RESEARCH NOTE - NOTA INVESTIGATIVA

XENIC CULTURING OF PLANT-PARASITIC NEMATODES: ARTIFICIAL SUBSTRATES BETTER THAN SOIL-BASED CULTURE SYSTEMS?

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ABSTRACT

Quénéhervé, Patrick, Marie-Luce Serge, Salmon Frédéric, and Barrière Virginie. 2010. Xenic culturing of plant-parasitic nematodes: artificial substrates better than soil-based culture systems? Nematropica 40:269-274.

Xenic culturing of plant-parasitic nematodes is a common laboratory technique to rear individuals on a plant host in a totally controlled environment. In 1999, Reversat *et al.* replaced sterilized soil by artificial substrate consisting of sand and a water absorbent polymer (SAP) allowing xenic culturing of tropical nematode species even if native soil was not available. The aim of this study was to demonstrate how the use of artificial substrate in xenic culturing can affect the multiplication of the most harmful pests of bananas: the burrowing nematode *Radopholus similis* and the lesion nematode *Pratylenchus coffeae*. The effects of native sterilized soil and SAP substrate on the growth of three cultivars of banana and on the multiplication rate of *R. similis* and *P. coffeae* were examined. The use of SAP substrate induced a faster development of root and aerial systems of bananas. Depending on cultivar, the population densities of *R. similis* were 43 to 1,102% higher when grown in SAP substrate than in native sterilized soil. Only in the case of one cultivar, the multiplication of *P. coffeae* was significantly higher on sterilized soil substrate than on SAP substrate. We conclude that xenic culturing performed with SAP substrate is a very convenient laboratory technique for multiplication of plant-parasitic nematodes in the absence of native soil and a practical way to standardize controlled experimental conditions.

Key words: Artificial substrate, Banana, Nematodes, *Pratylenchus coffeae, Radopholus similis,* Xenic cultivation.

RESUMEN

Quénéhervé Patrick, Marie-Luce Serge, Salmon Frédéric, and Barrière Virginie. 2010. Cultivo xénico de nematodos fitoparásitos: ¿Son mejores los sustratos artificiales que los sistemas de cultivo en suelo? Nematropica 40:269-274.

El cultivo xénico de nematodos fitoparásitos es una técnica de laboratorio común para mantener individuos en un hospedante vegetal en un ambiente totalmente controlado. En 1999, Reversat *et al.* remplazaron el suelo estéril con una mezcla de arena y polímero hidrofílico (SAP) que permite el cultivo de especies de nematodes tropicales cuando no se dispone de suelo nativo. El objetivo de este estudio es demostrar como el uso de sustrato artificial en el cultivo xénico puede afectar la reproducción de los nematodos más dañinos del banano: *Radopholus similis* y *Pratylenchus coffeae*. Se examinaron los efectos de suelo nativo esterilizado y de sustrato SAP en el crecimiento de tres culti-

vares de banano y en la reproducción de *R. similis* y de *P. coffeae*. El uso de sustrato SAP indujo desarrollo más rápido de los sistemas radicales y aéreo de la planta. Dependiendo del cultivar, las densidades de población de *R. similis* aumentaron un 43 a 1,102% cuando se usó sustrato SAP, comparado con el suelo nativo esterilizado. Tan solo en el caso de un cultivar, la reproducción de *P. coffeae* fue significativamente más alta en el sustrato de suelo esterilizado. Concluimos que el cultivo xénico en sustrato SAP es una técnica muy conveniente para el mantenimiento de nematodes fitoparásitos sin suelo native y una manera práctica de estandarizar condiciones experimentales controladas.

Palabras clave: Sustrato artificial, Banano, Nematodos, *Pratylenchus coffeae*, *Radopholus similis,* Cultivo xénico.

