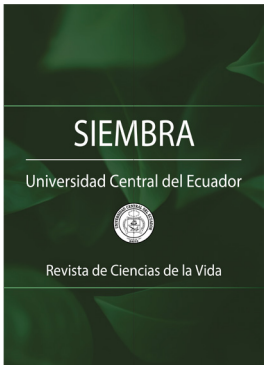
Using microorganisms with different antagonism mechanisms to reduce the severity of *Fusarium oxysporum f.* sp. *cubense race* 1 in Gros Michel bananas

Reducción de la severidad de Fusarium oxysporum f. sp. cubense raza 1 en banano Gros Michel mediante microorganismos con diferentes mecanismos de antagonismo



Miguel Hoyos[1], Pedro Terrero[2]

Siembra 12 (2) (2025): e8195

Received: 08/05/2025 / Revised: 23/06/2025 / 01/09/2025 / Accepted: 16/10/2025

[1] Instituto Nacional de Investigaciones Agropecuarias. Estación Experimental Tropical Pichilingue, Departamento de Protección Vegetal. Km 5 Vía Quevedo – El Empalme. EC120313. Mocache, Los Ríos, Ecuador.
✉ miguel.hoyos811@gmail.com
https://orcid.org/0009-0001-6993-419X

[2] Instituto Nacional de Investigaciones Agropecuarias. Estación Experimental Tropical Pichilingue, Programa de Banano, Plátano y otras musáceas. Km 5 Vía Quevedo – El Empalme. EC120313. Mocache, Los Ríos, Ecuador.
✉ pedro.terrero@iniap.gob.ec
https://orcid.org/0000-0002-4492-4577

[*] *Corresponding author:*
miguel.hoyos811@gmail.com

SIEMBRA
https://revistadigital.uce.edu.ec/index.php/SIEMBRA
ISSN-e: 2477-5788
Frequency: half-yearly
vol. 12, issue 2, 2025
siembra.fag@uce.edu.ec
DOI: https://doi.org/10.29166/siembra.v12i2.8195

Abstract

Fusarium vascular wilt of Musaceae, caused by strains of *Fusarium oxysporum* f. sp. *cubense* [Foc], has only been effectively managed by using genetically resistant varieties. In addition to genetic resistance, crop management strategies that focus on soil health and maintain long-lasting resistance can also impact the intensity of the epidemic. This study evaluated the effects of applying biological control agents with different antagonistic mechanisms on components of the *Fusarium* epidemic in Musaceae under greenhouse conditions. The study used the Foc race 1 [R1]-Gros Michel banana pathosystem as a model. Experiments under controlled conditions revealed that applying biological control agents to Gros Michel banana plants before and after *Fusarium* inoculation results in varying degrees of pathogen damage to the plant. When the biological control agents were applied to contaminated soil before planting, the onset of symptoms and the severity of the damage caused by the pathogen were delayed due to competition and antibiosis. While none of the treatments prevented infection by the pathogen, they delayed the timing of symptoms and reduced severity of damage. Therefore, under optimal management conditions, microorganisms may have the potential to reduce disease severity, but field evaluations are needed.

Keywords: vascular wilt, biological control, antagonism.

Resumen

La marchitez vascular por *Fusarium* de las musáceas, causada por cepas de *Fusarium oxysporum* f. sp. *cubense* [Foc], sólo se han manejado eficazmente con el uso de variedades con niveles de resistencia genética. Además de la resistencia genética, el uso de estrategias de manejo del cultivo enfocadas en la salud del suelo y aquellas que contribuyan a mantener duradera la resistencia disponible también son una opción con impacto en la reducción de intensidad de epidemia. Este estudio se realizó con el objetivo de evaluar el efecto de la aplicación de agentes de control biológico con diferente mecanismo de antagonismo en los componentes de la epidemia de *Fusarium* de las musáceas en condiciones de invernadero, utilizando como modelo el patosistema Foc raza 1 [R1]-banano Gros Michel. Los experimentos en condiciones controladas mostraron

