Response of cucumber to target spot as a function of plant age

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Abstract

The target spot of cucurbits, caused by *Corynespora cassiicola*, occurs in several cultivated species, being considered an important disease on cucumbers. The objetive was to determine the cucumber response to the target leaf spot as a function of plant age, seeking a better understanding of the pathosystem in order to avoid economic damages to producers. Cucumber 'Soldier' plants were inoculated at 20, 30 and 40 days after sowing. Disease severity was evaluated at three, six and nine days after inoculation of the pathogen; the number of leaves and plant height at intervals of 7 to 10 days after inoculation of the pathogen; the number disease severity was observed on younger leaves, on plants inoculated at 20 days of age from the sixth day of evaluation, with plants inoculated at 30 days being the most susceptible, reaching 64% disease severity after nine days of inoculation. The variables plant height, number of leaves and fruits, fruit production and fresh and dry mass of the plants are of the plant at the time of inoculation of the pathogen *C. cassiicola*, with the lowest values, in general, in the youngest inoculated plants, at 20 days, while the 40-day-old plants had the least affected vegetative development and production, evidencing the importance of delaying the entry of the disease in the area.

Keywords: Corynespora cassiicola, Cucumis sativus, phenological stage

Introduction

Cucumber production (*Cucumis sativus* L.) is affected by different diseases that can reduce productivity and increase production costs. Among the fungal diseases, the target spot, caused by *Corynespora cassiicola* (Berk. & Curt.) Wei, represents a challenge for cucumber growers (Fischer et al., 2021). Epidemics of the disease in cucumbers have already been reported in Brazil (Bezerra & Bentes, 2015; Fischer et al., 2021) and other countries such as China (Liu et al. 2019) and the United States (Sumabat et al. 2018).

The symptoms of the target spot begin with small necrotic spots that evolve to an angular shape, with a straw-colored center and a small yellow halo. Coalescing of spots results in plant defoliation (Pavan et al., 2016). The pathogen survives in crop residues and in more than 530 plant species (Dixon et al., 2009). Spores are spread mainly by wind and favorable conditions for the disease are high humidity and temperature between 25 and 35°C (Pavan et al., 2016; Zitter et al., 1996).

The phenological stage of plants is an important factor that directly affects the development of diseases (Ross, 2007). In tomato (MacKenzie et al., 2019) and tobacco (Fajola & Alasoadura, 1973) crops, greater susceptibility to target spot are seen on mature leaves. On rubber trees (Fernando et al., 2010) and African violets (Ross, 2007) there is a greater intensity of the disease in younger leaves, which is why chemical control of the disease is carried out mainly on rubber tree regrowth (Fernando et al., 2010).

Productivity and production quality are directly related to green or healthy leaf area, which makes early leaf senescence caused mainly by leaf diseases a factor that seriously affects yield potential (Navarini & Balardin, 2012). Therefore, the delay of the epidemic and consequently of the leaf senescence becomes a desirable agronomic characteristic. In the case of the target spot of cucurbits, it still lacks susceptibility studies in relation to the age of the plant and consequently the damage to production.

Recommended measures for the managing of target spot of cucurbits are crop rotation, adoption of more resistant genotypes and spraying with recommended fungicides for Alternaria and Cercospora spots (Galbieri et al., 2014; Pavan et al., 2016; Liu et al., 2017), since there are no products registered in Brazil to control the target spot disease on cucumbers (Agrofit, 2022).

Research on the target leaf spot on cucumber plants is scarce, making studies necessary to define disease management strategies and consequently the maintenance of the photosynthetic leaf area during fruiting, which are important for the economic viability of the crop. In accordance with what was exposed above, the objective was to determine the cucumber response to the target leaf spot according to the plant age under greenhouse conditions.

Material and methods

The experiments were conducted under greenhouse conditions with maximum temperature control of 30°C, from February to August 2021. Cucumber plants cultivar Soldier (Japanese group), considered a susceptible pattern (Fischer et al., 2021), were cultivated in plastic pots (10 L), containing commercial substrate Carolina Soil Padrão[®]. Two seeds were sown per pot, thinning the smallest seedling in case of emergence of two seedlings.

Irrigation was manual and daily, and fertilization was carried out three times a week via fertigation, applying 1g vase⁻¹ of the formulation containing (%): 15 N, $5 P_2O_5$, $10 K_2O$, 1 Ca, 1 Mg, 13 S, 0.2 Fe, 0.2 Zn, 0.06 B, 0.1 Mn, 0.05 Cu and 0.005 Mo.

The minimum and maximum temperatures inside the greenhouse were evaluated daily with a digital thermometer. The minimum, average and maximum temperatures obtained were 19.0°C, 24.6°C and 30.2°C, respectively, from February 23 to May 17, 2021, for the cucumber cultivation cycle in the first experiment, and 14.5°C, 22.3°C and 30.1°C, respectively, from May 18 to August 9, 2021 in the second experiment.

