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Strobilurins and carboxamides in physiology and management of *Alternaria* sp. of tomato

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ABSTRACT: The aim of this study was to evaluate the application effects of fungicides based on strobilurins and carboxamides on the physiology, management of *Alternaria* sp. and production of tomato fruits in a protected environment. Two experiments were carried out in Salto-SP in 2018 and 2019. The randomized block design was used, with seven treatments: inoculated control (application of water and inoculum with *Altenaria* sp.), absolute control (application of water), azoxystrobin, boscalid, boscalid + azoxystrobin, pyraclostrobin and boscalid + pyraclostrobin, and five replications. To verify the photosynthetic efficiency of plants, analysis of chlorophyll a fluorescence and leaf gas exchange was performed at 50, 95 and 120 DAT. It was also verified the disease severity (%) and finally and the total and commercial production of fruits. From the results, under this research conditions, the tested fungicide application promoted positive physiological responses in tomato plants, controlling early blight disease and increasing fruit production. The application of the fungicides boscalid and azoxystrobin + boscalid can be a management tool for *Alternaria* sp. for tomatoes, for controlling the progress of the disease as well as for promoting better physiological and productive responses.

Key words: fungicides; fluorescence; photosynthesis; *Solanum lycopersicum* L.

Estrobilurinas e carboxamidas na fisiologia e manejo de *Alternaria* sp. do tomateiro

RESUMO: O objetivo deste estudo foi avaliar o efeito da aplicação de fungicidas à base de estrobilurinas e carboxamidas na fisiologia, manejo de *Alternaria* sp. e produção de frutos de tomate em ambiente protegido. Dois experimentos foram realizados em Salto-SP em 2018 e 2019. O delineamento experimental foi em blocos casualizados, com sete tratamentos: controle inoculado (aplicação de água e inóculo com *Altenaria* sp.), Controle absoluto (aplicação de água), azoxistrobina, boscalida, boscalida + azoxistrobina, piraclostrobina e boscalida + piraclostrobina e cinco repetições. Para verificar a eficiência fotossintética das plantas, foram realizadas análises da fluorescência da clorofila a e das trocas gasosas foliares aos 50, 95 e 120 DAT. Verificou-se também a severidade da doença (%) e pôr fim a produção total e comercial dos frutos. Pelos resultados, nas condições desta pesquisa, a aplicação dos fungicidas testados promoveram respostas fisiológicas positivas em plantas de tomateiro, controlando a pinta-preta e aumentando a produção de frutos. A aplicação dos fungicidas boscalida e azoxistrobina + boscalida pode ser uma ferramenta de manejo para *Alternaria* sp. no cultivo do tomateiro, por controlar a evolução da doença, bem como para promover melhores respostas fisiológicas e produtivas.

Palavras-chave: fotossíntese; fungicidas; fluorescência; *Solanum lycopersicum* L.

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Introduction

Tomato (*Solanum lycopersicum* L.), is the main fresh-consumed vegetable (Hachmann et al., 2014). In 2017, world production was 180,301,395 tons, with China being the largest producer (32.64%) followed by India, Turkey, United States, Egypt, Iran, Italy, Spain, Mexico and Brazil (FAO, 2020).

However, the tomato production has high costs due to high need for inputs, irrigation and fertigation, monitoring and weekly control of pests and diseases, in addition to the intensive use of labor to conduct the crop.

With the growing expansion of this crop, several phytosanitary problems have arisen, emphasizing the diseases caused by fungi, mainly the early blight, caused by the pathogen *Alternaria solani*. This pathogen causes epidemics with temperatures between 25 and 30°C and high relative humidity, with great potential for leaf destruction (Inoue-Nagata et al., 2016).

Among the systemic fungicides registered for control the early blight in tomato, there are fungicides from the groups of strobilurins and carboxamides. However, these substances, in addition to fungitoxic action, can also provide positive physiological effects on plants.

According with the main results observed in these molecules uses, there are greater accumulations of biomass and productivity, photosynthetic increase, increased activity of the nitrate reductase enzyme, higher levels of photosynthetic pigments and reduction of oxidative stress (Amaro et al., 2020). This effect on plant physiology has been studied in different cultures such as rice (Debona et al., 2016), bananas (Lima et al., 2012), tomatoes (Marek et al., 2018), cucumbers (Amaro et al., 2018), carrots (Colombari et al., 2015). Other studies also demonstrate improvements in the post-harvest quality of tomato (Ramos et al., 2013) and melon fruits (Macedo et al., 2017).

The physiological effects evidenced in these studies, resulted improvements in growth, development and photosynthetic metabolism of the plant, culminating in greater productivity (Amaro et al., 2020). In addition, these effects are also observed in the antioxidative system of the plants, which may stimulate possible actions in the plant's defense responses (Lehmann et al., 2015).

Thus, applications of fungicides formulated based on strobilurins and carboxamides, become an important tool for producers in the management of early blight control in tomato, as they directly control the fungus that causes the disease, inhibiting its development, also helping in the improvement of plant metabolism boosting productivity, as well as activating the plant's defense arsenal. With additional effects besides antifungal action, it is of great interest to associate the products secondary activity, where at the same time that the disease is controlled, the photosynthetic system is stimulated, contributing to better productive responses.

Therefore, the aim of this study was to evaluate the application effect of strobilurins and carboxamides in physiology, management of *Alternaria* sp. and production of tomato plants.

