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Project: Microbial uptakes for sustainable management of major banana pests and diseases (H2020 MUSA - 727624)

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Field data for integration of fungi and bacteria-based formulations with susceptible/tolerant germplasm against RKN, BW and PD

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1. Introduction:

The management strategy of banana pests within the framework of the MUSA project involves the use of microbiological tools that can have a double role. On one hand, beneficial endophytes and biocontrol agents (EBCAs) can be considered as providing a direct attack to the disease causative organisms. They can be included in the category of biopesticides, taking into account the legal connotations that this term has, within current regulatory frameworks, differing among the regions of the Project. On the other hand there are also microorganisms that can stimulate plants growth so that, upon their effect, the yield losses induced by pathogens are lower than those observed in absence of the promoter EBCAs. In addition, there are some microorganisms that have a double role, both exercising direct control over pathogens and promoting root growth, by improving the overall plant metabolism and crop yield.

Within the management alternatives of black weevil (BW) and plant parasitic nematodes (PPN), two other concepts cannot be let aside, such as the resistance that characterizes some banana germplasm (especially to PPN and enhanced or not by associated EBCAs) or the use of traps to control BW. For the latter a management optimization is also necessary, to disseminate a combined strategy for producers that avoid the use of pesticides.

In this deliverable, field data derived by Project work related to the use of a number of EBCAs is presented. They are: *Azospirillum brasiliense* for plant growth promotion (University of Leuven, Belgium, partner 7), *Pochonia chlamydosporia* and *Trichoderma asperellum* (CENSA, Cuba, partner 13). Data arre then presented referring to the sustainable management of PPN populations contributed by CENSA, studying the presence of nematodes and of host weeds at the Cuban National collection of Musa spp. The effect of *Pochonia chlamydosporia* var. *catenulata* and *Trichoderma asperellum* (alone and in combination) on PPN populations is then presented, while EARTH University (partner 12), has developed an evaluation of germplasm for resistance to PPN in the field and experimental work on biological control of the burrowing nematode *Radopholus similis* using EBCAs. Finally, data from experimental field assays related to BW management are shown, mainly based on the use of pheromone aggregation traps for adult capture. They consider the evaluation of two types of traps for monitoring and control of weevils in banana fields (elaborated by CENSA), and the use of natural substances with repellent effects for BW (Coplaca, partner 4) in a collaboration study with the University of Alicante (partner 3).

2. Growth promotion of banana plants through the application of microorganisms

2.1 Trial to test the growth promotion effect of *Azospirillum brasilense* on banana plants grown under open-field conditions (KU Leuven, Belgium)

Location and climate

The plant growth promoting effect of the commercial product PTA 001 containing the beneficial rhizobacteria *Azospirillum brasilense* (from partner 11 Real IPM/Biobest Group NV; https://www.biobestgroup.com/) was tested on the plantation 'Paradise produce' of Fresh Fruit Holdings (https://freshfruitholdings.business.site/). The field trial was located in Guayubín, Monte Cristi, Dominican Republic (19°35'N 71°24'W) and consisted of 240 banana plants cv. Williams distributed in 12 by 20 rows, planted on June 21st 2018 (Fig. 1.1). Williams was selected for being a widely used cultivar in production areas in Latin America (Cavendish subgroup, AAA). The cultivar has been reported as sensitive to PPN and BW, while resistant to Panama disease race 1. The plant density in the trial was 1750 plants per hectare, using an inter-row spacing of 2.1 m and an interplant spacing of 2.7 m. The climatic conditions in Guayubín are tropical with a min. max. temperature range of 19 - 34 °C. The average precipitation is 693 mm per year and the humid/moist period stretches from April to the beginning of December (Fig. 1.2).

Figure 1.1 Field trial in Guayubín, Monte Cristi, Dominican Republic.
Source of the image: Paradise produce plantation.

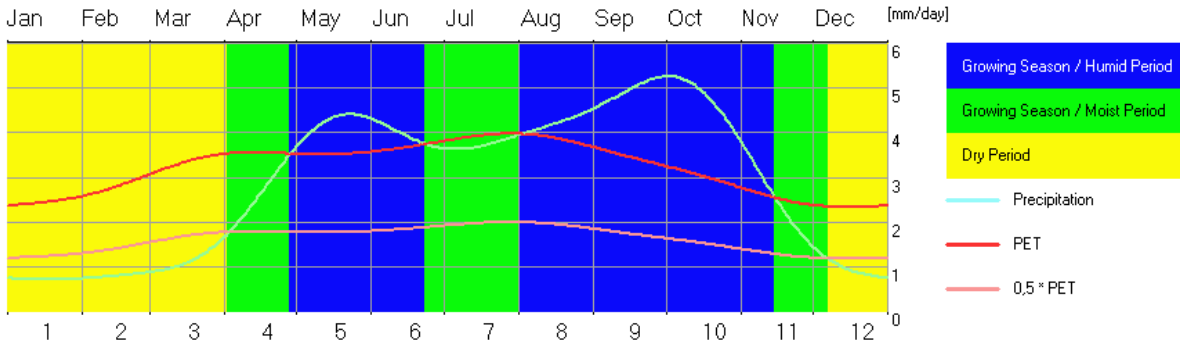


Figure 1.2 Vegetation period for Monte Cristi (Dominican Republic). Wet growing seasons (blue and green panels) stretch from beginning of April til early December. Dry season (yellow panel) stretches from December til the end of March.. Source: NewLocClim (data collected over 30 years), FAO (Gommes *et al.*, 2004).

Trial set-up

Commercial product PTA 001, which contains the nitrogen fixing rhizobacterium *A. brasilense* at $4 \cdot 10^8$ CFU/mL, was used at a final concentration of 10 mL/L. The plants were inoculated via a drench for the first time in the nursery on March 17th, 2018, 14 weeks before transplanting in the field. The following four drench applications were performed in the field at 2, 4, 9 and 11 weeks after planting (WAP). The treatment was applied to one block of 120 plants out of which 20 plants were selected for measurements (Fig. 1.3). Simultaneously, a control treatment was applied to plants planted at the same time in the same field but on another block, containing 120 plants. In that block 20 control plants were marked for the measurements. All plants were supplied with optimal irrigation and organic fertilizer (Orgamé Completo, Fertira; composition in appendix).

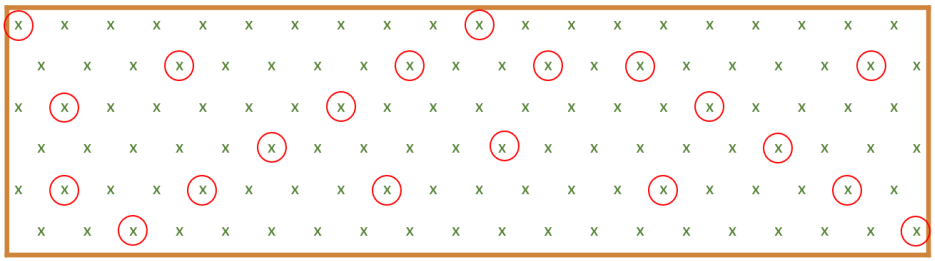


Figure 1.3 Scheme showing the distribution of the plants selected in each block. Each green cross represents a plant; those ones marked with a red circle were selected for the measurements.

Growth rate evaluation

Plant growth was assessed weekly through three measured parameters: pseudostem height (PsH), pseudostem diameter (PsD) and number of leaves (NL). The measurements were taken weekly between 6 and 16 WAP. The pseudostem height is

defined as the distance between the ground and the V formed by the two youngest leaves. To measure PsH, a tape measure (Stanley) was used. The girth of the pseudostem (PsG) was calculated by measuring the diameter of the pseudostem at 15 cm above the ground. This was done with a calliper (Duro, resolution: 0.03 mm) until the pseudostems became too big. From then on, a flexible tape measure was used to measure PsG directly. The measurements of PsH and PsD were combined into the pseudostem volume (PsV), assuming the pseudostem to be cylindric with the measured diameter as the cylinder diameter. The number of leaves was counted from 6 to 12 WAP. Later on, the leaf emission rate (LER) was determined more accurately by indicating the youngest leaf, with its candle stage on the plant.

Derived parameters

$$\mathrm{PsG} = 2\pi\left(\frac{\mathrm{PsD}}{2}\right)\ (\mathrm{cm})$$

$$\mathrm{PsV} = \mathrm{PsH}\,.\,\pi\left(\frac{\mathrm{PsD}}{2}\right)^{2}\ (\mathrm{cm}^3)$$

$$\mathrm{LER} = \frac{\mathrm{NL\ (time\ 2)} - \mathrm{NL\ (time\ 1)}}{\mathrm{time\ 2} - \mathrm{time\ 1}}\left(\frac{\mathrm{LE}}{\mathrm{week}}\right)$$

The percentage of difference between the treated and control plants for all plant growth parameters was calculated with the following formula:

$$\%\ \mathrm{diff.\ T\ vs\ C} = \frac{\mathrm{mean(treatment)} - \mathrm{mean(control)}}{\mathrm{mean(treatment)}}\ \mathrm{x}\ 100\ (\%)$$

Results

The effect of the treatment with *A. brasilense* (PTA 001) on the weekly measured growth parameters number of leaves (NL), pseudostem height (PsH), pseudostem girth (PsG) and the calculated variable pseudostem volume (PsV) on 'Williams' plants grown in open-field is shown in Table 1, for the period of 6 to 16 weeks after planting (WAP) and after 4 inoculations (2, 4, 9 and 11 WAP). Inoculations with PTA 001 had a significant effect allowing a 14-35% increase on pseudostem-related parameters between 6 and 8 WAP, in the field. At 6 WAP, PTA 001-treated plants had a taller pseudostem than the control plants. One week later, the PTA 001-treated plants had a taller and wider pseudostem and, thus, a significantly higher PsV than the control plants (35% of increase). At 8 WAP, the PsG and PsV of the treated plants were still significantly higher than those of the control plants (16 and 28% increase, respectively). However as of 9 WAP there were no treatment effects anymore.

Parameters NL at 11 to 16 WAP, PsH at all time points, PsG at all time points except for 9 WAP and PsV at 8, 15 and 16 WAP were analysed with ANOVA followed by a group

mean comparison with Fisher LSD test. The data were normally distributed according to the Shapiro-Wilk test ($p < 0.05$) and homoscedastic according to the Levene's test ($p < 0.05$; appendix D). All other parameters (NL at 6 to 10 WAP, PsG at 9 WAP and PsV at 7 and 9 to 14 WAP) were analysed with the Kolmogorov-Smirnov test.

Table 1.1 Effect of the inoculation with *A. brasilense* (PTA 001) on non-destructive plant growth parameters measured in Williams plants grown in open field (6 to 16 WAP). NL: number of leaves, PsH: pseudostem height, PsG: pseudostem girth, PsV: pseudostem volume; % diff. T *vs* C: percentage difference of treated versus control plants; KS: Kolmogorov-Smirnov. Ns: not significant; * = $p < 0.05$ ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. Results significantly different from control are highlighted in orange. Number of biological replicates (N treatment/control= 20/20).

Parameter		**Control (C)**		**PTA 001 (T)**			**Treatment effect**	
		Mean	**Std. Dev.**	**Mean**	**Std. Dev.**	**% diff. T *vs* C**	**ANOVA**	**KS**
6 WAP	NL	6.1	0.9	7.0	1.0	13		ns
	PsH (cm)	36.0	6.1	42.2	5.9	15	**	
7 WAP	NL	7.8	1.2	8.3	0.9	6		ns
	PsH (cm)	39.2	6.8	46.1	6.9	15	**	
	PsG (cm)	7.4	1.2	8.5	1.1	14	**	
	PsV (cm^3)	182	87	281	121	35		*
8 WAP	NL	8.8	1.2	8.9	1.1	1		ns
	PsH (cm)	43.8	8.6	45.8	6.8	4	ns	
	PsG (cm)	14.0	2.7	16.6	2.4	16	**	
	PsV (cm^3)	741	390	1030	373	28	*	
9 WAP	NL	9.9	1.1	9.9	1.0	0		ns
	PsH (cm)	53.3	9.9	53.6	8.4	1	ns	
	PsG (cm)	17.3	2.9	17.6	2.3	2		ns
	PsV (cm^3)	1367	715	1390	598	2		ns
10 WAP	NL	11.0	1.1	11.3	0.9	3		ns
	PsH (cm)	61.5	11.1	64.2	9.8	4	ns	
	PsG (cm)	20.3	3.3	20.4	3.3	0	ns	
	PsV (cm^3)	2176	1041	2267	1150	4		ns
11 WAP	NL	12.3	1.2	12.7	1.1	4	ns	
	PsH (cm)	72.0	14.6	73.8	12.92	2	ns	
	PsG (cm)	23.3	3.8	23.7	3.24	2	ns	
	PsV (cm^3)	3364	1710	3484	1556	3		ns
12 WAP	NL	13.7	1.2	14.1	1.0	3	ns	
	PsH (cm)	83.6	14.5	87.9	12.4	5	ns	
	PsG (cm)	26.7	4.3	27.5	3.7	3	ns	
	PsV (cm^3)	5089	2357	5557	2373	8		ns
13 WAP	NL	15.2	1.2	15.2	1.3	0	ns	

	PsH (cm)	99.0	17.6	100.1	13.5	1	ns	
	PsG (cm)	31.3	5.0	31.5	4.4	1	ns	
	PsV (cm³)	8278	3969	8286	3516	0		ns
14 WAP	NL	16.7	1.1	16.9	1.1	1	ns	
	PsH (cm)	114.4	17.1	115.7	14.7	1	ns	
	PsG (cm)	35.5	5.5	35.4	4.3	0	ns	
	PsV (cm³)	12208	5288	11992	4605	-2		ns
15 WAP	NL	18.0	1.1	18.1	1.1	1	ns	
	PsH (cm)	128.1	16.3	129.2	14.7	1	ns	
	PsG (cm)	40.1	6.7	39.2	4.7	-2	ns	
	PsV (cm³)	17232	6972	16344	5882	-5	ns	
16 WAP	NL	19.3	1.1	19.3	1.1	1	ns	
	PsH (cm)	143.4	17.6	143.0	14.4	0	ns	
	PsG (cm)	43.5	5.2	44.1	5.0	1	ns	
	PsV (cm³)	22384	7157	22846	7784	2	ns	

The PsH of PTA 001-treated plants was slightly taller than the control at 6 and 7 WAP (Fig. 1.4), but the significant difference was lost as of 9 WAP. PTA 001-treated plants had a significantly higher PsG and PsV at 7 and 8 WAP. However, the effect was lost as of 9 WAP (Fig. 1.5).

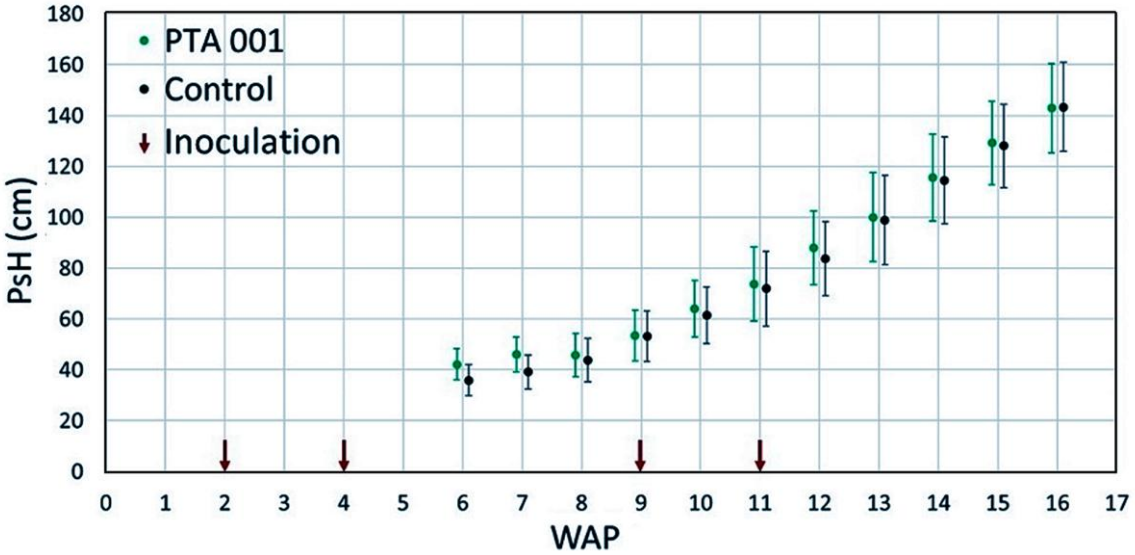


Figure 1.4 Effect of inoculation with *A. brasilense* (PTA 001) on pseudostem height (PsH) from 6 to 16 WAP in the field. Bars represent SD. N. treatment/control = 20/20.

All plant growth parameters recorded in the field trial were significantly positively correlated to each other based on a linear mixed-effects model taking into account the repetitiveness of the measurements (Fig. 1.6). In a partial least squares discriminant analysis (PLS-DA) of the parameters recorded at 7 WAP, the first component explained

20% and the second one less than 1% of the data variance. PsH, PsG and PsV had a high and almost equal share in determining the first component, whereas component 2 was mostly determined by NL and PsH (Fig. 1.6).

