



## Review

# Photosynthetic and productive increase in tomato plants treated with strobilurins and carboxamides for the control of *Alternaria solani*

Janaina Marek<sup>a,\*</sup>, Dione de Azevedo<sup>a</sup>, Elizabeth Orika Ono<sup>b</sup>, João Domingos Rodrigues<sup>b</sup>,  
 Cacilda Marcia Duarte Rios Faria<sup>a</sup>

<sup>a</sup> Universidade Estadual do Centro-Oeste (UNICENTRO), Campus Cedeteg, Departamento de Agronomia, CEP: 85040-080, Guarapuava, PR, Brazil

<sup>b</sup> Instituto de Biociências, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Campus de Botucatu, Departamento de Botânica, CEP: 18618-970, Botucatu, SP, Brazil



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## ABSTRACT

During two years (2015 and 2016), an experiment was carried out to investigate the effects of the application of strobilurin and carboxamides on the photosynthetic efficiency and yield of tomato plants inoculated with *A. solani* grown in greenhouse in southern Brazil. The experimental design was randomized blocks with five replicates and seven treatments: inoculated control (water + inoculum), absolute control (water), boscalid (0.075 g L<sup>-1</sup>), boscalid (0.100 g L<sup>-1</sup>) + kresoxim-methyl (0.050 g L<sup>-1</sup>), pyraclostrobin (0.100 g L<sup>-1</sup>), fluxapyroxad (0.058 g L<sup>-1</sup>) + pyraclostrobin (0.116 g L<sup>-1</sup>) and methyl (1.100 g L<sup>-1</sup>) + pyraclostrobin (0.100 g L<sup>-1</sup>), applied at 15 days after transplantation, at intervals of 15 days, totaling 6 applications. The effect on plant development was evaluated by plant height, leaf area, fresh and dry mass of leaves, stem and roots. To verify the photosynthetic efficiency, gas exchange, chlorophyll *a* fluorescence, content of photosynthetic pigments, nitrate reductase enzyme activity, carbohydrate content and yield were evaluated. All fungicides positively affected the evaluated parameters, improving photosynthetic efficiency and fruit production, in addition to providing efficient disease control. However, treatments using fluxapyroxad with pyraclostrobin, followed by metiram with pyraclostrobin stood out for promoting high photochemical yield due to the higher levels of photosynthetic pigments, activity of the nitrate reductase enzyme and, consequently, increase in the synthesis and translocation of photoassimilates. These results demonstrate the benefits of the use of these agrochemicals in the control of *A. solani* in tomato.

## 1. Introduction

Due to the growing expansion of tomato crop (*Solanum lycopersicum* L.), several phytosanitary problems have emerged, among them, many diseases, including black pint, caused by fungus *Alternaria solani*, capable of causing severe damages in the culture (Töfoli et al., 2014). Among strategies to control fungal diseases, the application of fungicides is the most frequent and vital for effective control (Petit et al., 2012; Bag et al., 2016). Many fungicides are registered for the control of black pint in tomato, such as those belonging to the chemical groups of strobilurins and carboxamides.

Fungicides of the strobilurin group, such as kresoxim-methyl and pyraclostrobin, act to inhibit mitochondrial respiration, blocking electron transfer in complex III and interfering in ATP production (Kanungo and Joshi, 2014). Similar to the mode of action of strobilurins,

carboxamides, such as boscalid and fluxapyroxad, act on complex II of mitochondrial respiration, inhibiting succinate dehydrogenase enzyme (SDHI) (Frac Code List ©\*, 2017), reducing the respiratory process and blocking the energy supply of the fungus (Van Dingenen et al., 2017).

Since the advent of these molecules, primarily strobilurins in the 1980s and more recently carboxamides, many researchers have observed that in addition to effective disease control, there are simultaneous effects on plant physiology, increasing crop yield (Fagan et al., 2010; Diaz-Espejo et al., 2012; Kanungo and Joshi, 2014; Van Dingenen et al., 2017). Numerous studies have shown positive effects of the application of these molecules on different crops such as soybeans, beans, corn, bananas, carrots, sunflowers, cucumbers, melons, tomatoes and grapes (Lima et al., 2009; Tsumanuma et al., 2010; Lima et al., 2012; Diaz-Espejo et al., 2012; Colombari et al., 2015; Ramos et al., 2015; Jadoski et al., 2015; Tsialtas et al., 2017; Macedo et al., 2017; Amaro

\* Corresponding author.

E-mail addresses: [janainamarek@gmail.com](mailto:janainamarek@gmail.com) (J. Marek), [dione.azevedo@hotmail.com](mailto:dione.azevedo@hotmail.com) (D. de Azevedo), [eoono@ibb.unesp.br](mailto:eoono@ibb.unesp.br) (E.O. Ono), [mingo@ibb.unesp.br](mailto:mingo@ibb.unesp.br) (J.D. Rodrigues), [criosfaria@hotmail.com](mailto:criosfaria@hotmail.com) (C.M.D.R. Faria).

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et al., 2018). As reported in these studies, the main effects observed are the activation of important enzymes in cell metabolism, such as NADH-nitrate reductase enzyme, increasing the assimilation of nitrogen and its incorporation in different vital molecules of the plant, such as chlorophyll, also reducing ethylene production, justifying the green effect, since in addition to the higher content of pigments, senescence is delayed. Another effect observed is the increase in CO<sub>2</sub> assimilation efficiency, increasing the photosynthetic rate and reducing the respiratory rate, culminating in higher net photosynthesis and productivity.

However, there are studies showing divergent results, as that by Petit et al. (2012), in which the application of strobilurins and even of fungicides of other chemical groups may negatively affect plant physiology and metabolism. These authors, after carrying out a large compilation of studies, pointed out that the intensity of the effect provided by different fungicides depends on the sensitivity of the plant species, vegetative phase, type and dose of the evaluated fungicide and environmental conditions. In addition, they reported that the disturbances caused by the use of fungicides in the cellular homeostasis of the plant can encode a situation of stress, generating metabolic expenditure, affecting metabolism.

In fact, there are reports in literature in which the application of pyraclostrobin did not affect the photosynthetic efficiency in corn plants in the absence of diseases (Junqueira et al., 2017) and of four soybean varieties (Swoboda and Pedersen, 2009). The final yield of common bean was not affected by the application of azoxystrobin and pyraclostrobin (Mahoney and Gillard, 2014). The application of azoxystrobin in wheat, barley and soybean (Nason et al., 2007), rice (Debona et al., 2016) and Japanese cucumber (Amaro et al., 2018), decreased the net CO<sub>2</sub> assimilation rate, transpiration, stomatal conductance and internal carbon dioxide concentration leading to a decrease in the photosynthetic efficiency of these plants.

Analyzing the world agricultural scenario and the constant search for high productivity, further studies should be carried out to verify the effects of these fungicides, promoting physiological effects on different crops and growing conditions.

The objective of this work was to evaluate the effects of the application of boscalid, kresoxim-methyl with boscalid, pyraclostrobin, fluxapyroxad with pyraclostrobin and metiram with pyraclostrobin on the photosynthetic efficiency and productivity of plants of hybrid tomato Conquistador in a greenhouse.

## 2. Material and methods

### 2.1. Plant material and cultivation system

Experiments were conducted in the period from January to May, in the years 2015 and 2016, in greenhouse belonging to the Department of Agronomy, “Universidade Estadual do Centro-Oeste (Unicentro)”, Cedeteg Campus, municipality of Guarapuava, State of Paraná, Brazil. The study site is located at latitude 25° 23'36"S and longitude 51° 27'1" W and 1,025 m above sea level, with humid mesothermic subtropical climate. Seeds of hybrid tomato Conquistador cv. (Sakata Seeds Sudamérica Ltda.) were seeded in polypropylene trays with 128 cells, filled with commercial substrate (Carolina Soil™) on December 2, 2015 (experiment I) and 2016 (experiment II). Transplanting was carried out 34 days after sowing in 10 dm<sup>3</sup> pots filled with typical Dusky Dystroferic Latosol (Embrapa - Empresa Brasileira de Pesquisa Agropecuária, 2013) with 1.0 x 0.5 m spacing. Soil correction and fertilization were carried out based on its chemical analysis. Throughout the crop cycle, drip irrigation was performed and weed control was manually performed and pest control was carried out according to recommendations for the crop.

Plants were conducted with a stem along the cycle, individually and vertically tutored. Shoots were removed when they reached 3–5 cm in length. Plants were cultivated up to the fifth bunch of fruits, when apical pruning was performed.

### 2.2. Experimental design and treatments

The experimental design was randomized blocks with 7 treatments and 5 replicates, each plot being composed of 4 plants. Treatments were the following: inoculated control (water + inoculum); absolute control (water); boscalid (0.075 g L<sup>-1</sup>); boscalid (0.100 g L<sup>-1</sup>) + kresoxim-methyl (0.050 g L<sup>-1</sup>); pyraclostrobin (0.100 g L<sup>-1</sup>); fluxapyroxad (0.058 g L<sup>-1</sup>) + pyraclostrobin (0.116 g L<sup>-1</sup>) and metiram (1.100 g L<sup>-1</sup>) + pyraclostrobin (0.100 g L<sup>-1</sup>).

The first application was performed 15 days after transplanting (DAT) and the others in 15-day intervals, totaling six applications in the entire plant using a pressurized CO<sub>2</sub> manual sprayer (0.3 kgf/cm<sup>2</sup>) and conical nozzles, using plastic curtain between treatments to avoid drift. Physiological and biochemical evaluations were performed after each treatment application.

### 2.3. Isolation and inoculation of the pathogen: *Alternaria solani*

*A. solani* isolate was obtained from leaf lesions of tomato plants at the Cedeteg University campus. The fungus was cultivated in PAD (potato agar dextrose) medium and the inoculation of plants was performed 24 h after the first application of treatments (16 DAT) with suspension containing  $1 \times 10^4$  conidia mL<sup>-1</sup>.

Plants, with the exception of the absolute control treatment, were inoculated 24 h after the first application of the treatments (16 DAT) with suspension containing  $1 \times 10^4$  conidia mL<sup>-1</sup>, on both sides of the leaflets of all the leaves of the plant, with hand spray until that drops were formed and began to drip from the ends of the leaflets. After inoculation of the plants the humidity and temperature of the greenhouse were monitored so that the ideal conditions for the development of the pathogen were maintained.

