Physiological Response of Cape Gooseberry Seedlings to Two Organic Additives and Their Mixture under Inoculation with *Fusarium oxysporum* f. sp. *physali*

José Luis Chaves-Gómez

Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá, Colombia

Alba Marina Cotes-Prado

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

Sandra Gómez-Caro and Hermann Restrepo-Díaz

Departamento de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Bogotá, Colombia

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Abstract. Vascular wilt caused by *Fusarium oxysporum* f. sp. *physali* is the most limiting disease in cape gooseberry crops. The use of natural products such as organic additives is a promising alternative for management of this disease. The present study sought to evaluate the physiological response of cape gooseberry plants infected with this pathogen and treated with the organic additives chitosan, burned rice husks, or their mixture. The test was conducted under greenhouse conditions and soil was inoculated with *F. oxysporum* f. sp. *physali* strain Map5. Chitosan was applied to seeds and seedlings at the time of transplantation, whereas burned rice husk was incorporated into the soil in a 1:3 ratio. Plants inoculated and not inoculated with the pathogen were used as controls. The following variables were evaluated: area under the disease progress curve (AUDPC), leaf water potential, stomatal conductance (g_s), leaf area (LA), dry matter accumulation, photosynthetic pigment contents, proline synthesis, and lipid peroxidation estimation [malondialdehyde (MDA)]. The results showed that cape gooseberry plants with vascular wilt and treated with chitosan had higher g_s , leaf water potential, LA, dry matter accumulation, and proline content values. In addition, the levels of vascular wilt severity decreased in comparison with pathogen-inoculated controls. The results suggest that chitosan applications on cape gooseberry plants may be considered as an alternative in the integrated management of the disease in producing areas, because they can mitigate the negative effect of the pathogen on plant physiology.

Cape gooseberry (*Physalis peruviana* L.) is a fruit bush native to the highlands of the Andean region of South America (Fischer et al., 2007). In Colombia, this crop is consid-

ered promising for export because of the high demand in European markets (Álvarez-Flórez et al., 2017; Cabrera et al., 2017) due to its flavor, color, and shape. In addition, it is a source of vitamins (A, C, and B complex), antioxidants (polyphenols), essential minerals [phosphorus (P), iron (Fe), potassium (K) and zinc (Zn)] and anti-inflammatory compounds (phytosterols) (Hassanien, 2011; Puente et al., 2011).

Cape gooseberry cultivation in Colombia faces different problems, with vascular wilt, caused by the pathogen *F. oxysporum* f. sp. *physali* (FOph), being one of the most limiting diseases in this crop (Enciso-Rodríguez et al., 2013; Osorio-Guarín et al., 2016; Simbaqueba et al., 2018). This fungus is characterized by the production of three types of propagules: macroconidia, microconidia, and chlamydospores (Okungbowa and Shittu, 2012). Microconidia are the main structures responsible for plant infection and pathogen dissemination. On the other hand, chlamydo-

spores are thick-walled resistance structures that allow the pathogen to survive under unfavorable conditions and remain in the soil for up to 10 years (Zhang et al., 2015; Gordon, 2017).

This fungus can affect plants at any development stage, from seedlings to reproductive stages. The main symptoms are root rot, marginal and complete chlorosis of mature leaves, stunted growth, and plant death (Enciso-Rodríguez et al., 2013; Joshi, 2018). Physiologically, the infection by this pathogen generates different alterations, such as plant growth reduction, and lower leaf gas exchange and water potential. In addition, the level of lipid peroxidation of cell membranes and amino acid contents such as proline increase (Dong et al., 2012; Nogués et al., 2002; Sun et al., 2017; Wang et al., 2012).

FOph management in cape gooseberry has been hampered by pathogen resistance to commercial fungicides and the survival of the pathogen in the soil for prolonged periods of time (Osorio-Guarín et al., 2016). Cultural practices, such as solarization, biofumigation, crop rotation, and crop waste management, have been insufficient to control vascular wilt in Colombia (Urrea et al., 2011). In addition, there is an increasing demand by consumers for food free of agrochemical residues and production processes that are friendlier to the environment. Because of these issues, alternatives to the use of agrochemicals, such as biological control and the application of organic additives, have gained interest recently (Moreno-Velandia et al., 2018; Pal and Gardener, 2006).