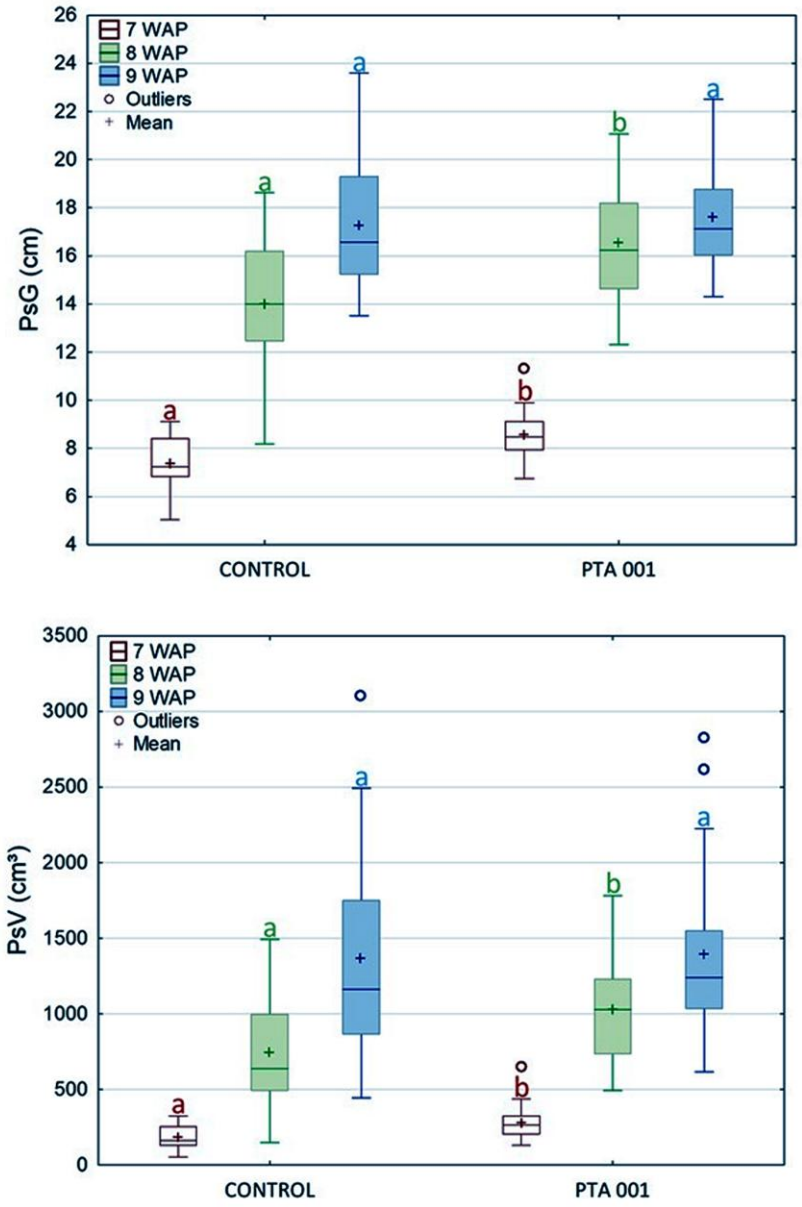


Figure 1.5 Effect of inoculation with *A. brasilense* (PTA 001) on pseudostem girth (PsG; top) and pseudostem volume (PsV; bottom) from 7 WAP to 9 WAP, in the field trial. Group mean comparisons of the same parameter (colour) by Fisher's LSD test or by the Kolmogorov-Smirnov test depending on the distribution of the parameter (a<b). Whiskers represent the non-outlier range. N treatment/control = 20/20.

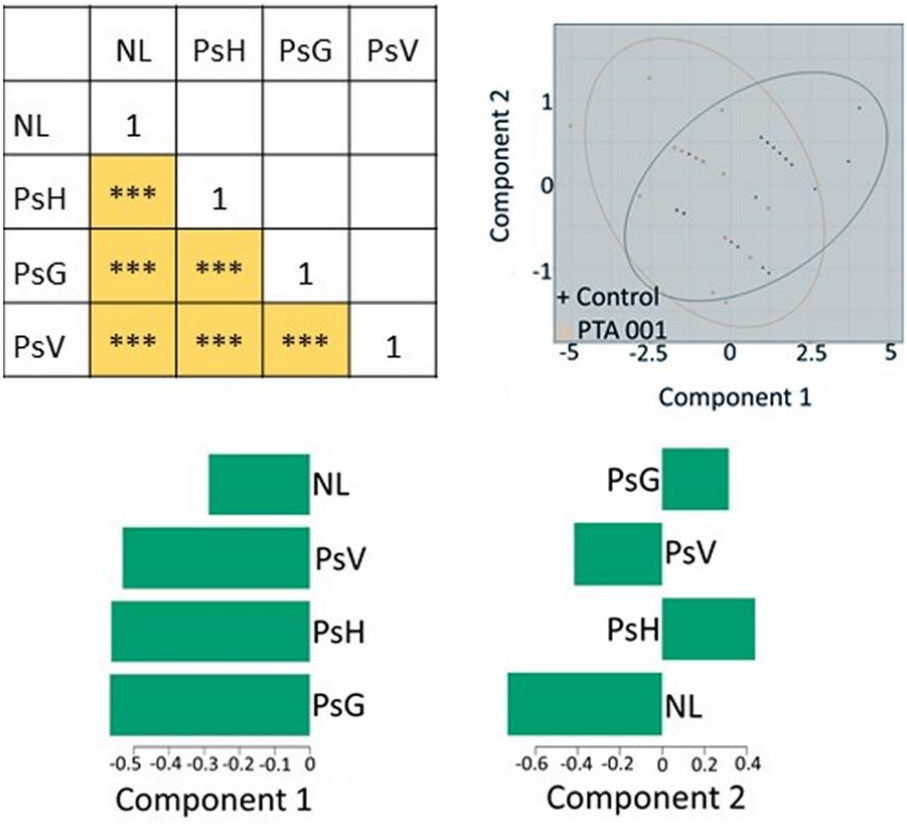


Figure 1.6 Correlation between the plant growth parameters recorded in the field trial: number of leaves (NL), pseudostem height (PsH), pseudostem girth (PsG), pseudostem volume (PsV). Significant positive correlations are highlighted in yellow (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Sample plot with the first two components of a partial least squares discriminant analysis (PLS-DA) on the axes. Ellipse contain 95% of the observations of each group: *A. brasilense* (PTA 001)-treated and control plants. The contribution of each parameter to the first and second component of the PLS-DA is also shown. N treatment/control = 20/20.

References

Gommes R., Grieser J., Bernardi M. (2004). FAO agroclimatic databases and mapping tools. *European Society for Agronomy Newsletter* 26: 1–5.

Appendix

Composition of the organic fertilizer Orgamé Completo (Fertira).

Composed organic mineral fertilizer: NPK 7-3-15
7.3 % organic nitrogen (2.5 % from blood meal, 2.5 % from feather meal, 2.3 % from bone meal); 3.5 % phosphorus anhydrid (P2O5) soluble in neutral ammonium citrate; 15.5 % potassium oxid (K2O) soluble in water; 46 % organic matter; 5 % CaO; 14 % SO3; 0.5 % Zinc (Zn).

2.2. Application of *Pochonia chlamydosporia* and *Trichoderma asperellum* in the process of adaptation of banana vitroplants (CENSA, Cuba)

In Cuba, banana/plantain represent food security crops grown in the whole country (land planes and mountains areas). Cuba has about 110,000 hectares of banana (*Musa* type AAB) and banana, harvesting in 2015, about 779,000 Tonnes. Some of the major pests of these crops are BW (*Cosmopolites sordidus*) and PPN such as the banana roundworm nematode (*Radopholus similis*), Root lesion nematode (*Pratylenchus coffeae*), reniform nematode (*Rotylenchulus reniformis*), root-knot nematode (*Meloidogyne incognita*) and the banana spiral nematode (*Helicotylenchus multicinctus*).

In addition, in *Musa* spp. plantations weeds coexist and can be hosts of nematode species that affect the main crop. Some weeds can sustain high nematode populations in absence of banana plantations, which makes their control difficult, due to the constant presence of alternative food sources (Guzmán et al. 2014). In Cuba, Casanueva et al. (2016) reported the presence of 30 weed species related to plantations of *Musa* spp. Several of these plants were hosts of the most important PPN species attacking the main crop.

On the other hand, the Cuban collection of *Musa* spp. germplasm, consisting of more than 300 accessions, is preserved in the field at the National Research Institute for Tropical Roots and Tuber Crops (INIVIT). The health status of the collection is an institutional responsibility. As part of pests management, it is necessary to determine the presence of PPN and host weeds in the area. At INIVIT, the presence of 10 weed species was reported, with a predominance of *Commelina diffusa* Burm. F. (Dávila et al. 2019). This species was previously reported in Cuba as a host for *R. reniformis* (Palenzuela et al. 1987).

Although progress has been made in Cuba in the improvement and selection of resistance to PPN and the BW, a complete and extensive resistant reaction has not been, however, achieved in combination with fruit acceptable for the consumer. In addition, given the climate evolution and expected changes, other potential solutions require more attention. The use of suitable germplasm lines, with a plant acceptable level of resistance/tolerance induction with EBCAs, and the application of essential oils as well, show a potential to improve the protection of the host crop.

The country has an historical background in the successful use of biological control agents for pest management. Among these prioritized products are the KlamiC® bionematicide, a harmless biological product developed at CENSA, from a native Cuban strain of *Pochonia chlamydosporia* var. *catenulata*. A further product is Sevetric (whose active component is an autochthonous strain of *Trichoderma asperellum*). CENSA also has experience in the use of essential oils for the management of different pests, a novel element to incorporate in the MUSA project.

Materials and Methods

The endophytic fungi *P. chlamydosporia* (commercial product KlamiC) and *T. asperellum* (commercial product SevetriC) from CENSA were applied alone or in combination at different times, during the acclimatization phase of banana cultivar FHIA-03 (ABB).

SevetriC biofungicide, formulated with the fungus *T. asperellum* (strain 13), was prepared in suspension at a dose of 20 g L^{-1} of colonized substrate ($4 \cdot 10^9$ spores $\cdot g^{-1}$), and inoculated by immersion of the vitroplants roots for five minutes, before planting in trays.

To obtain the spore suspension of *P. chlamydosporia* (IMI SD 187), 15.6 g of KlamiC were weighed per liter of water and stirred manually for one minute to release the chlamydospores from the substrate. Subsequently, it was filtered and poured into a 16 L spray backpack. KlamiC applications to vitroplants were carried out at different times: by roots immersion (five minutes) prior to planting, and in suspension at 3 and 20 days after sowing, applying 6 mL of suspension to each vitroplant, with a concentration of $5.6 \cdot 10^5$ chlamydospores $\cdot$ vitroplant^{-1}, as recommended by Hernández *et al.* (2016). In total, 12 treatments were carried out, including absolute control without application of endophytic fungi (Table 2.1).

Table 2.1 Treatments used in the experiment during Phase IV of acclimatization or ex vitro hardening of in vitro plants 'FHIA-03' (ABB).

No.	Treatments
1	Control (without fungi)
2	KlamiC® 1 application (3 DAS*)
3	KlamiC® 2 applications (3 and 20 DAS)
4	*Trichoderma* (immersion of roots for 5 min)
5	*Trichoderma* (immersion of roots for 5 min) + KlamiC (3 DAS)
6	*Trichoderma* (immersion of roots for 5 min) + KlamiC (3 and 20 DAS)
7	*Trichoderma* + KlamiC (immersion of roots for 5 min)
8	*Trichoderma* + KlamiC (immersion of roots for 5 min) + KlamiC (3 DAS)
9	*Trichoderma* + KlamiC (immersion of roots for 5 min) + KlamiC (3 and 20 DAS)
10	KlamiC® (immersion of roots for 5 min)
11	KlamiC® (immersion of roots for 5 min) + KlamiC (3 DAS)
12	KlamiC® (immersion of roots for 5 min) + KlamiC (3 and 20 DAS)

* DAS: Days after sowing.

The vitroplants were sown in polypropylene trays (47 x 69 cm) with 70 small alveoli (5 x 5 x 5 cm) (three repetitions per treatment, one plant in each alveolus), containing 65 g of substrate, conformed by leached Red Ferralitic soil (Nuricol Ródico Eútrico) (Hernández *et al*. 2005) and amendment, in a 1: 3 v / v ratio. FHIA-03 (ABB) cultivar banana plants were used in Phase III, at the end of the rooting phase in the Murashige and Skoog culture medium, supplemented with 1.3 mL of Indole Acetic Acid (IAA), 40 g sucrose and 2 mg L^{-1} thiamine.

The trays were located in the acclimatization area following an experimental design of random blocks, in semi-protected umbrellas in the roof and walls, cover by black shading meshes with 70% of the light intensity, where they remained for 30 days. In this conditions, two aerial irrigations were applied per day, through a micro sprinkler system to get 80% of field capacity in the substrate and relative environmental humidity between 85-95%.

The variables of the plant vegetative growth were measured twice (at 20 and 30 days). For evaluations, 25 plants of each random treatment were taken. The length of the plant (cm), diameter of the pseudostem (mm), number of active leaves, fresh and dry leaf mass (g), fresh and dry radical mass (g), were measured as described Hernández *et al.* (2016). Additionally, the increase in relation to the control of each of the treatments was calculated for all variables (except the number of active leaves), using the formula:

$$\text{Increment} = ((Mt / Mo) - 1) \times 100$$

where, Mt = variable treatment measure and Mo = absolute control measure

Results

The vitroplants achieved highest growth in the acclimatization phase at 30 days in all treatments. At 20 days, treatments 7 and 8, where both endophytic fungi were applied together by immersion of roots, the vitroplants showed a significantly lower height, compared to the control.

The other treatments showed no significant differences. Highest growth was observed in treatments 2 and 3 that received only one application of KlamiC, at 3 DAS. However, at 30 days the vitroplants of treatments 2, 3, 4, 5 and 11 (with one or both endophytes applied at different times), showed the highest growth, with significant differences from the control and other treatments (Fig. 2.1).

These results are similar to those obtained by Cassambai *et al.* (2012) who found that when inoculating vitroplants of Great Dwarf cultivar with endophytic fungi, a significant increase in plant height was promoted, compared to uninoculated plants or other controls.

No significant difference was observed for the vitroplants pseudostem diameters, at 20 days of growth in the trays (Treatment 1). After 30 days, treatments 2, 3 and 4 showed the largest diameters, although without significant differences when compared to treatments 1, 5, 10, 11 and 12 (Table 2.2).

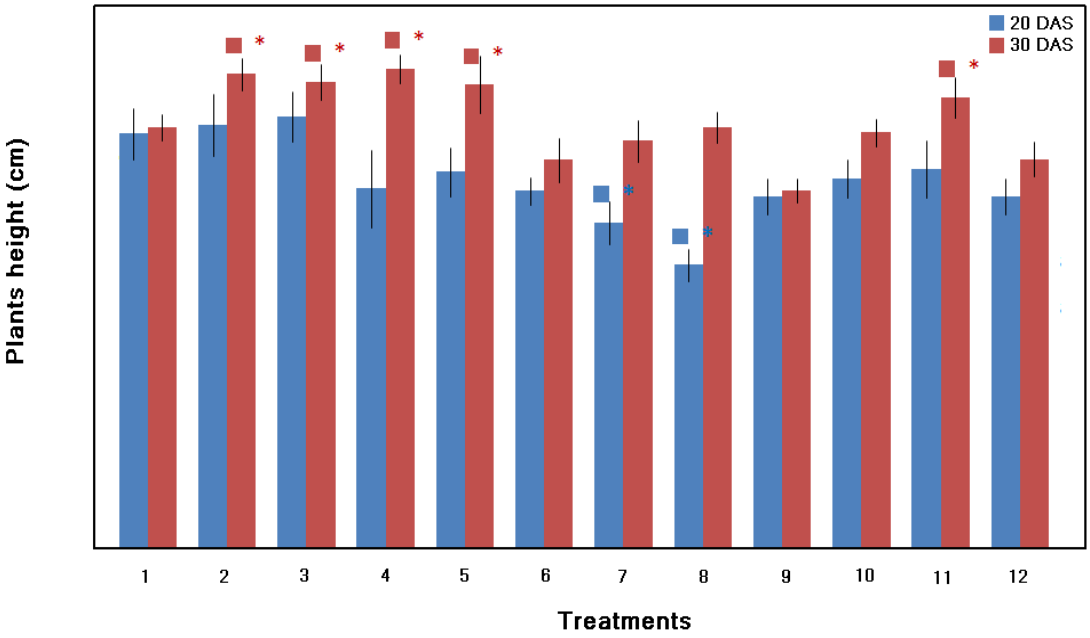


Figure 2.1 Growth of the banana plants FHIA-03 (ABB) at 20 and 30 days in acclimatization phase, with application of *P. chlamydosporia* and *T. asperellum* (for treatments see Table 2.1).

As concerns vitroplants growth, it was observed that treatments 2, 3, 4, 5 and 11 achieved an increase between 8.3 and 13.8 % of plant height, when compared to the control. Treatments 2 3 and 4 achieved an increase of the pseudo-total diameter between 2.5 and 12%, compared to control. The other treatments showed no effect on both plant length or pseudo-diameter (Fig. 2.2).

The growth promotion effect in terms of length and diameter of the pseudostem found in the present investigation are similar to those shown by Barrios (2006) who found significant differences in treatments with endophytic fungi, with height and pseudostem diameter values higher than control. Similarly, Meneses (2003) showed that eight weeks after protecting banana plants with endophytic fungi, they showed highly significant differences in growth promotion, compared to the control. Also Morales (2014), showed that banana plants inoculated with biological control agents reached a greater diameter and length of the radical system, compared to the control.

Table 2.2 Pseudostem diameters of banana plants FHIA-03 at 20 and 30 days in acclimatization phase, with application of *P. chlamydosporia* and *T. asperellum.*

Treatments	Pseudostem diameter (mm)		Differences*	
	20 days	30 days	20 days	30 days
1	4,33 ± 0,56 ns	5,14 ± 0,21 ab	1,21	2,02
2	5,00 ± 0,37 ns	5,60 ± 0,14 a	1,88	2,48
3	4,50 ± 0,43 ns	5,76 ± 0,23 a	1,38	2,64
4	4,83 ± 0,31 ns	5,27 ± 0,21 a	1,71	2,15
5	4,33 ± 0,56 ns	5,10 ± 0,33 ab	1,21	1,98
6	4,17 ± 0,31 ns	4,47 ± 0,24 bc	1,05	1,35
7	4,50 ± 0,22 ns	4,21 ± 0,27 c	1,38	1,09
8	5,00 ± 0,26 ns	4,18 ± 0,19 c	1,88	1,06
9	4,50 ± 0,34 ns	4,05 ± 0,26 c	1,38	0,93
10	5,33 ± 0,42 ns	5,05 ± 0,31 ab	2,21	1,93
11	5,33 ± 0,33 ns	5,10 ± 0,18 ab	2,21	1,98
12	4,83 ± 0,31 ns	5,11 ± 0,22 ab	1,71	1,99

* Difference between initial average of vitroplants pseudostem diameter and final measured at the conclusion of phase III. Different letters in the same column indicate significant differences ($p \leq 0.05$).

