Selection of resistance inducers for managing *Hemileia vastatrix* Berk. & Br. in coffee (*Coffea arabica* L.) seedlings

Selección de inductores de resistencia para el manejo de *Hemileia vastatrix* Berk. & Br. en plántulas de café (*Coffea arabica* L.)

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ABSTRACT

Coffee leaf rust (*Hemileia vastatrix*) is one of the most devastating coffee diseases, causing losses ranging from 23% to 50% of the crop. Chemical applications are the most employed control strategies in countries lacking resistant coffee varieties. In the search for new alternatives for integrated management, an *in vitro* and nursery evaluation protocol was developed for *Coffea arabica* cv. Caturra plants using the following resistance inducers: acibenzolar-*S*-methyl, salicylic acid, potassium phosphite, and Harpin protein. These compounds were tested at three concentrations, across three intervals between product application and pathogen inoculation, and in two response signaling pathways (local or systemic) to assess their effects on the *in vitro* germination of rust urediniospores and disease severity in 6-month-old coffee plants inoculated with the pathogen. In general, all compounds inhibited urediniospore germination and exerted disease control mediated mainly by the concentration of the product with biweekly application intervals and where local responses prevailed more than systemic ones. This study highlights the potential of these compounds as resistance inducers, especially for acibenzolar-*S*-methyl, where we observed the best effects on disease control. Our findings open new avenues for incorporating resistance inducers into integrated disease management programs to complement fungicide applications.

Key words: acibenzolar-*S*-methyl, coffee leaf rust, urediniospores, germination, disease severity.

RESUMEN

La roya del café (*Hemileia vastatrix*) es una de las enfermedades más devastadoras del café, causando pérdidas que van desde el 23% hasta el 50% del cultivo. Las aplicaciones químicas son las estrategias de control más comúnmente empleadas en países que carecen de variedades de café resistentes. En la búsqueda de nuevas alternativas para el manejo integrado, se desarrolló un protocolo de evaluación *in vitro* y en vivero para plantas de *Coffea arabica* cv. Caturra utilizando los siguientes inductores de resistencia: acibenzolar-S-metil, ácido salicílico, fosfito de potasio y proteína Harpin. Estos compuestos se probaron en tres concentraciones, en tres intervalos entre la aplicación del producto y la inoculación del patógeno, y en dos vías de señalización de respuesta (local o sistémica) para evaluar sus efectos sobre la germinación *in vitro* de las urediniosporas de la roya y la severidad de la enfermedad en plantas de café de 6 meses de edad inoculadas con el patógeno. En general, todos los compuestos evaluados inhibieron la germinación de las urediniosporas y ejercieron un control de la enfermedad mediado principalmente por la concentración del producto con intervalos de aplicación quincenal y en donde prevalecieron más las respuestas locales que las sistémicas. Este estudio destaca el potencial de estos compuestos como inductores de resistencia, especialmente del acibenzolar-S-metil, con el que se observaron los mejores efectos en el control de la enfermedad. Los hallazgos abren nuevas vías para incorporar inductores de resistencia en programas de manejo integrado de enfermedades como complemento a las aplicaciones de fungicidas.

Palabras clave: acibenzolar-S-metil, roya del café, urediniosporas, germinación, severidad de la enfermedad.

Introduction

Coffee leaf rust, caused by the fungus *Hemileia vastatrix* Berkeley & Broome (Basidiomycota, Pucciniales), remains the primary disease affecting coffee crops worldwide (Koutouleas *et al*., 2024; Sera *et al*., 2022; Zambolim, 2016). In Colombia, it causes losses exceeding 30% in susceptible varieties of *Coffea arabica* when preventive control measures are not implemented (Rivillas *et al*., 1999). This disease is closely linked to the physiological development of the crop, plant yield, and the region's rainfall distribution and amount (Rivillas *et al*., 2011). Damage from coffee rust is

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caused by a significant reduction in the photosynthetic area due to lesions and generalized defoliation caused by the pathogen (Avelino *et al*., 2015).

Infection of *H. vastatrix* begins with the germination of uredospores on the underside of the leaves, requiring optimal conditions of humidity, temperature, and low or an absence of light intensity (Haddad *et al*., 2009; Hernández-Amasifuen *et al*., 2023). Following germination, appressoria form on the stomata, allowing the penetrating hypha to enter the substomatal chamber. There, the hyphae develop haustoria, enabling intracellular colonization (Gichuru *et al*., 2012; Ramiro *et al*., 2009). During colonization, the hyphae intertwine within the substomatal cavities, eventually forming protosori. These mature into uredos over approximately three weeks, protruding through the stomata to form orange pustules that are visible structures characteristic of the infection on the leaf's underside (Salazar-Navarro *et al*., 2024; Talhinhas *et al*., 2017). Severe attacks result in excessive defoliation, exposing growth nodes on the plant branches and buds to direct radiation, leading to progressive cell death. This continuous cell death and necrosis of growth nodes affect potential plant yields during the current and subsequent harvest seasons (Kushalappa & Eskes, 2019).

Chemical control is a crucial component of integrated coffee rust management, particularly in *Coffea arabica* varieties such as Caturra and Typica, susceptible to the disease. In Colombia, chemical control is applied using cupric compounds or systemic fungicides such as triazoles and strobilurins (Rivillas *et al*., 2011). However, this approach can increase the presence of multiple rust races due to changes in pathogen virulence and dynamics in planting varieties with complete and partial resistance. This leads to new isolates infecting previously resistant materials (Rozo-Peña & Cristancho, 2010). The appearance of new races is often related to increased selective pressure on certain pathogenic races due to improper fungicide application, such as using higher or lower doses than recommended or increasing spraying frequency (Deising *et al*., 2008). Fortunately, until 2012, no rust races resistant to commonly used fungicides, such as triazoles, have been found in Colombia (Cristancho *et al*., 2012).

