



Application of defence inducers reduces the severity of Black Sigatoka (*Pseudocercospora fijiensis*) in *Musa acuminata* AAA Cavendish

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Abstract Banana production is threatened by Black Sigatoka disease caused by the fungus *Pseudocercospora fijiensis* (M. Morelet) Deighton, which is considered one of the most destructive diseases of this crop. Black Sigatoka control primarily relies on the use of chemical fungicides, which increases production costs and may have negative impacts on health and the environment when applied

inappropriately. In addition, their extensive use may select for resistant strains, causing reduced efficacy of fungicides. Therefore, alternative control options for Black Sigatoka are urgently needed. In the present study, we evaluated plant resistance inducers as an alternative for disease management. The effect of resistance inducers on the growth and development of *P. fijiensis* and on banana defence-related gene expression was measured. Banana plots were treated with five resistance inducers and the best three were included in a commercial programme of Black Sigatoka management. Each resistance inducer, either applied individually or mixed with standard fungicides, showed significant reductions of Black Sigatoka severity when compared to the control. It was confirmed that resistance inducers reduced *in vitro* growth and development of *P. fijiensis* as shown for other pathogens. Banana defence-related genes were found to be up-regulated after application of resistance inducers. These results suggest that the reduction of disease severity in banana crops after application of resistance inducers may be due to a direct effect on *P. fijiensis* in addition to the activation of plant responses. Resistance inducers are a potentially effective alternative to Black Sigatoka disease management.

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Introduction

Banana crops are one of the most important crops in the Colombian economy (AUGURA, 2021) and in banana export countries worldwide (FAO, 2023). However, fruit production faces limiting problems such as pests and diseases that constrain crop sustainability. The main disease in banana and plantain production systems worldwide is Black Sigatoka, caused by the fungus *Pseudocercospora fijiensis* (M. Morelet) Deighton. Despite cultural practices such as removing diseased leaves, managing weeds and maintaining drainage, the control of Black Sigatoka is mainly dependent on fungicide applications, representing the highest cost of banana production (Friesen, 2016).

In addition to economic issues, synthetic fungicides may have negative impacts on human health and natural ecosystems when applied inappropriately. Furthermore, inadequate applications may select fungicide-resistant pathogen populations, thus increasing the frequency of fungicide applications and ultimately leading to the withdrawal of some fungicides from management programmes (Chong et al., 2021; Churchill, 2011; FRAC, 2021).

In addition, The European Union Commission launched the Green Deal's Farm to Fork Plan to reduce pesticide usage by 50% to 2030 and this includes many fungicides currently used for Black Sigatoka control (Beckman et al., 2020). Therefore, it is crucial to investigate novel methods for Black Sigatoka disease management.

The ability of resistance inducers to activate plant defence responses has made them a research priority for many scientists worldwide (Vallad & Goodman, 2004). It has been observed that resistance inducers have several advantages over traditional chemical fungicides, including minimal impact on human health and the environment, and lower risk of resistance emergence in pathogen populations (Oliveira et al., 2016). As alternatives to chemical fungicides, several studies on different pathosystems have demonstrated the effectiveness of resistance inducers in protecting plants from disease. Hence, it is very important to perform research to implement them in commercial programmes of crop protection (Sandroni et al., 2020). However, some resistance inducers act as hormones, activating metabolic pathways with effects several physiological parameters of plant

growth and development and their effectiveness can depend on the plant genotype and environmental conditions (Reglinski et al., 2023). In addition, phytotoxicity has been observed for some resistance inducers, largely in a dose dependent manner (Castaño-Monsalve et al., 2015). Therefore, it is crucial for researchers and farmers to obtain prior knowledge of the potential phytotoxic effects of different doses of each resistance inducer in plants before their commercial application. Physiological and growth parameters such as leaf emission rate of plants treated with resistance inducers should also be measured, to avoid unintended effects on commercial banana crops (Ramírez et al., 2015; Sandroni et al., 2020). To the best of our knowledge, this crucial previous research has not been performed in banana crops. Recently, various strategies have been suggested to overcome potential limitations and maximize the benefits of resistance inducers, e.g., by changing the application frequency and doses to avoid phytotoxicity (Sandroni et al., 2020; Yassin et al., 2021).

Several studies in *Musa* spp. have demonstrated the potential of biotic and abiotic resistance inducers for managing diseases such as Black Sigatoka (Patiño-Hoyos, 2001; Mogollón-Ortiz & Castaño-Zapata, 2012; Elizalde-Largo, 2013; Wang et al., 2015; Zhu et al., 2016; Cheng et al., 2018; Dhakshinamoorthy et al., 2020; García-Bastidas et al., 2022; Sun et al., 2022). Resistance inducers activate plant defence responses, but they may also inhibit the growth and development of the fungus to some extent, as seen in *in vitro* studies of *P. fijiensis* treated with salicylic acid (SA), acibenzolar-S-methyl (ASM), 2,6-dichloroisonicotinic acid (INA), 3-aminobutanoic acid (BABA) and potassium phosphite (Mogollón-Ortiz & Castaño-Zapata, 2012).

Potassium phosphite is a known resistance inducer and was found to be equally as effective as the chemical fungicide propiconazole in controlling Black Sigatoka in young plantlets of the plantain cultivar Dominico Hartón (*Musa balbisiana* AAB) (Mogollón-Ortiz & Castaño-Zapata, 2011). In addition, the application of ASM and potassium phosphite alone or in combination with the fungicide propiconazole resulted in a higher level of control of Black and Yellow Sigatoka in a field crop of the plantain cultivar Dominico Hartón (Ramírez et al., 2012). ASM also led to a reduction in Black Sigatoka severity in the banana cultivar Grand Naine under field conditions when combined with standard chemical

fungicides (Patiño-Hoyos, 2001). Similarly, Zuluaga-Amaya et al. (2007) reported a reduction in Black Sigatoka severity in banana plantations when ASM and sodium salicylate were applied together or in rotation with fungicides.

The application of jasmonic acid (JA) in banana plantlets of cv. Grand Naine resulted in a reduction of Black Sigatoka disease severity (Pérez-Peláez, 2018). In addition, the application of JA and SA under field conditions reduced the severity of Black Sigatoka. Moreover, the application of SA led to an increase in the net weight of the banana bunches (Terrero-Yepey et al., 2020).

Previous studies have demonstrated the potential of resistance inducers to be integrated into Black Sigatoka management in banana crops, either alone or in combination with chemical fungicides and other management methods such as biological control using antagonistic microorganisms (Patiño-Hoyos, 2001). Most studies have been performed at the net-house level. However, the effectiveness of resistance inducers under field conditions depends on several edaphoclimatic and physiological parameters. In addition, appropriate doses, mixtures or rotations with other resistance inducers or chemical fungicides as well as studies of phytotoxicity should be determined prior to their application in a commercial programme of Black Sigatoka management.

In the present study, we examined the effect of several resistance inducers on the growth of *P. fijiensis* *in vitro* and investigated the development and severity of Black Sigatoka in banana plots treated with resistance inducers. Additionally, we evaluated the effect of integrating resistance inducers applied alone or in combination with chemical fungicides in a Black Sigatoka management programme in the field. Lastly, we studied the effect of several resistance inducers on the induction of expression of some pathogenesis-related genes, using quantitative PCR. Through these experiments, we aimed to investigate the potential of resistance inducers as a sustainable tool for managing Black Sigatoka disease in banana crops.

Materials and methods

Plants and pathogen

Net-house experiments were carried out using plants of *Musa* AAA cultivar Williams, obtained from the *in vitro* growth facilities of the Biotechnology unit from

Universidad Católica de Oriente, Rionegro, Antioquia-Colombia. Two-month-old plants were grown under net-house conditions at 29 °C and relative humidity above 95%, standard fertilization and irrigation and 12:12 h of light / darkness. Percent ascospore germination and germ tube length tests were performed with leaf samples collected in two plantations from Urabá, Antioquia, Colombia; one regularly treated with chemical fungicides for Black Sigatoka control and the other located in a buffer zone for prevention of spray drift and therefore not treated with chemical fungicides. Phytotoxicity and disease tests were conducted in a banana crop located in Urabá, Antioquia, Colombia, South America (7°48′09″ N 76°39′17″ W), using plants of cv. Williams that were 1.10 m in height with five functional leaves and managed with standard agronomic practices. *P. fijiensis* isolate C139 (monoascosporic, CIB microorganism collection, Medellín, Colombia) was grown on potato dextrose agar (PDA, Becton Dickinson, Franklin Lakes, NJ, USA) for use in the gene expression studies by qRT-PCR experiments (described below).

Effect of resistance inducers on percent ascospore germination and germ tube length of *P. fijiensis* on culture media

The length of the germ-tube is a standard measurement recommended by the Fungicide Resistance Action Committee (FRAC) to determine the sensitivity of fungal pathogens to a molecule. As frequent applications of fungicides may select for resistant strains of *P. fijiensis*, leaf samples intended for ascospore discharge were collected from the two plantations mentioned above, i.e., one treated and the other not treated with chemical fungicides, to examine whether or not treatments with resistance inducers could influence the ascospore germination and germ tube length. In the plantation with application of chemical fungicides, samples were collected at a minimum of five days after the application of fungicides took place.

Leaf fragments were used to discharge ascospores on Petri dishes containing water-agar (Difco™, Oxford, UK) supplemented with each resistance inducer: 10 mM methyl jasmonate (Aldrich® 392,707; Burlington, MA, USA) (MeJa), 4 g/L sodium salicylate (NaSa) (Merck®, Darmstadt, Germany), 1 g/L β-aminobutyric acid (Aldrich® A44207, Burlington,

MA, USA) (BABA), 10 mL/L potassium phosphite (Merck®, Darmstadt, Germany) (FosK), a control treatment with 10 ppm of the fungicide Boscalid (BASF®, Bogotá, Colombia) and a control with water-agar with no amendment. Boscalid was used as a control because, unlike contact fungicides, it is a systemic fungicide routinely monitored for risk of loss of sensitivity (FRAC, 2022). Contact fungicides usually do not develop or present a low risk of resistance in pathogen populations. Therefore, sensitivity tests are usually performed with systemic fungicides as Boscalid (FRAC, 2022). After 48 h of incubation, the germination of 30 ascospores of *P. fijiensis* and the length of 15 germ tubes were measured per Petri dish using a light microscope. Three Petri dishes were used per treatment and all measurements were repeated two times. Results are expressed as the percentage of ascospore germination and average germ tube length for each treatment. The whole trial was performed twice and repetitions were compared by Levene's analysis of homogeneity of variance ($p < 0.05$) (Levene, 1960). Data were combined if variances of repetitions were homogeneous.