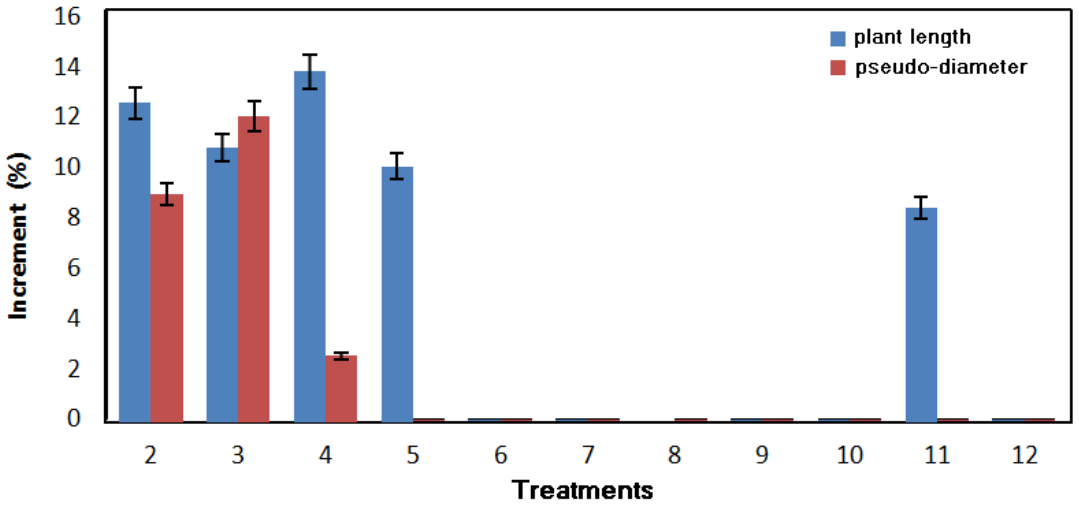


Figure 2.2 Percentage of increase in plants length and diameter of pseudostem in the treatments, in relation to control, at 30 days in banana FHIA-03 (ABB), with application of *P. chlamydosporia* and *T. asperellum* (for treatments see Table 2.1).

Considering the number of active leaves, there was no significant difference among the treatment, at 20 days of growth in the acclimatization phase. The highest average was 4.33 leaves, corresponding to treatments 6, 7 and 12, however not significantly different from control. At 30 days, the highest average number of leaves was 4.76 (treatment 2) not different, however, from treatments 3 and 5, but significantly different from control (Table 2.3).

Table 2.3 Number of active leaves of plants FHIA-03 (ABB) at 20 and 30 days, in acclimatization phase.

Treatment	Number of active leaves*	
	20 days	30 days
1	3,83 ab	4,32 bc
2	3,83 ab	4,76 a
3	4,00 ab	4,61 ab
4	3,83 ab	4,00 cd
5	4,17 ab	4,33 abc
6	4,33 a	3,82 cde
7	4,33 a	3,76 de
8	4,00 ab	3,50 e
9	3,67 b	4,1 cd
10	4,17 ab	4,15 cd
11	4,17 ab	4,14 cd
12	4,33 a	4,14 cd

* Different letters in the same column indicate significant differences ($p \leq 0.05$)

These results confirm those obtained by Menjivar (2005) in studies carried out in banana and vitroplants, where the vitroplants inoculation with endophytic fungi showed the same tendency as the control, as per the number of active leaves. However, Caballero (2011) found that several endophytic strains of *Trichoderma* spp., increased the number of banana leaves in Gros Michel (AAA) cultivar, with significant differences over control.

For treatments 2 and 3 (with one and two applications of KlamiC, respectively), increments were observed in fresh leaf (11.5 and 23.6 %), dry leaf (25 and 50 %) and radical dry (16.6 and 33.3 %) masses, with highest values in treatment 2. Treatment 4 (*Trichoderma*, 1 application) increased the radical dry mass to 16.6 % (Fig 2.3).

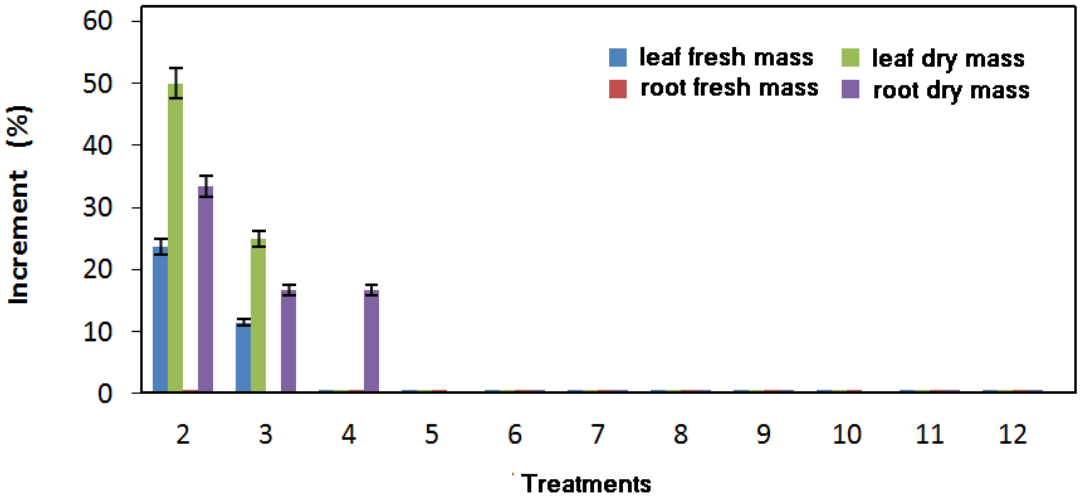


Figure 2.3 Increase percentages of different growth parameters in banana plants FHIA-03 (ABB) at 30 days in acclimatization phase, with application of *P. chlamydosporia* and *T. asperellum* (for treatments see Table 2.1).

The results obtained regarding the increase of the radical and foliar masses agree with data from Zum Felde (2006), where in vitro banana plants inoculated with isolates of *Trichoderma* and endophytic *Fusarium* increased the root mass and leaf area. Also, Chaves *et al.* (2009) showed an increase of total mass in plants protected with *T. atroviridae* of, compared to control (38 vs 34 g, respectively).

These results indicated that a time for the recovery of the plant after the transplant, before the inoculation of the endophytic fungi, may be necessary to achieve its recognition by the plant and the desired benefit in promoting plant growth. In addition, although both endophytes may be compatible, as Puertas *et al.,* (2006) demonstrated, it is important to take into account the time of application, since competition could occur during the saprophytic activity of both fungi.

In conclusion, both fungi can be incorporated into the banana vitroplants system, achieving a better adaptation and growth stimulation, while contributing to the early protection of the planting material.

The application of fungi incorporated directly into the substrate is recommended for better effectiveness. It is necessary to continue the evaluation of treated vitroplants in a period longer than 30 days measuring growth parameters, as well as the field behavior of inoculated planting material in presence of PPN populations.

3. Plant parasitic nematodes (PPN) management.

3.1.- Presence of PPN and host weeds at the Cuban National collection of Musa spp

Materials and methods

A survey was carried out in the *Musa* spp. germplasm collection (22.35 º N; 80.13 º O), located at the National Research Institute for Tropical Roots and Tuber Crops (INIVIT), Santo Domingo, Villa Clara Province, Cuba, with a Brown Fluffy Carbonated soil (Hernández *et al*. 2015). The collection has 355 accessions of *Musa* spp. planted at a distance of 3.6 x 2.0 m, in a total area of 2.12 ha.

FIgure 3.1 Location of INIVIT in Villa Clara Province and a view of the Germplasm bank.

The survey was carried out to determine the PPN associated with the most important genotypes and to establish pure populations of nematodes at CENSA, for further assays.

Given that *Trichoderma* spp. (provided by regional labs) had previously been used in the bank, the samples soil and roots were not analyzed for EBCAs. The selection of genotypes (Table 3.1) was carried out with the specialist responsible for the collection and national banana/plantain breeding program.

Samples of *C. diffusa* plants, roots and seedlings with high infestation were taken, also (Fig. 3.1). The samples were transferred to the Agricultural Nematology Laboratory of the National Center for Agricultural Health (CENSA) (23º N; 82º W), Mayabeque Province, Cuba.

Table 3.1 List of genotypes sampled at the INIVIT Germplasm bank.

Genotype	Sample number	Location			Resistance*
		Altitude (m.a.s.l)	north	west	
Burro CEMSA	M1	47	22.58623	080.22057	RN
ManzanoVietnamita	M2	57	22.58614	080.22065	
PisangCeilan	M3	52	22.58567	080.22101	
CEMSA 3/4	M4	55	22.58649	080.22056	SN
INIVIT PB 2011	M5	58	22.58721	080.22123	
INIVIT PV 2012	M6	57	22.58723	080.22128	
Yangambí	M7	57	22.58691	080.22072	ARR
Grand Naine	M8	58	22.58675	080.22094	SN
Gross Michel	M9	48	22.58722	080.22119	MRR
PisangHariBuaya	M10	54	22.58681	080.22133	RR
Calcuta 4	M11	51	22.58663	080.22145	
FHIA 18	M12	54	22.58592	080.22136	RR
INIVIT PV 0630 (Z30)	M13	59	22.58603	080.22092	SN
Enano Guantanamero	M14	57	22.58618	080.22092	SN
Macho 3/4	M15	56	22.58661	080.22072	SN
FHIA 21	M16	58	22.58634	080.22099	RN
SH3436L9	M17	57	22.58611	080.22124	RR
FHIA 01	M18	56	22.58611	080.22128	
FHIA 01-V1	M19	56	22.58600	080.22130	RR
SH3142	M20	56	22.58658	080.22149	
SH3362	M21	55	22.58654	080.22152	
FHIA 17	M22	54	22.58685	080.22568	

* RN: resistant to nematodes; SN: susceptible to nematodes; MRR: moderately resistant to *R. similis*; ARR: Highly resistant to *R. similis*; RR: resistant to *R. similis*

Figure 3.1 Plants of *C. diffusa* associated with germoplasm collection of *Musa* spp. in Cuba

The soil samples from the field, once at the laboratory, were spread in blankets of transparent polyethylene. The stones were removed, the sample were homogenized and passed through a 5 mm sieve. From each soil sample, 3 subsamples of 100 g each were processed by Whitehead trays protocol for nematodes extraction (Manzanilla-López, 2012). The nematode solution was collected after 72 hours. All the root samples were washed with running water, separating the functional roots from the non-functional ones. The selected roots were photographed and evaluated using the scale as indicated by Coyne *et al*. (2014). Finally, the roots were fractionated, homogenized and then 3 sub-units of 50 g each were taken, for processing through the shake + sieving method (Manzanilla-López, 2012; Coyne *et al.* 2014). The final suspension was collected, passed through a set of sieves, to collect the nematodes in a 38 µm sieve.

In addition, 25 adult females were extracted with their egg masses. Females were processed to obtain perineal patterns and pure populations were established using the egg masses (Hartmann and Sasser, 1985) for later studies. For all samples, number in the collection matched that of the original pattern.

Nematode Fixation

Nematodes removed from soil and roots were killed with gentle heat and fixed with TAF (Elmiligy and de Grisse, 1970; van Bezooijen, 2006). The nematodes in the suspensions were counted under a stereoscopic microscope (Zeiss STEMI DV4). A vertical wall PVC counting plate was used, with two compartments of different volume for quantification and generic identification.

Identification of nematode species of the genus *Meloidogyne*

The patterns were set on slides with lactic acid, sealed with paraffin and observed in a Axiostar Zeiss microscope, at 400 and 600 ×, in bright and interference fields, producing images with a digital camera. For diagnosis of *Meloidogyne* spp. associated

with the different banana and plantain genotypes as well as for *C. diffusa* samples, the keys and descriptions of species were used (Mai y Lyon, 1975; Andrássy, 1983).
The nematodes identified at the generic level were located in trophic groups, using the system proposed by Yeates *et al*. (1993). With the data related to genders and numbers of individuals by gender obtained, databases were built for corresponding analyses. The data were processed, statistically using Principal Component Analysis (PCA) with InfoStat 2016 Statistical Package (Di Rienzo *et al*., 2016).

Results

The dominant populations correspond to the nematodes belonging to the genera *Meloidogyne* (root knot nematode), *Pratylenchus* (lesion nematodes) and *Helicotylenchus* (spiral nematodes), in descending order. The presence of these populations is due not only to the capacity as hosts of these nematodes of most of the genotypes preserved in the collection, but also to the existence of weed plants that act as alternative hosts, such as *Commelina diffusa*.

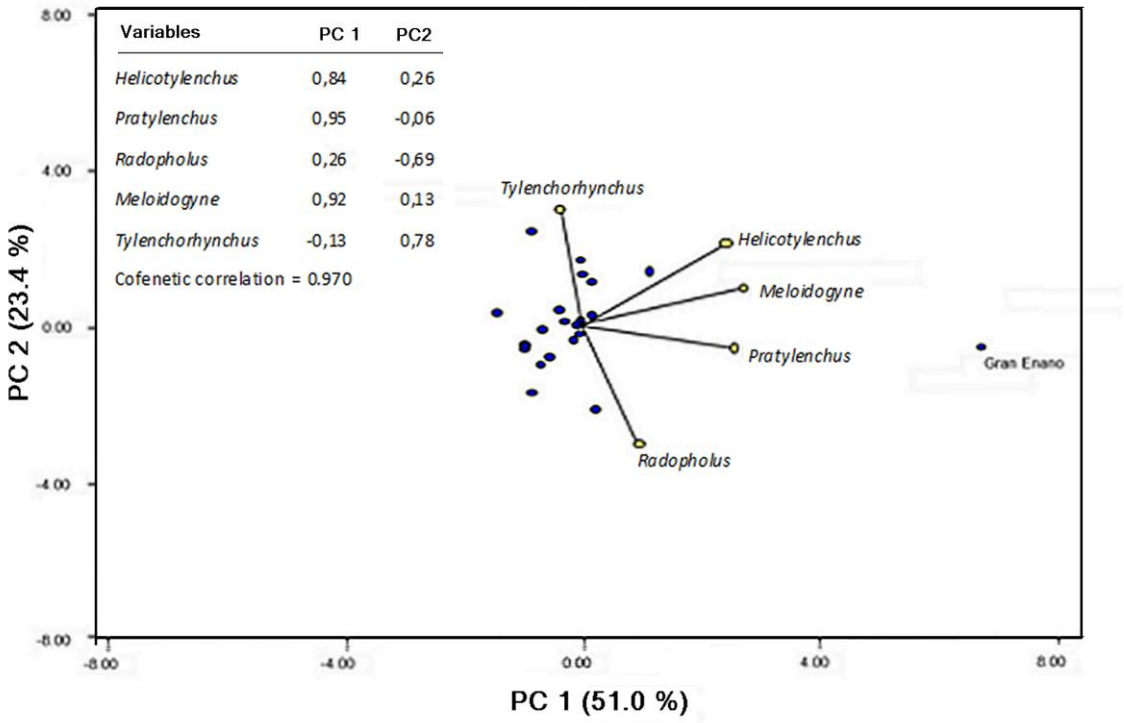


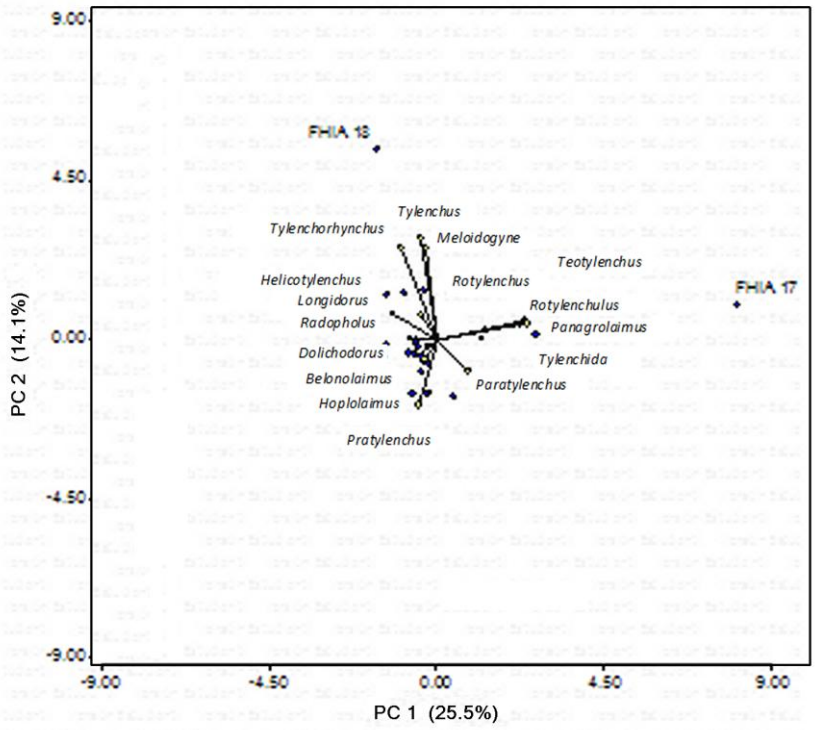
Figure 3.2 Dominant PPN associated with roots of 22 *Musa* spp. genotypes, preserved in the National Collection at INIVIT.

In the roots of most genotypes of *Musa* spp. the nematodes *Helicotylenchus, Pratylenchus, Radopholus, Meloidogyne* and *Tylenchorhynchus* were the dominant taxa, expressing a greater relationship of these genera with genotype Gran Enano.

In the soils of the national Musa collection at INIVIT, 16 PPN genera were found. The CEMSA ¾ genotype, one of the most distributed in the country, presented the largest

populations of *Helycotylenchus* and *Pratylenchus* in soil. FHIA 01 showed the largest populations of *Radopholus*, and FHIA 18 those of *Meloidogyne*.

PCA revealed that FHIA 17 genotype had a high correlation with all genders found.



Variables	CP 1	CP 2
Helicotylenchus	-0,44	0,2
Pratylenchus	-0,18	-0,51
Radopholus	-0,27	0,01
Meloidogyne	-0,16	0,81
Tylenchorhynchus	-0,37	0,73
Tylenchus	-0,11	0,73
Rotylenchus	0,5	0,08
Paratylenchus	0,32	-0,24
Rotylenchulus	0,9	0,16
Dolichodorus	-0,08	-0,05
Belonolaimus	-0,11	-0,15
Hoplolaimus	-0,19	-0,13
Longidorus	-0,15	0,19
Teotylenchus	0,86	0,12
Panagrolaimus	0,89	0,15
Tylenchida	0,92	0,14

Cofenetic Correlation = 0.806

Figure 3.3 PPN associated with soil and rhizosphere of 22 *Musa* spp. genotypes, preserved in the National Collection at INIVIT.

An earlier study by Fernández *et al.* (2015) indicated that nematodes associated to the different *Musa* spp. were *Radopholus similis*, *Pratylenchus coffeae, Helicotylenchus multicinctus* and *Meloidogyne* spp. Similarly, in the Province of Cienfuegos, were reported the presence of *Helicotylenchus* spp., *Pratylenchus coffeae, Meloidogyne* sp. and *R. similis* (Almarales *et al.* 2014).

