Physiological Response of Cape Gooseberry Seedlings to Three Biological Control Agents Under *Fusarium oxysporum* f. sp. *physali* Infection

José Luis Chaves-Gómez,¹ Cristian Camilo Chavez-Arias,¹ Alba Marina Cotes Prado,² Sandra Gómez-Caro,¹ and Hermann Restrepo-Díaz^{1,†}

¹ Facultad de Ciencias Agrarias, Universidad Nacional de Colombia sede Bogotá, Bogotá D.C., Colombia

² Corporación Colombiana de Investigación Agropecuaria AGROSAVIA, C. I. Tibaitatá, Bogotá D.C., Colombia

Abstract

Cape gooseberry (*Physalis peruviana*) fruit has gained recognition owing to its nutritional value and versatility to be consumed processed or as a fresh product. These characteristics have made it an important product in both national and international markets. One of the main limitations for this crop is Fusarium wilt caused by the fungus *Fusarium oxysporum* f. sp. *physalis*, for which biological control is emerging as an alternative to conventional management with chemical synthesis products. However, information on the effect that biological control agents have on the growth and development of plants is scarce. In this research, the physiological response of cape gooseberry plants (stomatal conductance, leaf water potential, growth parameters, total chlorophyll, carotenoid, and proline and malondialdehyde contents) to the treatment with three potential biocontrol agents (BCAs) *Trichoderma koningiopsis*, *Trichoderma virens*, and *Bacillus velezensis* was determined. The study was conducted

under greenhouse conditions; *F. oxysporum* was inoculated in the soil, and BCAs were soil drenched in the germination and transplanting stages. Plants inoculated with the pathogen and plants without inoculation were used as controls. It was found that the plants inoculated and treated with *T. virens* showed the lowest disease levels (area under the disease progress curve of 48.5 and disease severity index of 2.1). Additionally, they showed a lower water potential (−0.317 Mpa), a greater leaf area (694.7 cm²), and a higher stomatal conductance (110.3 mmol m^{−2} s^{−1}) compared with the control. Consequently, it can be concluded that *T. virens* can be a good candidate for the management of Fusarium wilt in the cape gooseberry crop.

Keywords: cultural and biological practices, disease management, fungi, tropical plants

Vascular wilts caused by pathogens of the genus *Fusarium* are a major concern in agriculture, because they cause great economic losses in a wide variety of crops (Okungbowa and Shittu 2012). In Colombia, Fusarium wilt caused by *Fusarium oxysporum* is a limiting disease in Andean fruit trees, such as lulo (*Solanum quitoense* Lamarck) and cape gooseberry (*Physalis peruviana* L.), that belong to the Solanaceae family (Ochoa et al. 2016; Osorio-Guarín et al. 2016). Cape gooseberry is a fruit of great economic importance that is commercialized in national and international markets. However, its production area has been reduced considerably by disease caused by *F. oxysporum* f. sp. *physali* (FOph) (Enciso-Rodríguez et al. 2013; Simbaqueba et al. 2018).

Plants infected with FOph show symptoms of chlorosis, leaf curl, and leaf senescence, whereas the water potential and gaseous exchange properties (stomatal conductance) decrease. In addition, increases in the malondialdehyde (MDA) and proline contents have also been reported in infected plants owing to cell damage caused by oxidative stress. Finally, plant death can be observed in severely infected plants (Chavez-Arias et al. 2019; Enciso-Rodríguez et al. 2013; Morkunas and Bednarski 2008; Villareal-Navarrete et al. 2017).

The presence of resistance structures (chlamydospores) of the pathogen in the soil has made it difficult to manage Fusarium wilt owing to the heterogeneous responses obtained with different control strategies (Alabouvette et al. 2009; Osorio-Guarín et al. 2016).

However, promising advances in the integrated management of this disease in different crops worldwide have been reported (Bubici et al. 2019; Cianchetta and Davis 2015; Jiménez-Díaz and Jiménez-Gasco 2011; Segura et al. 2018). The use of beneficial microorganisms (biocontrol agents [BCAs]) has shown positive results for crop protection against different diseases (O'Brien 2017; Syed Ab Rahman et al. 2018). Also, the application of BCAs can reduce the negative impacts generated by the use of chemical synthesis fungicides, because these agents have a low impact on the environment. Among the negative impacts of chemical synthesis fungicides, we can find resistance to active ingredients, residuality, and imbalance of the microbiota associated with the crop (Cawoy et al. 2011; O'Brien 2017; Syed Ab Rahman et al. 2018).

