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Banana Moko disease management with resistance inducers and chlorine dioxide

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Manejo del Moko en banano con inductores de resistencia y dióxido de cloro

Joaquín Guillermo Ramírez G.[1], Melissa Muñoz A.[1], Luis Fernando Patiño H.[2], and Juan Gonzalo Morales O.[1]

ABSTRACT

The plant disease Moko, caused by *Ralstonia solanacearum*, is the most important bacterial disease in banana and plantain crops worldwide. In the present study, chlorine dioxide and seven resistance inducers in banana plants (*Musa* sp.) infected with this bacterium were evaluated under greenhouse conditions. For the evaluation of chlorine dioxide, three doses were used (10, 30 and 50 mg L^{-1}). The evaluation of the resistance inducers included the following: sodium salicylate 0.4 g L^{-1}; hydrogen peroxide 1 mM; potassium phosphite 1.5 mL L^{-1}; 3-aminobutanoic acid 1.0 g L^{-1}; methyl jasmonate 0.2 g L^{-1}; acibenzolar-s-methyl 0.3 mL L^{-1} and chitosan 3.0 mg mL^{-1}. The results showed a significant reduction of 74% in the area under the disease progress curve (AUDPC) value, which was calculated for the disease development when the injected chlorine dioxide dose was 50 mg L^{-1}. The AUDPC value for the resistance inducers was reduced by 45.4% for chitosan, 75.5% for methyl jasmonate and 65.5% for 3-aminobutanoic acid. Therefore, the results indicated that these molecules have the potential to be used for control of the Moko disease.

Key words: banana bacterial pathogens, plant resistance inducers, disease management.

RESUMEN

La enfermedad denominada Moko, ocasionada por *Ralstonia solanacearum*, es la principal enfermedad de origen bacteriano en los cultivos de banano y plátano en todo el mundo. Este trabajo tuvo como objetivo la evaluación del dióxido de cloro y siete inductores de resistencia en plantas de banano (*Musa* sp.) infectadas con la bacteria bajo condiciones de invernadero. Para la evaluación del dióxido de cloro se utilizaron tres dosis (10, 30, 50 mg L^{-1}). Los inductores de resistencia utilizados y sus dosis fueron: salicilato de sodio: 0,4 g L^{-1}, peróxido de hidrógeno: 1 mM, fosfito de K: 1,5 g L^{-1}, ácido 3-aminobutanoico: 1,0 g L^{-1}, metil jasmonato: 0,2 g L^{-1}, acibenzolar-s-metil: 0,3 g L^{-1} y quitosano: 3,0 mg mL^{-1}. Los resultados muestran una reducción significativa del 74% en el valor del área bajo la curva del progreso de la enfermedad (ABCPE), cuando se utiliza dióxido de cloro en dosis de 50 mg L^{-1} aplicado por inyección. Para los inductores de resistencia se observa una reducción de 45,4% para quitosano, 75,5% para metil jasmonato y de 65,5% para el ácido 3-aminobutanoico en el ABCPE. Por lo tanto, los resultados indican que estas moléculas tienen el potencial para ser usadas en el control del Moko.

Palabras clave: patógeno bacterial del banano, inductores de resistencia en pantas, manejo de la enfermedad.

Introduction

Banana (*Musa* sp.) is an important economic, social and nutritional crop in the countries where it is produced or imported. This fruit is grown in countries with tropical climates and it has a wide variety of consumption possibilities that include fresh or processed products available for national consumption or export (Roldán *et al*., 2002; Sena, 2004). Colombia exports bananas more than any other fruit; therefore, bananas represent an important asset to the Colombian economy, not only for its contribution to the gross domestic product but also because it generates direct and indirect jobs (Augura, 2012).

The production of bananas, globally and in Colombia, faces several limitations, and one such limitation is disease, including "Moko disease or bacterial wilt," which is caused by *Ralstonia solanacearum* (Race 2) (Smith, 1993). In Colombia, this disease is important because of the ease of its dissemination, high aggressiveness, wide range of hosts, lack of effective management practices, high economic losses and eradication that requires long periods without cultivation or production. Additionally, misdiagnosis of the disease may lead to incorrect management (Buitrago, 2001; Arenas, 2004; Castañeda and Espinosa, 2005; Augura, 2012; Prieto *et al*., 2012).

New alternatives for the management of disease are constantly being researched, in particular for diseases of bacterial origin that exhibit difficult management (Agrios, 2005). Chlorine dioxide may be a novel option and it has

already been used in the field; however, systematic studies have not been performed that would provide scientific evidence of its benefits despite existing studies that have reported a reduction of the bacterial load in fruits and vegetables in the postharvest stage through the use of chlorine dioxide (Rodgers *et al.*, 2004). On the other hand, induced resistance can be used against a wide spectrum of diseases through the use of a plant's own resistance mechanisms and it has been widely studied (Herman *et al.*, 2007; Walters, 2009). This induced resistance is considered to be variable, with levels of disease control ranging from 4% to more than 90% (Vallad and Goodman, 2004; Walters *et al.*, 2005; Walters, 2009).

