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Agronomic, Physiological, and Post-Harvest Aspects of Different Blueberry Cultivars Treated with Silicon

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Abstract

The objective of this study was to evaluate the effect of foliar application of silicon in different blueberry (*Vaccinium myrtillus*) cultivars. The treatments were installed in a factorial design with six replications, and consisted of three different cultivars (Brightwell, Beckblue and Climax) with or without regular application of silicon via foliar, using silicon oxide at 1.5 g L^{-1}. It was evaluated the following plant attributes: leaf area, chlorophyll fluorescence, and phenylalanine ammonia lyase activity, meanwhile the following postharvest attributes were evaluated: fruit number, diameter and weight, yield, pH, content of soluble solids, titratable acidity, contents of phenolic compounds and anthocyanins, and activity of peroxidase. Vegetative development of blueberry plants in response to Si presented higher yield, leaf area, phenylalanine ammonia lyase activity, and higher chlorophyll fluorescence relationships such as maximum photochemical efficiency and PSII potential activity, as well as less stressed plants, especially in cultivars Climax and Brightwell. For post-harvest physicochemical characteristics, silicon foliar application improved fruit weight and diameter for cvs. Beckblue and Climax, and fruit number for cv. Brightwell. All cultivars, in response to silicon application increased the titratable acidity, the levels of phenolic compounds, the level of anthocyanins, whereas peroxidase activity decreased. The performance of the evaluated blueberry cultivars was better through silicon application, with greater performance of vegetative growth, fruit yield, and fruit quality, especially in cultivar Climax, which was the most suitable in terms in of fruit yield in this study.

Keywords: anthocyanins; phenolic compounds, plant nutrition, productivity, *Vaccinium myrtillus*

Introduction

The blueberry (*Vaccinium myrtillus*) fruits are important due its organoleptic characteristics and the benefits to human health, related to the prevention of diseases, such as cardiovascular (Eladwy et al., 2018), neurodegenerative (Debom et al., 2016), cancer (Davidson et al., 2018; Lin et al., 2019) and inflammation (Li et al., 2016; Kalt et al., 2020). Many of the benefits that blueberries can provide were assigned to a large amount of anthocyanin, which is one of the species with the highest content among the fruits and vegetables (Kalt et al., 2001; Gündüz et al., 2015; Kalt et al., 2020). Besides the fresh consumption, these fruits can be destined for the preparation of teas, pies, cakes, puddings, cookies, ice cream, jellies, jams, as well as processed foods (Pritts et al., 1992).

In 2020, world production was 851 thousand tons of blueberries (Faostat, 2022). The three largest producing countries in the 2020 season were the United States, Peru and Canada, which accounted for 35%, 21% and 17% of world production respectively (Faostat, 2022). In Brazil, there are still no official data on the cultivation of blueberry, however, it is esteemed that there are about 400 ha planted, divided between the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, and Minas Gerais (Cantuarias-Avilés et al., 2014). The main limiting factor for the expansion of the blueberry crop in Brazil is the cold requirement to overcome dormancy, which varies from 300 to 1100 hours per year (with temperatures ≤ 7.2 °C), depending upon the cultivar (Cantuarias-Avilés et al., 2014). In southern Brazil, some regions could meet these conditions, such as the region of Guarapuava, which on average, can reach about 300 hours of cold in winter season (Botelho et al., 2006), which is suitable for some blueberry cultivars being these cultivars Aliceblue, Beckblue, Bluegem, Brightwell, Climax, and Pownderblue

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(Rabteye) (Cantuarias-Avilés et al., 2014).

Regarding the better performance of plants, minerals can be used to influence the levels of organic compounds through their action in biochemical or physiological processes (Ferreira et al., 2006). Silicon (Si) in plants is a stress-protective mineral nutrient (Moradtalab et al., 2018), as it is a beneficial nutrient in plant physiology and development (Menegale et al., 2015). In plants, Si absorption was in the form of monosilicic acid (H_4SiO_4), mainly (Menegale et al., 2015). Si benefits metabolic functions in stressed plants, through the formation of an outer protective layer composed of silica, and the reactivity of the absorbed silicon with heavy metal ions and other plant compounds (Rodrigues & Datnoff, 2015).

The application of Si has beneficial effects on many fruit crops, but there is still little information about the effect of Si on blueberry. The application of Si in passion fruit (Passiflora edulis) increased the photosynthetic rate of the plant (Costa et al., 2018), and in strawberry (Fragaria × ananassa) its improved pulp firmness and increased the level of anthocyanins in fruits (Munaretto et al., 2018). In tomato (Solanum lycopersicum) a similar result was observed, where the application of Si increased pulp firmness, fruit yield, soluble solids, vitamin C, and lycopene contents (Marodin et al., 2014; Marodin et al., 2016).