Materials and Methods

The experiment was carried out in Salto (São Paulo) twice, latitude S 23'12"03º and longitude W 47'17"13º in two crop years 2018-2019, conducted in a protected environment covered with 150 µm low density polyethylene film, additive and closed on the sides with screen, the first period from July to November 2018 and the second from March to August 2019. The region's altitude is 550 m with Cfa climate according to the Köppen classification. During the experiments microclimate characteristics were monitored inside the environment: maximum, average and minimum temperatures in degrees centigrade and average relative humidity (RH %), in 2018 these data were 28.5; 21.9; 16.8°C and 72.1%, respectively, and in 2019 28.7; 22.4. 17.1°C and 72.9%.

The treatments used were: T1 - inoculated control (T INC); T2 - absolute control (T ABS); T3 - azoxystrobin 50 g ha^{-1} of the active ingredient (ai) (AZO); T4 - boscalid 50 g ha^{-1} of ai (BOS); T5 - boscalid 50 g ha^{-1} of ai + azoxystrobin 50 g ha^{-1} (BOS + AZO); T6 - pyraclostrobin 100 g ha^{-1} of ai (PYR); T7- boscalid 50 g ha^{-1} of ai + pyraclostrobin 100 g ha^{-1} of ai (BOS + PYR).

The experimental design used was in randomized blocks, with seven treatments and five blocks, with the experimental plot consisting of four useful plants.

The first treatments application was carried out at 15 days after transplantation (DAT) and the rest fortnightly, totaling eight applications. The applications were carried out via leaf, in the whole plant, using a manual pressurized CO_2 sprayer, with 0.3 kgf cm^{-2}, open conical nozzle, using a plastic curtain between treatments to avoid drift. For each application, calibration of the syrup volume was performed, applying only water to the controls until reaching the point of leaves runoff. The spray volume was increasing, using 100 L of water ha^{-1} in the first applications and 1,000 L of water ha^{-1} in the plant sprays after apical pruning.

For the inoculated control, water and *Altenaria* sp. isolate were applied, and in the absolute control only water. The products used in the treatments were: Amistar® containing 500 g kg^{-1} of azoxystrobin ai (group of strobilurins) manufactured by Syngenta; for boscalid (group of carboxamides) the product Cantus® containing 500 g kg^{-1} of ai and for pyraclostrobin (group of strobilurins) the product Comet® was used containing 250 g L^{-1} of ai both manufactured by BASF.

The inoculated *Alternaria* sp. isolate was obtained from leaf lesions of tomato plants in the region of Guarapuava-PR. For the maintenance of these isolates, the fungi were grown in PDA medium (potato, dextrose, agar) and incubated in BOD germination chamber at 25ºC (± 2°C) and photoperiod of 12 h, for 10 days. Afterwards, discs of these cultures were stored in Eppendorf microtubes (2 mL) containing sterile distilled water and preserved in BOD, under the same culture conditions.

Seedlings of the tomato hybrid COLT®, supplied by the company HM Clause, saladette type, of undetermined growth, indicated for sowing and transplanting at any time of the year to soil with the chemical characteristics were used: pH ($CaCl_2$) = 6.9; organic material (g dm^3) = 35.0; P resin (mg dm^3) = 248; H + Al ($mmol_c$ dm^3) = 8.0; K ($mmol_c$ dm^3) = 1.9; Ca ($mmol_c$ dm^3) = 156; Mg ($mmol_c$ dm^3) = 22; SB ($mmol_c$ dm^3) = 180; CTC ($mmol_c$ dm^3) = 188; V (%) = 96; B (mg dm^3) = 0.25; Cu (mg dm^3) = 20.2; Fe (mg dm^3) = 170; Mn (mg dm^3) = 32.6; Zn (mg dm^3) = 30.2. Fertilization was performed based on Trani et al. (2015), using the following dosages: N = 60 kg ha^{-1}; P_2O_5 = 200 kg ha^{-1}; K_2O = 150 kg ha^{-1}; B = 2.50 kg ha^{-1}; Cu = 0.5 kg ha^{-1}; Mn = 2.0 kg ha^{-1}; Zn = 1.0 kg ha^{-1} and no liming. The transplant took place on July 25, 2018 and March 22, 2019, for the first and second experiments, respectively.

The plants were conducted on a single stem, and tutored vertically with tape. The sprouting was performed weekly, when the shoots were 3 to 5 cm in length. Weed control was performed manually and pest control as recommended for tomato crop (Alvarenga, 2013).

Measurements of chlorophyll a fluorescence and gas exchange of plants were performed at 50 and 95 DAT in experiment I in 2018, and at 120 DAT in experiment II in 2019. For this, leaves completely expanded in middle third of the plant were selected; after the leaves were covered with aluminum foil for twenty minutes (so that all reaction centers were open, with the capacity to receive electrons), readings were carried out between 8:00 and 11:00 am, using equipment with an open system of photosynthesis with CO_2 analyzer and water vapor by infrared radiation (“Infra-Red Gas Analyzer - IRGA”, model LI-6400XT, LI-COR), coupled with a portable fluorometer, obtaining the characteristics: maximum quantum yield of (Photossystem II) PSII (Fv/Fm), effective photochemical efficiency (Fv’/Fm’), photochemical quenching (qP), non-photochemical quenching (qN) and relative electron transfer rate (ETR). CO_2 assimilation rate (A, µmolCO_2 m^{-2} s^{-1}), transpiration rate (E, mmol water vapor m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}) and internal CO_2 concentration in the leaf (Ci, µmolCO_2 mol^{-1}), in addition to the water use efficiency (WUE, µmolCO_2 (mmol H_2O^{-1}) determined through the relationship between CO_2 assimilation and transpiration rate and instant efficiency of carboxylation (A/Ci) determined through the relationship between CO_2 assimilation rate and internal CO_2 concentration in the leaf.