Potential phytotoxicity on banana plants

Several molecules used for agronomical practices may induce phytotoxicity when applied to crops. As no previous studies have described these effects, we performed evaluations with the resistance inducers tested to find appropriate doses for their application to banana plants. In a completely randomized design, 19 treatments with three plants per treatment of 20–24-week-old banana plants cv. Williams were applied to banana plots, including different concentrations of five resistance inducers (MeJa, NaSa, BABA, ASM, FosK), a treatment with the solvent (mixture of 10 mL Tween 20® (Merck®, Darmstadt, Germany), 100 mL 70% ethanol (v/v, Merck®, Darmstadt, Germany) and 890 mL sterile distilled water) as well as a control (water). The concentrations used were: MeJa (1, 2.5, 5, 10, 15 mM), NaSa (0.1, 1, 2, 4 g/L), BABA (0.01, 0.1, 1 g/L), ASM (5 mL/L), FosK (0.1, 1.5, 3, 10 mL/L). Approximately 18–20 cm³ was sprayed per leaf on the abaxial side of fully expanded leaves using a portable pressure pump sprayer, until run-off (Handy portable sprayer, Royal Condor®, Soacha, Cundinamarca, Colombia, coupled with a hollow cone nozzle with a discharge rate of 470 cc/min). The whole experiment was performed two times with the same number of replications and treatments.

Any morphological change or visible damage of plant tissues compared to the healthy control was registered as potential phytotoxicity. The response variables measured were Leaf Emission Rate (LER) and plant height, which are standard physiological parameters to indicate variations in plant growth and development for banana (Dita et al., 2021). The leaf emission rate (LER) of each plant is defined by the difference of leaf unfolding between two consecutive assessments, taking into account the loss of older or damaged leaves. LER was expressed on a weekly basis according to the unfolding degree of the leaf with values between 0.2 and 1, being 1 for complete leaf unfolding.

Development and severity of Black Sigatoka in banana plots sprayed with resistance inducers

For banana field experiments, 80 plants with an average height of 1.10 m (20–24-week-old) were selected. In a completely randomized design, eight treatments with ten plants per treatment (a total of 80 experimental units), including five resistance inducers (5 mM MeJa, 1 g/L BABA, 2 g/L NaSa, 5 mL/L ASM, 10 mL/L FosK), a fungicide control (5 g/L mancozeb, Corteva agriscience, Bogotá, Colombia), a treatment with the solvent (mixture of 10 mL Tween 20® (Merck®, Darmstadt, Germany), 100 mL 70% ethanol (v/v, Merck®, Darmstadt, Germany) and 890 mL sterile distilled water) as well as water (control), were sprayed, covering all leaves of each plant on the abaxial side until run-off. Mancozeb was used as a control because it is the most widely used fungicide for Black Sigatoka chemical management under field conditions, but its application has been phased out from the European Union (release G/SPS/N/EU/ 384 of 24 April 2020, Marín et al., 2003; FRAC, 2022). The whole experiment was performed two times with the same number of replications and treatments.

The response variables measured were percentage disease severity (DS %) and the stage of evolution of the disease (SED), in accordance with the guidelines reported by Fouré and Ganry (2008). Severity was measured weekly by adding the percentage of the foliar area affected of each leaf and the result of this sum was divided by the total number of leaves present on each plant tested. SED was measured to implement an early biological forecasting system to control Black Sigatoka disease, which monitors the development

of the disease allowing timely application of control measures (Fouré and Ganry, 2008). It is based on the early detection of new attacks through the calculation of SED, which represents the speed of development of the disease, reflecting the climatic conditions and the intensity of the infection. It is crucial to stop disease development before necrotic lesions develop and sexual sporulation takes place. If new infections in young leaves are controlled, their development toward coalescent necrosis will be blocked. When there are favourable climatic conditions for disease development, the first symptoms will appear on leaf no. 2. In contrast, if less favourable climatic conditions are present, the first symptoms will appear on leaf no. 3 or older. Therefore, there is a gradient of development of the disease from the top to the bottom of the banana plant. SED is calculated using LER and coefficients selected from Fouré and Ganry (2008), representing the speed at which the disease develops. The coefficients are values assigned to each different association of leaf number/stage of the disease of banana plants affected by Black Sigatoka and are available in Table II of Fouré and Ganry (2008).

For calculating SED, the gross sum of disease was estimated by the coefficients described before on leaves II, III and IV reported by Fouré and Ganry (2008). Severity and gross sum were normalised by subtracting the initial stage of each variable from the starting point of evaluation. The area under disease progress curve (AUDPC) was determined either using DS % or SED recorded at six weeks after inoculation (Van der Plank, 1963). AUDPC was calculated using the formula of the middle point, where t is the time of recording of each value in days, y is DS % or SED value at the i^{th} observation and n the numbers of evaluations.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Integration of resistance inducers applied alone or in mixture with chemical fungicides in a management programme of Black Sigatoka

Seventy 20–24-week-old plants located in a banana plot were selected for the field experiments, with a minimum distance of 80 cm from drainage channels.

The plants located at the border of the plot were excluded from the study. The solutions: 5 mM MeJa, 1 g/L BABA, 2 g/L NaSa and the mixture of resistance inducers 1:1:1 (v/v/v) of MeJa:BABA:NaSa, were applied using a portable sprayer (Handy portable sprayer (Royal Condor®, Soacha, Cundinamarca, Colombia), covering the upper portion of each experimental unit by applying 20 mL per plant (Table 1). The experiment comprised seven treatments with 10 plants per treatment. The standard programme of aerial spray of fungicides for the control of Black Sigatoka used by farmers in the Urabá region of Antioquia, Colombia was used as control (Table 1) (Sebastián Zapata, Director of the Banana Research Center, pers. Comm.). This programme of fungicide applications is part of a standard scheme of disease management in Urabá, and product selection depends on the criteria of a private company based on the monitoring of the disease. In addition, chemical fungicides are rotated to prevent fungicide resistance emergence in pathogen populations. Hence, as the whole experiment was repeated two times, the first repetition did not necessarily include the same chemical fungicides as the second repetition because they represent different times through the year (Table 1). Each treatment included eight applications spaced over a time period of 10 weeks. The application of resistance inducers or their mixture were interspersed with chemical fungicides for each treatment in the second, the fifth and the eighth application (Table 1).

The active ingredients of fungicides (doses and manufacturer in parentheses) used in the field experiments, and the doses recommended by the manufacturers, were: Mancozeb (1.5 kg/ha, Corteva agriscience, Bogotá, Colombia), Difenoconazole (0.4 L/ha, Syngenta, Bogotá, Colombia), Pyrimethanil (0.5 L/ha, Bayer, Soledad, Atlántico, Colombia), Fenpropimorph (0.7 L/ha, BASF, Bogotá, Colombia), Fenpropidin (0.6 L/ha, Syngenta, Bogotá, Colombia), Fenpicoxamid (0.385 L/ha, Corteva agriscience, Bogotá, Colombia), Boscalid (0.3 L/ha, BASF, Bogotá, Colombia), Flutriafol (0.2 L/ha, FMC, Bogotá, Colombia) (Table 1).

The trial was conducted for 10 weeks, corresponding to eight applications in a banana farm located in Carepa, Antioquia, Colombia, as indicated in Table 1. DS % and SED were evaluated following the protocols of Fouré and Ganry (2008).

Table 1 Programme for application of treatments for Black Sigatoka management

A. First experiment of application of treatments								
Application of treatments								
Treatment	First Application	Second Application	Third Application	Fourth Application	Fifth Application	Sixth Application	Seventh Application	Eighth Application
T1	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)
T2	Mancozeb	Difenoconazole	Fenpropimorph	Fenpropidin	Boscalid	Flutriafol	Fenpropimorph	Spiroraxamine
T3	Mancozeb	Methyl jasmonate (MeJa)	Fenpropimorph	Fenpropidin	Methyl jasmonate (MeJa)	Flutriafol	Fenpropimorph	Methyl jasmonate (MeJa)
T4	Mancozeb	β -aminobutyric acid (BABA)	Fenpropimorph	Fenpropidin	β -aminobutyric acid (BABA)	Flutriafol	Fenpropimorph	β -aminobutyric acid (BABA)
T5	Mancozeb	Sodium salicylate (NaSa)	Fenpropimorph	Fenpropidin	Sodium salicylate (NaSa)	Flutriafol	Fenpropimorph	Sodium salicylate (NaSa)
T6	Mancozeb	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*	Fenpropimorph	Fenpropidin	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*	Flutriafol	Fenpropimorph	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*
T7	Mancozeb	Methyl jasmonate	Fenpropimorph	Fenpropidin	β -aminobutyric acid (BABA)	Flutriafol	Fenpropimorph	Sodium salicylate (NaSa)
B. Second experiment of application of treatments								
Application of treatments								
Treatment	First Application	Second Application	Third Application	Fourth Application	Fifth Application	Sixth Application	Seventh Application	Eighth Application
T1	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)	Control (water)
T2	Mancozeb	Pyrimethanil	Difenoconazole	Fenpicoxamid	Fenpropimorph	Mancozeb	Fenpropidin	Difenoconazole
T3	Mancozeb	Methyl jasmonate (MeJa)	Difenoconazole	Fenpicoxamid	Methyl jasmonate (MeJa)	Mancozeb	Fenpropidin	Methyl jasmonate (MeJa)
T4	Mancozeb	β -aminobutyric acid (BABA)	Difenoconazole	Fenpicoxamid	β -aminobutyric acid (BABA)	Mancozeb	Fenpropidin	β -aminobutyric acid (BABA)
T5	Mancozeb	Sodium salicylate (NaSa)	Difenoconazole	Fenpicoxamid	Sodium salicylate (NaSa)	Mancozeb	Fenpropidin	Sodium salicylate (NaSa)
T6	Mancozeb	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*	Difenoconazole	Fenpicoxamid	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*	Mancozeb	Fenpropidin	Methyl jasmonate: β -aminobutyric acid:Sodium salicylate (MeJa:BABA:NaSa) (proportion 1:1:1)*
T7	Mancozeb	Methyl jasmonate (MeJa)	Difenoconazole	Fenpicoxamid	β -aminobutyric acid (BABA)	Mancozeb	Fenpropidin	Sodium salicylate (NaSa)