que la aplicación de agentes de control biológico en plantas de banano Gros Michel, antes y después de la inoculación de *Fusarium*, genera diferentes niveles de intensidad de daño del patógeno en la planta. Cuando se aplicaron agentes de control biológico a un suelo contaminado antes de la siembra de las plantas, la aparición de síntomas se retrasó, así como la severidad de daño causada por el patógeno debido a la competencia y antibiosis. Ningún tratamiento impidió la infección del patógeno, pero si retrasaron el tiempo de aparición de síntomas y la intensidad del daño, por tanto, el efecto de microorganismos puede ser potencial para disminuir la severidad de la enfermedad en condiciones de manejo óptimas, pero es necesario realizar evaluaciones en campo.

Palabras clave: marchitez vascular, control biológico, antagonismo.

1. Introductión

Fusarium Wilt of Banana [FWB] is the most significant yield-limiting disease in this tropical crop, caused by strains of the fungus *Fusarium oxysporum* f. sp. *cubense*. In the middle of the last century Foc race 1 1 [R1] of this pathogen devastated commercial Gros Michel banana plantations. Currently, tropical race 4 [TR4] poses a risk to Cavendish banana plantations, a resistant cultivar that was established in plantations that had been devastated by Foc R1 (Drenth & Kema, 2021; García-Bastidas, 2022). The most efficient strategy for managing this disease is genetic resistance (as evidenced in the last century); however, no variety with complete resistance to TR4 is currently available, that is accepted in the export market based on cultivars of the Cavendish group. Despite the availability of cultivars such as Formosana, promoted as tolerant to Foc TR4 and accepted in the market for being a Cavendish type (Munhoz et al., 2024), different levels of susceptibility have been reported under experimental conditions after the first production cycle, thus raising doubts about its usefulness as a replacement for highly susceptible Cavendish cultivars (Viljoen et al., 2020).

In the case of cultivars with quantitative resistance (Formosana), reducing the inoculum level to a threshold they can withstand, or establishing biotic and abiotic factors that allow them to maintain their resistance can help maintain their production for more than one cycle in infected soils (García-Bastidas et al., 2024; Munhoz et al., 2024). The use of biological control agents [BCAs] appears to be an increasingly important disease management strategy aimed at suppressing the pathogen, both because of the trend toward reducing the use of chemicals, and because of the limited options available for effective disease control. Bubici et al. (2019) conducted an extensive review of the use of BCM against *Fusarium* wilt in bananas. In this review, they highlight the control effects of promising species under controlled conditions, but also address the need to develop strategies for the use of microorganisms under specific crop conditions. The suppression of the disease is associated with the presence of BCAs in the soil, which hinders or prevents the establishment of pathogens in the soil (Huang et al., 2019).

The use of BCAs is one of the options that has generated promising results in experiments conducted under controlled conditions; the compounds produced by microorganisms have been effective in *in vitro* assays against Foc in banana cv. Williams. However, the results have not been replicated in the field (Bubici et al., 2019). Bubici et al. (2019) question whether the effectiveness observed in individual microorganism-based strategies, such as the case of *Trichoderma* for controlling TR4, will be applicable under productive field conditions. The use of BCAs has not been useful in completely suppressing the disease, but under controlled conditions, a reduction in incidence has been observed with the use of species from the genera *Trichoderma* or *Pseudomonas* (Hernández-Melchor et al., 2023; Nel et al., 2006). The combined use of *Bacillus subtilis* and Pseudomonas fluorescens showed a synergistic effect in controlling *Fusarium* infection in banana, reducing the severity of wilting by 60% compared to an untreated control (Solórzano et al., 2025).

The severity of Foc epidemics in bananas is generally determined by three components: i) pathogen dispersal, ii) amount of primary inoculum, and iii) infection rate and disease development (Román Jeri, 2012). Practices aimed at preventing the accumulation of inoculum in the soil (mainly through the effect of BCAs) may be sufficient to block residual inoculum through cultivar resistance (Parlevliet, 1993; Zadoks, 1993). In addition to considering the epidemiological components on which the application of BCAs will have an effect, it is also important to study the mechanisms that BCAs have on pathogens and plants (Bubici et al., 2019). Since vascular wilt caused by *F. oxysporum* has no cure, management must be approached preventively. Therefore, it is necessary to study biotic suppression comprehensively, within a disease management program, seeking productive rehabilitation in affected plantations (García-Bastidas et al., 2024; Munhoz et al., 2024).