Based on the above information, we hypothesize that Si application can potentially improve blueberry production and quality. The objective of this study was to evaluate the effect of Si on the physiological, agronomic, and on postharvest attributes of different blueberry cultivars.

Material And Methods

Site description

The experiment was carried out in Guarapuava, Paraná, Brazil (25°23'S, 51°27'W, and altitude of 1,120 m) under humid subtropical mesothermic climate (Cfb-Köppen) (Alvares et al., 2013), and the average chilling accumulation in the region of Guarapuava is about 308.6 hours (Botelho et al., 2006). The soil of the experimental area is a very clayey Typic Hapludox (USDA Soil Survey), corresponding to Latossolo Bruno according to the Brazilian soil classification system (Embrapa, 2013).

Blueberry plants with 3 years old in a 3 × 1 m spacing were drip irrigated. The weeds were removed and controlled periodically. Phytosanitary control was conducted using Bordeaux mixture, calcium polysulfide (lime sulfur), and Neem oil, whenever necessary.

Experimental design and evaluations

The experimental design consisted of blocks in a randomized factorial 3 × 2 (cultivars × Si treatment), which consisted of three cultivars (Beckblue, Climax e Brightwell) and two Si treatments (with and without Si application), with six replications and one plant per experimental unit.

Silicon (Si) applications were conducted weekly with an electric backpack sprayer, between November 2018 and March 2019, totaling 21 applications. Si source was silicon oxide (SiO_2) (98% of SiO_2), applied at a concentration of 1.5 g L^{-1} in aqueous solution. Fruit harvesting began on Dec. 18, 2018 (after 7 Si applications), being conducted every two days.

Soon after harvest, fruit diameter, average fruit mass and productivity per plant were determined. Then, they were stored at -18 °C for further physicochemical analyses. To evaluate the single leaf area, ten leaves of each plant were taken randomly and measured with leaf area meter (LI-3100; Li-COR Biosciences Inc.). The chlorophyll fluorescence was measured on fully expanded leaves three weeks after the final Si application in the morning of sunny days (between 08 to 11h a.m.). A Portable Chlorophyll Fluorometer (PAM-2500; Walz) were used. Prior to this measurement, plants were dark adapted for 20 minutes and measurements were performed. From the fluorescence in response to the first light pulses, the following parameters were obtained: F_O (initial fluorescence), F_M (maximum fluorescence), F_V (variable fluorescence), and the following parameters were calculated: F_V/F_o (PSII potential activity) and F_V/F_M (maximum photochemical efficiency).

Protein concentration was determined in leaves and fruits by the method of Bradford, 1976). Phenylalanine ammonia lyase (PAL) activity was evaluated based on the difference in absorbance resulting from the conversion of phenylalanine to trans-cinnamic acid based on the method of (Umesha, 2006). For the evaluation of Protein and PAL, expanded leaves of plants from each experimental plot were collected in the morning, wrapped in aluminum foil, cooled in liquid nitrogen, placed in a Styrofoam box with ice for transport to the laboratory, and subsequently stored in a freezer at -80°C, samples were collected after the last application of silicon. Pulp was macerated and was analyzed for soluble solids content (SS) with digital refractometer model Pal-1 (Atago). The titratable acidity (TA) was determined as follow: 10 g of the blueberry pulp were diluted in 50 ml of distilled water, and this solution was titrated with a standard solution of 0.1 mol L^{-1} NaOH until it reached pH 8.2. Polyphenol levels of fruit peels was analyzed according to method of (Singleton et al., 1999) using Folin Ciocalteu reagent and calibration curve with gallic acid. Absorbance readings was obtained at 740 nm (UV 1800, Shimadzu).

The evaluations of anthocyanin contents were conducted in the fruit peel using the differential pH method (Giusti & Wrolstad, 2001; 2003). Delphinidin-3-O-glucoside was the major anthocyanin in blueberry. Peroxidase enzyme (POD) activity on fruit pulp was determined according to (Kar and Mishra, 1976), with determination of the enzymatic activity constituted the conversion of guaiacol into tetraguaicol.

In **Figure 1** is presented the data of rainfall and maximum and minimum temperatures during the experiment. Meteorological data were obtained from a meteorological station (SIMEPAR/Brazil) located around 100 m far from the experiment.