*Mixture of the resistance inducers MeJa, BABA and NaSa in proportion 1:1:1 (v/v/v). Resistance inducers were applied after every two applications of chemical fungicides. As control, the standard programme of aerial spray of fungicides for the control of Black Sigatoka used by farmers in the Urabá region of Antioquia, Colombia, was used. As the whole experiment was repeated two times, the first repetition not necessarily include the same chemical fungicides as the second repetition because correspond to different times through the year

Determination of the relative expression levels of genes related to banana defence responses

Previous studies have shown a role for different plant hormones such as jasmonates in the banana responses

to Black Sigatoka (Rodríguez et al., 2020). Hence, we selected the pathogenesis-related gene 1, *PR-1* as indicator of the salicylate pathway and *LOX* and *MYC2* as indicators of the activation of the jasmonate pathway (Rodríguez et al., 2016; Rodríguez et al.,

2020). Banana plantlets of cv. Williams, obtained from *in vitro* culture, were acclimatized for nine weeks and then transferred to a net-house, where the temperature was maintained at 29 °C and relative humidity above 98%. Resistance inducers, MeJa (5 mM), SaNa (2 g/L), and BABA (1 g/L), were sprayed on the abaxial side of all leaves of each plant using a portable sprayer (Handy portable sprayer (Royal Condor®, Soacha, Cundinamarca, Colombia), applying approximately 2 mL per leaf. Two hours after the application of the resistance inducers, a fungal suspension of *P. fijiensis* isolate C139 at a concentration of 10^6 mycelial fragments per mL was applied. Mycelial fragment concentration was measured using a haemocytometer (Boeco, Hamburg, Germany). Three whole leaves from each of two plants (totally six leaves) were collected per treatment at each time point (0, 24, 72 and 144 h after application of resistance inducers). The three leaves of each individual plant were immediately macerated in liquid nitrogen and mixed and pooled per plant, which was stored at –80 °C for further analysis. Three independent RNA extractions and purifications were performed from every pool, after infection of banana leaves was verified by conventional PCR.

Verification of infection of banana leaves by P. fijiensis.

To verify infection of banana leaves by *P. fijiensis* by conventional PCR, DNA was isolated and purified using the CTAB method (Doyle & Doyle, 1987) from leaf samples of each treatment at 20 days after inoculation with *P. fijiensis* isolate C139, to perform conventional PCR using *P. fijiensis* -specific primers MF137 (5'-GGCGCCCCGGAGGCCGTCTA-3') and R635 (5'-GGTCCGTGTTTCAAGACGG-3'). Conventional PCR was performed for 35 cycles consisting of a denaturation step at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. Three replications were performed for each sample (Leiva-Mora et al., 2013).

Gene expression studies

After verification of infection of banana leaves of cv. Williams by *P. fijiensis*, total RNA was extracted from the pools obtained from leaves, using the CTAB method as described by White et al. (2008). Samples were treated with DNase I (Thermo Scientific, Wilmington, DE, USA)

to remove any residual DNA. RNA concentration was determined by spectrophotometry in a Multiskan Skyhigh Microplate Spectrophotometer (Thermo Fisher Scientific, Wilmington, MA, USA). Three independent RNA purifications from each pool were mixed for the synthesis of cDNA. Total RNA (400 ng) was used to synthesize the first cDNA strand using the Thermo Scientific Maxima Reverse Transcriptase kit (Thermo Scientific, Wilmington, DE, USA) as per the manufacturer's instructions. The quality of the cDNA was verified in a Bioanalyzer 2100 instrument (Agilent, Waldbronn, Germany). For each qRT-PCR reaction, 200 ng cDNA was used in a CFX96 Touch™ thermal cycler (Biorad, Foster City, CA, USA) with the Maxima SYBR Green qPCR Master Mix (2X) kit (Thermo Scientific, Wilmington, DE, USA) according to the manufacturer's instructions. The ribosomal subunit 26S was used as a reference gene (forward primer 5'-ACATTGTCAGGTGGGGAGTT-3', reverse primer 5'-CCTTTTGTTCACACGAGATT-3') (Rodríguez et al., 2016). The relative expression levels of the following genes were studied: *LOX* (forward primer 5'-ACTACTTGC TCTTCCCCAGCGG-3', reverse primer 5'-CGT CCCTCACACCACACGC-3') (Rodríguez et al., 2020), *MYC2* (forward primer 5'-CTCCCGCCC GCTCGTTCAAG-3', reverse primer 5'-GCTCGC CCTGGATGTCGCTC-3') (Rodríguez et al., 2020) and *PR-1* (forward primer 5'-CGCTTCGGCTTT GGTGTGTG-3', reverse primer 5'-CGCTCGTTG GCGTAGTTCTG-3') (designed in the present work using Primer 3 software using default parameters (Koressaar & Remm, 2007; Untergasser et al., 2012). Expression was determined by the $2^{-\Delta\Delta CT}$ method according to Livak and Schmittgen (2001). qRT-PCR was performed using the following programme: 2 min pre-warming at 50 °C, 10 min denaturation at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 58–60 °C for 30 s and extension at 72 °C for 30 s. cDNA was quantified by qRT-PCR in triplicate. The whole experiment was repeated two times.

Statistical analysis

Data was analysed by ANOVA and post hoc analysis at a confidence level of 95% ($p < 0.05$). The

assumption of normality of data was verified by the Shapiro–Wilk test and the coefficient of skewness. Data that did not meet assumptions of normality were analysed by non-parametric tests (Kruskal–Wallis) and post hoc analysis with Bonferroni adjustments. Differences between means were determined by the Tukey HSD test ($p < 0.05$). *In vitro* tests and field experiments were conducted two times. Repetitions were compared by Levene's analysis of homogeneity of variance ($p < 0.05$) (Levene, 1960). If variances of repetitions were homogeneous, data were combined. A completely random design was used for banana field experiments. The software RStudio, version 1.4.1717 (Posit, Boston, MA), was used for all analyses.

Results

In vitro effect of resistance inducers on *P. fijiensis* isolates

The control treatment with water (91.6%) showed the highest value of the percentage of ascospore germination after 48 h of incubation ($p = 5.64 \times 10^{-16}$, Table 2). Media supplemented with potassium phosphite at 10 mL/L (80.0%), sodium salicylate at 4 g/L (78.9%), or methyl jasmonate at 10 mM (72.2%), were not significantly different from the water control (91.6%). Treatment with

the resistance inducer β -aminobutyric acid—BABA [1 g/L] showed a significantly lower percentage of ascospore germination (71.6%) than the control (water). The lowest value of the percentage of ascospore germination was observed for the fungicide Boscalid (3.9%).

The fungicide Boscalid showed the shortest average germ tube length of 7.1 μm for germinating ascospores followed by medium supplemented with BABA (30.4–35.9 μm) in samples collected from fungicide treated and untreated banana crops, respectively (Table 2). All resistance inducers resulted in shorter germ tube length compared to the control (water) ($p < 2 \times 10^{-16}$). There were no differences in the results between samples collected from the two contrasting plantations, treated and non-treated with chemical fungicides.

Phytotoxicity and physiological effects of resistance inducers in banana plots

The solvent for methyl jasmonate (mixture of 10 mL Tween 20, 100 mL 70% ethanol (v/v) and 890 mL sterile distilled water), the treatment with FosK and the control (water) did not show symptoms of phytotoxicity (Fig. 1A) at the concentrations tested. Leaf atrophy, necrosis, curling, wrinkling, premature unfolding, rupture of leaf blades and coiling of the flag leaf were identified in 100% of plants treated with ASM at 5 mL/L, suggesting phytotoxicity (Fig. 1B).

Table 2 Percent ascospore germination and average germ tube length of ascospores of *P. fijiensis* grown on water agar amended with resistance inducers or fungicide (Boscalid)

Treatment	Percentage ascospore germination (%)	GT length (μm) + F *	GT length (μm) – F *
Control (Water, agar with no amendment)	91.6 ^a	45.5 ^a	55.8 ^a
Methyl jasmonate [10 mM]	72.2 ^{ab}	39.0 ^c	48.7 ^b
β -aminobutyric acid—BABA [1 g/L]	71.6 ^b	30.4 ^d	35.9 ^e
Sodium salicylate [4 g/L]	78.9 ^{ab}	42.8 ^b	41.2 ^d
Potassium phosphite [10 mL/L]	80.0 ^{ab}	41.7 ^b	47.1 ^c
Boscalid [1.0 ppm]	3.9 ^c	7.1 ^e	7.0 ^f
<i>p</i> -value	5.64×10^{-16}	$< 2 \times 10^{-16}$	$< 2 \times 10^{-16}$

*GT length (μm) + F and GT length (μm) – F: Germ tube length in μm of samples collected from banana crops with or without prior application of fungicides, respectively. Data are shown as the average for each treatment ($n = 30$). The Levene test showed no differences in homogeneity of variances by between experimental repetitions for the percentage of ascospore germination ($p = 0.535$), for the germ tube length measurements of ascospores collected from banana leaves from crops that had been treated (Fungicide application (+F): $p = 0.548$) and not treated (No fungicide application (–F): $p = 0.926$) with chemical fungicides. Therefore, data were combined for the individual experiments

With NaSa at 4 g/L, 67% of the plants exhibited symptoms related to phytotoxicity such as leaf burning (Fig. 1C). Similarly, 67% of plants treated with MeJa at 10 mM and 100% of plants treated with MeJa at 15 mM showed symptoms of phytotoxicity. In addition, abnormal gelatinous secretions were observed in plants treated with MeJa at 50 mM (Fig. 1D).

The lowest LER (0.12 leaves per week) was observed in banana plants treated with NaSa [4 g/L], followed by ASM [5 mL/L] (0.35 leaves per week) and MeJa [15 mM] (0.40 leaves per week) and these treatments showed significantly lower values compared to the water control (0.78 leaves per week, $p < 2e^{-16}$) (Table 3). MeJa [15 mM] and NaSa [4 g/L] showed significantly lower plant height after six weeks (first repetition, $p < 2e^{-16}$, second repetition, $p < 2.85e^{-08}$) compared to the control (water) (Table 3).