Because of the importance of the Moko disease (bacterial wilt) in the *Musaceae* family and the limited number of effective control strategies that currently exist, this study aimed to identify alternative strategies for the establishment of an integral disease management plan by evaluating several doses of chlorine dioxide and seven resistance inducers in the banana plant (*Musa* sp.) cultivar Williams infected with the bacterium *R. solanacearum* (Race 2) under greenhouse conditions.

Materials and methods

Location

The present study was performed in the greenhouse and Molecular Studies Laboratory of the Faculty of Agricultural Sciences in the Universidad Nacional de Colombia, Medellin (6º15'N, 75º35'W, 1,495 m a.s.l.).

Plant material

In-vitro banana seedlings cv. Williams (AAA) were obtained from the laboratory of Universidad Católica de Oriente, (Rionegro, Colombia). The seedlings were transferred to pots containing 3.5 kg of substrate (in a 3:1:1 ratio of soil, rice husk and quartz) that was previously autoclaved (103.42 kPa and 121ºC for two cycles of 1 h each). The seedlings were kept under greenhouse conditions (mean temperature 22ºC, relative mean humidity 85%, soil humidity 50% of the maximum humidity retention capacity and fertilization with Hoagland nutritive solution). The experiments started when the seedlings had 3 months of age.

Microorganisms

A strain of *R. solanacearum* (race 2) was provided by Centro de Investigaciones del Banano (CENIBANANO, Uraba-Colombia). The strain was replicated in the laboratory using a semi-selective culture medium, Kelman-TZC-Agar (K-TZC-A) (1 g 2,3,5-triphenyl-2H-tetrazolium chloride,

10 g dextrose, 10 g peptone, 1 g casamino acids and 18 g agar dissolved in 1 L H_2O) (Kelman, 1954).

Pathogen inoculation

The inoculation process was conducted following the method of Obregón *et al.* (2008), in which pure colonies grown in an K-TZC-A medium (Kelman, 1954) were re-grown in this same liquid medium (K-TZC) under constant shaking (150 rpm) for 24 h at 25ºC. The inoculation was performed using a 5 mL hypodermic syringe (Nipro, Osaka Japan) filled with a 2.5 mL solution containing an approximate concentration of $1 \cdot 10^8$ colony forming units (cfu) per mL, measured by spectrophotometry at 640 nm (NanoDrop 2000, spectrophotometer Thermo Scientific®, Wilmington, DE) (Smith, 1993; French *et al.*, 1995).

Evaluated treatments

Chlorine dioxide

Phytotoxicity test on the banana seedlings. To analyze the phytotoxicity, the pseudostem of the seedlings at 12 weeks of age was injected at the lower end with 50, 30 and 10 mg L^{-1} chlorine dioxide (Clodos®) using a hypodermic syringe. Each treatment was performed on five banana seedlings under greenhouse conditions and a control without treatment was included. Possible toxicity symptoms such as discoloration, sagging, abnormal growth, leaf burn and wilt and the height and diameter at the base of the stem were recorded weekly. Each treatment was applied weekly on the same plants for 3 weeks (days 0, 7 and 14).

In vitro **inhibition test of chlorine dioxide on *R. solanacearum*.** The test in a liquid medium was performed in 50 mL Falcon tubes containing 15 mL of K-TZC medium (Kelman, 1954) under shaking at 150 rpm and at 25-30ºC. For the growth test in a solid media, five petri dishes for each chlorine dioxide concentration tested that contained 15 mL of K-TZC-A medium (Kelman, 1954) were placed under conditions of 12 h light and 12 h darkness at 25-30ºC. In each medium type, five chlorine dioxide doses (0, 10, 30, 50 and 70 mg L^{-1}) were evaluated for *R. solanacearum*. To analyze the effect of the different doses in the liquid media, the bacterial concentration was measured by spectrophotometry (640 nm) at 3, 6, 9, 12, 18, 24, 30 and 36 h after inoculation. For the solid media, the effect of the different doses was evaluated using cfu counts over the 6 d after the start of the experiment.

Chlorine dioxide tests on the banana seedlings infected with *R. solanacearum*. Based on the *in vitro* evaluation and phytotoxicity tests, three chlorine dioxide doses (10, 30 and 50 mg L^{-1}) were injected into the stem (I) and drenched

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(D) in the soil as previously described. The treatment was applied when the first external symptoms were visualized corresponding to the necrosis of the candle leaf, according to the disease rating scale reported by Silva *et al.* (2000).