Xenic culturing of plant-parasitic nematodes, extensively reviewed by De Ley and Mundo-Ocampo (2004), may be necessary to obtain larger nematode populations for many systematic or experimental studies. As obligate parasites, these nematodes must feed on host cells for development and reproduction. As they thrive in the soil and rhizosphere environment, their development is strongly influenced not only by abiotic and biotic soil factors such as temperature, pH, moisture content, host requirements but also other micro-organisms. For experimental purposes, the challenge is thus to minimize the number of unknown soil factors involved in the development of the target species. Wang *et al.* (1997) had demonstrated that *R. similis* can reproduce on anthurium grown in different soilless media. The most common techniques used to rear Pratylenchidae monoxenically involve callus tissue, usually carrot (Reise *et al.*, 1987). However these techniques do not enable the mass propagation of large population of nematodes and some questions about pathogenicity may be raised when the nematode populations are artificially maintained in the laboratory outside of their natural host plants for many years (e.g. *Radopholus similis* and *Musa* spp.). Consequently, most laboratories use xenic culturing. The objective of this experiment was to determine whether the burrowing nematode *R. similis* and the lesion nematode *Pratylenchus coffeae* could be mass propagated aseptically in mesocosms on rooted banana plantlets growing on pure artificial substrate to avoid any interference from the soil.

The method used was originally developed by Reversat *et al.* (1999). The plant material was propagated on MS medium, maintained in the dark in a cooled incubator at 22 ± 0.5°C and regenerated as needed in test tubes on modified MS medium including active charcoal in a culture incubator at 25 ± 0.5°C with continuous light for 6-8 weeks. Plantlets were then transferred to PVC culture tubes (4.5 cm in diameter; 17.5 cm in length; 237 cm^3 in volume), placed on a rack, inserted through a grid of circular openings in a growth chamber. The culture tubes were filled with either steam-sterilized Andosol (1 hour at 100°C) chosen as the reference soil for banana cultivation in Martinique (pH 6.2, organic matter content 7.3%, CEC 10.3 meq/100g soil) or with an artificial substrate made of a mixture of pure silica sand and water-absorbent synthetic polymer (SAP) as described by Reversat *et al.* (1999) and allowed to grow for 6-7 weeks in a growth chamber at 24-28 ± 1°C (thermoperiod night/day) with 14 h of light. Irrigation was automated as required (Gardena®, Ulm, Germany). Plants were watered every three days with a nutrient solution (Mairol® 14:12:15 + oligo-elements, GmbH & Co, Germany).

Two cultivars of Grande Naine (*Musa* AAA, Cavendish subgroup) cv 902 and cv William and a new CIRAD banana hybrid

FB924 with resistance to Yellow Sigatoka and Black Leaf Streak and lower nematode susceptibility (Quénéhervé *et al.*, 2009) were micropropagated. The burrowing nematode *R similis* and the lesion nematode *P. coffeae* were originally isolated from banana plants in Martinique and maintained in the laboratory both on carrot tissue culture and banana plants (cv. Grande Naine). Following extraction of nematodes reared on carrot discs, each culture tube was inoculated with about 400 individuals (adults and juveniles) carefully placed in a hole near the banana plantlet as previously described (Quénéhervé *et al.,* 2006). The 12 treatment combinations (3 banana cultivars, 2 nematode species and 2 substrates) were replicated 10 times. At the end of the experiment, 45 days after inoculation with the nematodes, all banana plantlets were uprooted, carefully washed to remove adhering sand and soil, and aerial parts and root systems were weighed. For each cultivar, the effect of substrate on root and aerial biomass was analyzed using a Kruskal - Wallis test for testing equality of population among data groups.

Root and aerial growth of bananas appeared to be closely linked with the substrate used. According to the Kruskal-Wallis test, only the final root weight of the FB924 bananas did not appear to vary with the substrate (P_value > 0.05). Except for this cultivar, both root and aerial growth were better when bananas were grown in SAP substrate than in Andosol soil (Fig. 1). There was an increase in the average root weight of 34 and 42% for cv William and cv 902, respectively. While the average weight of the aerial parts increased by 16, 27 and 53% in the FB924, cv William and cv 902, respectively in the SAP substrate.

