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Research Article

Inhibition of mycelial growth, conidial germination, and *Botrytis cinerea* Pers.:Fr colonization in begonia with biocompatible products

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ABSTRACT: This study evaluated the effects of potassium and sodium carbonate and bicarbonate, *Bacillus subtilis* (Cohn, 1872) QST-713, *Bacillus pumilus* (Meyer & Gottheil, 1901) QST-2808, and crude and roasted coffee oils on the inhibition of mycelial growth and conidial germination in *Botrytis cinerea* Pers.:Fr and the colonization of begonia (*Begonia elatior* Hort. ex Steud) leaf discs by *B. cinerea* inoculated before, simultaneously and after with these alternative products. The assays were carried out using the Baladin begonia cultivar. The inhibition of *B. cinerea* mycelial growth and conidial germination was proportional to increases in the concentration of all the products. The inhibition of conidial germination was directly proportional to the concentrations of *B. pumilus* QST-2808 and *B. subtilis* QST-713. Coffee oils were less efficient in inhibiting germination than the other products. The crude and roasted coffee oils, potassium and sodium carbonates and bicarbonates, and *B. pumilus* and *B. subtilis* sprayed 24 h before, simultaneously, or 24 h after pathogen inoculation inhibited the colonization of begonia leaf discs by *B. cinerea*. The positive results for the suppression of *B. cinerea* by the alternative products tested herein merit scrutiny. There is a pressing need to evaluate these products in the management of gray mold, as the severity of this disease is usually high under favorable conditions in greenhouses.

Keywords: biocontrol, gray mold, salts, alternative products

Introduction

Gray mold (*Botrytis cinerea* Pers.:Fr) is the most severe disease currently threatening begonia (*Begonia elatior* Hort. ex Steud) causing lesions on all of the aerial organs over the entire plant cycle (Daughtrey et al., 1995; Alexandre and Duarte, 2007; Fujinawa et al., 2020). This fungus displays abundant sporulation that serves as inoculum and has spread widely throughout the environment (Kersies et al., 1997). The environmental conditions appropriate for greenhouse cultivation exacerbate the severity of the disease (Rosa and Moorman, 2018) due to difficulties in controlling it (Daughtrey et al., 1995; Carisse, 2016). Currently, in Brazil, gray mold in begonia is controlled with thiophanate-methyl (MAPA, 2020). However, the problem of *Botrytis* fungicide resistance has long been recognized (Ghini and Kimati, 1990; Elad et al., 1992). In addition to fungicide sprays, sanitation, roguing, removal of crop debris, and control of environmental conditions are recommended (Daughtrey et al., 1995; Hausbeck and Moorman, 1996; Morandi et al., 2003). The frequent use of fungicides on greenhouse begonia crops requires intense labor and can lead to worker safety issues from possible exposure. In addition, it is imperative a specific period be determined prior to workers re-entering the area to avoid exposure.

These consequences intensify the necessity to develop further effective alternative products that are generally regarded as safe. Alternatives to *Botrytis* control in greenhouses have been studied due to its importance to this environment (Paulitz and Bélanger, 2001; Morandi et al., 2003; Lee et al., 2006). Deliopoulos et al. (2010) reviewed the use of inorganic salts to control plant pathogens, such as potassium and sodium bicarbonate. The effectiveness of crude and roasted coffee oils in inhibiting the germination of *Phakopsora pachyrhizi* (Sydow and Sydow.) and controlling soybean rust has also been evaluated (Dorighello et al., 2015; Dorighello, 2020). Considering its characteristics, *Bacillus* spp. could also be considered an important source of microbial bioprotectants for controlling gray mold in several crops (Lee et al., 2006; Ongena and Jacques, 2008; Xu et al., 2016; Mousavi et al., 2017; Jiang et al., 2018; Calvo-Garrido et al., 2019), and biocontrol of gray mold in begonias has been one such alternative that has been studied in recent years (Fujinawa et al., 2020). We evaluated the potential of crude and roasted coffee oils, potassium and sodium carbonate and bicarbonate, and *Bacillus* spp. to inhibit the mycelial growth and germination of *Botrytis* conidia and the colonization of begonia leaf discs by *B. cinerea*.

