



## **Nematoregulatory Effect in Industrial Banana Plantations of Service Plants, *Crotalaria retusa*, *Cajanus cajan*, *Brachiaria decumbens* and *Panicum maximum* in Fallow Land in the South Comoé Region of Côte d'Ivoire**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author YG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KKFJM managed the analyses of the study and improved the manuscript according to journal requirements. Author NGPH managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** In the context of promoting alternative cropping systems with less chemical inputs, studies on the introduction of service plants to reduce pesticide use are a major research effort. The pest caused by plant parasitic nematodes is a major constraint in banana cultivation. One of the main alternatives to the use of chemical nematicides is the rational use of service plants with nematoregulatory properties in crop rotation.

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**Study Design:** The experiment was carried out following Complete randomized design (CRD) with three replications.

**Place and Duration of Study:** In industrial banana plantations located in the town of Ayamé, 40 km from the town of Aboisso in the forest area of southeast Côte d'Ivoire, between March to October 2016.

**Methodology:** Among the candidate species, *Crotalaria retusa*, *cajanus cajanus*, *Brachiaria decumbens* and *Panicum maximum* were evaluated because of their nematoregulatory ability, promoting better sanitation of banana plots infested with nematodes. The experiment tested the behaviour of four service plant species in the field over a period of 8 months during a banana fallow (*Musa* spp.) populated by nematodes (*Radopholus similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus* and *Meloidogyne* spp.).

**Results:** The results obtained showed that all the service plants used had a purifying effect on nematodes except *Cajanus cajan*. Soil and root analyses of host plants show the nematoregulatory action of *Crotalaria retusa*, *Cajanus cajan*, *Brachiaria decumbens* and *Panicum maximum* by reducing the number of nematodes after 8 months of fallow. Fallows of poacea are the ones that reduce the most *Radopholus similis* and *Pratylenchus coffeae* rates. A reduction in the length of fallow time was also noted.

**Conclusion:** This research has made it possible to improve the management of banana fallow land. This will ultimately help to define, under Côte d'Ivoire environmental conditions, a permanent living cover in association and/or rotation with the banana tree, in order to sustainably improve banana cultivation.

**Keywords:** *Radopholus similis*; *Pratylenchus coffeae*; *Helicotylenchus multicinctus* and *Meloidogyne* spp.; pest management; service plants; fallow.

## 1. INTRODUCTION

Banana (*Musa* spp.), native to South Asia, is grown in more than 120 countries around the world [1]. There are two subgroups of banana cultivars: raw fruit bananas consumed raw, dessert type, and plantain bananas consumed after cooking [2]. Dessert bananas represent the fourth major fruit harvest [3] and play an essential role in global food security. In Côte d'Ivoire, with an estimated annual production of 1.7 million tonnes, plantain bananas are the third most important food crop after yams and cassava [4].

However, banana production faces several constraints. These are generally of an agronomic (poor application of agricultural routes), climatic (insufficient rainfall and drought), soil (loss of physical, chemical or biological fertility of the soil) and health (diseases caused by viruses, fungi, bacteria and pests) nature. These constraints lead to a decrease in yield after a few years of cultivation. The low productivity of banana growing systems is mainly due to the increase in populations of terrestrial bioaggressors, particularly plant parasitic nematodes. Indeed, the latter are responsible for significant economic losses for a wide variety of crops worldwide [5-7]. The fight against these bioaggressors is essentially carried out through the often abusive use of synthetic products. These are sources of

environmental contamination (soil, water, air pollution and loss of biodiversity) and human health risks [8-11]. It is therefore necessary to promote agriculture that respects the environment and human well-being. The aim is to design more sustainable cropping systems that integrate service plants (SP) to naturally regulate or control the development of plant pests. Indeed, service plants that are plants installed in an agroecosystem to benefit from the ecosystem services they provide, but which are not harvested, seem to be an effective solution because their use could contribute to the reduction or even elimination of chemical pesticides. It is with this in mind that service plants have been used to control banana pests by growing them on fallow plots. The overall objective of this work was to evaluate the nematoregulatory effect of candidate service plants, which achieves better sanitation of nematode infested plots. The specific objectives were (i) to determine the effect of service plants on the population dynamics of banana nematodes in the soil after fallow and (ii) to identify alternative host plants for plant parasitic nematodes on fallow plots.