Resistance inducers

Phytotoxicity test on the banana seedlings. To evaluate possible problems associated with high doses of inducers, phytotoxicity tests were performed for the banana seedling 'Williams'. Symptoms such as discoloration, sagging, abnormal growth, leaf burn and wilt were evaluated and the height and diameter at the base of the stem were measured. The tests included two or three doses of each inducer applied to the leaves as follows: sodium salicylate (SaNa) (0.4, 0.8 and 1.2 g L^{-1}), hydrogen peroxide (H$_2$O$_2$) (1.2 and 3.0 mM), potassium phosphite (FosK) (1.5, 2.0 and 2.5 mL L^{-1}), 3-aminobutanoic acid (BABA) (1.0, 1.5 and 2.0 g L^{-1}), methyl jasmonate (MeJa) (0.2, 0.4 and 0.6 g L^{-1}), acibenzolar-s-methyl (ASM) (0.3, 0.6 and 0.9 mL L^{-1}) and chitosan (Qt) (3.6 and 9.0 mg mL^{-1}).

Effect of different resistance inducers on the banana seedlings infected with *R. solanacearum*. Based on the phytotoxicity tests, the following doses of resistance inducers were applied: SaNa 0.4 g L^{-1}; H$_2$O$_2$ 1.0 mM; FosK 1.5 mL L^{-1}; BABA 1.0 g L^{-1}; MeJa 0.2 g L^{-1}; ASM 0.3 mL L^{-1}; and Qt 3.0 mg mL^{-1}. The products were applied on the leaves using a manual pressure sprayer (10.34 kPa) until run-off, 24 h prior to the inoculation and on the 10th d after the start of treatment.

Variables evaluated for the chlorine dioxide and resistance inducer experiments. For both experiments, the development of the disease was evaluated using the external symptoms rating scale reported by Silva *et al.* (2000), which establishes five stages: 1) no symptoms, 2) necrosis of the candle leaf, 3) yellowing of 2-3 leaves, 4) buckle of the petiole, 5) death of the plant. Based on the data obtained from the evaluations, the area under the disease progress curve (AUDPC) was calculated (Madden *et al.*, 2007) and its dynamics were recorded over time. At the end of the experiment, the dry biomass was measured (dried in an oven at 60°C for 72 h). The data were recorded every three days until the inoculated controls reached stage 5 of the disease corresponding to death of the plants. At the end of the experiments, the presence of the pathogen was verified by dissecting the tissue and re-isolating it on K-TZC-A (Kelman, 1954).

Statistical analysis

A completely randomized experimental design was employed, with one plant as the experimental unit and five replicates used for each treatment. Two replicates in time were performed for each experiment. The homoscedasticity and normality of the data were analyzed ($P \leq 0.01$) using the Levene and Kolmogorov-Smirnov tests, respectively. Subsequently, an analysis of variance (ANOVA) and mean separation test were performed using Tukey's test with a significance level of 99%. The data were analyzed using the statistical software SAS® v.9.1.

Results and discussion

Chlorine dioxide

Chlorine dioxide phytotoxicity test on the banana seedlings. No phytotoxicity symptoms were found when the seedlings were treated with all three chlorine dioxide doses (10, 30 and 50 mg L^{-1}). In fact, the seedlings treated with chlorine dioxide had improved growth as compared to the control ($P \leq 0.01$), showing the higher values at 30 mg L^{-1} (Fig. 1A). Although Cl$^-$ is not essential to plants and is not required in high concentrations, it is considered beneficial because it may help with photosynthesis and water regulation in the plant (Rodgers *et al.*, 2004). However, the dynamics of Cl$^-$ within the plant and dose applications in the field must be assessed so that chlorine dioxide may be correctly used with no adverse effects.

***In vitro* inhibition test of chlorine dioxide on *R. solanacearum*.** The results obtained in the *in vitro* inhibition test in the liquid media showed that the treatments using 50 and 70 mg L^{-1} chlorine dioxide had an immediate bactericidal effect, because an increase in the optical density was not observed for these doses, as compared to the non-treated control, which may indicate that no bacterial growth occurred (Fig. 1B). However, the 10 and 30 mg L^{-1} doses had an inhibitory effect that was only limited to the 1st h of treatment. At 9 h after the start of the treatment, an increase in bacterial concentration was observed and these results were confirmed in the solid media test (Tab. 1); the 50 and 70 mg L^{-1} dose treatments showed no bacterial growth, which was indicated by the absence of cfus, whereas microbial growth was evident in the 30, 10 mg L^{-1} and control treatments (Fig. 1C).