This study combined an experimental approach using race 1 of Foc as a model in Gros Michel banana plants with the application of BCAs under different epidemiological conditions to explore the impact on the components of the Foc R1 epidemic. Different BCAs antagonism mechanisms were also tested to reduce the intensity of Foc R1 symptoms under controlled conditions.

2. Materials and Methods

2.1. Collection of fungal and bacterial strains

Plantations of Gros Michel bananas exhibiting symptoms of infection by *Fusarium oxysporum* f. sp. *cubense* [Foc R1] were identified in the provinces of Pichincha, Imbabura, Bolívar, Los Ríos, Guayas, and Manabí. During visits to the farms between June 2023 and August 2024, samples of rhizosphere soil and plant tissue were collected from plants showing symptoms of *Fusarium* wilt. The samples were then transferred to the Plant Protection Department's [DPV] Plant Pathology Laboratory, which belongs to the Estación Experimental Tropical Pichilingue [EETP] of the Instituto Nacional de Investigaciones Agropecuarias [INIAP].

To isolate microorganisms in the laboratory, 5 mm fragments of plant tissue were cut and washed for two minutes with a 10% sodium hypochlorite solution, then rinsed three times with sterile distilled water and seeded in PDA and Nutrient Agar (DIFCO, USA) culture media. Twenty grams of soil from each sample were suspended in 180 ml of distilled water and shaken for five minutes, followed by four serial dilutions (1/10). One hundred microliters of each dilution were seeded in the same culture media, and incubated in the dark at 25°C. After five days, pure cultures were obtained in sterile medium and incubated under the same conditions.

2.2. Characterization of microorganisms

The isolated microorganisms were identified and grouped according to their morphological and microscopic characteristics (Delgado-Baquerizo et al., 2018; Watanabe, 2002). The original colonies are preserved in the Plant Pathology Laboratory of the EETP at INIAP. Pathogenic microorganisms were discarded, and pathogenicity was tested on Gros Michel banana plants artificially inoculated by pouring spore suspension onto the soil and injecting it into plant tissue. All isolates that caused lesions in the plants were discarded.

First, the mode of antagonism was identified following the classification proposed by Avis et al. (2008), which is summarized in Table 1. All direct antagonism tests were performed with Foc R1 (isolate SC-Fo-043, preserved at the Estación Experimental Santa Catalina - EESC).

Table 1. Classification of antagonism mechanisms, adapted from Avis et al. (2008).

Effect type	Mode	Mechanism
Direct	Competition	Carbohydrate consumption
		Root infection sites
	Predation	Mycoparasitism
		Lytic enzyme production
Indirect	Antibiosis	Volatiles and non-volatiles secondary metabolites
	Plant strengthening	Induction of systemic resistance
		Growth stimulation

The direct effects of antagonism were identified using culture medium tests in Petri dishes (Villavicencio-Vásquez et al., 2025). For the competition mode, a dual growth technique was used, confronting the antagonistic microorganism with Foc R1 (Ghanbarzadeh et al., 2014). For the predation mode, the interactions between the hyphae of the pathogenic and antagonistic fungi, that grew on the same plate as in the competition mode experiment, were observed using a phase contrast microscope (Eclipse Si E200, Nikon, USA) (Guzmán-Guzmán et al., 2019). The indirect effects of antagonism were identified using an observation test on three-week-old Gros Michel banana plants, planted in a sterile substrate composed of soil and sand in a 4:1 ratio.