Induced resistance against plant pathogens represents an alternative disease control method, activating latent defense mechanisms in plants. This resistance can be triggered by biotic agents (plant extracts, microorganisms, or parts of these organisms) or abiotic agents (chemicals) (Cavalcanti *et al*., 2005; Reglinski *et al*., 2023; Resende *et al*., 2006). Induced resistance (IR) is divided into systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Van Loon, 1997). In IR, resistance operates systemically or locally in response to a pathogen causing necrotic lesions (hypersensitivity reaction) or through exogenous application of synthetic compounds primarily mediated by the salicylic acid (SA) metabolic pathway. Unlike SAR, ISR is mediated by jasmonic acid and ethylene metabolism triggered by beneficial or endophytic plant microorganisms or by the exogenous application of synthetic compounds (Flors *et al*., 2024; Pieterse *et al*., 2001). According to Gust *et al*. (2012), initiating plant defense processes against possible infection or colonization by microorganisms requires molecular dialogue between the organisms involved, considering 1) the perception of pathogen-associated molecular patterns (PAMPs) by plant pattern recognition receptors (PRRs) or 2) the perception of microbial pathovar-specific proteins by the receptors of the plant immune system (Akhter *et al*., 2021; Chisholm *et al*., 2006; Dangl & Jones, 2001; Spoel & Dong, 2012).

The success of induced resistance depends not only on the compound itself but also on factors such as 1) the concentration of the molecule, achieving the best effects without affecting the biology of the pathogen, 2) the prolonged duration of the inducing effect, and 3) the type of signaling for the immune response (Hönig *et al*., 2023).

Extensive research has been conducted to find and implement such inducers in integrated coffee disease management. Examples include extracts of rust-infected coffee leaves (Amaral *et al*., 2005, 2007; Barguil *et al*., 2005), suspensions of inactive spores from *H. vastatrix* (Costa *et al*., 2007; Leonel & Barros, 2013), foliar fertilization with phosphites or potassium silicates (Carré-Missio *et al*., 2009, 2012; Costa *et al*., 2007; Lopes *et al*., 2013; Mehta *et al*., 2022; Pereira *et al*., 2009), or the foliar application of exogenous molecules such as ASM (Fernandes *et al*., 2013; Galdeano *et al*., 2010; Ito *et al*., 2024; Marchi *et al*., 2002; Patrício *et al*., 2008), harpin proteins (Chuang *et al*., 2014; de Capdeville *et al*., 2003; Galdeano *et al*., 2010; Sands *et al*., 2022), xanthan gum (Guzzo *et al*., 1993; Hassanisaadi *et al*., 2025), and SA (Berumen *et al*., 2015; McLaughlin *et al*., 2024; Mogollón Ortiz & Castaño Zapata, 2023), etc.

Despite using conventional management strategies, such as the recurrent application of fungicides and the planting of resistant varieties, disease control remains a challenge due to the genetic variability of the pathogen and the favorable environmental conditions for its development. In this context, resistance-inducing products represent a promising alternative within integrated management programs. However, even though these types of exogenous

compounds have been widely studied and evaluated in a wide variety of pathosystems in Colombia, the information on the efficacy of these products in coffee cultivation is limited and poorly verified; this makes it challenging to integrate and adopt them in integrated coffee rust management programs at a national level. Therefore, this research seeks to generate knowledge by evaluating four molecules currently marketed in the country as resistance inducers and their efficiency in controlling coffee rust. The results will contribute to the development of innovative and sustainable strategies for disease management, benefiting producers by reducing the use of agrochemicals and strengthening the resilience of coffee plantations in the face of this crucial phytosanitary problem.

Materials and methods

Four molecules with high potential for induced resistance were selected with documented results for managing and controlling foliar diseases in economically important crops. All evaluation parameters of these molecules were designed according to selection criteria proposed by Steiner and Schönbeck (1995). They classified as inducing agents all compounds that met the following characteristics: 1) absence of direct toxic effects by the inducer on pathogen germination, 2) increase in the magnitude of resistance, regardless of the applied concentration, 3) prolonged duration of the inducing effect between the expression of resistance and plant exposure to the pathogen, and 4) systemic response. The protocol development was proposed in the following two stages:

Effect of the inducer on *H. vastatrix* urediniospore germination

Aqueous solutions of each inducer treatment were prepared at three different doses, considering the concentration of the active ingredient in the commercial product (Tab. 1). The pH of the water used ranged between 7.05 and 7.10. The source products were ASM (BION 50WG®, Syngenta Crop Protection LLC, Greensboro, NC, USA), SA (Re-Leaf®, Stoller International Inc., Houston, TX, USA), Potassium phosphite (PhytoGard Phosphyte®, Stoller International Inc., Houston, TX, USA), and Harpin protein (Messenger®, Plant Health Care, Holly Springs, NC, USA).

Coffee leaves (*Coffea arabica* var. Caturra) were collected from experimental plots at the Naranjal Central Station of Cenicafé (Chinchiná, Caldas) to obtain the rust inoculum. The leaves exhibited characteristic symptoms and signs of the disease. They were free of biological control agents such as *Simplicillium* sp. and *Lecanicillium* sp. mycoparasites of rust uredospores that are visually easy to identify due to the whitish color of the rust pustules. Subsequently, the leaves were stored in paper bags and taken to the Phytopathology Laboratory at the National Research Center of Coffee (Cenicafé), where they were placed for 48 h in moist chambers at 25°C in the dark to preserve the inoculum. Once the lesions showed abundant sporulation, the urediniospores were harvested by scraping the undersides of the leaves with a #22 scalpel blade and then storing them in gelatin capsules at room temperature for no longer than 24 h.