The disease severity (%) was assessed from the appearance of first symptoms, which occurred five days after inoculation. Evaluations were carried out in five leaflets previously identified, in 10 plants per treatment, by three evaluators, at intervals of 7 days, by Azevedo’s diagrammatic scale (Azevedo, 1997).

The fruits harvest of experiment I (2018) started on October 21, and on June 18 for experiment II (2019), with the first five clusters being harvested in both experiments, at the moment that the fruits achieve a commercial color parameter. The harvested fruits were classified and weighed, in this way the total and commercial production was computed in kg m^{-2}, through the sum of the fruits weight of all harvested bunches.

For statistical analysis, the data were previously submitted to Anderson Darling homogeneity test, using the Minitab program. After checking the data normality, analysis of variance was performed (F test), comparing means by the Tukey test at 5% probability, using the AGROESTAT® program.

Results and Discussion

Analyzing the results of chlorophyll a fluorescence evaluations and gas exchanges, performed at 50 and 95 DAT in the 2018 experiment (Table 1), significant differences were observed for some variables on certain evaluation dates. In the first assessment at 50 DAT, a significant difference was observed, for ETR and WUE where BOS+AZO, PYR and BOS+PYR showed better results, while at 95 DAT, Fv’/Fm’, qP, qN, ETR and A, gs, E, A/Ci, showed significant differences, with BOS+AZO and PYR manifesting superior results in most of these variables.

The fluorescence analysis of chlorophyll a is capable of describing physiological state of plant regarding energy dissipation through electron transport, in addition to relating stresses and damage caused to the plants photosynthetic apparatus through the results of this evaluation.

Sunlight on plants is absorbed by chlorophylls that become excited, because they move from their basal state to a stimulus state. However, to maintain the homeostasis of photosynthetic metabolism, chlorophylls need to return to basal state, but for this it is necessary that the absorbed energy is dissipated (Taiz & Zeiger, 2013). The dissipation of this energy by plants can be photochemical, fluorescence or heat; in plants without stress indexes, energy is directed towards photosynthesis, thus increasing the potential quantum efficiency of photosystem II (PSII, lower Fv/Fm), photochemical extinction coefficient (qP) and electron transport rate (ETR).

Plants treated with BOS + AZO, PYR, BOS+PYR presented higher ETR, at 50 DAT. Fungicide application showed more

Table 1. Average values of photochemical quenching (qP) and apparent electron transport rate (ETR), water use efficiency (WUE- µmolCO_2 (mmol $H_2O)^{-1}$), of tomato plants subjected to application of fungicides with physiological effect at 50 DAT, Salto, SP, Brazil (2018).

50 DAT (2018)			
Treatments	qP	ETR	WUE
T INC	0.30 b	110.44 c	2.18 b
T ABS	0.38 ab	119.92 bc	2.83 ab
AZO	0.36 ab	116.66 bc	2.78 ab
BOS	0.34 ab	107.45 c	2.48 b
BOS + AZO	0.41a	131.08 ab	3.44 a
PIR	0.44a	145.85 a	3.43 a
BOS + PIR	0.45a	141.61ab	3.50 a
General mean	0.38	124.72	2.95
CV (%)*	9.95	8.04	15.02

Averages followed by the same lowercase letter in the column and in the evaluation period, do not differ by Tukey test at 5% probability. T INC - absolute control; T ABS - absolute control; AZO - azoxystrobin; BOS - boscalid; BOS+AZO - boscalid + azoxystrobin; PYR - pyraclostrobin; BOS+PYR - boscalid + pyraclostrobin. * CV (%) - Coefficient of variation.

variables of chlorophyll a fluorescence and gas exchange with statistical difference at 95 DAT than 50 DAT. At 95 DAT, plants treated with AZO, BOS+AZO and PYR showed, qP and ETR. On the other hand, T INC plants had the lowest A (1.84 µmol m^{-2} s^{-1}), and A/Ci (0.0064), for the T ABS plants were observed the same behavior of T INC.

The stress caused by the incidence of Altenaria sp. in the plant physiology, by the inoculated and absolute control treatments, can be observed from the lowest values of qP and ETR at 50 DAT (Table 1). It is more evident at 95 DAT, showed by Fv'/Fm', qP, qN and ETR (Table 2). In general, the incidence of *Altenaria* sp. in T INC possibly reflected in lower values for qP, ETR and A. The light energy intercepted by these plants was more directed towards dissipation in a non-photochemical way and less directed towards the photochemical process. These losses may have been due to the damage caused to the plants leaves with the disease incidence, since the species of the genus *Alternaria* are producers of prolific toxins, which facilitate their necrotrophic life, causing cell death, and consequently damage to plant tissue (Lawrence et al., 2008).