The C. cassiicola isolated sample used was obtained from cucumber cv. Soldier, located in the county 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" from APTA, Instituto Biológico de São Paulo. The pathogen inoculum propagation was performed in tomato juice (4.5 g of $CaCO_3$, 15 g of agar, 200 mL of commercial tomato juice and 800 mL of distilled water), contained in Petri dishes, incubated for 15 days at 25 °C in climatized B.O.D. type chambers, under continuous fluorescent light. The conidia suspension in distilled water was adjusted to a concentration of 1x10⁴ conidia mL⁻¹ with the aid of a Neubauer chamber.

Inoculation was carried out by spraying the conidia suspension on all leaves, on both sides, until the point of runoff, on plants at 20, 30 and 40 days after sowing, when the 2nd and 3rd true leaves were young (not fully expanded), semi-mature (fully expanded) and mature, respectively. Plants at three different ages were obtained by sowing on three successive dates intercalated for 10 days. Uninoculated plants were sprayed with distilled water. Then, the plants were placed individually inside a transparent polyethylene plastic bag for 24 hours, aiming at the formation of a humid chamber.

Disease severity (% of leaf area affected) was evaluated in 36 cm² (6 x 6 cm) digitized photographs of the median region of the 2nd and 3rd true leaves, using the ImageJ computer program, after three, six and nine days of pathogen inoculation. The fresh and dry mass of the aerial parts of the plants were evaluated at 60 days after sowing; the number of fruits and the fresh mass of fruits considered commercial (>20 cm in length) (Sediyama et al., 2014); the number of leaves and plant height at 20, 30, 40, 50 and 60 days after sowing.

The experimental design used was in completely randomized blocks, in a factorial scheme of 3 (plant ages) x 2 (inoculated and non-inoculated plants), with four replications, with the plot consisting of one plant. The results were subjected to analysis of variance and the means of treatments compared by the Tukey test at 5% significance, using the computer program Systems for Analysis of Variance - SISVAR (Ferreira, 2019). The experiment was repeated once.

Results and Discussion

The average results of the two experiments were presented in joint analysis since no differences were observed between the variables in the experiments (p>0.05). Differences in the target leaf spot severity among the three plant ages were observed in plants inoculated with *C. cassiicola* from the evaluation carried out six days after inoculation, with a greater severity in plants inoculated 30 days after sowing and a lesser severity in the plants inoculated 20 days after sowing, a result that is maintained at nine days of evaluation, with plants inoculated at 40 days occupying an intermediate severity position (**Table 1**). Table 1. Severity (%) of target spot on cucumbers after three, six and nine days of inoculation or not with *Corynespora cassiicola*, in plants of different ages (20, 30 and 40 days after sowing), under greenhouse conditions

Plant age (days)	Inoculated with C. cassiicola	Not inoculated with C.cassiicola	
	Three days after inoculation		
20	1.3 aA	0.1 aB1	
30	1.8 aA	0.1 aB	
40	1.4 aA	0.2 aB	
CV(%)	24.4		
	Six days after inoculation		
20	3.3 cA	0.8 aB	
30	25.3 aA	0.2 aB	
40	17.6 bA	0.5 aB	
CV(%)	14.6		
	Nine days after inoculation		
20	6.6 cA	3.7 aA	
30	64.0 aA	0.4 aB	
40	39.6 bA	2.2 aB	
CV(%)	15.3		

CV(%) 15.3 'Average data followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey's test, at 5% probability. Statistical analysis with data transformed into root x+0.5.

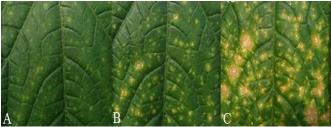


Figure 1. Target spot severity on cucumber leaves 30 days after sowing, after three (A), six (B) and nine (C) days after inoculation with Corynespora cassiicola.

The severity of the disease showed a linear growth ($R^2 \ge 0.98$) until the ninth day of evaluation (**Figure 1**), for the three inoculation ages, and was higher in the inoculated plants in relation to the non-inoculated ones, except for plants with 20 days to nine days after inoculation, in which the disease severity was below 7% on the inoculated plants. On the non-inoculated plants, the severity was below 4% until the ninth day of evaluation, with no differences between the ages of the plants (Table 1). This occurrence of the target leaf spot on uninoculated plants is possibly because the disease is endemic in the cucumber crop in the Central West region of São Paulo (Fischer et al., 2021), in addition to the hundreds of other host species of the pathogen (Dixon et al., 2009) likely to be a source of inoculum for the pathogen.