Materials and Methods

Coffee oils were obtained by compressing crude or roasted coffee beans in a cold press expeller. Subsequently, oils were separated from the coffee bean mass using a filter press. The biofungicides Serenade™ (*Bacillus subtilis* (Cohn, 1872) QST-713) and Sonata™ (*Bacillus pumilus* Meyer & Gottheil QST-2808) were provided by AgraQuest (Davis, CA, USA). Sodium and potassium carbonates and bicarbonates were obtained from Dinâmica Química Contemporânea Ltda.

Inhibition of *B. cinerea* mycelial growth

The effects of crude or roasted coffee bean oils, potassium carbonate and bicarbonate, and sodium carbonate and bicarbonate on mycelial growth were evaluated *in vitro*. *Botrytis cinerea* discs (diameter = 0.8 cm) were transferred to the center of Petri dishes (diameter = 9 cm) containing potato-dextrose-agar (PDA) with 0, 1, 10, 100, 1,000, and 10,000 mg L^{-1} crude and roasted coffee oils, potassium carbonate and bicarbonate, and sodium carbonate and bicarbonate. The pH of the culture medium was adjusted to the same as that of PDA before sterilization. The plates were incubated at 22 ± 2 °C in the dark. The diameters of the colonies were measured daily until the mycelial growth of the control plates covered the Petri dish. The assays were set up with five replications in a completely randomized design, each consisting of one Petri dish. The experiment was repeated twice.

Inhibition of *B. cinerea* conidial germination

The effects of crude or roasted coffee beans oils, potassium carbonate and bicarbonate, and sodium carbonate and bicarbonate on conidial germination were evaluated *in vitro* in Petri dishes. Four 20 µL droplets of *B. cinerea* conidial suspension (1×10^5 conidia mL^{-1}) obtained by washing ten-day colonies on plates with sterile distilled water plus Tween 20 (0.05 %) were transferred to four different positions on the Petri dishes (diameter = 9 cm) containing water agar with 0, 1, 10, 100, 1,000, and 10,000 mg L^{-1} of crude or roasted coffee oils, potassium carbonate and bicarbonate, and sodium carbonate and bicarbonate. The pH of the culture medium was adjusted to the same as that of the control before sterilization. After incubation for 8 h at 22 ± 2 °C in the dark, germination was interrupted by adding 10 µL of lactophenol cotton blue dye to each droplet. For each droplet, under a light microscope with 200 × magnification, 100 conidia were examined. Conidia were considered germinated when the germ tubes were at least one-half the length of their greatest diameter. The rate of conidial germination was calculated as a percentage. The assays were set up in a completely randomized experimental design, with four replicates, each consisting of one Petri dish with four droplets. The experiment was repeated twice. To evaluate the effects of *Bacillus*-based products, 10 µL of each product in suspension was deposited onto four different point-one Petri dishes containing water agar together with 10 µL of *B. cinerea* conidial suspension (1×10^5 conidia mL^{-1}). The same incubation conditions and evaluation methods described below were put in place.

