots from Balatana Farm, Costa Rica (CR1), La Rita Research Station, Costa Rica (CR5), and Santa Rosa Farm, Honduras (H1). Burrowing nematodes were extracted from the cultures (Kaplan and Davis, 1990), quantified, concentrated and resuspended in sterile deionized water (~40 nematodes/ml). Five ml of the nematode suspension containing about 200 juveniles and adults were added to the base of each plant and covered with sand. Inoculated plants were maintained for an additional 8 weeks at ~27°C and 80% RH in the greenhouse.

Eight weeks after inoculation, plant height was measured from the base of the plant to the insertion of the cigar leaf. Fresh shoot and root weights were then measured (data not included). Total root necrosis and primary root necrosis (0-100%) also were determined on a visual (subjective) basis and used to assess damage potential. Primary-root necrosis was not measured in the first experiment.

Roots were cut in 1-cm pieces and incubated in water in jars at 25°C for 7 days to extract nematodes. Roots were washed on a set of 24- and 35-mesh sieves, and the nematodes were collected on a 38-µm pore sieve. Nematodes were resuspended in 100 ml of water, and a 10-ml aliquot was taken for quantification of each sample. The

number of nematodes per plant (P_f) was estimated and used as an indication of reproductive fitness. The nematode reproductive factors [= final population (P_f)/initial population (P_i)] also were calculated, but detailed data are not included herein.

Each experiment was designed and analyzed as a randomized complete block design with 10 replicates. The first experiment was repeated once over time. All data were subjected to analysis of variance, and all nematode and root necrosis data were transformed to $\log_{10}$ (x + 1) and square root of (x) respectively, before statistical analyses. Each variable was subjected to Waller-Duncan k-ratio (k = 100) t-tests. Statistical analyses were performed using SAS (release 6.11, SAS Institute, Cary, NC.).

RESULTS

Resistance of selected hybrids to CR1. The number of *R. similis* extracted from the banana genotypes differed (Fig. 1A). The final population (P_f) levels for the hybrids FHIA-01 and FHIA-03 were not significantly different from those in Grande Naine, the susceptible control. The nematode population from Costa Rica (CR1) reproduced poorly in the resistant control Pisang Jari Buaya.

Total root necrosis was not different between the two hybrids; root necrosis in Grande Naine was not statistically different ($P > 0.05$) to FHIA-01 (Fig. 1B). Pisang Jari Buaya showed the least necrosis; however, it was relatively high (~50%) considering the number of nematodes recovered.

Resistance to selected-nematode populations. Statistical analyses were performed for each hybrid individually because the interaction factor "cultivar × nematode population" was significant for total root ($P = 0.01$) and primary-root ($P < 0.001$) necrosis. This source of variation was not significant for the final number of nematodes ($P = 0.37$). The most consistent

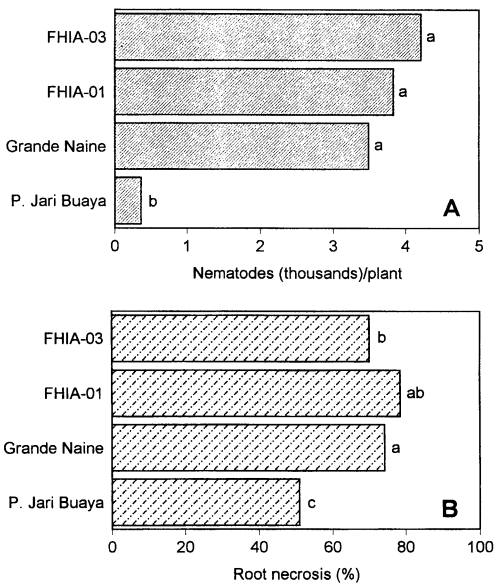

Fig. 1. Number of *Radopholus similis* extracted (A) and total root necrosis (B) of different banana genotypes inoculated with *R. similis* under greenhouse conditions. Bars followed with different letters are statistically different, according to Waller-Duncan k-ratio (k = 100) t-test.

effect evaluated was "nematode population", which had a $P < 0.001$ for the three variables determined.

The P_f for H1 and CR5 differed in FHIA-01 and FHIA-21, but was not different in FHIA-02 and FHIA-03 (Fig. 2A). In spite of different P_f's, the total-root necrosis was not different between nematode populations tested for all hybrids except FHIA-03 (Fig. 2B). Background root necroses in the non-inoculated controls were similar among hybrids (~20%).

Primary-root necrosis (Fig. 2C) differed between nematode populations on FHIA-01 and FHIA-03. The population H1 induced more necrosis in roots of FHIA-01 than CR5; however, for the other hybrids tested, CR5 had not only the highest P_f's but also induced more necrosis than H1. The amount of primary-root necrosis was ~10% in the non-inoculated controls.

