Severity of target spot and gas-exchange in tomato cultivated under colored films[1]

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ABSTRACT

The tomato target spot caused by *Corynespora cassiicola* may have an important economic impact on tomato production in tropical regions. The objective of this work was to study the effect of different colored plastic films on tomato target spot severity, gas-exchange, and fruit yield. Disease severity, photosynthetic rate, stomatal conductance, transpiration rate, relative chlorophyll content and fruit yield were evaluated in tomato, grown in individual mini-greenhouses covered with transparent plastic films of different colors (clear, red, blue, green, and yellow). At the beginning of the target spot epidemic, the plants under red and blue covers had lower leaf disease infection than those under other colored films ($p = 0.013$). However, as the disease progressed over time, the effect of the colored films on disease severity become non-significant ($p = 0.82$), and at the end of the experimental period, the target spot infection was about 70% in all treatments. Photosynthetic rates, stomatal conductance, and transpiration rate were not affected by the plastic film covers; neither the fruit number or fruit production. This study showed that colored films may reduce the infection by target spot, but only in the early stages of disease development (up to ~2% severity).

Keywords: *Corynespora casssicola*; *Solanum lycopersicum*; plant disease management; greenhouse cultivation.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the main vegetables produced worldwide, and in Brazil it represents the second largest harvested area, behind only to potato (*Solanum tuberosum* L.) (IBGE, 2019). Hot and humid environments favor disease incidence, pest attack and impair pollination of tomato. Several diseases, mainly those caused by fungal pathogens, constrain tomato productivity. Among them, the fungus *Corynespora cassiicola* (Berk. and Curt.) Wei, the causal agent of tomato target spot (Lopes & Reis, 2011), is one of the most important pathogens of this crop and causes severe yield losses, mainly in tropical regions.

So far, there is no tomato cultivars resistant to the target spot (MacKenzie *et al.*, 2018), hence, fungicides are often applied preventatively for reducing the effect of the target spot in tomato in other countries (MacKenzie *et al.*, 2018). In Brazil, however, no fungicides are registered for controlling this disease (Agrofit, 2021). Furthermore, it has been reported that *C. cassiicola* has become not only more aggressive but also progressively less sensitive, tolerant or resistant to fungicides (Rondon & Lawrence, 2019).

Greenhouse cultivation is widely used in North Region of Brazil to minimize the effect of excessive rainfall intensity. The use of colored covering materials in greenhouses can be useful to improve crop production, as the covers can reduce the effects of pest and diseases. Plastics or shade screens are filters that modify the solar radiation spectrum that reaches the plant. Specific wavelengths may promote physiological responses in plants, and improve yield, fruit quality characteristics (Holcman *et al.*, 2017) and plant defense mechanisms (Nagendran & Lee, 2015).

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Red light can induce resistance in plants. Leaf infection caused by *Alternaria tenuissima* (Kunze) Wiltshire in broad bean (*Vicia faba* L.) can be completely suppressed in red light illuminated leaflets, irrespective of pathogen strain or spore concentration (Rahman *et al.*, 2003). While the exposure of bell pepper (*Capsicum annuum* L.), tomato and pumpkin (*Cucurbita moschata* L.) to red light can induce resistance against damping-off caused by *Phytophthora capsici* Leon. (Islam *et al.*, 2002). Light quality affects plant defense response against various plant-pathogens, such as powdery mildew (*Leveillula taurica* (Lév.) Arn.) in bell pepper and *Sphaerotheca fuliginea* (Schltdl.) Pollacci in cucumber (*Cucumis sativus* L.) (Wang *et al.*, 2010). The exposure to red light can also reduce target spot severity caused by *Corynespora cassiicola* in cucumber (Rahman *et al.*, 2010), perhaps because light quality can affect fungus spore germination. In *Corynespora cassiicola*, for example, diameter and mass of colonies can be inhibited by light at wavelengths between 390-620 nm (Paixão *et al.*, 2018).