Reduction in *B. cinerea* colonization on leaf discs

The effects of crude or roasted coffee bean oils, potassium and sodium carbonate and bicarbonate, and *B. pumilus* QST-2808 and *B. subtilis* QST-713 on the colonization of begonia leaf discs by *B. cinerea* were evaluated. The leaf discs (1-cm diameter) of begonia plants (cv. Baladin) were surface sterilized in 70 % ethanol (1 min) followed by 2 % sodium hypochlorite (1 min) and rinsed three times in sterile distilled water. These leaf discs were air-dried in filter paper overnight under a laminar flow hood. Next, 55 leaf discs were placed on a disposable plate (diameter = 150 mm, Pleion) on two layers of humidified (with 5 mL of sterilized water) sterile absorbent paper. Subsequently, the 55 leaf discs contained in these plates were sprayed with 0, 1, 10, 100, 1,000, and 10,000 mg L^{-1} crude or roasted coffee beans, potassium carbonate and bicarbonate, sodium carbonate and bicarbonate; *B. pumilus* QST-2808 and *B. subtilis* QST-713 at 0, 10^7, 10^8, and 10^9 CFU mL^{-1} (obtained through serial dilution of commercial Sonata™ and Serenade™ products). All the products were sprayed on the discs at three-time points: 24 h before and after *B. cinerea* inoculation and simultaneously with *B. cinerea* inoculation. Each disc was inoculated with an aliquot of *B. cinerea* (10 µL – 10^5 conidia mL^{-1}). These discs were then transferred to paraquat-chloramphenicol agar medium (PCA) in Petri dishes (diameter = 90 mm) (Peng and Sutton, 1991), with 11 discs per plate. The growth and sporulation of the pathogen were estimated after the tissues were incubated at 22 ± 2 °C (12 h light – Grolux and fluorescent lamps of 40 W at 40 cm of distance/12 h dark) for four, seven, and ten days. The control treatment included inoculation of *B. cinerea* onto leaf discs treated with water. The evaluation was completed by using a scale scoring system for the area of the discs covered with *B. cinerea* conidiophores, as follows: 0 = 0 % (0 %), 1 = 2 % (1–3 %), 2 = 5 % (4–6 %), 3 = 10 % (7–12 %), 4 = 20 % (13–26 %), 5 = 40 % (27–53 %), 6 = 65 % (54–76 %), and 7 = 90 % (77–100 %) (Peng and Sutton, 1991).

Experimental design and data analysis

All assays were repeated twice for different periods. The data from the two experimental repetitions were grouped for analysis after verifying that the variances between the experiments were no more significant than three (Pimentel-Gomes and Garcia, 2002), and subjected to analysis of variance (F, $p \leq 0.05$). Where significant differences between treatments were observed, they were compared with the control (concentration zero) by Dunnett's test ($p \leq 0.05$). The percent inhibition of pathogen development was estimated, and the effect of different concentrations was determined using regression analysis. Statistical analyses were performed using SAS software (Statistical Analysis System, version 9.4), and graphics were created with Excel 2011 (Office for Mac).

Results

Inhibition of *B. cinerea* mycelial growth

All the concentrations of crude and roasted coffee oils, and potassium and sodium carbonates and bicarbonates

($p \leq 0.05$) inhibited the mycelial growth of *B. cinerea* compared to the control with water. The crude and roasted coffee oils at 100 mg L^{-1} and 1,000 mg L^{-1} reduced mycelial growth by more than 57 % and 73 % and 53 % and 61 %, respectively. However, at 10,000 mg L^{-1}, both oils inhibited 100 % mycelial growth (Table 1). Potassium bicarbonate and sodium and potassium carbonates at 10,000 mg L^{-1} were effective, with inhibition over 80 %. Sodium bicarbonate inhibited 100 % of mycelial growth at 10,000 mg L^{-1} (Table 1). However, potassium and sodium carbonates and bicarbonates at 1,000 mg L^{-1} inhibited approximately 20 % of *B. cinerea* mycelial growth (Table 1).

Inhibition of *B. cinerea* conidial germination

The crude and roasted coffee oils were evaluated at 0, 10, 100, and 1,000 mg L^{-1} because at 10,000 mg L^{-1} it was impossible to visually track conidial germination under the assay conditions. The inhibition of conidial germination with crude and roasted coffee oils at 1,000 mg L^{-1} were 37 % and 26 %, respectively (Table 2). Sodium and potassium carbonate and bicarbonate at more than 1,000 mg L^{-1} reached almost 100 % inhibition of conidial germination (Table 2). Crude and roasted coffee oils, and potassium and sodium carbonates and bicarbonates at 1,000 mg L^{-1} ($p \leq 0.05$) all inhibited conidial germination of *B. cinerea* compared to control with water (Table 2).