### 2.4. Properties evaluated

#### 2.4.1. Plant development analysis

Plant height evaluations were performed at 14 (before the first application of treatments) and at 21, 28, 35, 42, 49, 56 and 65 DAT (days after transplanting). The last evaluation occurred at 65 DAT, at which time apical pruning was performed. For measurements, a graduated ruler was used and the results were expressed in cm<sup>-1</sup> plant.

At 120 DAT (harvest term), five plants from each treatment were collected, separated in leaf, stem and root, and fresh and dry mass was measured. The dry mass was obtained by drying leaves, stems and roots in a forced circulation oven at constant temperature of 60 °C until reaching constant mass, and results were expressed in grams (g). The leaf area was measured using LiCor® Area Meter, Model LI-3100 Area Meter and results were expressed in square centimeters (cm<sup>2</sup>).

#### 2.4.2. Gas exchanges and chlorophyll *a* fluorescence

Gas exchange and chlorophyll *a* fluorescence evaluations were performed at 34 and 94 DAT (5th day after the second and sixth treatments, respectively), in completely expanded leaves, located in the middle third of the plant. Readings were performed in duplicate, from 9:00 AM to 11:00 AM. For the readings of gas exchanges, equipment with open system with photosynthesis with CO<sub>2</sub> analyzer and water vapor by infrared radiation (“*Infra Red Gas Analyser* – IRGA”, model LI-6400XT, LI – COR) was used and results were calculated from the difference between the CO<sub>2</sub> concentration and the reference air water vapor (value present in the chamber without leaves) and the sample (value with the presence of leaves in the chamber), obtaining the water vapor and CO<sub>2</sub> concentrations that were released (transpiration - water vapor) and assimilated (CO<sub>2</sub> assimilation) through the stomata.

The characteristics of gas exchanges analyzed were: net CO<sub>2</sub> assimilation rate ( $A_{net}$ , μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), transpiration rate ( $E$ , mmol water vapor m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance ( $g_s$ , mol m<sup>-2</sup> s<sup>-1</sup>) and internal CO<sub>2</sub> concentration in leaves ( $C_i$ , μmol CO<sub>2</sub> mol<sup>-1</sup> air). These

characteristics were calculated by the data analysis program present in the gas exchange measuring equipment, which uses the general gas exchange equation of [Von Caemmerer and Farquhar \(1981\)](#).

The water use efficiency ( $WUE$ ,  $\mu\text{mol CO}_2$  ( $\text{mmol H}_2\text{O}$ ) $^{-1}$ ) was determined by the relationship between  $\text{CO}_2$  assimilation rate and transpiration rate and the carboxylation efficiency of the ribulose 1,5-diphosphate carboxylase (Rubisco) enzyme, which was determined by the relationship between  $\text{CO}_2$  assimilation rate and internal  $\text{CO}_2$  concentration in leaves ( $A_{\text{net}}/C_i$ ).

The chlorophyll *a* fluorescence readings were performed with a Portable Chlorophyll Fluorometer (PAM-2500), simultaneously with gas exchange measures. Slow kinetics measurements were performed, with leaf adaptation to dark for 20 min (so that all reaction centers were open, with ability to receive electrons). Basal fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) parameters were obtained, from which fluorescence ( $F_v$ ) and maximum quantum yield of PSII ( $F_v/F_m$ ) were calculated. The responses of effective photochemical quantum yield of PSII, Y(II) and relative electron transfer rate ( $ETR$ ) were also obtained. Fluorescence data obtained were automatically stored in the PAM memory and later transferred to the computer via Wincontrol computer software.

#### 2.4.3. Activity of the nitrate reductase enzyme and content of photosynthetic pigments

To evaluate the activity of the nitrate reductase enzyme (NR, EC 1.6.6.1) and the content of photosynthetic pigments, 8 leaves were collected at 15, 20, 35, 50, 65, 80, 95 and 110 DAT, corresponding to the following moments:

- Time 0 h before treatment (15 DAT);
- 5th day after the first treatment application (20 DAT);
- 5th day after the second treatment application (35 DAT);
- 5th day after the third treatment application (50 DAT);
- 5th day after the fourth treatment application (65 DAT);
- 5th day after the fifth treatment application (80 DAT);
- 5th day after the sixth and last treatment applications (95 DAT);
- 15 days after the last treatment application (105 DAT).

For the determination of the activity of the NR enzyme, leaves were collected between 10 and 11 A M, packed in foil and stored in styrofoam box with ice (keeping the material fresh, not freezing). Subsequently, the collected material was taken to the laboratory and the activity of the NR enzyme was determined according to methodology proposed by [Streeter and Bosler \(1972\)](#), where 1 g of sample was weighed into light protected tubes (paper coated aluminum). Then, 8 mL of buffer composed of 0.1 M phosphate buffer (KPi) pH 7.5 + 0.2 M  $\text{KNO}_3$  were added, the reaction was conducted at 37 °C for 1 h. After this period, 1 mL of 1% sulfanilamide in 1.5% HCl + 1 mL of 0.2%  $\alpha$ -Naphthyl was added. After 5 min, the sample was filtered in cotton and read in spectrophotometer at 540 nm. Samples were collected and analyzed on the same day of collection and the activity of the NR enzyme was expressed in  $\mu\text{g min}^{-1}$  of fresh nitrite mass  $\text{g}^{-1}$ .

To determine the pigment content, collection occurred simultaneously with the collection to determine the activity of the NR enzyme, however, after collection, leaves were immediately frozen in liquid nitrogen to stop all reactions. The content of photosynthetic pigments was determined by the spectrophotometric method proposed by [Lichtenthaler and Wellburn \(1983\)](#), using 0.030 g of macerated vegetable material with 8 mL of 80% acetone and 0.01 g of calcium carbonate ( $\text{Ca}(\text{CO}_3)_2$ ) and centrifuged for 20 min at 13,500 g. The supernatant was read in spectrophotometer at wavelengths of 470.0, 646.8 and 663.2 nm.

For calculations of the content of chlorophyll pigments (chl *a*, *b* and total) the following formulas were used:

$$\text{Chlorophyll } a \text{ (}\mu\text{g/mL)} = 12.1 A_{663} - 2.81 A_{646}$$

$$\text{Chlorophyll } b \text{ (}\mu\text{g/mL)} = 20.13 A_{646} - 5.03 A_{663}$$

$$\text{Chls total} = 17.76 A_{646.6} + 7.34 A_{663.6}$$

$$\text{Carotenoids (}\mu\text{g/mL)} = ((1000A_{470} \cdot 3.27[\text{Chl } a] - 104[\text{Chl } b]))/229.$$

The levels of chlorophyll and carotenoid pigments were expressed as  $\mu\text{g mL}^{-1}$  g fresh mass $^{-1}$ .

#### 2.4.4. Carbohydrate analysis

To determine the carbohydrate content (reducing sugars and soluble sugars), two destructive evaluations were carried out during plant development, the first was carried out at 50 DAT and the second at 120 DAT (the last day of harvest). Five plants were collected per treatment, which were separated into leaves, stem and fruits and the carbohydrate content was determined in these organs. The extraction of soluble sugars was performed according to methodology proposed by [Garcia et al. \(2006\)](#) using 0.1 g of fresh plant material.

The extract obtained was separated into microtubes and stored at  $-20$  °C until the determination of carbohydrates. For the quantification of total soluble sugars, the methodologies of [Morris \(1948\)](#) and [Yemm and Willis \(1954\)](#) were used. Reading was performed in spectrophotometer at 620 nm, using standard curve of glucose solution. The content of total soluble sugars was expressed as  $\text{mg g}^{-1}$  of fresh mass. The quantification of reducing sugars was performed according to methodology proposed by [Miller \(1959\)](#). Reading was performed in spectrophotometer at 540 nm, using standard curve of glucose solution. Reducing sugars were expressed as  $\text{mg g}^{-1}$  of fresh mass.

#### 2.4.5. Assessment of the severity of *Alternaria solani*

The severity of the disease was evaluated at the onset of the first symptoms, occurring five days after inoculation (20 DAT). The evaluations were carried out in five previously identified leaflets, by three evaluators, in 10 plants per treatment, at intervals of 7 days. Eight severity assessments were performed from January 28 to March 17 in both experiments. For the severity evaluation, the diagrammatic scale of [de Azevedo \(1997\)](#) was used. The methodology proposed by [Campbell and Madden \(1990\)](#) was used to calculate the area under the progression curve of the disease (AUPCD).

#### 2.4.6. Production

In the experiment conducted in 2015, fruit harvest began on March 27, 2015 (79 DAT) and extended until May 5, 2015 (118 DAT), while experiment conducted in 2016, fruit harvest started on March 30, 2016 (82 DAT) and extended until May 7, 2016 (120 DAT). The harvest period comprised on average 37 days and was performed weekly, as fruits reached the stage of light red maturation. All fruits were weighed in analytical scale, and results were expressed in grams (g); diameter measured with pachymeter and values expressed in millimeters (mm).

Total and commercial production of tomato fruits ( $\text{kg m}^{-2}$ ), number of fruits  $\text{m}^{-2}$ , average fruit mass (g), diameter and length of fruits were evaluated. To evaluate production, fruits were selected as "non commercial" and "commercial", considering fruits with diameter less than 40 mm as "non commercial" and greater than 40 mm and without defects (symptoms of diseases, plagues, physiological disorders and/or physical damages) as "commercial".

#### 2.5. Statistical analysis

Data normality was verified by the Shapiro-Wilk test and homogeneity of variance by Cochran C test, before each parametric statistical test and analysis of variance (ANOVA), and means were compared by the Tukey test, both at 5% significance level. Gas exchange and chlorophyll *a* fluorescence data were not normal and were transformed by the Box-Cox method. Meanwhile, data on non-commercial fruit percentage were transformed by  $\sqrt{x}$  (%). All statistical tests were

**Table 1**

Height (cm) at 14, 21, 28, 35, 42, 49 and 56 days after transplanting (DAT) and development variables at 120 days after transplanting (DAT) of hybrid tomato plants Conquistador cv. cultivated in greenhouse and treated with different fungicides with physiological effects and inoculated with *Alternaria solani*, in the years 2015 and 2016.

