Pathogenicity on maize and banana among isolates of *Radopholus similis* from four producing countries of Africa and Asia

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ABSTRACT
A study was conducted to investigate the host status of maize to *Radopholus similis* and the pathogenicity on maize and banana of four populations of *R similis* from Kenya, Uganda, Sri Lanka and Cameroon. The host plants were maize seedlings *(Zea mays)* and *in vitro* banana (*Musa* sp cv Grande Naine). Plants were inoculated with nematodes cultured on excised maize roots. Nematodes were recovered from the roots of both plants 4 weeks after inoculation. There was high variability among isolates concerning the extent of plant damage, rates of nematode development and multiplication, and host range.

Étude du pouvoir pathogène de quatre isolats de *Radopholus similis* d'Afrique et d'Asie sur maïs et bananier.

RÉSUMÉ
Le statut hôte du maïs vis-à-vis de *Radopholus similis* a été étudié et le pouvoir pathogène de quatre populations de *R similis* du Kenya, de l'Ouganda, du Sri Lanka et du Cameroun a été comparé par inoculation sur maïs *(Zea mays)* et sur vitroplants de bananier (*Musa* sp cv Grande Naine). Les nématodes inoculés avaient été élevés sur racines de maïs excisées. Quatre semaines après inoculation, les nématodes ont été extraits des racines des deux plantes. L'observation du taux de lésions racinaires, de la vitesse de développement et de multiplication ainsi que la gamme d'hôtes indique une grande variabilité entre les isolats.

Estudio del poder patógeno de cuatro aislamientos de *Radopholus similis* de Africa y de Asia en el maíz y en el banano.

RESUMEN
Un estudio fue llevado a cabo para determinar el poder patógeno de cuatro poblaciones de *R similis* de Kenia, Ouganda, Sri Lanka y Camerún. Las plantas hospederas fueron el maíz (*Zea mays*) y vitroplantas de banano (*Musa* sp cv Grande Naine). Las plantas fueron inoculadas con nemátodos cultivados sobre raíces de maíz. Cuatro semanas después de la inoculación, los nemátodos fueron extraídos de las raíces de las dos plantas. Las observaciones de la tasa de lesiones en las raíces y la velocidad de infección y de multiplicación indicaron gran variabilidad entre los aislamientos.

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KEYWORDS
Musa, *Zea mays*, plant nematodes, *Radopholus similis*, infestation, pathogenicity, damage, host pathogen relations.

MOTS CLÉS
Musa, *Zea mays*, nématode des plantes, *Radopholus similis*, infestation, pouvoir pathogène, dégât, relation hôte pathogène.

PALABRAS CLAVES
Musa, *Zea mays*, nemátodos de las plantas, *Radopholus similis*, infestación, poder patógeno, daños, relaciones huésped patógeno.

introduction

Banana is a staple food of a significant proportion of the world population and is considered to be the most important tropical fruit (PURSEGLOVE, 1972). It is attacked by a wide range of pests and diseases including nematodes.

Worldwide, more than 151 nematode species have been reported to feed on banana roots (GOWEN and QUÉNÉHERVÉ, 1990), and *R similis* is considered to be the most important of these pests (BLAKE, 1961, 1966; GOWEN and QUÉNÉHERVÉ, 1990). However, there has been little progress in determining efficient techniques for estimating soil population densities of this nematode. Poor estimates are often obtained when using direct methods (SARAH, 1988, 1991). Maize seedlings have been used to assess soil populations of *R similis,* but the technique has not yet been standardized (SARAH, 1988). Moreover, there is substantial variability in the extent of damage caused by this nematode worldwide (PINOCHET, 1988).

The objective of the present study was to investigate the host status of maize as compared to *in vitro* banana plantlets, and to study the pathogenicity on maize and banana of four *R similis* populations from different ecological areas.

materials and methods

maize host status

The experiment was conducted with one maize cultivar (cv CMS) from Cameroon. The banana cultivar (cv Grande Naine) was used as the reference crop. Three *R similis* inoculum levels of 10, 100 and 1 000 nematodes per pot were used, along with a noninoculated control. Pots (24 for each plant species) were arranged on a bench in the glasshouse (22-28°C, 12L/12D) according to a completely randomized design. Plants were harvested 4 weeks after inoculation.

pathogenicity among isolates of *R similis*

The study was conducted on three maize cultivars (CMS, Ndock, ATP) from Cameroon and one banana cultivar (Grande Naine-AAA). The four isolates came from four producing countries: Cameroon, Uganda, Kenya and Sri Lanka. Each pot was inoculated with 300 *R similis* nematodes. The sex ratio of the inoculum was determined before inoculation (table I). A fifth population was added, made up of a mixture of nematode specimens from the four axenic populations that were reared on artificial media. Roots were assessed 6 weeks after inoculation. An experimental randomized block design was used. Replication was 3-fold.