Organic additives are substances of natural origin that can act as biostimulants in plants (Ab Rahman et al., 2017; Mesa et al., 2017). One of these substances, chitosan, has been used as an ecofriendly biopesticide because it is biodegradable, nontoxic, and biocompatible (Hassan and Chang, 2017). The use of organic amendments to soil, such as compost, organic fertilizers, and burned rice husk, also have had a positive effect on the control of soil pathogens and have decreased disease incidence (Akhtar and Malik, 2000; Alabouvette et al., 2009; Bonanomi et al., 2015; Eo et al., 2018).

Chitosan is a natural polycationic biopolymer derived from the deacetylation of chitin and can be obtained from crustacean shells by either chemical or microbiological processes (Al-Hetar et al., 2011). This biopolymer is involved in pathogen attack responses in dicots and monocots due to its antimicrobial and biostimulant activity and the ability to induce plant defense mechanisms (El Hadrami et al., 2010; Hassan and Chang, 2017; Lárez, 2008). Under in vitro conditions, chitosan completely inhibited *F. oxysporum* f. sp. *cubense* race 4 and *F. oxysporum* f. sp. *albedinis* growth, causal agents of vascular wilt in banana and palm, respectively (Al-Hetar et al., 2011; El Hassni et al., 2004). Berger et al. (2016) observed that bean plants treated with chitosan and inoculated with *F. oxysporum* f. sp. *tracheiphilum* showed a reduction in the disease severity index. Likewise, chitosan treatment in seeds and seedlings at the time of transplanting significantly reduced the number of

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H.R.-D. is the corresponding author. E-mail: hrestrepod@unal.edu.co.

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root lesions caused by *F. oxysporum* f. sp. *radicis-lycopersici* in tomato plants (Benhamou et al., 1994).

Chitosan also shows physiological, biochemical, and plant growth effects (Pichyangkura and Chadchawan, 2015). It has been reported that chitosan treatment of wheat seeds under drought conditions promotes root development, antioxidant enzyme activity, MDA reduction, and chlorophyll content increase (Zeng and Luo, 2012). Mahdavi et al. (2011) found that the application of chitosan to safflower (*Carthamus tinctorius* L.) seedlings increased proline contents and reduced MDA content when they were subjected to different osmotic potential (ψ_s). In addition, foliar chitosan applications in strawberry (*Fragaria × ananassa*) promoted carotenoid synthesis and an increase in dry matter accumulation (Abdel-Mawgoud et al., 2010; Rahman et al., 2018).

Organic amendments are applied in agricultural systems to improve soil conditions (Lazarovits, 2001). Their use also has effects on the management of soilborne diseases (Bailey and Lazarovits, 2003; Bonanomi et al., 2007, 2010). One of the most commonly used amendments is burned rice husk, which has been applied for the management of *Cylindrocarpon destructans* and *Fusarium solani* in ginseng (*Panax ginseng*) plants, reducing root rot incidence (Eo et al., 2018). In addition, the application of this amendment also resulted in a reduction of the area of foliar lesions caused by *Colletotrichum dematium* in tomato (*Lycopersicon esculentum* L.) plants (Somapala et al., 2016). Burned rice husk has been used in mixtures for substrate to avoid the incidence of vascular wilt caused by *F. oxysporum* in horticultural crops (Quintero et al., 2012); however, this product has not been reported as an organic amendment for *F. oxysporum* management.

Some of the effects of the use of burned rice husk as an amendment include an increase in dry matter content in stems and roots, LA, and relative chlorophyll content in corn plants (Saranya et al., 2018). Likewise, the application of this product favored chlorophyll synthesis in bitter melon (*Momordica charantia* L.) plants affected by downy mildew, and decreased the disease severity index (Ratnayake et al., 2018). In addition, it has been found that rice husk, when composted, favors the synthesis of chlorophyll, accumulation of carbohydrates, and growth of sunflower (*Helianthus annuus* L.) plants (Badar and Qureshi, 2014).

The search for management alternatives based on biological control, particularly the application of biocontrol agents and their effect on the disease, has become very important in the cape gooseberry-*F. oxysporum* pathosystem in Colombia (Díaz et al., 2013; Moreno-Velandia et al., 2018). However, information on the use of products of natural origin, such as chitosan and burned rice husk, on vascular wilt and the physiological response of cape gooseberry plants is still scarce. Therefore, the objective of this

study was to examine the effects of the application of organic additives, chitosan, burned rice husk, and their mixture, on the physiological response of cape gooseberry plants to FOph.

Materials and Methods

Plant material and growth conditions.

