REPRODUCTIVE FITNESS AND PATHOGENICITY OF EIGHT *RADOPHOLUS SIMILIS* ISOLATES ON BANANA PLANTS (*MUSA* AAA CV. POYO)

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ABSTRACT

Fallas, G. A., J. L. Sarah, and M. Fargette. 1995. Reproductive fitness and pathogenicity of eight *Radopholus similis* isolates on banana plants (*Musa* AAA cv. Poyo). Nematropica 25:135-141.

Eight isolates of *Radopholus similis* were compared for their reproductive fitness and pathogenicity on banana (*Musa* AAA cv. Poyo) under controlled conditions in a climatic chamber. Five isolates were originally collected from African countries (Uganda, Cameroon, Nigeria, Ivory Coast, and Guinea) and the 3 others from Guadeloupe, Queensland, and Sri Lanka. The African isolates, and particularly the one collected in Uganda, had the highest reproductive fitness. Strong negative correlations were observed between nematode numbers in roots 6 and 8 weeks after inoculation and fresh root and shoot weights 12 weeks after inoculation. These results suggest that differences in pathogenicity on banana among *R. similis* isolates are a direct consequence of differences in reproductive fitness on banana tissue, and consequently that the relative virulence is roughly similar for all isolates considered. This study increases our knowledge of the biological intraspecific diversity of *R. similis* throughout the world and strengthens the hypothesis that the high pathogenicity among African populations of *R. similis* due mainly to better reproductive fitness, which could explain greater field damage generally observed in these regions.

Key words: banana, biological diversity, *Musa* sp., pathogenicity, *Radopholus similis*, reproductive fitness.

RESUMEN

Fallas, G. A., J. L. Sarah y M. Fargette. 1995. Capacidad reproductiva y patogenicidad de ocho poblaciones de *Radopholus similis* inoculadas sobre plántulas de banano (*Musa* AAA cv. Poyo). Nematropica 25:135-141.

La capacidad reproductiva y la patogenicidad de ocho poblaciones de *Radopholus similis* inoculadas sobre banano (*Musa* AAA cv. Poyo) fueron evaluadas bajo condiciones controladas. Cinco de las poblaciones estudiadas provienen de paises productores de Africa (Uganda, Camerún, Nigeria, Costa de Marfil y Guinea), mientras que las tres restantes son originarias de Guadalupe, Queensland y Sri Lanka. Las poblaciones africanas, y sobre todo la de Uganda, presentaron la mayor capacidad reproductiva. Se observaron fuertes correlaciones negativas entre el numero de nematodos a las 6 y 8 semanas y los pesos frescos de la raíz y del tallo a las 12 semanas después de la inoculación. Estas correlaciones confirman que la patogenicidad de *R. similis* sobre banano es una consecuencia directa de su capacidad reproductiva y que la virulencia es similar entre las poblaciones. El presente estudio aumenta nuestro conocimiento sobre la diversidad biológica del nematodo a nivel mundial y a la vez refuerza la hipótesis de que las poblaciones africanas presentan un alto grado de patogenicidad el cual explica los severos daños observados a nivel de campo.

Palabras claves: banano, diversidad biológica, *Musa* sp., patogenicidad, *Radopholus similis*, capacidad reproductiva.

INTRODUCTION

Early investigations conducted in Central America and the Caribbean suggested the existence of different pathogenic forms of the *Radopholus similis* (Cobb, 1893) Thorne 1949 on bananas (8,12). Recent studies comparing isolates from a broader geographic origin indicated an even larger intraspecific biological diversity of this nematode species (2,10). These recent studies showed that pathogenicity on banana plants and *in vitro* reproductive fitness (11) on carrot disc cultures are fairly well correlated: the greater the pathogenicity on banana roots, the higher the multiplication rate on carrot discs. Genomic diversity among *R. similis* isolates also was shown at the DNA level (4), and molecular markers for pathogenicity are being sought. The present study compares 8 *R. similis* isolates originating from different regions of the world for their relative reproductive fitness and pathogenicity on banana plants.