In both experiments, nematodes were isolated from banana roots, sterilized and kept for multiplication on excised maize roots in petri dishes (HOOPER, 1986). Three maize seeds were sown in each pot (1 l). The banana plants were young *in vitro* plantlets, about 25-30 cm in height with two leaves (leaves of at least 10 cm width). The plants were grown on John Innes 2 compost.

Nematodes were extracted from the roots by blending 25 g of roots and pouring the suspension through a set of sieves of different pore sizes (250, 63 and 32 µm). Nematodes were collected from the two sieves with the smallest pore size. Root damage was assessed using the root lesion index (LORIDAT and GANRY, 1991).

The results were statistically assessed by an analysis of variance with the INSTAT software package. Differences between means were analysed with the Duncan's Multiple Range Test.

results

maize host status

Plants were harvested for direct observations 4 weeks following inoculation. No lesions were found on roots of the two crops at *R similis* inoculum levels of 10 and 100 nematodes per plant. For plants inoculated with 1 000 *R similis* nematodes, necroses were observed in both crops, with 2% of the total root length lesioned in banana. Although lesions were observed on maize roots (Pi = 1 000 *R similis*/plant), there were fewer (< 0.5% of total root length) than on banana roots.

There were no significant differences between final nematode populations recovered from plants inoculated with 10 and 100 *R similis* nematodes, on both maize and banana 4 weeks

Table I
Percentage of males, females and juveniles in the inoculum for each isolates.

Isolates	*Females*	*Males*	*Juveniles*
Kenya	20	20	60
Uganda	28	22	50
Cameroon	75	25	00
Sri Lanka	53	20	27
Mixed population	80	12	08

after inoculation (fig 1). However, final *R similis* populations recovered from plants inoculated with 1 000 nematodes per plant were significantly higher than those from plants inoculated with 10 and 100 nematodes per plant ($P < 0.05$). Nematode populations recovered from banana plantlets inoculated with 1000 *R similis* nematodes were significantly higher than populations recovered from maize seedlings inoculated with 1000 *R similis* nematodes (fig 1). No effect of inoculum concentration on plant growth was observed 4 weeks after inoculation. This could have been due to the fact that the population build up was not enough to have a significant effect on these parameters, or to the low inoculum level used in the experiment.

pathogenicity among isolates of *R similis*

The root lesion index was used to estimate the level of damage. On banana, all four isolates attacked roots but the extent of necrosis varied between isolates. The mixed population caused the highest level of damage to banana roots (12.3%), followed by isolates from Cameroon and Sri Lanka with 3.6 and 3.1% necrosis, respectively (fig 2). The percentage necrosis caused by isolates from Kenya and Uganda was significantly lower than that caused by isolates from Cameroon and Sri Lanka. Damage caused by the mixed population was significantly higher than that caused by the four axenic populations.

Figure 3 shows the nematode densities/g roots for each host and each isolate. All four isolates and the mixed population were recovered from banana and from one maize cultivar (cv Ndock). On ATP, isolates from Kenya and Uganda were not found, while on cultivar CMS the isolate from Cameroon which had the highest nematode density on cultivar ATP was not recovered. The mixed population was the most abundant on cultivars Ndock, CMS and Grande Naine.

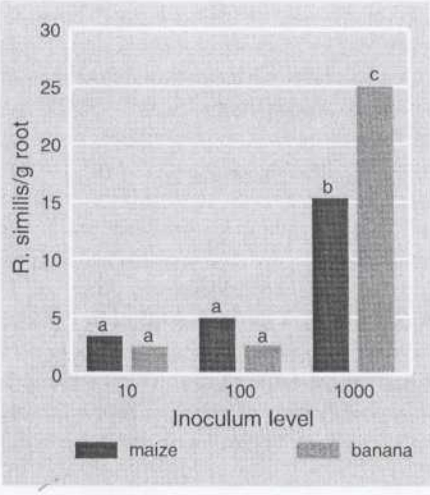

Figure 1
Final Radopholus similis *populations on maize and banana 4 weeks following inoculation with different inoculum levels; 10, 100 and 1000 nematodes per pot (*R similis *populations with different letters [abc] were significantly different at 5% level).*

discussion

Maize and banana roots were successfully invaded by *R similis* at different inoculum levels even though lesions were not observed at inoculum concentrations of 10 and 100 *R similis* nematodes per plant. The sex ratio analysis showed