Inoculum solutions were prepared at a rate of 0.3 mg ml^{-1} urediniospores in sterile distilled water, yielding a final concentration of approximately 4000 urediniospores ml^{-1}. These solutions were homogenized by ultrasound for 30 s at 50 Hz and maintained under constant stirring. Ten reading units were subsequently evaluated, each consisting of 4 µl of the inducing compound solution plus 4 µl of the suspension containing homogenized urediniospores. These tests were performed on slides covered with Parafilm® M. Once all the slide covers were prepared, they were placed in moist chambers at a temperature of 22°C in complete darkness. After 4 h, 2 µl of lactophenol cotton blue dye was added to each slide to stop germination and facilitate counting germinated and non-germinated spores using an Axio Lab. A1 stereomicroscope (Zeiss Company, Oberkochen, Germany) located in the Phytopathology Laboratory at Cenicafé. All processes of counting germinated and non-germinated spores were supported by Carl Zeiss® ZEN image acquisition software and processed using an algorithm designed for this purpose in ImageJ® (version 1.49).

TABLE 1. Products and doses used.

Inducer (treatment)	Active ingredient per ml of water		
	Low dose	Recommended dose*	High dose
Acibenzolar-*S*-methyl (ASM)	50 µg	200 µg	800 µg
Salicylic acid (SA)	0.3 µl	1.2 µl	5.0 µl
Potassium phosphite	0.2 µl	0.8 µl	3.3 µl
Harpin	7.5 µg	30 µg	120 µg

*According to the product manufacturer.

Spores were considered germinated when they exhibited a germinative tube measuring more than one-third of the diameter of the urediniospore (Kushalappa & Eskes, 1989). The germination rate variable was calculated by randomly applying the treatment doses to the experimental units (slide mounts) and the doses of each inducer as the treatments. Since each molecule was evaluated separately, a daily evaluation of the absolute control (germination only in distilled water) was necessary to determine the quality of the inoculum. If the germination rate was lower than 50%, the treatment readings were not taken, and the trial was discarded. The percentage of inhibition in germination was obtained by percentage difference, considering the germination rates obtained for the absolute control of each treatment.

Effects of inducers on the control of coffee leaf rust

The coffee plants were obtained from the harvested seeds of self-fertilized *C. arabica* cv. Caturra populations to avoid genetic variability. These plants, located at the Naranjal Central Station of Cenicafé, are highly susceptible to all physiological races of *H. vastatrix*. The seedlings were transplanted into 17 x 23 cm seedbed plastic bags filled with soil supplemented with organic matter and placed in a mesh house under 40% shade, an average temperature of 25°C, and an average relative humidity of 70% until they reached six months of age or developed five pairs of fully expanded leaves. The application of fungicides or other products that could interfere with disease development was restricted. The seedbed was fertilized before treatment application with 2 g of diammonium phosphate (DAP) every two months, and irrigation was scheduled twice weekly.

When the plants reached the required number of leaves (four pairs of fully extended leaves), aqueous solutions of each inducer treatment were prepared at three different concentrations, as shown in Table 1. Each treatment was applied using a Gast® spray pump (Gast Manufacturing Inc., Benton Harbor, MI, USA) at a pressure of 5 psi, with a volume of 0.5 ml/leaf, maintaining a 30 cm distance between the applicator nozzle and the plant. For evaluations of the local and systemic response of the inducers, the second and fourth pairs of leaves were protected with a waterproof plastic cover to avoid direct contact during spraying (systemic response). The first and third pairs of leaves were applied on upper and lower surfaces to ensure uniform distribution (local response). Control treatments were applied using only distilled water, following the same spraying method.

Each treatment had an exposure time interval between product application and pathogen inoculation of 15, 30, and 45 d. Inoculation with *H. vastatrix* was performed on the same day for all intervals to avoid variations. As previously described, an inoculum solution of approximately 4 x 104 spores ml^{-1} was prepared. The inoculation was applied to the first through fourth pairs of leaves from the apex, including those previously protected using a Gast® spray pump at a constant pressure of 5 psi, with a volume of 0.5 ml/leaf, maintaining a 30 cm distance between the applicator nozzle and the plant. Once inoculated, the plants were placed in a dark room for 72 h at 23°C with a relative humidity of 90% or more, maintained by a Bahnson® humidifier (DnB Humidifier Manufacturing Inc., Winston-Salem, NC, USA), to favor pathogen germination and penetration. Afterward, the plants were returned to the mesh house under the same conditions (40% shade, average temperature 25°C, and average relative humidity 70%) until the first symptoms appeared.

The test was completely randomized with a 3x3x2 factorial arrangement (three doses x three-time intervals x two response types). The experimental unit was the seedling or nursery plant. The treatments were the interaction of the three doses (low, recommended, and high), the time intervals (15-, 30-, and 45-d post-inducer application), and the response signaling pathways (local and/or systemic). Each treatment, including the control, had six plants as replicates, each with eight leaves as reading units (n = 48). After 60 d post inoculation (the date on which the control reached maximum pustule development), the evaluation process of each treatment began. To do this, each leaf corresponding to the reading units was removed with the help of a scalpel, trying not to cause disturbance or loss of the generated pustules. The leaves were then placed in previously labeled trays and taken to the Phytopathology laboratory of Cenicafé.

Each treatment was evaluated by recording the percentage of the affected area as disease severity using a UMAX Powerlook® scanner (Model 2100XL). Each image was processed at 500 dpi on a black background with a quadratic area-preserving map. Images were individually processed with ImageJ® (version 1.49) software, calibrated to identify each lesion's minimum and maximum visible spectral ranges to determine the final severity on each reading unit. The percentage of control was obtained by percentage difference, considering the final severity obtained for the

absolute power. The test duration was 6 months and was not repeated over time.

Statistical analysis

For both the *in vitro* germination rate of urediniospores and the disease severity in the leaves, we checked for normal distribution and homogeneity of variance with the Shapiro-Wilk and Levene test. Then, a descriptive analysis of variance (ANOVA) was performed using the statistical arithmetic mean, standard deviation, and coefficient of variation. Variables showing significant differences were subjected to Duncan's means comparison test ($\alpha = 0.05$). Data analysis was performed with a Dunnett's means comparison test ($\alpha = 0.05$) for the germination rate variable since each treatment was individually compared to the absolute control. All statistical analyses were conducted using R version 3.3.2 (R Core Team, 2023).