Differences in susceptibility to C. cassiicola based on the leaf age have already been reported for other plant species, as well as involving other pathosystems. Depending on the disease, host tissue susceptibility may increase or decrease over time (Furtado et al., 2009). Different from what was found in the present work, greater susceptibility to target leaf spot on African violet and rubber trees were observed in young leaves, decreasing the size of the lesions or the amount of the disease with leaf maturity (Ross, 2007; Fernando et al., 2010). However, a lower severity was observed in the mature leaves of cucumber in relation to the semi-mature ones, which may be associated with a greater thickness of the cuticle and/or amount of antifungal compounds such as phenolic compounds as also hypothesized by Ross (2007) on African violet. Ingram & Nawrath (2017) state that once leaves mature and reach their maximum size, cuticle formation accompanies expansion, and phenolic substances increase in the palisade parenchyma layers of the leaf blade (Carneiro & Isaias, 2015).

In tomato crops, target leaf spot is most found on mature leaves (MacKenzie et al., 2018), with young leaves considered less susceptible (MacKenzie et al., 2019). On tobacco higher target leaf spot severity was also observed on leaves of mature plants compared to younger plants (Fajola & Alasoadura, 1973). In a study of soybean leaves susceptibility to asian rust (*Phakopsora pachyrhizi*), the oldest trifoliate showed greater severity among the first four inoculated trifoliate of the plant, while in the evaluation of leaves of soybean plants in three phenological stages (V3, R1 and R5), the leaves of plants at the most advanced phenological stage (R5) were, contrary to the age of the leaves, the least affected (Furtado et al., 2009) showing that susceptibility is also variable with plant age.

According to Menzies & Belanger (1996), the difficulties encountered in the management of the target leaf spot in a greenhouse are due to the environmental conditions that favor the development of this disease. In the present study, the use of a humid chamber for 24 hours and average temperatures of 22.3°C and 24.6°C favored the development of the disease.

Long periods of dew and high temperatures are known to increase the severity of the disease (Kwon et al., 2003). The temperature and leaf wetness period also influenced the severity, number of lesions and diameter of target spot lesions in soybean; with higher values obtained at 27°C and 48 hours of leaf wetness (Mesquini, 2012). The age of tomato plants has already been associated with a more favorable microclimate for the occurrence of the target spot (Barbosa, 2016), in which older plants with a denser canopy, impairing the incidence of light and air circulation, resulted in greater disease index.

In the different plant's height and number of leaves, the treatments differed from each other (**Table 2**), with plants inoculated at 40 days showing greater height and number of leaves in relation to plants inoculated at 30 days and those at 20 days with the lowest values. A similar result was observed in plants not inoculated by the pathogen, except for plant height which although higher in plants from the 40-day treatment, did not significantly differ from the 30-day treatment.

Plants inoculated at 20 and 30 days of age were more affected, with significant differences in the number of leaves in relation to non-inoculated plants (Table 2). Progressive reduction in plant height and number of leaves have already been observed after 10 or 20 days of inoculation of the pathogen, when comparing the three ages of inoculation of the plants, also showing that the earlier the disease occurs, the greater the damage is (**Figure 2**).

Table 2. Height and number of cucumber leaves after 60days of sowing in plants inoculated or not with Corynesporacassiicola, at different ages (20, 30 and 40 days after sowing)under greenhouse conditions

Plant age	Plant height (cm)		Number	of leaves
(days)	Inoculated	Not inoculated	Inoculated	Not inoculated
20	147.4 cA	165.1 bA	30.7 cB	41.9 cA ¹
30	181.9 bA	198.0 aA	50.6 bB	90.1 bA
40	228.7 aA	219.9 aA	110.0 aA	104.9 aA
CV (%)	8	3.9	8	.0

¹Average data followed by the same letter, lowercase in the column and uppercase in the row, do not differ from each other by Tukey's test, at 5% probability.

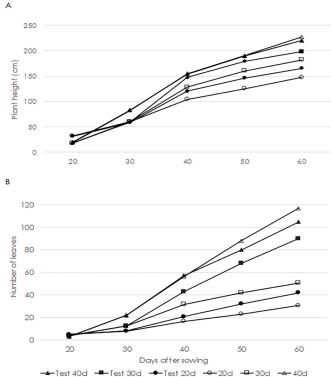


Figure 2. Plant height (A) and number of leaves (B) of cucumber plants inoculated with *Corynespora cassiicola* at different ages (20, 30 and 40 days after sowing) during 60 days of cultivation under greenhouse conditions (Test = not inoculated).

Comunicata Scientiae, v.14: e4007, 2023

The fresh mass and dry mass variables of the aerial part of the plants were significantly affected by the age of the plant at the time of inoculation of *C*. *cassiicola* differing from each other with the plants inoculated later, at 40 days after sowing, presenting the highest masses: those with 30 days an intermediate position and those with 20 days the most affected by the target spot (**Table 3**).