The experiment was conducted under greenhouse conditions at the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia in Bogotá (lat. 4°35'56"N, long. 74°04'51"W, altitude 2557 m) between Dec. 2016 and Mar. 2017. The climatic conditions during the test were as follows: natural photoperiod of 12 h (photosynthetically active radiation $1500 \mu\text{m}^{-1}\cdot\text{s}^{-2}$ at noon), $\approx 70\%$ relative humidity and an average temperature of 25.2 °C (Fig. 1). Commercial cape gooseberry ecotype 'Colombia' seeds (Semicol S.A., Bogotá, Colombia) were initially subjected to surface disinfection with a 70% (v/v) ethanol solution for 60 s, 3% sodium hypochlorite (v/v) for 20 min in agitation, and finally three rinses with sterile distilled water before germination and the establishment of treatments. In addition, the seeds destined to the chitosan treatment were subjected to an additional immersion in a chitosan solution at a concentration of 0.1% (w/v) for 20 min in constant agitation (150 rpm).

The disinfected seeds were initially sown in 70-cell germination trays containing peat without any nutrients (Klasmann; Klasmann-Deilmann GmbH, Saterland, Germany) as substrate. After seed germination [30 d after sowing (DAS)], each seedling was irrigated every 3 days with 10 mL of a nutrient solution containing a complete liquid fertilizer (N, P, K, and micronutrients) (Nutriponic; Walco S.A., Bogotá, Colombia) at a concentration of 3 mL per liter of water until transplanting at 45 DAS (seedlings with four fully expanded leaves). Seedlings were subsequently

transplanted into 2-L capacity plastic containers containing the corresponding substrate according to the treatment and FOph presence or absence.

Organic additives treatments and inoculation of soil with *F. oxysporum*. The organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] were incorporated at the time of substrate preparation before transplanting. Four different types of substrates were obtained for the establishment of treatments: 1) mix of soil and rice husk (3:1 v/v) (control substrate), 2) mix of soil and burned rice husk (3:1 v/v), 3) mix of soil and rice husk (3:1 v/v) with the addition of chitosan, and 4) mix of soil and burned rice husk (3:1 v/v) with the addition of chitosan. Each substrate was either inoculated or not with FOph. The absence of *F. oxysporum* in the soil used for the preparation of substrates was previously confirmed using the microbial count by soil dilution technique by Gamliel and Katan (1991) with modifications. A total of 45 mL sterile water agar (0.1%) supplemented with $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ (0.1%) were 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 d in petri dishes with Czapek Dox agar medium. Inoculation of soil with *F. oxysporum* was performed by incorporating the propagules (microconidia) into the substrate (Hao et al., 2009; Purwati et al., 2008). Inoculation of substrates with FOph strain Map5 (Agricultural Microbiology Laboratory of Agrosavia, Mosquera, Colombia) was performed using 100 mL of conidia suspension per kilogram of substrate, whereas control substrates were treated with 100 mL of distilled water. Finally, eight treatments were tested in this study.

Chitosan (Sigma Aldrich, St. Louis, MO) application was carried out at two moments: after surface disinfection of seeds as previously described, and at the time of

Fig. 1. Climatic conditions [day T°, night T°, average T°, and % relative humidity (RH)] in the greenhouse during the experiment.

Table 1. Statistical probability of F ($P > F$) of the evaluated physiological variables due to the effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on cape gooseberry plants with or without *Fusarium oxysporum* f. sp. *physali* (FOph⁺).

	Abbreviation	Samplings				
		10 DAI	20 DAI	30 DAI	40 DAI	50 DAI
Stomatal conductance	g_s	NS	0.0001	0.0000	0.0000	0.0000
Leaf water potential	Ψ_{wf}	NS	0.0008	0.0000	0.0000	0.0000
Leaf area	LA	0.0000	0.0006	0.0000	0.0000	0.0000
Dry weight aerial	DWA	NS	0.0005	0.0000	0.0000	0.0000
Dry weight root	DWR	NS	0.0003	0.0000	0.0000	0.0000
Dry weight total	DWT	NS	0.0005	0.0000	0.0000	0.0000
Aerial part/root ratio	AP/R	NS	0.0000	0.0000	0.0000	0.0000
Total chlorophyll	Chl Total	ND	ND	ND	ND	0.0000
Carotenoids	Cx + c	ND	ND	ND	ND	0.0000
Malondialdehyde	MDA	ND	ND	ND	ND	0.0000
Proline		ND	ND	ND	ND	0.0000

DAI = days after inoculation; NS = not significant at $\alpha = 0.05$; ND = nondetermined parameter.