To identify the mode of plant strengthening, three applications of a suspension of microorganisms ($1x10^6$ spores ml^{-1}) were carried out on two groups of plants, one group inoculated with Foc R1, and another group without inoculation of any pathogen. In addition, the ability of the selected microorganisms to establish themselves in the soil was evaluated. To do so, soil samples were taken two months after the last application, and the spores present per gram of soil were quantified. For the antibiosis mode, a suspension of Foc R1 spores was exposed to a suspension of the antagonist microorganism, then, after five minutes of exposure, dilutions of the treatment were sown in PDA to quantify the colonies that germinated from the pathogen (Rashad & Abdel, 2020).

Finally, molecular identification of the selected microorganisms was performed by amplifying two genetic regions with Sanger sequencing. For fungi, the translation elongation factor 1-a (TEF1), and the ITS1 region of ribosomal DNA were used, whereas for bacteria, the small subunit of rDNA (16s) and subunit b of rRNA (rpoB) were used.

2.3. Biological control trials

For the in vivo tests, four BCAs were selected that were isolated from the natural environment and identified in the laboratory. Each microorganism selected is representative of a mode of antagonism described in Table 1. Gros Michel banana plants were used as plant material. They were planted in one-liter plastic bags containing black soil, pomina, and clay in a 4:1:1 ratio and acclimated for 15 days until the treatments were applied. The plant experiments were conducted in the Plant Pathology greenhouse at the EETP.

The treatments resulted from implementing the application of BCAs with different modes of antagonism as a strategy for managing *Fusarium* wilt of musaceae under two epidemiological scenarios: one with the presence of the disease, and one without it (Dita et al., 2018). In these two scenarios, it was proposed that each mode of antagonism may influence the components of the *Fusarium* epidemic (incubation period, incidence, severity, and level of vascular colonization) in Musaceae (Román Jeri, 2012; Shen et al., 2019).

After inoculation, the plants were monitored every five days using an improved method for phenotyping *Fusarium* wilt in bananas (García-Bastidas et al., 2019). The incubation period was established as the number of days from inoculation to yellowing of the oldest leaf (considered an initial symptom of wilt) in two of the three plants comprising each experimental unit. Incidence was recorded as the percentage of diseased plants relative to the number of inoculated plants, and the value was expressed for each experimental unit. Severity was assessed using a scale that classifies the percentage of wilted leaves on a scale of 1 to 4, where 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100%. Vascular colonization was assessed using a destructive method: the plants were washed and cut transversely at the height of the rhizome, and the percentage of necrotic tissue area was recorded using a visual scale with 10% intervals.

The biological control trial was conducted under a completely randomized design [CRD] with five observations, each experimental unit being represented by three plants. Nine treatments were applied, consisting of the interaction between the two epidemiological scenarios and the four modes of antagonism, plus a negative control inoculated with Foc R1 without the application of microorganisms (Table 2). The data for the variables were analyzed using ANOVA, and for variables with statistical differences, the least significant difference [LSD] was sought, the data were recorded in a Microsoft 365 spreadsheet and processed with the STATAMP 17 statistical package.

Foc R1 inoculation was performed using the spore pouring method (García-Bastidas et al., 2019). All plants were inoculated with a suspension of 1x106 spores ml-1 of the SC -Fo-043 isolate of Foc R1. The spore suspension was prepared by adding Foc R1 spores to potato dextrose broth (DIFCO, USA) culture medium and incubating at 25°C in an orbital shaker at 120 rpm for five days.

BCAs was applied two weeks apart for the presence (treatments 1-4) and absence of disease (treatments 5-8) conditions, after and before Foc inoculation, respectively. In the case of *Trichoderma* sp., 50 ml of a suspension of $1x10^6$ spores ml^{-1} was applied to each plant. The concentration was determined by counting the spores in a hemocytometer using an optical microscope. In the case of *Bacillus* sp., 25 ml of a suspension of 1x107 cells ml-1 was applied to each plant, the concentration being determined with a visible light spectrophotometer (SP-MUV8000T, Bioevopeak, China) at a wavelength of 600 nm, and an absorbance of 0.1. Both the application of fungi and bacteria was repeated in the same way seven days after the first application.

Table 2. Treatments implemented in the biological control test.