Effect of resistance inducers on development and severity of Black Sigatoka disease on banana plots

After six weeks, the highest severity of Black Sigatoka was recorded for treatment FosK [10 mL/L] as well as for the control (water) and solvent control (Table 4). In contrast, the lowest values of Black Sigatoka severity were observed in banana plants treated with MeJa [5 mM], ASM [5 mL/L] and the commercial fungicide mancozeb [4 g/L] (AUDPC calculated using DS %, $p = 9.48e^{-10}$; AUDPC calculated using SED, $p = 0.0122$). However, despite the low severity of Black Sigatoka observed on plants treated with ASM, negative effects were detected on growth and development such as a decrease in LER compared to the water control and leaf malformation (Table 3, Fig. 1B).

Fig. 1 Phytotoxicity observed after treatment of banana cultivar Williams with resistance inducers. **A.** Healthy plants. **B.** Symptoms after application of 5 mL/L ASM: 1. Leaf curling and wrinkling; 2. First leaf atrophy and necrosis; 3. First leaf atrophy. **C.** Leaf burning after application of 4 g/L NaSa. **D.** Abnormal gelatinous secretion after application of 50 mM MeJa on banana plots

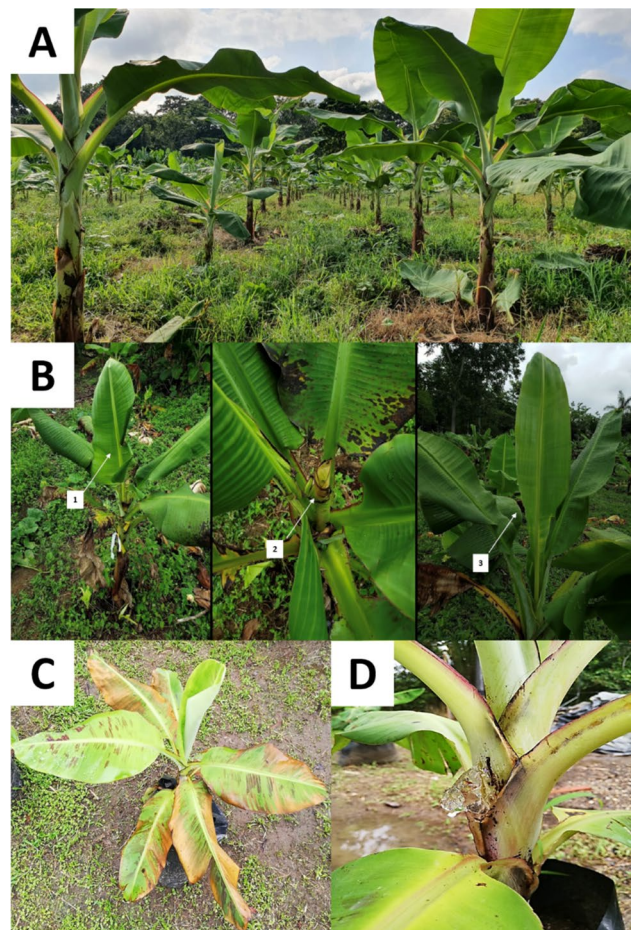


Table 3 Phytotoxicity and physiological effects of inducers on banana plots. Banana cv. Williams plants were sprayed with different doses of resistance inducers. Significant changes of the physiological parameters LER and plant height during

10 weeks in plants treated with resistance inducers compared to the control (water) were considered as indications of phytotoxicity

Treatment	Concentration	Leaf emission rate (LER)**	Change in plant height during 10 weeks (cm). First Repetition***	Change in plant height during 10 weeks (cm). Second Repetition****
Control (Water)	-	0.78	11.3	3.9
Methyl jasmonate	1 mM	0.72	10.5	3.6
Methyl jasmonate	2.5 mM	0.74	10.2	4.3
Methyl jasmonate	5 mM	0.75	11.6	3.0
Methyl jasmonate	10 mM	0.55	6.1	4.1
Methyl jasmonate	15 mM	0.40	5.4	1.6
Sodium salicylate	0.1 g/L	0.76	12.5	3.6
Sodium salicylate	1 g/L	0.70	12.1	3.7
Sodium salicylate	2 g/L	0.70	12.5	4.0
Sodium salicylate	4 g/L	0.12	5.1	0.6
β -aminobutyric acid	0.01 g/L	0.74	10.8	3.4
β -aminobutyric acid	0.1 g/L	0.76	8.7	3.7
β -aminobutyric acid	1 g/L	0.79	15.8	3.9
Acibenzolar-S-methyl	5 mL/L	0.35	8.5	3.3
Potassium phosphite	0.1 mL/L	0.72	11.5	3.4
Potassium phosphite	1.5 mL/L	0.80	11.9	3.6
Potassium phosphite	3 mL/L	0.74	13.9	3.4
Potassium phosphite	10 mL/L	0.74	13.4	2.2
Solvent for methyl jasmonate*	*	0.85	11.1	3.7
<i>p</i> -value		$< 2e^{-16}$	$2e^{-16}$	$< 2.85e^{-08}$

*Solvent for methyl jasmonate: mixture of 10 mL Tween 20, 100 mL 70% ethanol (v/v) and 890 mL sterile distilled water. No variance inhomogeneity was observed between repetitions for the variable leaf emission rate (LER, $p=0.0714$) and therefore data were combined. **LER is given in leaves per week. LER data are shown as the average for each treatment ($n=42$, $p < 2e^{-16}$). Data represents total change in plant height growth during the 10 weeks of the experiment, expressed in cm. Analysis for variance homogeneity showed differences between repetitions for the variable plant height ($p=0.0449$) and therefore data of each repetition are shown separately for the two experiments. ***Plant height (cm) first repetition ($p < 2e^{-16}$). ****Plant height (cm) second repetition ($p < 2.85e^{-08}$). Plant height data are shown as the average for each treatment in each repetition of the experiment ($n=21$). Means followed by different letters are significantly different between treatments according to Kruskal Wallis test's and Post hoc analysis with Bonferroni adjustments

Integration of resistance inducers in a programme for Black Sigatoka disease management in banana plots in Urabá, Antioquia, Colombia

AUDPC values obtained using two different methods for calculation (SD% and SED) showed significant differences between all treatments containing fungicides and/or resistance inducers, when compared to the control where only water was applied ($p=4.06e^{-15}$ for analysis of AUDPC calculated using DS %; $p=8.08e^{-14}$ for analysis of AUDPC calculated

using SED, Table 5). Treatments with three applications of each of the resistance inducers (MeJa, BABA and NaSa) interspersed with five applications of fungicides showed similar results to the control using standard chemical fungicides (Table 5). Furthermore, three applications of a mixture of resistance inducers (1:1:1, v/v/v, BABA, MeJa, NaSa) interspersed with five applications of fungicides also showed similar results to the control of standard chemical fungicides (Table 5). The treatment with MeJa, BABA and NaSa interspersed with fungicides, in the second, the fifth

Table 4 Black Sigatoka severity on banana plots treated with resistance inducers. The Area Under Disease Progress Curve was calculated using the percentage of Disease Severity (AUDPC, DS %) or the Stage of Evolution of the Disease (AUDPC, SED)

Treatment	Concentration	AUDPC (calculated with DS %)**	AUDPC (calculated with SED)***
Mancozeb	4 g/L	17.3 ^c	18.8 ^b
Control (Water)	100%	29.1 ^a	194.5 ^a
Methyl jasmonate	5 mM	19.1 ^{bc}	164.5 ^{ab}
Sodium salicylate	2 g/L	24.3 ^{abc}	170.4 ^{ab}
Potassium phosphite	10 mL/L	30.8 ^a	176.8 ^{ab}
Acibenzolar-S-methyl	5 mL/L	17.2 ^c	189.5 ^a
β -aminobutyric acid	1 g/L	26.3 ^{ab}	167.0 ^{ab}
Solvent for methyl jasmonate*	*	30.9 ^a	229.6 ^a
<i>p</i> -value		9.48e ⁻¹⁰	0.0122

*Solvent for methyl jasmonate: mixture of 10 mL Tween 20, 100 mL 70% ethanol (v/v) and 890 mL sterile distilled water. No differences in variance homogeneity were found between repetitions of the experiment ($p=0.4779$) and therefore data were combined. Response variable values are shown as average for each treatment ($n=20$). Means followed by different letters are significantly different according to Tukey's test ($p<0.05$). **AUDPC: area under disease progress curve calculated using the percentage of disease severity (DS %) at six weeks. ***AUDPC area under disease progress curve calculated using the stage of evolution of the disease (SED) at six weeks

and the eighth application, respectively, exhibited the lowest AUDPC value (calculated as DS%) (182.52) at 11 weeks after the first application, compared to the control of the standard chemical fungicide application programme. Interestingly, the lowest AUDPC value (calculated as SED) was observed for the treatment with three applications of BABA interspersed with five applications of fungicides (Table 5).

Molecular confirmation of *P. fijiensis* infection by conventional PCR

Twenty days after application, chlorosis and necrosis were observed in the treatment inoculated with *P. fijiensis* only (Fig. 2A). Using the *P. fijiensis*-specific primers Mf1375' and R635, a PCR-amplified product of ~1018 bp was obtained, which is consistent with the reported fragment, indicating infection by *P. fijiensis* of banana leaves (Fig. 2B). After infection was verified in banana leaves, RNA was purified for gene expression analysis (Fig. 2C).

Relative expression of defence-related genes in banana plants after induction of resistance inducers

Expression of the defence-related banana genes *PR-1*, *LOX* and *MYC2* was studied at three time points: 24, 72 and 144 h after individual application of resistance inducers BABA, MeJa, NaSa. *P. fijiensis* was

inoculated two hours after application of resistance inducers and thus, the time points corresponded to 22, 70 and 142 h after pathogen inoculation (Fig. 3). The gene expression level for each treatment and time point was compared to the corresponding expression level of the water control. Thus, each expression level value reflects the effect of treatment and time point.