Fourteen weeds have been found associated to *Musa* spp. in the National Collection in Cuba: *Alcalipha havaniensis, Amaranthus spinosus, Commelina* spp*., Cynodon dactylon, Cyperus rotundus, Digitaria ciliaris, Echinochloa colonum, Eleusine indica, Euphorbia hirta, Ipomoea* spp*., Macroptilium lathyroides, Portulaca oleraceae, Rottboellia exaltata* and *Sorghum halepense.* Some of them are PPN hosts in banana plantations in Caribbean (Quénehérvé *et al*. 2006) and Cuba (Casanueva *et al*. 2016). One management tactic suggested to the authorities in charge in Cuba of the National Musa Collection was to eliminate the weeds hosting plant parasitic nematodes.

It was found that *Commelina diffusa* plants had numerous galls of various sizes with well-formed adult females, with egg masses containing eggs, indicative of the reproduction of the nematode in this plant (Fig.s 3.4 - 3.5).

The perineal patterns of *Meloidogyne* adult females extracted from the roots of *C. diffusa* had the following characteristics: gently wavy cuticle lines, dorsal arch, generally, from low to medium, in some cases trapezoidal, but generally round. Phasmids visible, lateral lines present and visible, interrupting the cuticle lines on one or both sides of the perineum, producing the so-called "shoulder pads". The general pattern configuration was rounded or alike.

It is important that researchers and producers possess this information. Together with the previous knowledge about the hosts species for *R. reniformis* (a nematode that also affects *Musa* spp.), we suggest to eliminate this weed from the field where the collection of genotypes and banana plantations are located.

Figure 3.4 Galls, females (in circle) and egg masses of *Meloidogyne* sp. in roots of *C. diffusa* from Nationa *Musa* collection in Cuba

Peraza *et al.* (2018) reported *Commelina* spp. as PPN hosts in Costa Rica, in particular for the presence of *Meloidogyne* spp. in roots of *Commelina erecta* L. Fernández *et al.* (2015) reported that *Commelina elegans* H.B.K. represents a host of *Meloidogyne incognita* (Kofoid & White) Chitwood.

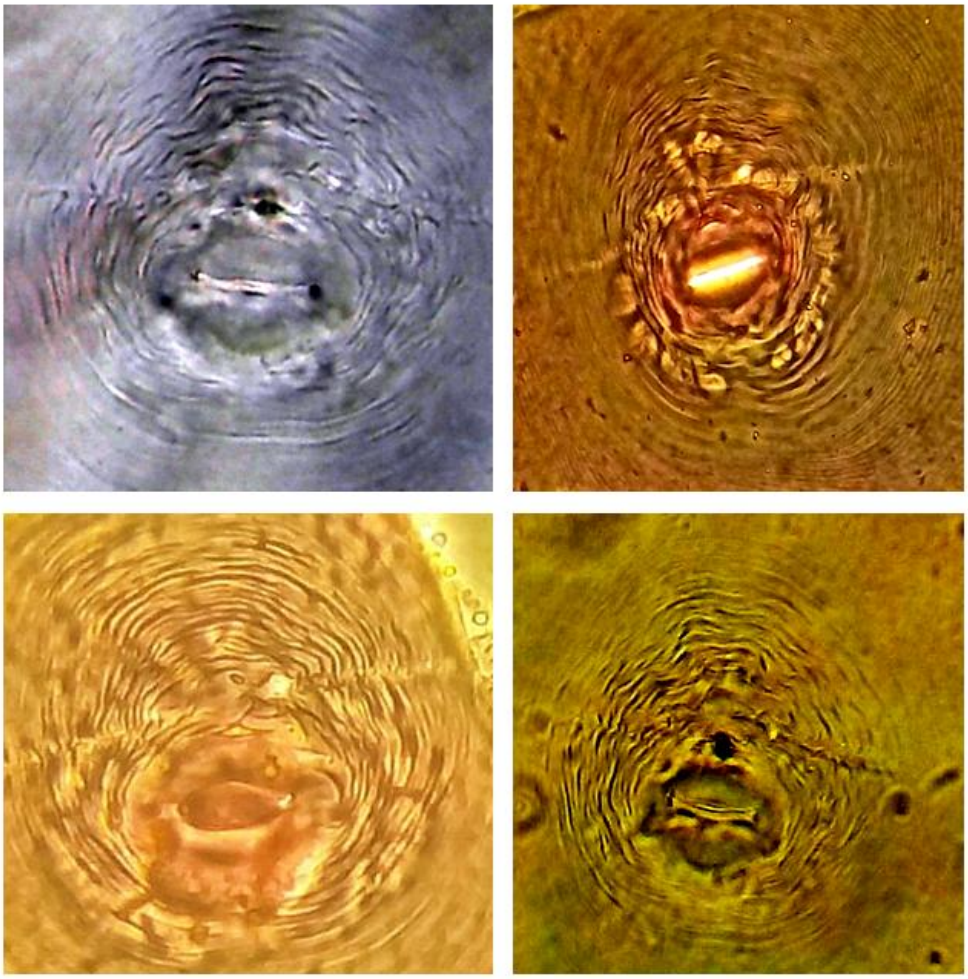


Figure 3.5. Perineal patterns of *Meloidogyne* females associated to *Commelina diffusa* in Cuban National *Musa* collection.

3.2 Effect of *Pochonia chlamydosporia* var. *catenulata* and *Trichoderma asperellum* (alone and in combination), on the populations of PPN

Materials and methods

Selection, geographic location and edaphoclimatic characterization of the study area

The present investigation is being carried out in a selected agricultural scenario of the Nueva Paz Municipality, in the province of Mayabeque, Cuba (Fig. 3.6, 3.7).

Figure 3.6 Geographical location of the province and municipality.

The field experiment is being carried out in a space within the 6.5 ha area of the Capitolio farm belonging to the Basic Unit of Cooperative Production (UBPC) "Antonio Maceo", between the town of Nueva Paz and Los Palos, with access by roads in good condition. Geographically, the area is located at 22° 46' 49.5" N and 81° 45' 0.4'' W, at 25 meters above sea level.

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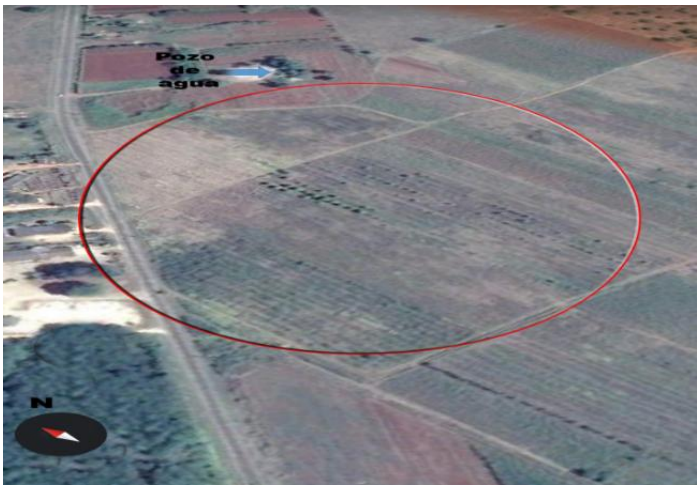


Figure 3.7 Exact geographical location of the area where the experiment is executed. Scale 1:50 m (taken from: Google maps and Google earth,on 4/26/2019, at 11:13 am)

The climate in the municipality presents the following characteristics: annual rainfall close to 1415 mm, average temperature = 24.9 ° C and relative humidity ranging between 74 - 76 %. The area has a source of drinking water supply for crop irrigation, carried out by gravity, following rules established for this activity (ACTAF, 2008). The surface has a flat or almost flat topography, with light slopes between 0 - 3%. According to the Soil Classification System of Cuba, the experiment is on a hydrated Ferralitic Red soil (Hernández *et al.* 1999; FAO 1999). It has horizons A + B of 82 cm and total depth greater than 100 cm, with content in organic matter between 2 and 3%. Due to its agroproductivity the soil is classified as very productive with good drainage

Experimental development

The soil preparation tasks were carried out as established by the Technical Instructions for the crop (ACTAF 2008). The selected plantation was 6 months old.

Before set up the experiment, different samples were taken from the selected area for chemical, microbiological (presence of *Pochonia* and *Trichoderma* spp.) and nematological characterization (initial sampling).

The chemical analysis for soil characterization is being carried out in the National Institute of Agricultural Sciences (INIA), through the analytical methods established by the Cuban Standards (NRAG 1987; 1988) and Paneque *et al*. (1998; 2000), with the following variables:

pH in H_2O: potentiometry. Soil ratio - solution: 1: 2.5
Organic matter (%)
N assimilable ($kg \cdot ha^{-1}$)
P_2O_5: ($mg \cdot kg^{-1}$)
Interchangeable cations: Ca, Mg, Na and K ($cmol \cdot kg^{-1}$).

Nematological analyses

For nematode extraction, killing, fixation and identification from root and soil samples the protocols described before were applied.

Mycological analysis

The presence in soil of *Pochonia* spp. was determined by the technique of dilution and growth in semi-selective medium established by Kerry *et al.* (1993). For detection of *Trichoderma*, a serial dilutions method was used, combined with growth in PDA.

In addition, the following measurements were taken on mother and son banana plants, selected and identified as experimental units: height, diameter of the pseudostem, number of functional leaves and number of new shoot. These measurements were made at 6 months after plantation establishment and just before the application of the KlamiC and SevetriC bioproducts.

Subsequently, the experiment was set up in March 2019. As a plant material, 1 m tall needles of banana "*Burro*", cultivar CEMSA ¾ (ABB) were used. The seeds (corms) were acquired from plantations adapted to the climatic conditions of the area, which previously were identified as productive, vigorous and healthy.

For plantation, the corms were spaced in simple rows at a distance of 4 x 4 m (wide street by lines), for a population density of 625 plantas $\cdot ha^{-1}$.

FIgure 3.8 View of plantation of *plátano Burro*, cv CEMSA ¾ (ABB), established in the experimental area.

The banana clone Burro CEMSA ¾ (ABB) has normal foliar habit, height between 2.20 - 3.80 m, light green pseudostem, green fruits, position of the curved fruits upwards, duration of first vegetative cycle (sowing-flowering) between 240-280 days, duration of first productive cycle (flowering-harvest) between 90-120 days, bunch weight between 15 - 23 kg and 63 - 95 fingers per bunch. This cultivar is one of the most widespread in Cuba, due to its characteristics of rusticity and ability to adapt to different environments (Fig. 3.9).

Figure 3.9 Cluster of banana *Burro*, cultivar CEMSA ¾ (ABB).

The experiment was established in an area of 2 ha, under a block design totally randomized, consisting of 4 treatments and 6 replications of treatments (the seedling being the experimental unit, composed of the mother plant and the new shoot 1). It was tutored to lead the plantation leaving 3 and 4 children followers.

Each treatment has an area of 0.5 ha (6 rows with 52 plants each). For sampling and measurements, the 4 central rows were selected. The experimental units (replicates)

spaced at 16 m (every 4 seedlings), were randomly selected leaving the 2 outer grooves as edge areas. The furrows selected for each treatment were marked and identified as well as the plants selected for sampling (Fig. 3. 10).

Figure 3.10 Identification and marking of the experimental units (plants).

The treatments were:

Treatments		Abbreviation	Observations
1	Control	C	Traditional cultivation without application of EBCAs
2	KlamiC*	K	
3	SevetriC	S	
4	KlamiC + SevetriC	K + S	

* KlamiC = *P. chlamydosporia* strain IMI SD 187. SevetriC = *T. asperellum* strain 13.

After defining the areas for each treatment, the bioproducts were applied alone or in combination around all seedlings in the 4 central furrows, at a distance of 30 cm from the mother plant. KlamiC was applied at a dose of 42 g · new plant^{-1}

Samples were taken at 45 days, three and six months after application of the EBCAs. In each treatment and sample, the presence and populations of PPN, EBCAs and other plant physiological variables will be analyzed, as described for the initial sampling. In addition, at the time of harvest, the cluster weight (kg), number of hands and number

of fingers per hand will be measured. Percentage of plants overturned by experimental plot will also be included.

KlamiC bioproduct used in the experiment proceeded from batch No. 030219 and was obtained within a quality management system under ISO 9000 standards, in the Research Unit Development and Production of Biological Control Agents. Final concentration was $1.3 \cdot 10^7$ chlamydospores $\cdot g^{-1}$ (viability: 93.75%), without contamination (Fig 3.12A). SevetriC bioproduct used in the experiment proceeded from lot No.010419. Final concentration was $3 \cdot 10^9$ conidia $\cdot mL^{-1}$, viability (93.25%, and 100% purity), without contamination (Fig 3.12B).

Figure 3.11 Products applied in the experimental trials: Klamic (AB) and SevetriC (B).

The corresponding sampling was carried out at 45 days and 3 months post inoculation. The sampling of soil and roots to determine the presence of nematodes and of the applied EBCAs was carried out following established protocols for banana cultivation. The volume of soil extracted and the root fractions were packed in black polypropylene bags completing 1 kg. The bags containing the samples were transferred to the laboratories where they were processed and analyzed.

Figure 3.13 EBCAs application in the field.

Figure 3.14 Measurements and countings of growth variables in plants, field data recording androcessing of soil and root samples in the laboratory.

Results

The mycological determinations carried out at the initial sampling confirmed the absence of fungal structures of genera *Pochonia* and *Trichoderma* in soil, so it can be stated that the fungi found and their effects after applications should be referred to the inoculated products only.
The initial study of field soil, where the field experiment was established, showed that bacteriophages and predatory nematodes predominated, mainly with generes *Cephalobus* and *Pelodera* (bacteriophagous) and *Mononchus* (predator). Among the PPN, *Helicotylenchus* stands out with highest number of specimens in 100 g soil, followed by *Pratylenchus* and *Tylenchorhynchus*, respectively (Table 3.2).

Table 3.2 Nematode groups and densities in banana rhizosphere, at Nueva Paz (in red non parasitic grops).

Nematode Genera	**Total individuals in 100 g soil**
Pelodera	1454
Mononchus	1678
Cephalobus	1590
Meloidogyne	120
Tylenchorhynchus	125
Helicotylenchus	663
Pratylenchus	265
Radopholus	10

Identification of *Meloidogyne* spp.

The perineal patterns morphology of the *Meloidogyne* females suggested the presence of *Meloidogyne incognita, M. javanica* and *M. arenaria* the *Musa* spp. genotypes (Fig. 3.15). However, the intraspecific variability in morphological characters presented by *M. incognita* and *M. arenaria* impliee that a definitive diagnosis had to be established with the help of molecular techniques, investigations to be undertaken in next project stage. Pure populations *M. javanica* were established, whereas a higher number of patterns, with male and second stage juveniles (J2) morphometrics, are under examination. The results for soil chemical analyses are shown in the Table 3.3

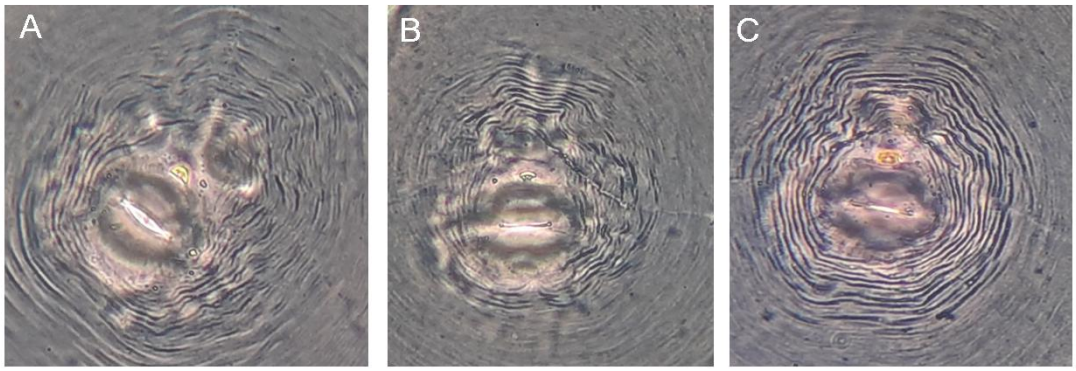


Figure 3.15 Perineal patterns of adult females of root knot nematodes from New Paz areas, exhibiting characteristics indicative of *Meloidogyne incognita* (A), *M. javanica* (B) and *M. arenaria* (C).

Table 3.3 Chemical characterization of soil (depth 0-25 cm).

Soil	Na	K	Ca	Mg	P_2O_5	M.O	pH in H_2O
	$cmol_c\ Kg^{-1}$				($mg100g^{-1}$)	(%)	
M1	0,07	0,84	20,0	4,0	42,59	2,75	7,1
M2	0,10	1,06	15,5	4,5	41,22	2,61	6,9
M3	0,09	1,23	14,0	7,0	44,65	3,04	6,9
M4	0,08	1,02	17,5	5,5	53,13	2,93	7,1
M5	0,08	0,85	18,0	5,0	30,69	2,86	7,1
X	**0,084**	**1,00**	**17,00**	**5,20**	**42,45**	**2,84**	**7,02**

The evaluation of soil fertility showed presence of neutral pH, with low content of organic matter, assimilable phosphorus and exchangeable potassium, as well as adequate values of Na, Ca and Mg, for the development of the crop.

Trichoderma analysis

At 45 days post inoculation with the EBCAs, it was possible to identify colonies of *T. asperellum* from soil and endophytic growth in the banana root fragments. In treatments T3 (*Trichoderma*), and T4 (*Trichoderma* + *Pochonia*), a colonization of $1.58 \cdot 10^5$ CFU g^{-1} and $1.25 \cdot 10^5$ CFU g^{-1} were observed, respectively. Such results indicate that the fungus was able to establish and develop, in a satisfactory way.
After three months of application, once again soil analysis of treatments T3 and T4 showed the same levels of colonization, indicating that the fungus remained viable over time.
Six months after application, *Trichoderma* colonization increased to $2.17 \cdot 10^5$ CFU g^{-1} in treatment 3 and $1.58 \cdot 10^5$ CFU g^{-1} in treatment 4, indicating that the fungus remained latent in soil, with abundant growth of colonies. Data showed that *Trichoderma* was able to endure and multiply, a performance that may also be related to the crop (Fig. 3.16).