Table 2. Area under the disease progress curve (AUDPC) and severity index of vascular wilt caused by *Fusarium oxysporum* f. sp. *physali* (FOph⁺) in cape gooseberry plants with or without organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application at 50 d after inoculation.

Treatment	AUDPC	Disease severity index
FOph ⁺	67.1 a ^z	3.6 a
Chi/FOph ⁺	56.3 c	2.5 c
Rh/FOph ⁺	63.0 ab	3.2 ab
ChiRh/FOph ⁺	59.1 bc	2.8 bc
Significance	***	**
cv (%)	4.22	10.53

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

cv = coefficient of variation.

, *Significant at $P \leq 0.01$ or 0.001, respectively.

transplantation. A total of 30 mL of a chitosan solution at a concentration of 0.1% (w/v) in sterile distilled water was drench-applied per pot. Finally, the treatments were arranged in a completely randomized design with four repetitions (each repetition was a cape gooseberry plant). In total, each treatment had 20 seedlings and four plants were used at each evaluation time [10, 20, 30, 40, and 50 d after inoculation (DAI)].

Disease severity analysis. Vascular wilt severity was determined every 3 d from the moment of inoculation (45 DAS) to the end of the experiment (95 DAS) for each of the treatments. Visual evaluations were performed using the six-level scale proposed by Moreno-Velandia (2017) based on the characteristic symptoms of the disease (epinasty, chlorosis, loss of turgor in leaves, and defoliation until total plant wilting). The severity index was calculated using Eq. [1] described by Chiang et al. (2017):

$$\text{Disease severity index} = \sum(nv) / V, \quad [1]$$

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

Finally, the severity of the disease in terms of the AUDPC was estimated in each

treatment following the trapezoidal integration method (Campbell and Madden, 1990):

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

where n is the number of evaluations, y_i 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.

g_s and leaf water potential. g_s and leaf water potential (Ψ_{wf}) were estimated on sunny days at 10, 20, 30, 40, and 50 DAI between 0900 and 1200 HR. The estimation of g_s was performed on a fully expanded leaf randomly taken from the upper third of the canopy using a steady-state porometer (SC-1; Decagon Devices Inc., Pullman, WA). Then, Ψ_{wf} was determined with a Schollander pressure chamber (Model 615; PMS, Albany, OR) on the same leaf used to estimate g_s .

Growth parameters. Dry weight was determined by separately collecting leaves, stems, and roots of plants at 10, 20, 30, 40, and 50 DAI. LA was estimated from digital images in TIFF (Tagged Image File Format) format (D3300; Nikon, Bangkok, Thailand) which were analyzed using a Java image-processing program (Image J; National Institute of Mental Health, Bethesda, MD).

Chlorophyll and carotenoid content. Leaf samples of 0.03 g were collected from the middle canopy of each plant for each of the treatments, which were macerated with liquid nitrogen and homogenized in 4 mL of 80% acetone. Samples were then centrifuged (Model 420101; Becton Dickinson Primary Care Diagnostics, Sparks Glencoe, MD) at 5000 rpm for 10 min to remove particles. Acetone was added to the supernatant to a final volume of 6 mL. Then, a spectrophotometer reading was performed (Spectronic BioMate 3 ultraviolet-vis; Thermo, Madison, WI) using 663- and 646-nm wavelengths for chlorophyll and 470 nm for carotenoids. These values were used in the equations described by Lichtenthaler (1987) to estimate chlorophyll and carotenoid content. The variables were determined at 50 DAI.

MDA and proline content. Lipid oxidation (MDA) determination was based on the thiobarbituric acid method (Hodges et al.,

1999). Leaf samples of 0.3 g from the middle third of plants of each of the treatments were collected at 50 DAI. The plant material was macerated and stored in liquid nitrogen until analysis; then, samples were centrifuged at 5000 rpm for 10 min and the absorbances were estimated at 440, 532, and 600 nm with a spectrophotometer (Spectronic BioMate 3 ultraviolet-vis; Thermo). Finally, the extinction coefficient ($157 \text{ M}^{-1}\text{mL}^{-1}$) was used to obtain MDA concentration.

