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Defence-related components in cucumber susceptibility to target spot

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Abstract

Target spot is an important disease in cucumber culture. Due to differences in the susceptibility of cucumber cultivars to the disease, the objective was to evaluate the behavior of three cvs. in terms of leaf trichome density and biochemical variables in the interaction with the pathogen *Corynespora cassiicola*. The density of trichomes was evaluated on both sides of the leaves. The activity of peroxidase and polyphenol oxidase enzymes and the levels of free and bound phenolic compounds were evaluated one, three and five days after inoculation or not with the pathogen. Disease severities in Safira, Taiko and Soldier cucumber cvs. reached 0.9%, 2.1% and 4.7%, respectively. Higher density of trichomes was found in the Safira cv., with no differences between Soldier and Taiko cvs. The adaxial side of the leaves had a greater number of trichomes than the abaxial side. The enzymatic activity was higher in the Safira cv., less susceptible to the disease, in relation to the Taiko and Soldier cvs. Inoculated plants showed higher peroxidase activity, *demonstrating* that the response to infection was accompanied by the synthesis of this enzyme, and it increased with the days after inoculation. The levels of phenolic compounds varied according to the cvs. and as a function of time, with the highest levels in the Safira and Soldier cvs. No direct relationships were observed between biochemical and trichome variables and disease severity in cucumber cvs., since the Soldier cv., the most susceptible to the disease, did not differ in relation to 'Taiko' in enzymatic activities, free phenolic compounds and number of trichomes, including higher levels of bound phenolic compounds.

Keywords: *Cucumis sativus, Corynespora cassiicola*, resistance mechanisms

Introduction

Target spot, caused by *Corynespora cassiicola*, occurs in several species of cucurbits, with greater importance in cucumber, being very frequent in tropical regions. Symptoms are angular, yellowish leaf spots that grow, become circular, with a light brown center and dark edges (Pavan et al. 2016). Both immature and mature leaves are affected, leading to massive defoliation and consequently growth delay and yield losses (Silva et al., 2023).

Among the alternatives to control plant diseases, the use of resistant cultivars can be considered the most efficient and sustainable, which is due both to its economic and environmental advantages (Sankar et al., 2021). In this sense, some works indicate that cucumber cultivars have different levels of susceptibility to the disease, showing the importance of using resistant cultivars. In this context, it is worth mentioning the work carried out by Fischer et al (2021) evaluating different cucumber cultivars, which identified three groups of cultivars with different levels of susceptibility: one group that was less affected by the disease, represented by Safira and Diplomata cvs.; a second group showing intermediate behaviour, including Valent, Eureka, Taiko, Yamarashi and Darlington cvs., and a third group that was most susceptible to the pathogen and was represented by Soldier, Vulcano and Exocet cvs. Safira and Diplomata cvs. showed low level of susceptibility to target spot and can be recommended for areas with high disease intensity. Bezerra & Bentes (2015) also demonstrated levels of susceptibility to target spot in different cucumber cvs. However, in these works, the possible causes of variability in the behavior of cvs. in relation to the disease were not evaluated.

Plants respond to pathogen infections through many different defence mechanisms, which include the synthesis of antimicrobial compounds such as defense

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enzymes and phenolics (Oliveira et al., 2016; Nishad et a., 2020). According Fortunato et al. (2015), the defence-related enzyme activities increased upon *C. cassiicola* infection, regardless of the basal level of resistance of the soybean cv. However, in a soybean cv. resistant to target spot, a higher activity of the enzyme polyphenol oxidase, a higher concentration of phenolic compounds and lignin-thioglycolic acid derivatives (Fortunato et al., 2015) and an apparent higher density of trichomes (Fortunato et al., 2017) were observed, in relation to susceptible cv. Trichomes can protect plants, forming a barrier to the adhesion of infective structures of pathogens to the plant surface. But on the other hand, can facilitate the infection of pathogens, possibly by providing a certain level of moisture and nutrients for microbial development (Simplício et al., 2022). Cucumber trichomes belong to the multicellular nonglandular type of trichome (Chen et al., 2014).