Significant differences were identified for the expression level of each individual gene between the different time points and between treatments with resistance inducers within each time point evaluated. Significantly higher expression of *PR-1* was observed at 22 h after pathogen inoculation in plants treated with BABA and NaSa ($p=2.24e^{-14}$, Fig. 3). The lowest significant expression of *PR-1* was observed in plants treated with MeJa and NaSa at 142 h after pathogen inoculation (Fig. 3). *LOX* exhibited significantly ($p=3.41e^{-14}$) higher up-regulation in plants treated with MeJa at 22, 70 and 142 h after pathogen inoculation (Fig. 3). The lowest significant expression of *LOX* was observed in plants treated with BABA, NaSa, *P. fijiensis* and water at 22 h after pathogen inoculation, BABA, *P. fijiensis* and water at 70 h after pathogen inoculation and NaSa and water at 142 h after pathogen inoculation (Fig. 3). *MYC2* was significantly ($p=1.35e^{-08}$) up-regulated in plants treated with MeJa at 22, 70 and 142 h after pathogen inoculation (Fig. 3). The lowest expression of *MYC2* was recorded in plants treated with water at 22 h after

Table 5 Resistance inducers integrated in a commercial programme for Black Sigatoka disease management in banana plots located in Urabá, Antioquia, Colombia. Each treatment included eight applications. The application of resistance inducers or their mixture were interspersed with chemical fungicides for each treatment in the second, the fifth and

the eighth application. As control, eight applications of water was used. As control of standard chemical fungicides, the usual programme performed by farmers of aerial spray for the control of Black Sigatoka in the Urabá-Colombia region was implemented

Treatment	Applications	AUDPC (DS %)*	AUDPC (SED)**
Control (Water)	Eight	358.2 ^a	2368.3 ^a
Fungicide control (standard in the Urabá-Colombia region)	Eight	233.3 ^{bc}	1194.5 ^b
Methyl jasmonate	Three applications of MeJa interspersed with five applications of fungicides in the following order: fungicide-MeJa-fungicide-fungicide-MeJa-fungicide-fungicide-MeJa	205.6 ^{bc}	831 ^{bc}
β -aminobutyric acid	Three applications of BABA interspersed with five applications of fungicides in the following order: fungicide-BABA-fungicide-fungicide-BABA-fungicide-fungicide-BABA	214.6 ^{bc}	216.2 ^c
Sodium salicylate	Three applications of NaSa interspersed with five applications of fungicides in the following order: fungicide-NaSa-fungicide-fungicide-NaSa-fungicide-fungicide-NaSa	247.9 ^b	746.1 ^{bc}
Mixture (1:1:1, v/v/v) of methyl jasmonate: β -aminobutyric acid: sodium salicylate	Three applications of the mixture of resistance inducers interspersed with five applications of fungicides in the following order: fungicide-mixture-fungicide-fungicide-mixture-fungicide-fungicide-mixture	236.6 ^b	803.5 ^{bc}
Rotated (methyl jasmonate / β -aminobutyric acid / sodium salicylate)	Application of each resistance inducer interspersed with five applications of fungicides in the following order: fungicide-MeJa-fungicide-fungicide-BABA-fungicide-fungicide-NaSa	182.5 ^c	952.5 ^b
<i>p</i> -value		4.06e ⁻¹⁵	8.08e ⁻¹⁴

No differences in variance homogeneity were seen between repetitions of the experiment ($p=0.194$) and therefore, data were combined. Response variable values are shown as the average for each treatment ($n=20$). Means followed by different letters are significantly different according to Tukey's test ($p=0.05$). *AUDPC: area under disease progress curve calculated using the percentage of disease severity (DS %) at six weeks. **AUDPC area under disease progress curve calculated using the stage of evolution of the disease (SED) at six weeks

pathogen inoculation and 70 h after pathogen inoculation and NaSa and water at 142 h after pathogen inoculation (Fig. 3).

Discussion

Here, we show for the first time a comprehensive model of an integrated management programme for Black Sigatoka in banana in commercial plots, testing the resistance inducers methyl jasmonate, β -aminobutyric acid and sodium salicylate, alone, in mixture or in combination with synthetic fungicides.

We demonstrate the usefulness of resistance inducers to manage the Black Sigatoka under field conditions. In addition, we describe symptoms of phytotoxicity on banana plants when resistance inducers were applied at high doses, highlighting the importance of previous and careful determination of concentration thresholds, timing and other factors to prevent unwanted effects during their practical commercial use.

Previously, several inducers of resistance in *Musa* spp. against the fungal pathogen *P. fijiensis* were tested (García-Bastidas et al., 2022; Patiño-Hoyos, 2001; Sun et al., 2022). Additionally, plant hormones,

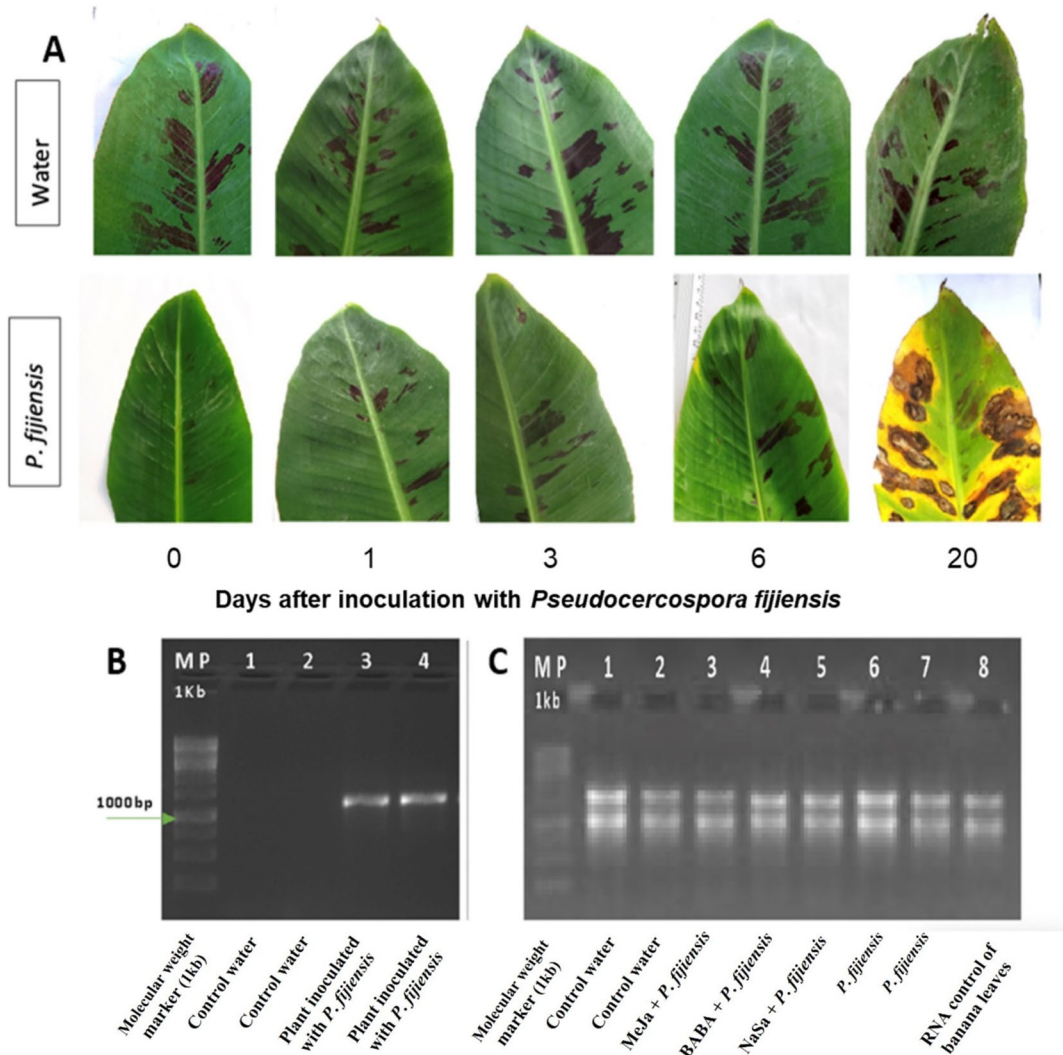


Fig. 2 Black Sigatoka on banana plants cv. Williams inoculated with *P. fijiensis*. **A.** Top row: control leaves only treated with water at 0, 1, 3, 6 and 20 days after application. Bottom row: symptoms of Black Sigatoka in banana leaves at 0, 1, 3, 6 and 20 days after inoculation with *P. fijiensis*. **B.** Agarose

gel electrophoresis (1%) showing a specific PCR-amplification fragment of *P. fijiensis*. **C.** Agarose gel electrophoresis (1.5%) showing RNA purified from banana plants treated with resistance inducers and *P. fijiensis*

such as jasmonates, have been found to play key roles in the defence response of the Black Sigatoka-resistant banana cultivar Calcutta 4 (Torres et al., 2012; Rodríguez et al., 2016; Rodríguez et al., 2020). These studies identified new potential tools to be included in the management of the Black Sigatoka, such as the use of resistance-inducers.

Previous research suggested that resistance inducers activate plant defence mechanisms, but do not inhibit pathogen growth directly. However, recent

findings (Mogollón-Ortíz & Castaño-Zapata, 2012) suggested that some resistance inducers might exert a direct inhibitory effect on plant pathogens as was observed in the current study, where all resistance inducers at high concentration reduced the length of the germ tube of *P. fijiensis*. In addition to reducing germ tube length, Mogollón-Ortíz & Castaño-Zapata (2012) found that BABA also decreased *in vitro* sporulation and colony size indicating a direct inhibitory effect on growth and development of *P. fijiensis*. Our