Light quality also affects biomass production and physiological variables, such as photosynthetic rates and stomatal conductance (Arena *et al.*, 2016; Choong *et al.*, 2018). For instance, *Dysosma versipellis* (Hance) M. Cheng grown under yellow and blue films may have lower photosynthetic rates than under white or red films (He *et al.*, 2018), while stomatal conductance can be higher in tomato plants grown under red, green and blue light than under white light (Arena *et al.*, 2016). Whereas, *Perilla frutescens* (L.) Britt grown under blue, red and green films can produce less biomass than control plants under natural light (Grbic *et al.*, 2016). The objective of this work was to evaluate the effect of transparent colored film covers on the target spot and photosynthetic traits of tomato plants grown in mini-greenhouses.

MATERIAL AND METHODS

The study was carried out in 2014, at the Instituto Nacional de Pesquisas da Amazônia, in Manaus, Amazonas, Brazil (3º05'29.1"S, 59º59'34.3"W). Tomato plants *cv* Yoshmatsu L-3-5, resistant to bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, were grown in greenhouses, in 5 L pots, containing soil and organic material (3:1, v/v). After 45 days from germination, the plants were taken to individual mini-greenhouses and covered with transparent colored polyvinyl chloride films (Figure 1). The plants were inoculated by spraying a conidial suspension (4.75 x10^4 conidia mL^{-1}). We used conidia from a mixture of three *C. cassiicola* isolates from tomatoes collected at Iranduba and Manaus, AM (isolates Inpa 2668, 2669, and 1833). The conidial suspension, obtained after incubation for seven days in potato-dextrose-agar, was sprayed late in the afternoon on leaves, using a hand sprayer, until runoff.

The individual mini-greenhouse (0.6 m x 0.6 m x 2.5 m) were made of polyvinyl chloride – PVC pipes (20 mm diameter), and with bamboo sticks used as plant support to prevent bending. The plants were watered daily and no phytosanitary treatment was used for pest and disease control. The experimental unit was the individual mini-greenhouse with one tomato plant in a 5-L pot, and the treatment, the five film colors (red, blue, green, yellow and clear a transparent colorless film). A diagrammatic scale, with eight disease severity levels, generated using the software Severity-Pro (Nutter Jr., 1998), was used to assess disease severity, which was expressed as a percentage of the infected area per leaf. Starting seven days after inoculation, the severity of target spot was evaluated twice a week for 44 days (i.e. at 2, 6, 9, 13, 16, 20, 23, 27, 30, 33, 37, 41, and 44 days after inoculation). On each evaluation three leaves were randomly selected from the lower, middle and upper third part of the plant, to obtain a mean value. Disease progress curves were plotted for each treatment and the area under disease progress curve (AUDPC) were calculated using the equation (Campbell & Madden, 1990):

$$AUDPC = \sum_{i=1}^{n_i-1} \frac{y_i+y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where n = *number* of observations, y_i disease severity (%) at the ith observation and t_i the time in days at the ith

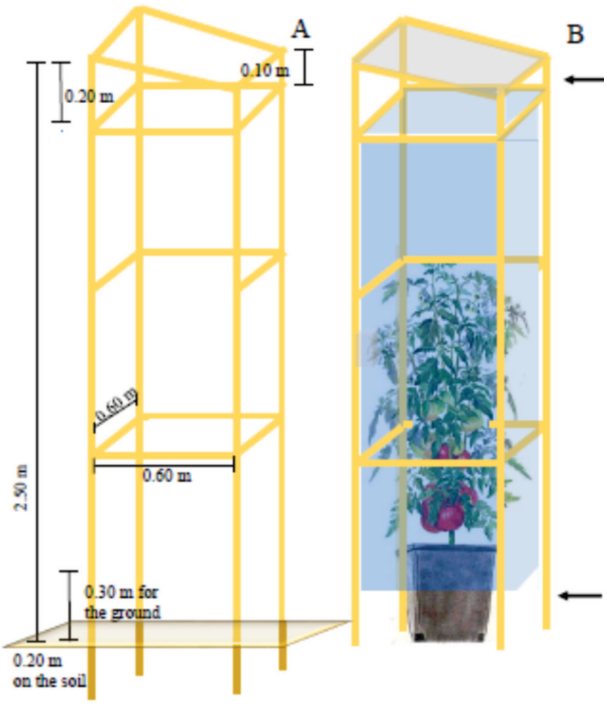

Figure 1: Schematic of greenhouse used for growing tomatoes with dimensions (A) and clear cover arrangement and plant location (B). The arrows indicate the openings for warm air outlet.