Banana roots were placed in a mist chamber for 2 weeks to extract the nematodes from the roots (Seinhorst, 1950). The nematodes were counted in a 5-ml aliquot of homogenized suspension under a stereomicroscope and expressed as the number of nematodes per gram of fresh root. The multiplication rate was calculated as the ratio of the final endoparasitic nematode population divided by the inoculum density. We analyzed the variances of substrate, root biomass and their interactions on the abundance of each nematode species expressed as the number of nematodes per gram of roots using a generalized linear model (GLM), while overdispersion was accounted for using a Quasi-Poisson instead of the Poisson model (R©, 2009).

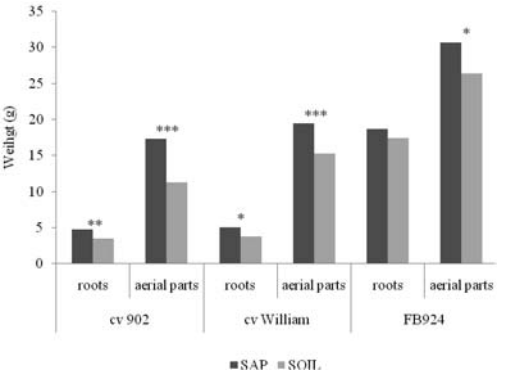

Fig. 1. Effect of substrate on banana growth. The weight of roots and aerial parts (in grams) are shown for each cultivar (means of 10 replicates). * = P_value < 0.05; ** = P_value < 0.01; ***= P_value < 0.001.

The development of the *R. similis* population was seen to depend on the substrate in all cultivars evaluated. In all cases, the SAP substrate resulted in better growth of the *R. similis* populations (Fig. 2). The average densities of nematodes increased by 43% in the FB924, by 82% in the cv 902 and up to 1,102% in the cv William. In the cv William and FB924, the final concentration of *R. similis* was also influenced by root weight (Table 1). Otherwise, the interaction between the two predictors was only significant for cv William (P_value < 0.05). Consequently, it showed that the effect of the substrate on the growth of the nema-

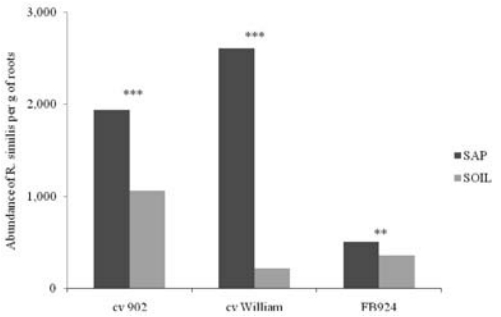

Fig. 2. Effect of SAP substrate (Sand and water-Absorbent synthetic Polymer) or sterilized soil on final population densities of *R. similis*, expressed as the quantity of nematodes per gram of roots for each cultivar (means of 10 replicates). * = P_value < 0.05; ** = P_value < 0.01; *** = P_value < 0.001.

tode populations was not due to its effect on root growth of banana plantlets, at least for cv 902 and cv FB924.

However, the substrate only affects population densities of *P. coffeae* on cultivar 902 (Table 1). Their average concentration was 170% of that in the soil substrate than SAP (Table 2). Population densities of *P. coffeae* were not affected by root weight, nor substrate.

Finally, major differences of pseudostem colors between seedlings grown on soil substrate or SAP substrate were observed. Therefore, we measured the color of pseudostems of cultivar FB924 that showed the most contrasting differences among treatments. For each treatment, the color of four plantlets of hybrid FB924 was measured with a chroma meter (CR 400, Minolta, Japan). The color is described based on the values of L*, a* and b*. The values of a* and b* define color in a two-dimensional chromatic space, indicating the green-red and blue-yellow axis, respectively, while L* is a measure of lightness. The results revealed significant differences (Fig. 3), which were strongest on axis a* (green-red) with opposite values. Seedlings grown on sand were mostly green whereas those grown on the soil were mostly red. Several biotic and abiotic factors, including UVB, water stress, injuries, lack of nutrients (nitrogen and phosphorus), some plant hormones and pathogens (Chalker-Scott, 1999) may be responsible for an increase in anthocyanin content leading to red coloration of the plantlets. However, in this experiment, which was conducted under controlled environment, the only difference in treatments was the nature of substrate used.