2015													
Treatments	Plant height (cm)							Development variables					
	Days after transplanting (DAT)							Leaf area (cm <sup>2</sup> )	Fresh leaf mass (g)	Dry leaf mass (g)	Fresh stem mass(g)	Dry stem mass (g)	Fresh root mass (g)
	14	21	28	35	42	49	56						
Inoculated control (inoculum + water)	26.3a	35.5c	42.3c	65.9c	85.6c	120.8c	167.5b	3298.32e	111.65e	17.26e	418.99c	42.32c	60.18b
Absolute control (water)	27.6a	33.7c	45.8c	62.8c	86.4c	124.2c	166.2b	6247.84d	211.84d	21.03d	423.56c	44.51c	63.93b
Boscalid (0.075 g L <sup>-1</sup> )	26.3a	39.2b	49.6b	69.9b	92.3b	139.6b	174.2a	8141.22c	275.37c	26.41c	443.25b	51.47b	81.03a
Boscalid (0.100 g L <sup>-1</sup> )	27.2a	38.6b	50.3b	68.3b	93.2b	140.2b	174.6a	8037.21c	270.12c	26.97c	440.24b	48.94b	78.91a
+ Kresoxim methyl (0.050 g L <sup>-1</sup> )													
Pyraclostrobin (0.100 g L <sup>-1</sup> )	27.0a	39.4b	52.4b	69.9b	95.6b	141.2b	175.2a	8119.04c	273.98c	26.24c	442.08b	49.12b	79.31a
Fluxapyroxad (0.058 g L <sup>-1</sup> )	26.9a	41.2a	57.6a	72.9a	106.5a	152.3a	175.6a	10,452.11a	330.95a	39.09a	502.34a	56.98a	82.01a
+ Pyraclostrobin (0.116 g L <sup>-1</sup> )													
Metiram (1.100 g L <sup>-1</sup> )	27.4a	40.6a	55.2a	70.1ab	104.9a	149.7a	175.3a	9914.29b	309.71b	30.42b	495.36a	55.09a	80.96a
+ Pyraclostrobin (0.100 g L <sup>-1</sup> )													
CV (%)	12.4	14.5	16.4	18.7	18.1	19.2	12.5	12.65	13.45	14.72	12.35	10.84	12.72

2016													
Treatments	Plant height (cm)							Development variables					
	Days after transplanting (DAT)							Leaf area (cm <sup>2</sup> )	Fresh leaf mass (g)	Dry leaf mass (g)	Fresh stem mass (g)	Dry stem mass (g)	Fresh root mass (g)
	14	21	28	35	42	49	56						
Inoculated control (inoculum + water)	26.8a	35.3c	43.6c	65.7c	90.7c	124.1c	168.1b	3098.12e	108.69e	15.86d	401.32e	41.28c	61.39b
Absolute control (water)	27.9a	33.4c	44.8c	63.3c	88.2c	122.5c	167.7b	6384.17d	208.49d	19.91c	418.74d	43.38c	62.91b
Boscalid (0.075 g L <sup>-1</sup> )	26.5a	37.1b	49.9b	70.4b	95.4b	140.2b	175.0a	8172.24c	276.57c	28.96b	442.58c	50.79b	80.59a
Boscalid (0.100 g L <sup>-1</sup> )	26.8a	37.1b	50.4b	69.9b	96.2b	140.8b	174.9a	8019.45c	271.21c	27.40b	438.16c	47.26b	78.27a
+ Kresoxim methyl (0.050 g L <sup>-1</sup> )													
Pyraclostrobin (0.100 g L <sup>-1</sup> )	27.3a	36.8b	50.1b	70.9b	96.9b	141.4b	174.2a	8121.25c	274.23c	28.19b	441.92c	49.98b	79.48a
Fluxapyroxad (0.058 g L <sup>-1</sup> )	27.1a	39.9a	55.2a	74.1a	107.9a	154.1a	176.9a	10,311.51a	329.32a	34.55a	501.12a	56.24a	80.94a
+ Pyraclostrobin (0.116 g L <sup>-1</sup> )													
Metiram (1.100 g L <sup>-1</sup> )	27.0a	39.1a	53.9a	71.6ab	105.8a	151.0a	176.1a	9889.77b	314.18b	32.97a	488.67b	54.73a	79.99a
+ Pyraclostrobin (0.100 g L <sup>-1</sup> )													
CV (%)	10.3	12.2	9.9	15.2	13.5	12.9	14.2	9.56	12.32	11.25	10.59	12.56	8.78

Mean (n = 5) followed by different letters in the column differ significantly from each other by the Tukey test at 5% probability ( $P \leq 0.05$ ).

performed using Environment R software (R Development Core Team, 2016).

### 3. Results

#### 3.1. Development of tomato plants

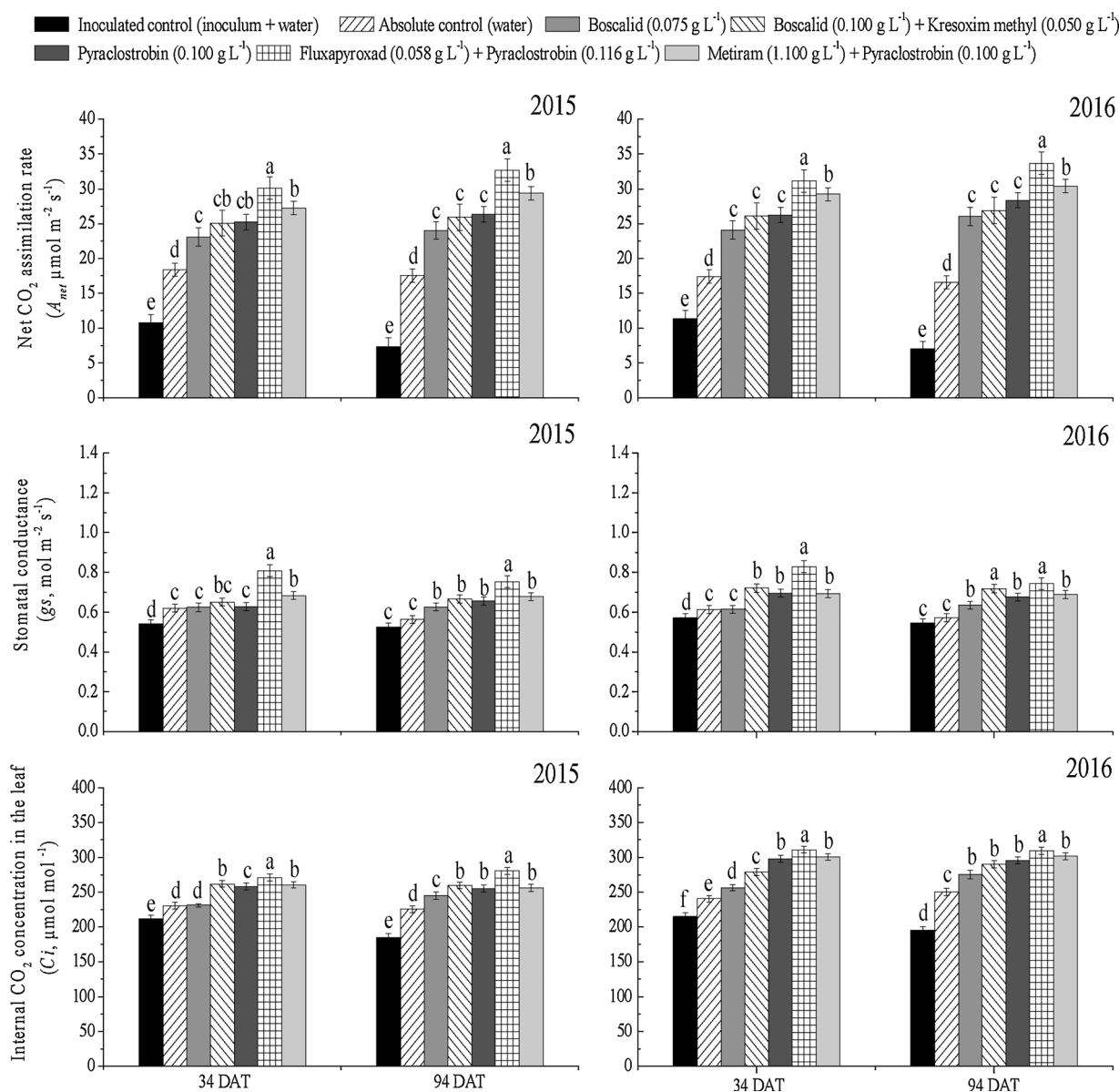
Analyzing the initial development of tomato plants submitted to different treatments: inoculated control (Inoc-control), absolute control (Abs-control), boscalid (Bosc), boscalid with kresoxim methyl (Bosc + Kre), pyraclostrobin (Pyra), fluxapyroxad with pyraclostrobin (Flux + Pyra) and metiram with pyraclostrobin (Me + Pyra), significant differences ( $P \leq 0.05$ ) were observed among treatments, presenting similar behavior in 2015 and 2016 (Table 1).

At 14 DAT (evaluation performed before the first application of treatments, which occurred at 15 DAT), there was no significant difference among treatments ( $P \leq 0.05$ ). From 21 DAT extending up to 49 DAT, the highest plant height values were observed in Flux + Pyra and Me + Pyra treatments. The height of plants at 56 DAT did not differ significantly among fungicides; however, they were higher than

controls, which had the lowest mean height values.

Significant differences ( $P \leq 0.05$ ) were observed among treatments for the following variables: leaf area (cm<sup>2</sup>), fresh and dry mass of leaves, stems and roots (Table 1). The highest leaf area values were verified in the Flux + Pyra treatment (10,452.1 and 10,311.5 cm<sup>2</sup> of plant<sup>-1</sup> leaf area, in years of 2015 and 2016, respectively) followed by Me + Pyra treatment (9914.3 and 9889.8 cm<sup>2</sup> of plant<sup>-1</sup> leaf area, in years 2015 and 2016, respectively). In contrast, the lowest mean leaf area values were identified in Inoc-control treatment (3298.3 and 3098.1 cm<sup>2</sup> of plant<sup>-1</sup> leaf area, in years 2015 and 2016, respectively) and Abs-control (6247.8 and 6384.2 cm<sup>2</sup> of plant<sup>-1</sup> leaf area, in years 2015 and 2016, respectively).

Flux + Pyra followed by Me + Pyra treatments showed the highest accumulations of fresh and dry biomass with significant increases, differing from the other treatments ( $P \leq 0.05$ ). However, there was no significant difference among fungicides for the fresh and dry mass accumulation of roots, differing only from controls.



**Fig. 1.** Net CO<sub>2</sub> assimilation rate ( $A_{net}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) and internal CO<sub>2</sub> concentration in the leaf ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ) of hybrid tomato plants Conquistador cv. cultivated in greenhouse, treated with different fungicides with physiological effects and inoculated with *Alternaria solani*, at 34 and 94 days after transplanting (DAT), in years 2015 and 2016. Means ( $n = 5$ ) followed by different letters, in the same evaluation, differ by the Tukey test at 5% probability level ( $P \leq 0.05$ ). Bars indicate standard deviation of the mean.