Among the BCAs with the greatest potential are several fungal species of the genus *Trichoderma* (El Komy et al. 2015; Gava and Pinto 2016; Howell 2003; Jogaiah et al. 2018; Martínez et al. 2013; Michel et al. 2001; Ramesh and Singh 2017; Rey et al. 2000) and bacteria of the genus *Bacillus* (Cawoy et al. 2011; Jacobsen et al. 2004; Shafi et al. 2017). Seeds treated with *Trichoderma virens* suppressed the development of Fusarium wilt symptoms in cotton (Howell 2006). Seed treatment with *Trichoderma koningiopsis* according to the technique by Cotes et al. (2001) also decreased Fusarium wilt (*F. oxysporum* f. sp. *lycopersici*) incidence by 35% in tomato (Jaimes et al. 2009). Preliminary research showed that *T. koningiopsis* application also reduced Fusarium wilt incidence and severity on cape gooseberry plants (Cotes et al. 2012). Finally, one of the advantages of the use of the genus *Trichoderma* is that it can be applied in different modes, such as root dipping, soil drench, and inoculation (Bubici et al. 2019).

The use of *Bacillus amyloliquefaciens* has been widely reported in the control of fungal diseases, because it favors the production of secondary metabolites with antifungal and antibacterial activity and the induction of plant defense mechanisms (Chowdhury et al. 2015; Gowtham et al. 2018; Ji et al. 2013). In banana, soil application of *B. amyloliquefaciens* strain W19 decreased Fusarium wilt caused by *F. oxysporum* f. sp. *cubense* through the production of volatile antifungal compounds and lipopeptides (Wang et al. 2012).

[†]Corresponding author: H. Restrepo-Díaz; hrestrepod@unal.edu.co

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The use of BCAs has positive effects on the plant's physiological parameters and its association with plant defense responses to pathogen attack (Cordo et al. 2007; Doni et al. 2014; Maksimov and Khairullin 2016; Radhakrishnan et al. 2017). Plant growth stimulation generated by BCAs of the genera *Trichoderma* and *Bacillus* has been widely reported (Hermosa et al. 2013; Samaniego-Gómez et al. 2016; Sivasakthi et al. 2014; Stewart and Hill 2014). In this regard, the positive effect of the use of BCAs on growth stimulation in plants infected by vascular pathogens has been documented. Nosir (2016) observed that the dipping of tuberose (*Polianthes tuberosa*) bulbs in a *Trichoderma harzianum* (strain T22) suspension generated a higher biomass production in plants inoculated with *F. oxysporum* f. sp. *tuberosi*. Similarly, *B. amyloliquefaciens* application also promoted growth of banana plants affected with *F. oxysporum* f. sp. *cubense* (Yuan et al. 2013).

The use of BCAs affects osmolyte production and lipid peroxidation of membranes associated with biotic stress conditions. The use of *T. harzianum* or *Trichoderma viride* reduced the lipid peroxidation represented as MDA in tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* (Tayal et al. 2011; Zehra et al. 2017). Treatment with *Bacillus* species, such as *Bacillus cereus* and *Bacillus subtilis*, also showed a decrease in the MDA content in tomato and banana plants, respectively, affected by Fusarium wilt caused by *F. oxysporum* (Din et al. 2018; Ferraz et al. 2014). Additionally, treatment of tomato plants with *B. amyloliquefaciens* promoted proline synthesis in plants inoculated with *F. oxysporum* f. sp. *lycopersici* compared with inoculated plants and plants without the BCAs (Shahzad et al. 2017). Finally, the application of *Trichoderma* species can favor the synthesis of photosynthetic pigments (chlorophylls [Chls] and carotenoids) in Fusarium wilt-infected peanut (*Arachis hypogaea*) plants (Rajeswari 2014).

The use of BCAs and their effect on the disease level have become important in the cape gooseberry–*F. oxysporum* pathosystem in Colombia (Díaz et al. 2013; Moreno-Velandia et al. 2016, 2019). However, information about the characterization of potential BCAs application (the genera *Trichoderma* and *Bacillus*) on the physiological response of plants infected with FOph is still scarce. Therefore, the objective of this research was to study the potential effect of the application of three BCAs *T. koningiopsis* (Th003), *T. virens* (Gl006), and *B. amyloliquefaciens* (Bs006) on the physiological response of *F. oxysporum*-inoculated and noninoculated cape gooseberry plants.

Materials and Methods

This study was carried out between December 2016 and April 2017 in the greenhouses of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia in Bogotá (4°35'56" N, 74°04'51" W, altitude of 2,557 meters above sea level [m.a.s.l.]) with a natural photoperiod of 12 h, 50 to 70% relative humidity, and ±24°C and 18°C average day and night temperature, respectively.