More detailed research on the behavior of cucumber genotypes to diseases is important, aiming to direct breeding studies to obtain resistant cvs. Due to differences in the susceptibility of cucumber cvs. in relation to the target spot, the objective was to evaluate the behavior of three cvs. in terms of leaf trichome density and biochemical variables, in plants inoculated or not with *C. cassiicola*, under greenhouse conditions.

Material And Methods

The *C. cassiicola* isolate was obtained from a 'Soldier' cucumber crop with symptoms of target spot, located in the municipality of Avaí-SP, coordinates 22□03'12.1"S, 49°15'31.5"W, being preserved with the denomination MMBF 01/20, in the mycoteca "Mário Barreto Figueiredo" of APTA, Instituto Biológico.

The trichome density and the biochemical response of cucumber to infection by *C. cassiicola* were evaluated in three cucumber cvs., considered highly susceptible (Soldier), moderately susceptible (Taiko) and slightly susceptible (Safira) to target spot (Fischer et al., 2021).

Plants were grown individually in plastic pots containing 5 liters of commercial substrate composed of pine bark (Substrato Para Plantas Carolina II, Carolina Soil) under greenhouse conditions. Minimum and maximum temperatures inside the greenhouse were daily assessed with a digital thermometer (K29-7070®, KASVI). The minimum, mean and maximum temperatures obtained were 19.2 °C, 24.6 °C and 30.1 °C, respectively, from October 11th, 2019 to November 14th, 2019 for cucumber cultivation cycle. Irrigation was manual and daily, while fertilization was carried out twice a week via fertirrigation with the application of 1 g pot^{-1} formulation containing (%): 15 N, 2.2 P, 8.3 K, 1 Ca, 1 Mg, 13 S, 0.2 Fe, 0.2 Zn, 0.06 B, 0.1Mn, 0.05 Cu e 0.005 Mo.

Trichome density

To determine the density of trichomes, areas measuring 3.53 mm in diameter were demarcated with a cork borer, in the median region of the abaxial and adaxial surface of the epidermis of the second and third fully expanded definitive leaves, 30 days after sowing. The count of trichomes was performed using a stereoscopic microscope, taking five readings from each side of the leaf, at a magnification of 30X. From the diameter of the circular field, the area of the circle was calculated in mm^2 and by rule of three calculated the number of trichomes per mm^2.

The experimental design was completely randomized, adopting a factorial scheme of 3 cucumber genotypes x 2 leaf sides. Four replications were adopted, with each experimental unit consisting of one plant and each plot represented by two true leaves. Mean results were subjected to analysis of variance and treatment means compared by Tukey's test at 5% significance. The relationship between trichome density and disease severity in the three cucumber cvs. was determined by Person's linear correlation.

Biochemical responses of cucumber cultivars inoculated with Corynespora cassiicola

The inoculum of *C. cassiicola* was multiplied in Petri plates containing tomato juice medium (4.5 g $CaCO_3$, 15 g agar, 200 mL commercial tomato juice, and 800 mL distilled water), which were incubated for 15 days at 25 °C in B.O.D. chamber, under continuous fluorescent light. The conidial suspension in distilled water was adjusted to the concentration of 10^4 conidia mL^{-1} with a Neubauer chamber.

Plant inoculation was carried out by spraying the conidial suspension onto both surfaces of the second and third completely expanded true leaves until runoff, after 30 days of sowing. Then, the leaves were kept inside a high-density polyethylene bag during 24 h in order to obtain a humid chamber. Target spot severity, expressed as a percentage of affected leaf area, was evaluated at five days after inoculation of the pathogen, in a 36-cm^2 (6 x 6 cm) area photographed from the central region of each leaf, using the software ImageJ®.