Number	Treatment	Description
1	A1 (Ta) - C	Inoculation of Foc R1 before application of microorganism with competition
2	A2 (Th) - D	Inoculation of Foc R1 before application of microorganism with predation
3	A3 (Bs) - A	Inoculation of Foc R1 before application of microorganism with antibiosis
4	A4 (B) - F	Inoculation of Foc R1 before application of microorganism with plant strengthening
5	B1 (Ta) - C	Inoculation of Foc R1 after application of microorganism with competition
6	B2 (Th) - D	Inoculation of Foc R1 after application of predatory microorganism
7	B3 (Bs) - A	Inoculation of Foc R1 after application of antibiotic microorganism
8	B4 (B) - F	Inoculation of Foc R1 after application of plant-strengthening microorganism
9	Control	Inoculation of Foc R1 without application of microorganisms

3. Results and Discussion

3.1. Characterization and selection of microorganisms

Morphological characterization allowed the grouping of fungal and bacterial isolates (according to their microscopic and macroscopic characteristics in culture medium), and the genera *Trichoderma* and *Bacillus* were preliminarily identified. Molecular confirmation was performed by amplifying and sequencing the ITS1 and TEF1 regions for fungi, and 16s rRNA and rpoB for bacteria, whose fragments showed between 97.6 and 99.3% similarity with sequences deposited in GenBank (data pending publication). The molecular identification service was performed by the IDGEN laboratory, which provided sequencing results and similarity analysis with the GenBank database.

In the collections made in areas of Musaceae production, nine fungi and nine bacteria with different morphological characteristics were isolated. Of the 18 microorganisms that underwent qualitative antagonism tests (data not shown), four microorganisms were selected, one for each mode (Table 3), for plant trials.

Table 3. Modes of antagonism of the microorganisms selected for the in vivo test.

Code	Identity	Competition	Predation	Antibiosis	Plant strengthening	Establishment in the soil
LR-1.3	*Trichoderma asperellum*	X	X			
I-1.1	*Trichoderma harzianum*		X		X	X
LR-1.4	*Bacillus subtilis*			X		
B-1.6	*Bacillus* sp.				X	

The 18 microorganisms showed at least two modes of antagonism. Those with low antagonistic effects were discarded, and those that showed a mode of antagonism with a noticeable effect were selected as the aim was to study the individual effect of the antagonistic mechanisms of microorganisms in the Foc epidemic (Zheng et al., 2024). In the selection process, microorganisms that easily establish themselves in the soil were excluded because they can have an ecological impact on the environments into which they are introduced. If an environmental impact study is not carried out before the application of foreign microorganisms, it is possible that native microorganisms that contribute to higher levels of suppressiveness than in artificial microbial consortia may be displaced (Gomes et al., 2020).

The antagonistic mechanisms expressed by the BCAs selected in this study, as well as those summarized in the review by Bubici et al. (2019), are diverse, complex, and in most cases occur in combination. In *in vitro* experiments, the multiple response observed in most microorganisms is a common phenomenon (Vinale and Sivasithamparam, 2020), but it is possible that the tests proposed are not the most appropriate for quantitatively identifying the interaction between microorganism and pathogen. For example, Keswani et al. (2014) indicate that the production of secondary metabolites in *Trichoderma* species is higher when the microorganism grows

under stressful conditions. In the experiments to identify the mechanism of antagonism, the conditions were optimal for the growth of microorganisms in culture medium, which may have prevented the full potential for the production of secondary metabolites in fungi from being expressed.

Traditionally, *in vitro* tests have been used as a criterion for selecting and classifying microorganisms with biological control potential (Mayorga Morejón et al., 2024). In these cases, working with the saprophytic phase of microorganisms will probably not always yield the same response as the parasitic or pathogenic phase (Vinale et al., 2008). In this study, in vitro experiments were conducted for the sole purpose of identifying the antagonism mechanisms present in microorganisms, biological control experiments were conducted *in vivo* to represent the reality of the Foc more closely R1 - Gros Michel pathosystem.