Growth conditions. Cape gooseberry ecotype Colombia (Semicol S.A.) commercial seeds were subjected to surface disinfection with a 70% (vol/vol) ethanol solution for 60 s, 3% (vol/vol) sodium hypochlorite for 20 min in agitation, and finally, three rinses with sterile distilled water. Then, seeds were sown in 70-cell germination trays containing peat without nutrients (Klasmann; Klasmann-Deilmann GmbH). After germination (at 30 days after sowing [DAS]), seedlings were fertilized every 3 days with 10 ml of compound liquid fertilizer (Nutriponic) at a concentration of 3 ml/liter of water. At 45 DAS, when the seedlings showed four fully expanded leaves, they were transplanted to 2-liter plastic pots containing a mixture of pathogen-free soil and rice husk (3:1 vol/vol). Absence of *F. oxysporum* in the soil used as substrate was determined using the microbial count by soil dilution technique with modifications. Forty-five milliliters of sterile water agar (0.1%) supplemented with MgSO₄·7H₂O (0.1%) was added to three 5-g soil samples. The mix was shaken for 15 min and then, serially diluted. Samples of 0.2 ml were taken and incubated in the dark at 28°C for 7 days in petri dishes with Czapek Dox agar medium (Gamliel and Katan 1991).

Treatments with biological control agents. *T. koningiopsis* (Th003), *T. virens* (Gl006), and *Bacillus velezensis* (Bs006; previously *B. amyloliquefaciens*) were selected for this study based on their biocontrol potential of plant pathogens (Benitez et al. 2004; Jaimes et al. 2009). Four groups of treatments were established: (i) cape gooseberry seedlings inoculated with *F. oxysporum* and separate application of the three BCAs (Gl006 + *F. oxysporum*, Th003 + *F. oxysporum*, and Bs006 + *F. oxysporum*); (ii) seedlings not inoculated with *F. oxysporum* and treated separately with the three BCAs (Gl006, Th003, and Bs006); (iii) seedlings only inoculated with *F. oxysporum* (pathogenic control [FOph⁺]), and (iv) seedlings without any inoculation (absolute control [FOph⁻]).

Each biocontrol treatment was prepared in a suspension in sterile distilled water adjusted to the following concentrations: 1 × 10⁶ conidia ml⁻¹ for Th003 and Gl006 and 1 × 10⁸ cells ml⁻¹ for Bs006. Drench application of 5 ml of each BCA suspension per cell of the germination trays was done at 30 DAS and 30 ml per pot at the time of transplanting (45 DAS). *F. oxysporum* inoculation was carried out incorporating the pathogen's propagules (microconidia) at the time of the substrate preparation before transplantation (Hao et al. 2009; Purwatti et al. 2008). For every 1.0 kg of substrate used (soil + rice husk), 100 ml of *F. oxysporum* strain Map5 suspension in sterile distilled water was added at a concentration of 1 × 10⁶ conidia ml⁻¹. All of the microorganisms used in this study were supplied by the Agricultural Microbiology Laboratory of Agrosavia.

Disease severity analysis. Fusarium wilt severity was determined every 3 days from 45 DAS (which corresponded to transplanting time) until the end of the experiment (95 DAS) in each treatment. Visual evaluations of the characteristic symptoms of the disease, such as hyponastic response, chlorosis, loss of turgor in leaves, and defoliation until complete wilting of the plant, were quantified using the scale proposed by Moreno-Velandia (2017). Subsequently, the disease severity index was calculated using Equation 1 described by Chiang et al. (2017):

$$\text{disease severity index} = \left(\sum (nv) / V \right) \quad (1)$$

where n is the level of infection according to the scale, v is the number of plants present in each level, and V is the total number of plants evaluated.

The severity of the disease in each treatment was estimated by calculating the area under the disease progress curve (AUDPC) following the trapezoidal integration method (Campbell and Madden 1990):

$$\text{AUDPC} = \left\{ \sum_{i=1}^{n-1} [(y_i + y_{i+1})/2] \times (t_{i+1} - t_i) \right\} \quad (2)$$

where n is the number of evaluations; y_i , y , and y_{i+1} are the values of the severity scale that were obtained at every evaluation time; and $(t_{i+1} - t_i)$ is the time interval between evaluations.

Table 1. Area under the disease progress curve (AUDPC), disease severity index, and control efficacy (percentage) of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *physali* (FOph⁺) in cape gooseberry plants with the application of biocontrol agents *Trichoderma virens* (Gl006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006)

Treatment	AUDPC	Disease severity index	Efficacy (%)
FOph ⁺	66.6 a ^y	3.2 a	–
Gl006/FOph ⁺	48.5 b	2.1 b	32 a
Th003/FOph ⁺	62.1 ab	2.7 ab	14 b
Bs006/FOph ⁺	58.3 ab	2.8 ab	12 b
Significance ⁺	At $P \leq 0.05$	At $P \leq 0.01$	At $P \leq 0.01$
CV (%) ^z	11.15	12.58	38.72

^y Values within one column followed by different letters are significantly different according to the Tukey test.

