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Review

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

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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^{-1}), boscalid (0.100 g L^{-1}) + kresoxim-methyl (0.050 g L^{-1}), pyraclostrobin (0.100 g L^{-1}), fluxapyroxad (0.058 g L^{-1}) + pyraclostrobin (0.116 g L^{-1}) and methyl (1.100 g L^{-1}) + pyraclostrobin (0.100 g L^{-1}), 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

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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_2 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_2 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^3 pots filled with typical Dusky Dystroferric 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^{-1}); boscalid (0.100 g L^{-1}) + kresoxim-methyl (0.050 g L^{-1}); pyraclostrobin (0.100 g L^{-1}); fluxapyroxad (0.058 g L^{-1}) + pyraclostrobin (0.116 g L^{-1}) and metiram (1.100 g L^{-1}) + pyraclostrobin (0.100 g L^{-1}).

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_2 manual sprayer (0.3 kgf/cm^2) 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 1×10^4 conidia mL^{-1}.

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 1×10^4 conidia mL^{-1}, 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 maintaine.

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^{-1} 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^2).

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_2 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_2 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_2 concentrations that were released (transpiration - water vapor) and assimilated (CO_2 assimilation) through the stomata.

The characteristics of gas exchanges analyzed were: net CO_2 assimilation rate (A_{net}, μmol CO_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 leaves (*Ci*, μmol CO_2 mol^{-1} air). These