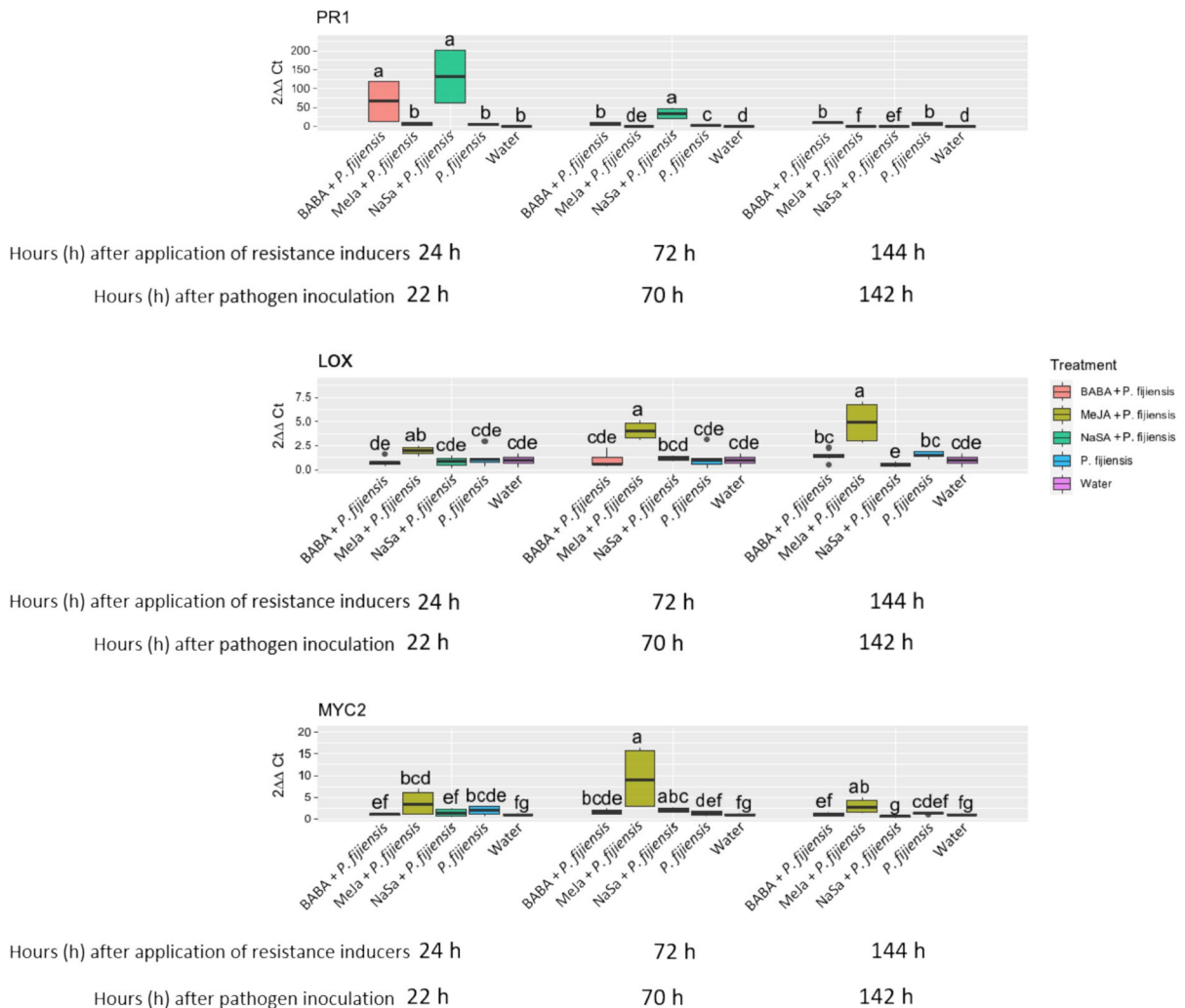


Fig. 3 Relative expression of defence-related genes *PR-1* of banana treated with resistance inducers + *P. fijiensis*. Samples were treated with MeJA [5 mM], BABA [1 g/L], NaSA [2 g/L], *P. fijiensis* [10^6 mycelial fragments per mL], with water as control. Gene expression was evaluated at 24, 72 and 144 h after application of resistance inducers, which corresponded to 22, 70 and 142 h after pathogen inoculation. No differences in the

homogeneity of variances were observed between independent repetitions for the variable $2\Delta\Delta C_t$ ($p=0.08$) and therefore, data were combined. Values represent the mean of six replications for each gene relative to the reference gene 26S. Means with different letters indicate statistical differences between treatments according to the Kruskal Wallis test's and Post hoc analysis with Student's T-test ($p<0.05$)

results confirm that resistance inducers exert a detrimental direct effect on *P. fijiensis* in addition to activating plant resistance mechanisms.

It has been found that the application of resistance inducers to crops may cause some negative side effects on growth, development and production as well as causing phytotoxicity (Sandroni et al., 2020). Negative side effects depend on a number of factors such as the plant genotype, pathogen, environment, type of resistance inducer, mode of application, dose etc.,

suggesting that rigorous testing should be performed under every particular condition (Castaño-Monsalve et al., 2015; Sandroni et al., 2020; Walters et al., 2013). Therefore, caution should be taken before their widespread use in commercial plantations, and preliminary experiments should be mandatory to determine the appropriate doses that do not induce negative effects on banana production. In the present study, we tested several concentrations of MeJA, NaSA, BABA and FosK to find the concentration that could be

applied to induce effective resistance against *P. fijiensis* without causing adverse effects on physiological parameters of production of banana plants.

All resistance inducers produced symptoms of phytotoxicity and reduced LER (Leaf Emission Rate) and plant growth when applied at the highest doses tested. To our knowledge, this is the first report of such symptoms in banana treated with resistance inducers. Morphological changes in the banana plant, caused by phytotoxicity, may reduce fruit yield (Aeberli et al., 2023; Batte et al., 2021; Guimarães et al., 2021; Israeli et al., 1993; Jayasinghe et al., 2022; Woomer et al., 1999). However, fruit yield measurement was out of the scope of this research and therefore, further studies should be performed to analyse fruit quality and yield to observe whether Black Sigatoka control based on or including resistance inducers may affect banana production and postharvest quality negatively. Nevertheless, when resistance inducers are applied at lower doses, they do not cause symptoms, suggesting that with appropriate management, they can be included in an integrated disease management programme. Hence, the information obtained is crucial to determine concentrations to be applied in banana crops. Further research must include determination of the frequency of application of each resistance inducer to control the disease, but without causing phytotoxicity.

Based on the information obtained, we used low doses of the resistance-inducers that did not cause adverse effects on banana plants for further field experiments. The first and second repetition of the experiment to measure phytotoxicity showed different plant heights, but with a similar tendency between repetitions. It has been observed that banana plant growth rate depends on edaphoclimatic and physiological conditions such as precipitation, temperature and others (Turner, 1995). In the Urabá region, these factors are variable in a way that probably favours a higher plant growth rate during the second half of the year (from July to December, Sánchez & Mira, 2013). This may partially explain why the two independent experiments to measure plant height showed differences, because the second repetition was performed during the first half of the year (from January to June) and the first repetition of the experiment was performed during the second half of the year (from July to December), resulting in higher plants as observed in Table 3.

Previous research has shown that phosphonates are effective resistance inducers in various pathosystems, including the banana-*P. fijiensis* pathosystem (Patiño-Hoyos, 2001; Mogollón-Ortiz & Castaño-Zapata, 2011; Mogollón-Ortiz & Castaño-Zapata 2012; Ramírez et al., 2012). However, in the present research, banana plants treated with FosK showed the same level of Black Sigatoka severity as the control (water) or solvent controls. In contrast to FosK, MeJa and ASM were found to be as effective in reducing Black Sigatoka severity as the chemical fungicide mancozeb. Additionally, incorporation of applications of MeJa, BABA and NaSa in a programme of Black Sigatoka control, exhibited similar results as the commercial programme of chemical fungicide applications. Importantly, treatment T7 (Table 1), which consisted in the following eight applications spaced over ten weeks – the first with mancozeb, second with MeJa, third with difenoconazole, fourth with fenpicoxamid, fifth with BABA, sixth with mancozeb, seventh with fenpropidin and eighth with NaSa – was found to be the most effective in reducing Black Sigatoka severity, suggesting that rotating chemical fungicides with resistance inducers may be a good approach to control the disease.

In several pathosystems, resistance inducers have been tested in rotation or combined with chemical fungicides for integrated disease management (Patriocio et al., 2007; Liljeroth et al., 2010; Monsalve et al., 2012; Cohen et al., 2016). The synergistic effects between resistance inducers and fungicides on plant protection has been attributed, at least in part, to the anatomical and biochemical changes observed in plants after product application (Li et al., 2018). The results obtained in the present study suggest that incorporating resistance inducers in commercial programmes for Black Sigatoka control may reduce the number of chemical fungicide applications needed.

Complex molecular and biochemical signalling has previously been identified in the *Musa* spp.-*P. fijiensis* interaction (Noar et al., 2022; Rodríguez et al., 2016). Understanding the molecular and biochemical signalling pathways involved in this plant-pathogen interaction is very important to understand the key factors determining plant resistance and in designing novel or improved strategies for Black Sigatoka management. A set of genes, which indicate the onset of the defence response have been described (Rodríguez et al., 2016; Rodríguez et al., 2020).

Here, we measured the relative expression of some of those genes to test whether they were activated after the application of resistance inducers in banana plants inoculated with *P. fijiensis*. Our results showed increased relative expression of defence-related genes *PR-1*, *LOX* and *MYC2*, dependent on the treatment and time point tested, suggesting that the defence responses in banana plants were effectively activated against *P. fijiensis* by the resistance inducers applied. This finding constitutes an advancement in the understanding of the plant defence activation after application of resistance inducers. This knowledge that may be useful to improve or design novel strategies for the Black Sigatoka disease management.

In the present study, *PR-1* was up-regulated during the biotrophic phase of the infection in plants treated with NaSa and BABA and down-regulated in plants treated with MeJa. Menéndez et al. (2020) found a similar association of *PR-1* to defence responses regulated by the hormone methyl-salicylate. The *PR-1* protein shows antimicrobial activity by sequestration of sterols from pathogens, and evidence suggests an involvement in systemic acquired resistance (Gamir et al., 2017; Han et al., 2023). The *PR-1* protein is also significantly up-regulated after infection of *Musa* sp. by several pathogenic fungi including *P. fijiensis* (Portal et al., 2011; Rodríguez et al., 2016; Anuradha et al., 2022). *P. fijiensis* requires sterol for membrane biosynthesis and growth (Cañas-Gutiérrez et al., 2009). In fact, azole fungicides such as the DMI fungicides imidazoles and triazoles act through the inhibition of sterol 14 α -demethylase (CYP51) and are widely used for Black Sigatoka control (Cañas-Gutiérrez et al., 2009; Chong et al., 2021; Lepesheva & Waterman, 2004). Plant *PR-1* may target pathogen sterols, as do bacteria, with the production of antimycotic polyene antibiotics that bind to ergosterols in fungal membranes to cause cellular leakage and azole fungicides that act as sterol biosynthesis inhibitors (Gamir et al., 2017; Han et al., 2023).

In the present study, it was found that application of MeJa up-regulated the expression of *LOX* and the transcription factor *MYC2*, which are known to be associated to jasmonate-mediated signalling (Rodríguez et al., 2016; Rodríguez et al., 2020). Similarly, *MYC2* was up-regulated in cv. Calcutta 4 after infection with *P. fijiensis*, which in turn may activate *PR*-proteins and defence-related transcription factors of the WRKY family (Rodríguez et al., 2016; Rodríguez

et al., 2020). Pérez-Peláez (2018) showed that jasmonic acid and salicylic acid reduced Black Sigatoka severity and, in addition, the application of SA increased the banana bunch net weight. These results are consistent with the molecular pathways apparently activated in our research (Rodríguez et al., 2016; Rodríguez et al., 2020; Terrero-Yepez et al., 2020).