Biochemical responses in plants were evaluated 1, 3 and 5 days after inoculation (dai) of the pathogen. Proportional samples of the second and third definitive leaves were macerated in a mortar with a pestle in liquid

nitrogen. Then they were homogenized in potassium phosphate buffer 0.01 M (pH 6.6) in a 1:4 ratio (weight/volume) plus polyvinylpyrrolidone (PVP) 0.5% (weight/volume). Then, the homogenate was centrifuged at 20,000 g for 20 minutes at 2°C, and the supernatant (protein extract) was used to determine the total protein content using the Bradford method (1976) and the activities of peroxidases and polyphenol oxidases enzymes.

Peroxidase activity was determined by converting guaiacol to tetraguaiacol, at 30 °C, using the direct spectrophotometric method (Lusso & Pascholati, 1999). The reaction mixture consisted of 100 µL of diluted protein extract and 2.9 mL of reaction solution (250 µL guaiacol, 306 µL hydrogen peroxide, and 100 mL potassium phosphate buffer 0.01 M, pH 6.6). The protein extract was diluted twice in extraction buffer (50 µL of the extract in 100 µL of the buffer) to reduce the activity, which was very high. In the reference cuvette, 2.9 mL of reaction solution and 100 µL of phosphate extraction buffer 0.01 M (pH 6.6) were used. Peroxidase activity was expressed as an absorbance unit min^{-1} mg protein $^{-1}$.

Polyphenol oxidase activity was quantified according to the methodology described by Duangmal & Apenten (1999). The assay was based on the measurement of the oxidation of catechol converted into quinone, a reaction mediated by the enzyme under study. The enzyme substrate was prepared with 110.1 mg of catechol dissolved in 50 mL of sodium phosphate buffer 0.01 M (pH 6.0), forming a catechol solution 0.02 M. The reaction was carried out by mixing 450 µL of substrate and 50 µL of protein extract. The results obtained were expressed in absorbance units min^{-1} mg $protein^{-1}$.

For the quantification of phenolic compounds, 0.5 g of plant tissue was macerated in liquid nitrogen and the powder was resuspended in 4 mL of methanol 50% and left in a water bath for 1.5 h at 80°C. The extract was cooled and centrifuged at 20,000 rpm for 15 minutes at 2°C. The supernatant was collected to determine the content of free phenols. Two ml of NaOH 0.5 M was added to the pellet and incubated for approximately 24 h for saponification of phenols bound to the cell wall. The reaction was neutralized with 9.5 mL of HCl 2M and the extract was centrifuged at 20,000 rpm for 15 minutes at 2°C. To 150 µL of the supernatants (free and wall-bound phenols) was added 3 mL of Na_2CO_3 2% (m/v) and 150 µL of Folin-Ciocateu reagent diluted in water (1:1 v/v) (Kofalvi & Nassuth 1995). The reading was carried out in a spectrophotometer at 750 nm. Phenol concentration was expressed as chlorogenic acid equivalents (mg) per g of fresh tissue.

The experimental design was completely randomized, adopting a factorial scheme of 3 cucumber genotypes x 3 sampling periods x 2 with or without pathogen inoculation. Three replications were adopted, with each experimental unit consisting of one plant and each plot represented by two true leaves. The mean results of each variable per plot were submitted to analysis of variance and the means of treatments compared by Tukey's test at 5% significance. For disease severity data, only the plants of the three inoculated cucumber cultivars were analyzed, since disease did not occur in the non-inoculated plants. The relationship between biochemical variables and disease severity was determined by Person's linear correlation.

Results and Discussion

Trichome density

Differences in trichome density were observed as a function of cucumber cvs. and leaf sides, with no significant interaction between cucumber cvs. and leaf sides (**Table 1**). A greater number of trichomes was found in the Safira cv., with no differences between the Soldier and Taiko cvs. The adaxial side had a greater number of trichomes than the abaxial side. Unlike the present study, Leite et al. (2006) did not observe differences in trichome density between the abaxial and adaxial sides of cucumber cv. Jewel (Agroceres). No correlation was observed between the number of trichomes and target spot severity among the three cucumber cvs. (r= -0.51, p>0.05).