The ninhydrin acid method (Bates et al., 1973) was used to determine leaf proline concentration. Leaf samples of 0.3 g from the middle third of cape gooseberry plants were macerated with liquid nitrogen; 10 mL of 3% sulfosalicylic acid was added to the samples, which were then filtered through Whatman paper (No. 2). Subsequently, 2 mL of the filtrate was 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 the reaction was stopped in ice. The resulting solution was dissolved in 4 mL of toluene by shaking the test tubes vigorously using a vortex mixer (V-1; BOECO, Hamburg, Germany) and the absorbance was determined at 520 nm with the spectrophotometer. Proline content was calculated using the fresh weight of the sample by means of a standard calibration curve [Eq. (3)].

$$\begin{aligned} & \frac{\mu\text{mol proline}}{\text{g fresh plant material}} \\ &= \frac{\left[\frac{\mu\text{g proline}}{\text{mL}} \times \text{mL Toluene} \right]}{\left[\frac{115.5 \mu\text{g}}{\mu\text{mol}} \right]} \times \left[\frac{\text{g of sample}}{5} \right] \quad [3] \end{aligned}$$

Experiment design and data analysis.

Data were analyzed using a completely randomized design in which each treatment consisted of five plants per repetition. An analysis of variance (ANOVA) was performed, and when significant differences ($P \leq 0.05$) were found, a Tukey post hoc test was used for the comparison of means. A principal components analysis was performed using biplot graphs with the purpose of visualizing the relationships between treatments. Data were analyzed using Statistix v. 9.0 program (Analytical Software, Tallahassee, FL). Figures were made with SigmaPlot (Systat Software, San Jose, CA), and the InfoStat v 2018 program (National University of Córdoba, Córdoba, CBA, Argentina) was used for the biplot analysis.

Results

The ANOVA showing the effect of organic additives [chitosan (Chi), burned rice husk (Rh) or chitosan + burned rice husk (ChiRh)] application on the evaluated variables in cape gooseberry plants with or without *F. oxysporum* f. sp. *physali* (FOph⁺) is summarized in Table 1.

Vascular wilt development. All FOph⁺ inoculated plants had a 100% incidence of the disease. The presence or absence of the pathogen was confirmed by isolates from the base of the plant stem in PDA medium (Leslie and Summerell, 2006) (data not shown).

Differences between the evaluated treatments were observed on the AUDPC ($P = 0.0004$) and severity index ($P = 0.0025$) at 50 DAI (Table 2). Cape gooseberry plants inoculated with the pathogen and without additive treatments (FOph⁺) had the highest AUDPC value (67.1). Intermediate values were recorded in plants treated with burned rice husk and the mixture of chitosan + burned rice husk (63.0 and 59.1, respectively). Chitosan use showed the lowest values (56.3). Finally, similar results also were observed on the severity index in plants treated with the different organic additives and inoculated with FOph.

g_s and leaf water potential. Differences were not observed in leaf g_s between treat-

ments at 10 DAI. However, differences ($P = 0.0001$) were observed from 20 DAI, with lower g_s in the group of FOph⁺ inoculated plants compared with plants without inoculation ($\approx 205.51 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $\approx 255.14 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively). The values of g_s continued to decrease in FOph⁺ inoculated plants through the experiment; however, the treatments with organic additives (Chi, Rh, or ChiRh) slightly favored g_s at the end of the experiment ($\approx 72.1 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (50 DAI) (Table 3).

Similarly, differences were not observed in leaf water potential at 10 DAI between the evaluated treatments. However, differences were recorded ($P = 0.0008$) at 20 DAI. FOph⁺ inoculated plants and cape gooseberry plants

treated with chitosan and *F. oxysporum* (Chi/FOph⁺) showed more negative Ψ_{wf} values (32%) compared with plants with no inoculation or treatment (FOph⁻). At 30 and 40 DAI, the group of plants without pathogen inoculation (FOph⁻) had a better water status ($\Psi_{wf} \sim -0.2 \text{ Mpa}$) vs. plants with FOph⁺ ($\Psi_{wf} \sim -0.4 \text{ Mpa}$). Finally, the treatment with chitosan (-0.58 Mpa) and the mixture of chitosan and burned rice husk (-0.52 Mpa) favored water status (Ψ_{wf}) recovery in plants with FOph⁺, reaching values observed in control plants without inoculation (-0.44 Mpa) at 50 DAI (Table 3).

Growth parameters. In this study, statistical differences between the different treatments were observed on growth parameters

Table 3. Effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on stomatal conductance (g_s) and leaf water potential (Ψ_{wf}) of cape gooseberry plants with (FOph⁺) and without (FOph⁻) *Fusarium oxysporum* f. sp. *physali* inoculation at 10, 20, 30, 40, and 50 d after inoculation (DAI).