## 2. MATERIALS AND METHODS

The experiment was carried out on the Akressi site owned by an industrial dessert banana company located in the town of Ayamé, 40 km

from the town of Aboisso in the forest area of southeast Côte d'Ivoire.

During the experiment two legumes (*Crotalaria retusa* and *Cajanus cajan*) and two poacea (*Brachiaria decumbens* and *Panicum maximum*) were used. The test was carried out on a plot with a 7-year history of banana cultivation. After eight months of fallow land with service plants, the plots were replanted again with banana trees and the nematode population was monitored after three (3) and six (6) months.).

## 2.1 Experimental Preparation and Design

This study was conducted from March to October 2016. The experimental device adopted was a Fischer block, with three repetitions of five treatments. Each block measured 10 m wide by 100 m long. The blocks were separated by drains 1.2 m deep and 1 m wide. Each block was composed of five elementary plots, one of which represented the control (fallow without SP) and the other four the treatments (fallow with service plants). The four varieties of service plants (SP) were sown manually on the plots in rows, with a spacing of 20 cm x 20 cm. During the first week, daily irrigations guaranteed optimal water conditions for germination. The experiment was carried out on a total area of 3000 m<sup>2</sup> with a previous banana tree cultivation.

## 2.2 Assessment of Nematode Populations in Banana Root Crops

The roots were collected using the Tabarant method [12] 30 to 40 cm deep and at a distance from the plant between 0 and 50 cm. A fraction of 50 g of banana root was collected per sample for nematode extraction. The nematodes were extracted from the roots by the centrifugation-flotation technique. The identification and enumeration of each species was carried out under an AMSCOPE mini optical microscope of a camera using the method described by Siddiqi [13,14]. The density of nematodes in roots is expressed for each species as the number of individuals per 100 g of fresh roots. The nematode population was assessed before fallowing, at the end of fallow and after replanting with banana.

## 2.3 Assessment of Nematode Populations in Soil

Soil sampling consisted of taking five incremental soil samples from the same plot using an auger,

following the diagonals. Each sample was homogenized and crumbled by manual stirring. Then, the various samples were cleared of plant debris and stones. A fraction of 50 g of soil was collected per sample for nematode extractions. This method uses the principle of migration of nematodes by positive geotropism to the wettest environment [15]. Morphological identification and enumeration of each species was performed under an AMSCOPE mini optical microscope of a camera using the method described by Siddiqi [13,14]. The density of nematodes in the roots is expressed for each species as the number of individuals per 100 g of soil.

## 2.4 Assessment of Nematode Populations in the Roots of Alternative Host Plants

In order to research and quantify nematode populations on fallow plots of host plants of different banana nematode species were collected and the roots analysed. Thus, *Chromolaena odorata*, *Phyllanthus amarus*, *Eulesine indica* and *Passiflora* sp. were used as indicator plants for the presence of these parasites. Sampling consisted in manually removing the likely host plants of nematodes found on each plot. The roots of the same variety on the same plot were collected in a bin and homogenized to obtain a single composite sample. A fraction of 50 g of alternative nematode host plant roots was used for extraction. The nematodes were extracted from the roots by the centrifugation-flotation technique according to Coolen et al. [16]. The identification and enumeration of each species was carried out under an AMSCOPE mini optical microscope of a camera using the method described by Siddiqi [13,14]. The density of nematodes in roots is expressed for each species as the number of individuals per 100 g of fresh roots.