Resistant cultivars of banana, such as cv. Calcutta 4, displayed a stronger and earlier response of jasmonate regulated defence-related genes, compared to the susceptible cv. Williams (Rodríguez et al., 2016; Rodríguez et al., 2020). The effect of resistance inducers on banana plants found in the present study apparently resembles the response of resistant banana genotypes such as cv. Calcutta 4 described by Rodríguez et al. (2020).

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Author contributions Diana C. Henao-Ochoa: formal analysis, conceptualization, investigation, writing original draft, the present paper was written with results of the thesis to obtain the Master of Science degree, visualization; Vicente E. Rey-Valenzuela: conceptualization, supervision; Sebastián Zapata-Henao: resources, conceptualization, supervision, project administration, funding acquisition; Rafael E. Arango-Isaza: resources, conceptualization, supervision, project administration, funding acquisition, writing original draft; Héctor A. Rodríguez-Cabal: conceptualization, methodology, supervision, project administration, writing original draft, formal analysis, investigation; Juan Morales: conceptualization, methodology, supervision, project administration, writing original draft, resources, funding acquisition.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on request.

Declarations

Research involving human participants and/or animals Not applicable. The research involved no human participants or animals.

Informed consent The research involved no human participants, and no animals so that the statement on the welfare of animals is not required.

competing interest The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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References

- Aeberli, A., Phinn, S., Johansen, K., Robson, A., & Lamb, D. W. (2023). characterisation of banana plant growth using high-spatiotemporal-resolution multispectral UAV imagery. *Remote Sensing*, 15, 679. <https://doi.org/10.3390/rs15030679>
- Anuradha, C., Chandrasekar, A., Backiyarani, S., Thangavelu, R., Giribabu, P., & Uma, S. (2022). Genome-wide analysis of pathogenesis-related protein 1 (PR-1) gene family from *Musa* spp. and its role in defense response during stresses. *Gene*, 821, 146334. <https://doi.org/10.1016/j.gene.2022.146334>.
- AUGURA. (2021). Asociación de Bananeros de Colombia, Medellín. *Coyuntura Bananera*, 2021, 1–55.
- Batte, M., Swennen, R., Uwimana, B., Akech, V., Brown, A., Geleta, M., & Ortiz, R. (2021). Traits that define yield and genetic gain in East African highland banana breeding. *Euphytica*, 217, 193. <https://doi.org/10.1007/s10681-021-02920-x>
- Beckman, J., Ivanic, M., Jelliffe, J., Baquedano, F., & Scott, S. (2020). Economic and food security impacts of agricultural input reduction under the European Union green deal's farm to fork and biodiversity strategies, eB-30, U.S. Department of Agriculture, Economic Research Service. November, *Economic Brief* No. (EB-30) 59 pp.
- Cañas-Gutiérrez, G. P., Angarita-Velásquez, M. J., Restrepo-Flórez, J. M., Rodríguez, P., Moreno, C. X., & Arango, R. (2009). Analysis of the CYP51 gene and encoded protein in propiconazole-resistant isolates of *Mycosphaerella fijiensis*. *Pest Management Science*, 65, 892–899. <https://doi.org/10.1002/ps.177>
- Castaño-Monsalve, J., Ramírez-Gil, J. G., Patiño-Hoyos, L. F., & Morales-Osorio, J. G. (2015). Alternativa para el manejo de *Phytophthora infestans* (Mont.) de Bary en *Solanum betaceum* Cav. Mediante inductores de resistencia. *Revista De Protección Vegetal*, 30, 204–212.
- Cheng, Z., Yu, X., Li, S., & Wu, Q. (2018). Genome-wide transcriptome analysis and identification of benzothiadiazole-induced genes and pathways potentially associated with defense response in banana. *BMC Genomics*, 19, 454. <https://doi.org/10.1186/s12864-018-4830-7>
- Chong, P., Essoh, J.N., Arango Isaza, R.E., Keizer, P., Stergiopoulos, I., Seidl, M.F., Guzman, M., Sandoval, J., Verweij, P.E., Scalliet, G., Sierotzski, H., de Lapeyre de Bellaire, L., Crous, P.W., Carlier, J., Cros, S., Meijer, H.J.G., Peralta, E.L., & Kema, G.H.J. (2021). A world-wide analysis of reduced sensitivity to DMI fungicides in the banana pathogen *Pseudocercospora fijiensis*. *Pest Management Science*, 77, 3273–3288. <https://doi.org/10.1002/ps.6372>.
- Churchill, A. C. (2011). *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: Progress towards understanding pathogen biology and detection, disease development, and the challenges of control. *Molecular Plant Pathology*, 12, 307–328. <https://doi.org/10.1111/j.1364-3703.2010.00672>
- Cohen, Y., Vaknin, M., & Mauch-Mani, B. (2016). BABA-induced resistance: Milestones along a 55 year journey. *Phytoparasitica*, 44(4), 513–538. <https://doi.org/10.1007/s12600-016-0546>
- Dhakshinamoorthy, D., Sundaresan, S., Iyadurai, A., Subramanian, K.S., Janavi, G.J., Paliyath, G., & Subramanian, J. (2020). Hexanal vapor induced resistance against major postharvest pathogens of banana (*Musa acuminata* L.). *Plant Pathology Journal*, 36(2), 133–147. <https://doi.org/10.5423/PPJ.OA.03.2019.0072>.
- Dita, M., Teixeira, L., Li, C., Zheng, S., O'Neill, W., Daniels, J., Pérez-Vicente, L., Carreel, F., Roussel, V., Carlier, J., Abadie, C., Carpentier, S.C., Iyyakutty, R., Kissel, E., van Wesemael, J., Chase, R., Tomekpe, K., Roux, N. (eds.) (2021). Practical guidelines for early screening and field evaluation of banana against Fusarium wilt, Pseudocercospora leaf spots and drought. Montpellier (France): Biodiversity International. 83 p.
- Doyle, J., & Doyle, J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19, 11–15.
- Elizalde-Largo, C.G. (2013). Uso de inductor de resistencia en mezcla con fungicidas convencionales para el control de Sigatoka negra *Mycosphaerella fijiensis* morelet. Undergraduate Thesis. UTMACH, Unidad Académica de Ciencias Agropecuarias, Machala, Ecuador.
- FAO. (2023). *Banana Market Review – Preliminary results 2023*. Food and Agriculture Organization of the United Nations.
- Fouré, É., & Ganry, J. (2008). A biological forecasting system to control black leaf streak disease of bananas and plantains. *Fruits*, 63(5), 311–317. <https://doi.org/10.1051/fruits:2008029>
- FRAC. (2021). Mode of Action Group. Fungicide Resistance Action Committee. <https://www.frac.info/fungicide-resistance-management/by-frac-mode-of-action-group>.
- FRAC. (2022). The minutes of the Banana FRAC Working Group Meeting in Miami / Florida, May 9th - May10th, 2022.

