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Research Paper

Effects of the fungicides azoxystrobin, pyraclostrobin and boscalid on the physiology of Japanese cucumber

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ABSTRACT

Strobilurins are fungicides with beneficial physiological effects on crop yield due to promotion of net carbon assimilation, nitrate reductase enzyme activity, stress tolerance and hormonal balance. The carboxamides complement the action of the strobilurins when applied alternately or together. This study aimed to evaluate the effect of application of pyraclostrobin, azoxystrobin and boscalid on grafted and ungrafted Japanese cucumber (*Cucumis sativus* L.) in order to analyze the effects of these fungicides on plant physiology and increased fruit production. The experimental design was completely randomized in a 2 × 5 factorial scheme, with grafted and ungrafted cucumber plants and 5 fungicide treatments: control; azoxystrobin 60 g a.i ha^{-1}; boscalid 50 g a.i ha^{-1}; pyraclostrobin 50 g a.i ha^{-1}; and boscalid 100 g a.i ha^{-1} + pyraclostrobin 50 g a.i ha^{-1}, applied 18 days after transplanting and then at intervals of seven days. The effect of the treatments was evaluated based on the average yield and fruit mass, in addition to observations of gas exchange, lipid peroxidation, and the activities of peroxidase, superoxide dismutase, catalase and nitrate reductase. Based on the results obtained, it was concluded that the fungicide treatments resulted in different responses between the grafted and ungrafted plants. The physiological benefits were more evident in the grafted plants treated with pyraclostrobin or boscalid alone, and these benefits manifested in terms of fruit production and increased the activity of the antioxidative system, thereby reducing stress. The higher productivity probably occurred due to the better physiological performance of these plants, mainly at the beginning of development, presenting greater activity of the enzyme nitrate reductase, in addition to the higher net CO_2 assimilation and carboxylation efficiency.

1. Introduction

Until recently, disease control was the only purpose of fungicide use; however, the physiological benefits of strobilurins opened a new concept for the use of these products (Venancio et al., 2003). Due to the large capacity of the plant to absorb them, these fungicides have positive physiological effects on the yields of crops, causing alterations in metabolism and growth (Koehle et al., 2002). This effect was observed even without any alterations caused by pathogenic fungi; the plants treated with these substances showed greater vigor and higher yield than untreated plants. The carboxamides have also been included in this group of fungicides with positive physiological effects, but because they have spent less time on the market, there is little information or experimental results regarding this family.

The physiological effect of strobilurins results from the net photosynthesis increase due to the temporary reduction of plant respiration, which causes less carbon loss, thereby generating more energy for the plant. In addition, strobilurins increase the activity of nitrate reductase and antioxidant enzymes, which leads to greater stress tolerance; additionally, increased synthesis of indoleacetic acid (IAA), isopentenyl adenine (I6-ADE) and abscisic acid (ABA) and reduced ethylene production leads to a better hormonal balance, delayed senescence and prolonged photosynthetic activity, the so-called "green effect" (Bartlett et al., 2002; Ypema and Gold, 1999; Zhang et al., 2010).

Most of the results in the literature have been obtained in experiments on field crops, such as soybean (Fagan et al., 2010; Joshi et al., 2014; Soares et al., 2011), corn (Barbosa et al., 2011; Blandino et al., 2012), wheat (Grossmann and Retzlaff, 1997; Inagaki et al., 2009;

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Ishikawa et al., 2012; Koehle et al., 2002; Ypema and Gold, 1999), bean (Jadoski et al., 2015; Kozlowski et al., 2009) and barley (Jabs et al., 2002), and there are few reports on the physiological effects of these fungicides on vegetables, although they are also used preventively in this group, mainly in the Solanaceae and Cucurbitaceae families. Studies on the physiological changes in the plant are scarce, there is much controversy on this subject, and there few data on the effects on disease-free plants.

The Japanese cucumber (*Cucumis sativus* L.) is among the crops that are most widely cultivated in a protected environment. The use of this technique allows year-round production of fruits with excellent commercial quality, reducing losses and increasing productivity (Mohammadi and Omid, 2010). However, this practice causes problems such as increased incidence of diseases and nematodes, requiring the use of grafting in resistant plants for preventive purposes to control diseases and improve the absorption of nutrients (Lee and Oda, 2010).

Agricultural practices aim to increase productivity and the quality of the final product, so it is increasingly important to understand the physiological changes that occur after the application of these important groups of fungicides. The aim of this study was to evaluate the effect of the application of pyraclostrobin, azoxystrobin and boscalid on grafted and ungrafted Japanese cucumber plants in order to analyze the effects of these fungicides on plant physiology, as well as on the increase of fruit production.

2. Material and methods

2.1. Plant material and application of fungicides

The experimental area was located at the Faculdade de Ciências Agronômicas (FCA), Universidade Estadual Paulista (UNESP), located in São Manuel city, São Paulo state, Brazil. The study site is located at 22° 44′ S latitude, 47° 34′ W longitude, and an altitude of 750 m. The climate is humid subtropical mesothermic with drought conditions in the winter season. We used an arc-type greenhouse with the following characteristics: 30 m length, 7 m width and 3 m height, covered with low-density polyethylene film (150 μm) with the lateral sides covered with a 75% shade cloth.