Plants treated with AZO, AZO+BOS and PYR, on the other hand, showed higher values of qP and ETR, when compared to other treatment plants. This indicates that the plants of these treatments used incident energy more efficiently and increased electron transport between photosystems II and I; thus, it can be said that in these plants there was also a greater formation of ATP and NADPH + H used to fix atmospheric CO_2, when compared to plants from other treatments.

The increase in CO_2 assimilation by plants treated with BOS, BOS+AZO and PYR may be related to greater stomatal opening (gs) also observed in these treatments without inoculation, however, differing from the application of BOS+PYR. Nonetheless, the applications of BOS, BOS+AZO and PYR treatments enabled the plant to diffuse more CO_2 into the sub-stomatal chamber, which was later used in the assimilation of CO_2. Stomatal opening is related to control of water loss and gas exchange by the plant, which reflects in the assimilation of CO_2 and maintenance of photosynthetic rate (Taiz & Zeiger, 2013), reason why the inoculated and absolute control treatments showed lower values of A, because they markedly reduced the stomatal conductance of the plants and perhaps, reducing also the disponibility of CO_2.

Analyzing transpiration (E) at 95 DAT (Table 2), it is possible to observe higher values for the BOS and PYR treatments applied in isolation, associated with gs, and the increased transpiration may result in less efficient water use; meanwhile, for this variable there was no significant difference between treatments. The carboxylation efficiency (A/Ci) was superior in AZO, BOS, BOS+AZO and PYR treatments, where the higher carboxylation efficiency observed in these treatments reflects the efficient functioning of the enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco), an essential enzyme in reduction of CO_2 in the photosynthetic process, related to greater stomatal opening and high values of CO_2 assimilation.

As a complementary form, chlorophyll a fluorescence analysis and gas exchange at 120 DAT were performed in the experiment conducted in 2019 (Table 3). For the fluorescence variables at 120 DAT, the same behavior is observed in the control plants, inoculated at 95 DAT in 2018, higher qN value, showing with the disease's advance the stress caused in the plant and that fungicides, besides controlling this stress, cause increases in the treated plants physiology. For this evaluation date, the treatment that stood out from the others was the application of BOS with higher values of A, gs and A/Ci. The application of boscalid in tomato plants Giuliana cv. also increased the rate of CO_2 assimilation (Ramos et al., 2015), but it reduced stomatal conductance and transpiration of plants, increasing efficiency in the use of water, different to observed in the present research. Even with low efficiency in water use, caused by increased transpiration, plants treated with boscalid showed high carboxylation efficiency, which reflected in plant productivity.

It was found that the application of strobilurins and carboxamides, caused different physiological effects on tomato plants infected by *Alternaria* sp., linked to different product's formulations and to the application forms, whether isolated or together.

The application of strobilurins, such as kresoximmetil, pyraclostrobin, azoxystrobin, trifloxystrobin and picoxystrobin,

Table 2. Mean values of quantum antenna efficiency (Fv'/Fm'), photochemical quenching (qP), non-photochemical quenching (qN), apparent electron transport rate (ETR), CO_2 assimilation rate (A-µmol m^{-2} s^{-1}); stomatal conductance (gs-mol m^2 s^{-1}), transpiration rate (E-mmol m^{-2} s^{-1}), carboxylation efficiency (A/Ci), of tomato plants submitted to application of fungicides with physiological effect at 95 DAT, Salto, SP, Brazil (2018).

	95 DAT (2018)							
Treatments	**Fv'/Fm'**	**qP**	**qN**	**ETR**	**A**	**gs**	**E**	**A/Ci**
T INC	0.446 bc	0.149 c	1.93 ab	39.88 b	1.84 c	0.034 cd	0.95 c	0.0064 b
T ABS	0.445 abc	0.175 bc	1.94 ab	47.14 b	1.53 c	0.022 d	0.81 c	0.0062 b
AZO	0.448 abc	0.183 abc	2.01 ab	61.49ab	6.09 b	0.076 bcd	2.33 bc	0.0250 a
BOS	0.554 a	0.156 c	1.37 c	53.18 b	7.65 ab	0.170 ab	4.80 a	0.0260 a
BOS+AZO	0.506 ab	0.270 ab	1.69 bc	84.09a	9.08 ab	0.138 abc	3.15 b	0.0345 a
PIR	0.497 ab	0.280 a	1.72 bc	84.84a	10.40a	0.225 a	5.28 a	0.0356 a
BOS+PIR	0.387 c	0.140 c	2.12 a	34.30 b	1.65 c	0.025 ab	0.88 c	0.0061 b
General mean	0.469	0.199	1.83	57.85	5.46	0.099	2.60	0.0199
CV(%)*	8.07	17.98	7.27	16.98	23.05	24.02	21.04	18.94

Averages followed by the same lowercase letter in the column and in the evaluation period, do not differ by the Tukey test at 5% probability. T INC - absolute control; T ABS - absolute control; AZO - azoxystrobin; BOS - boscalid; BOS+AZO - boscalid + azoxystrobin; PYR - pyraclostrobin; BOS+PYR - boscalid + pyraclostrobin. * CV (%) - Coefficient of variation.