The results suggested that chlorine dioxide is a highly reactive and efficient product in the initial stages of the plant-pathogen interaction, but if the correct dose is not delivered, the product cannot eliminate subsequent growth of the bacterial population. The action of chlorine dioxide on bacteria is considered to be direct and its chemical form allows it to penetrate the bacterial membrane and kill the organism (Junli *et al.*, 1997).
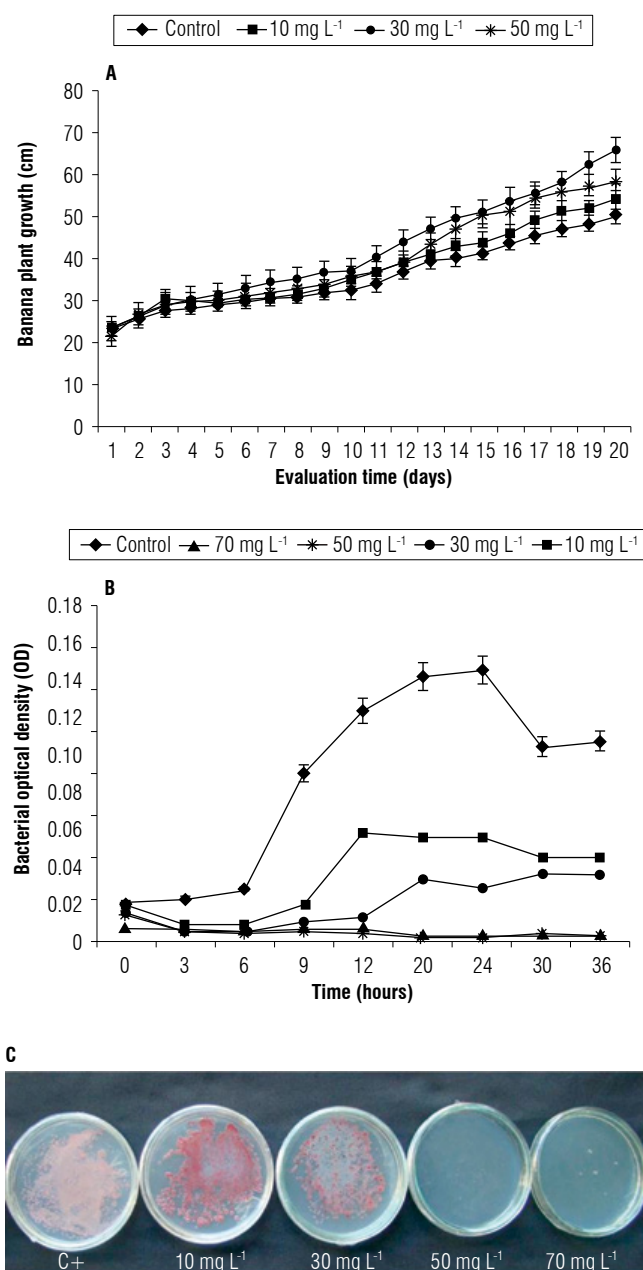
FIGURE 1. Effect of different concentrations of chlorine dioxide on the banana seedlings and the *R. solanacearum* isolate. A, height (cm) of *Musa* sp. treated with three doses of chlorine dioxide; B, optical density measured by NanoDrop spectrophotometer of *Ralstonia solanacearum* liquid cultures supplemented with four doses of chlorine dioxide; C, *Ralstonia solanacearum* growth in TZC solid medium supplemented with five different doses of chlorine dioxide. Error bars represent the standard error (*n* = 5).

Chlorine dioxide tests on the banana seedlings infected with *R. solanacearum*. Significant differences ($P \leq 0.01$) were found between the treatments with respect to the AUDPC (Fig. 2A), with which Tukey's test clustered the treatments into four different groups. The first group corresponded to the inoculated control (C+) that had the highest disease development. The second group corresponded to the 10 and 30 mg L^{-1} injections of chlorine dioxide and the 10, 30 and 50 mg L^{-1} chlorine dioxide drenched soil that had a medium-level AUDPC value. The third group corresponded to the 50 mg L^{-1} injections of chlorine dioxide and it had the lowest disease development. Finally, the last group was formed by the non-inoculated control, where no disease was found.

The inoculated control exhibited a significantly ($P \leq 0.01$) higher disease severity at the second week of evaluation than the other treatments (Fig. 2B). This same tendency occurred up to week five, where no statistically significant differences were found ($P \leq 0.01$) between the inoculated controls and plants subjected to the 10 and 30 mg L^{-1} chlorine dioxide drenched soil treatments. At week seven, the plants treated with chlorine dioxide at 10, 30 and 50 mg L^{-1} in drenched soil, 10 mg L^{-1} injections and inoculated controls showed no statistically significant differences ($P \leq 0.01$) in the dynamics of the disease severity, which was evaluated up to plant death. However, the 30 and 50 mg L^{-1} injections at the pseudostem showed that the speed of disease growth was significantly slower ($P \leq 0.01$), as compared with the other treatments. Here, the 50 mg L^{-1} chlorine dioxide injection in the stem showed the lowest response, confirming the data found for the AUDPC variable (Fig. 2A).