Bubici et al. (2019) point out that the protocols used in several countries to test the effect of BCAs against Foc TR4 have produced different and incomparable results, and that the next steps are to optimize bioformulation technologies, microbial consortia, and metabolite exploitation. To this, we can add the application of microorganisms targeted at a specific objective in the development of the Foc epidemic, as it was done in this research.

3.2. Biological control trials

In the experiment with plants inoculated with Foc R1, different levels of damage were observed among the treatments applied (Table 4). When BCAs with different modes of antagonism were applied after Foc inoculation, only microorganisms that exhibited antibiosis (T3) and plant strengthening (T4) generated differences in the timing and intensity of symptom expression. When microorganisms were applied before Foc inoculation, microorganisms that showed competition (T5) and plant strengthening (T8) delayed the onset of symptoms and reduced the intensity of disease symptoms. The BCAs that induces plant strengthening was useful in delaying the expression of pathogen infection symptoms, regardless of whether it was applied before or after pathogen inoculation.

Table 4. Incubation period, incidence, severity and vascular colonization of Foc R1 on Gros Michel banana under nine treatments.

No.	Treatment	Incubation period	Incidence (%)	Severity (%)	Vascular colonization (%)
1	A1 (Ta) - C	40 ± 2.1 d	100 ± 0.0 c	70 ± 4.5 c	100 ± 5.0 c
2	A2 (Th) - D	40 ± 2.4 d	100 ± 0.0 c	50 ± 5.0 b	70 ± 4.2 b
3	A3 (Bs) - A	60 ± 1.8 b	100 ± 0.0 c	70 ± 4.0 c	70 ± 4.8 b
4	A4 (B) - F	55 ± 2.0 b	80 ± 5.2 b	50 ± 4.8 b	90 ± 4.3 bc
5	B1 (Ta) - C	50 ± 2.2 c	80 ± 4.7 b	50 ± 3.9 b	70 ± 4.9 b
6	B2 (Th) - D	45 ± 2.5 c	60 ± 6.0 a	70 ± 5.1 c	70 ± 5.0 b
7	B3 (Bs) - A	40 ± 2.0 d	100 ± 0.0 c	70 ± 4.3 c	90 ± 4.1 bc
8	B4 (B) - F	65 ± 1.9 a	60 ± 6.1 a	30 ± 3.5 a	50 ± 3.7 a
9	Control	40 ± 2.0 d	100 ± 0.0 c	70 ± 4.7 c	100 ± 4.5 c

The effect of the treatments was analyzed based on the incubation period and the percentage of vascular colonization, as these were the variables with the widest range of responses and are perhaps the most relevant in the study of Foc epidemics. Under these experimental conditions, incidence was not an informative variable because it was determined from external symptoms according to a visual scale, but when the plants were cut to measure vascular colonization, it was observed that there was no correspondence between internal and external symptoms.

The lowest disease severity (assessed exclusively by chlorotic leaf area) was obtained with the application of BCAs that induce plant growth, but the greatest effect was obtained when the BCAs was applied before inoculation with the pathogen. This result fits well with a prevention scenario, in which the application of BCAs reduces the probability of the pathogen infecting the plant and perhaps establishing itself in the soil (Hernández-Melchor et al., 2023). On the other hand, the effect of BCAs exhibiting antibiosis, competition, and predation is not different between plants before and after inoculation when analyzing the severity of *F. oxysporum* infection.

The competitive mode exhibited by *Trichoderma asperellum* under controlled conditions was not useful in reducing the level of vascular colonization or delaying Foc R1 infection when applied to inoculated plants (theoretically after infection had taken place). In contrast, when applied to healthy plants (prior to Foc R1 infection), vascular colonization was reduced by 30% and symptom onset was delayed by 20%. The difference observed between healthy and diseased plants is possibly due to the antagonist's ability to occupy root infection sites, in addition to the classic consumption of carbohydrates in the BCAs (Castillo et al., 2019; Mon et al., 2021).