Results and discussion

Effect of the inducer on *H. vastatrix* urediniospore germination

None of the treatments were statistically equal to the germination rates obtained for the absolute control (without product application) across the three evaluated doses (Fig. 1). On average, the germination rate for the absolute control was 67.9%, followed by potassium phosphite treatment (15.7%), ASM (15.1%), harpin (6.3%), and SA (0%). The treatment with the highest inhibition rate was SA (100%), followed by harpin (90.9%), potassium phosphite (76.4%), and ASM (74.5%).

According to the ANOVA, there were significant differences among treatments in terms of urediniospore germination after 4 h of exposure to the three different doses ($F = 230.9$, $df = 4$, *P-value* <0.0001). None of the evaluated molecules exhibited germination rates close to those of the absolute control (water alone without product application), which ranged from 59.2% to 76.2%. The germination inhibition displayed an inversely proportional relationship between the dose and the urediniospore germination rate. However, this effect did not occur with SA, which prevented spore germination even at the lowest dose, demonstrating the compound's marked direct inhibitory effect (Fig. 2).

Germination at the low dose (0.2 µl ml^{-1}) was only 27.9% germination, followed by the recommended dose (0.8 µl ml^{-1}) with 19.1% germination, and finally, the high dose (3.3 µl ml^{-1}) where germination did not occur. These findings are consistent with Costa *et al.* (2007), who evaluate the biocidal effect of 7.5 µl ml^{-1} potassium phosphite on the *in vitro* germination of *H. vastatrix* urediniospores, reporting 100% inhibition compared to the absolute control, indicating a significant toxic effect of this molecule on pathogen germination. Similarly, Fernandes *et al.* (2013), studying the efficacy of copper and manganese phosphates at a concentration of 5 µl ml^{-1} on the in vitro germination of *H. vastatrix* urediniospores, observes low germination rates of 22.8% and 20.8%.

Likewise, ASM showed adverse effects compared to those obtained in the absolute control. Germination at low doses (50 µg ml^{-1}) was only 29.9%. This was followed by the recommended dose (200 µg ml^{-1}) with 15.4% germination, and finally, the high dose (800 µg ml^{-1}) where germination did not occur. These results differ from those Guzzo *et al.* (2001) reported, who assessed ASM's biocidal effects on urediniospore germination and appressorium formation

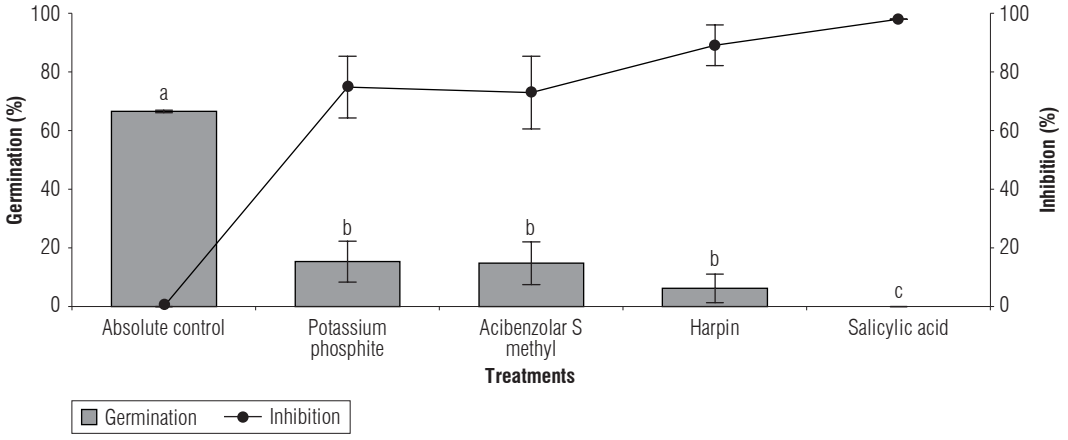

FIGURE 1. The mean germination rate of urediniospores was exposed to three doses of potassium phosphite, Acibenzolar S methyl, harpin, and salicylic acid for 4 h, compared to the absolute control. The vertical lines on the bars and dots indicate 95% confidence intervals (± SE, n = 30). According to Duncan's multiple comparison test at 5%, different letters on the vertical lines indicate significant differences.