### 3.2. Photosynthetic efficiency

In gas exchange evaluations performed at 34 and 94 DAT (Figs. 1 and 2), significant differences ( $P \leq 0.05$ ) were observed among treatments, in the two years evaluated, presenting similar behavior. Plants treated with Flux + Pyra obtained the highest CO<sub>2</sub> assimilation rates ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) and internal CO<sub>2</sub> concentration on leaf ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ) (Fig. 1), statistically differing from the other treatments ( $P \leq 0.05$ ).

For the transpiration rate ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ) of plants at 34 and 94 DAT, it was observed that there was no statistical difference among fungicides tested for this characteristic (Fig. 2).

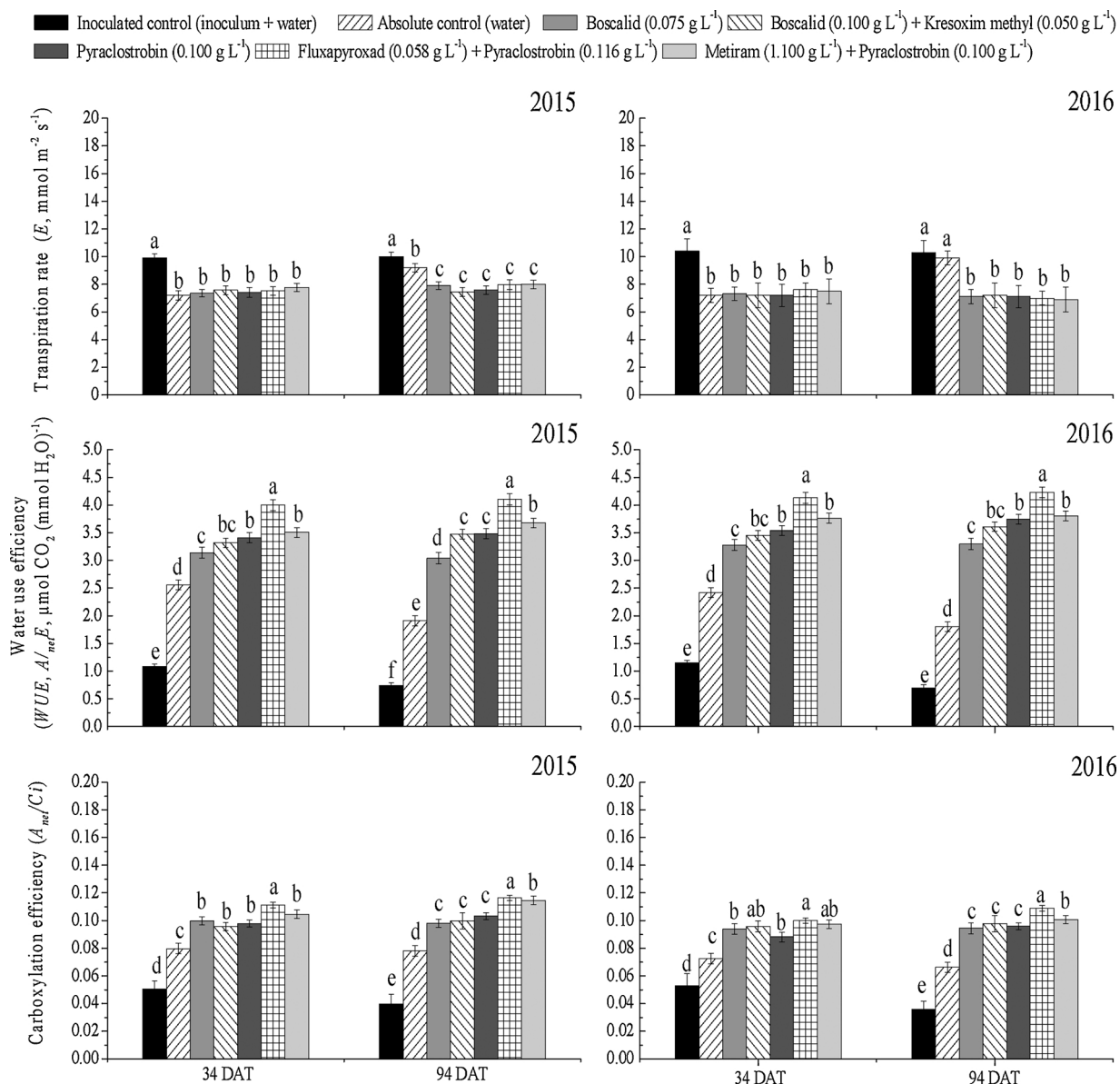
However, the transpiration of plants in these treatments is smaller when compared to controls, significantly differing from them ( $P \leq 0.05$ ). Evaluating water use efficiency ( $WUE$ ,  $A_{net}/E$ ,  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) and carboxylation efficiency ( $A_{net}/C_i$ ) (Fig. 2), Flux + Pyra treatment again stands out and statistically differs ( $P \leq 0.05$ ) from

the other treatments, at 34 and 94 DAT.

Plants treated with Flux + Pyra showed higher  $WUE$  ( $A_{net}/E$ ) at 34 DAT (3.99 and 4.13  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$  in 2015 and 2016, respectively) and at 94 DAT (4.10 and 4.22  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$  in 2015 and 2016, respectively). In contrast, Inoc-control plants had the lowest  $WUE$  ( $A_{net}/E$ ), between 0.70 and 1.14  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ , in the two-year experiment. Abs-control plants also presented lower values for the evaluated gas exchanges characteristics when compared to the different fungicides tested, but superior to Inoc-control.

Fungicide application also showed significant effects ( $P \leq 0.05$ ) on the chlorophyll fluorescence parameters evaluated at 34 and 94 DAT. Variables fluorescence ( $F_v$ ), maximum efficiency of PSII ( $F_v/F_m$ ), photochemical quantum yield of PSII,  $Y(II)$  and relative electron transfer rate ( $ETR$ ) showed similar behavior in the two years evaluated (Fig. 3).

The  $F_v/F_m$  values were significantly different ( $P \leq 0.05$ ) among treatments, but there was no statistical difference for plants treated with the different fungicides, which had higher quantum yield values of



**Fig. 2.** Transpiration rate ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), water use efficiency ( $WUE, A_{net}/E$ ,  $\mu\text{mol CO}_2 (\text{mmol H}_2\text{O})^{-1}$ ) and carboxylation efficiency ( $A_{net}/C_i$ ) of hybrid tomato plants Conquistador cv. cultivated in greenhouse, treated with different fungicides with physiological effects and inoculated with *Alternaria solani*, at 34 and 94 days after transplanting (DAT), in years 2015 and 2016. Means ( $n = 5$ ) followed by different letters, in the same evaluation, differ by the Tukey test at 5% probability level ( $P \leq 0.05$ ). Bars indicate standard deviation of the mean.

PSII, Y (II) at 34 and 94 DAT, with values varying between 0.79 and 0.86.

In Inoc-control plants, lower  $F_v/F_m$  values were observed at 34 and 94 DAT, with a decrease from 0.64 to 0.43, showing a marked decline from one evaluation to another, possibly indicating the presence of damages in the photosynthetic apparatus. In the Abs-control treatment, the  $F_v/F_m$  values at 34 and 94 DAT were 0.73 and 0.62, respectively. In relation to the  $F_v$  parameters and effective quantum yield of PSII, Y(II), a significant difference was observed among treatments (Fig. 3), with Flux + Pyra treatment showing the highest  $F_v$  values at 94 DAT, however, without differing from the other fungicides at 34 DAT.

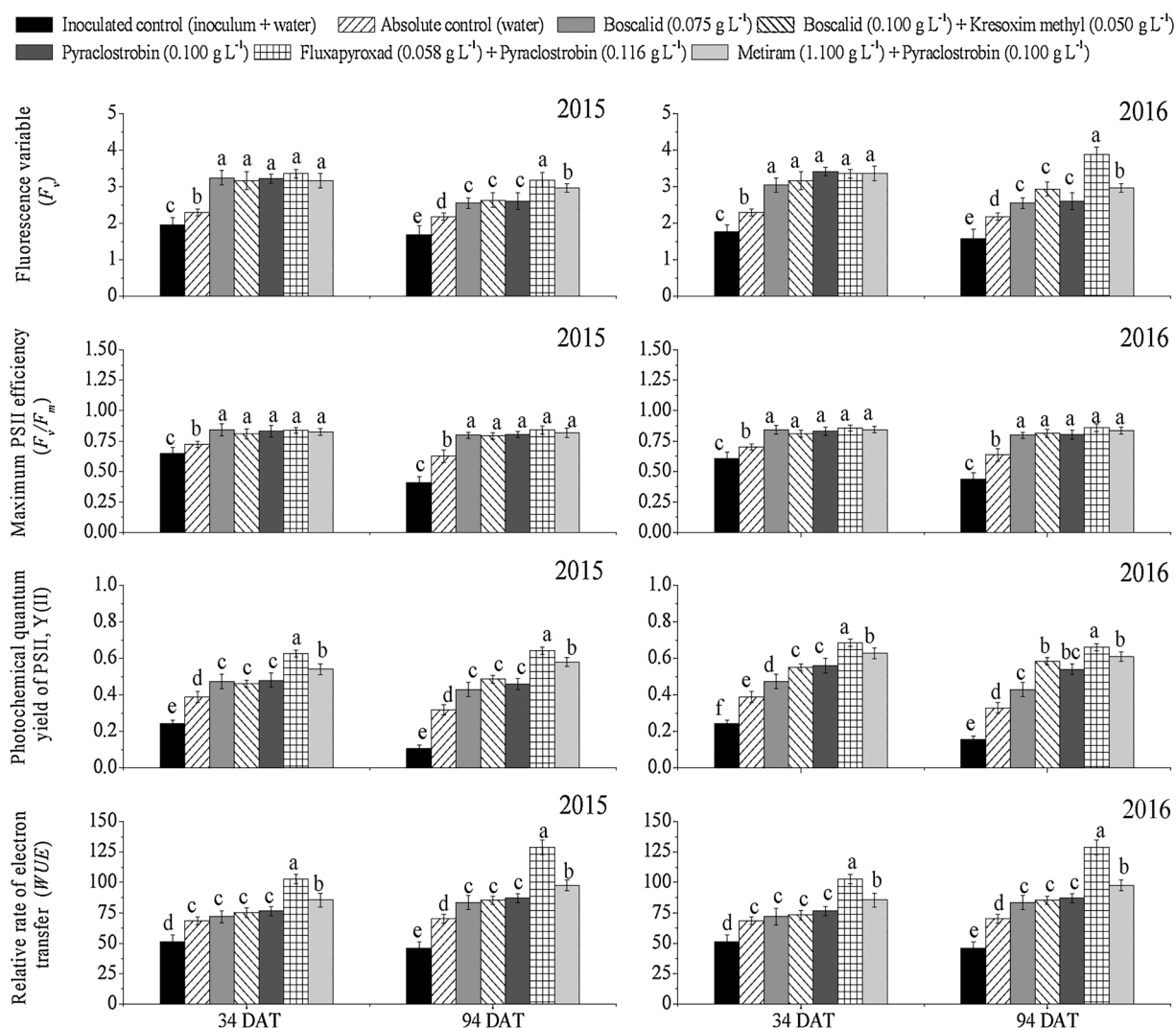
The  $ETR$  results (Fig. 3) were significantly different among treatments ( $P \leq 0.05$ ), being higher in treatments with different fungicides (Flux + Pyra > Me + Pyra > Pyra > Bosc + Kre > Bosc). For all evaluated parameters, the lowest values were observed in Inoc-control plants, followed by Abs-control.