MATERIALS AND METHODS

Isolates of *R. similis* were originally collected on banana roots (*Musa* sp.) in 8 production areas of the world and maintained monoxenically on carrot discs at 27°C (± 0.5°C) (3,6). Five of these isolates originated from African countries: Uganda (Labbubbo-Kyadondo), Cameroon (Island 5, Sanaga River), Nigeria (Port Harcourt), Ivory Coast (Anguédédou), and Guinea (Balikouré). Three other isolates were collected in Guadeloupe (Neufchâteau), Queensland (MacKay State, Tuley, Australia), and Sri Lanka (Hantane). The Ivory Coast, Guadeloupe, and Sri Lanka isolates have already been studied for their pathogenicity on banana plants (10). These 3 isolates plus those from Guinea and Queensland have been included in a former study about reproductive fitness on carrot discs in relation to temperature (2).

Banana plantlets (*Musa* sp. AAA cv. Poyo) were micropropagated through *in vitro* culture. They were transferred and progressively adapted to ambient conditions in a climatic chamber (27-28°C, 75-80% relative humidity, 12hr/12hr photoperiod) for 3 weeks. Plantlets were then transplanted into 800 cm^3 of a ready-made substrate containing an autoclaved (120°C, 1 bar for 20 min) mixture of peat and mold (20% organic matter, pH 6.0-6.5). Slow-release fertilizer (2 g/plant, N-P-K-Mg: 15-12-13-2%) was added at the beginning of the adaptation period. Plants were irrigated daily during the three-week adaptation period, and then 3 times a week until the end of the experiment. Experimental climatic conditions remained the same as for the acclimation period until the end of the experiment.

Following the adaptation period, plants were inoculated with nematodes. Carrot discs from each monoxenic culture were cut into small pieces, and put onto absorbing paper placed on a 200-µm mesh sieve. Sieves were placed in large beakers, covered with water, and aerated for 2-3 hours, after which nematodes were collected. Suspensions of 100 ± 10 *R. similis* (adults and juveniles) were inoculated at the surface of the soil substrate, at a distance of 2.5-3.0 cm around the plant.

The 27 treatment combinations (8 nematode isolates plus 1 non-inoculated control times 3 harvest dates for observations) were replicated 9 times. The plant arrangement was completely randomized.

Observations were made 6, 8, and 12 weeks after inoculation. Plant parameters measured included fresh shoot and root weight and plant height. Plant height was measured from the basal part of the corm to the intersection point of the petioles of the two youngest leaves. Nematode num-

bers were evaluated from root tissue to assess the reproductive fitness. Roots were cut into 1-cm pieces and macerated in 200 ml of water in a blender for 3-5 seconds at full speed. The macerated suspension was poured onto a column of sieves with different pore sizes (250, 80, 50, and 32 µm). The nematodes and tissue debris collected on the last 3 sieves were separated using the centrifugation/flotation technique (1).

Data were subjected to analysis of variance. Nematode counts were transformed to $\log_{10}(x+1)$ for analysis and means were compared using the Newman & Keuls test ($P \leq 0.05$).

RESULTS

Nematode population increase: Six and 8 weeks after inoculation, reproduction of the isolate from Sri Lanka was significantly lower than that of all other isolates (Table 1). In contrast, the five African isolates produced the highest numbers of nematodes per plant, with the Uganda isolate significantly highest at 8 weeks after inoculation. Six weeks after inoculation, nematode numbers produced by the Guadeloupe isolate were not significantly different from those of the Queensland and Cameroon isolates, but were lower than those of the 4 other isolates from Africa. Two weeks later, population increase of the Guadeloupe isolate was similar to those of the African isolates (with the exception of the Uganda isolate), and higher than the Queensland isolate.

Between 8 and 12 weeks, population increase of the African isolates slowed down sharply, their multiplication rates ranged from 1.2 up to 2.6 (Table 1). In contrast, multiplication rates of the isolates from Guadeloupe and Queensland were higher during this period (5.1 and 6.8 respectively). Therefore, numbers of nematodes of these latter 2 isolates reached their highest values 12 weeks after inoculation, although these values were not signif-

Table 1. Reproductive fitness of 8 *Radopholus similis* isolates in banana roots (*Musa* AAA cv. Poyo) at 6, 8, and 12 weeks after inoculation with 100 nematodes per plant.