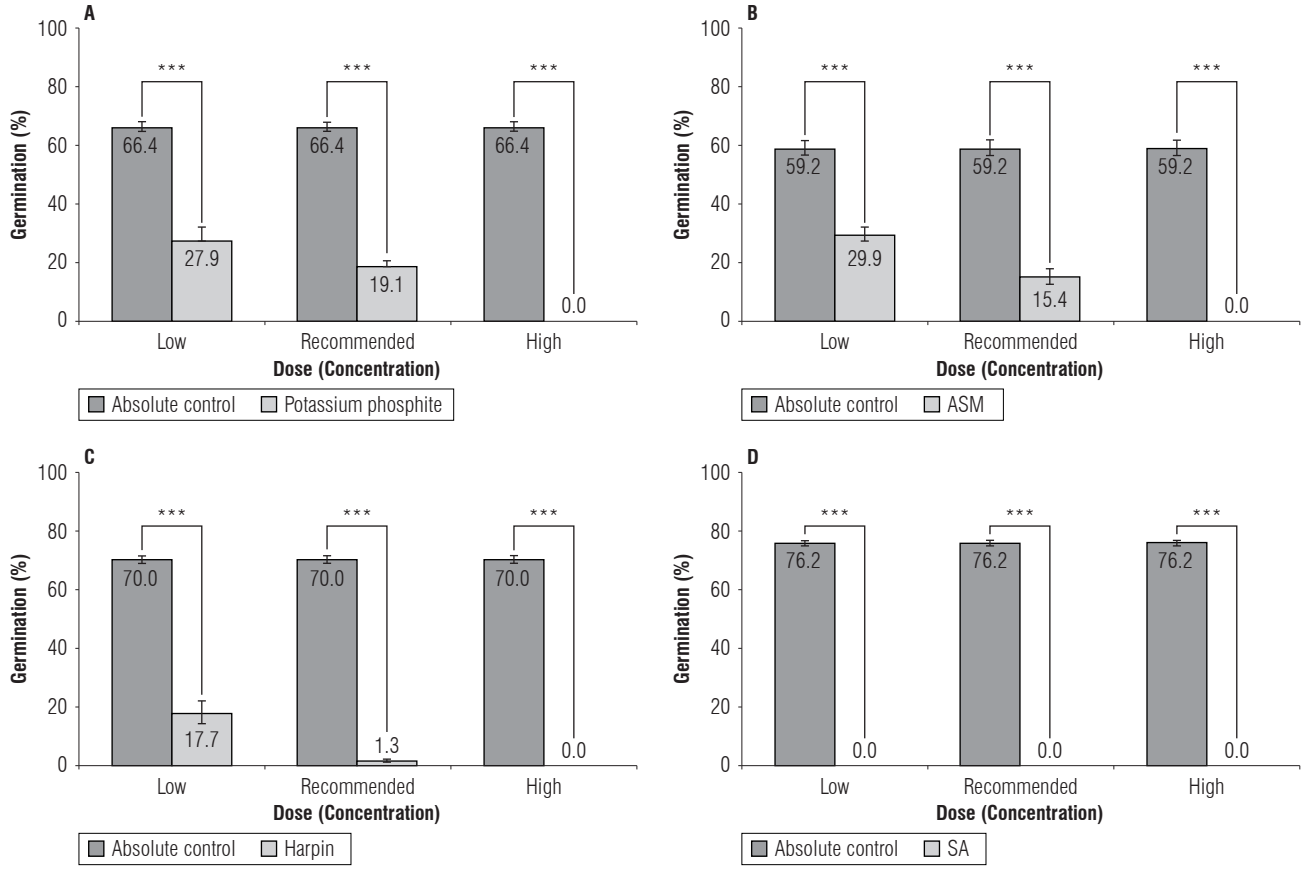

FIGURE 2. The germination rate of urediniospores exposed to three different doses of A) potassium phosphite, B) ASM - Acibenzolar S methyl, C) harpin, and D) SA - Salicylic acid for 4 h. The vertical lines on the bars indicate 95% confidence intervals (± SE, n = 10). Asterisks connected by solid lines indicate the significance levels according to Dunnett's multiple comparison tests at 5% between the dose (light bars) and the absolute control (dark bars), significance $P<0.05$ *, $P<0.01$ **, and $P<0.001$ ***.

in *H. vastatrix* using fluorescence microscopy. Their study found no significant inhibition of germination or appressorium formation across all ASM concentrations tested (10 to 400 µg ml^{-1}). Similarly, Marchi *et al*. (2002) evaluated ASM across different concentrations (1 to 1000 µg ml^{-1}) on coffee leaves. They found no fungitoxic effects on the pathogen.

Harpin also showed adverse effects compared to those obtained in the absolute control. Germination at the low dose (7.5 µg ml^{-1}) was only 17.7%, followed by the recommended dose (30 µg ml^{-1}) with 1.3% germination, and finally at the high dose (120 µg ml^{-1}) where germination did not occur. These findings contrast with those reported by Jesus (2009), who found no significant differences in the toxic effects of harpin on *H. vastatrix* urediniospore germination across a range of concentrations from 250 to 4000 µg ml^{-1}, results that were comparable to the absolute control. However, Galdeano *et al*. (2010) investigated harpin's effects on *Cercospora coffeicola*. They observed a stimulating effect on conidial germination and mycelial growth at concentrations ranging from 7.5 to 120 µg ml^{-1}. These discrepancies suggest that harpin may not exert a direct toxic effect on pathogen development but could instead stimulate growth, a phenomenon whose significance in disease control remains debated. Unlike our study, Jesus (2009) and Galdeano *et al*. (2010) applied harpin directly to leaf surfaces, allowing for absorption over approximately 6 h before pathogen inoculation (*H. vastatrix* and *C. coffeicola*), indicating that the molecule was not in direct simultaneous contact with pathogen spores.

Furthermore, it is essential to consider the pH of the aqueous dilutions used with Harpin treatment, which ranged between 5.40 and 5.57, representing the most acidic conditions in our experiment. Carré-Missio *et al*. (2012) investigated the effects of potassium silicate applications at different concentrations on *H. vastatrix* urediniospore germination across pH values of 5.5, 7.5, and 10.5. They report that at a dose of 8 g L^{-1}, germination inhibition rates are 13.9%, 15.6%, and 20.8%, respectively, indicating

varying effects of the product with spores germinating more effectively under acidic conditions than neutral pH. Finally, SA showed inhibitory effects on germination for all evaluated doses, even at the lowest dose (0.3 µl ml^{-1}).

Although most authors did not report adverse effects on the germination of *H. vastatrix*, our results suggest a toxic effect on the germination processes of the spores, mainly depending on a high concentration of the applied product. Because of this, and in light of our results, it is not possible to ensure that the control of the disease corresponds to the direct activation of the plant's defense mechanisms but to the adverse effects that the molecules have on the germination of the spores, an essential aspect if we consider that this type of research seeks the selection of molecules without direct toxic effects (Steiner & Schönbeck, 1995), due to the implications that this has on the selection pressure of the pre-existing populations of the pathogen or the generation of a more significant number of resistant isolates.