Bacillus pumilus QST-2808 (Sonata™) showed more efficacy in inhibiting conidial germination than *B. subtilis* QST-713 at 10^7 CFU mL^{-1} and 10^8 CFU mL^{-1} (Table 3). At a concentration of 10^9 CFU mL^{-1}, *B. pumilus* QST-2808 (Sonata™) and *B. subtilis* QST-713 (Serenade™) inhibited conidial germination by almost 80 % (Table 3).

Reduction of *B. cinerea* colonization on leaf discs

For the concentrations of salts (sodium and potassium carbonates and bicarbonates), coffee oils (crude and roasted), and biocontrol agents [*B. pumilus* QST-2808 (Sonata™) and *B. subtilis* QST-713 (Serenade™)], an effect was observed in the inhibition of the colonization of begonia discs by *Botrytis* (Table 4). On the other hand, the application time of these products affected the effectiveness of potassium bicarbonate and coffee oils. However, no effects were observed for the interaction of the application time and concentration of the products (Table 4). Due to the points mentioned above, analyses were carried out combining all application times.

The inhibition of colonization of begonia discs by *B. cinerea* was 75 %, 71 %, and 62 % for *B. pumilus* QST-2808 (Sonata™), and 65 %, 60 %, and 61 % for *B. subtilis* QST-713 (Serenade™), when applied before, simultaneously and after pathogen inoculation, respectively (Table 5). *Bacillus pumilus* QST-2808 showed more efficacy in inhibiting colonization than *B. subtilis* QST-713. At a 10^9 CFU mL^{-1}, *B. pumilus* QST-2808

Table 1 – Mycelial growth (cm) of *Botrytis cinerea* in response to the addition of different salts and oils in the culture medium.

Concentration	Potassium Bicarbonate	Potassium Carbonate	Sodium Bicarbonate	Sodium Carbonate	Roasted Coffee Oil	Crude Coffee Oil
0 mg L^{-1}	8.59	8.63	9.00	8.57	8.81	9.00
1 mg L^{-1}	6.98* (19 %)	7.46* (14 %)	8.42* (6 %)	7.23* (16 %)	7.40* (16 %)	8.83* (2 %)
10 mg L^{-1}	6.83* (20 %)	7.54* (13 %)	8.55* (5 %)	6.81* (21 %)	7.04* (20 %)	6.70* (26 %)
100 mg L^{-1}	6.98* (19 %)	7.08* (18 %)	8.39* (7 %)	7.26* (15 %)	4.15* (53 %)	3.90* (57 %)
1,000 mg L^{-1}	6.91* (20 %)	7.26* (16 %)	7.15* (21 %)	6.54* (24 %)	3.40* (61 %)	2.44* (73 %)
10,000 mg L^{-1}	1.61* (81 %)	1.70* (80 %)	0.00* (100 %)	0.91* (89 %)	0.00* (100 %)	0.00* (100 %)
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV[1]	7.95	8.60	7.69	7.73	8.74	6.49

[1]Coefficient of variation. *Different from the control (Dunnett test, $p \leq 0.05$); Values in parentheses indicates the % of inhibition of mycelial growth compared to the control; Mycelial growth was evaluated after incubation for five days at 22 ± 2 °C in the dark.

Table 2 – Percentage of conidial germination of *Botrytis cinerea* in response to the addition of different salts, and oils in the culture medium.

Concentration	Potassium Bicarbonate	Potassium Carbonate	Sodium Bicarbonate	Sodium Carbonate	Roasted Coffee Oil	Crude Coffee Oil
0 mg L^{-1}	76.66	77.91	77.03	78.75	76.47	80.31
1 mg L^{-1}	74.23 (3 %)	75.81(3 %)	76.02 (1 %)	77.49 (1 %)	75.61(1 %)	77.44 (4 %)
10 mg L^{-1}	25.91* (66 %)	75.66 (3 %)	75.85 (1 %)	57.75* (27 %)	74.95 (20 %)	77.40 (4 %)
100 mg L^{-1}	25.69* (66 %)	76.81 (1 %)	3.31* (96 %)	36.31* (54 %)	74.80 (22 %)	76.13* (5 %)
1,000 mg L^{-1}	0.22* (99 %)	7.47* (90 %)	0.06* (99 %)	0.03* (99 %)	56.56* (26 %)	50.97* (37 %)
10,000 mg L^{-1}	0.00*	0.00*	0.06*	0.00*	–	–
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV[1]	7.68	5.79	6.86	8.95	2.83	4.46