Table 3. Average values of maximum quantum yield of PSII (Fv/Fm), quantum antenna efficiency (Fv'/Fm'), photochemical quenching (qP) non-photochemical quenching (qN) and apparent electron transport rate (ETR), CO_2 assimilation rate (A-μmol m^{-2} s^{-1}); stomatal conductance (gs-mol m^2 s^{-1}), transpiration rate (E-mmol m^{-2} s^{-1}), water use efficiency (WUE- μmolCO_2 (mmol $H_2O)^{-1}$), carboxylation efficiency (A/Ci), of tomato plants submitted to application of fungicides with physiological effect at 120 DAT, Salto, SP, Brazil (2019).

	120 DAT (2019)									
Treatments	Fv/Fm	Fv'/Fm'	qP	qN	ETR	A	gs	E	WUE	A/Ci
T INC	0.977 ab	0.513 c	0.202 a	1.83 a	63.29 a	8.25 b	0.069 cd	1.38 c	6.07 a	0.035 ab
T ABS	0.969 ab	0.602 ab	0.139 ab	1.18 b	50.28 ab	9.36 ab	0.141 bc	4.43 ab	2.42 b	0.033 ab
AZO	0.979 a	0.596 abc	0.128 ab	1.12 b	47.05 ab	8.78 ab	0.161 b	3.43 abc	2.77 b	0.028 bc
BOS	0.971 ab	0.548 bc	0.205 a	1.34 ab	66.74 a	12.71 a	0.338 a	5.06 a	2.56 b	0.045 a
BOS+AZO	0.967 b	0.581 abc	0.076 b	1.31 ab	27.06 b	4.12 c	0.059 d	1.65 c	2.45 b	0.013 c
PIR	0.971 ab	0.604 ab	0.091 ab	1.10 b	33.76 ab	7.72 bc	0.107 bcd	3.41 abc	2.26 b	0.023 bc
BOS+PIR	0.965 b	0.631 a	0.138 ab	1.06 b	53.76 ab	7.63 bc	0.134 bcd	2.40 bc	3.31 ab	0.022 bc
General mean	0.971	0.582	0.140	1.32	48.85	8.51	0.144	3.11	3.12	0.0289
CV(%)*	1.89	4.97	11.29	15.65	25.01	16.44	19.57	25.50	12.58	18.89

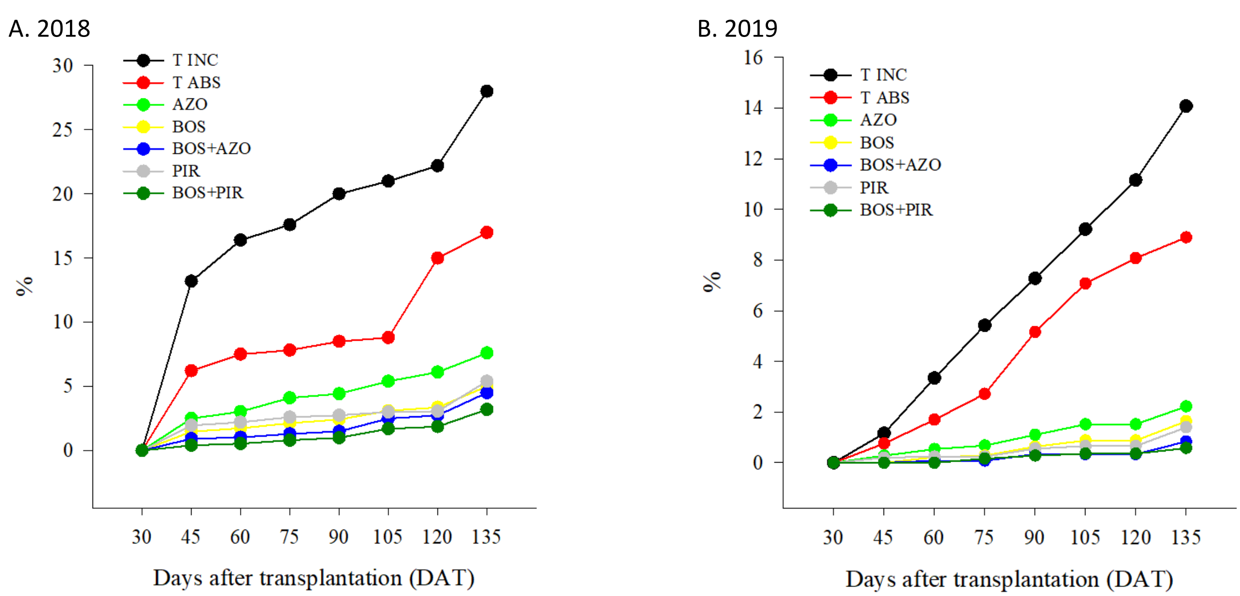
Averages followed by the same lowercase letter in the column and in the evaluation period, do not differ by the Tukeytest at 5% probability. T INC - absolute control; T ABS - absolute control; AZO - azoxystrobin; BOS - boscalid; BOS+AZO - boscalid + azoxystrobin; PYR - pyraclostrobin; BOS+PYR - boscalid + pyraclostrobin. * CV (%) - Coefficient of variation.

reduced the net CO_2 assimilation rate, transpiration rate and stomatal conductance in wheat, barley and soybeans (Nason et al., 2007). These authors state that these effects occurred due to reduction in production of ATP caused by blocking of electron transport in the cytochrome bc1 complex, which may have impaired the normal metabolism in guard cells, reducing turgor and, consequently, stomatal opening, since the guard cell's osmotic potential is limited by the production of ATP. This behavior was also observed in the present research for the PYR treatment plants, indicating that these strobirulins in the used dosage directly inhibited photosynthesis, causing stomatal closure and decreased CO_2 assimilation in tomato plants.