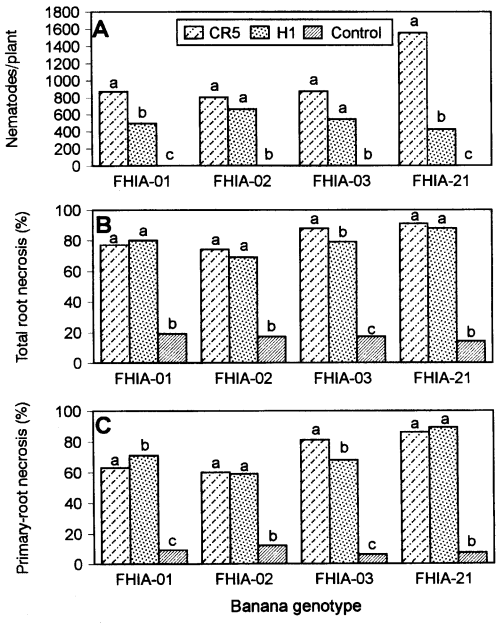

Fig. 2. Number of nematodes extracted (A), total root necrosis (B), and primary root necrosis (C) of different banana genotypes inoculated with 2 populations of *Radopholus similis* under greenhouse conditions. Bars followed with different letters are statistically different, according to Waller-Duncan k-ratio (k = 100) t-test. Analyses were performed for each genotype separately. Key for genotypes in "A" applies for A-C. Nematode populations were from Costa Rica (CR5) and Honduras (H1). Control treatment consisted of plants not infected by nematodes.

FHIA-01 and FHIA-02 showed higher necrosis in the non-inoculated controls than FHIA-03 and FHIA-21.

DISCUSSION

In our tests, nematode reproduction in FHIA-01 and FHIA-03 did not differ from the susceptible control, Grande Naine. The result obtained for FHIA-03 was not surprising; however, it was expected that FHIA-01 would show a high level of resistance to *R. similis* because the diploid SH-3142 was in its parentage (Rowe and Rosales, 1993). Stanton (1994) and Binks and Gowen (1996) also reported a susceptible reaction of FHIA-01, comparable to the susceptible Cavendish controls, in field experiments. Burrowing nematodes reproduced poorly in Pisang Jari Buaya which is evidence of the resistance of this diploid to *R. similis*, as reported by Pinochet and Rowe (1978, 1979) and Wehunt and Hutchinson (1965).

The amount of root necrosis was associated somewhat with the final population levels (P_f) of the nematode populations tested. However, the resistant control, Pisang Jari Buaya, had high levels of necrosis in spite of a low P_f. Based on results in unrelated greenhouse tests, most of the background root-necrosis encountered in the experiments described herein could be circumvented by growing banana plants in a soil-sand mix in larger, well drained containers such as 15-cm diam clay pots (Barker, K. R., unpubl. data). Still, short-term greenhouse tests may not be sufficiently sensitive to detect some types of useful host resistance to nematodes, especially where quantitative traits are involved. The use of tissue-culture plants also may have influenced phenotypic responses to nematodes. Therefore, if reproductive fitness and damage potential are to be evaluated simultaneously, a clear understanding of the germplasm evaluation system is required.

In the second experiment, the four hybrids tested did not show evidence of resistance to the *R. similis* populations used. Although resistant and susceptible controls were not available for this experiment, the previous experiment demonstrated that FHIA-01 and FHIA-03 are as susceptible as Grande Naine.

The reproductive potential of CR5, as measured by P_f, was generally greater than H1 for all hybrids tested, although the difference was significant only for FHIA-01 and FHIA-21. This result provides additional evidence for differences in aggressiveness observed between these two populations, as described by Marin (1997). FHIA-01, FHIA-

02, and FHIA-21 have SH-3142 in their pedigree, and were considered as potentially resistant genotypes to *R. similis*. Nevertheless, FHIA-01 and FHIA-21 had given a susceptible response under field conditions in Honduras (Binks and Gowen, 1996).

Pinochet (1996) suggested that plant maturity may influence the results of assessments of nematode resistance. Selection of other parameters for experiments dedicated to identification of nematode-resistant bananas also will likely influence results, particularly if multiple mechanisms of resistance are in play. Some reactions that are incompatible with nematode development may be based upon maturation of root tissues, whereas others may be based upon physiological responses to nematode attack. It appears that resistance in Pisang Jari Buaya to burrowing nematodes is not dependent upon plant maturity as young plants were resistant to burrowing nematodes in our experiments.

Plant age is only one of several factors that can influence the interaction of burrowing nematodes with banana. FHIA-01 was determined to be susceptible to burrowing nematodes when tested under field conditions in Honduras (Binks and Gowen, 1996). However, FHIA-01 plants were considered to be resistant to burrowing nematodes when 8-month-old seedlings were infected with burrowing nematodes under controlled conditions in Australia (Stanton, J., 1996, pers. comm.). Such discrepencies may reflect differences in plant maturity, differences in the aggressiveness of discrete burrowing nematode populations, or presence of other organisms (field studies) that may influence the interaction of nematode and banana root.

The banana germplasm SH-3142 was resistant to burrowing nematodes and, therefore, was considered an important parent line (Pinochet, 1996). However, it is not known if the original resistant features have been inherited by subsequent generations of hybrids. None of the SH-3142 hybrids tested in our study were resistant to burrowing nematodes. Our results suggest that identification of banana germplasm and hybrids for burrowing nematode-resistance will require both short- and long-term analyses involving multiple burrowing nematode populations.

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