## 2.5 Statistical Analysis

Nematological test data obtained before the set-aside was carried out were analysed with the Quantitative Sisa software where an X2 test was carried out. In addition, those obtained at the end of the experiment were analysed using STATISTICA 7.1 software. The one-factor ANOVA test was used to assess the effect of service plants on nematode populations. In the event of a significant difference, the Newman-Keuls test at the 5% threshold was performed to classify the averages into homogeneous groups.

### 3. RESULTS

#### 3.1 Assessment of Nematode Populations in Banana Roots before Fallowing

Analysis of banana root samples prior to fallowing the plot revealed three types of plant parasitic nematodes, namely *Radopholus similis*, *Pratylenchus coffeae*, and *Helicotylenchus multicinctus*. The X2 test showed a difference between the populations of *Radopholus similis*, *Pratylenchus coffeae* and *Helicotylenchus multicinctus* (Fig. 1). The genus *Radopholus* was the most extracted from the roots collected, with 8000 individuals in 100 g of the sample. As for *Pratylenchus*, 4600 individuals were identified in the same extract. *Helicotylenchus* with 1500 individuals and *Meloidogyne* sp. were the least represented nematodes with an absence in the roots.

#### 3.2 Evaluation of the Effect of Service Plants on the *Radopholus similis* Population

After eight months of fallow with service plants (Fig. 2), nematological tests carried out on soil samples showed a greater reduction in the population of *Radopholus similis* on fallows with *Crotalaria retusa* covers (1 individual / 100 g soil), *Panicum maximum* (1 individual / 100 g soil) and *Brachiaria decumbens* (2 individuals / 100 g soil) compared to fallows without service plants cover (4 individuals / 100 g soil). However, the population of *R. similis* has increased considerably on fallows with the coverage of *Cajanus cajan* (19 individuals / 100 g soil).

#### 3.3 Evaluation of the Effect of Service Plants on the *Pratylenchus coffeae* Population

The results of nematological tests carried out on soil samples at the end of the eight months of fallow with the different service plants presented in Fig. 2, revealed that the population of *Pratylenchus coffeae* on fallows in plots without service plants (6 individuals / 100 g of soil) was much higher than that of fallows with service plants (1 and 3 individuals / 100 g soil respectively for *Brachiaria decumbens*, *Panicum maximum* and *Crotalaria retusa* covers), with the exception of *Cajanus cajan* (20 individuals / 100 g soil).

#### 3.4 Evaluation of the Effect of Service Plants on the *Helicotylenchus multicinctus* Population

After eight months of cultivation of the service plants, nematological analyses of the different soil samples showed an increase in the population of *Helicotylenchus multicinctus* on the plots with service plants (2; 4 and 17 individuals / 100 g soil respectively for *Brachiaria decumbens*, *Crotalaria retusa* and *Cajanus cajan*) compared to those without service plants (1 individuals / 100 g soil). However, soil samples treated with the *Panicum maximum* service plants coverage shown in Fig. 2 showed a slight decrease in the population of the nematode *Helicotylenchus multicinctus* (0.66 individuals / 100 g soil).

#### 3.5 Evaluation of the Effect of Service Plants on the Population of *Meloidogyne* spp.

No variation in the number of *Meloidogyne* spp. was observed on fallow land of *Panicum maximum* (5 individuals / 100 g soil) and *Cajanus cajan* (5 individuals / 100 g soil) compared to those without service plants (5 individuals / 100 g soil) after eight months of cultivation. In fallows covered by *Crotalaria retusa* plants, the population of *Meloidogyne* spp. increased (8 individuals/100 g soil), while these nematodes were totally absent on fallows covered by *Brachiaria decumbens* (Fig. 2).