Application of chemical fungicides is a widespread method in the agricultural sector for diseases control, as they present rapid results (Amorim et al., 2011). Beyond the physiological effect, the tested fungicides showed a reduction in disease severity caused by *Alternaria* sp. in the two years of crops and in different evaluation date (Figure 1). In the experiment conducted in 2018, the disease severity reached 15% at 45 DAT, reaching levels greater than 25% at 135 DAT in the inoculated control.

In this research the fungicides were able to control the disease, also observed that the plants of absolute control also presented severity indexes superior to those of the treatments with fungicides, demonstrating that after inoculation there was disease transmission from the inoculated control to the other treatments.

The disease severity did not exceed 5% in 2018, and 2% in 2019 for all treatments where fungicides were applied, these are then effective for the management of *Alternaria* sp. However, regarding the physiological parameters evaluated, there was a significant difference between the fungicides, the application of BOS, BOS+AZO and PYR was efficient to increase



T INC - absolute control; T ABS - absolute control; AZO - azoxystrobin; BOS - boscalid; BOS+AZO- boscalid + azoxystrobin; PYR - pyraclostrobin; BOS+PYR - boscalid + pyraclostrobin.

Figure 1. Disease severity (%) in the 2018 (A) and 2019 (B) experiment, of tomato plant plants submitted to application of fungicides with physiological effect at 30, 45, 60, 75, 90, 105, 120 and 135 DAT, Salto, SP, Brazil (2018-2019).

CO_2 assimilation and the productivity of tomato plants. From this, it is possible to isolate the beneficial effect of these treatments on the plant's physiology since the severity was similar to the other fungicides applied.

For the tested fungicides, the BOS + PYR plants showed the lowest disease severity in the two years of cropping. Relating the severity data and gas exchange analyzes to 120 DAT, the BOS treatment controlled the disease progress while increasing the rate of CO_2 assimilation, demonstrating its antifungal and physiological effect.

The action of fungicides in the disease control can be perceived from 30 DAT; however, the physiological effect is more noticeable at 95 and 120 DAT, possibly showing that the effect on plant photosynthesis is accentuated with a greater number of biweekly applications. Therefore, the inclusion of these products in phytotechnical management of tomato crop, can control the disease and positively influence the plant's physiology.

Due to the early blight's pathogen presence in plants, *Alternaria* sp., there was biotic stress that reflected in the tomato photosynthetic efficiency, mainly after 90 DAT. The presence of this stressor was observed mainly in chlorophyll fluorescence related to the disease's progress, by increasing the severity (%) in the inoculated control treatment plants.

The results related to photosynthetic parameters of chlorophyll a fluorescence and gas exchanges, indicate that the plants infected by *Alternaria* sp. and treated with fungicides presented better conditions when compared to controls, mainly after a greater number of applications and also days after transplanting. Physiological effects of fungicides in have been reported in tomato (Marek et al., 2018) and melon (Macedo et al., 2017), this is because the application of strobilurins causes changes in the CO_2 compensation point and decreases the respiration that these compounds provide to the plant, reflecting the increase in photosynthetic rate (Grossmann et al., 1997), the application of carboxamides has a similar function, however there is still a lack of information to explain the beneficial physiological action of carboxamides (Amaro et al., 2020).

These fungicides can also promote increases in productivity, with a significant effect observed between treatments in the two consecutive cycles in 2018 and 2019 (Table 4). Therefore, application of fungicides had a positive influence on crop productivity, since for the year 2018 the plants that received application of AZO, BOS and BOS+AZO presented the highest values for total production, with increments of 6.9, 8.7 and 5% in relation to the inoculated control, respectively. While for the cultivation of 2019 this same variable was superior in the BOS and BOS+AZO treatments, with increments of 33.0 and 22.3% comparing to the inoculated control respectively.

The application of fungicides had a positive influence on photosynthesis, increasing the rate of CO_2 assimilation which directly influenced the fruit production by tomato plants. As for commercial production, the plants of the BOS and BOS + AZO treatments in 2018 stood out with values of 8.87 and 8.45 kg m², respectively. In 2019, only the BOS treatment plants emerged with the best result of 9.51 kg m², this behavior being the result of higher CO_2 assimilation rate measured in these treatments plants.

The results show that the application of carboxamides and strobilurins, alone or together, provided an increase in tomato productivity, when compared to controls. Corroborating these results, the application of strobilurins increased the total and commercial production of tomato by 14.2 and 19.1%, respectively (Cantore et al., 2016); other crops also obtained increases in production and physiological efficiency of plants when subjected to application of these fungicides, such as carrots (Colombari et al., 2015), beans (Jadoski et al., 2015), corn (Shetley et al., 2015) and cucumbers (Amaro et al., 2018).

In fact, the CO_2 assimilation rate and carboxylation efficiency were higher in plants treated with boscalid at 120 DAT, with this treatment showing greater total and commercial production. Likewise, the plants treated with boscalid + azoxystrobin, showed higher A, A/Ci and ETR at 95 DAT, and higher total production as well as the treatment plants with only boscalid applied.