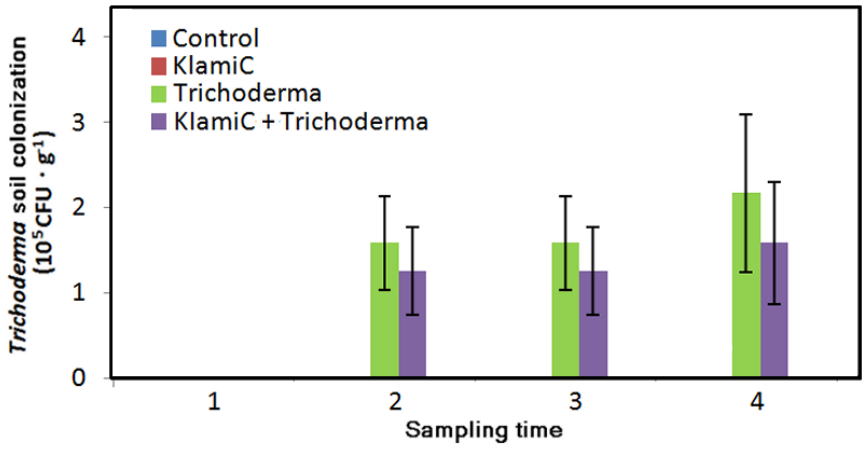


Figure 3.16 *Trichoderma* colonization in soil rhizosphere of *Burro* CEMSA ¾ genotype. Samplings are: initial (1), and 45 days (2), three months (3) and six months (4) after inoculation.

45 days after application of *Trichoderma* only one endophytic colonization was observed in a roots of a seedling from the *Trichoderma* and KlamiC + *Trichoderma* treatments. In the 3rd sampling (3 months), *Trichoderma* colonization of roots reached $1.67 \cdot 10^4$ CFU g^{-1} in the *Trichoderma* alone treatment, and $4.17 \cdot 10^4$ CFU g^{-1} in the KlamiC + *Trichoderma* application, the latter with significant differences from the other treatments. At the 4th sampling (6 months) the same level of colonization was

maintained in the *Trichoderma* treatment, while in the KlamiC + *Trichoderma* colonization decreased to 1.33 · 10^4 CFU g^{-1} (Fig. 3.17).

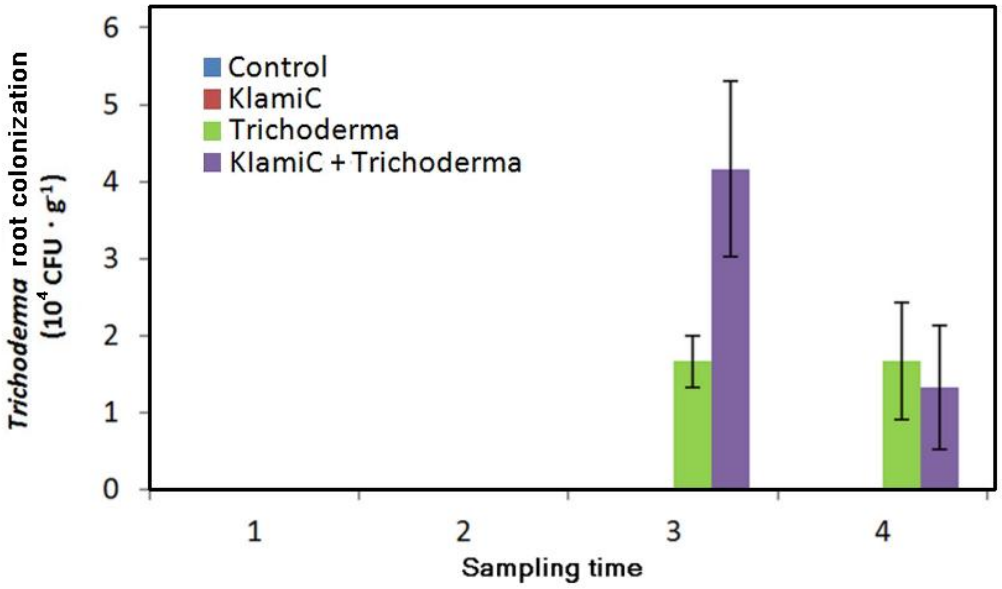


Figure 3.17 *Trichoderma* colonization in roots of Burro CEMSA ¾ genotype. Samplings are: initial (1), and 45 days (2), three months (3) and six months (4) after inoculation.

Despite what has been observed, it is important to consider some facts which could affect the colonization such as the crop ages. The fungus colonizes younger roots, because they are less lignified and emit more exudates, which provides the fungus the nutrients necessary for its development.

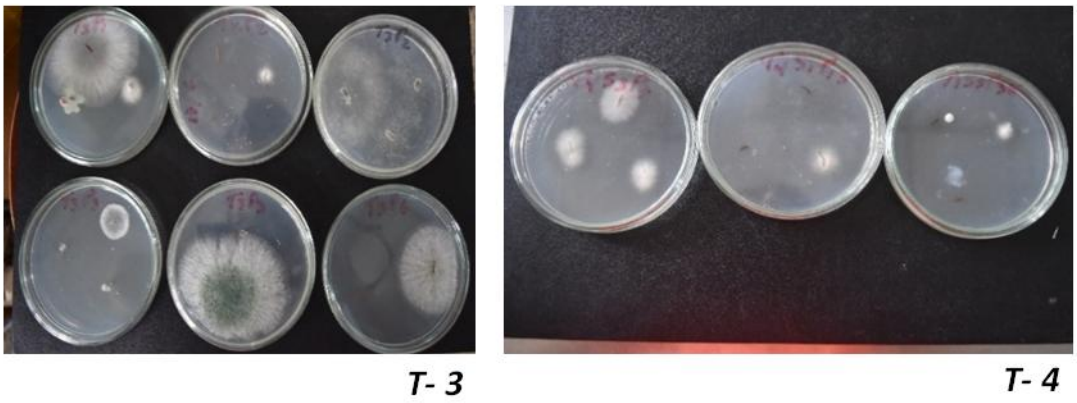


Figure 3.18 Endophytic growth of *T. asperellum* strain Ta.13, from banana roots.

***Pochonia* analysis**

In the second sampling, 45 days after application, the highest populations of *P. chlamydosporia* in soil were obtained in treatments with KlamiC applied (alone and combined with *Trichoderma*), with 6.23 · 10^3 CFU g^{-1} and 4.68 · 10^3 CFU g^{-1}, respectively.

There was a decrease in soil colonization in the third and fourth sampling (3 and six months), likely related to the course of warmer months. In the treatment with KlamiC, soil colonization varied from 6.23 · 10^3 to 4.58 · 10^2 CFU g^{-1}. In the treatment KlamiC + *Trichoderma* it varied from 4.68 · 10^3 to 1.25 · 10^3 CFU g^{-1}. At each time of evaluation, there were no significant differences in colonization in treatments between KlamiC and KlamiC + *Trichoderma* (Fig. 3.19).

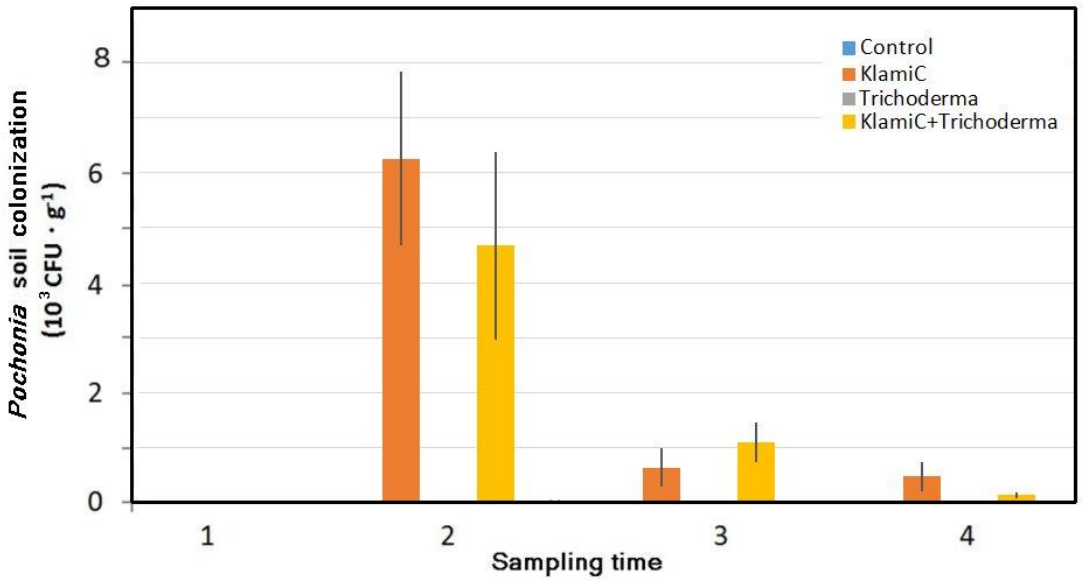


Figure 3.19. *Pochonia* colonization in soil. Burro CEMSA ¾ genotype. Samplings are: initial (1), and 45 days (2), three months (3) and six months (4) after inoculation.

In the roots, the colonization of *P. chlamydosporia* was 4.17 and 4.93 · 10^2 CFU g^{-1} in the treatment with KlamiC and KlamiC + Trichoderma, respectively, in the 2nd sampling, 45 days after application (Table 3.4). In the rest of the evaluation moments, the presence of the fungus in the roots was not detected.

Table 3.4 Colonization of *P. chlamydosporia* on banana roots of *Burro* CEMSA ¾, under field conditions, 45 days after application.

Treatment	Roots colonization (CFU g^{-1})
Control	0.00
KlamiC	4.17
Trichoderma	0.00
KlamiC+Trichoderma	492.5
Esx	82.06
CV	323.77

Roots endophytic colonization by *P. chlamydosporia* in treatment with KlamiC showed the highest value (9.71 %) 45 days after application (sampling 2), corresponding to the moment of evaluation when highest colonization was observed in soil and roots. In the KlamiC + *Trichoderma* treatment the endophytic colonization was verified from the 3rd sampling and increased from 0.69 to 2.08% (Table 3.5).

Table 3.5. Endophytic colonization by *P. chlamydosporia* of banana roots of *Burro* CEMSA ¾ under field conditions, at different evaluation times for 6 months (see Fig. 3.19 for sampling times).

Treatments	Samplings			
	1	2	3	4
KlamiC	0.0	9.71	0.69	2.77
KlamiC+*Trichoderma*	0.0	0.0	0.69	2.08

The colonization of soil by *P. chlamydosporia* was $6.25 \cdot 10^3$ CFU.g^{-1} (T2) and $4.67 \cdot 10^3$ CFU.g^{-1} (T4). Roots colonization was 4.17 CFU $\cdot$ g^{-1} (T2) and $4.92 \cdot 10^2$ CFU $\cdot$ g^{-1} (T4). Maximum endophytic colonization observed from the root fragments was 9.7% (Table 3.5).

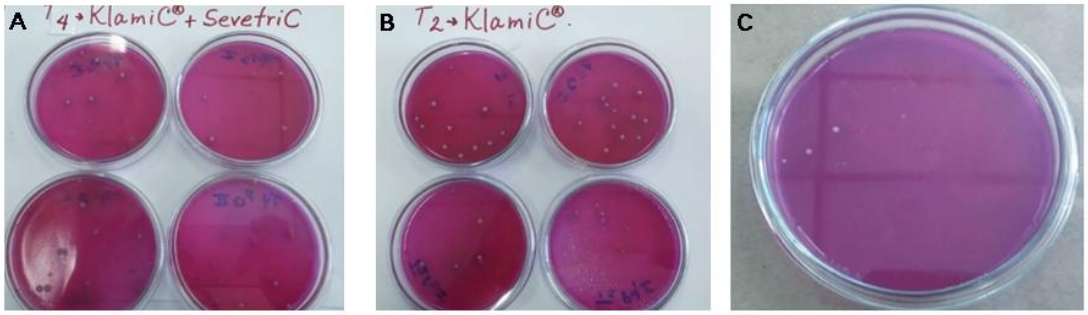


Figure 3.20 Colonization by *P. chlamydosporia* of soil (A, B) and banana roots (C) at 45 days.

Nematological analyses

In general, up to the present evaluation, PPN populations in roots and soil increased in all treatments, following the crop development and the emission of new roots. No treatment managed to reduce populations, since they showed the same tendency as the control without EBCAs.

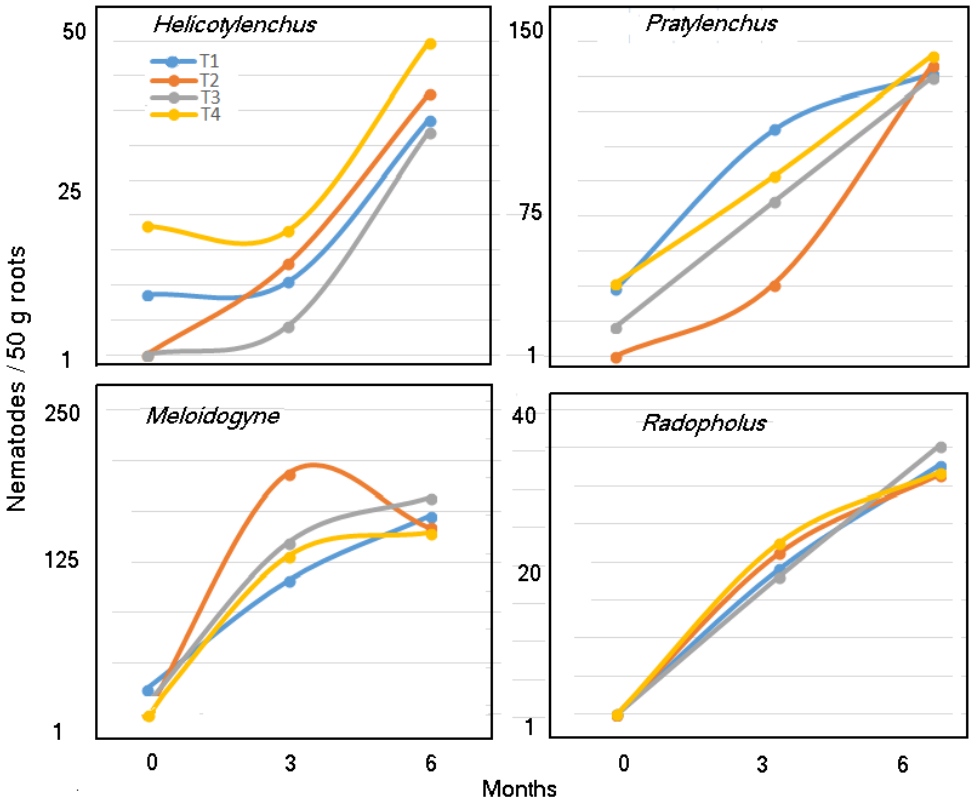


Figure 3.21 Nematode density changes at three months sampling intervals, from 50 g roots of *Burro* CEMSA3 in production areas from Nueva Paz Municipality, Mayabeque, Cuba. Treatments: T1 = Control, T2= KlamiC, T3= SevetriC; T4 = KlamiC + Sevetric.

These results coincide with what was reported in a study carried out by Vargas *et al.* (2015), during 20 months, showing that the application of *Trichoderma* and *Purpureocillum lilacinus*, at a dose of 10^9 CFU / ml (100 ml per plant) + 0.05 % Tween 20, no significantly decreased the number of nematodes. However, the plants treated with these fungi showed greater cluster weight ($p = 0.0375$) and circumference ($p = 0.0402$) of the mother plant, compared to those without application.

From the results of these first evaluations derives the need to assess higher application doses and design a field experiment with other alternatives and combinations, for example:

- Application of EBCAs from the hardening phase in the biofactory + mycorrhizae
- Use in that phase of biochar + organic fertilizers + oils or plant material with effect *vs* nematodes in banana / plantain + mycorrhizae
- In case of use of corms for planting, test heat treatments and ten essentials oils
- Soil preparation + solarization + bioinfection
- Application of EBCAs in the transplant hole
- Application of biochar "loaded" in the hole
- Use of treatments with cover plants with nematicidal effect
- Use of growth promoting bacteria

3.3. Evaluation of germplasm for resistance to PPN in the field (Univ EARTH, Costa Rica)

1. Objective

To evaluate four banana cultivars and their performance vs PPN as well as to evaluate the growth of the cultivar under the field conditions.

2. Methodology

Four cultivars of banana were tested for their performance towards *R. similis* in the field conditions of the Musa project at EARTH University. The cultivars evaluated were Baby Banana (AA), Grande Naine (AAA), Red Macabu (AAA) and Plantain (AAB). The four cultivars were planted on February 2019 in a field infested with PPN and three bi-monthly samplings were conducted, in order to test the performance of the cultivars. The extraction were performed using roots maceration, collecting the nematodes in a sieve of 500 mesh, for identification and countings. In addition to the population dynamics data, plant growth parameter such as height and girth of the pseudostem were assessed every month. This study is part of a B. Sc. Thesis of two students at EARTH University.

3. Results

Four cultivars of banana were tested for their performance towards *R. similis* and other PPN in field conditions. Baby Banana (AA), Grande Naine (AAA), Red Macabu (AAA) and Plantain (AAB) were planted in fields infested with *R. similis*, with 3 evaluations every 2 months. The results indicate that the population of *R. similis* was significantly higher on plantain and Grande Naine, in comparison with Red Macabu and Baby Banana (Table 4.1.)

Table 3.6 Comparison of mean PPN density (nematodes / 100 g roots) on four banana cultivars during three bi-monthly samplings. *

Cultivar	*R. similis*	*M. incognita*	*H. multicinctus*	FNT
Baby Banana	6250 a	7500 b	1083 a	14 833 ab
Red Macabú	3750 a	2417 a	1167 a	7333 a
Gran Enano	18 833 b	43 33 ab	1083 a	24 250 b
Plátano	40 000 c	15 500 c	2250 a	57 750 c

* Means with a letter in common are not significatively different according to Duncan's multiple range test (p>0,05).

Concerning the the dynamic of population of *R. similis* during the three sampling the number of nematodes increased during time on plantain, differing from the other cultivars in all samplings. On the other hand, cultivar Red Macabu registered the lowest number of *R. similis* in all sampling (Fig. 3.22).