The experiment was conducted with grafted and ungrafted Japanese cucumber plants (*Cucumis sativus* L.). The Japanese cucumber hybrid 'Taisho' (scion) was grafted onto the pumpkin hybrid 'Excitte Ikki' (root-stock) using the tongue approach grafting method. To ensure that both hypocotyl diameters were similar, allowing proper grafting, the pumpkins were sown 4 days before the cucumbers. The grafting was performed 10 days after sowing the cucumbers, and the plants were transplanted into pots 4 days after grafting. Thereafter, the seedlings were kept in a moist chamber until they were suitable for transplantation.

A spacing of 1.0 × 0.5 m was used between seedlings. The seedlings were guided conducted with one stem oriented vertically to avoid damage to fruit production or quality. We removed all shoots and eliminated all buds and flowers from the 1 st node through the 5th node, allowing the side branches to grow starting from the 6th node; the side branches were topped and tailed after the 3rd internode.

The experimental design was completely randomized in a 2 × 5factorial scheme, with grafted and ungrafted cucumber plants and 5 treatments with fungicides: control; azoxystrobin 60 g a.i ha^{-1}; boscalid 50 g a.i ha^{-1}; pyraclostrobin 50 g a.i ha^{-1}; and boscalid 100 g a.i ha^{-1} + pyraclostrobin 50 g a.i ha^{-1}, applied 18 days after transplanting and then at intervals of seven days.

The azoxystrobin source (strobilurin) was the product Amistar® containing 500 g kg^{-1} a.i.; for boscalid, the product was Cantus®, containing 500 g kg^{-1} a.i.; for pyraclostrobin (strobilurin), the product used was Comet®, containing 250 g L^{-1} i.a.; and for the mixture of boscalid and pyraclostrobin, the product used was Bellis®, containing 200 g kg^{-1} boscalid and 100 g kg^{-1} pyraclostrobin.

The fungicide applications were carried out via foliar spraying using a manual pressurized CO_2 sprayer with 0.3 kg f cm^{-2} with conical nozzles and using a plastic curtain between treatments to avoid drift. Healthy plants were used so that only the physiological effect, rather than the antifungal effect, could be observed.

The first application of the treatments was carried out 18 days after transplanting (DAT), when the seedlings had 6 completely expanded leaves, and subsequent applications were at seven days intervals, with four replicates and six plants per plot, assuming four useful plants.

Physiological and biochemical evaluations were performed at the beginning of the harvest (35 DAT), the peak of the harvest (57 DAT) and the end of the harvest (80 DAT); at each time point, the second fully expanded leaf that was healthy and without signs of senescence was selected and standardized.

2.2. Gas exchange

Gas exchange was measured with an infrared CO_2 and water vapor analyzer (LI-6400, Li-Cor Inc., Lincoln NE, USA). The measurements were performed from 9:00 am until 11:00 am on a sunny day. The net assimilation rate (A_{net}, μmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) were measured. The water use efficiency (*WUE*, μmol CO_2 (mmol H_2O)$^{-1}$) was determined by the relationship between net assimilation rate and transpiration, and the apparent carboxylation efficiency (A_{net}/C_i) was determined by the relationship between the net assimilation rate and the intercellular CO_2 concentration (C_i, μmol CO_2 mol^{-1} air).

To ensure that the experimental conditions were consistent, the PPFD was standardized through the use of a light-emitting diode coupled to a photosynthesis chamber, and the light-emitting diode emitted 1500 μmol m^{-2} s^{-1}, as this is the saturating luminosity, according to a light curve previously performed (Amaro et al., 2014). The reference CO_2 concentration that was used during the evaluation was the ambient value, which ranged from 380 to 400 μmol mol^{-1} of air.

2.3. Biochemical analyses

The leaf blades were collected and placed in plastic bags, wrapped in aluminum and immediately frozen in liquid nitrogen to stop all metabolic reactions. The leaf blade samples were stored in a −80 °C freezer for further analysis.

2.3.1. Measurement of nitrate reductase activity

For the determination of the activity of the nitrate reductase enzyme (EC 1.6.6.1), leaf blades (1 g) were sliced and placed in a dark vial containing 8 mL of potassium phosphate buffer (0.1 M, pH 7.0) and KNO_3 (0.02 M). Thereafter, the vials were incubated for one hour at 37 °C in the absence of light. After this period, 1 mL of sulfanilamide solution (1%) and 1 mL of N-Naphthyl solution (0.02%) were added and then incubated at 37 °C in the dark for 5 min. After this period, the nitrate reductase activity was determined spectrophotometrically at 540 nm. A nitrite solution was used to construct the standard curve (Streeter and Bosler, 1972).

2.3.2. Measurement of antioxidant enzyme activities and lipid peroxidation

For enzyme extracts, the leaf blades (300 mg) were pulverized in liquid nitrogen and homogenized in 4 mL of pre-cooled potassium phosphate buffer (0.1 M, pH 6.8) and 200 mg PVP. The homogenates were centrifuged at 10,000 × g for 10 min at 4 °C, and the resulting supernatants were used for enzyme assays (Kar and Mishra, 1976). The soluble protein content was determined using casein as a standard (Bradford, 1976). The supernatant from the extraction was used to determine the activities of the enzymes superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6).

The SOD activity was determined by spectrophotometrically