Results by Lopes et al. (2018) suggest the application of pyraclostrobin in sugarcane plants as a management strategy aimed at disease control along with a set of practices to achieve high yields. Thus, and according to the results obtained in this

Table 4. Average values of total production rate (kg m^{-2}) and commercial (kg m^{-2}) of tomato plants submitted to application of fungicides with physiological effect in two years of cropping, Salto, SP, Brazil (2018-2019).

Treatments	2018		2019	
	Total production	Commercial production	Total production	Commercial production
	(kg m^{-2})			
T INC	10.57 b	7.27 c	9.27 c	6.99 d
T ABS	9.91 b	7.43 b	8.68 cd	6.68 e
AZO	11.30 a	7.53 b	10.23 bc	7.72 c
BOS	11.50 a	8.87 a	12.36 a	9.51 a
BOS+AZO	11.01 a	8.45 a	11.34 ab	8.72 b
PIR	10.25 b	7.43 b	10.97 b	8.42 b
BOS+PIR	10.08 b	7.38 b	9.26 c	6.99 d
General mean	10.1	7.81	10.30	7.86
CV (%)*	8.27	9.22	7.90	6.98

Averages followed by the same lower case letter in the column and in the year of harvests, do not differ by the Tukey test at 5% probability. T INC- absolute control; T ABS - absolute control; AZO - azoxystrobin; BOS - boscalid; BOS+AZO - boscalid + azoxystrobin; PYR - pyraclostrobin; BOS+PYR - boscalid + pyraclostrobin. * CV (%) - Coefficient of variation.

work, it is possible to prospect the use of fungicides, mainly boscalid, in an application for phytosanitary prevention and the others for productivity gain of tomato culture.

Conclusions

The application of strobilurins and carboxamides provide positive responses in the physiology of tomato plants, and controls early blight disease.

The application of the fungicides boscalid and azoxystrobin + boscalid can be a management tool for *Alternaria* sp. for tomatoes in a protected environment, for controlling the progress of the disease as well as for promoting better physiological and productive responses of tomato plants.

Compliance with Ethical Standards

Author contributions: Conceptualization: EOO, JDR; Data curation: ESA, AKLF; Formal analysis: ESA, AKLF; Investigation: WJJ; Methodology: WJJ; Project administration: WJJ, AKLF; Supervision: EOO; JDR; Validation: EOO; JDR; Visualization: EOO; JDR; Writing - original draft: WJJ, ESA; Writing - review and editing: JDR.

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Literature Cited

Alvarenga, M.A.R. Tomate: produção em campo, casa de vegetação e hidroponia. Lavras: UFLA, 2013. 457p.

Amaro, A.C.E.; Baron, D.; Ono, E.O.; Rodrigues, J.D. Physiological effects of strobilurin and carboxamides on plants: an overview. Acta Physiologiae Plantarum, v. 42, n.3, article 4, 2020. https://doi.org/10.1007/s11738-019-2991-x.

Amaro, A.C.E.; Ramos, A.R.P.; Macedo, A.C.; Ono, E.O.; Rodrigues, J.D. Effects of the fungicides azoxystrobin, pyraclostrobin and boscalid on the physiology of Japanese cucumber. Scientia Horticulturae, v. 228, n. 4, p.66-75, 2018. https://doi.org/10.1016/j.scienta.2017.10.016.

Amorim, L.; Bergamin Filho, A.; Camargo, L.E.A.; Rezende, J.A.M. Manual de fitopatologia: doenças das plantas cultivadas. São Paulo: Editora Agronômica Ceres, 2011. 774p.

Azevedo, L.A.S. Manual de quantificação de doenças de plantas. São Paulo: Ciba Agro, 1997. 48p.

Cantore, V.; Lechkar, O.; Karabulut, E.; Sellami, M.H.; Albrizio, R.; Boari, F.; Stellacci, A.M.; Todorovic, M. Combined effect of deficit irrigation and strobilurin application on yield, fruit quality and water use efficiency of "cherry" tomato (*Solanum lycopersicum* L.). Agricultural Water Management, v. 167, n.12, p.53-61, 2016. https://doi.org/10.1016/j.agwat.2015.12.024.

Colombari, L.F.; Baldini, L.F.G.; Baldini, V.; Cardoso, A.I.I.; Goto, R. Efeito fisiológico de fungicidas sistémicos em parâmetros agronómicos da cenoura. Revista de Ciências Agrárias, v. 38, n.4, p.366-371, 2015. https://revistas.rcaap.pt/rca/article/view/16941/13796. 18 Jan. 2021.

Debona, D.; Nascimento, K.J.T.; Gomes, J.G.O.; Aucique-Perez, C.E.; Rodrigues, F.A. Physiological changes promoted by a strobilurin fungicide in the rice- *Bipolaris oryzae* interaction. Pesticide Biochemistry and Physiology, v.130, n.12, p.8–16, 2016. https://doi.org/10.1016/j.pestbp.2015.12.006.

Food and Agriculture Organization of the United Nations – FAO. FAOSTAT. Countries by commodity 2018. http://fao.org/faostat/en/#rankings/coutries_by_commodity. 27 Dec 2020.