Effects of inducers on the control of coffee leaf rust

According to the ANOVA, there were significant differences among treatments regarding disease control, regardless of the dose, application interval, and type of response signaling, compared to the absolute control ($F = 26.08$, $df = 4$, *P-value* <0.0001). The severity of the absolute control was 11.6%, followed by treatments with potassium phosphite (2.7%), harpin (2.5%), SA (1.6%), and ASM (1.3%). The treatment with the best control was ASM, achieving 88.6% control compared to the absolute control, followed by SA (86.1%), harpin (78.6%), and potassium phosphite (76.6%) (Fig.3).

Regarding the dose effects, only SA and ASM showed significant differences in disease control at high doses. Potassium phosphite, SA, and ASM exhibited substantial differences compared to harpin at recommended doses. In contrast, harpin, SA, and ASM showed significant differences compared to potassium phosphite at low doses. Regarding the effect of application intervals, harpin, SA, and ASM displayed substantial differences at a 15 d interval. Additionally, SA and acibenzolar-S-methyl (ASM) showed significant differences at 30 and 45 d application intervals. Concerning the effect of type of response signaling, SA and ASM showed substantial differences for local signaling. Furthermore, ASM was the only compound showing significant differences compared to the others for systemic signaling (Fig. 4).

Potassium phosphite achieved the best results when applied at the high dose (3.3 µl ml^{-1}), followed by the recommended dose (0.8 µl ml^{-1}), and then the low dose (0.2 µl ml^{-1}), with disease control percentages of 83.4%, 82%, and 64%. Optimal disease control was observed when treatments were applied at a 15-d interval, followed by 30 and 45-d intervals, resulting in control percentages of 82.6%, 75.5%, and 71.2%. The predominant signaling pathway was more local (83.7%) than systemic (69.2%), indicating that disease control is more effective with higher doses applied at intervals not exceeding 15 d, achieving control rates ranging from 80% to 86% (Fig. 5A). These results contrast with Costa *et al.* (2007), who demonstrates effective disease control (57-65%) with 7.5 µl ml^{-1} potassium phosphite applied at intervals close to 30 d. Similarly, Fernandes *et al.* (2013) found that manganese phosphite provided higher disease

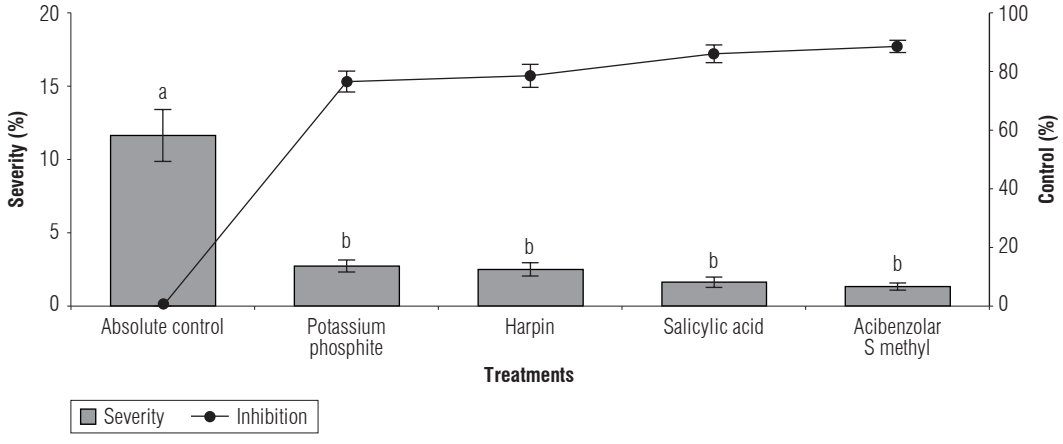

FIGURE 3. Mean severity index at three doses, three different time intervals between product application and pathogen inoculation, and two signaling pathways in the responses to potassium phosphite, harpin, SA, and ASM compared to the absolute control. The vertical lines on the bars indicate 95% confidence intervals (± SE, n = 54). According to Duncan's multiple comparison test at 5%, different letters on the vertical lines indicate significant differences.