In agreement with what was observed in the present study, in soybean, a greater number of trichomes on the adaxial leaf surface was found in the cv. resistant to target spot (Fundacep 59), in comparison with the susceptible cv. (TMG 132), and according to the authors (Fortunato et al., 2017) trichomes may have impaired the deposition and germination of *C. cassiicola* conidia on the leaf surface, as well as contributing to the lower growth of the pathogen's hyphae and, consequently,

Table 1. Number of trichomes per mm^2 on the abaxial and adaxial surfaces of leaves of three cucumber cultivars

Cultivar	Leaf side		Mean
	Adaxial	Abaxial	
Safira	1.62	1.45	1.54 a[1]
Soldier	1.40	1.32	1.36 b
Taiko	1.41	1.16	1.28 b
Mean	1.48 A	1.31 B	
CV(%)		9.2	
F (cultivar)		8.0**	
F (leaf side)		10.3**	
F (cultivar*side)		0.9^{ns}	
F (blocks)		2.3^{ns}	

[1]Data followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other (Tukey, p<0.05).

to the reduction of the target spot . In the leaf sheaths of rice plants of a resistant cv., hyphae of *Rhizoctonia solani* grew less; consequently, sheath blight symptoms were reduced (Schurt et al. 2015). Trichome density was negatively correlated with necrotized leaf area (%), caused by *Didymella bryoniae*, in five species of cucurbits, with watermelon and zucchini showing 1.11 and 8.28 trichomes per mm^2 of leaf and 99.7 and 3.74% of necrotic area, respectively (Rennberger et al., 2017).

Biochemical responses of host-pathogen interaction

The three cucumber cvs. showed differences regarding target spot severity (F=8.155; P<0.05), with Safira presenting 0.9% of disease severity, lower than Soldier, with 4.7%, and Taiko occupying an intermediate position (2.1%), not differing from the others.

Greater peroxidase activity was found in the Safira cv. compared to Taiko; in inoculated plants compared to non-inoculated ones, showing that the response to infection was accompanied by the synthesis of these enzymes, and it increased with the days of inoculation, with greater activity five days after inoculation compared to the first day. No significant interactions were found between the three variables analyzed for peroxidase enzyme activity (**Table 2** and **Figure 1**).

As in the present study, plants of the Soldier cv. showed an increase in peroxidase enzyme activity five days after pathogen inoculation, in a study of the effect of phosphites and chitosan on the disease (Fischer et al., 2022). Plants of the Safira cv. also showed an increase in peroxidase enzyme activity when infected with *C. lagenarium*, seven days after pathogen inoculation (Silva et al., 2011). In studies with Trichoderma harziaum, Yedidia et al. (1999) verified that the biocontrol agent induced an increase in peroxidase after intervals of less than seven days, while Silva et al. (2011) did not find an increase at 7 and 14 days of plant treatment, showing that the activity of this enzyme tends to decrease after a week of treatment with this inducer.

Peroxidase activity in the roots of pepper plants was higher in Phytophthora *blight* resistant and partially resistant cvs. (*P. capsici*) than in the susceptible cv. (Zhang et al. 2013). Likewise, Leite et al. (2014) reported that peroxidase activity was more significant in plants of a resistant common bean genotype in response to *Sclerotinia sclerotiorum* infection than in a susceptible genotype. In the present study, greater peroxidase activity was also found in the cucumber cv. with greater resistance to target spot and although the peroxidase activity did not differ between cvs. considered susceptible and intermediate, the hypothesis of a protective effect

Table 2. Peroxidase activity (abs. unit min^{-1} mg $protein^{-1}$) in three cucumber cultivars, after 1, 3 and 5 days of plants being or not inoculated with *Corynespora cassiicola*