Isolate	Weeks after inoculation			Multiplication rate (12/8 weeks)
	6	8	12	
Uganda	7740 a[z]	47310 a	58480 ab	1.2
Ivory Coast	6220 a	25410 bc	59430 ab	2.3
Nigeria	5730 a	26360 bc	64860 ab	2.5
Guinea	6520 a	23710 bc	62520 ab	2.6
Cameroon	4020 ab	29440 b	77620 ab	2.6
Guadeloupe	2910 b	22130 bc	112460 a	5.1
Queensland	2390 b	13740 c	93970 ab	6.8
Sri Lanka	1260 c	4470 d	27540 b	6.2

[z]Data are geometric means of 9 replicates. Original data are presented, but they were transformed to $\log_{10}(x+1)$ for analysis of variance. Means in the same column followed by the same letter do not differ according to the Newman & Keuls test ($P \leq 0.05$).

icantly different from those reached by the five African isolates. Numbers of the Sri Lanka isolate 12 weeks after inoculation were significantly lower than those of the Guadeloupe isolate.

Growth and development of plants: No significant differences could be found in plant height, regardless of the isolate or observation date. Differences in fresh shoot and root weights were only observed 12 weeks after inoculation, and differences were greater for roots than for shoots (Table 2). Differences in fresh shoot weight were not significant among the 5 African isolates. Only the Uganda isolate was associated with a fresh shoot weight significantly lower than those from the non-African isolates and the non-inoculated control. For fresh root weight, statistical analysis allowed us to distinguish two main groups (Table 2). The first group with lower root weight included all of the African isolates, among which the Uganda isolate was associated with the most reduced root system. The second group included the non-inoculated control and the non-African isolates. In this second group, only the Guadeloupe isolate showed a root weight which was not significantly higher than 4 of the 5 African isolates.

Relationships between nematode population and plant growth: The geometric mean of nematode numbers at 6 and 8 weeks after inoculation was negatively correlated with fresh root weight (Fig. 1, $r^2 = 0.96$) and fresh shoot weight (Fig. 2, $r^2 = 0.973$) measured 12 weeks after inoculation. Along the regression lines, isolates ranged from the Uganda isolate with the highest reproduction and most severe plant weight reduction to the Guadeloupe, Queensland, and Sri Lanka isolates with medium or low nematode numbers and almost no noticeable damage on shoots and little damage on roots. The other African iso-

Table 2. Effect of 8 *Radopholus similis* isolates on fresh root and shoot weight of banana plants (*Musa* AAA cv. Poyo) at 12 weeks after inoculation with 100 nematodes per plant.

Isolate	Root weight (g)	Shoot weight (g)
Control	37.8 a[z]	119.2 a
Sri Lanka	33.8 a	115.3 a
Queensland	31.8 a	115.7 a
Guadeloupe	30.5 ab	120.2 a
Guinea	24.8 bc	100.0 ab
Cameroon	23.2 bc	104.4 ab
Nigeria	23.5 bc	97.1 ab
Ivory Coast	23.0 bc	94.9 ab
Uganda	17.7 c	73.0 b

[z]Data are arithmetic means of 9 replicates. Means in the same column followed by the same letter do not differ according to the Newman & Keuls test ($P \leq 0.05$).