Treatment	Sampling days									
	g_s (mmol·m ⁻² ·s ⁻¹)					Ψ_{wf} (-Mpa)				
	10 DAI	20 DAI	30 DAI	40 DAI	50 DAI	10 DAI	20 DAI	30 DAI	40 DAI	50 DAI
FOph ⁻	204.3 a ^z	275.4 a	247.5 a	219.7 b	233.6 ab	0.18 a	0.18 b	0.22 c	0.26 bc	0.44 c
FOph ⁺	202.3 a	197.9 b	123.9 c	50.0 d	45.0 d	0.22 a	0.26 a	0.36 a	0.42 a	0.90 a
Chi/FOph ⁻	217.6 a	260.3 a	255.0 a	249.7 a	252.3 a	0.15 a	0.17 b	0.23 c	0.24 c	0.51 bc
Chi/FOph ⁺	217.7 a	228.4 ab	161.3 b	94.2 c	78.9 c	0.19 a	0.26 a	0.38 a	0.35 ab	0.55 bc
Rh/FOph ⁻	215.6 a	238.3 ab	231.3 a	224.4 ab	213.8 b	0.17 a	0.17 b	0.25 bc	0.27 bc	0.60 b
Rh/FOph ⁺	203.1 a	195.9 b	134.2 bc	72.6 c	59.9 c	0.20 a	0.21 ab	0.33 ab	0.35 ab	0.90 a
ChiRh/FOph ⁻	213.1 a	246.4 ab	233.1 a	219.9 b	226.5 b	0.14 a	0.22 ab	0.22 c	0.23 c	0.48 bc
ChiRh/FOph ⁺	207.1 a	199.6 ab	135.2 bc	70.8 c	77.3 c	0.18 a	0.22 ab	0.36 a	0.41 a	0.58 bc
Significance	NS	***	***	***	***	NS	***	***	***	***
cv (%)	3.04	9.58	6.40	7.91	7.08	15.95	14.85	13.30	14.47	9.72

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

cv = coefficient of variation.

, *Significant at $P \leq 0.01$ or 0.001 , respectively.

Fig. 2. Effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on (A) leaf area (LA), (B) dry weight aerial part (DWA), (C) dry weight root (DWR), (D) dry weight total (DWT), and (E) aerial/root ratio (AP/R) of cape gooseberry plants with (FOph⁺) and without (FOph⁻) *Fusarium oxysporum* f. sp. *physali* inoculation at 50 d after inoculation. Bars represent the mean of four values $\pm$ SE. Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level.

Fig. 3. Effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on total chlorophyll content (A) and carotenoids (B) of cape gooseberry plants with (FOpH⁺) and without (FOpH⁻) *Fusarium oxysporum* f. sp. *physali* inoculation at 50 d after inoculation. Bars represent the mean of four values $\pm$ SE. Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level. FW = fresh weight.

[LA, accumulation of dry matter in aerial part (DWA), root (DWR), total (DWT), and aerial part-root ratio (AP/R)] from 20 DAI (Table 1). The most marked effect was observed at 50 DAI (Fig. 2). In general, the results at the end of the experiment showed a lower plant growth in FOpH⁺ inoculated plants compared with noninoculated plants, although less reduction was observed when plants were treated with organic additives. Chitosan addition stimulated LA, DWA, DWR, and DWT in both groups (cape gooseberry plants with and without vascular wilt). Finally, the use of organic additives and their mixture had no effect on AP/R in both studied groups of plants (FOpH⁺ and FOpH⁻), except for plants with vascular wilt and treated with burned rice husk (Rh/FOpH⁺), which presented the lowest AP/R values (0.71).

Chlorophyll and carotenoid content. Significant differences ($P = 0.0000$) were observed between the evaluated treatments for photosynthetic pigments [total chlorophyll (Chl total) and carotenoids (Cx + c)] at 50 DAI. Lower Chl total values were recorded in cape gooseberry plants only infected with *F. oxysporum* f. sp. *physali* (FOpH⁺) [823.2 $\mu\text{g}\cdot\text{mg}^{-1}$ fresh weight (FW)]. Likewise, the use of organic additives and their mixture increased the total Chl content in plants with FOpH⁺ (49%). The application of additives in

plants with vascular wilt showed higher values than in inoculated control plants (FOpH⁺) (Fig. 3A). Regarding Cx + c, the application of burned rice husk and the mixture with chitosan in inoculated plants also had a positive effect on the synthesis of these pigments (165.96 $\mu\text{g}\cdot\text{mg}^{-1}$ FW and 152.93 $\mu\text{g}\cdot\text{mg}^{-1}$ FW, respectively). On the other hand, the mixture of additives reduced carotenoid content in plants without pathogen inoculation (ChiRh/FOpH⁺) (Fig. 3B).