Regarding the total biomass variable (Fig. 2C), the inoculated control plants, plants treated with chlorine dioxide at 10 and 30 mg L^{-1} (both injected and in drenched soil) and plants treated with 50 mg L^{-1} drenched soil showed a significantly lower total biomass ($P \leq 0.01$) compared to the non-inoculated controls and 50 mg L^{-1} injection treatment. The 50 mg L^{-1} injection treatment achieved an increase in biomass despite the bacterial infection. The results obtained for the AUDPC, disease dynamics and biomass are visually confirmed in Fig. 3 A, B and C, where the best health stage can be observed in the plants injected with 50 mg L^{-1} chlorine dioxide at the pseudostem.
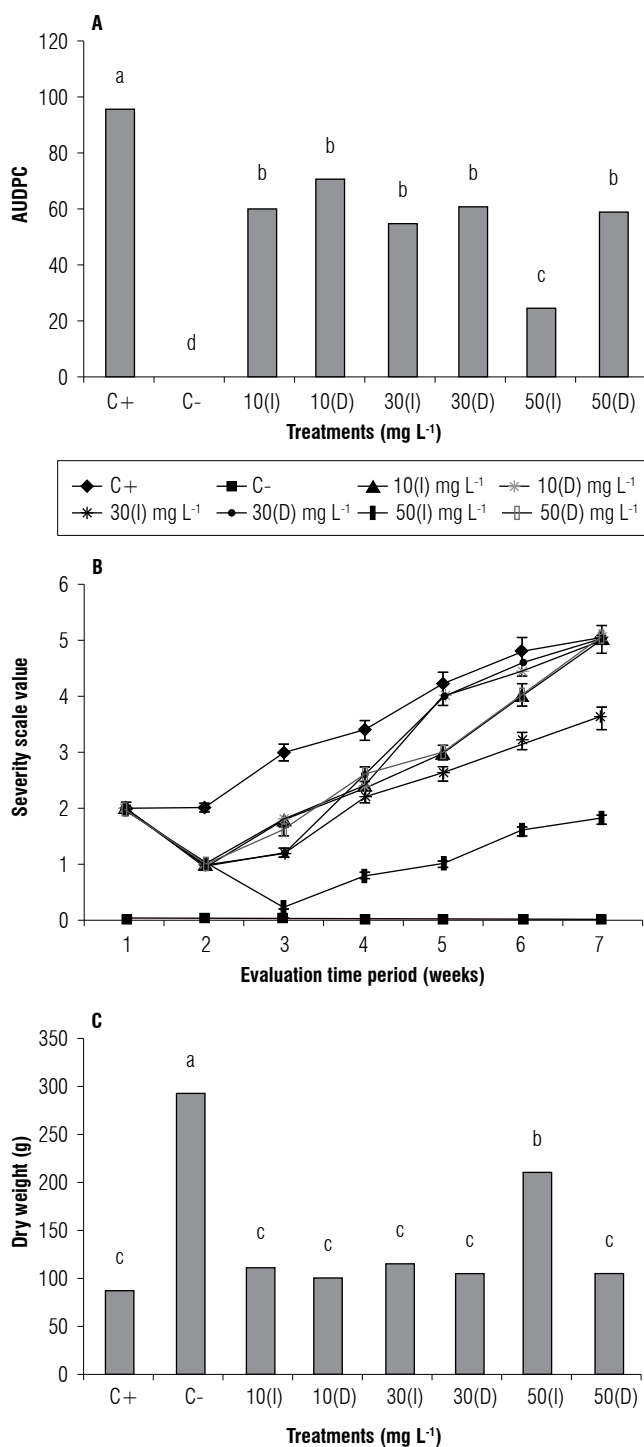
TABLE 1. *In vitro* growth of *R. Solanacearum* treated with several doses of chlorine dioxide.

Treatment	Control +	10	30	50	70
			mg L^{-1}		
Colony forming units	$1 \cdot 10^7$ a	$1 \cdot 10^6$ a	$1 \cdot 10^2$ b	0 c	0 c

The isolates were incubated at 25-30ºC under 12 h of light and 12 h of darkness. The cfu were recorded at 6 d after the start of the experiment. Average values were calculated using five replicates. Different letters represent statistical differences according to the Tukey test ($P \leq 0.01$).

FIGURE 3. Banana plants inoculated with *R. solanacearum* and treated with different chlorine dioxide doses. A, healthy plants control (C-); B, plants injected with different doses of chlorine dioxide; C, plants drenched with different doses of chlorine dioxide. C+, plants inoculated with *R. solanacearum* without chlorine dioxide. Pictures represent the observed effect on five replicates.