- Friesen, T. L. (2016). Combating the Sigatoka disease complex on banana. *PLOS Genetics*, 12(8), e1006234. <https://doi.org/10.1371/journal.pgen.1006234>
- Gamir, J., Darwiche, R., Van't Hof, P., Choudhary, V., Stumpe, M., Schneider, R., & Mauch, F. (2017). The sterol-binding activity of PATHOGENESIS-RELATED PROTEIN 1 reveals the mode of action of an antimicrobial protein. *Plant Journal*, 89(3), 502–509. <https://doi.org/10.1111/tbj.13398>
- García-Bastidas, F.A., Arango-Isaza, R., Rodríguez-Cabal, H.A., Seidl, M.F., Cappadona, G., & Segura, R. (2022). Induced resistance to Fusarium wilt of banana caused by tropical race 4 in Cavendish cv. Grand Naine bananas after challenging with avirulent *Fusarium* spp. *PLoS ONE*, 17(9), e0273335. <https://doi.org/10.1371/journal.pone.0273335>
- Guimarães, B. V. C., Donato, S. L. R., Aspiázú, I., & Azevedo, A. M. (2021). Yield prediction of 'Prata Anã' and 'BRS Platina' banana plants by artificial neural networks. *Pesquisa Agropecuária Tropical*, 51, e66008. <https://doi.org/10.1590/1983-40632021v5166008>
- Han, Z., Xiong, D., Schneider, R., & Tian, C. (2023). The function of plant PR1 and other members of the CAP protein superfamily in plant-pathogen interactions. *Molecular Plant Pathology*, 24(6), 651–668. <https://doi.org/10.1111/mpp.13320>
- Israeli, Y., Shabi E., & Slabaugh, W.R. (1993). Effect of banana spray oil on banana yield in the absence of Sigatoka (*Mycosphaerella* sp.). *Scientia Horticulturae*, 56(2), 107–117.
- Jayasinghe, S. L., Ranawana, C. J. K., Liyanage, I. C., & Kaliyadasa, P. E. (2022). Growth and yield estimation of banana through mathematical modelling: A systematic review. *The Journal of Agricultural Science*, 160(3–4), 152–167. <https://doi.org/10.1017/S0021859622000259>
- Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics*, 23(10), 1289–1291.
- Leiva-Mora, M., Portal, O., Alvarado-Capó, Y., Acosta-Suárez, M., Cruz-Martínez M., Sánchez-García, C., Rojas, L. E., & Roque, B. (2013). Identificación molecular de aislados cubanos de *Mycosphaerella fijiensis* Morelet. *Revista de Protección Vegetal*, 28, 229–231. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522013000300012&nrm=iso
- Lepesheva, G. I., & Waterman, M. R. (2004). CYP51 the omnipotent P450. *Molecular Cellular Endocrinology*, 215, 165–170. <https://doi.org/10.1016/j.mce.2003.11.016>
- Levene, H. (1960). Robust tests for equality of variances. In I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, & H. B. Mann (Eds.), *Contributions to probability and statistics: Essays in honor of Harold Hotelling* (pp. 278–292). Stanford University Press.
- Li, C., Wang, P., Menzies, N., Lombi, E., & Kopittke, P. M. (2018). Effects of methyl jasmonate on plant growth and leaf properties. *Journal of Plant Nutrition and Soil Science*, 181, 409–418. <https://doi.org/10.1002/jpln.201700373>
- Liljeroth, E., Bengtsson, T., Wiik, L., & Andreasson, E. (2010). Induced resistance in potato to *Phytophthora infestans*-effects of BABA in greenhouse and field tests with different potato varieties. *European Journal of Plant Pathology*, 127(2), 171–183. <https://doi.org/10.1007/s10658-010-9582-4>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, 25(4), 402–408. <https://doi.org/10.1006/meth.2001.1262>
- Marín, D. H., Romero, R. A., Guzmán, M., & Sutton, T. B. (2003). Black Sigatoka: An increasing threat to banana cultivation. *Plant Disease*, 87(3), 208–222. <https://doi.org/10.1094/PDIS.2003.87.3.208>
- Menéndez, E., Navarro, J., López, J., & Dalmau, A. (2020). Mecanismos de defensa en plantas. Proteínas relacionadas con la patogenicidad. *Revista de Investigaciones de la Universidad Le Cordon Bleu*, 7(2), 98–109. <https://doi.org/10.36955/RIULCB.2020v7n2.010>
- Mogollón-Ortiz, Á., & Castaño-Zapata, J. (2011). Efecto de inductores de resistencia en plántulas de plátano dominico hartón (*Musa balbisiana* AAB) contra *Mycosphaerella* spp. *Revista De La Academia Colombiana De Ciencias*, 35(137), 463–471.
- Mogollón-Ortiz, Á., & Castaño-Zapata, J. (2012). Evaluación *in-vitro* de inductores de resistencia sobre *mycosphaerella fijiensis* morelet. *Revista Facultad Nacional De Agronomía, Medellín*, 65(1), 6327–6336.
- Monsalve, J.V., Rosero, S.E.V., & Cárdenas, N.J.R. (2012). Efecto del fosfito de potasio en combinación con el fungicida metalaxyl + mancozeb en el control de mildew vellosa (*Peronospora destructor* Berk) en cebolla de bulbo (*Allium cepa* L.). *Revista Facultad Nacional de Agronomía Medellín*, 65(1), 6317–6325.
- Noar, R., Thomas, E., & Daub, M. (2022). Genetic characteristics and metabolic interactions between *Pseudocercospora fijiensis* and banana: Progress toward controlling Black Sigatoka. *Plants*, 11, 948. <https://doi.org/10.3390/plant11070948>
- Oliveira, M., Varanda, C., & Félix, M. (2016). Induced resistance during the interaction pathogen x plant and the use of resistance inducers. *Phytochemistry Letters*, 15, 152–158. <https://doi.org/10.1016/j.phytol.2015.12.011>
- Patino-Hoyos, L. (2001). Efecto de una fuente de energía, tres inductores de resistencia y un sustrato foliar sobre Sigatoka negra en banano. Master of Science Thesis. CATIE, Turrialba, Costa Rica. pp. 75–84. <https://repositorio.catie.ac.cr/handle/11554/4912>.
- Patrício, F., Almeida, I. M., Barros, B. C., Santos, A., & Frare, P. M. (2007). Effectiveness of acibenzolar-S-methyl, fungicides and antibiotics for the control of brown eye spot, bacterial blight, brown leaf spot and coffee rust in coffee. *Annals of Applied Biology*, 152, 29–39. <https://doi.org/10.1111/j.1744-7348.2007.00187.x>
- Pérez-Peláez, S. (2018). Efecto del ácido jasmónico, etefón y benzoiladiazol exógeno en la respuesta del cultivar 'Gran naine' (*Musa AAA*) a la Sigatoka negra. Undergraduate thesis. Trabajo de diploma. Universidad Central "Marta Abreu" de Las Villas, Facultad de Ciencias Agropecuarias, Departamento de Biología. Santa Clara. Villa Clara. Cuba.
- Portal, O., Izquierdo, Y., De Vleeschauwer, D., Sánchez-Rodríguez, A., Mendoza- Rodríguez, M., Acosta-Suárez, M., Ocaña, B., Jiménez, E., & Hofte, M. (2011). Analysis of expressed sequence tags derived from a compatible *Mycosphaerella fijiensis*-banana interaction. *Plant Cell Reports*, 30(5), 913–928.

- Ramírez, C., Castaño, J., Villegas, B., & Aristizábal, M. (2012). Effect of chemical inducers of disease resistance to Black(*Mycosphaerella fijiensis* Morelet) and yellow Sigatoka (*Mycosphaerella musicola* Leach) in plantain. *Revista U.D.C.A Actualidad & Divulgación Científica*, 15(12), 363–371.
- Ramírez, G., Muñoz, M., Patiño, L., & Morales, J. (2015). Banana Moko disease management with resistance inducers and chlorine dioxide. *Agronomía Colombiana*, 33(2), 194–202. <https://doi.org/10.15446/agron.colomb.v33n2.48663>
- Reglinski, T., Havis, N., Rees, H. J., & de Jong, H. (2023). The practical role of induced resistance for crop protection. *Phytopathology*, 113(4), 719–731. <https://doi.org/10.1094/PHYTO-10-22-0400-IA>
- Rodríguez, H. A., Rodríguez-Arango, E., Morales, J. G., Kema, G., & Arango, R. E. (2016). Defense gene expression associated with the biotrophic phase of *Mycosphaerella fijiensis* M. Morelet Infection in Banana. *Plant Disease*, 100, 1170–1175. <https://doi.org/10.1094/PDIS-08-15-0950-RE>
- Rodríguez, H. A., Hidalgo, W. F., Sánchez, J. D., Menezes, R. C., Schneider, B., Arango, R. E., & Morales, J. G. (2020). Differential regulation of jasmonic acid pathways in resistant (Calcutta 4) and susceptible (Williams) banana genotypes during the interaction with *Pseudocercospora fijiensis*. *Plant Pathology*, 69(5), 872–882. <https://doi.org/10.1111/ppa.13165>
- Sánchez, J., & Mira, J. (2013). Principios para la nutrición del cultivo de banano. Asociación de Bananeros de Colombia AUGURA. <http://hdl.handle.net/20.500.12324/12593>.
- Sandroni, M., Liljeroth, E., Mulugeta, T., & Alexandersson, E. (2020). Plant resistance inducers (PRIs): Perspectives for future disease management in the field. *CAB Reviews*, 15(1), 1–10. <https://doi.org/10.1079/PAVSNNR202015001>
- Sun, Y., Huang, B., Cheng, P., Li, C., Chen, Y., Li, Y., Zheng, L., Xing, J., Dong, Z., & Yu, G. (2022). Endophytic *Bacillus subtilis* TR21 improves banana plant resistance to *Fusarium oxysporum* f. sp. *cubense* and promotes root growth by upregulating the jasmonate and brassinosteroid biosynthesis pathways. *Phytopathology*, 112(2), 219–231. <https://doi.org/10.1094/PHYTO-04-21-0159-R>.
- Terrero-Yepe, P.I., Peñaherrera-Villafuerte, S.L., Bustamante-Gonzales, A.J., Cedeño-García, G.A., Solórzano-Alcívar, R.F., & Cedeño-García, G.A. (2020). Induction of resistance to *Mycosphaerella fijiensis* Morelet and its relationship with yield of banana plants (*Musa* AAA) cv. Williams. *Revista ESPAMCIENCIA*, 11(2), 80–87. https://doi.org/10.51260/revista_espamciencia.v11i2.230
- Torres, J., Calderón, H., Rodríguez-Arango, E., Morales-Osorio, J. G., & Arango-Isaza, R. (2012). Differential induction of pathogenesis-related proteins in banana in response to *Mycosphaerella fijiensis* infection. *European Journal of Plant Pathology*, 133, 887–898. <https://doi.org/10.1007/s10658-012-0012-7>
- Turner, D.W. (1995). The response of the plant to the environment. In S. Gowen (Ed.), *Bananas and Plantains*. World Crop Series (pp. 206–229). Springer, Dordrecht. https://doi.org/10.1007/978-94-011-0737-2_9
- Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research*, 40(15), e115.
- Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science*, 44(6), 1920–1934. <https://doi.org/10.2135/cropsci2004.1920>
- Van der Plank, J. E. (1963). *Plant Diseases: Epidemics and Control*. Academic Press.
- Walters, D. R., Ratsep, J., & Havis, N. D. (2013). Controlling crop diseases using induced resistance: Challenges for the future. *Journal of Experimental Botany*, 64(5), 1263–1280. <https://doi.org/10.1093/jxb/ert026>
- Wang, Z., Jia, C., Li, J., Huang, S., Xu, B., & Jin, Z. (2015). Activation of salicylic acid metabolism and signal transduction can enhance resistance to *Fusarium* wilt in banana (*Musa acuminata* L. AAA group, cv. Cavendish). *Functional & Integrative Genomics*, 15(1), 47–62. <https://doi.org/10.1007/s10142-014-0402-3>.
- White, E. J., Venter, M., Hiten, N. F., & Burger, J. T. (2008). Modified cetyltrimethylammonium bromide method improves robustness and versatility: The benchmark for plant RNA extraction. *Biotechnology Journal*, 3, 1424–1428. <https://doi.org/10.1002/biot.200800207>
- Woomer, P. L., Bekunda, M. A., & Nkalubo, S. T. (1999). Estimation of banana yield based on bunch phenology. *African Crop Science Journal*, 7(4), 341–347.
- Yassin, M., Ton, J., Rolfé, S. A., Valentine, T. A., Cromey, M., Holden, N., & Newton, A. C. (2021). The rise, fall and resurrection of chemical-induced resistance agents. *Pest Management Science*, 77(9), 3900–3909. <https://doi.org/10.1002/ps.6370>
- Zhu, X., Lin, H., Si, Z., Xia, Y., Chen, W., & Li, X. (2016). Benzothiadiazole-mediated induced resistance to *Colletotrichum musae* and delayed ripening of harvested banana fruit. *Journal of Agricultural and Food Chemistry*, 64(7), 1494–1502. <https://doi.org/10.1021/acs.jafc.5b05655>
- Zuluaga-Amaya, C. M., Patiño-Hoyos, L. F., & Collazos-Villa, J. C. (2007). Integración de inducción de resistencia con bacterias quitinolíticas en el control de la Sigatoka negra (*Mycosphaerella fijiensis* Morelet) en banano. *Revista Facultad Nacional De Agronomía Medellín*, 60(2), 3891–3905.

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