#### 3.6 Growth Capacity of Alternative Nematode Host Plants on the Different Fallows of the Potatoes after Eight Months of Cultivation

After eight months of fallow from the potatoes, four alternative host plants of plant nematodes, namely *Chromolaena odorata* (Asteraceae), *Phyllanthus amarus* (Phyllanthaceae), *Eulesine indica* (Poaceae) and *Passiflora* sp. (Passifloraceae) were recorded on plots without service plants. All these plants housed nematodes. Except *Passiflora* sp., all these alternative host plants have also been identified on the fallows of the two legumes *Crotalaria retusa* and *Cajanus cajan*. However, the rate of nematodes found in the fallow of *C. cajan* was higher than that of *C. retusa* (Table 1). *Passiflora* sp. was the only nematode host plant found on fallow land in the *Panicum maximum* poacea. However, no alternative nematode host plants

have been identified on fallow land of the poacea *Brachiaria decumbens* (Table 1).

### 3.7 Follow-up after Replanting of the Cleaned Plots of Land

Three months after replanting on the different plots, the assessment of nematode populations in banana tree roots remained below 500 individuals / 100 g of roots on plots cleaned with *Crotalaria retusa*, *Panicum maximum* and *Brachiaria decumbens* plants. On plots cleaned with *Cajanus cajan* cover and fallow land without service plants, populations of *Radopholus similis*, *Pratylenchus coffeae* and *Meloidogyne* spp. were

greater than 500 individuals / 100 g of roots (Fig. 4). Banana trees replanted on fallow plots without service plants showed the highest number of parasitic nematodes in their roots. Repopulation on these plots was earlier than the sanitized plots with *Crotalaria retusa*, *Panicum maximum*, *Brachiaria decumbens* and *Cajanus cajan*.

Six months after replanting, plots treated with *Crotalaria retusa*, *Panicum maximum* and *Brachiaria decumbens* retained their nematoregulatory effect on the main nematodes infested with bananas (*Radopholus similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus*