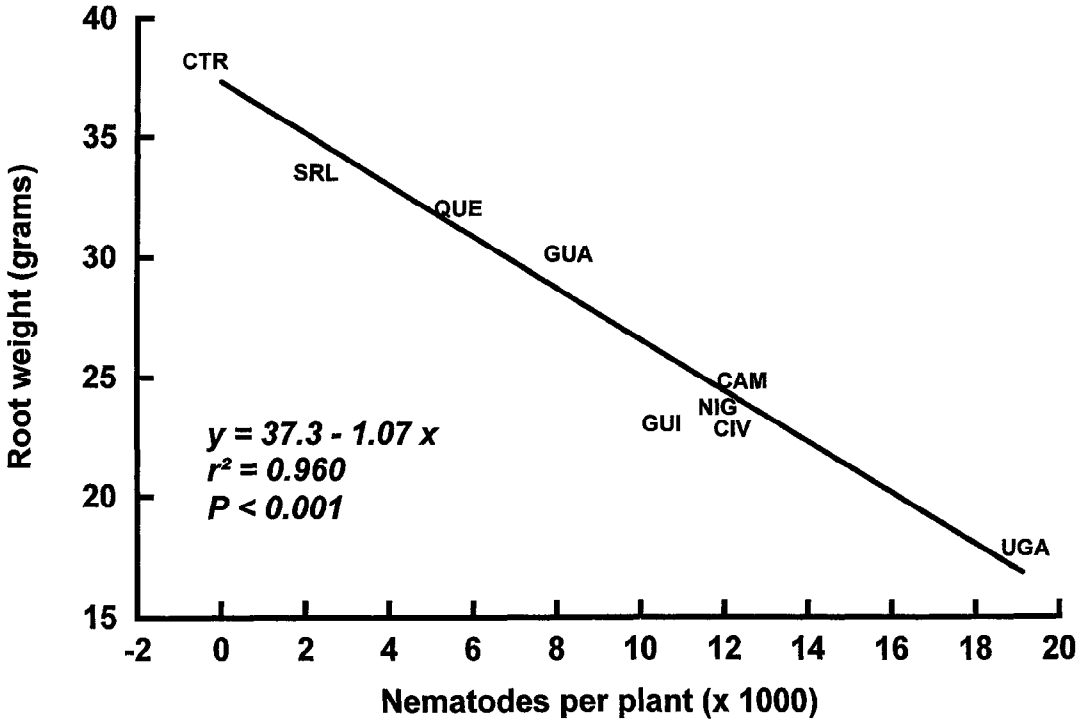

Fig. 1. Relationship between the geometric mean of nematode numbers per plant at 6 and 8 weeks and fresh root weight at 12 weeks after inoculation of banana plants (*Musa* AAA cv. Poyo) with 100 *Radopholus similis*. Abbreviations on figure represent nematode populations from Sri Lanka (SRL), Queensland (QUE), Guadeloupe (GUA), Cameroon (CAM), Nigeria (NIG), Guinea (GUI), Ivory Coast (CIV) and Uganda (UGA). CTR indicates untreated control.

lates were in between with high nematode numbers and intermediate reduction in root and shoot growth.

DISCUSSION

Under these experimental conditions, plant height was not a reliable criterion for evaluation of *R. similis* pathogenicity on bananas, confirming previous (10) and parallel (5) results. In contrast, shoot weight and root weight are good indicators of *R. similis* impact on the banana plant, provided that the experiment is conducted for a long enough time (i.e., 12 weeks with initial inoculum of 100 *R. similis* under our experimental conditions). Root weight is most affected since nematodes first damage the roots before affecting growth of other plant parts.

Isolates originating from Africa produced high nematode numbers at 6 and 8 weeks after inoculation. This high reproductive fitness at an early stage induced strong reduction in root weight which in turn limited space and food available, and consequently, decreased multiplication rates observed later in the experiment. The Uganda isolate had the highest reproductive fitness during the first 8 weeks of the experiment and correlatively, induced the highest damage in plants, confirming a result obtained by Hahn *et al.* (5). The 3 "non-African" isolate, particularly the Sri Lanka isolate, generally showed lower reproductive fitness at the early stages.