### 3.3. Photosynthetic pigments and activity of the nitrate reductase enzyme

The content of photosynthetic pigments in leaves analyzed at 15, 20, 35, 50, 65, 80, 95 and 110 DAT (Fig. 4) showed a significant difference ( $P \leq 0.05$ ) among treatments in the two years evaluated.

In the first evaluation (15 DAT), before the application of fungicides, there was no significant statistical difference ( $P \leq 0.05$ ) among treatments, however, from the 20 DAT, plants treated with Flux + Pyra presented the highest chlorophyll  $a$  and  $b$  levels ( $\mu\text{g g fresh matter}^{-1}$ ), with an expressive increase with the advancement of evaluations. This behavior is related to the sum of the effects of applications, since evaluations between 20 and 95 DAT were always carried out on the 5th day after application, so at 20, 35, 50, 65, 80 and 95 DAT we have: 5th day after 1st, 2nd, 3rd, 4th, 5th and 6th treatments, respectively.

It was also observed that with the advancement of evaluations, Me + Pyra treatment showed similar effect to Flux + Pyra, not statistically differing in chlorophyll  $a$  and  $b$ , in some evaluations. Again, the lowest photosynthetic pigment contents were observed in Inoc-control



**Fig. 3.** Fluorescence variables chlorophyll *a* ( $F_v$ ), maximum PSII efficiency ( $F_v/F_m$ ), photochemical quantum yield of PSII,  $Y(II)$  and relative electron transfer rate ( $ETR$ ) of hybrid tomato plants Conquistador cv. cultivated in greenhouse, treated with different fungicides with physiological effects and inoculated with *Alternaria solani*, at 34 and 94 days after transplanting (DAT), in years 2015 and 2016. Means ( $n = 5$ ) followed by different letters, in the same evaluation, differ by the Tukey test at 5% probability level ( $P \leq 0.05$ ). Bars indicate standard deviation of the mean.

treatments, followed by Abs-control, in all evaluations performed at 15 DAT.

Regarding the carotenoid contents, higher values were observed for plants treated with Flux + Pyra, followed by Me + Pyra, significantly differing ( $P \leq 0.05$ ) from each other and from other treatments in evaluations performed from 20 to 110 DAT in the two years evaluated.

According to Fig. 5, the physiological effects on the activity of the nitrate reductase enzyme at 15, 20, 35, 50, 65, 80, 95 and 110 DAT were evaluated (same days in which the content of photosynthetic pigments were evaluated). In general, plants treated with the different fungicides showed higher activity of the nitrate reductase enzyme throughout the evaluations, with a significant difference ( $P \leq 0.05$ ) among treatments, in both evaluated years. Plants that received application of Flux + Pyra and Me + Pyra were those that presented greater activity of the nitrate reductase enzyme (NR) in all collections.

However, two peaks of NR enzyme activity occurred in these treatments, the first at 50, 65 and 80 DAT and the second peak at 95 and 110 DAT. In contrast, the lower levels of these pigments and NR enzyme activity were observed for Inoc-control plants, followed by Abs-control treatment in all evaluations.

### 3.4. Carbohydrate synthesis and translocation

Since carbohydrate production is the product of photosynthesis, relationships between source-drain organs are also influenced by photosynthetic efficiency. In this way, the translocation of photoassimilates in tomato plants at 50 and 120 DAT (Table 2) was evaluated and it was observed that the total sugar contents in leaves, stem and fruits, both at 50 and 120 DAT, in all plants analyzed, was in the following order: fruits > stem > leaves.

However, there was a significant difference ( $P \leq 0.05$ ) in the concentration of total sugars among different organs, which was also observed for the content of reducing sugars. However, Flux + Pyra and Me + Pyra treatments presented the highest concentrations of these carbohydrates in leaves, stems and fruits, in both evaluations (50 and 120 DAT), significantly differing ( $P \leq 0.05$ ) from the other treatments, with similar behavior in both evaluated years.

Verifying carbohydrate levels in Bosc, Bosc + Kre and Pyra treatments at 50 and 120 DAT, it was observed that there was no statistical difference between them for the content of total and reducing sugars in the evaluated organs. However, these three treatments significantly differed from the others, presenting mean values of these characteristics higher than those of controls (Inoc-control and Abs-control) and lower

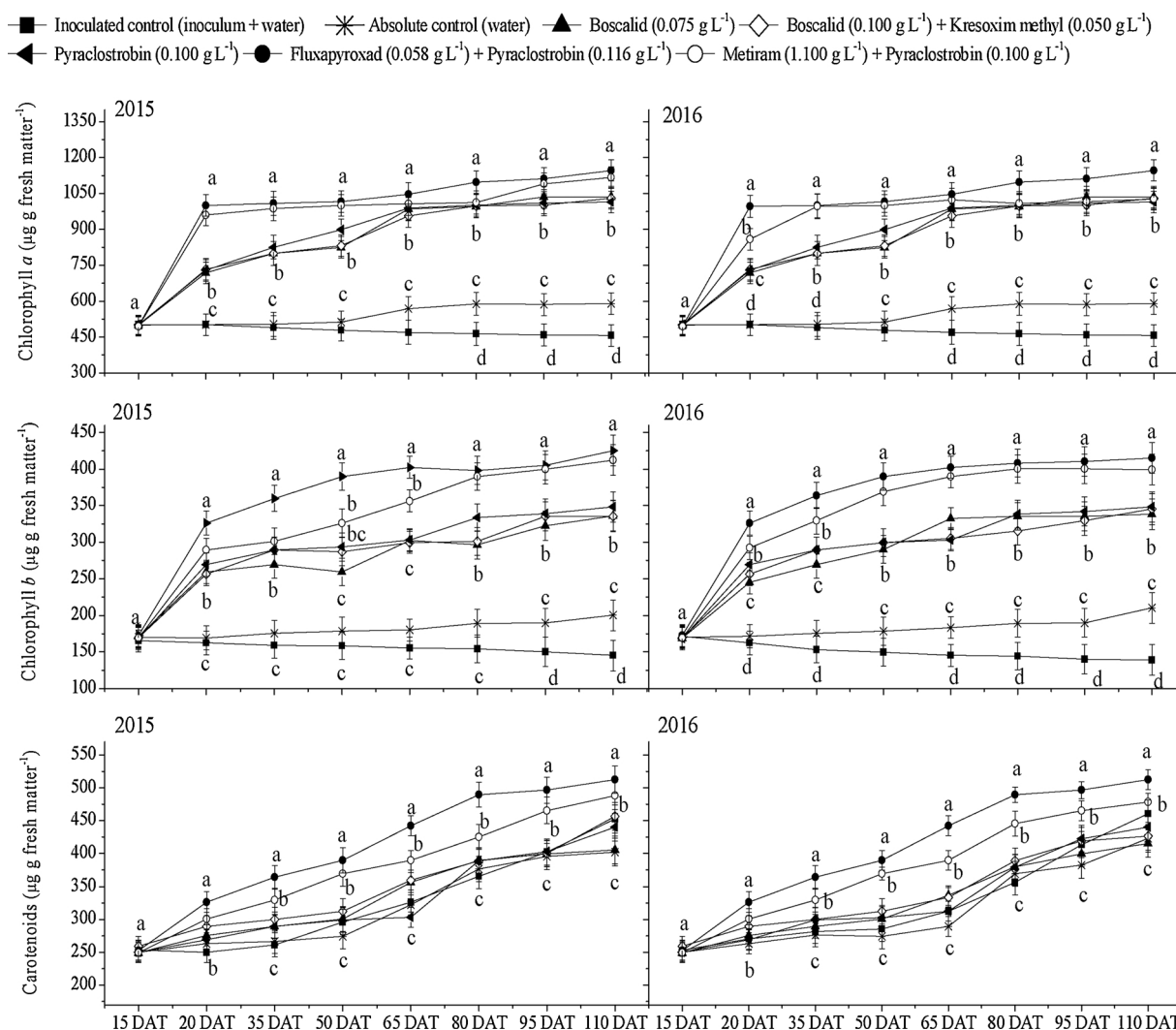


Fig. 4. Chlorophyll a ( $\mu\text{g g fresh matter}^{-1}$ ), chlorophyll b ( $\mu\text{g g fresh matter}^{-1}$ ) and carotenoids ( $\mu\text{g g fresh matter}^{-1}$ ) in hybrid tomato plants Conquistador cv. cultivated in greenhouse, treated with different fungicides of physiological effects and inoculated with *Alternaria solani* at 15, 20, 35, 50, 65, 80, 95 and 110 days after transplanting (DAT) in years 2015 and 2016. Means ( $n = 5$ ) followed by distinct letters in the same evaluation differ by the Tukey test at 5% probability level ( $P \leq 0.05$ ). Bars indicate standard deviation of the mean.

than those observed for Flux + Pyra and Me + Pyra, both at 50 DAT and at 120 DAT.

As for the concentration of reducing sugars, it was observed that the lowest values were found in the leaves of plants treated with Flux + Pyra and Me + yira, significantly differing ( $P \leq 0.05$ ) from the other treatments.

The main drain in tomato plants from flowering are fruits, as observed for Flux + Pyra and Me + Pyra treatments, which presented the highest concentrations of total and reducing sugars at 120 DAT, significantly differing ( $P \leq 0.05$ ) from the other treatments.