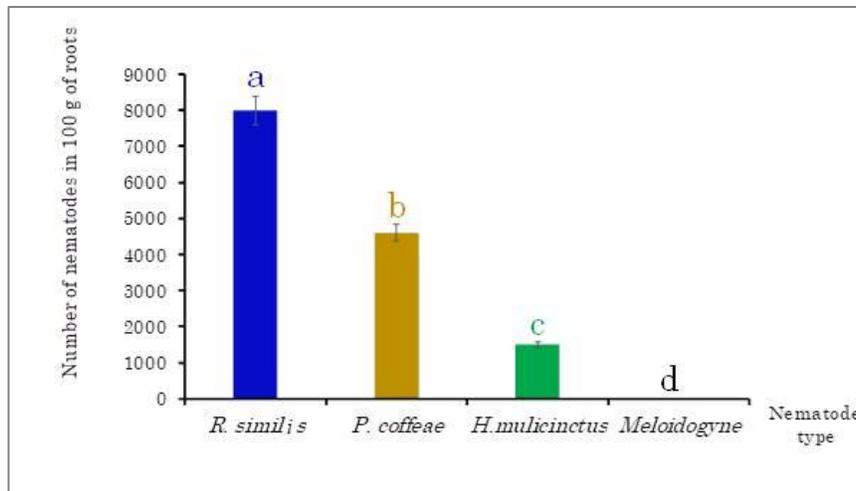


Fig. 1. Initial population of plant nematodes in banana roots before fallowing

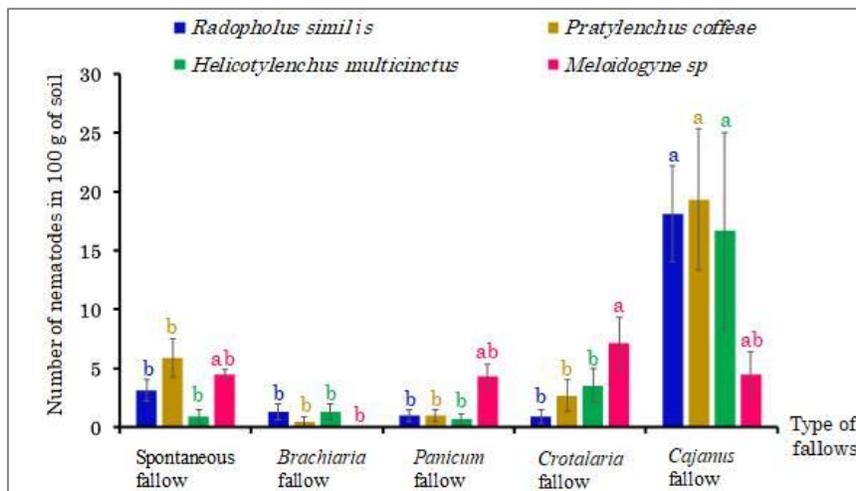


Fig. 2. Nematode population in soil after eight months of cultivation of service plants

and *Meloidogyne* spp.) with infestation rates per species well below 3000 individuals / 100 g of roots. On fallow land without service plants, the populations of *Radopholus similis* and *Pratylenchus coffeae* reached 7333 and 3000 nematodes / 100 g of roots respectively (Fig. 4).

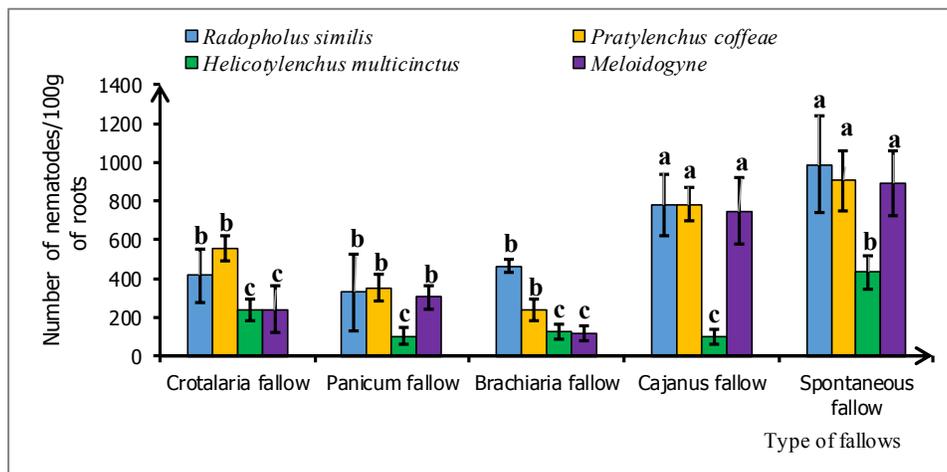
**4. DISCUSSION**

*Brachiaria decumbens* poacea is a plant known for its nematicidal properties [17]. It reduces the parasitic pressure of nematodes in the soil, particularly in intercultural situations. The

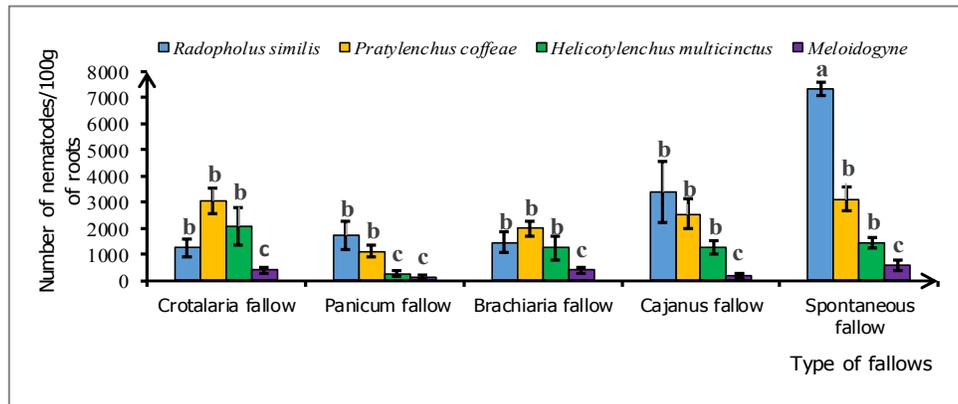
reduction in the populations of *Pratylenchus coffeae* and *Radopholus similis* and the suppression of *Meloidogyne* spp. on fallow land of *Brachiaria decumbens* is linked to its nematoregulatory effect. Our results are similar to those obtained by Dias-Arieira [18] who effectively observed a considerable reduction in the population of *Meloidogyne incognita* and *Meloidogyne javanica* by inserting *Brachiaria brizantha* into a tomato rotation. As for the *Helicotylenchus multicinctus* population, a slight increase in population level was observed. Our results are contrary to those of Machado [19]