^z CV, coefficient of variation.

Finally, Abbott's formula was modified to calculate the efficacy for each of the BCAs:

$$\text{Efficacy (\%)} = (X - Y) / X \times 100 \quad (3)$$

where X is the severity index of the pathogen control and Y is the severity index of each treatment (Abbott 1925).

Stomatal conductance and leaf water potential. Stomatal conductance (g_s) was estimated on a fully expanded leaf randomly taken from the upper part of the plant using a steady-state porometer (SC-1; Decagon Devices Inc.). Then, the leaf water potential (Ψ_{wf}) was determined using a Schollander pressure chamber (PMS, Model 615, OR) on the same leaf used to estimate g_s . Measurements of Ψ_{wf} and g_s were made between 0900 and 1100 h on

Fig. 1. Stomatal conductance (g_s) of cape gooseberry plants with and without *Fusarium oxysporum* f. sp. *physali* inoculation (F'Oph⁺ and F'Oph⁻, respectively) and the application of three different biocontrol agents *Trichoderma virens* (Gl006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) at **A**, 10, **B**, 20, **C**, 30, **D**, 40, and **E**, 50 days after inoculation. Bars represent the mean of four values $\pm$ standard error. Different letters indicate significant differences between treatments according to the Tukey test ($P \leq 0.05$). N.S., not significant.

totally sunny days at 10, 20, 30, 40, and 50 days after inoculation (DAI).

Growth parameters. Leaves, stems, and roots of each plant per treatment were collected separately at 10, 20, 30, 40, and 50 DAI to determine their dry weight. Leaf area was estimated from digital images in tagged image file format (D3300; Nikon) and analyzed

using an image processing software by Java (ImageJ; National Institute of Mental Health).

Chl and carotenoids content. A 0.03-g leaf sample from the middle third of the plant was collected and homogenized in 4 ml of 80% acetone. Then, the samples were centrifuged (model 420101; Becton Dickinson Primary Care Diagnostics) at 5,000 rpm for 10 min to

Fig. 2. Leaf water potential (Ψ_{wf}) of cape gooseberry plants with and without *Fusarium oxysporum* f. sp. *physali* inoculation (FOpht⁺ and FOpht⁻, respectively) and the application of three different biocontrol agents *Trichoderma virens* (Gl006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) at **A**, 10, **B**, 20, **C**, 30, **D**, 40, and **E**, 50 days after inoculation. Bars represent the mean of four values $\pm$ standard error. Different letters indicate significant differences between treatments according to the Tukey test ($P \leq 0.05$). N.S., not significant.

remove particles. The supernatant was diluted with acetone to a 6-ml final volume. Then, a reading was performed with a spectrophotometer (Spectronic BioMate 3 UV-vis Thermo) using 663- and 646-nm wavelengths for Chl and a 470-nm wavelength for carotenoids. To estimate their content, these values were used in the equations described by Lichtenthaler (1987). These variables were determined at 50 DAI.

MDA and proline content. The methods based on thiobarbituric acid (Hodges et al. 1999) and ninhydrin acid (Bates et al. 1973) were used to estimate the membrane lipid peroxidation (MDA content) and the leaf proline concentration at 50 DAI, respectively.

In both tests, a 0.5-g leaf sample from the middle third of the plant was macerated and stored in liquid nitrogen until analysis. For the MDA determination, samples were centrifuged at 5,000 rpm for 10 min, and the absorbances at 440, 532, and 600 nm were estimated with a spectrophotometer (Spectronic BioMate 3 UV-vis Thermo). Finally, the extinction coefficient (157 M ml^{-1}) was used to obtain the MDA concentration.

Proline concentration was determined by adding 10 ml of 3% sulfosalicylic acid to the stored samples and then, filtering them with Whatman paper (no. 2). Two milliliters of this filtrate were incubated with 2 ml of ninhydrin acid and 2 ml of glacial acetic acid in a water bath at 90°C for 1 h, and then, it was stopped in ice. The resulting solution was dissolved in 4 ml of toluene by shaking the test tubes vigorously using a vortex shaker, and the absorbance readings were determined at 520 nm with a spectrophotometer. The proline content was calculated using the fresh weight of the sample by means of a standard calibration curve (Equation 3):

$$\frac{\mu\text{mol proline}}{\text{g fresh plant material}} = \frac{\left[\frac{\mu\text{g proline} \times \text{ml toluene}}{\text{ml}} \right]}{\left[\frac{115.5 \mu\text{g}}{\mu\text{mol}} \right]} \quad (4)$$

Experiment design and data analysis. The cape gooseberry seedlings were arranged in a completely randomized design with four repetitions per treatment. In total, each treatment had 20 seedlings, and four plants were used at each evaluation time (10, 20, 30, 40, and 50 DAI). The experiment lasted 95 days. An analysis of variance was performed, and when significant differences ($P \leq 0.05$) were found, a Tukey post hoc test was used for the mean comparison. The percentage values were transformed using the arcsine function. A main component analysis was performed using biplot graphs with the purpose of visualizing the relationships between the treatments. The data were analyzed using the Statistix v 9.0 software (Analytical Software). The figures were done using SigmaPlot software (Systat Software), and the biplot analysis was carried out with the InfoStat v 2018 software (Universidad Nacional de Córdoba).