observation. At the beginning of fruiting stage, photosynthesis, respiration, and transpiration rates were measured using a portable gas exchange measurement system a Li-6400XT (Li-Cor, NE, USA). These measurement were made in six healthy leaves per plant (three in the middle third and three in the upper third of the plant), at ambient temperature and using a photosynthetically active radiation (PAR) of 1000 µmol $m^{-2}s^{-1}$ and 400 ppm of CO_2. In the same leaves, three measurements of the relative chlorophyll content (RCI) were taken using a portable chlorophyll meter (SPAD-502, Minolta, Osaka, Japan), and then a mean values was obtained. The fruits were collected twice a week and the fruit number and fruit mass per plant (fresh weight) were determined. We also recorded the number of days to beginning of flowering and fruit production. In one of the mini-greenhouse from each treatment, selected at random, relative humidity (RH) and air temperature were measured daily with thermo-hygrometers coupled to a data logger (HT-400, Icel, Manaus, Brazil).

The layout of experiment was a completely randomized design with five replications. The classic one way ANOVA was used to assess the effect of treatments on leaf and fruiting traits (we used a significant level of p = 0.05). Whereas to evaluate the effect of treatments on disease severity a repeated measures ANOVA was used. When required data were log transformed prior to statistical analysis in order to fulfill the assumptions of analysis of variance. Statistica 7.0 (Stat Soft Inc., 2004) was used for the statistical analysis.

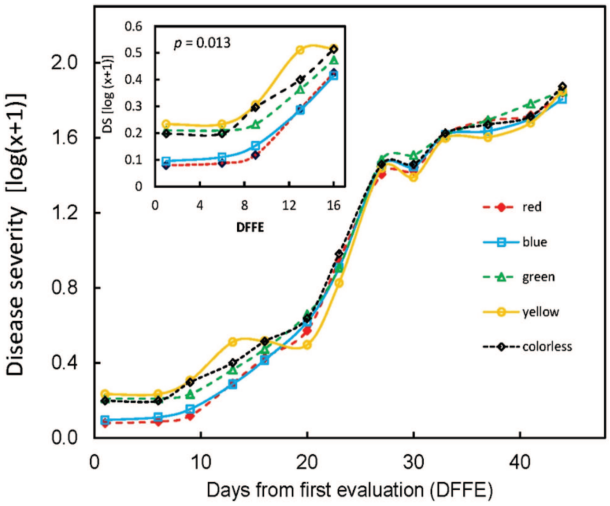

Figure 2: Target-spot disease progress curves in tomato plants grown in mini-greenhouses under colored plastic covers. The inset shows the disease severity (DS) during the first 16 days (DFFE). The effect of the plastic cover on DS was significant during the first 16 days (inset, p = 0.013), but not afterward (days 20–44, p = 0.819). Note that the data were log(x+1) transformed; hence, 0.5 corresponds to 2.16%, 1.0 a 9.0% and 2.0 to 99% in the original scale. In Y-axis, the x represents disease severity in percent.

RESULTS AND DISCUSSION

The first symptoms of the target spot were observed seven days after inoculation and by the seven weeks after inoculations, the disease had infected about 70% of the leaf area (Figure 2). During the first five disease severity measurements, there was a significant effect of the film color on the severity of the target spot, being the disease less severe in plants grown under red and blue films (inset in Figure 2, p = 0.013, Table 1-Appendix). Afterwards, the effect of the film color on disease severity became non-significant (days 20-44 in Figure 2, p = 0.819, Table 2- Appendix). It seems that the positive effect of the film cover occurred too early during plant growth, as to have an influence on the agronomic traits of tomato plants.