FIGURE 2. Effect of different doses of chlorine dioxide on the banana plants inoculated with *R. solanacearum*. A, area under disease progress curve (AUDPC) of banana plants inoculated with *R. solanacearum* injected (I) or drenched (D) with different concentrations of chlorine dioxide; B, severity of the Moko disease on plants injected (I) or drenched (D) with different concentrations of chlorine dioxide; C, dry weight of banana plants (g) inoculated with *R. solanacearum* injected (I) or drenched (D) with different concentrations of chlorine dioxide. C+, banana plants inoculated with *R. solanacearum* without chlorine dioxide applications; C- healthy banana plants. Means with different letters indicate significant differences according to the Tukey test ($P \leq 0.01$) ($n = 5$).
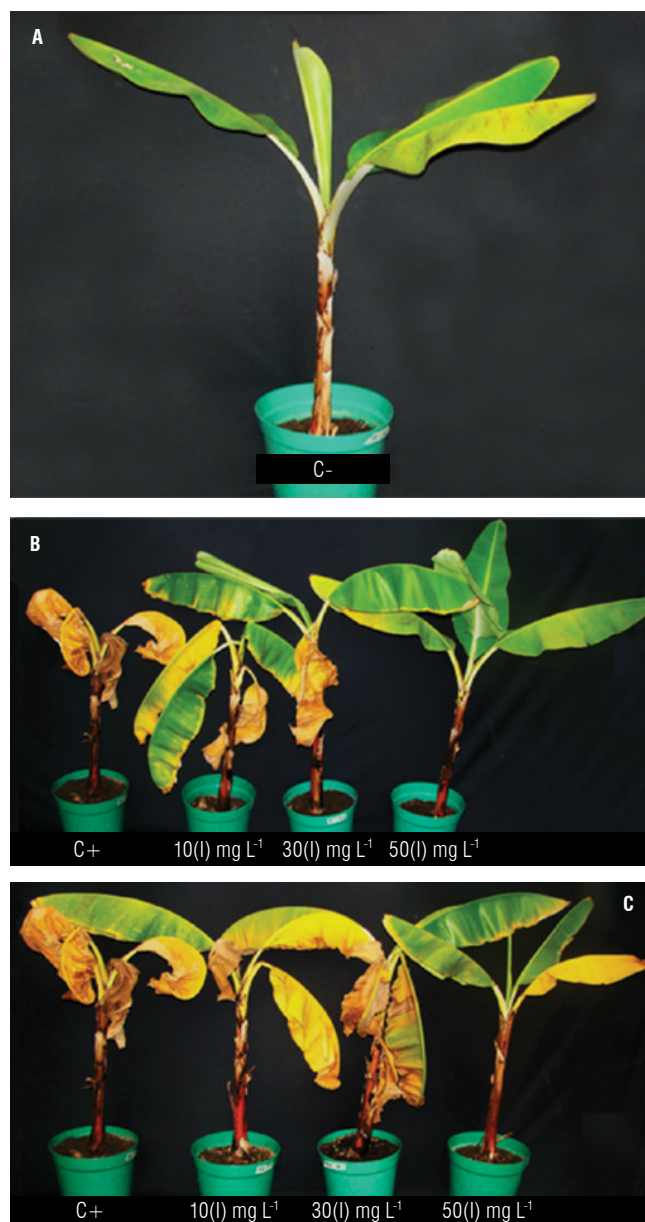
The results found in this study suggest a potential use for chlorine dioxide as a bactericidal agent when 50 mg L^{-1} is injected in the pseudostem of banana plants infected with *R. solanacearum*. This compound achieves a reduction in the disease development and improves a plant's growth by increasing its biomass. Therefore, this product can be considered an alternative for the integral management of this crop; however, these results must be verified under field conditions to establish the efficacy of the treatment. In

addition, experiments with repeated doses over time must be performed to determine if the total eradication of the bacteria can be achieved in sick plants. Also, it is important to evaluate the treatment with drenched soil because, as this study demonstrated, this chlorine dioxide treatment was not particularly effective and its broad spectrum action can affect native bacterial populations in the soil, which might lead to an imbalance with negative consequences for the agroecosystem.

Studies reporting the direct action of chlorine dioxide in the management of plant diseases are scarce because, in most situations, this product has been evaluated in the post-harvest stage as a disinfectant, where it produces excellent results, such as those reported by Singh *et al.* (2002), who managed to reduce the bacterial load of *Escherichia coli* 0157:H7 in lettuce leaves using this compound. These results are consistent with the results reported by Zhang and Faber (1996), who demonstrated that 5 mg L^{-1} chlorine dioxide can eliminate a higher load of *Listeria monocytogenes* in lettuce leaves, as compared to water washing. However, Rodgers *et al.* (2004) reported that chlorine dioxide at 3 and 5 mg L^{-1} had a direct effect on *Escherichia coli* O157:H7 and *Listeria monocytogenes* in apple, lettuce and strawberry. In banana Ramirez *et al.* (2014) reported the use of Chlorine dioxide in doses of 10, 20 and 30 mg L^{-1} to reduce the incidence and severity of the soft rot of the pseudo-stem cause by Dickeya sp.