Grossmann, K.; Kwiatkowski, J.; Retzlaff, G. Regulation of phytohormone levels, leaf senescence and transpiration by the strobilurin kresoxim-methyl in wheat (*Triticum aestivum*). Journal of Plant Physiology, v.154, n.4, p.805-808, 1997. https://doi.org/10.1016/S0176-1617(99)80262-4.

Hachmann, T.L.; Echer, M.M.; Dalastra, G.M.; Vasconcelos, E.S.; Guimarães, V.F. Cultivo do tomateiro sob diferentes espaçamentos entre plantas e diferentes níveis de desfolha das folhas basais. Bragantia, v.73, n.5, p.399-406, 2014. https://doi.org/10.1590/1678-4499.0163.

Inoue-Nagata, A.K.; Lopes, C.A.; Reis, A.; Pereira, R.B.; Quezadoduval, A.M.; Pinheiro, J.B.; Lima, M.F. Doenças do Tomateiro. In: Amorim, L.; Rezende, J.A.M.; Bergamin Filho, A.; Camargo, L.E.A. (Eds.). Manual de fitopatologia: doenças das plantas cultivadas. Ouro Fino: Agronômica Ceres, 2016, v.2, p. 123-165.

Jadoski, C.J.; Rodrigues, J.D.; Soratto, R.P.; Santos, C.M.; Ribeiro, E. Ação fisiológica da piraclostrobina na assimilação de CO_2 e enzimas antioxidantes em plantas de feijão condicionado em diferentes tensões de água no solo. Irriga, v.20, n.2, p.319-333, 2015. https://doi.org/10.15809/irriga.2015v20n2p319.

Lawrence, C.B.; Mitchell, T.K.; Craven, K.D.; Cho, Y.R.; Cramer, R.A.; Kim, K.H. At death's door: Alternaria pathogenicity mechanisms. The Plant Pathology Journal, v.24, n.2, p.101-111, 2008. https://doi.org/10.5423/PPJ.2008.24.2.101.

Lehmann, S.; Serrano, M.; L'Haridon, F.; Tjamos, S.E.; Metraux, J.P. Reactive oxygen species and plant resistance to fungal pathogens. Phytochemistry, v.112, n.8, p.54-62, 2015. https://doi.org/j.phytochem.2014.08.027.

Lima, J.D.; Moraes, W.D.S.; Silva, S.H.M.G.D. Respostas fisiológicas em mudas de bananeira tratadas com estrobilurinas. Semina: Ciências Agrárias, v.33, n.1, p.77-86, 2012. https://doi.org/10.5433/1679-0359.2012v33n1p77.

Lopes, A.M.; Schumacher, P.V.; Martínez, A.T.P.; Costa Netto, A.P.; Chalfun-Junior, A. Insights into the positive effect of pyraclostrobin on sugarcane productivity. Agronomy, v.122, n.4, p.1-11, 2018. https://doi.org/10.3390/agronomy8070122.

Macedo, A.C.; Amaro, A.C.E.; Ramos, A.R.P.; Ono, E.O.; Rodrigues, J.D. Strobilurin and boscalid in the quality of net melon fruits. Semina: Ciências Agrárias, v.38, n.2, p.543-550, 2017. https://doi.org/10.5433/1679-0359.2017v38n2p543.

Marek, J.; Azevedo, D.; Ono, E.O.; Rodrigues, J.D.; Farias, C.M.D.R. Photosynthetic and productive increase in tomato plants treated with strobilurins and carboxamides for the control of *Alternaria solani*. Scientia Horticulturae, v.242, n.7, p.76-89, 2018. https://doi.org/10.1016/j.scienta.2018.07.028.

Nason, M.A.; Farrar, J.; Barlett, D. Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress. Pest Management Science, v.63, n.14, p.1191-1200, 2007. https://doi.org/10.1002/ps.1443.

Ramos, A.R.P.; Amaro, A.C.E.; Macedo, A.C.; Sugawara, G.S.A.; Evangelista, R.M.; Rodrigues, J.D.; Ono, E.O. Qualidade de frutos de tomate “Giuliana” tratados com produtos de efeitos fisiológicos. Semina. Ciências Agrárias, v.34, n.5, p.3543-3552, 2013. https://doi.org/10.5433/1679-0359.2013v34n6supl1p354.

Ramos, R.P.; Amaro, A.C.E.; Macedo, A.C.; Souza, E.R.; Rodrigues, J.D.; Ono, E.O. Acúmulo de carboidratos no desenvolvimento de tomateiro tratado com produtos químicos. Semina: Ciências Agrárias, v.36, n.2, p.705-718, 2015. https://doi.org/10.5433/1679-0359.2015v36n2p705.

Shetley, J.; Nelson, K.A.; Stevens, W.G.; Dunn, D.; Burdick, B.; Motovalli, P.P.; English, J.T.; Dudenhoeffer, C.J. Corn yield response to pyraclostrobin with foliar fertilizers. Journal of Agricultural Science, v.7, n.7, p.18-34, 2015. https://doi.org/10.5539/jas.v7n7p18.

Taiz, L.; Zeiger, E. Fisiologia vegetal. Porto Alegre: Artmed, 2013. 918p.

Trani, P.E.; Kariya, E.A.; Hanai, S.M.; Anbo, R.H.; Bassetto Júnior, O.B.; Purqueiro, L.F.V.; Trani, A.L. Calagem e adubação do tomate de mesa. Campinas: IAC, 2015. 35p.