MDA and proline content. Lipid peroxidation of cell membranes expressed as MDA production showed statistical differences ($P = 0.0000$) between treatments at 50 DAI. FOpH⁺ inoculation caused a higher MDA production despite the addition of organic additives ($\approx 10.82 \mu\text{mol}\cdot\text{g}^{-2}$ FW) in comparison with noninoculated plants (FOpH⁻) ($\approx 7.78 \mu\text{mol}\cdot\text{g}^{-2}$ FW) (Fig. 4A). On the other hand, proline synthesis was generally inhibited by FOpH⁺ presence with the exception of inoculated plants treated with chitosan. The application of this additive caused a reduction in the production of this amino acid in FOpH⁻ plants, whereas it favored its content in FOpH⁺ plants (Fig. 4B).

Biplot analysis of physiological responses to FOpH management with organic additives. The principal components analysis shows that the evaluated variables explained 79.2%

of the physiological response of cape gooseberry plants subjected to organic additive treatments at 50 DAI. The vectors of g_s , Ψ_{wf} , growth (LA and DWT), and biochemical variables (Chl total and proline) present angles close to the origin, showing that there is a high correlation between the physiological response of plants and these variables. Likewise, it was observed that cape gooseberry plants without pathogen inoculation are grouped in the same sector, indicating that they have a similar physiological response (group I). Plants inoculated only with FOpH⁺ (group IV) are located in the sector opposite to group I, showing an antagonistic effect of the pathogen on the physiological response of plants. On the other hand, the application of organic additives had two differential effects in the analysis. Plants treated with Rh/FOpH⁺ and ChiRh/FOpH⁺ (group III) did not show a differential behavior compared with group IV (FOpH⁺), whereas the treatment with chitosan favored the physiology of FOpH-inoculated plants (group II), bringing it closer to the physiological response registered in control plants (group I) (Fig. 5).

Discussion

In the present study, the effect of the application of organic additives showed differential behaviors in both disease development and the physiological response of cape gooseberry plants affected by vascular wilt. Plants treated with chitosan or its mixture with burned rice husk showed lower severity and disease index caused by FOpH. This study confirms previous observations that suggested that chitosan reduced the severity of some root diseases caused by phytopathogenic fungi. Studies carried out by Bell et al. (1998) and Berger et al. (2016) also found a reduction in the incidence and severity of vascular wilt caused by *F. oxysporum* f. sp. *apii* and *F. oxysporum* f. sp. *tracheiphilum* by $\approx 30\%$ in celery and cowpea bean plants, respectively, with applications of this biopolymer. The reduction of disease severity in the treatments with chitosan may be due to the antifungal activity of this biopolymer associated with the ability to induce defense responses in plants and the production of callose and protease inhibitors that negatively affect fungus growth (De Oliveira, 2016; Lárez, 2008; Vander et al., 1998).

The use of organic additives decreased the negative effects of the pathogen by favoring physiological responses in cape gooseberry plants affected by vascular wilt. Chitosan treatments reduced chlorophyll degradation in FOpH-inoculated plants. An increase in the chlorophyll content as a result of chitosan application has been reported by several authors under biotic or abiotic stress conditions. Salachna and Zawadzinska (2014) observed an increase in chlorophyll content in leaves of *Freesia* sp. treated with chitosan under water stress conditions. Likewise, it has been observed that the use of this biopolymer favored chlorophyll synthesis in plants exposed to pathogens (Sakornyen

et al., 2010; Zahid et al., 2014). The increase in chlorophyll content as a result of chitosan application in cape gooseberry plants affected by FOph can be caused by the ability of this biopolymer to favor nutrient and water uptake under stress conditions (Farouk et al., 2011; Farouk and Amany, 2012).

On the other hand, it has been reported that the use of burned rice husk amendments combined with fertilizer application improves the chlorophyll content in maize plants (Saranya et al., 2018). Ratnayake et al. (2018) found that foliar applications of burned rice husk favored chlorophyll content in bitter melon plants. In this study, the use of this amendment increased carotenoid content in cape gooseberry plants with and without pathogen presence. Othman et al. (2014) reported that environmental factors such as temperature, light, salinity, irrigation, and nutrient uptake have an impact on the synthesis of these pigments. In addition, it is known that burned rice husk has significant amounts of P, K, S, Fe, Ca, Mg, and Na (Thind et al., 2012), which could favor the synthesis of this pigment.