Evaluation of resistance inducers in plants inoculated with *R. solanacearum*

Phytotoxicity test on the banana seedlings. The two higher doses of all of the resistance inducers used caused a degree of phytotoxicity that presented burned, curling and spotted leaves and delayed growth; therefore, the lower doses were used.

Effect of resistance inducers in the banana plants inoculated with *R. solanacearum*. Based on the Tukey test, no statistically significant differences (*P*≤0.01) were found for the AUDPC of the plants that were treated with FosK, Sana, H$_2$O$_2$ and ASM; these inducers only managed to reduce the expression of the pathogen compared with that of the inoculated control (Fig. 4A). However, the Qt treatment showed a significant reduction (*P*≤0.01) in the disease compared with the inoculated control and the inducers SaNa and FosK. However, this reduction was lower than that of the MeJa and BABA inducers, which produced the highest decrease in the AUDPC (Fig. 4A). In the non-inoculated control plants, a pathogen development was not observed. The results obtained for the AUDPC variable
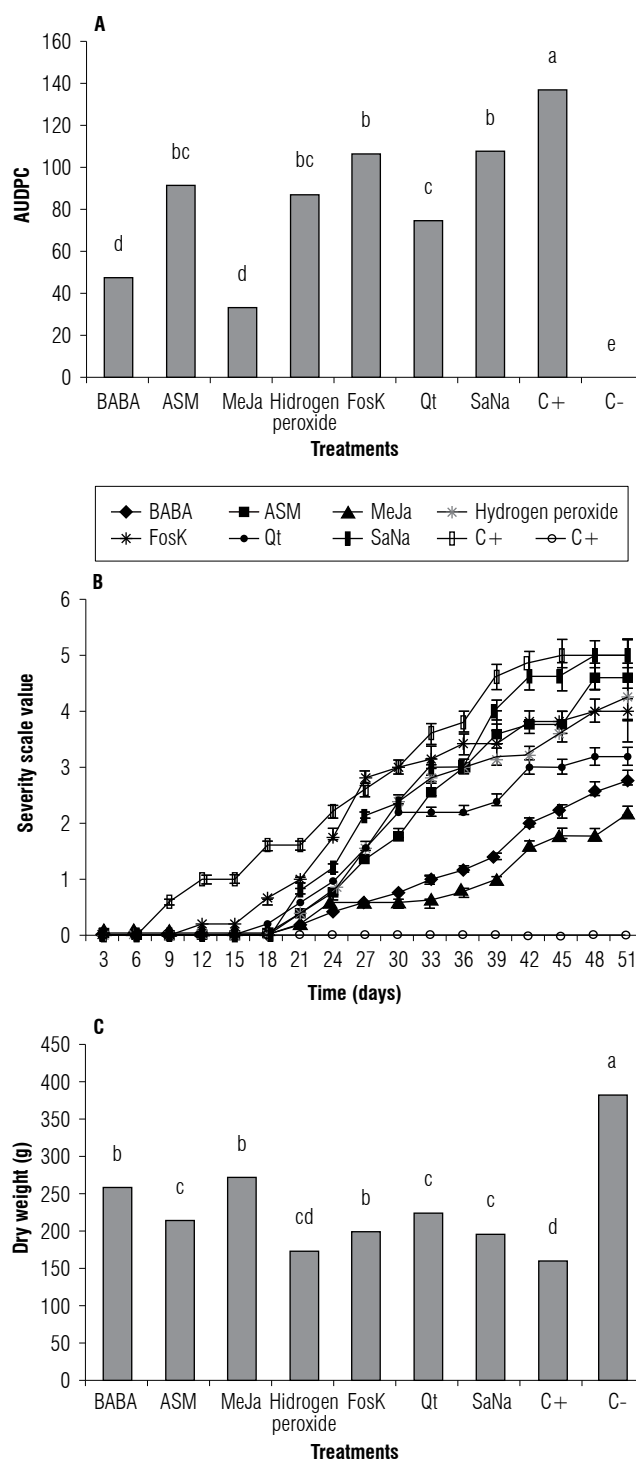