[1]Coefficient of variation. *Different from the control (Dunnett test, $p \leq 0.05$); Values in parentheses indicates the % of inhibition of conidial germination compared to the control; At a concentration of 10,000 mg L^{-1}, it was impossible to evaluate the conidial germination for the roasted and crude coffee oil treatments; Conidia germination was evaluated after incubation for 8 h at 22 ± 2 °C in the dark.

inhibited colonization by approximately 100 %, while at the same concentration, *B. subtilis* QST-713 inhibited colonization by approximately 90 % (data not shown).

We present the begonia leaf disc colonization by *B. cinerea* in response to applying different salts, oils, and bacterial suspensions before, simultaneously, and after with pathogen inoculation in Table 5. The application of all the products reduced the colonization of the discs by *B. cinerea* independent of the application time (Table 5). At concentrations > 100 mg L^{-1}, roasted coffee oil was more efficient than crude coffee oil at all application times; and sodium carbonate was more efficient than sodium bicarbonate at lower concentrations (data not shown). Considering the three application times of the products, potassium carbonate (75.7 %) was the most efficient of the evaluated products in reducing begonia leaf disc colonization by *B. cinerea*, followed by potassium bicarbonate (69.3 %), *B. pumilus* QST-2808 (69.3 %), *B. subtilis* QST-713 (62 %), sodium carbonate (59.6 %), roasted coffee oil (59 %), sodium bicarbonate (52 %) and crude coffee oil (48.3 %) (Table 5).

Table 3 – Percentage of conidial germination of *Botrytis cinerea* in response to the addition of bacterial suspensions of *Bacillus subtilis* QST-713 (Serenade™) and *Bacillus pumilus* QST-2808 (Sonata™) to the culture medium.

Concentration (CFU mL^{-1})	*B. subtilis* QST-713	*B. pumilus* QST-2808
0	77.67	75.42
1×10^7	71.25 (8 %)	44.83* (41 %)
1×10^8	66.00* (15 %)	26.75* (64 %)
1×10^9	16.67* (78 %)	13.42* (82 %)
Pr > F	< 0.0001	< 0.0001
CV[1]	13.43	18.73

[1]Coefficient of variation. * Different from the control (Dunnett test, $p \leq 0.05$); Values in parentheses indicates the % of inhibition of conidial germination compared to the control; Conidia germination was evaluated after incubation for 8 h at 22 ± 2 °C in the dark.

Table 4 – Effect of application time and concentration of different salts, oils and bacterial suspensions on leaf disc colonization by *Botrytis cinerea*.

Treatments	Application time[1]	Concentration	Application time × Concentration
Potassium bicarbonate	0.0015[2]	< 0.0001	0.0636
Potassium carbonate	0.4177	< 0.0001	0.5472
Sodium bicarbonate	0.0568	< 0.0001	0.7983
Sodium carbonate	0.0975	< 0.0001	0.6900
Roasted coffee oil	0.0039	< 0.0001	0.2342
Crude coffee oil	0.0081	< 0.0001	0.0828
Bacillus subtilis QST-713	0.9972	< 0.0001	0.9701
Bacillus pumilus QST-2808	0.7066	< 0.0001	0.9582

[1]It was evaluated before, simultaneously, and after the pathogen inoculation. [2]*p*-value.

Discussion

Begonias are exposed to various plant pathogenic fungi throughout their cycle, with gray mold being the most serious and limiting. The environmental conditions in greenhouses are ideal for cultivation, although they also accentuate the severity of the disease, causing significant losses. In the present study, we investigated whether these biocompatible products inhibit mycelial growth and conidial germination of *B. cinerea* and colonization of begonia leaf discs by *B. cinerea*, intending to determine the potential of these alternative products to control gray mold at early stages of infection. These studies are essential because of problems with *Botrytis* resistance to fungicides (Ghini and Kimati, 1990; Elad et al., 1992) in high-selection pressure environments and the intense management of the crop during the cycle. This intense management in greenhouses serves intensifies the need for the development of GRAS (Generally Recognized as Safe) products as effective alternatives.