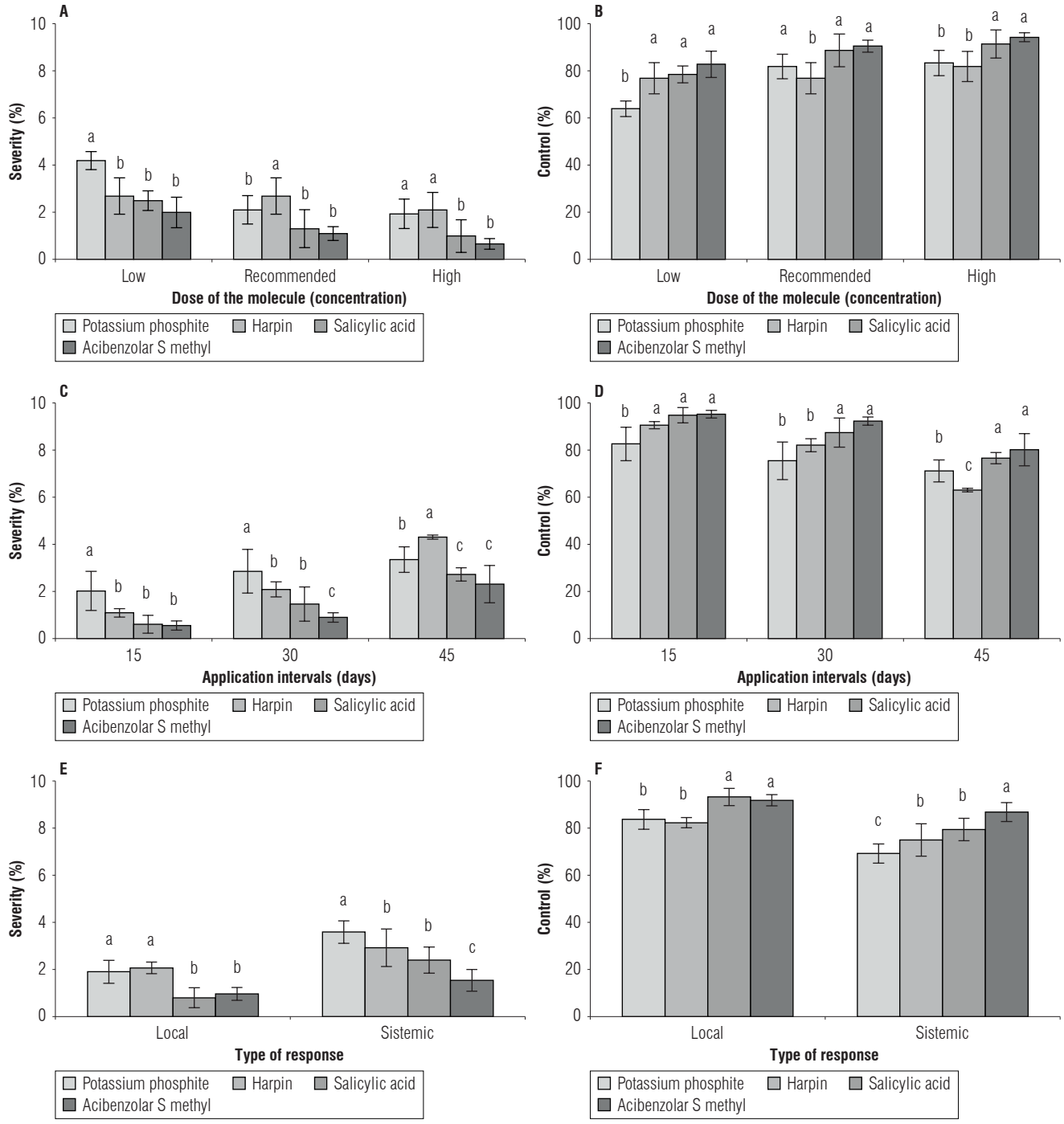

FIGURE 4. Mean severity index (left) and percent control (right) of variables. A and B) The molecule doses, C and D) The time intervals between product application and pathogen inoculation, E and F) The types of responses to potassium phosphite, harpin, SA, and ASM. The vertical lines on the bars indicate 95% confidence intervals (± SE, n = 18). According to Duncan's multiple comparison test at 5%, different letters on the vertical lines indicate significant differences.

control (70%) than copper phosphite (56%) during longer application intervals, including 40 d against coffee rust.

Harpin exhibited the most effective results when applied at the highest dose (120 µg ml^{-1}), followed by the recommended (30 µg ml^{-1}), and then the lowest dose (7.5 µg ml^{-1}), achieving disease control of 81.9%, 76.9%, and 76.9% (Fig. 5B). Optimal disease control outcomes were observed with application intervals of 15 d, followed by 30 and 45 d, resulting in controls of 90.6%, 82.1%, and 63.2%, respectively, with a predominant signaling effect more local (82.3%) than systemic (74.9%).