Cucumber cultivar	Peroxidase activity
Safira	65.0 a
Soldier	52.0 ab
Taiko	41.5 b
Pathogen response	Peroxidase activity
With inoculation	78.3 a
Without inoculation	27.3 b
Days after inoculation	Peroxidase activity
1	38.3 b
3	56.6 ab
5	63.5 a
CV(%)	28.19
F (cultivar)	4.3*
F (day)	4.3*
F (inoculation)	56.1**
F (cultivar x day)	0.9^{ns}
F (cultivar x inoculation)	2.9^{ns}
F (day x inoculation)	0.4^{ns}
F (cultivar x day x inoculation)	1.7^{ns}
F (blocks)	1.5^{ns}

Data followed by the same letter in the column for each variable do not differ from each other (Tukey, p<0.05). ^{ns}=not significant at 5% probability; *significant at 5% probability; **significant at 1% probability. Statistical analysis with data transformed into √ x.

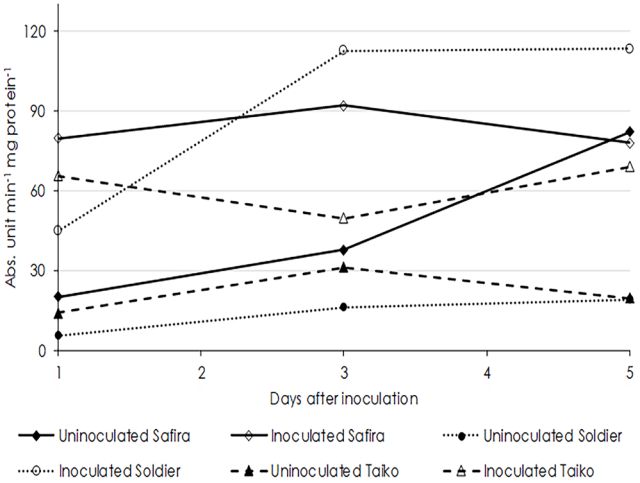

Figure 1. Peroxidase activity in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*.

is reinforced, based on the fact that one of the main defense mechanisms in cucumber during SAR (Systemic Acquired Resistance) is lignification which, in turn, is associated with the activity of peroxidases (Begović et al., 2017). Therefore, according to Di Piero & Pascholati (2004), the increase in the level of peroxidase activity, alone, does not represent the plant's defense response, as this is a multicomponent characteristic, but opens the perspective that other mechanisms may have been activated, since the various metabolic pathways interact and it is expected that alterations in peroxidase activity also involve alterations in other enzymes present in the same metabolic pathway (Labanca, 2002).

As with peroxidase, differences were found

between cvs. for the enzyme polyphenol oxidase, with a significant interaction between cvs. and days after inoculation. Higher polyphenol oxidase activity was observed in the Safira cv. one and five days after pathogen inoculation, with no differences between the Taiko and Soldier cvs. The Safira cv. showed a lower polyphenol oxidase activity three days after inoculation, compared to the other days (**Table 3** and **Figure 2**).

Polyphenol oxidase, which exists mainly in the cytoplasm in the free form or bound to chloroplasts, mitochondria and other subcellular organelles, is the main phenolic oxidation enzyme (Quarta et al. 2013), associated with the increase of fungitoxic quinones, positively correlated with plant disease resistance (Taranto et al. 2017). Ramezani et al. (2018) associated the resistance of cucumber plants to downy mildew (*Pseudoperonospora cubensis*) with a 21% increase in polyphenol oxidase activity in cucumbers, after 4 days of treatment with K phosphite. According to the authors, polyphenol oxidase is associated with plant cell wall thickening, which could form a stronger physical barrier against invading pathogens. In the present study, the highest polyphenol oxidase activity in the Safira cv. was independent of *C. cassiicola* infection (Table 3 and Figure 2).