Crude and roasted coffee oils, as well as sodium and potassium carbonate and bicarbonate concentrations, inhibited *B. cinerea* mycelial growth (Table 1). All alternative products inhibited conidial germination (Table 2). Coffee oils were less efficient at inhibiting germination than the other evaluated products (Table 2). While crude and roasted coffee

Table 5 – Leaf disc colonization (%) by *Botrytis cinerea* in response to the application of different salts, oils and bacterial suspensions before, simultaneously and after pathogen inoculation.

Treatment	Before[1]	Simultaneously	After
Potassium bicarbonate	11.16 (76 %) A	13.41(71 %) A	17.72 (61 %) B
Potassium carbonate	10.18 (78 %)	11.66 (76 %)	12.07 (73 %)[NS]
Sodium bicarbonate	19.17 (59 %) A	24.58 (47 %) B	22.46 (50 %) B
Sodium carbonate	16.77 (64 %)	21.07 (54 %)	17.43 (61 %)[NS]
Roasted coffee oil	19.22 (59 %) AB	20.63 (55 %) B	16.62 (63 %) A
Crude coffee oil	24.19 (49 %) B	20.54 (55 %) A	26.45 (41 %) B
Bacillus subtilis QST-713	16.49 (65 %)	18.31 (60 %)	17.29 (61 %)[NS]
Bacillus pumilus QST-2808	11.92 (75 %)	13.33 (71 %)	17.08 (62 %)[NS]
Water (control)	47.27	46.02	44.91 [NS]

[1]It was evaluated before, simultaneously, and after the pathogen inoculation. The average values of leaf disc colonization for each product were obtained without the zero concentration (control). Means followed by the same uppercase in the row do not differ statistically from each other (Dunnett's test ($p \leq 0.05$)). Values in parentheses indicates the % of inhibition of leaf disc colonization compared to the control. NS = not significant.

oils inhibited mycelial growth by 73 % and 61 % at a 1,000 mg L^{-1}, the inhibition of conidial germination at this concentration was 37 % and 26 %, respectively. These results indicate that the oils were more efficient in inhibiting the mycelial growth of the pathogen than conidial germination. On the other hand, the salts had more uniform effects concerning the inhibition of mycelial growth and the germination of conidia of the pathogen. Potassium bicarbonate inhibited the mycelial growth of several pathogens of tomato (*Solanum lycopersicun* L.) plants (*Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen, *F. oxysporum* f. sp. *radicis-lycopersici* (Jarvis & Shoemaker), *Fusarium solani* (Mart.) Sacc., *Verticillium dahliae* Kleb, *Colletotrichum coccodes* (Walir.) S. Hughes, *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) De Bary, *Pythium aphanidermatum* (Eds.) Fitzp., *Alternaria solani* (Ell. & Mart.) Jones & Grout and *B. cinerea*), and the highest concentration presented the most significant inhibitory effect (Jabnoun-Khiareddine et al., 2016). These authors observed that at a concentration of 0.1 M, potassium bicarbonate completely inhibited *B. cinerea* mycelial growth, and in the present study, 0.1 M potassium carbonate and bicarbonate inhibited mycelial growth by 81 % and 80 %, respectively (Table 1). Sodium carbonate and bicarbonate inhibited mycelial growth by 100 % and 89 %, respectively (Table 1). The activity of sodium carbonate and sodium bicarbonate in inhibiting mycelial growth and conidial germination of *B. cinerea* has also been observed by Nigro et al. (2006).