The same result was observed in plants not inoculated by the pathogen, as they were infected by the secondary inoculum produced in the inoculated plants and disseminated by the wind (Pavan et al., 2016) to the non-inoculated plants. Rapid progress of the target spot is observed in susceptible varieties such as Soldier with incubation and latent disease periods of 2 and 5 days, respectively (Fischer et al., 2021), with the period of exposure of the plant to the pathogen being directly proportional to the damage expressed by the reduction in fresh and dry mass.

Analyzing plants inoculated and not inoculated with the pathogen at each plant age, no differences were observed in fresh and dry mass only in the 40-day treatment, in which the plants were more developed at the time of inoculation, while in the 20 and 30-day treatments, the inoculated plants showed a reduction in at least one of the masses in the inoculated plants (Table 3).

Higher numbers of fruits and production were observed in the 40-day treatment, regardless of the inoculation of the pathogen, as well as the higher values in non-inoculated plants at 30 days in relation to those at 20 days (**Table 4**). This result is justified by the stage in which the infection occurs in the treatments, demonstrating a decrease in production as the disease is inoculated in treatments with ages below the fruiting stage, whose period started from 35 to 40 days. Higher numbers of fruits and production were obtained in plants not inoculated at 30 and 40 days in relation to those inoculated at the respective ages (Table 4).

Table 3.Fresh and dry mass of cucumbers after60 days of sowing, in plants inoculated or not withCorynespora cassiicola at different ages (20, 30 and40 days after sowing), under greenhouse conditions

Plant age	Fresh plant mass (g)		Dry plant mass (g)	
(days)	Inoculated	Not inoculated	Inoculated	Not inoculated
20	88.7 cA	129.5 cA	11.7 cB	17.5 cA ¹
30	164.5 bB	385.0 bA	23.2 bB	49.5 bA
40	541.2 aA	583.0 aA	62.0 aA	58.2 aA
CV (%)		11.6	8	3.9
Average	data followed	by the same letter,	lowercase in	the column and

uppercase in the row, do not differ from each other by Tukey's test, at 5% probability.

Although the defoliation was not evaluated on the cucumber plants, visually the plants inoculated at 20 days showed an early defoliation (**Figure 3**A) also observed in the non-inoculated plants of the same age as evidenced by the lower fresh and dry mass of the aerial part compromising plant production.

It is noteworthy that even non-inoculated plants from the 20-day treatment were severely affected by the disease contaminated by plants inoculated from 25 days of age, considering a latent period of the disease of 5 days (Fischer et al., 2021), according to what has already been mentioned. The disease occurring in younger plants ended up resulting in less photosynthetic aerial at fruiting stage (Figure 3) and, consequently, the plants produced fewer flowers and fruits.

Likewise, Igarashi et al. (2010) report results that demonstrate that the losses in soybean productivity gradually reduce, as the establishment of powdery mildew (*Microphaera diffusa*) occurs in the most advanced stages of development where plants that

Table 4.Number and production (g) of cucumber fruitsduring 60 days of cultivation, in plants inoculated or notwith Corynespora cassiicola, at different ages (20, 30and 40 days after sowing), under greenhouse conditions

Plant age	Number of fruits		Fruit production (g)	
(days)	Inoculated	Not inoculated	Inoculated	Not inoculated
20	0.2 bA	0.3 cA	27.0 bA	36.2 cA1
30	1.1 bB	2.7 bA	66.8 bB	333.7 bA
40	5.7 aB	8.3 aA	838.2 aB	1287.7 aA
CV (%)	32.2		29.7	



Figure 3. Cucumber plants inoculated with Corynespora cassiicola at 20 (A), 30 (B) and 40 (C) days after sowing, at 60 days of cultivation in a greenhouse.

were in the stages R5.1 – R5.2 and R5.3 – R5.4 presented smaller leaf affected area by the disease and obtained smaller yield and production losses in relation to plants R1-R2 and R2-R3. At working with the cucumber crop, the reduction in fruit production and quality was proportional to the level of loss of leaf area with plants supporting up to 25% of defoliation without a decrease in production (Nomura & Cardoso, 2000) which makes early leaf senescence, caused mainly by leaf diseases, a factor that seriously affects the productive potential (Navarini & Balardin, 2012).

Conclusion

Plants infected later, 40 days after sowing, were less affected by the target leaf spot showing greater development and fruit production, evidencing the importance of preventively managing the disease from the initial stages of cultivation to prevent early leaf senescence. As for disease susceptibility based on the leaf age, younger leaves were less affected while fully expanded leaves presented more severity.

Acknowledgements

The authors are grateful to the São Paulo State Research Support Foundation (Process FAPESP 2020/13494-5) for funding the work.

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