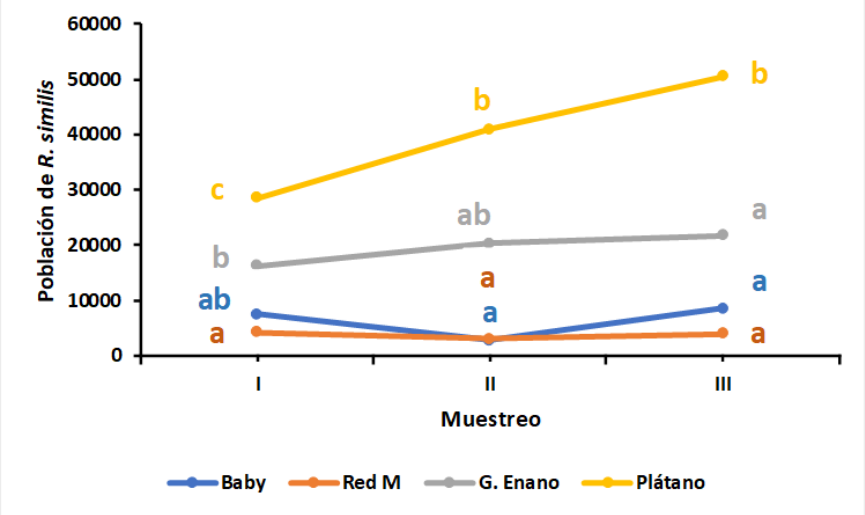


Figure 3.22 Population dynamics of *R. similis* during the three field samplings.

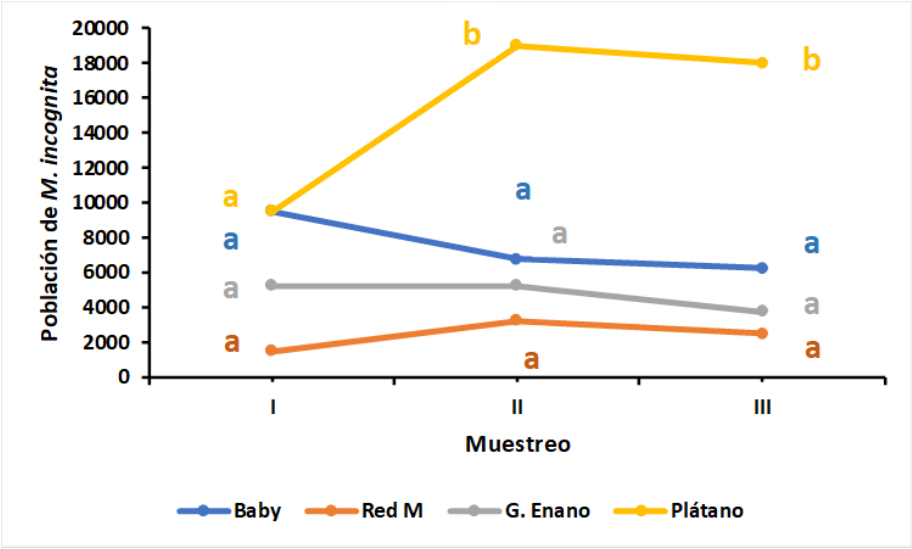


Figure 3.23 Population dynamics of *M. incognita* during the three field samplings.

Concerning the population dynamics of *M. incognita* the most susceptible cultivar was also the plantain, detecting significant differences in comparison to the other cultivars, with the Red Macabu as most resistant (Fig. 3.23).

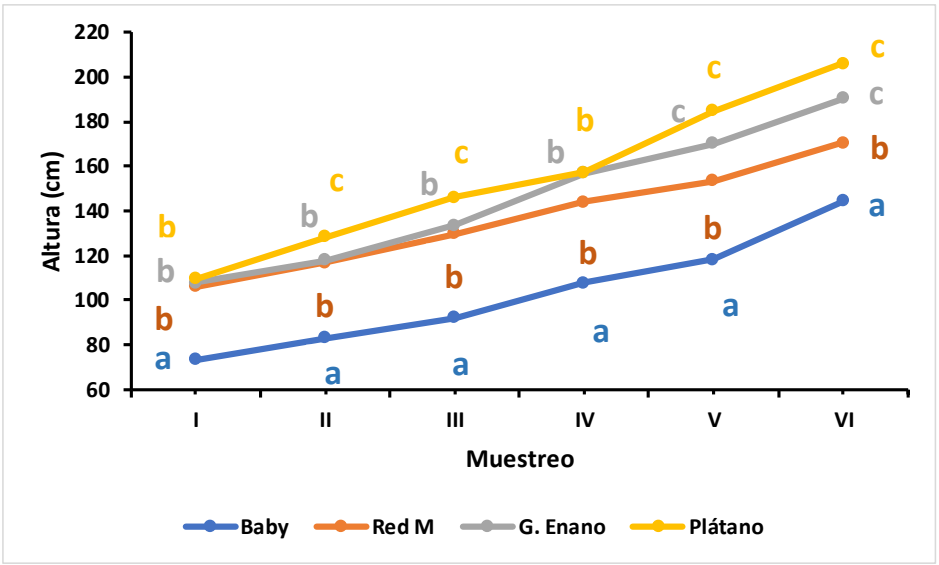


Figure 3.24 Effect of banana cultivars on the plant height during six months.

Data indicate that plantain registered the highest value on height in all the sampling with Baby Banana and Red Macabu as lowest (Fig. 3.24) At the end of the six months sampling plantain still registered the highest value as well as Grande Naine and baby banana with Red Macau as lowest.

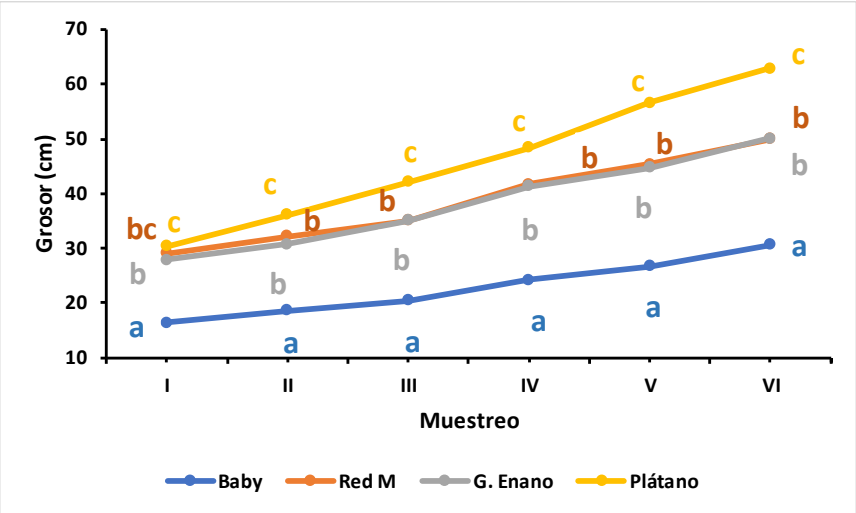


Figure 3.25 Effect of banana cultivars on the pseudostem girth during six months.

Results showed significant differences also for the pseudostem girth of the cultivars tested, with plantain showing the highest values and baby banana the lowest (Fig. 3.25).

4. Conclusions

The most important nematode affecting all the cultivars were *R. similis*, followed by *Meloidogyne incognita* and *Helicotylenchus multicinctus*.

The most resistant cultivar to *R. similis* under the field conditions was Red Macabu and Baby Banana registering significant differences, in comparison with Grande Naine and Plantain

The most susceptible cultivar to *R. similis* was plantain followed by Grande Naine, reaching population higher than 10000 *R. similis* / 100 g roots, largely overpassing its damage threshold.

The highest values on pseudostem girth as well as for plant height were registered in plantain, and the lowest in Baby Banana.

3.4.- Biological control of burrowing nematode *Radopholus similis* using endophytes (Univ. EARTH, Costa Rica)

Objective

To evaluate the effect of four strains of *Trichoderma asperellum* and two commercial products (Soilset and Trichomax) on penetration rate as well as final population of *R. similis* in greenhouse conditions.

Methodology

Banana cultivar

Tissue cultured plants of the cv Grande Naine in the phase 4 of a micropropagation system were used for both experiments: penetration and final population of *R. similis.*

Nematodes

Monoxenic cultures of *R. similis* were reproduced on carrot discs over 6 six weeks. The nematode suspension was collected and 1000 nematodes were inoculated in single plants, in both experiments.

Endophytes

Four strains of *T. asperellum* as well as two commercial products, Soilset and Trichomax, were tested for biocontrol activity on the *R. similis* (burrowing nematode) under greenhouse conditions (Fig. 3.26)

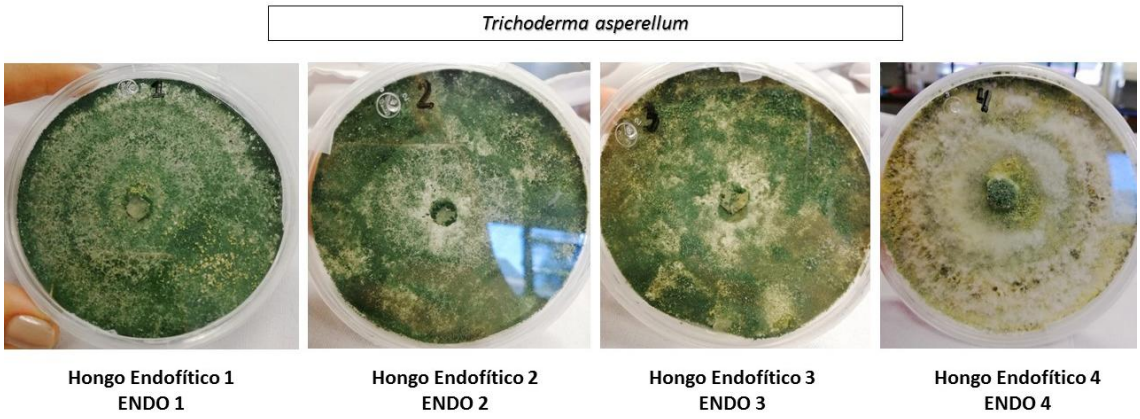


Figure 3.26 Four strains of Trichoderma asperellum growing in PDA media were used for protecting plants of Grande Naine for their biocontrol activity to R. similis

Protection of plants with endophytes

Tissue culture plants of Grande Naine in stage 4 of micropropagation were inoculated with four strains of *Trichoderma asperellum* as well as two commercial products, Soil set and Trichomax (commercial products based on T. asperellum from Biotor) were tested for penetration and reproduction rate of *R. similis* in two experiments, in

greenhouse conditions. The inoculation of the fungus was by dipping the plants for 5 minutes in a spore suspension of 10^7 cfu / ml (Fig. 3.27).

Figure 3.27 Inoculation of tissue cultured plants by dipping in a spore suspension of four strains of *T. asperellum* and two commercial products.

Penetration and reproduction experiment

The methodology used for the assay of penetration and reproduction was the same. Each plants was inoculated with 1000 *R. similis*. For the penetration experiment, the plants were scarified 10 days after inoculation with nematodes. For the reproduction test, 60 days after inoculation with *R. similis*.

Conclusion

All the endophytes used reduced the penetration of *R. similis* in the root system. The endophyte *Trichoderma asperllum* Endo 4 reduced over 97% the penetration of *R. similis* in comparison with control. All endophytes used as well as Soilset and Trichomas reduced significantly the final population of *R. similis* with Endo 4 as the most effective. Although there was no difference in plant weights among treatments and control, Trichomas obtained the highest values.

4. Black Weevil management

4.1. Evaluation of two types of traps for monitoring and control of weevils in banana fields (CENSA, Cuba)

Evaluation of two types of traps for monitoring and control of weevils in banana fields.

Materials and methods

The study was conducted in areas of Finca Santa Elena 2, belonging to the Agricultural Company of Nueva Paz, geographical references in the Mayabeque province. The

predominant soil type is Ferralitic red. The banana varieties used in the experiment were FHIA 25 and Yangambi, with 10 years of planting both of them.

Two treatments (types of traps) were established with five replicates each, for a total of 10 traps per hectare, previously identified, using a randomized complete block design. The traps were reviewed every 7 days and renewed every 28 days. The investigation lasted 56 days, beginning on March 8 and ending on May 22, 2019.

Direct observation in the field was used as a sampling method, the capture was performed manually and the number of adults of *Cosmopolites sordidus* (BW) and *Metamasius hemipterus* was quantified for each trap. The traps were made using fractions of the pseudo total of plants that were already cooked.

Modified disk trap: made by cutting the pseudostem at height of 15 cm, then cuts were made in the form of grids and covered with leaves, until the time of observation.

Wedge-type trap: made by cutting fractions of the wedge-shaped pseudostem, leaves were placed between one fraction and another to facilitate the entry of the insect.

The population density in each type of trap and variety was compared using a Bifactorial Analysis of Variance; the means were contrasted by the Duncan multiple range comparison test for a 0.05 significance level. The statistical package InfoStat version 2016 was used.

Modified disk trap (TDM)

Wedge-type trap (TTC)

Interspecific competition was determined with the data obtained from traps. The Lotka – Volterra model of interspecific competition was used to describe the competitive interaction between both species, where:

$$\frac{dN1}{dt} = r1.N1(1 - \frac{N1+W1.N2}{K1}) \qquad (1)$$

$$\frac{dN2}{dt} = r2.N2(1 - \frac{W2.N1+N2}{K2}) \qquad (2)$$

K1: maximum population density or carrying capacity of *C. sordidus*

K2: maximum population density or carrying capacity of *M. hemipterus*

r1: intrinsic rate of increase of *C. sordidus* in these specific conditions

r2: intrinsic rate of increase of *M. hemipterus* in these specific conditions

w1: competition coefficient of *C. sordidus* (represents the per capita effect of this species on *M. hemipterus*)

w2: competition coefficient of *M. hemipterus* (represents the per capita effect of this species on *C. sordidus*)

In our study K1, K2, r1, r2 were determined using the logistic model

$$Y = \frac{K}{1 + be^{-rx}}$$

W1 = W2 = 0.5 assuming that both insects can be in the field in equal proportion.

Subsequently, the isoclines graph was made

$$Y_1 = \frac{-1}{W_1}X + \frac{K_1}{W_1} \quad , Y_2 = -w_2X + K_2$$

Results

Statistical analysis showed significant differences between the types of traps and the varieties evaluated. The TDM in the FHIA 25 variety captured the greatest number of *M. hemipterus*, followed by the TTC in the Yangambi variety. In all the variants the quantity of BW was always higher than *M. hemipterus*.

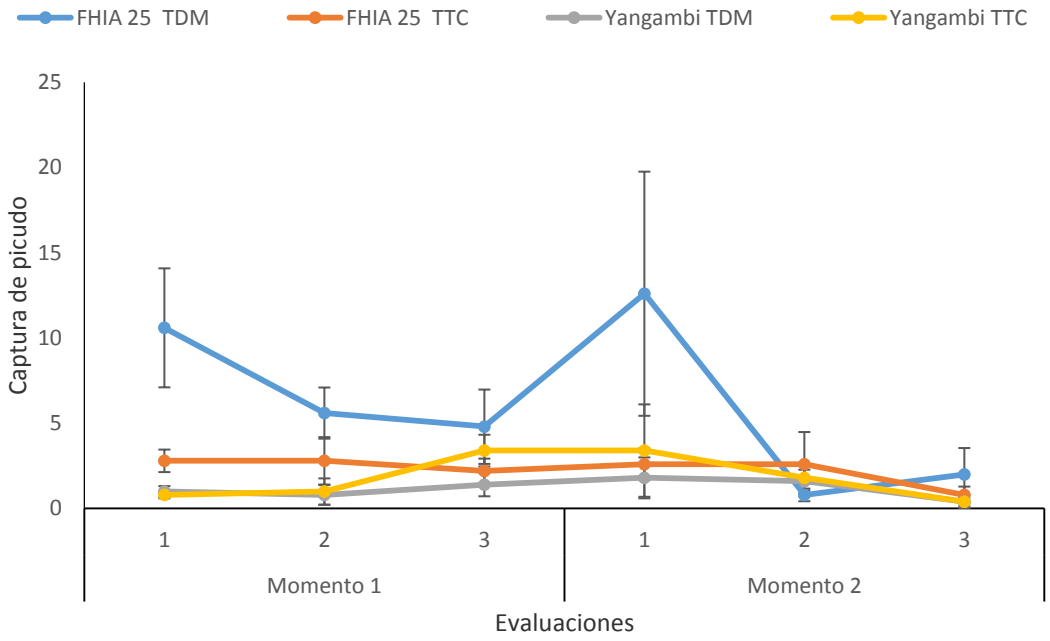
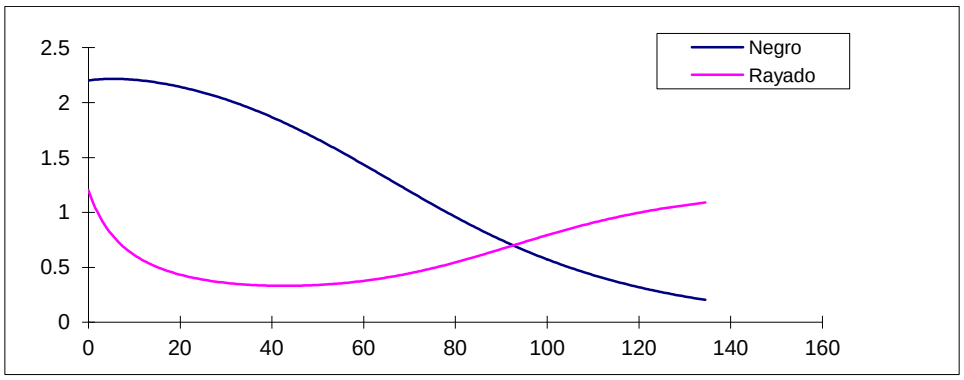
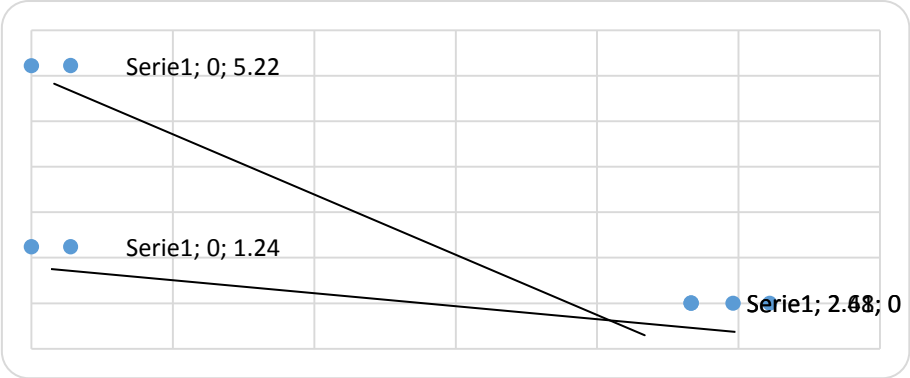


Figure 4.1 Comparison in the capture of BW and *M. hemipterus* with the two types of traps in the FHIA 25 and Yangambi varieties.