Results

Disease severity. Cape gooseberry plants inoculated with FOph had a 100% incidence of *Fusarium* wilt, where the presence of the pathogen was confirmed by obtaining characteristic colonies in potato dextrose agar (PDA) medium according to Leslie and Summerell (2006) (data not shown). Regarding the disease severity values (AUDPC and severity index), Table 1 shows that significant differences ($P \leq 0.01$) on these variables were obtained between the different treatments evaluated. The AUDPC showed higher values in plants inoculated with FOph⁺ (66.6). Intermediate values were recorded in plants with FOph and plants inoculated with *T. koningiopsis* or *B. velezensis* (62.1 and 58.3, respectively); plants inoculated with FOph and treated with *T. virens* showed the lowest AUDPC (48.5). The severity index showed similar trends to the ones observed for the AUDPC, with the lowest values for plants inoculated with FOph and treated with *T. virens* (disease severity index of 2.1). Finally, plants treated with this BCA showed the highest efficacy in the control of *Fusarium* wilt (32%).

Stomatal conductance and leaf water potential. The stomatal conductance of the leaf (g_s) is shown in Figure 1. Differences were not observed between treatments evaluated at 10 DAI (Fig. 1A). However, differences ($P = 0.0007$) began to be observed from 20 DAI, when the cape gooseberry plants inoculated with FOph and treated with *B. velezensis* (Bs006/FOph⁺) showed a lower g_s ($217.62 \text{ mmol m}^{-2} \text{ s}^{-1}$) compared with the other treatments ($\sim 253.29 \text{ mmol m}^{-2} \text{ s}^{-1}$) (Fig. 1B). At 30 DAI, plants without *F. oxysporum* (FOph⁻) in general showed a greater g_s ($\sim 239.87 \text{ mmol m}^{-2} \text{ s}^{-1}$) compared with plants with FOph⁺ ($\sim 170.59 \text{ mmol m}^{-2} \text{ s}^{-1}$) (Fig. 1C). At 40 DAI, a significant reduction (67%) of g_s was observed mainly in the treatments of plants inoculated only with FOph⁺ and plants with Bs006/FOph⁺ compared with plants without FOph and BCAs (FOph⁻). *T. virens* (Gl006/FOph⁺) and *T. koningiopsis* (Th003/FOph⁺) application increased (93%) g_s in plants with *Fusarium* wilt compared with plants inoculated only with FOph⁺ (Fig. 1D). At the end of the experiment (50 DAI), the g_s was slightly favored by *T. virens* application in plants with the presence of the pathogen (Gl006/FOph⁺) (Fig. 1E).

The leaf water potential (Ψ_{wf}) is shown in Figure 2. Differences ($P = 0.0003$) began to be observed between the treatments from 10 DAI, when plants treated only with FOph⁺ showed a more negative Ψ_{wf} (-0.25 megapascals [Mpa]) (Fig. 1A). At 20 DAI, cape gooseberry plants without the pathogen (FOph⁻) showed a better water status (Ψ_{wf} approximately -0.173 Mpa) than plants with FOph⁺ (approximately -0.248 Mpa). However, *T. virens* favored a slight increase in Ψ_{wf} (-0.213 Mpa) in the seedlings with FOph (Gl006/FOph⁺) (Fig. 2B). At 30 DAI, there was a reduction of Ψ_{wf} (42%) in all plants with FOph⁺ compared with plants without the pathogen (FOph⁻) (Fig. 2C). At 40 DAI, plants inoculated only with FOph⁺ and treated with *T. koningiopsis* and *B. velezensis* showed the most negative Ψ_{wf} . However, *T. virens* favored Ψ_{wf}

Table 2. Effect of the application of biocontrol agents *Trichoderma virens* (Gl006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) and *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *physali* (FOph⁺) on the foliar area, dry weight of the aerial part (DWA), dry weight of the root (DWR), total dry weight (DWT), and aerial part/root ratio (AP/R) in cape gooseberry plants