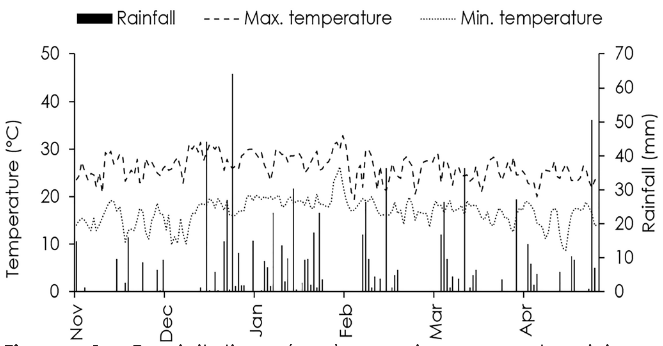

Figure 1. Precipitation (mm), maximum, and minimum temperature (°C) recorded on 2018/2019 growing season in Guarapuava, Paraná, Brazil.

Statistical analysis

The data were submitted to the Shapiro-Wilk test ($p \leq 0.05$) to verify the normality and homoscedasticity of the variance, and when they did not meet this condition, they were transformed according to Box-Cox test and then subjected to analysis of variance (ANOVA). Data was submitted to analysis of variance ($\alpha = 0.05$) in a factorial scheme (3 × 2). When significant, treatments means were classified by the Tukey test ($\alpha = 0.05$).

Results and Discussion

Leaf applications of Si increased leaf area in all cultivars evaluated (**Figure 2**A). The leaf area of cv. Climax was higher compared to other cultivars for both treatments (Figure 2A). The initial fluorescence (F_O) presented an interaction between the cultivars and the application of Si, where within cv. Blueberry the treatment with Si reduced the F_O, while there was no difference in the other cultivars (Figure 2B). The cultivar Beckblue presented the highest initial fluorescence emission (F_O) (Figure 2B).

Variable fluorescence (F_V) presented interaction between cultivars and Si application (Figure 2C). The Beckblue cultivar, compared to the other cultivars, presented the highest F_V, but this attribute did not differ in response to Si, whereas other cultivars presented an increase in F_V when treated with Si (Figure 2C).

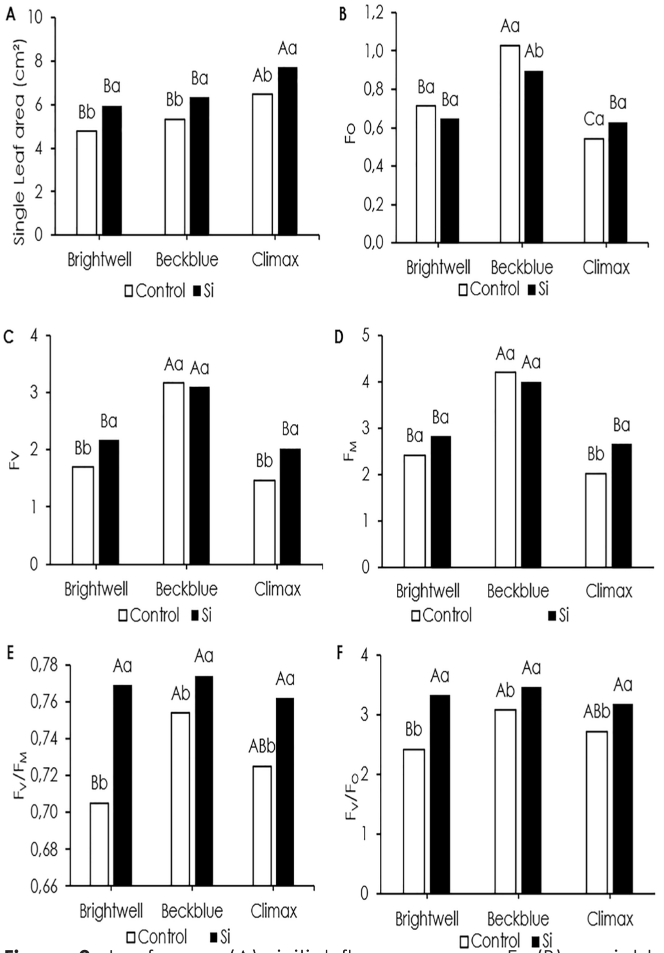

Figure 2. Leaf area (A), initial fluorescence - F_O (B), variable fluorescence - F_V (C), maximum fluorescence - F_M (D), maximum primary photochemical efficiency - F_V/F_M (E), PSII potential activity - F_V/F_O (F) of different blueberry cultivars treated or not with silicon in Guarapuava, Paraná, Brazil. *Same lowercase letters mean no differences between silicon treatments; and same uppercase letters means no differences between blueberry cultivars.