The effects of potassium and sodium bicarbonate in the control of powdery mildew have been widely studied in several cultures (Deliopoulos et al., 2010). However, comparatively speaking, there are few studies on *Botrytis* control. Field applications of sodium carbonate and sodium bicarbonate reduced gray mold postharvest (Nigro et al., 2006), although Jabnoun-Khiareddine et al. (2016) did not observe any reduction in the diameters of lesions caused by *B. cinerea* with the application of potassium bicarbonate. In this study, the effect of bicarbonate and carbonate sodium and potassium on the inhibition of *B. cinerea* colonization on begonia leaf discs was observed (see Table 5) when sprayed 24 h before, simultaneously, or after *Botrytis* inoculation.

Bacillus pumilus QST-2808 showed more efficacy in inhibiting conidial germination and colonization of the leaf discs by the pathogen than *B. subtilis* QST-713 (Tables 3 and 5). At concentrations of 10^8 and 10^9 CFU mL^{-1}, *B. pumilus* QST-2808 inhibited germination by 64 % and 82 %, respectively, while *B. subtilis* QST-713 inhibited germination by 15 % and 78 %, respectively (Table 3). The inhibition of pathogen spore germination by *B. subtilis* QST-713 (Serenade™) and B. *pumilus* QST-2808 (Sonata™) has been recognized previously (Marrone, 2002; Wszelaki and Miller, 2005; Lahlali et al., 2011). The effect of *B. pumilus* and *B. subtilis* found in this study regarding *B. cinerea* conidial germination is related to the action of the metabolites present in the *Bacillus*-based products Serenade™ and Sonata™.

At concentrations of 10^8 and 10^9 CFU mL^{-1}, *B. pumilus* QST-2808 (Sonata™) inhibited the colonization of the discs by the pathogen by 75 %, 71 %, and 62 %, respectively, while *B. subtilis* QST-713 (Serenade™) inhibited colonization by 65 %, 60 %, and 61 %, respectively (Table 5), when applied before, simultaneously and after pathogen inoculation (Table 5). The effect of both products on the colonization of leaf discs by *B. cinerea* is also probably related to the action of the *Bacillus* metabolites, which may act systemically and persist in the plant for some time (Bottone and Peluso, 2003; Wagacha et al., 2007) and induce resistance. The results obtained with the begonia leaf discs indicate the potential of certain *Bacillus* spp. isolates to exert a protective influence on the crops.

For all the alternative and biological products evaluated in this study, the efficacy was similar for the three application times (Table 5). However, in general, the preventive application was more efficient than the simultaneous application or application after the inoculation of the pathogen to colonize the begonia leaf discs with *B. cinerea*. These results indicate that these products need to be used preventively and may induce host resistance. However, this statement needs to be verified in further studies with other hosts and under typical cultivation conditions for ornamental plants.

The positive results in suppressing *B. cinerea* with the crude and roasted coffee oils, potassium and sodium carbonates and bicarbonates, and *B. pumilus* and *B. subtilis* evaluated in this study merit scrutiny. There is also a need to evaluate these products as part of the management strategy of gray mold, as the severity of this disease is usually high under greenhouse conditions. Furthermore, the results obtained in the present study suggest the possibility of using alternative products with sanitation, roguing, elimination of crop debris, and control of the environmental conditions, in addition to fungicide sprays, to reduce the use of non-natural pesticides.

The use of alternative products as related to cost is of particular importance. Bacillus-based products are marketed as biofungicides; therefore, their costs are compatible with current chemical fungicides. Prices of the salts (potassium and sodium carbonate and bicarbonate) range between US$ 2 and US$ 5 kg^{-1}, which do not impact the cost of production. On the other hand, coffee oils cost approximately US$ 180 L^{-1}, which make them expensive for agricultural use. Apart from the cost, growers should also consider the efficacy of different biocompatible products and their concentrations.

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Authors' Contributions

Conceptualization: Bettiol, W.; Piermann, L. **Data acquisition**: Piermann, L.; Galvão, J.A.H.; Fujinawa, M.F. **Data analysis**: Piermann, L.; Pontes, N.C.; Bettiol, W. **Design of methodology**: Bettiol, W.; Piermann, L. **Writing and editing**: Bettiol, W.; Pontes, N.C.; Piermann, L.

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