Interspecific Competition. If isocyclines do not cross, one species is excluded by another. In the FHIA 25 variety, if there is interspecific competition, one species displaces the other.

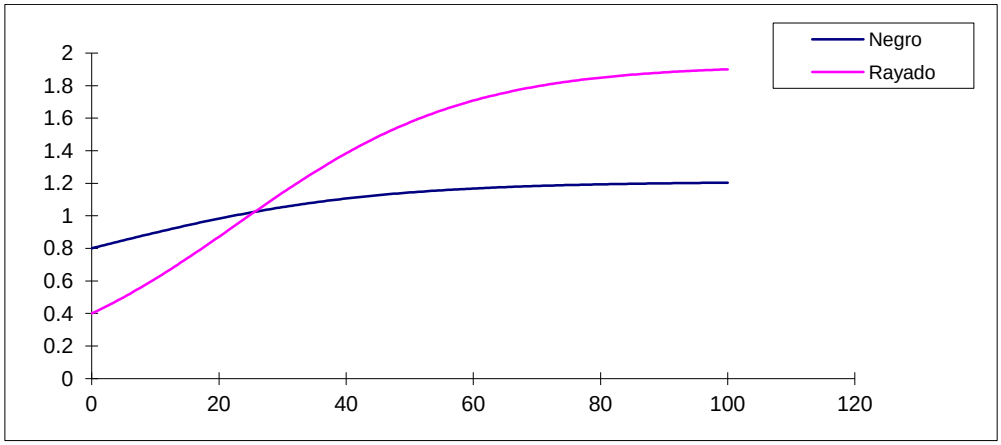
FHIA 25

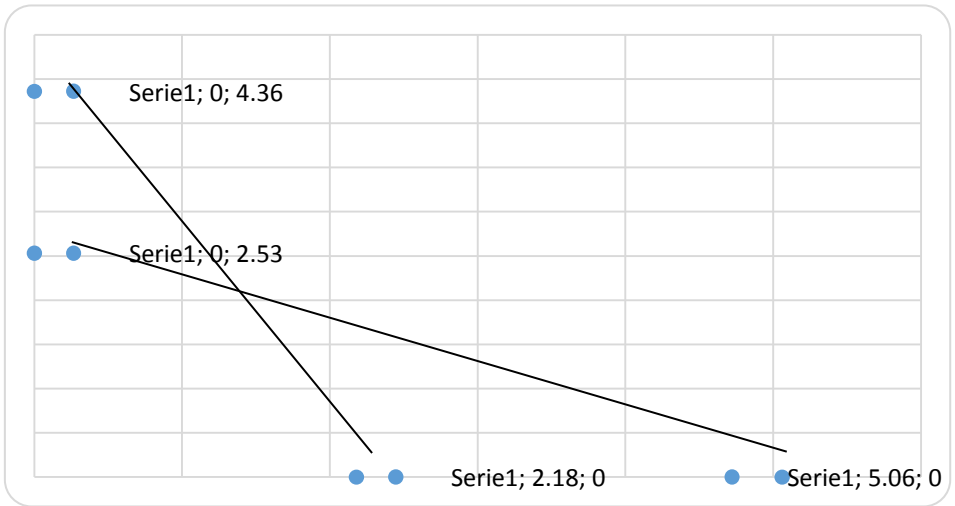




Yangambi

If the isoclines cross, then there is a balance in which the species coexist. In the trap TTC Yangambi genotype both species coexist, and there is a time when they are in equal density.





4.2.- Use of natural substances with repellent effect for BW (Univ. Alicante and COPLACA, Spain)

The main method of BW control is the capture of adults using traps in which aggregation pheromones are placed, which simulate the "chemical call" that indicates the presence of food to the insect. Taking advantage of this sensitivity of the BW to chemical substances, a test is carried out in which a pheromone trap design is placed in two plots of a banana plantation in Tenerife (Canary Islands), in some of which a repellent substance is also incorporated. This was chosen out of the two that in the laboratory demonstrated greater efficacy in olfactometer tests, to determine their effectiveness in the field by masking the attractive signal emitted by the pheromone. Aim was proposing a possible use of them as repellents in new plantations or for the perimeter protection of plots, in which the presence of this pest has not been detected.

Field tests

To validate the activity on the BW mobility the field, some substances from those selected were tested in commercial banana fields in Tenerife. Two consecutive tests were conducted in the same fields, following the same experimental scheme (detailed below). The first test was conducted from April 23, 2019 until June 4, 2019. The second test was conducted from June 4, 2019 until July 16, 2019. In this second test, unlike of the first, half of the tests were replaced three weeks after the start of the experiment to the dispensers, both the pheromone and the VOCs.

Compounds C1 and C2 are the only repellent candidates tested in the field, in order to be compared with the data provided by J. Jalinas (2016), which evaluated the repellent action of these same compounds in the lab against another Curculionidae, the red palm weevil. These compounds, tested in the laboratory, have been in the field to ascertain whether these substances modify the behaviour of *C. sordidus* even in agronomical canditions. Two banana plantations were identified to conduct these tests in the Island of Tenerife (Canary Islands). These two fields, a larger one (0.35 ha) (28 ° 23 '39.72' 'N - 16 ° 39' 18.97 '' W; Z = 68 m above s.l.) (BF =Big Field) and a smaller one (0.06 ha) (28 ° 23 '40.14' 'N - 16 ° 39' 16.55 '' W; Z = 65 m above s.l.) (SF = Small Field) are adjacent (Fig. 4.2).

Twenty-nine traps were placed (Table 4.1) in a randomized block (Fig. 4.3). Of these, 10 contained BW pheromone only (Fig. 12). Further, another 10, in addition to the pheromone, each contained a C1 dispenser (2 g of silica gel and 0.5 ml of C1) (Fig. 7.3). The remaining, another 9 traps, in addition to the pheromone, each contained a C2 dispenser (2 g of silica gel and 0.5 ml of C2) (Fig. 4.4).

Figure 22 Banana fields selected for the VOCs assays, in Tenerife (Canary Islands Spain)

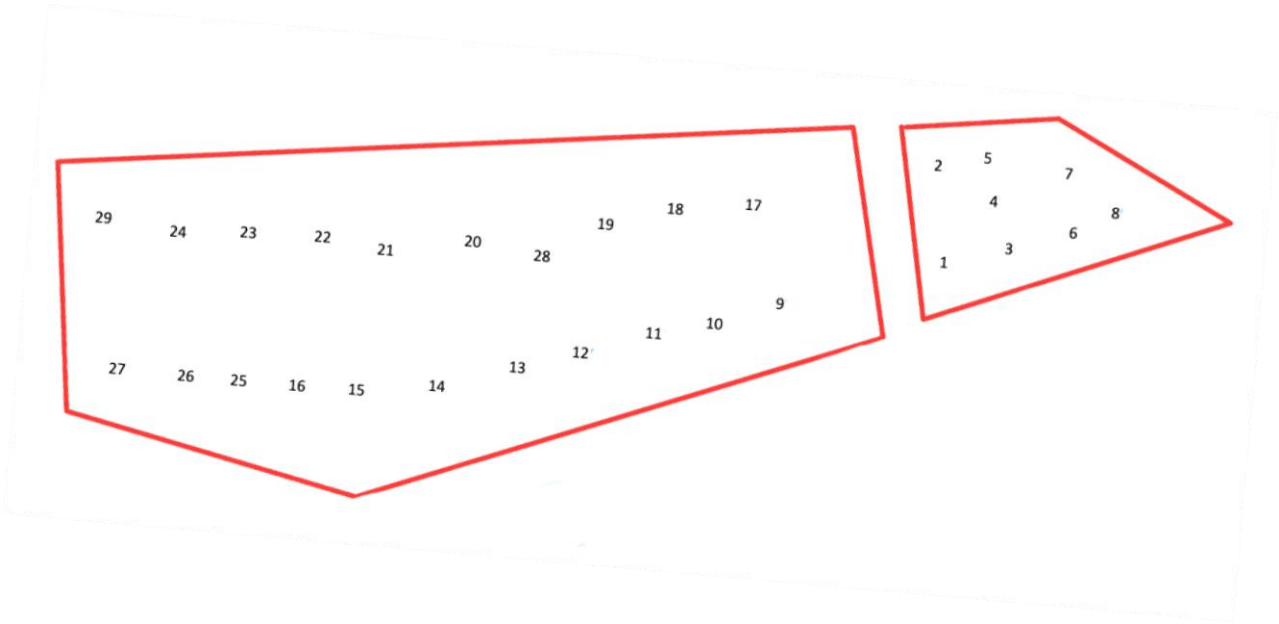


Figure 4.3 Arrangement of the different BW traps between the two fields, according to the randomized block

Figure 4.43 BW traps used in the field: a) ECOSordidina60 dispenser; b) miracloth dispenser of compounds C1 and C2; c) base of the trap activated with ECOSordidina60 and C1/C2; d) trap positioned in the field

Table 4.1 Treatments and position of the traps. *F* = pheromone; *FC1* = pheromone + C1; *FC2* = pheromone + C2.

N. progressive	Content	Coordinates	N. progressive	Content	Coordinates
1	F	28°23'39.9"N 16°39'16.6"W	16	FC1	28°23'40.1"N 16°39'18.0"W
2	FC1	28°23'40.4"N 16°39'16.9"W	17	FC2	28°23'40.0"N 16°39'18.4"W
3	FC2	28°23'40.0"N 16°39'16.3"W	18	F	28°23'39.9"N 16°39'19.0"W
4	FC1	28°23'40.3"N 16°39'16.5"W	19	FC2	28°23'39.9"N 16°39'19.4"W
5	F	28°23'40.1"N 16°39'16.1"W	20	FC1	28°23'39.8"N 16°39'19.7"W
6	F	28°23'40.4"N 16°39'16.2"W	21	F	28°23'39.9"N 16°39'20.0"W
7	FC1	28°23'40.2"N 16°39'16.0"W	22	FC2	28°23'39.8"N 16°39'20.2"W
8	FC2	28°23'39.7"N 16°39'17.4"W	23	FC1	28°23'39.1"N 16°39'19.8"W
9	F	28°23'39.8"N 16°39'17.8"W	24	F	28°23'39.2"N 16°39'20.1"W
10	FC1	28°23'39.4"N 16°39'18.1"W	25	F	28°23'39.2"N 16°39'20.4"W
11	F	28°23'39.3"N 16°39'18.4"W	26	FC1	28°23'39.9"N 16°39'18.7"W
12	FC2	28°23'39.1"N 16°39'19.1"W	27	FC2	28°23'40.5"N 16°39'16.6"W
13	FC1	28°23'39.1"N 16°39'19.3"W	28	FC1	28°23'39.3"N 16°39'18.7"W
14	F	28°23'39.1"N 16°39'19.6"W	29	FC2	28°23'39.8"N 16°39'20.6"W
15	FC2	28°23'40.2"N 16°39'17.6"W			

The count of the BW captured in the traps were scored weekly and removed.

On the collected data, histograms have been created with the accumulated value of the catches per treatment each day of both tests. Furthermore, the data were analyzed with the ANOVA tests using the Rstudio statistical software.

Results

Effect of VOCs on BWs under field conditions

Captures of BWs in traps with VOCs

Compared to the control (F), in the first test it can be inferred that in the C1 (FC1) catches were fluctuating, showing a lower number of catches only in the second week (blue bar) (Fig. 4.5). In the rest of the surveys, the captures of this treatment are higher than the control.

As regards C2 (FC2), the catches were always lower than the control, except for the third catch (green bar) (Fig. 4.6).

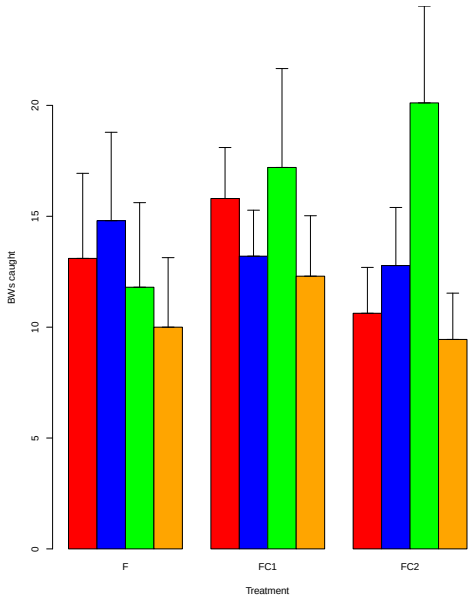


Figure 4.5 First test: trend of the catches for the treatments in the different weeks of the test. In red the catches are represented from 16 to 23 April 2019; in blue the catches from 24 to 30 April 2019; in green the catches from 1 to 15 May 2019; in orange the catches obtained from 16 to 22 May 2019.

In the second trial, conducted from 4 June to 16 July 2019, the catches were relatively lower than the previous test. This total decrease in catches is attributable to the rise in temperatures in the period in which this second test was conducted. High temperatures reduce the mobility of the BW in the field.

Compared to the control, FC1 showed lower catches in the first two weeks of the test and the last 3. Only in the third week, there was a higher capture than the control (Fig. 4.7). FC2, compared to the control, showed only lower catches compared to the control from the third to the fifth week of testing (Fig. 4.8).

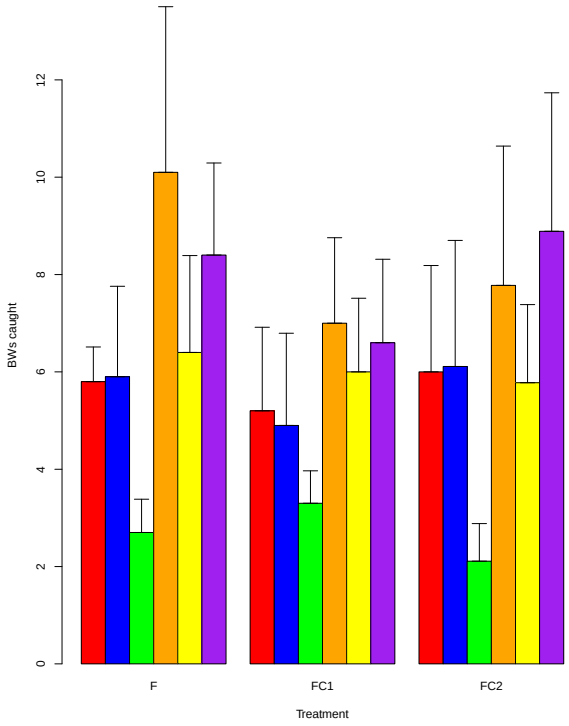


Figure 4 Second test: trend of the catches for the treatments in the different weeks of the test. In red the catches are represented from 4 to 11 June 2019; in blue the catches from 12 to 18 June 2019; in green the catches from 19 to 25 June 2019; in orange the catches from 26 June to 2 July 2019; in yellow from 3 to 9 July 2019; while in violation from 10 to 16 July 2019

In this test, as previously mentioned, the stimuli of half of the FC1 and FC2 treatments were renewed in the third week. As regards FC1, the renewed traps have captured more (108 BWs) than those in which the stimuli were not changed (87 BWs). Instead, FC2 showed a different trend. The renewed traps have captured less (94 BWs) than those to which the stimuli have not been renewed (108 BWs).

The catches of both the first and second trials were compared with the trends of average temperature, average HR and the precipitations of the periods concerned to understand the progress and mobility of the BW population.

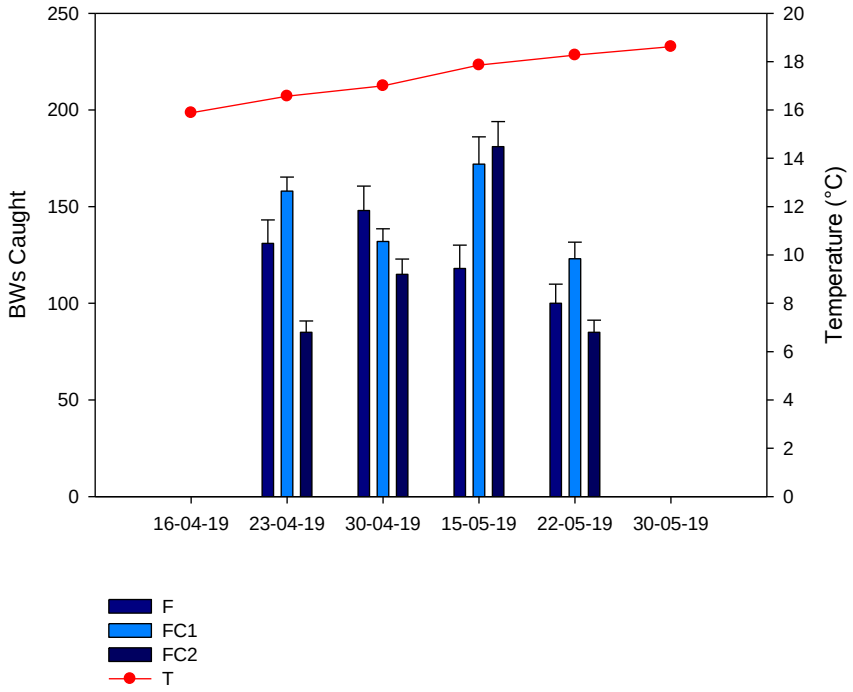


Figure 4.7 Comparison of data relating to the catches of the first test and the average weekly temperature.

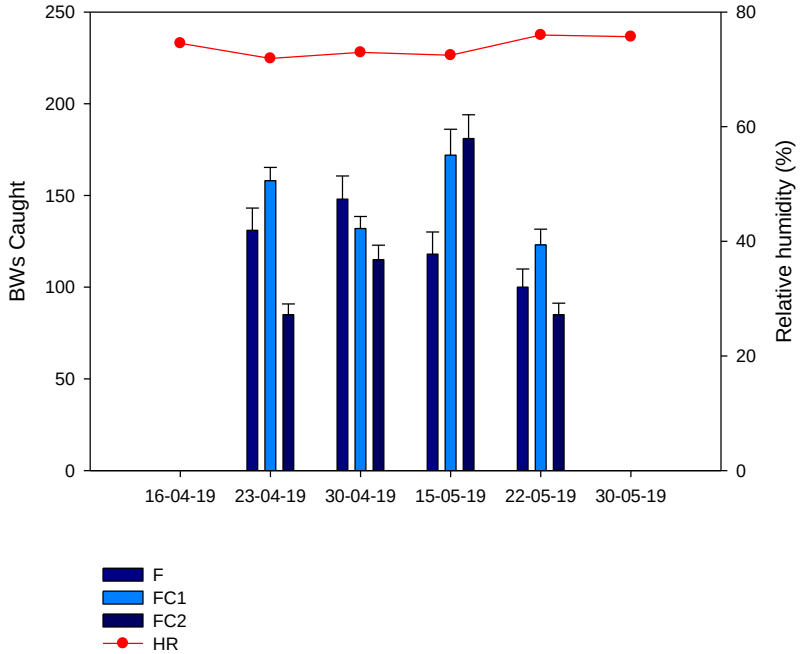


Figure 4.8 Comparison of the data relating to the catches of the first test and the average weekly relative humidity.