Maximum fluorescence (F_M) presented interaction between cultivars and Si application (Figure 2D). The cultivar Beckblue presented the highest F_M, but no difference in Si treatment, meanwhile the cultivars Climax and Brightwell presented higher F_M with the application of Si.

The F_V/F_M ratio presented interaction between cultivars and Si application (Figure 2E). The F_V/F_M ratio increased in the Si application for all cultivars. Within control treatment, F_V/F_M ratio presented the following order between cultivars: Beckblue ≥ Climax ≥ Brightwell, meanwhile within Si treatment there was no significant difference between the cultivars (Figure 2E).

The F_V/F_O ratio presented interaction between cultivars and Si application (Figure 2F). The F_V/F_O ratio increased in the Si application for all cultivars. Within control treatment, F_V/F_O ratio presented the following order between cultivars: Beckblue ≥ Climax ≥ Brightwell, meanwhile within Si treatment there was no significant difference between the cultivars (Figure 2F).

The PAL enzyme activity presented interaction between Si application and cultivars (**Figure 3**A). The Si application improved PAL activity in cvs. Beckblue and Climax. Within control treatment PAL enzyme activity presented the following order between cultivars: Brightwell = Climax > Beckblue; meanwhile within Si treatment presented the following order between cultivars: Climax > Beckblue > Brightwell (Figure 3A).

The number of fruits presented interaction between Si application and cultivars (Figure 3B). The Si application improved fruit number only for cv. Brightwell (+25%). Within control treatment, the fruit number presented the following order between cultivars: Climax > Beckblue = Brightwell; meanwhile within Si treatment the decreased sequence was Climax ≥ Brightwell ≥ Beckblue (Figure 3B).

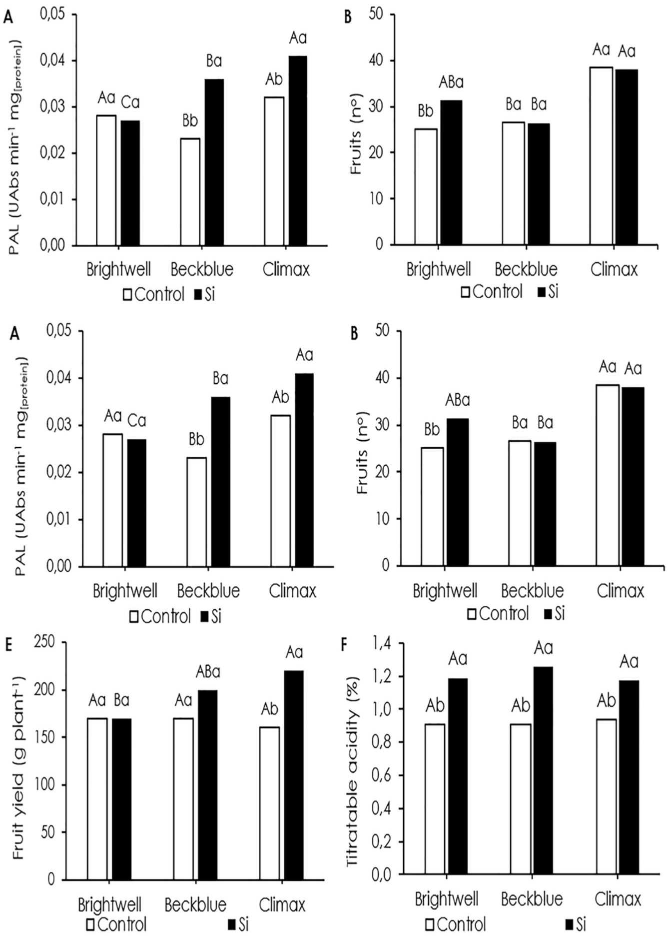

Figure 3. PAL (A), number of fruits (B), fruit diameter (C), fruit weight (D), yield (E), titratable acidity (F) of different blueberry cultivars treated with silicon in Guarapuava, Paraná, Brazil. *Same lowercase letters mean no differences between silicon treatment; and same uppercase letters means no differences between blueberry cultivars.

The fruit diameter presented interaction between Si application and cultivars (Figure 3C). The application of Si increased the fruit diameter of cvs. Beckblue and Climax, at 10% and 14.5%, respectively, while in cv. Brightwell there was no difference (Figure 3C). Within control treatment the diameter presented no differences between cultivars, meanwhile within Si treatment the diameter presented the following order between cultivars: Beckblue ≥ Climax ≥ Brightwell (Figure 3C).