It is likely that the availability of mineral nutrients triggered the production of anthocyanins in the banana seedlings planted in sterilized soil. One major drawback of soil sterilization is the alteration of soil components (e.g. organic matter) and structure. This will result in the loss of structure stability of the clay-humus complex (this stability is usually maintained by

Table 1. Probability results of the analyze of variances performed on *R. similis* and *P. coffeae* data for each cultivar, using the Generalize Linear Model (R©, 2009). The model includes three predictors: root weight, substrate type and interaction.

	Radopholus similis			*Pratylenchus coffeae*		
	902	William	924	902	William	924
Roots weight	ns	***	**	***	ns	***
Substrate	***	***	**	***	ns	ns
Interaction	ns	*	ns	ns	ns	**

* = P_value < 0.05. ** = P_value < 0.01. *** = P_value < 0.001.

Table 2. *Radopholus similis* and *P. coffeae* densities in roots of 3 cultivars of banana, 45 days after inoculation with 400 individuals (means of 10 replicates).

		Soil substrate (Nematodes/g of root fw)	SAP substrate (Nematodes/g of root fw)
Radopholus similis	902	1065	1937
	William	217	2608
	FB924	357	511
Pratylenchus coffeae	902	3238	1198
	William	945	748
	FB924	173	79

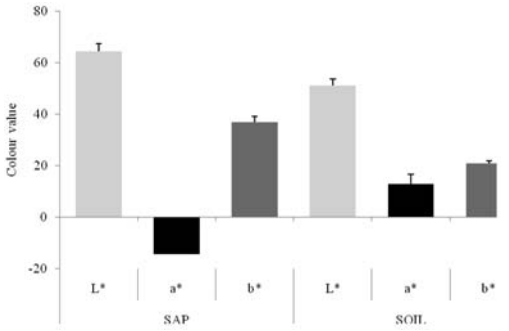

Fig. 3. Average color values of pseudostems of FB924 plantlets (means of 4 replicates), according to the substrate used (SAP or sterilized soil). Bar on top of each column is standard deviation.

living organisms such as earthworms and fungi). As a consequence, the cation exchange capacity of these clay-humus complexes could be weakened and no longer play its role as a nutrient reservoir for plant nutrition (De Deyn *et al.*, 2004). In contrast, the artificial substrate SAP contains a polymer that can absorb and retain water and nutrients. This certainly permits better nutrition of the banana plantlets: the plants are less stressed and thus produce fewer anthocyanins (Chalker-Scott, 1999) allowing *R. similis* to develop easily. In contrast, these artificial growing conditions appeared to be slightly less favorable to the lesion nematode *P. coffeae* on cv 902, while similar results were observed on the other cultivars (Table 2).

Various authors (Valette *et al.* 1998; Collingborn and Gowen 2000; Wuyts *et al.* 2003) have suggested that the amount of secondary phenolic metabolites (condensed tannins, flavonoids which are precursors of anthocyanins) in *Musa* roots plays a role in resistance of banana to nematodes. By reducing the nutritional stress imposed on plants cultivated in controlled conditions, we hypothesized that a reduced amount of anthocyanins in plant roots allowed better development of *R. similis* populations. Xenic culturing of nematodes on host plants is commonly used in laboratories with sterilized soil, native or not. As mentioned by Reversat *et al.* (1999), xenic culturing of plant-parasitic nematodes is suitable for many physiological studies aimed at measuring the fine effect of isolated factors related to plant nutrition using mineral nutrients. However, this practice largely depends on the soil type (sand and clay content, organic matter content) and of the sterilization procedure (moist or dry heat). By replacing the native growing soil with a totally artificial and standardize substrate, we have showed that it was possible i) to overcome the hidden constraints related to natural soil characteristics and heterogeneity and ii) to obtain

equal or better nematode multiplication. Moreover, the use of standardized nematode cultivation should allow a better comparison of biological traits among separate experiments whatever the laboratory facilities and geographical localization.

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