### 3.5. Control of *Alternaria solani*

To verify the effect on *A. solani* control, the area under the progression curve of the disease (AUPCD) (Fig. 6) was evaluated in the two years analyzed and it was observed that there was a reduction of the AUPCD for the different fungicides evaluated in relation to the Inoc-control. It can be observed that the Flux + Pyra, Me + Pyra, Bosc, Pyra and Bosc + Kre fungicides reduced the values in 92.88, 89.54, 56.38, 65.16 and 68.20% for AUPCD in the year of 2015, and 90.23, 85.68, 48.84, 59.09 and 63.15% in the year 2016, respectively, in relation to the inoculated control.

However, the Flux + Pyra and Me + Pyra treatments had the lowest AUPCDs, differing significantly ( $p < 0.01$ ) from the other treatments, with similar results in the two years evaluated. The fungicides Bosc, Bosc + Kre and Pyra were also efficient in reducing the severity of the disease, when compared to the Inoc-control.

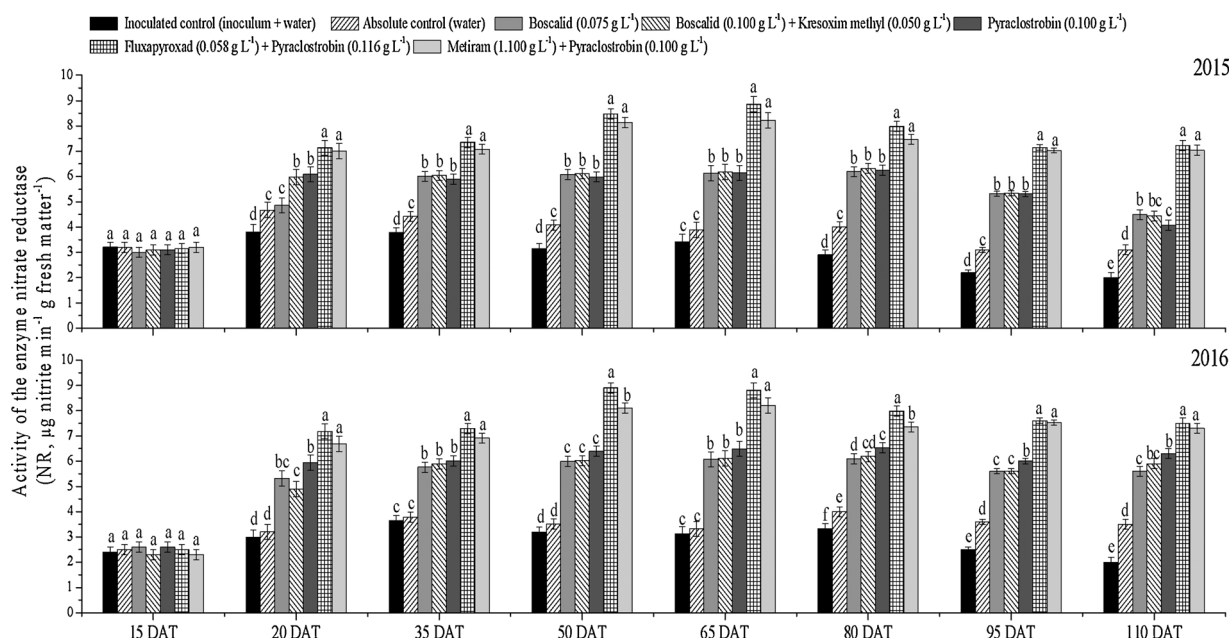
The Abs-control treatment plants, which were not inoculated at the beginning of the experiment, also showed a certain percentage of disease severity in the last evaluations, so they presented AACPD indices of 248 and 329 in the years of 2015 and 2016, respectively. Due to the ideal conditions observed for the development of the disease, high temperatures and relative humidity (data not shown), in addition to the presence of inoculum (diseased plants: Inoc-control).

### 3.6. Production

According to data in Table 3, it can be observed that there was a significant difference ( $P \leq 0.05$ ) among treatments in the production characteristics and fruit yield. The results were similar for both experiments (years 2015 and 2016).

In general, it was observed that the application of fungicides positively influenced the total fruit production ( $\text{kg m}^{-2}$ ), with an increase of 104.2, 98.5, 63.4, 64.7 and 56.2% in relation to Inoc-control, for





**Fig. 5.** Activity of the nitrate reductase enzyme (NR,  $\mu\text{g nitrite min}^{-1} \text{g fresh matter}^{-1}$ ) in hybrid tomato plants Conquistador cv. cultivated in greenhouse, treated with different fungicides with physiological effects and inoculated with *Alternaria solani* at 15, 20, 35, 50, 65, 80, 95 and 110 days after transplanting (DAT), in years 2015 and 2016. Means ( $n = 5$ ) followed by different letters, in the same evaluation, differ by the Tukey test at 5% probability level ( $P \leq 0.05$ ). Bars indicate standard deviation of the mean.

Flux + Pyra, Me + Pyra, Pyra, Bosc + Kre and Bosc, respectively, in year 2015 and 124.8, 115.4, 75.6, 73.3 and 72.0% in year 2016.

An increase of 76.4, 71.5, 41.2, 42.3 and 35.0% was also observed in relation to Abs-control for Flux + Pyra, Me + Pyra, Pyra, Bosc + Kre and Bosc treatments, respectively, in year 2015 and 92.6, 84.5, 50.5, 48.5 and 47.4% in year 2016. The largest increases in production observed in 2016, compared to 2015, are due to the fact that controls presented lower production in 2016, due to the greater severity of the disease (data not shown).

Treatment with the highest fruit yield was Flux + Pyra (12.2 and 11.7  $\text{kg m}^{-2}$  in 2015 and 2016, respectively) followed by Me + Pyra treatment (11.9 and 11.27  $\text{kg m}^{-2}$  in 2015 and 2016, respectively), not significantly differing from each other. These treatments also presented the highest number of total fruits  $\text{m}^{-2}$ , with higher fresh fruit mass ( $\text{g fruit}^{-1}$ ) and, consequently, lower percentage of non-commercial fruits.

#### 4. Discussion

All plants at the beginning of development (at 14 DAT) had the same developmental pattern, however, during treatment applications, it was generally observed that plants treated with strobilurin and carboxamides presented greater initial development and biomass accumulation when compared to controls. Several studies have reported the physiological effects of these substances on maize (Lima et al., 2009), soybean (Tsumanuma et al., 2010), common bean (Jadoski et al., 2015), carrot (Colombari et al., 2015), sunflower (Tsiatas et al., 2017) and Japanese cucumber (Amaro et al., 2018), where positive effects on biomass accumulation and productivity were observed. In *Arabidopsis thaliana* Van Dingenen et al. (2017) observed that the application of strobilurins promoted greater plant growth, confirming the positive effects of these substances.

The magnitude of the effects was different for each of the five fungicides tested, with Flux + Pyra treatment, followed by Me + Pyra being those that provided greater leaf development, allowing these plants greater photosynthetic capacity (Kluge et al., 2014). In addition to better development and productivity gains, since 90% of the dry mass of the plant is formed by photoassimilates that have been converted into biomass (Popov et al., 2003).

Different results were observed in Inoc-control and Abs-control plants, since development evaluations were performed at 120 DAT (after the last fruit harvest), at the end of the crop cycle, Test-Inoc plants were more severely affected (*A. solani* inoculated at 16 DAT). Due to its high aggressiveness, the incidence of *A. solani* reduces plant vigor and development, as it causes damage to the photosynthetically active area and defoliation in more advanced stages of the disease (Töfoli et al., 2014).

This explains, in part, the smaller leaf area observed in Inoc-control plants, because Abs-control plants, which were not inoculated at the beginning of experiments, also presented lower leaf development, being significantly different from the other treatments. These results indicate that these fungicides, in addition to controlling the disease efficiently, promote a positive effect on leaf expansion, as also observed by Fagan et al. (2010) in soybean.

All fungicides applied increased the photosynthetic efficiency at 34 and 94 DAT in relation to controls, however, Flux + Pyra presented the best effects on photosynthetic efficiency in tomato plants. The results of other studies demonstrate an increase in the  $\text{CO}_2$  assimilation rate and elevation in the net photosynthetic rate after application of strobilurins, such as pyraclostrobin and kresoxim-methyl, and carboxamides, such as boscalid, in tomato plants (Ramos et al., 2015), Japanese cucumber (Amaro et al., 2018) and soybean (Fagan et al., 2010).

The photosynthetic parameters of wheat plants were higher with the application of strobilurins (Nason et al., 2007) however, these authors observed greater stomatal conductance together with higher transpiration rate, unlike results observed in tomato plants. Plants treated with Flux + Pyra presented lower  $E$  value and, consequently, higher  $WUE$  ( $A_{net}/E$ ) in both evaluations, indicating greater capacity to assimilate  $\text{CO}_2$  with low water loss, generating greater water savings and higher synthesis of photoassimilates.

Similar effects were observed in tomato plants with application of pyraclostrobin plus boscalid (Ramos et al., 2015) and azoxystrobin (Giuliani et al., 2010). In beans, the application of pyraclostrobin improved the maintenance of  $g_s$  in plants under water stress, which is advantageous (Jadoski et al., 2015). These authors point out that the maintenance of  $g_s$  in the increase of water tension depends on the dynamic balance among the three carboxylation factors:  $A_{net}/C_i$ ,  $E$  and

**Table 2**

Mean of total sugars, sugars, reducing sugars ( $\mu\text{g g fresh matter}^{-1}$ ) in leaves, stem and fruits at 50 and 120 days after transplanting (DAT) of hybrid tomato plants Conquistador cv. cultivated in greenhouse and treated with different fungicides of physiological effects and inoculated with *Alternaria solani*, in the years 2015 and 2016.