In the period of the first trial, the weekly T average has gradually grown. In Fig. 4.8 it is possible to see how, in the last week, the catches have decreased for all treatments together with a slight increase in average temperature. HR remained almost stable, between 73 and 75%. Therefore, the decrease found cannot in any way be ascribed to the recorded hygrometric trend. Even in the case of precipitation, there seems to be no link with the decrease in catches. In fact, in the considered period, the precipitations were not almost wholly absent (Fig. 4.9).

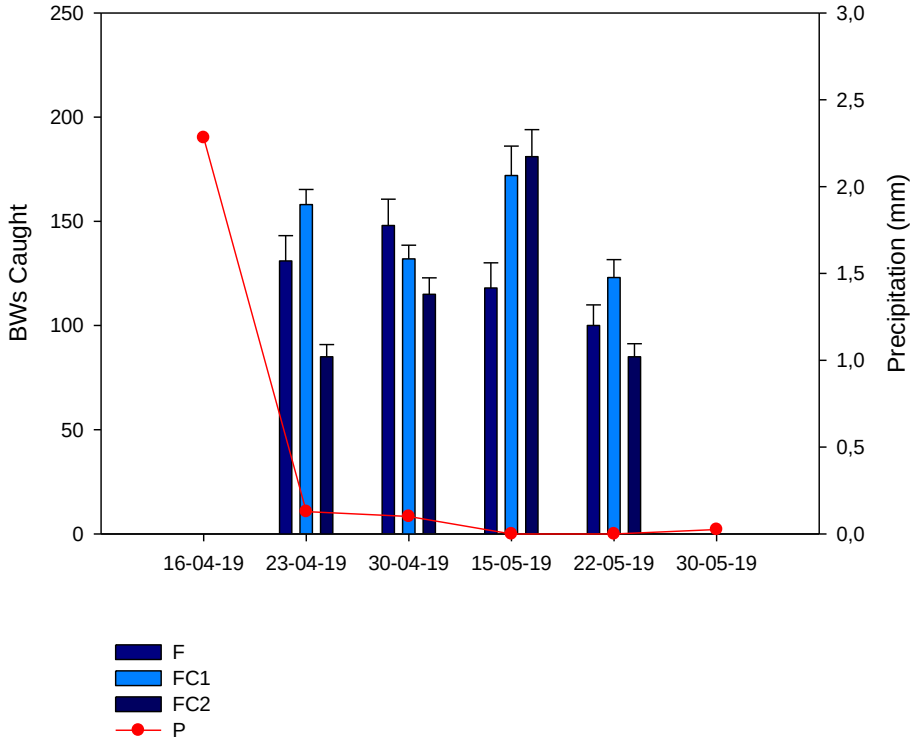


Figure 4.9 Comparison of the data relating to the catches of the first test and the average weekly precipitation.

In the second trial, the recorded weekly average Ts showed an average progressive increase in the current season (Fig. 4.10). The catches during this period were lower than in the previous test period. The most evident drop in catches occurred in the week between 19 and 25 June 2019. At the same time, there was also a considerable average HR and rainfall (1.2 mm) (Fig. 4.11 and 4.12), which could have affected the BW ethology, concerning mobility in the field.

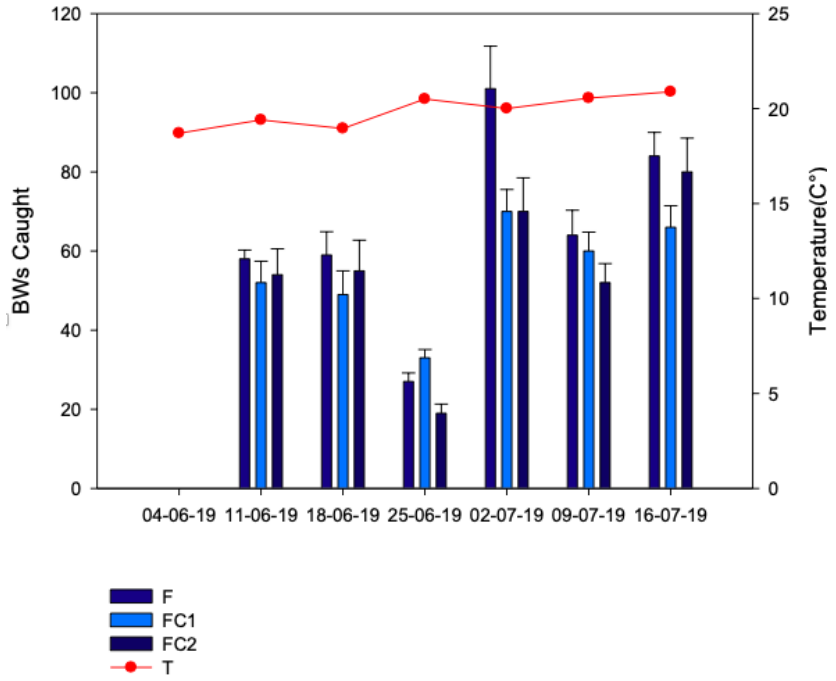


Figure 4.10 Comparison of data relating to the catches of the second test and the average weekly temperature.

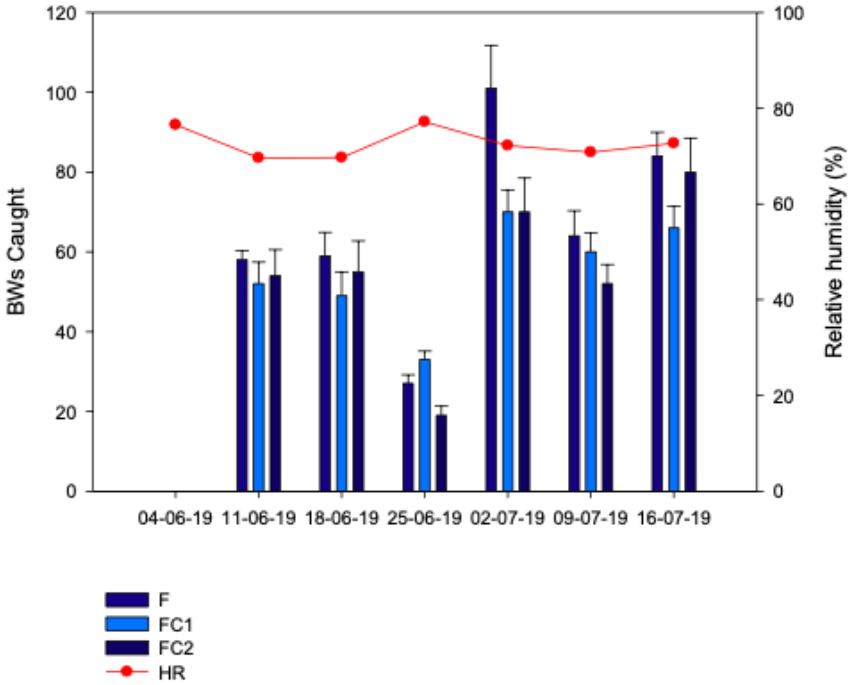


Figure 4.11 Comparison of data relating to the catches of the second test and the average weekly relative humidity

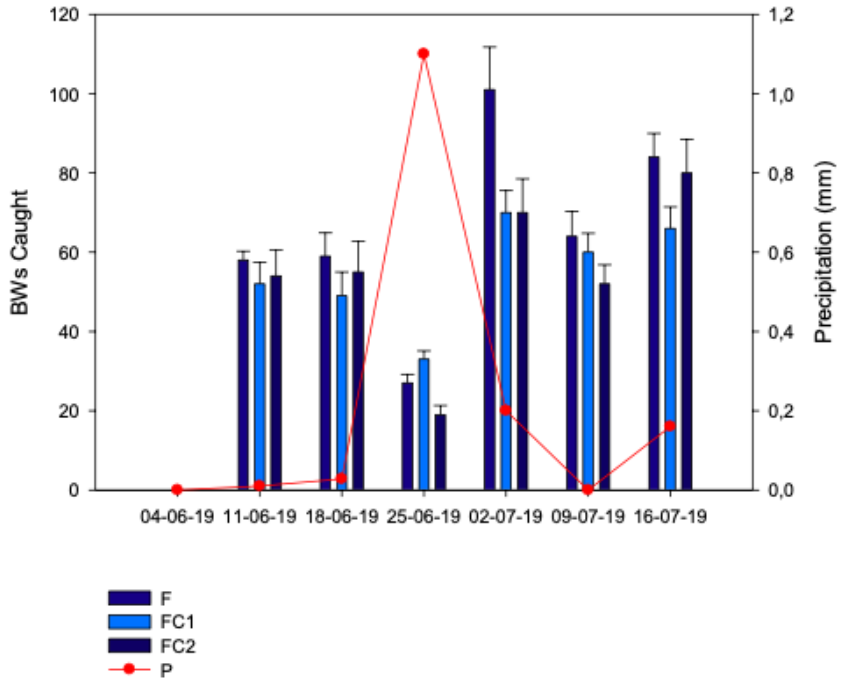


Figure 4.12 Comparison of data relating to the catches of the second test and the average weekly precipitation.

3.3.2 Statistical analysis

The catches found in the first test do not appear to be conditioned by the stimuli with which the traps are activated. From the multifactorial ANOVA analysis, it also emerged that the recorded catches do not seem to be related to the time variable as the stimuli ($p\text{-}value_{VOCs}$ = 0,625; $p\text{-}value_{weeks}$ = 0,226; $p\text{-}value_{VOCs:weeks}$ = 0,658).

As for the catches found in the second test, they also do not seem to be influenced by the stimuli with which the traps were loaded. However, these captures seem to be influenced by the time taken for the test ($p\text{-}value_{VOCs}$ = 0,63568; $p\text{-}value_{weeks}$ = 0,00941; $p\text{-}value_{VOCs:weeks}$ = 0,99735).

3.3.3 Heat maps of BWs field distribution

Data of the catches collected for each trap, it was possible to generate heat maps to monitor the mobility of *C. sordidus* under the conditions to which it was subjected in the field.

In the temporal sequence of the heat maps generated by the captures of the first trial and shown in Fig. 4.13, it can see how the specimens moved during the experiment.

Initially, it can be seen that in the SF (small field) there is only one focus (trap 5-F), while in the BF (big field) there are two well-marked foci (traps 23-FC1 and 11-F).

Following the temporal sequence, it is possible to observe how, over the weeks, the population of BWs present in the field has moved. The population gradually shifted, focusing more on the lower part of the BF. It can be seen from the colouration shown by the capture points on the May 22nd heat map.

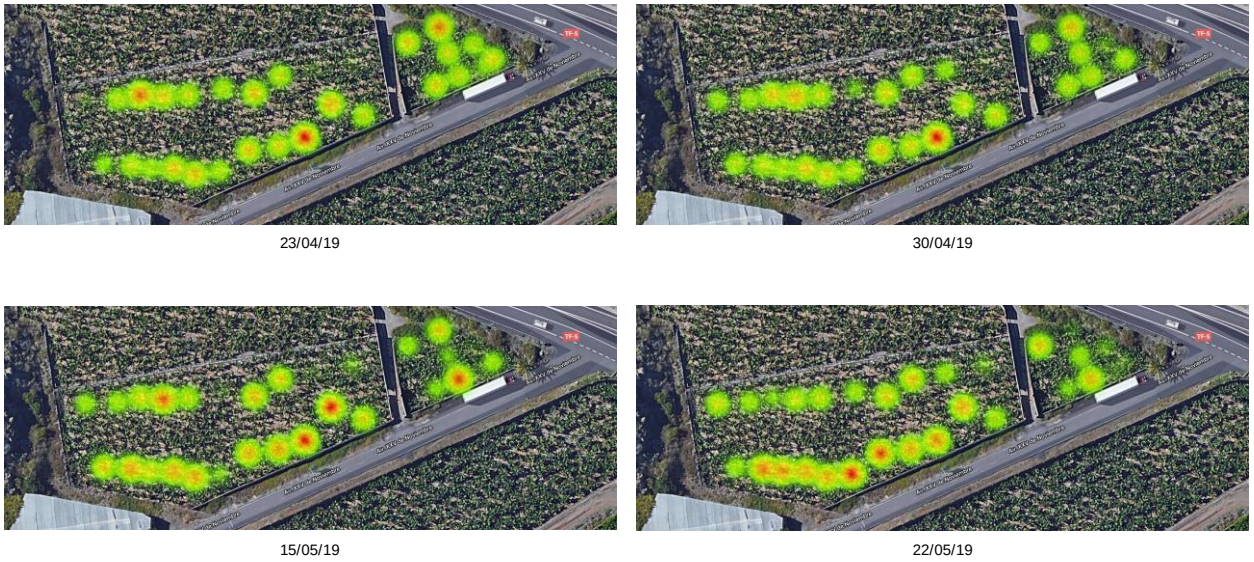


Figure 4.13 Weekly Heat Maps of the first test conducted in the field.

With the accumulated data of all catches per trap, it was possible to generate an overall heat map (HM) of the test (Fig. 4.14). In this HM, it is possible to see a single outbreak that is nothing but the trap that has recorded the most catches. This trap is the 11, loaded only with the pheromone.

Figure 4.14 Total HM, cumulative catches of the first test conducted.

From the HMs obtained with the weekly catches of the second test, they are shown in Fig. 4.15.

After three weeks, the data from the June 11th sampling show how the situation has changed. On this date, it is possible to note that in the SF there is an outbreak in correspondence of the trap 3 (FC2); while in the BF there is only one outbreak in correspondence of the trap 23 (FC1).

After two weeks, on June 22nd, it can be seen that the resident population has moved more towards the northern part of the BF. In this portion of the plantation, several hot foci are observed.

Over time, the population has shown an increasingly intense polarization towards the north-west sector of the BF. On July 16th, we can see how the only hot spots are concentrated only in this area of the experimental field.

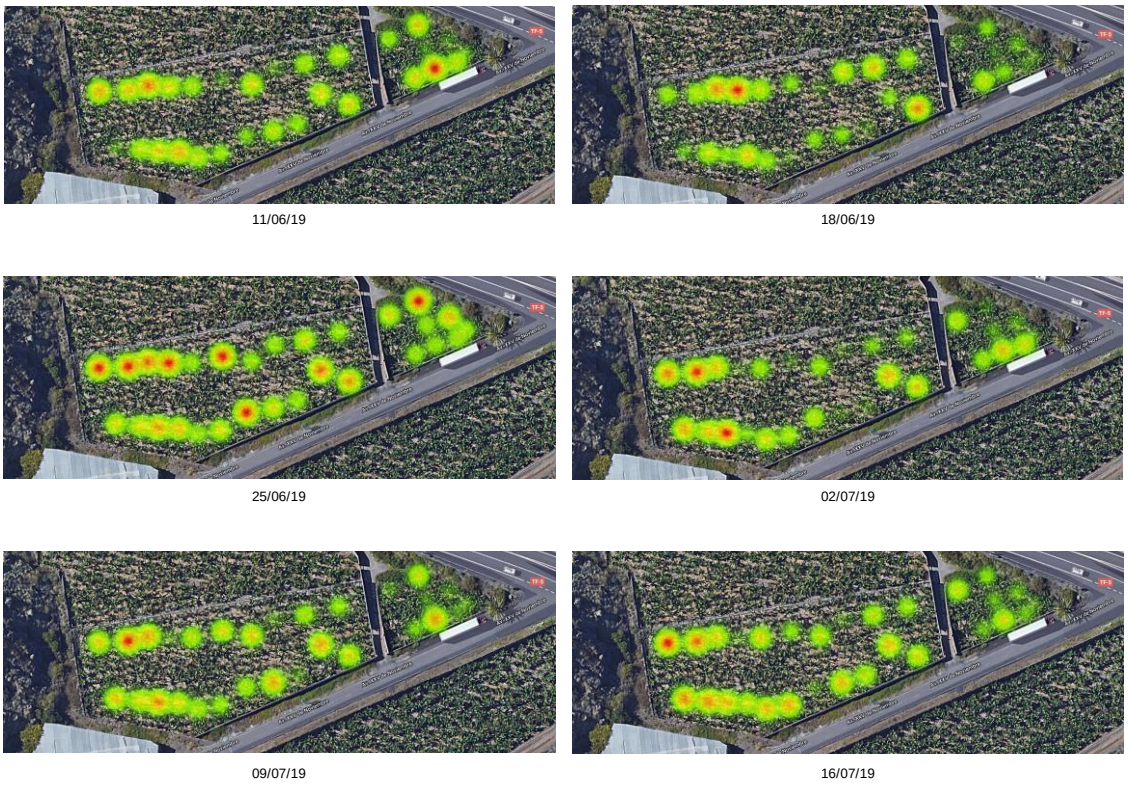


Figure 4.15 Weekly HMs of the second test conducted in the field

With the accumulated data of all catches per trap, it was possible to generate an overall HM of this test (Fig. 4.16). The points with the highest amount of BWs captures are represented by different traps (29, 24, 23 and 25), loaded with the three

treatments tested. Among these traps, the one with the hottest outbreak was the 24, loaded only with the pheromone (F).

Figure 56 Total HM, cumulative catches of the second test conducted

Conclusion

Compounds C1 and C2 (DC154), already known as repellents of *R. ferrugineus* (Jolinas, 2016), were also repellent for *C. sordidus* and are considered two *soft-repellents* for the latter. Reduce the attractiveness expressed by *pabulum* and pheromone against BW. These two substances, tested in vitro, showed considerable repellent action. In the field tests, however, both do not seem to perform the same repellent action shown in the laboratory. In fact, in none of the two tests carried out in the banana plantations was there a clear reduction in catches in the presence of these two soft-repellents. Only initially, in the first two weeks of the two tests, slight drops in the catches were observed in the presence of both compounds. This phenomenon could be ascribed more to the more complex environmental conditions of the *in vivo* tests than those *in vitro*, but also to the non-optimal dispersion technology used for the tests. Furthermore, the presence of different phytophagous foci and its distribution in the field may have altered the activity of the compounds tested.