FIGURE 4. Effect of resistance inducers in the banana plants inoculated with *R. solanacearum*. A, AUDPC values for banana plants under the different applied treatments; B, severity of the Moko disease on the plants treated with resistance inducers; C, dry weight of the banana plants (g) inoculated with *R. solanacearum* treated with resistance inducers. C+, banana plants inoculated with *R. solanacearum* without resistance inducers; C-, non-inoculated healthy banana plants. Means with different letters indicate significant differences according to the Tukey test (*P*≤0.01) (*n* = 5). BABA, 3-aminobutanoic acid; ASM, acibenzolar-s-methyl; MeJa, methyl jasmonate, hydrogen peroxide; FosK, potassium phosphite; Qt, chitosan; SaNa, sodium salicylate.

were consistent with the results found for the dynamics of the disease over time (Fig. 4B), in which it was evident that the treatments with MeJa (75.5% reduction in the AUDPC value) and BABA inducers (65.5% reduction in the AUDPC value) were more effective at controlling *R. solanacearum* because they caused a lower progression of the disease. The Qt inducer was in the middle (45.4% reduction in the AUDPC value), whereas FosK, H₂O₂ and ASM were only inferior compared with the inoculated control, which showed no statistical differences with the SaNa inducer. The plants under the MeJa and BABA treatments reached a higher dry biomass (*P*≤0.01) than the inoculated control and the other inducers tested, but had a lower dry biomass than the healthy non-inoculated control (Fig. 4C), suggesting partial compensation for the detrimental effects of Moko disease.

Despite the inducers, FosK, H₂O₂, SaNa and ASM showed less disease (*P*≤0.01), as compared with the inoculated control plants; the disease dynamics indicated that there was a rapid increase of disease severity in the plants, which indicates that the effect of these inducers in delaying the pathogen was minimal and that they did not increase biomass.

The treatments with the Qt, MeJa and BABA inducers reduced *R. solanacearum* in the banana plants and demonstrated the potential use of these products in the management of this complex disease. Qt has been used as an inducer for other diseases and its effect on the reduction of diseases such as *Botrytis cinerea* in grapevine, *P. infestans* and *P. nicotianae* in potato and tobacco and *R. solanacearum* in red-ripe tomato has been identified (Ait Barka

FIGURE 5. Banana plants inoculated with *R. solanacearum* and treated with different resistance inducers. A, methyl jasmonate (MeJa); B, 3-amino-butanoic acid (BABA); C, chitosan (Qt); D, acibenzolar-s-methyl (ASM); E, potassium phosphite (FosK); F, hydrogen peroxide; G, sodium salicylate (SaNa). Pictures represent the observed effect on five replicates. C+, *R. solanacearum* inoculated plants without resistance inducer; C-, healthy plants.

et al., 2004; Ozeretskovskaya *et al.*, 2009; González *et al.*, 2009; Falcón-Rodríguez *et al.*, 2011). Regarding the Baba inducer and its effect on the reduction of *R. solanacearum* in banana, its action may be caused by its association with the accumulation of Pathogenesis-Related proteins in tissues treated before contact with the pathogen (Jakab *et al.*, 2001; Siegrist *et al.*, 2000). In practical studies, the BABA inducer has presented an effect on the control of *Fusarium solani* in potato (Olivieri *et al.*, 2009; Yin *et al.*, 2010). Also, the MeJa inducer has been the subject of fewer studies and its effect is considered to be the result of the rapid induction of hydrogen peroxide (H_2O_2) during the early stages of the infection process (Zhu and Tian, 2012), which would explain its ability to decrease the pathogenic effect of *R. solanacearum* in inoculated banana plants.

It is important to understand that the action of resistance inducers is highly variable and depends on multiple factors (Walters, 2009). Therefore, the products that did not work under the conditions of this study cannot be discarded because, under different conditions, their effect may improve. Based on the results of this study, to define and promote the use of Qt, Meja and Baba in the control of *R. solanacearum*, the mechanisms by which these products achieve the observed effects must be identified. In addition, it is necessary to identify the basic aspects for their application in the field, including the correct dose and application frequency and their effects on growth and development, because these products require additional energy expenditures by the plant through the activation of several metabolic pathways (Walters, 2009). However, the effect of these products on sick plants could be analyzed without such energy expenditures because evaluations of the inducers in this study were performed 24 h prior to inoculation. This process would allow for assessments of a plant's healing ability and not the disease prevention effect that this study demonstrated.

Conclusions and perspectives

Chlorine dioxide, in 50 mg L^{-1} doses, injected in the banana seedlings (*Musa* sp.) under greenhouse conditions significantly reduced the development of the Moko disease caused by *R. solanacearum*. In addition, the potential use of chitosan, methyl jasmonate and 3-aminobutanoic acid was observed for the management of the disease. Field tests must be performed to validate the results found in this study and to determine the applicability of the treatments that showed favorable management of *R. solanacearum* as possible alternative strategies for integrated management of the disease.

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