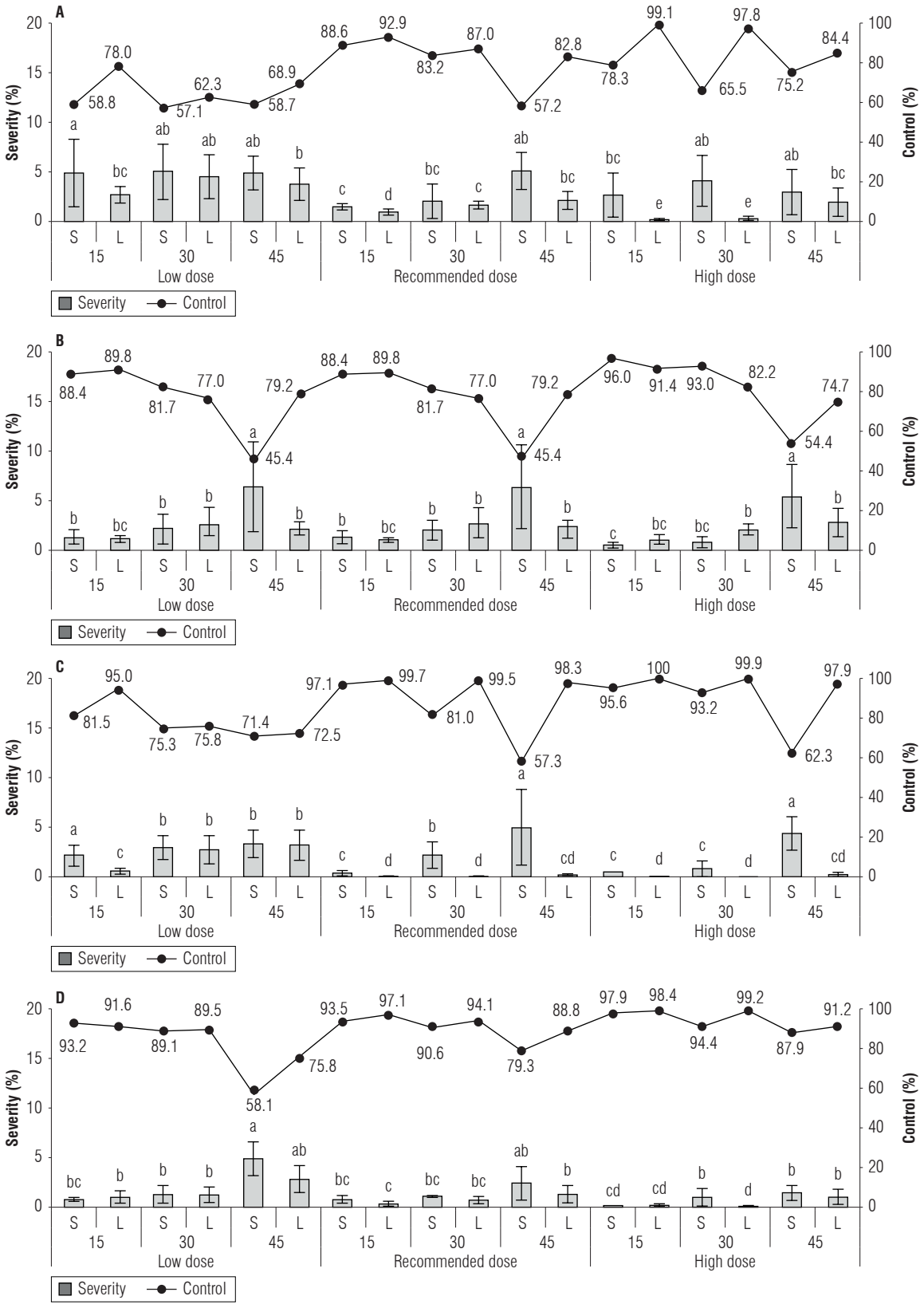

FIGURE 5. Severity index at three different doses (low, recommended, and high), at three different time intervals (15, 30, and 45 d) between the product application and pathogen inoculation, with the response signaling pathway being (L), a local effect, and (S), a systemic effect of A) potassium phosphite, B) harpin, C) SA, and D) ASM. The vertical lines on the bars indicate 95% confidence intervals (± SE, n = 3). According to Duncan's multiple comparison test at 5%, different letters on the vertical lines indicate significant differences.

SA demonstrated optimal results at high doses (5 µl ml^{-1}), followed by the recommended (1.2 µl ml^{-1}), and then the lowest dose (0.3 µl ml^{-1}), achieving disease controls of 91.5%, 88.8%, and 78.6%, respectively (Fig. 5C). The most effective disease control occurred with application intervals of 15 d, followed by 30 and 45 d, achieving controls of 94.8%, 87.5%, and 76.6%, respectively, with a predominant local signaling effect (93.2%) over systemic (79.4%).

ASM showed optimal results at high doses (800 µg ml^{-1}), followed by the recommended dose (200 µg ml^{-1}). Then, the low dose (50 µg ml^{-1}) achieved 94.3%, 90.6%, and 82.9% control, respectively (Fig. 5D). The best disease control occurred at 15 d intervals (95.3%), followed by 30 and 45 d (92.3% and 80.2% control, respectively), predominantly through local (91.7%) rather than systemic (86.8%) signaling. Similar results were reported by Guzzo *et al.* (2001), who observes ASM's protective effects ranging from 66% to 97% locally and 83% to 94% systemically at higher concentrations (400 µg ml^{-1}). However, effectiveness significantly decreases when intervals between ASM application and pathogen inoculation exceeded 49 d, even at high doses. To study their effects on coffee rust, Sancho and Diaz (2006) conducted greenhouse experiments at Cenicafé using ASM and other resistance-inducing biological products at varying concentrations. Applying the BION product (ASM) at concentrations of 1, 10, 20, 50, and 100 µg ml^{-1} effectively reduces the number of leaf lesions, with optimal application intervals observed at 8 d to induce robust plant resistance and minimize disease development time.

According to the results, treatments with these compounds consistently reduced disease severity and improved disease control compared to untreated controls. The efficacy of disease control was mainly influenced by the concentration of the product, which dictated the optimal frequency of application to avoid dilution of the treatment effects, with biweekly application intervals (every 15 d) being the best for disease control at a recommended dose. However, it is essential to note that in many of the compounds evaluated, the increase in concentration did not necessarily translate into longer application intervals (every 30 or 45 d), which led to adjusting the application intervals according to the product selected and the dose used.

In terms of signaling, local responses prevailed over systemic ones, and this behavior was primarily governed by the intrinsic properties of the molecule rather than other factors such as dose or application intervals. These types of local responses have economic and operational implications for preventive disease management plans since, as far as possible, the application process of the product should be carried out directly on the leaves to achieve better coverage, which increases application times and, therefore, the costs associated with management.

Conclusions

The direct impact of ASM, SA, potassium phosphite, and harpin on *H. vastatrix* urediniospore germination indicated varying degrees of toxicity against the pathogen. These inhibitory effects are dose-dependent, suggesting that higher concentrations of these inducers can totally suppress urediniospore germination. These results somewhat disputed the impact of this type of inducer on disease management since their responses may be more influenced by the low germination capacity of the pathogen when it comes into contact with the molecule than by the activation of the plant defense mechanisms due to its application. An undesirable effect in preventive disease management is to reduce the selection pressure on the pathogen currently exerted by chemical molecules.

Regardless of these effects, treatments with these compounds consistently reduce disease severity and enhance disease control compared to untreated controls. In general, disease control efficacy is mainly influenced by the concentration of the product, which dictates the optimal application frequency to prevent dilution of treatment effects. In terms of signaling, local responses prevail over systemic ones, and this behavior is primarily governed by the intrinsic properties of the molecule rather than other factors such as dosage or application intervals.

The present study highlights the practicality and high potential of integrating resistance-inducing compounds like ASM, SA, harpin, and potassium phosphite into disease management strategies in commercial seedbeds, particularly for managing foliar diseases such as rust. However, using these compounds may require a higher labor cost due to the requirements regarding the quality and frequency of application. For this reason, the success of implementing this type of molecule will depend more on the efficiency in controlling the disease than on its already proven effectiveness.

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Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

JML, ALG, and CAA formulated the research aims, and JML applied statistical and computational techniques to analyze the study data. JML conducted the research and investigation process, explicitly performing the experiments; JML and CAA designed the methodology; JML and CAA wrote the initial draft; MAC translated the initial draft; JML and MAC carried out the critical review, commentary, and revision of the whole manuscript. All authors approved the final version of the manuscript.

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