Treatment	Leaf area, cm ²	DWA, g dry matter	DWR, g dry matter	DWT, g dry matter	AP/R
FOph ⁻	1,015.8 a ^y	1.09 a	0.96 abc	2.05 a	1.13 a
FOph ⁺	505.4 e	0.81 d	0.77 c	1.58 c	1.06 ab
Gl006/FOph ⁻	906.9 b	0.92 bc	0.76 c	1.68 c	1.23 a
Gl006/FOph ⁺	694.7 c	0.80 d	0.78 c	1.59 c	1.03 ab
Th003/FOph ⁻	1,052.6 a	1.06 a	0.87 bc	1.92 ab	1.23 a
Th003/FOph ⁺	604.5 d	1.05 a	1.05 ab	2.10 a	1.01 ab
Bs006/FOph ⁻	1,016.0 a	0.94 b	0.75 c	1.69 bc	1.26 a
Bs006/FOph ⁺	889.8 b	0.85 cd	1.16 a	2.01 a	0.74 b
Significance	At $P \leq 0.001$	At $P \leq 0.001$	At $P \leq 0.001$	At $P \leq 0.001$	At $P \leq 0.001$
CV (%) ^z	4.25	3.67	11.39	5.71	13.50

^y Values within one column followed by different letters are significantly different from $P \leq 0.05$ according to the Tukey test.

^z CV, coefficient of variation.

(25%) in plants with FOph (GI006/FOph⁺) (Fig. 2D). Finally, at 50 DAI, the application of all BCAs caused the water status (Ψ_{wf}) in plants with FOph⁺ to be higher, because *T. virens* continued mitigating the negative impact of the pathogen (GI006/FOph⁺) on this variable (−0.317 Mpa) (Fig. 2E).

Growth parameters. The statistical differences observed on growth parameters (leaf area, accumulation of dry weight in the aerial part [DWA], dry weight in the root [DWR], and total dry weight [DWT], and aerial part/root ratio [AP/R]) among treatments of BCAs at the end of the trial (50 DAI) are summarized in Table 2. The leaf area showed the lowest values in cape gooseberry plants inoculated with FOph⁺ (505.4 cm²). The application of the BCAs favored the leaf area in plants with Fusarium wilt in which *B. velezensis* (Bs006/FOph⁺) and *T. virens* (GI006/FOph⁺) caused significant increases of this parameter (889.8 and 694.7 cm², respectively).

The DWA showed lower values in cape gooseberry plants inoculated with FOph⁺ (0.81 g), GI006/FOph⁺ (0.80 g), and Bs006/FOph⁺ (0.85 g) compared with cape gooseberry plants without the pathogen (FOph[−]; 1.09 g). The application of *T. koningiopsis* generated a positive effect on the DWA in both inoculated and noninoculated plants (1.05 and 1.06 g, respectively), showing statistically similar values to the control plants (FOph[−]). The application of the BCAs did not cause any differences in the DWR in plants inoculated and not inoculated with the pathogen compared with the plants without any application (FOph[−]); however, seedlings with Bs006/FOph⁺ had a higher DWR (1.16 g).

An effect similar to that observed in the DWA was obtained in the DWT in plants with FOph⁺ (1.58 g) and GI006/FOph⁺ (1.59 g), which was reflected in a highly significant decrease for this variable compared with the control plants (FOph[−]). Similarly, the DWT of cape gooseberry plants with GI006/FOph[−] (1.68 g) and Bs006/FOph[−]

Fig. 3. A, Total chlorophyll and **B,** carotenoids content of cape gooseberry plants with and without *Fusarium oxysporum* f. sp. *physali* inoculation (FOph⁺ and FOph[−], respectively) and the application of three different biocontrol agents *Trichoderma virens* (GI006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) at 50 days after inoculation. Bars represent the mean of four values $\pm$ standard error. Different letters indicate significant differences between treatments according to the Tukey test ($P \leq 0.05$). N.S., not significant; FW, fresh weight.

(1.69 g) showed a reduction in this parameter. However, plants of Th003/FOph⁺ (2.10 g), FOph⁻ (2.05 g), Bs006/FOph⁺ (2.01 g), and Th003/FOph⁻ (1.92 g) showed the highest dry matter accumulation. Finally, the application of BCAs had no effect on AP/R in FOph-inoculated and noninoculated plants; the lowest AP/R values were observed in the plants with *B. velezensis* and inoculated with the pathogen (Bs006/FOph⁺; 0.74).

Chl and carotenoids content. The content of the photosynthetic pigments (total Chl and carotenoids) is shown in Figure 3. Highly significant differences ($P = 0.0000$) were observed in the different treatments evaluated at 50 DAI for these two variables. Higher total Chl values were recorded in cape gooseberry plants without *F. oxysporum* (FOph⁻; $\sim 2,286.58 \mu\text{g}^{-1}$ mg fresh weight [FW]) compared with inoculated plants (FOph⁺; $\sim 1,096.21 \mu\text{g}^{-1}$ mg FW) (Fig. 3A). Regarding carotenoids, control plants without *F. oxysporum* or treated with BCAs (FOph⁻) showed the highest concentrations of these pigments ($245.22 \mu\text{g}^{-1}$ mg FW). Finally, the cape gooseberry plants of GI006/FOph⁺ showed a higher increase (62%) compared with the plants inoculated only with FOph⁺ (Fig. 3B).