Table 3. Polyphenoloxidase activity (abs. unit min^{-1} mg $protein^{-1}$) in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*

Cucumber culltivar	Days after *Corynespora cassiicola* inoculation			
	1	3	5	Mean
Safira	1.87 aA	0.56 aB	1.28 aA	1.24 a
Soldier	0.50 bA	0.41 aA	0.40 bA	0.44 b
Taiko	0.48 bA	0.73 aA	0.74 bA	0.65 b
Mean	0.95 A	0.57 A	0.81 A	
CV(%)	29.24			
F (cultivar)	12.67**			
F (day)	1.95^{ns}			
F (inoculation)	1.90^{ns}			
F (cultivar x day)	3.93*			
F (cultivar x inoculation)	1.02^{ns}			
F (day x inoculation)	0.15^{ns}			
F (cultivar x day x inoculation)	1.35^{ns}			
F (blocks)	1.13^{ns}			

Data followed by the same letter, lowercase in the column and uppercase in the line, do not differ from each other (Tukey, p<0.05). ^{ns}=not significant at 5% probability; *significant at 5% probability; **significant at 1% probability. Statistical analysis with data transformed into the root of x.

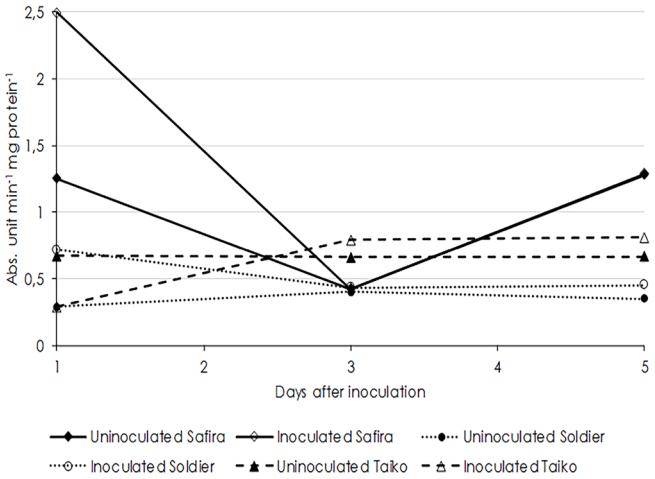

Figure 2. Polyphenoloxidase activity in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*.

The levels of free and bound phenolic compounds varied depending on the cucumber cvs. and on the basis of sampling time (**Tables 4** and **5**; **Figure 3**). The Taiko cv. had a lower content of free phenolic compounds in relation to Safira and a lower content of bound phenolic compounds in relation to Soldier. As a function of the days, despite the inoculation not having significantly influenced, lower levels of free phenolic compounds were observed at three days and, in general, of bound phenolic compounds at five days. Significant interaction was observed between time and inoculation for bound phenolic compounds, with higher levels being observed with pathogen inoculation in Safira and Soldier cvs., one day after inoculation. In a previous study with the Soldier cv., Fischer et al. (2022) had already observed no alteration in the levels of polyphenol oxidase and free phenolic compounds due to the inoculation of *C. cassiicola*.

Plant phenolics include several substances such as phenolic acids, flavonoids, polyphenols, and stilbenoids, which may be free or bound to the cell wall. Among the functions in the plant, phenolic compounds are an important part of plant protection against fungi

Table 4. Activity of free phenolic compounds (mg equiv. chlorogenic acid g fresh $tissue^{-1}$) in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*

Cucumber cultivar	Days after *Corynespora cassiicola* inoculation			
	1	3	5	Mean
Safira	11.2	8.0	12.5	10.6 a
Soldier	9.0	7.1	12.1	9.4 ab
Taiko	9.1	6.3	8.8	8.1 b
Mean	9.8 A	7.1 B	11.1 A	
CV(%)	19.0			
F (cultivar)	8.9**			
F (day)	23.6**			
F (inoculation)	4.1^{ns}			
F (cultivar x day)	1.5^{ns}			
F (cultivar x inoculation)	0.5^{ns}			
F (day x inoculation)	1.9^{ns}			
F (cultivar x day x inoculation)	1.3^{ns}			
F (blocks)	0.7^{ns}			

Data followed by the same letter, lowercase in the column and uppercase in the line, do not differ from each other (Tukey, p<0.05). ^{ns}=not significant at 5% probability; *significant at 5% probability; **significant at 1% probability.