**Table 1. Nematode populations in the roots of alternative nematode host plants recorded on fallow land**

Service plants	Host plants	Individuals of nematodes / 100 g of roots			
		<i>Radopholus similis</i>	<i>Pratylenchus coffeae</i>	<i>Helicotylenchus multicinctus</i>	<i>Meloidogyne</i> spp.
Spontaneous Fallow	<i>Phyllantus amarus</i>	13,33	29,02	148,78	6,06
	<i>Chromolaena odorata</i>	0,89	14,72	1,65	6,34
	<i>Eleusine indica</i>	50,56	8,33	2,22	29,17
	<i>Passiflore</i> sp.	0,00	0,00	0,00	3,45
	<i>Phyllantus amarus</i>	0,00	34,28	104,76	8,73
<i>Cajanus cajan</i>	<i>Chromolaena odorata</i>	3,75	15,00	6,74	5,68
	<i>Eleusine indica</i>	75,00	0,00	44,45	52,78
	<i>Phyllanthus amarus</i>	0,00	4,17	14,17	12,13
	<i>Chromolaena odorata</i>	0,00	0,00	0,00	1,70
<i>Crotalaria retusa</i>	<i>Eleusine indica</i>	19,96	0,00	0,00	11,29
	<i>Passiflore</i> sp.	0,00	0,00	0,00	19,05
<i>Panicum maximun</i>					
<i>Brachiaria decumbens</i>	No host plant	0,00	0,00	0,00	0,00



**Fig. 3. Number of nematodes in 100 g of roots three months after planting**



**Fig. 4. Number of nematodes in 100 g of roots six months after planting**

who revealed a decrease in the *Helicotylenchus dihystrera* population by introducing *Brachiaria ruziziensis* into a soybean rotation. In addition, the absence of an alternative nematode host plant on *Brachiaria decumbens* fallows after eight months of experimentation shows that this poacea could also be used to control weed development. Indeed Achard [20] at the end of their experiments showed that *Brachiaria decumbens* produces a high biomass and therefore covers the soil strongly. This prevents the proliferation of weeds.

The decline in the populations of *Radopholus similis* and *Pratylenchus coffeae*, as well as the increase in the population of *Helicotylenchus multicinctus* and *Meloidogyne* spp. on fallows of *Crotalaria retusa*, indicate that the latter has had a nematicidal effect on the populations of *Radopholus similis* and *Pratylenchus coffeae*. However, *Crotalaria retusa* was particularly host to *Helicotylenchus multicinctus* and *Meloidogyne* spp. Our results are consistent with those of Wang et al [21,22] who showed that *crotalaria* are weak hosts of many plant parasitic nematodes. These authors also stated that the nematoregulatory effect of *crotalaria* is short-lived. Indeed, according to Wang et al. [21], the regulation of nematodes by *crotalaria* is limited to only three months. Beyond that, they can become host to certain nematodes. However, nematological soil analyses were carried out eight months after the setting up of the various fallows. The loss of the nematoregulator effect of *Crotalaria retusa* would therefore be at the origin of the high population size of *Helicotylenchus multicinctus* and *Meloidogyne* spp. Moreover, this increase could be explained by the fact that *Crotalaria retusa*, being a slow-growing shrub legume [22], has allowed the development of nematode pool weeds on its fallows. This would

explain the presence of the three host plants of plant nematodes (*Chromolaena odorata*, *Phyllanthus amarus* and *Eleusine indica*) which have hosted more or less important populations of nematodes.