2015												
Treatments	Concentration of carbohydrates – 50 DAT						Concentration of carbohydrates – 120 DAT					
	Total Sugars ( $\mu\text{g g fresh matter}^{-1}$ )			Reducing sugars ( $\mu\text{g g fresh matter}^{-1}$ )			Total Sugars ( $\mu\text{g g fresh matter}^{-1}$ )			Reducing sugars ( $\mu\text{g g fresh matter}^{-1}$ )		
	Leaves	Stem	Fruits	Leaves	Stem	Fruits	Leaves	Stem	Fruits	Leaves	Stem	Fruits
Inoculated control (inoculum + water)	85.2cC	110.6cB	298.3cA	48.6aC	78.9aB	65.9cA	63.2cC	102.3cB	302.4cA	28.4aC	50.2cB	72.6cA
Absolute control (water)	82.1cC	113.2cB	299.3cA	50.2aC	81.8aB	67.2cA	60.7cC	99.8cB	307.2cA	29.6aC	52.6cB	74.9cA
Boscalid (0.075 g L <sup>-1</sup> )	92.4bC	136.7bB	336.5bA	39.3bC	61.9bB	105.6bA	80.8bC	126.6bB	382.1bA	21.2bC	42.7bB	125.6bA
Boscalid (0.100 g L <sup>-1</sup> ) + Kresoxim methyl (0.050 g L <sup>-1</sup> )	94.9bC	134.6bB	325.8bA	40.2bC	63.5bB	102.8bA	80.2bC	131.6bB	376.9bA	20.1bC	43.5bB	118.3bA
Pyraclostrobin (0.100 g L <sup>-1</sup> )	95.6bC	138.5bB	330.5bA	39.8bC	60.5bB	99.3bA	79.3bC	130.6bB	399.7bA	21.8bC	39.8bB	120.7bA
Fluxapyroxad (0.058 g L <sup>-1</sup> ) + Pyraclostrobin (0.116 g L <sup>-1</sup> )	126.4aC	154.6aB	385.9aA	28.1cC	49.6cB	123.5aA	99.6aC	152.3aB	435.6aA	13.3cC	22.3aB	166.0aA
Metiram (1.100 g L <sup>-1</sup> ) + Pyraclostrobin (0.100 g L <sup>-1</sup> )	124.1aC	149.7aB	372.1aA	27.7cC	52.9cB	120.9aA	98.4aC	150.6aB	429.7aA	15.6cC	22.7aB	162.2aA
CV (%)	8.6	9.2	7.8	5.9	8.7	8.5	10.7	9.3	9.2	8.7	9.1	7.6

2016												
Treatments	Concentration of carbohydrates – 50 DAT						Concentration of carbohydrates – 120 DAT					
	Total Sugars ( $\mu\text{g g fresh matter}^{-1}$ )		Reducing sugars ( $\mu\text{g g fresh matter}^{-1}$ )		Fruits	Total Sugars ( $\mu\text{g g fresh matter}^{-1}$ )		Reducing sugars ( $\mu\text{g g fresh matter}^{-1}$ )		Fruits	Total Sugars ( $\mu\text{g g fresh matter}^{-1}$ )	
	Leaves	Stem	Leaves	Stem		Leaves	Stem	Leaves	Stem		Leaves	Stem
Inoculated control (inoculum + water)	89.5cC	121.9cB	299.9cA	45.5aC	84.6aB	60.3cA	68.0cC	99.3cB	322.9cA	31.9aC	56.2cB	78.1cA
Absolute control (water)	86.3cC	124.2cB	303.1cA	48.2aC	85.7aB	62.4cA	66.8cC	103.6cB	318.9cA	32.6aC	55.0cB	79.4cA
Boscalid (0.075 g L <sup>-1</sup> )	96.2bC	143.1bB	342.5bA	37.3bC	66.2bB	95.3bA	80.2bC	126.6bB	384.1bA	25.1bC	40.7bB	123.7bA
Boscalid (0.100 g L <sup>-1</sup> ) + Kresoxim methyl (0.050 g L <sup>-1</sup> )	98.8bC	147.8bB	338.3bA	36.4bC	65.6bB	96.8bA	78.4bC	123.2bB	385.9bA	23.9bC	44.6bB	126.9bA
Pyraclostrobin (0.100 g L <sup>-1</sup> )	99.1bC	146.4bB	337.4bA	37.9bC	68.2bB	93.4bA	79.9bC	124.0bB	387.2bA	26.5bC	41.2bB	125.7bA
Fluxapyroxad (0.058 g L <sup>-1</sup> ) + Pyraclostrobin (0.116 g L <sup>-1</sup> )	129.1aC	160.2aB	388.9aA	25.5cC	55.6cB	121.3aA	89.7aC	139.6aB	447.2aA	14.4cC	27.4aB	158.1aA
Metiram (1.100 g L <sup>-1</sup> ) + Pyraclostrobin (0.100 g L <sup>-1</sup> )	126.9aC	159.9aB	389.0aA	24.9cC	54.8cB	118.9aA	91.9aC	141.3aB	446.1aA	11.9cC	28.6aB	162.0aA
CV (%)	9.6	8.7	7.2	6.4	7.4	8.4	9.4	8.7	10.4	9.7	8.4	6.8

Mean (n = 5) followed by different letters, lowercase in column and upper case for each characteristic in the row, significantly differ by the Tukey test at 5% probability (P ≤ 0.05). 50 DAT = beginning of fruiting; 120 DAT = end of harvest.

**WUE.**

The higher photosynthetic efficiency observed in plants treated with Flux + Pira is related to the higher  $g_s$ , which allowed greater CO<sub>2</sub> diffusion to the substomatic chamber increasing  $A_{net}$  and, consequently, the amount of photoassimilates produced, allowing greater plant development. The higher the  $A_{net}$  value, the greater the photosynthetic gain, as the increase in liquid photosynthesis is a result of the increase of gross photosynthesis and reduction of respiration (Kluge et al., 2014).

In addition to this factor, it is noteworthy that both strobilurins and carboxamides act in the complex III (QoI) and complex II (SDHI) of the fungus mitochondrial respiration, respectively, irreversibly reducing the synthesis of NADH and ATP, blocking energy production (Kanungo and Joshi, 2014). Therefore, smaller AUPCDs, were observed in the plants treated with these fungicides, in contrast to the Inoc-control indicating that these molecules present efficient control during the germination of *A. solani* conidium due to their effect on the electron transport chain in the mitochondria, affecting the respiration of the fungus.

Some studies have indicated that these molecules act reversibly in the plant, causing transient blockade of electron transport in chloroplasts and mitochondria, limiting the production of ATP, which could lead to lower  $g_s$  and reduction of  $F_v/F_m$  values (Nason et al., 2007).

However, the opposite was observed in this experiment, and a possible explanation could be that there was a temporary reduction of cellular respiration due to the limited ATP production, in addition to the increase in the CO<sub>2</sub> compensation point, which resulted in an increase in liquid photosynthesis, as already reported by Grossmann and Retzlaff (1997).

Plants development depends on the processes of cell division and expansion, which are sensitive to the concentration of assimilated CO<sub>2</sub> (Tsutsumi et al., 2014). This justifies the lower development of Inoc-control and Abs-control plants, because the reduction of the stomatal opening restricted the CO<sub>2</sub> fixation rate, with consequent reduction of its concentration in the substomatic cavities and in the intercellular spaces ( $C_i$ ), reducing  $A_{net}$ , directly reflecting on the net photosynthesis of plants (Xu et al., 2015). The progress of the disease causes falls in photosynthetic efficiency, as it is a stressful factor. The plant reflects

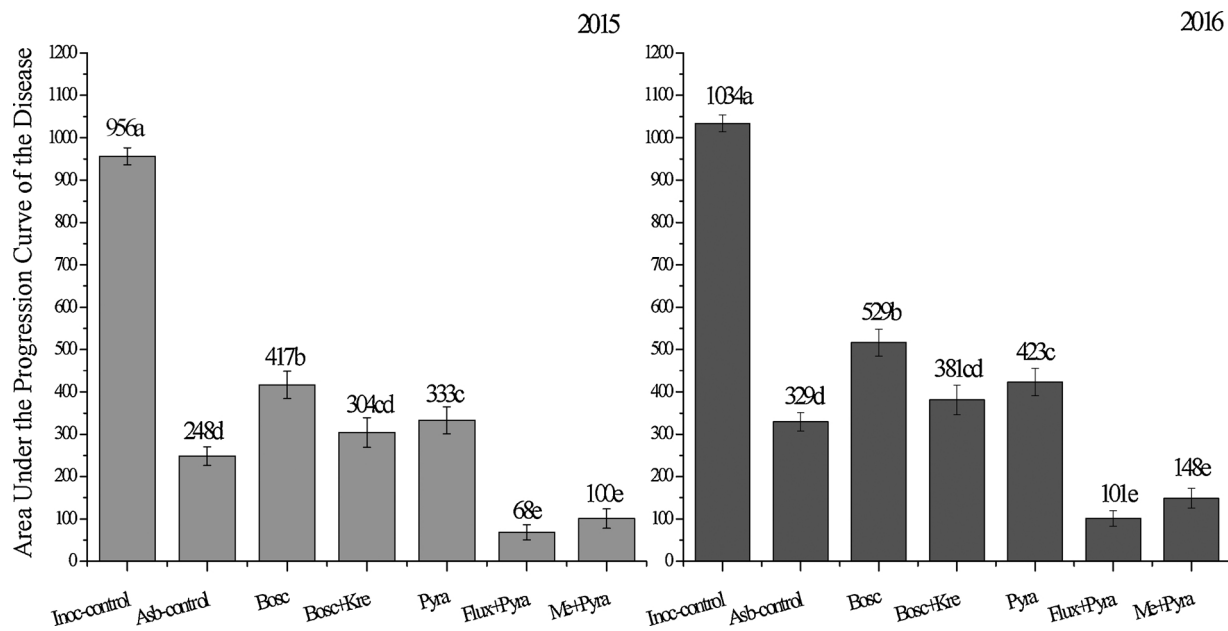


Fig. 6. Effect of different treatments with fungicides in the area under the progression curve of the disease (AUPCD), tested on the control of *Alternaria solani* in plants of hybrid tomato Conquistador, grown in greenhouse, in the years 2015 and 2016. Treatments: inoculated control (Inoc-control), absolute control (Abs-control), boscalid (Bosc), boscalid with kresoxim methyl (Bosc + Kre), pyraclostrobin (Pyra), fluxapyroxad with pyraclostrobin (Flux + Pyra) and metiram with pyraclostrobin (Me + Pyra). Means (n = 10) followed by distinct letters differ by the Tukey test at the 1% probability level (p < 0.01). Bars indicate and standard deviation of the mean.

Table 3

Total production (TP, kg m<sup>-2</sup>), number of fruits (NF, fruits m<sup>-2</sup>), fresh fruit mass (FWF, g fruit<sup>-1</sup>) and percentage of non-commercial fruits (NCF, %) of hybrid tomato plants Conquistador cv. cultivated in greenhouse and treated with different fungicides of physiological effects and inoculated with *Alternaria solani*, in the years 2015 and 2016.