Table 5. Activity of bound phenolic compounds (mg equiv. chlorogenic acid g fresh tissue^{-1}) in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*

Cucumber cultivar	Inoculation of *C. cassiicola*	Days after *C. cassiicola* inoculation			Mean
		1	3	5	
Safira	Without	2.5 bAB	3.7 aA	1.1 aB	2.5ab
	With	4.3 aA	3.0 aA	0.4 aB	
Soldier	Without	2.7 bAB	3.6 aA	1.5 aB	2.8a
	With	4.2 aA	4.4 aA	0.7 aB	
Taiko	Without	2.3 aA	3.3 aA	0.5 aB	2.0b
	With	2.1 aA	3.5 aA	0.4 aB	
Day means		3.0 A	3.6 A	0.8 B	2.4
CV(%)			28.9		
F (cultivar)			6.3**		
F (day)			78.6**		
F (inoculation)			1.2^{ns}		
F (cultivar x day)			1.2^{ns}		
F (cultivar x inoculation)			0.5^{ns}		
F (day x inoculation)			5.6**		
F (cultivar x day x inoculation)			2.5^{ns}		
F (blocks)			0.3^{ns}		

Data followed by the same letter, uppercase in the row and lowercase in the column (within each cultivar in the inoculation comparison), do not differ from each other (Tukey, $p<0.05$). ^{ns}=not significant at 5% probability; *significant at 5% probability; **significant at 1% probability.

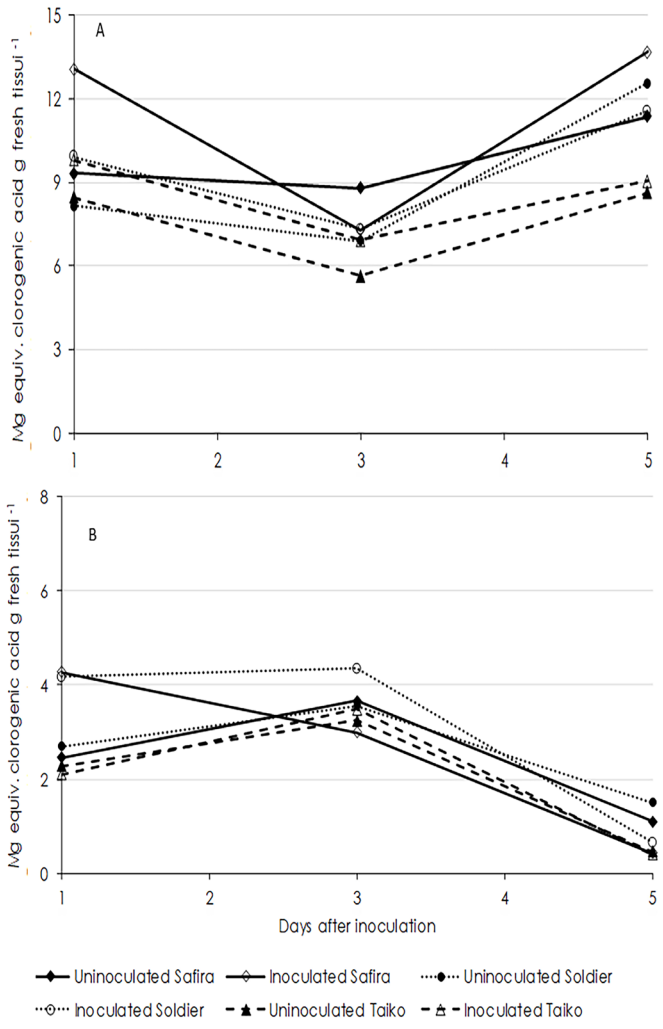

Figure 3. Contents of free (A) and bound (B) phenolic compounds in three cucumber cultivars, after 1, 3 and 5 days of the plants being or not inoculated with *Corynespora cassiicola*.