Inside the mini-greenhouse, temperature and RH were similar over treatments (Figure 3). The temperature was well above that considered optimal for tomato growth. For this crop, the optimal temperature is between 21 °C and 27 °C. Under adequate soil moisture, however, tomato plants can tolerate temperatures up to about 38 °C, but fruit set is adversely affected at high temperatures (Strange *et al.*, 2000). The optimal temperature for *C. cassiicola* and tomato target spot development is between 20 °C and 28 °C (Jones & Jones, 1984) and the relative humidity above 70%. Teramoto *et al.* (2013) observed that the optimum temperature for mycelial growth was

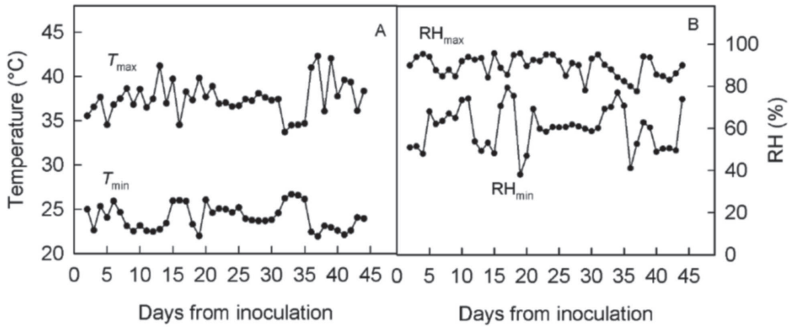

Figure 3: Average values of maximum and minimum temperature (A) and air relative humidity, RH (B) inside tomato mini-greenhouses.

28 °C, but its growth is reduced at 35° C. Relative humidity and temperature during the experimental period favored the pathogen development, and hence disease severity increased rapidly (Figure 2).

In comparison with the green, yellow and the clear colorless film, photosynthetic rates (P_n), stomatal conductance (g_s) and transpiration tended to increase in plants growing under red and blue films, but overall the film covering had no significant effect on P_n, g_s and transpiration (Table 2, $p > 0.10$). Our results are in disagreement with those reported by He *et al.* (2018) and Arena *et al.* (2016), who found an effect of light quality on photosynthetics traits. The discrepancy with those findings can be ascribed, at least in part, to the biotic (leaf infection by target-spot) conditions during the experimental period.

The number of days to flowering and beginning to fruit production was 51.9 and 65.8 days after emergence, respectively, with no effect of the film colors on these variables (Table 1, $p \geq 0.10$). There was also no significant influence of the film covering ($p > 0.05$) on both fruit number ($p = 0.12$) and mass of fruits ($p = 0.31$, Table 1). Iliæ *et al.* (2012) reported an increase in tomatoes yield under red nets, which is nor consistent with the results reported in this study (Table 1). The difference can be attributed to the high disease severity observed in our experiment. The tomato target spot is a disease that initiate in older leaves and spreads rapidly upwards. Then, the leaves turn yellow and collapse. Therefore the disease negatively impacts photosynthesis by causing loss of leaf area, and thereby biomass allocation to plant parts. Thus, when infection occurs before fruit development, the yield is very low. In the experiment, the disease developed rapidly in all treatments and, consequently fruit yield was severely reduced.

Cultivation under red net has been often reported to improve plant production. Iliæ & Fallik (2017) observed that the light quality under the photo-selective shade nets exerts a positive effect on yield, quality variables and phytochemical contents of commonly consumed vegetables such as tomatoes, sweet peppers (*Capsicum annuum* L.), lettuce (*Lactuca sativa* L.), and aromatic herbs at harvest and after storage. With respect to the chlorophyll content, we found that there was no difference in relative chlorophyll content among the treatments ($p = 0.87$, Table 1). Chlorophylls are essential for photosynthesis, and this respect, the amount of light absorbed by leaf at a given wavelength depend not only on the light quality itself but also of light intensity (Marenco & Lopes 2009, Liu & Van Iersel 2021).