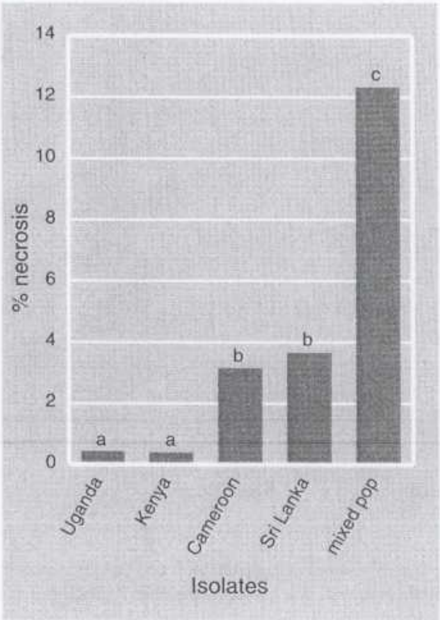

Figure 2
Necrosis on banana roots caused by different R similis *populations (from Uganda, Kenya, Cameroon, Sri Lanka and mixed isolates) 4 weeks after inoculation with 300 individuals per pot. Isolates with the same letter (abc) were not significantly different at 5% level (mixed pop = mixed population).*

that maize and banana are good hosts for *R similis*. The presence of males is a good indication that breeding has occurred since they are unable to invade roots (BLAKE, 1972); this confirms the interest of using maize as a bioassay crop for estimating soil populations of *R similis*, as suggested by SARAH (1988). However, RIVAS and ROMAN (1985) reported that a *R similis* strain from Puerto Rico was unable to reproduce on maize roots. Moreover, maize was reported to be a *R similis* host plant in Panama, but was found to be a nonhost in Honduras. In this study, the maize cultivar ATP was found to be a poor host in comparison to the 2 other maize cultivars. These observations suggest differences in *R similis* races from banana-producing areas worldwide.

The variability in damage observed between the four isolates used in this experiment suggests that these isolates behave differently on the differentials. The mixed *R similis* population was able to parasitize three of the four hosts used. However, the extent of necrosis varied between hosts. The high level of damage caused by the mixed population suggested that each isolate causes a different type of damage, thus resulting in higher overall damage. Moreover, the mixed population had the highest number of females (80% of the population) in the inoculum as compared to the other isolates. The banana cultivar and the maize Ndock cultivar were found to be better hosts than the maize cultivars ATP and CMS. The Kenya isolate failed to invade ATP but was recovered from Ndock, CMS and Grande Naine. On CMS, the isolate from Cameroon was not found, whereas high numbers of this isolate were recovered from banana roots, and also from ATP roots (but at low levels). Isolates from Cameroon and Sri Lanka seemed to be more pathogenic than populations from Uganda and Kenya. These preliminary observations may help to explain differences in *R similis* attacks between banana-growing areas.

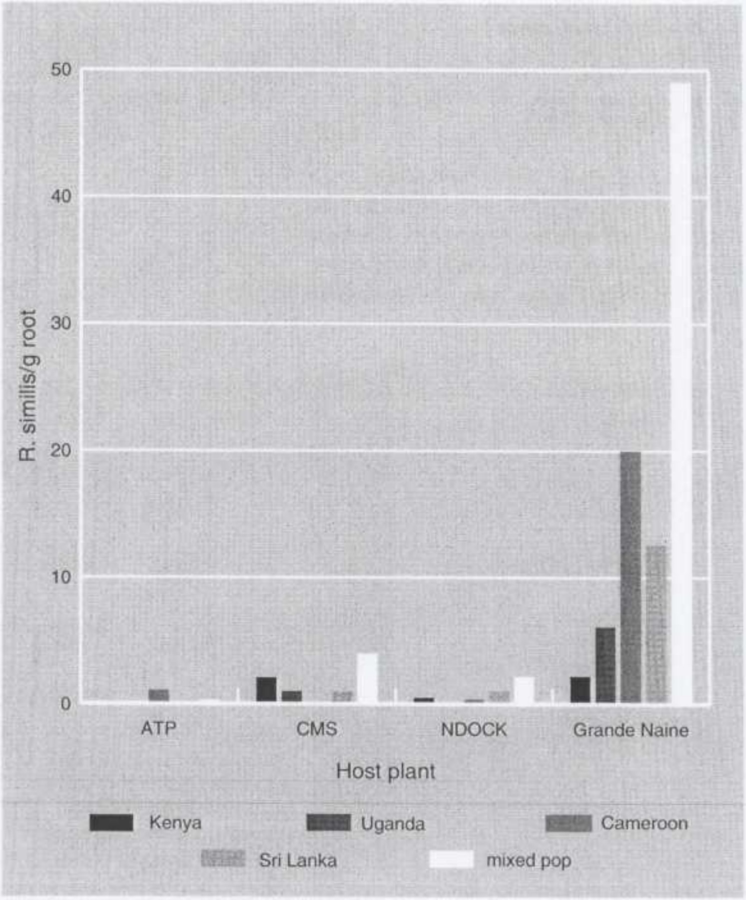

Figure 3
Final population from four host plants (three maize cultivars: ATP, CMS, NDOCK and the banana cultivar Grande Naine) inoculated with five different R similis *populations (mixed pop = mixed population).*

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