MDA and proline content. The MDA and proline contents are shown in Table 3. Lipid peroxidation of cell membranes expressed as MDA production was similar in almost all treatments, with a higher production in FOph⁺ ($11.49 \mu\text{mol g}^{-2}$ FW) plants. Also, proline synthesis showed highly significant differences ($P = 0.0000$) among the treatments evaluated, in which plants affected by Fusarium wilt presented the lowest values ($\sim 10.52 \mu\text{mol g}^{-2}$ FW) compared with plants without FOph⁻ ($\sim 4.61 \mu\text{mol g}^{-2}$ FW).

Biplot analysis of physiological responses to FOph management with BCAs. The analysis of main components showed that the variables evaluated, such as g_s , Ψ_{wf} , leaf area, DWT, total Chl, carotenoids, MDA, and proline, explained 83% of the physiological response of cape gooseberry plants subjected to different treatments at 50 DAI (Fig. 4). The biplot analysis also showed that there is a high correlation between these variables and the physiological behavior of plants, because the vectors corresponding to each of these variables have angles close to the origin. Similarly, it was observed that the plants without FOph inoculation were grouped in the same sector, indicating that they have a similar physiological response (group I). Regarding plants only inoculated with *F. oxysporum* (group IV), these were located in the sector opposite to group (I), showing an FOph⁺ antagonistic effect on the physiology of cape gooseberry seedlings. In contrast, the addition of BCAs showed two differential effects in the analysis, in which plants treated with Th003/FOph⁺ and Bs006/FOph⁺ (group III) did not show a differential behavior compared with group IV (FOph⁺); also, *T. virens* addition favored the physiology of plants inoculated with FOph (group II), bringing its response

closer to the physiological behavior registered in control plants (group I).

Discussion

The use of BCAs belonging to the genera *Trichoderma* and *Bacillus* has reduced the incidence and severity of vascular diseases in different crops (Cawoy et al. 2011; El Komy et al. 2015; Jacobsen et al. 2004; Jogaiah et al. 2018; Ramesh and Singh 2017). In this study, the application of BCAs had a positive effect on the reduction of the disease, and *T. virens* (GI006) had the lowest severity index and AUDPC in cape gooseberry plants inoculated with *F. oxysporum*. Rozlianah et al. (2007) also observed that the addition of *T. virens* decreased the severity index and AUDPC in tomato plants inoculated with *F. oxysporum*.

The positive response of the BCAs application in the management of diseases is also related to their effect on the plant's physiology (O'Brien 2017; Syed Ab Rahman et al. 2018). The reduction in g_s and Ψ_{wf} is one of the main responses to biotic stress generated by *F. oxysporum* (Dong et al. 2012; Nogués et al. 2002; Sun et al. 2017; Wang et al. 2015). In this study, the application of the three groups of biological control agents (*T. virens*, *T. koningiopsis*, and *B. amyloliquefaciens*) improved the water status (expressed as Ψ_{wf}) in cape gooseberry plants with Fusarium wilt. Additionally, the use of *T. virens* increased the g_s in the plants with *F. oxysporum*. Studies by Segarra et al. (2009) and Doni et al. (2014) showed that the use of different *Trichoderma* isolates favored g_s and Ψ_{wf} in tomato and rice plants, respectively. The use of BCAs can improve the water status or gas exchange properties of plants through better water use efficiency or the overexpression of integral membrane proteins that facilitate the transport of water and solutes, such as aquaporins (Gül et al. 2008; Vieira et al. 2017).

Other positive aspects of the use of biological agents for disease control are their effect on the promotion of plant growth (Samaniego-Gómez et al. 2016; Sivasakthi et al. 2014). In this study, the application of *T. virens*, *T. koningiopsis*, and *B. amyloliquefaciens* caused a higher biomass accumulation in FOph-inoculated plants. Similar results were obtained in the work by Yuan et al. (2013), in which banana plants treated with *B. amyloliquefaciens* and inoculated with *F. oxysporum* had a greater dry matter accumulation attributed mainly to the production of auxins and gibberellins. Nosir (2016) also reported that the application of *T. harzianum* caused greater dry matter accumulation in tuberose plants affected with *F. oxysporum* f. sp. *tuberosi* owing to greater nutrient availability. Similarly, BCAs of the genus *Trichoderma* stimulate the production of secondary metabolites, which act as growth promoters (azaphilone, butenolide, harzianopyridone, harzianolide, 1-hydroxy-3-methylanthraquinone, 1,8-dihydroxy-3-methyl-anthraquinone, and 6-*n*-pentyl-6H-pyran-2-one) (Stewart and Hill 2014).