2015				
Treatments	TP (kg m <sup>-2</sup> )	NF (fruits m <sup>-2</sup> )	FWF (g fruit <sup>-1</sup> )	NCF <sup>1</sup> (%)*
Inoculated control (inoculum + water)	5.98d	52.00d	115.00d	30.20d
Absolute control (water)	6.92c	56.00c	123.57c	8.95c
Boscalid (0.075 g L <sup>-1</sup> )	9.34b	61.00b	153.11b	4.62b
Boscalid (0.100 g L <sup>-1</sup> )	9.85b	64.00b	153.91b	5.21b
+ Kresoxim methyl (0.050 g L <sup>-1</sup> )				
Pyraclostrobin (0.100 g L <sup>-1</sup> )	9.77b	63.00b	155.08b	4.99b
Fluxapyroxad (0.058 g L <sup>-1</sup> )	12.21a	67.00a	182.24a	2.84a
+ Pyraclostrobin (0.116 g L <sup>-1</sup> )				
Metiram (1.100 g L <sup>-1</sup> )	11.87a	66.00a	179.85a	2.56a
+ Pyraclostrobin (0.100 g L <sup>-1</sup> )				
CV (%)	16.54	14.23	10.78	9.56
2016				
Treatmentscheme	TP (kg m <sup>-2</sup> )	NF (fruits m <sup>-2</sup> )	FWF (g fruit <sup>-1</sup> )	NCF (%)
Inoculated control (inoculum + water)	5.21d	48.00d	108.54d	27.08d
Absolute control (water)	6.08c	52.00c	116.83c	9.61c
Boscalid (0.075 g L <sup>-1</sup> )	8.96b	61.00b	146.85b	4.91b
Boscalid (0.100 g L <sup>-1</sup> )	9.03b	62.00b	145.56b	6.45b
+ Kresoxim methyl (0.050 g L <sup>-1</sup> )				
Pyraclostrobin (0.100 g L <sup>-1</sup> )	9.15b	60.00b	152.53b	5.00b
Fluxapyroxad (0.058 g L <sup>-1</sup> )	11.71a	65.00a	180.18a	3.07a
+ Pyraclostrobin (0.116 g L <sup>-1</sup> )				
Metiram (1.100 g L <sup>-1</sup> )	11.22a	64.00a	175.36a	1.56a
+ Pyraclostrobin (0.100 g L <sup>-1</sup> )				
CV (%)	14.23	9.56	12.6	10.4

<sup>1</sup>NCF = discard of fruits smaller than 40 mm in diameter and with defects (rot, deformed, physiological disorders etc.). \* Data transformed into arc sine of √x (%). Means (n = 5) followed by the same letter in the columns do not significantly differ from each other by the Tukey test at 5% probability (P ≤ 0.05).

this damage by the concentration of pigments or by the higher fluorescence emission of chlorophyll *a* (Brestic and Zirvcak, 2013).

The fact that gas exchange measurements and chlorophyll *a* fluorescence analyses were carried out simultaneously helped understanding the severity of the damage caused by the presence of pathogen *A. solani*. Studies have indicated that any change in photosynthetic rate or heat dissipation will reflect in higher fluorescence emission (Rousseau et al., 2013), allowing identifying the sanity of the photosynthetic apparatus of the plant at the moment of evaluation. Changes in the emission kinetics of chlorophyll *a* fluorescence can detect and infer the presence of damage in the photosynthetic apparatus due to damage caused by stressors (Yusuf et al., 2010).

The  $F_v/F_m$  values observed in plants treated with the different fungicides tested showed that they were healthy, being in agreement with studies carried out by Björkman and Demming (1987), who evaluated different  $C_3$  cycle species and observed typical  $F_v/F_m$  values in healthy leaves and, under adequate lighting conditions, around 0.83. Already values below this standard indicate that the plant presents some type of stress, causing damages to its photosynthetic apparatus, as observed in Inoc-control and Abs-control plants.

Stress conditions are detected by the decrease in the  $F_v/F_m$  ratio (Baker, 2008; Rousseau et al., 2013), reflecting lower photosynthetic capacity and photochemical efficiency (Casierra-Posada et al., 2011). Species of the genus *Alternaria* produce proliferative toxins, which facilitate their *necrotrophic* life, causing damage to plant tissues (Lawrence et al., 2008). Therefore, in addition to the decrease in the  $F_v/F_m$  ratio, the presence of the pathogen *A. solani* also caused the imbalance between PSII and *PSI* photosystems, as can be observed by the lower *ETR* values in Inoc-control plants. This could trigger the pseudo-cyclic transport of electrons (Mehler's reaction) to form superoxide anion ( $\cdot O_2^-$ ) and other reactive oxygen species (ROS), such as  $\cdot O_2$  (Karuppanapandian et al., 2011), intensifying damage to the photosynthetic apparatus.

Plants submitted to the application of strobilurin and carboxamides presented higher  $F_v$  values, effective quantum yield of PSII, *Y(II)* and *ETR* when compared to controls, with emphasis on Flux + Pyra treatment, indicating greater capacity to assimilate  $CO_2$ , since this parameter indicates the transference capacity of PSII and electron between PSII and *PSI* for the generation of  $NADPH_2$  and ATP. Culminating in higher photosynthetic efficiency due to high *ETR* values (Baker, 2008), as verified in the gas exchange parameters at 34 DAT, in the vegetative phase, as at 94 DAT in the reproductive phase.

The concentration of photosynthetic pigments was higher in plants treated with strobilurin and carboxamides, indicating that the increase in photosynthetic efficiency observed in these treatments, has a great relation with the amount of chlorophyll *a* and *b*, and carotenoids present in *PSI* and PSII (Casierra-Posada and Peña-Olmos, 2015). Resulting in greater absorption of sunlight and, consequently, greater *ETR* values (Rodrigues et al., 2016), as observed for Flux + Pyra, followed by Me + Pyra treatments. Increases in the content of photosynthetic pigments were also reported in tomato with the application of pyraclostrobin plus boscalid (Ramos et al., 2015) and coffee with application of pyraclostrobin (Honorato Júnior et al., 2015), reflecting higher photosynthetic efficiency of plants, in addition to the increase of the repair and protection of the photosynthetic apparatus by carotenoids (Nisar et al., 2015).

The highest content of photosynthetic pigments reflected in the efficiency of the photochemical metabolism, being strongly influenced by the activity of the NR enzyme, which is the key enzyme in the regulation of nitrogen metabolism (Viana and Kiehl, 2010), main constituent of the chlorophyll molecule (Kluge et al., 2014). In fact, the activity of the NR enzyme was influenced by the application of strobilurin and carboxamides, especially Flux + Pyra and Me + Pyra treatments, with enzyme expression peaks occurring at 50, 65 and 80 DAT (transition between vegetative phase and early reproductive period) and at 95 and 110 DAT (fruit growth and maturation phase).

The effect of NR enzyme activity during the plant life cycle was observed in soybean (Soares et al., 2011), corn (Barbosa et al., 2011) and Japanese cucumber plants (Amaro et al., 2018), related to the phases of higher demand for nitrogen by the plant. Some studies have demonstrated that there is an increase in biomass and production, even in plants that are not affected by fungi, so they are interesting agricultural practices in the agricultural scenario in the management of nitrogen fertilization (Kanungo and Joshi, 2014).

It was observed that at 50 and 120 DAT, regardless of treatment, all plants presented effective translocation of carbohydrates to fruits. However, the highest concentrations of total sugars and reducing sugars were observed in fungicide-treated plants, especially Flux + Pyra and Me + Pyra treatments. It is evident that productivity increases in tomato plants treated with such fungicides due to the better physiological performance that allowed greater synthesis and translocation of carbohydrates to fruits. Evaluating the application of pyraclostrobin and boscalid, isolated and combined with other chemicals in tomato plants, Ramos et al. (2015) also observed that in all treatments, the concentrations of total and reducing sugars were higher than in control plants. In addition to verifying that the fruit was statistically superior to leaves and stem, evidencing that these treatments are effective regarding the greater translocation of these sugars, promoting greater tissue development, and increasing tissue drainage capacity.

Studies have shown that strobilurins, such as pyraclostrobin, alter the hormonal balance of the plant, promoting the synthesis of auxins and abscisic acid (ABA) and reducing the ethylene production (Kanungo and Joshi, 2014). A possible hypothesis in this study may be related to the improvement in the hormonal balance, influencing the regulation of source-drain relations, promoting greater drainage tissue growth and, thus, influencing the translocation of photoassimilates to these organs (Albacete et al., 2014). Another possible effect of these substances on transport and accumulation of sugars was identified by Van Dingenen et al. (2017) in *A. thaliana* plants treated with kresoxim-methyl (strobilurin). The authors observed that the transcribed genes involved in the transport of sugars were differentially expressed in plants treated with strobilurins, although no difference in sucrose content was observed. However, carbohydrates translocated at long distances in the phloem are non-reducing sugars, because they are less reactive (Dinant and Lemoine, 2010). Sucrose is the sugar predominantly translocated via phloem, but other mobile sugars as raffinose and stachyose are also present, in addition to sugar alcohols such as sorbitol and mannitol (Van Bel and Hess, 2008).

At the end of the crop cycle (120 DAT) Flux + Pyra and Me + Pyra treatments presented the highest concentrations of total sugars in all evaluated organs. As a result, these plants presented higher fruit yield and, concurrently, more commercial fruits. These results show that the application of strobilurins and carboxamides increased tomato productivity and are consistent with Giuliani et al. (2010) and Cantore et al. (2016), who reported that the application of azoxystrobin and pyraclostrobin, respectively, in tomato plants promoted higher marketable fruit yield. Previous studies have also demonstrated productivity increases by these fungicides in other crops such as grapevine (Diaz-Espejo et al., 2012), soy (Joshi et al., 2014), beans (Jadoski et al., 2015), corn (Shetley et al., 2015), carrot (Colombari et al., 2015) and Japanese cucumber (Amaro et al., 2018). Such productive gains are associated with side effects on plant physiology and improved disease control efficacy (Diaz-Espejo et al., 2012). The application of fungicides that promote physiological effects can be considered as part of managements that maximize the photosynthetic process and provide greater amount of energy to increase plant productivity.

## 5. Conclusion

Based on the results obtained and the experimental conditions, it could be concluded that the applications of boscalid, boscalid with kresoxim methyl, pyraclostrobin, fluxapyroxad with pyraclostrobin and

metiram with pyraclostrobin provided positive effects on the photosynthetic efficiency and yield of tomato plants, besides efficient control of *A. solani*. The highest fruit development and yield was observed in plants treated with fluxapyroxad with pyraclostrobin, followed by metiram with pyraclostrobin, which showed high photochemical yield due to the higher levels of photosynthetic pigments, high activity of the nitrate reductase enzyme and increased synthesis and translocation of photoassimilates.

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