Table 1: Area under the disease progress curves (AUDPC) of target-spot, number of days after emergence to the beginning of flowering and fruiting, fruit yield and fruit mass (fresh weight) per plant, and relative chlorophyll content (in SPAD values) in tomato plants grown in individual mini-greenhouses covered with a colored or clear plastic film. The coefficient of variation (CV) and the *p* values of ANOVA are also shown. The AUDPC data were log-transformed before data analysis

Film color	AUDPC (rel. un.)	Days to flowering	Day to fruiting	Fruit (number plant^{1})	Fruit mass (g plant^{-1})	SPAD values (rel. un.)
Red	875.3	52.4	66.0	11.4	283.8	36.0
Blue	848.7	52.0	65.8	6.8	208.2	33.1
Green	952.0	51.6	64.6	8.0	184.0	34.4
Yellow	824.0	51.2	66.0	12.2	267.0	35.3
Clear	910.1	52.2	66.4	12.4	328.0	34.2
CV (%)	1.9	3.69	5.89	39.5	45.3	13.2
p-value	0.89	0.10	0.10	0.12	0.31	0.87

Table 2: Photosynthesis (P_n), stomatal conductance (g_s), and transpiration rates (E) of tomato plants gown under different plastic film colors (each value represents the mean, $n = 5$). The coefficient of variation (CV) and *p* values of ANOVA are also shown

Film color	P_n (µmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})
Red	4.96	0.159	2.80
Blue	5.35	0.146	2.57
Green	3.94	0.084	1.66
Yellow	3.31	0.061	1.36
Clear	2.88	0.066	1.50
CV (%)	64.5	70.9	63.8
p - value	0.54	0.14	0.28

CONCLUSIONS

The effect of film coverings on disease severity was significant only at early stages of the epidemic (up to about 16 days from inoculation), and at this phase, the severity of the disease under red and blue films was lower than in the other treatments. As the disease severity increased, the initial benefit of the plastic film waned, and hence after that initial protection the film cover did not affect the disease development, and ultimately the disease severity reached about 70% in all treatments. Therefore, covering the mini-greenhouses with the plastic film was not enough to reduce the severity of target spot, which was particularly high. The severe disease infection may also have contributed to the lack of an effect of the film coverings on photosynthetic traits and fruit production. The results of this research add information on the search of alternative methods for the control of the target spot in tomato in the Amazon region.

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Table 1 - Appendix: Repeated measures analysis of variance of the effect film color (FC) on the target-spot disease progress curves in tomato plants grown in mini-greenhouses evaluated at 2, 6, 9, 13 and 16 days after inoculation. Data were log-transformed prior to statistical analysis. Abbreviations: DF, degree of freedom; MS, mean square; *F*, the fisher ratio, and *p*, the probability value

Source of variation	**DF**	**MS**	***F***	***p***
Film color (FC)	4	0.107101	4.1167	0.013581
Error	20	0.026016		
Evaluation (Ev)	4	0.436398	64.1311	< 0.00001
FC × Ev	16	0.003393	0.4986	0.940959
Error	80	0.006805		

Table 2 - Appendix: Repeated measures analysis of variance of the effect film color (FC) on the target-spot disease progress curves in tomato plants grown in mini-greenhouses evaluated at 20, 23, 27, 30, 33, 37, 41, and 44 days after inoculation. Data were log-transformed [*log* (*x* + 1)] prior to statistical analysis. Abbreviations: as described in Table 1A

Source of variation	**DF**	**MS**	***F***	***p***
Film color (FC)	4	0.0522	0.380	0.819900
Error	20	0.1371		
Evaluation (Ev)	7	4.5839	446.585	< 0.00001
FC × Ev	28	0.0060	0.581	0.952572
Error	140	0.0103	-	-