The decrease in Ψ_{wfs} , water content, and relative water content in cucumber (*Cucumis sativus*) plants inoculated with *F. oxysporum* f. sp. *cucumerinum* is an effect of the stress condition (Sun et al., 2017). Wang et al. (2015) also found a reduction in g_s , water uptake, hydraulic conductance, and leaf water content in this pathosystem. The results in this study showed higher g_s in diseased cape gooseberry plants and treated with chitosan compared with FOph-inoculated plants without any treatment. Better g_s in treatments

Fig. 4. Effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on malondialdehyde (MDA) (A) and proline (B) content of cape gooseberry plants with (FOph⁺) and without (FOph⁻) *Fusarium oxysporum* f. sp. *physali* inoculation at 50 d after inoculation. Bars represent the mean of four values $\pm$ SE. Different letters indicate significant differences among treatments. Multiple comparisons of means were performed using Tukey's test at the 0.05 significance level. FW = fresh weight.

Fig. 5. Effect of organic additives [chitosan (Chi), burned rice husk (Rh), or chitosan + burned rice husk (ChiRh)] application on the biplot analysis of cape gooseberry plants with and without *Fusarium oxysporum* f. sp. *physali* inoculation (FOph⁺ and FOph⁻) on dry weight total (DWT), leaf area (LA), proline, total chlorophyll (Chl total), carotenoids (Cx + c), malondialdehyde (MDA), stomatal conductance (g_s), and leaf water potential (Ψ_{wfs}) at 50 d after inoculation. PC = principal component.

with chitosan can be because the application of this biopolymer stimulates the gas exchange properties as a result of the increase in chlorophyll content and photosynthetic efficiency (Xu and Mou, 2018). In addition, chitosan application increased the thickness of the leaf mesophyll under conditions of abiotic stresses (water stress) (Farouk and Amany, 2012). The beneficial effect of chitosan treatments on Ψ_{wf} , especially in FOPh-inoculated cape gooseberry plants, could be explained by the ability of this biopolymer to promote root development, strengthen water uptake, and stimulate low osmotic adjustment under drought conditions (Bistgani et al., 2017; Zeng and Luo, 2012). Similar results were obtained by dos Reis et al. (2018), who observed that foliar chitosan application in maize plants favored Ψ_{wf} and increased water use efficiency.

It has also been reported that chitosan is a growth promoter because it favors the leaf gas exchange properties in plants under different types of biotic or abiotic stress (Asghari-Zakaria et al., 2009; Xu and Mou, 2018). The increase in the growth of strawberry plants with foliar or edaphic chitosan application was associated with the increase in leaf length and width, canopy diameter, leaf number, plant height, and fresh and dry weight of roots and aerial part, which was also reflected in yield (Abdel-Mawgoud et al., 2010; Rahman et al., 2018). Chitosan applications increased yield by 20% and reduced the level of powdery mildew in tomato plants (Walker et al., 2004). Similarly, in this study, chitosan mitigated the impact that the pathogen had on growth parameters in cape gooseberry plants with vascular wilt.

Lipid peroxidation of cell membranes is usually detected with the measurement of MDA, as it is a widely used marker of oxidative lipid damage caused by stress (Kong et al., 2016). Among the obtained results, the use of organic additives did not reduce the oxidative damage by FOPh infection expressed as MDA content. These results are opposite to those found by Mahdavi et al. (2011), who observed that chitosan application decreased MDA content in safflower plants subjected to different ψ_s . Likewise, wheat seeds treated with chitosan reduced MDA contents in seedlings under drought conditions (Zeng and Luo, 2012). Proline content is one of the main adaptive responses of plants subjected to different conditions of biotic and abiotic stress (Hayat et al., 2012). In this study, chitosan application increased proline content in cape gooseberry plants with vascular wilt, which was reflected in lower AUDPC values. Similar responses were obtained in thyme (*Thymus daenensis*) plants subjected to drought stress, in which chitosan application was related to a higher content of this amino acid (Bistgani et al., 2017).

Conclusion

The results obtained in the present study showed that the use of organic additives in *P.*

peruviana improved the physiological response of FOPh-affected plants, with chitosan showing the lowest disease levels. Chitosan also promoted higher g_s , Ψ_{wf} , foliar area, dry matter accumulation, and proline content values. These results suggest that chitosan application in cape gooseberry plants could be considered as a complementary tool for the integrated management of vascular wilt.

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