Predation or mycoparasitism is a phenomenon that has been little studied due to the complexity of pathogen-antagonist and plant-pathogen interactions. In this study, the application of *Trichoderma harzianum*, identified with the mode of antagonism of predation, did not generate differences in infection time or vascular colonization level. The methodology used to identify the mode of action of predation does not allow us to discern whether it was mycoparasitism or the production of lytic enzymes, or possibly, the *in vitro* behavior of the antagonist is not replicated under natural field conditions (Rajeswari, 2019). Other studies have found that the suppression of Foc TR4 is the result of crop intercropping, allowing for an increase in predatory protists of filamentous fungi such as Foc (Ren et al., 2024). In specific studies with species of the genus *Trichoderma* that have a mycoparasitism mechanism (a type of predation), Foc R1 control efficacy of between 50 and 60% has been found (Chaves et al., 2016; Vijayasanthi et al., 2022).

Species of the genus *Bacillus* are frequently studied for their antibiotic capacity. The *Bacillus subtilis* strain used in this study delayed the onset of symptoms by 30% and reduced the level of vascular colonization by 30% when applied to infected plants without external symptoms (onset of infection). When applied to healthy plants prior to inoculation with Foc R1, no differences were observed compared to control plants. The results observed for control with bacteria that produce secondary metabolites are similar to those observed in other pathosystems and with other strains of Foc TR4 (Dadrasnia et al., 2020; Yadav et al., 2021). The use of the genus *Bacillus* is perhaps the most effective in controlling Foc R1, with 100% efficacy reported in trials with the Prata variety (Vieira et al., 2020) and 75% in Gros Michel (Izquierdo-García et al., 2024)

Along with antibiosis, plant strengthening is the most studied phenomenon in BCAs, and it is more common in fungi; genetic and biosynthesis studies do not allow us to differentiate whether the secondary metabolites produced by endophytic fungi or bacteria have more effect on the pathogen or on the host plant (Nandhini et al., 2020). In plants treated with *Bacillus* sp., the onset of symptoms was delayed by 60% and the percentage of vascular colonization was reduced by half in plants inoculated after application of the antagonist (Figure 1). In this experiment, it was not possible to differentiate whether the mechanism by which the antagonist operates is systemic resistance induction or growth stimulation; but, in light of these results, it is clear that the differences in infection are attributable to the effect of the pathogen.

Figure 1. Banana plants inoculated with Foc R1 and treated with antagonists. From left to right: plant strengthening, competition, predation, antibiosis, and control (without treatment).

4. Conclusions

Microorganisms with different modes of antagonism (competition, antibiosis, predation, and plant strengthening) were identified and evaluated. The results highlight that microorganisms with specific effects on the control of Foc R1 showed variable efficacy, depending on whether they were applied before or after pathogen infection. Plant strengthening and antibiosis stood out as effective mechanisms for delaying the onset of symptoms and reducing vascular colonization in infected plants.

Bacillus subtilis and *Bacillus sp.* significantly reduced vascular colonization and delayed the onset of symptoms when applied to infected plants, demonstrating the efficacy of antibiosis. On the other hand, *Trichoderma asperellum* and *Trichoderma harzianum* showed that the context of application (before or after infection) is crucial for their effectiveness, with competition being most effective in healthy plants. These findings reinforce the importance of targeting the application of antagonists according to the stage of pathogen infection, the phase of plant growth, and the epidemiological condition.

The response observed to microorganisms represents a specific observation. To clearly discern the behavior of other antagonists as biological control agents, the mechanisms of antagonism must be further studied and the trials replicated in field plants.

Acknowledgments

We would like to thank the AGROSAVIA researchers for sharing protocols and communication necessary to carry out the research activities.

Funding

This work was carried out within the framework of project ATN/RF-18761-RG, Fortalecimiento de capacidades para la prevención y el manejo de la marchitez por Fusarium de las Musáceas en América Latina y el Caribe, which received financial support from the Fondo Regional de Tecnología Agropecuaria [FONTAGRO] and the Inter-American Development Bank [IDB].

Contributor roles

- Miguel Hoyos: conceptualization, investigation, methodology, writing – review & editing.
- Pedro Terrero: project administration, supervision.

Ethical implications

Ethics approval not applicable.

Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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