The increase in populations of *Pratylenchus coffeae*, *Radopholus similis* and *Helicotylenchus multicinctus* on fallow land of the *Cajanus cajan* legume is believed to be due to the fact that the latter has not completely covered the soil. Indeed, since *Cajanus cajan* is a shrub legume, the spacing between the plants of the latter has allowed the host plants of nematodes (*Phyllanthus amarus*, *Chromolaena odorata* and *Eleusine indica*) to proliferate freely. This has created a favourable environment for the reproduction of these nematodes. Also, according to Siambi et al [23,24] *Cajanus cajan* is more involved in improving soil structure than in sanitation. Indeed, *Cajanus cajan* because of its extensive root system allows the soil to soften, significantly increasing its fertility. This plant can fix up to 235 kg of nitrogen per hectare of crop and is therefore very interesting for environmentally friendly agriculture [25]. As for nitrogen residues left by crops, they are around 40 kg/ha, which increases soil fertility for rotational crops [22].

The regression of populations of *Radopholus similis*, *Pratylenchus coffeae* and *Helicotylenchus multicinctus* on fallow land in the *Panicum maximum* poacea is linked to its nematoregulatory effect. In addition, unlike *Cajanus cajan* it has considerably covered the ground. This has therefore prevented the proliferation of the majority of alternative nematode host plants listed on fallow land without SP. Our results are similar to those of Mollot et al. [26] who found fewer plant nematodes in treatments with *Paspalum notatum*

poacea than in treatments with legumes. They conclude that plants of the poacea family are more favourable to the regulation of plant nematodes than those of the legume family, thanks to the higher carbon/nitrogen (C/N) ratio for Poacea.

The history of the plot also influences nematode populations [21,27]. Indeed, a previous host will induce an increase in pathogenic populations. The next crop will then be at greater risk of nematode attack if it is susceptible. On the other hand, a non-hosted precedent will reduce the risk of contamination for the crop. This could explain the nematode populations recorded in banana tree roots on replanted plots that have not received service plants. These values are well above the threshold of 4000 *R. similis* [28]. Similarly, the threshold of *coffea* is exceeded with 3133 individuals / 100 g of roots against 3000 *P. coffea* [29]. The cultivation of non-hosted service plants in the intercropping phase has made it possible to prevent the multiplication of plant parasites associated with bananas. The ecosystem use of *Crotalaria retusa*, *Cajanus cajan*, *Brachiaria decumbens* and *Panicum maximum* nematodes has been highlighted. This service plants activity is probably carried out through different isolated or conjugated mechanisms of disruption of the nematode development cycle, or by emitting biochemical compounds or precursors toxic or repellent for nematodes. This measure made it possible to limit the reinfestation of replanted plots by more than six months. This saves the producer a nematicidal treatment.

## 5. CONCLUSION

This study on the regulation of banana parasitic nematode populations by four service plants showed that, with the exception of *Cajanus cajan*, all the service plants used had a cleansing effect. However, the *Brachiaria decumbens* poacea was the most effective. It was followed by *Panicum maximum* and *Crotalaria retusa* legumes respectively. Also, the ability of these service plants to clean up the soil varies according to the type of nematode.

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This study discovers that the use of service plants also makes it possible to consider limiting the use of nematicides, herbicides and heavy tillage. The control of banana plant nematodes can be ensured in particular by the use of service plants for the sanitation of plots in the inter-crop

phase. However, it remains to be fully understood the mechanisms of action of each of these plants.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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