(Wallis & Galarneau, 2020), and titres are often elevated following infection (Fortunato et al., 2015), as observed in the present study for bound phenolic compounds after one day of *C. cassiicola* inoculation. The increased phenolic levels provide an adequate substrate for oxidative reactions catalyzed by polyphenol oxidases, which consume oxygen and produce fungitoxic quinones inside plant tissues, reducing tissue colonization by pathogens (Taranto et al. 2017).

The activity of the enzymes peroxidase, polyphenol oxidase, and the concentration of thioglycolic acid lignin derivatives significantly increased in two soybean cvs. infected with *C. cassiicola*, regardless of the resistance level of the cv., while the activity of lipooxygenases decreased. According to the authors (Fortunato et al., 2015), target spot resistance of cv. FUNDACEP 59 was associated with higher polyphenol oxidase activity and higher concentrations of derivatives of phenolic compounds and thioglycolic acid lignin in the early stages of infection (4 and 6 days after pathogen inoculation), compared to cv. susceptible (GMT 132). Sahoo et al. (2009) also reported an increase in the concentration of phenolic compounds and polyphenol oxidase activity in the leaves of resistant yam genotypes compared to a susceptible genotype after *Phytophthora colocasia* infection.

Target spot control with copper, zinc and manganese phosphites has been associated with plant resistance induction (Fischer et al., 2022). The enzyme peroxidase was induced by copper phosphite; polyphenol oxidase increased with zinc, manganese and copper phosphites, and the levels of free phenolic compounds were higher with copper phosphite. In a study carried out with cucumber plants submitted to the resistance inducer *Saccharomyces cerevisiae* and subsequent inoculation with *C. lagenarium*, the increase in resistance to anthracnose was accompanied by an increase in peroxidase activity, without, however, alteration in the concentration of free and bound phenols (Labanca , 2002).

There was a positive correlation between peroxidase and polyphenol oxidase activities, as well as the levels of phenolic compounds, with resistance to anthracnose in four common bean cvs. (Campos et al., 2004). In the evaluation of the effect of phytoplasma infection on peroxidase, β-1,3 glucanase and chitinase activity in maize, the activities of the enzymes were, in general, higher in the susceptible hybrid when compared to the resistant one (Junqueira et al., 2011). According to Ryals et al. (1996), the speed and intensity of production

of these enzymes may or may not determine plant resistance to pathogens. To understand the role of these enzymes in relation to the defense responses of corn plants inoculated with phytoplasma, it would be interesting to know the profile of their respective isoenzymes (Junqueira et al., 2011).

Target spot severities on the three cucumber cvs. were similar to those observed by Fischer et al. (2021), evaluating 10 cucumber cvs., with Safira, Taiko and Soldier cvs. showing 1.2; 2.0 and 4.1% of disease severity, after five days of inoculation. As with the trichome density, no direct relationships were observed between biochemical variables and disease severity in the three cucumber cvs., five days after inoculation, since the Person correlation coefficients ($-0.68 \leq r \leq 0.51$) were not significant ($p>0.05$). The highest enzymatic activities and levels of free phenolic compounds were found in the Safira cv., less affected by the disease, however, while the Soldier cv. was the most susceptible to the disease, it did not differ in relation to Taiko, even presenting higher bound phenolic compounds.

Conclusions

The cucumber cv. Safira, less affected by target spot, showed higher activity of peroxidase and polyphenoloxidity enzymes, higher content of free phenolic compounds and higher trichome density in relation to cvs. with intermediate behavior (Taiko) and more susceptible (Soldier) to the disease . However, no direct relationship was established between biochemical and trichome variables and disease severity in cucumber cvs., as the two most susceptible cvs. did not differ from each other for most of the analyzed variables.

In the interaction with the pathogen, there was an increase in the activity of the enzyme peroxidase in the cucumber cvs. and in the content of phenolic compounds bound in the Safira and Soldier cvs., at the beginning of the infectious process.

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