The synthesis of proline, MDA, or photosynthetic pigments plays an important role in the characterization of the plant's physiological response to biotic and abiotic stresses. In this work, the proline production in general was lower in all *F. oxysporum*-inoculated plants, except for those treated with *T. virens*, which favored the synthesis of this amino acid with and without the presence of the pathogen. In this regard, Rajeswari (2015) observed that the use of *T. viride* and *T. harzianum* also increased the content of this amino acid in leaves of peanut plants inoculated with *F. oxysporum* and stated that the accumulation of phenol and proline compounds acts as adaptive mechanism in the host plant against the fungal infection, causing a restriction of pathogen development.

In this study, lipid peroxidation of cell membranes expressed in terms of MDA production in the cape gooseberry plants of the different treatments did not show any significant differences compared with control plants (FOph⁻). El-Zahaby et al. (1995) and Siddique et al. (2014) also found no increase in the MDA contents in cotton and barley plants susceptible to the cotton leaf curl Burewala virus and the fungus *Erysiphe graminis* f. sp. *hordei*, respectively. These authors argue that the low MDA production can be owing to the induction of antioxidant processes by an elicitor effect of the pathogen, which causes a suppression of lipid peroxidation through the

Table 3. Effect of the application of biocontrol agents *Trichoderma virens* (GI006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *physali* (FOph⁺) on the malondialdehyde (MDA) production and leaf proline content in cape gooseberry plants

Treatment	MDA, $\mu\text{mol g}^{-2}$ FW ^x	Proline, $\mu\text{mol g}^{-2}$ FW
FOph ⁻	8.61 ab ^y	10.82 b
FOph ⁺	11.49 a	4.60 d
GI006/FOph ⁻	9.31 ab	13.58 a
GI006/FOph ⁺	9.24 ab	6.92 c
Th003/FOph ⁻	7.92 ab	7.93 c
Th003/FOph ⁺	11.17 a	2.17 e
Bs006/FOph ⁻	6.16 b	9.78 b
Bs006/FOph ⁺	10.82 a	4.75 d
Significance	At $P \leq 0.01$	At $P \leq 0.001$
CV (%) ^z	18.52	9.97

^x FW, fresh weight.

^y Values within one column followed by different letters are significantly different from $P \leq 0.05$ according to the Tukey test.

^z CV, coefficient of variation.

Fig. 4. Biplot analysis of cape gooseberry plants with and without *Fusarium oxysporum* f. sp. *physali* inoculation (FOPh⁺ and FOPh⁻, respectively) and the application of three different biocontrol agents *Trichoderma virens* (Gl006), *Trichoderma koningiopsis* (Th003), and *Bacillus velezensis* (Bs006) at 50 days after inoculation. Chl, chlorophyll; MDA, malondialdehyde; DWT, dry weight total; LA, leaf area; PC, principal component.

expression of some enzymes, such as glutathione *S*-transferase, that can diminish the harmful effects of lipid peroxidation.

The production of toxins by *F. oxysporum*, such as fusaric acid, is involved in the alteration of the plant's metabolism and the degradation of photosynthetic pigments (Wu et al. 2008). In this study, the application of BCAs did not reduce the Chl degradation generated by FOPh in cape gooseberry plants. These results contrast with those obtained by Patel and Saraf (2017) in tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* and treated with *Trichoderma asperellum*, in which an increase in the total Chl content was observed. However, *T. virens* application in plants inoculated with the pathogen promoted the synthesis of carotenoids compared with plants with *F. oxysporum* exclusively. Similar results were also obtained by John et al. (2010) and Rajeswari (2014) in soybean and peanut plants inoculated with *F. oxysporum* and treated with two species of the genus *Trichoderma* (*T. viride* and *T. harzianum*) that promoted the synthesis of carotenoids as a possible response to the stress condition.

In summary, the results obtained in this study showed that the inoculation of BCAs in *P. peruviana* favored the physiological response of plants affected by Fusarium wilt, mainly *T. virens* (Gl006), which generated a higher *g_s* and a lower Ψ_{wf} . In addition, this BCA obtained the lowest disease severity compared with plants only inoculated with *F. oxysporum*; it also showed the highest efficacy in relation to the other treatments. Similarly, different studies have shown *T. virens* as a mycoparasite that produces secondary metabolites that induce rapid defense responses with antifungal activity, which can reduce disease severity (Hermosa et al. 2013; Howell 2006; Jogaiah et al. 2018). In conclusion, the results obtained suggest that *T. virens* (Gl006) application can be considered as a complementary tool for the integrated management of Fusarium wilt in cape gooseberry plants.

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