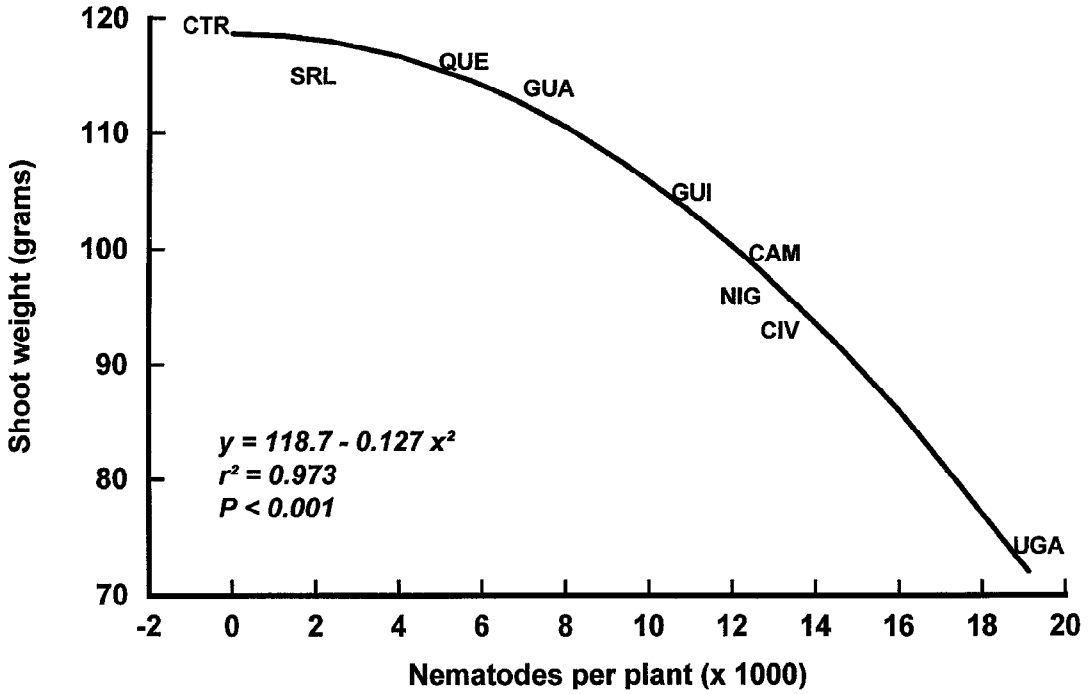

Fig. 2. Relationship between the geometric mean of nematode numbers per plant at 6 and 8 weeks and the fresh shoot weight at 12 weeks after inoculation of banana plants (*Musa* AAA cv. Poyo) with 100 *Radopholus similis*. Abbreviations on figure represent nematode populations from Sri Lanka (SRL), Queensland (QUE), Guadeloupe (GUA), Cameroon (CAM), Nigeria (NIG), Guinea (GUI), Ivory Coast (CIV) and Uganda (UGA). CTR indicates untreated control.

Therefore, root weight was less reduced by these isolates and plant growth not significantly affected during the experiment. As a consequence, the multiplication rate is apparently not affected during the last stage of the experiment, and nematode numbers reached those of the African isolates.

The different degrees of pathogenicity observed among the Ivory Coast, Guadeloupe, and Sri Lanka isolates in the present study are consistent with a previous experiment (10). Pathogenicity on the banana plant also correlates with *in vitro* multiplication rates on carrot discs, with the exception of the Sri Lanka isolate, which reproduces faster than the Queensland isolate on carrot discs (2). According to Shaner *et al.* (11), pathogenicity on a given host is the consequence of two independent parameters: reproductive fitness (comparative multiplication rate of different isolates on a given host) and virulence (ability to induce damage). In the present study, pathogenicity of the different *R. similis* isolates on banana plants appeared to be a direct consequence of reproductive fitness. Consequently, we assume that virulence may be similar for all the isolates tested. The high levels of pathogenicity of the African isolates tested here probably explain why important field damage has been reported in these countries (7,9,10). Specific environmental and agronomic conditions may have induced evolution of types of *R. similis* with higher reproductive fitness on banana.

The separation which was made between African and "non-African" isolates in terms of pathogenicity is artificial since

the three "non-African" isolates examined here are obviously not representative of all the "non-African" populations of the world, particularly those from the "non-African" tropical humid area. For example, in Central America, high pathogenic and low pathogenic forms of *R. similis* are found in similar humid tropic environments. The more damaging of these pathogenic forms also are more fit and tend to prevail in Panama and Costa Rica where uproot losses and field populations (nematode root densities) are considerably higher than in other banana producing areas where the less damaging forms occurs, such as the Sula Valley in Honduras and in Belize (8). It would be useful to include such isolates in further studies.

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