The average fruit weight presented interaction between Si application and cultivars (Figure 3D). Foliar application of Si increased the average fruit weight for cultivars Beckblue and Climax, by 22% and 34%, respectively, while in Brightwell there was no difference (Figure 3D). Within the control treatment, the average fruit weight had no difference among cultivars, meanwhile with Si application, the average fruit weight presented the following order: Climax ≥ Beckblue ≥ Brightwell (Figure 3D).

The fruit yield presented interaction between Si application and cultivars (Figure 3E). The application of Si improved fruit yield Climax and Beckblue cultivars (Figure 3E). Within control treatment, fruit yield presented no difference among cultivars, meanwhile within Si treatment was as follows: Climax ≥ Beckblue ≥ Brightwell (Figure 3E). The titratable acidity did not differ between cultivars both with and without Si application, however, the application of Si increased acidity for all cultivars (Figure 3F).

The content of phenolic compounds differed between cultivars and with the application of Si (**Figure 4**A). Within the cultivars, the content of phenolic compounds was as follows: Brightwell = Climax > Beckblue (Figure 4A). The application of Si increased the content of phenolic compounds by 20%, 21% and 24% for the cultivars Brightwell, Beckblue and Climax, respectively (Figure 4A). There were no significant differences between cultivars for anthocyanin content in the control treatment (Figure 4B). The application of Si increased the concentration of anthocyanins in the fruit epidermis for all cultivars. Among the cultivars, the anthocyanin content presented the following order: Climax = Brightwell > Beckblue (Figure 4B).

POD activity presented interaction between Si application and cultivars (Figure 4C). Within cultivars, Si application reduced POD activity only for the cvs. Climax and Beckblue in 54% and 60%, respectively (Figure 4C). Without Si application, cultivars presented the following order: Climax > Beckblue > Brightwell; meanwhile with Si application was as follows: Climax > Beckblue = Brightwell (Figure 4C).

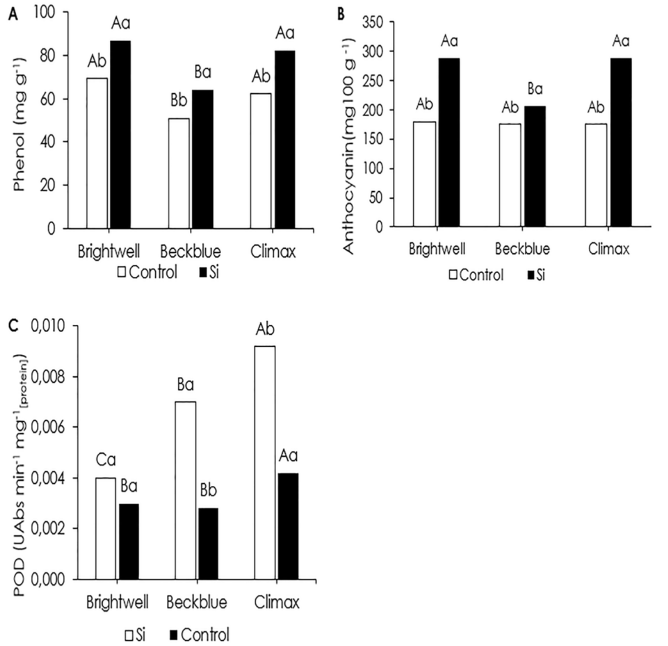

Figure 4. Total phenols (A), anthocyanins (B) and POD activity (C) of different blueberry cultivars treated with silicon. *Same lowercase letters mean no differences between silicon treatment; and same uppercase letters means no differences between blueberry cultivars.

The greater leaf area in response to Si may be related to the deposit of this element below the cuticle, which can provide a more erect plant architecture, thus improve light interception and, therefore, with greater potential for photosynthesis (Silva et al., 2013). Si accumulation below the cuticle can also reduce water loss through transpiration, which benefits net photosynthesis (Silva et al., 2013). The application of Si through various sources was also reported to increase the leaf area of sugarcane (*Saccharum officinarum*) (Sobral et al., 2011), physalis (*Physalis angulata*) (Assis et al., 2013) and passion fruit (*Passiflora edulis*) (Costa et al., 2018).

The greater leaf area in response to Si in all of the cultivars can be related to the deposit of this element below the cuticle, which can provide a more erect plant architecture, thus improving light interception and, therefore, more potential for photosynthesis (Silva et al., 2013). Si accumulation below the cuticle can also reduce water loss through transpiration, which benefits photosynthesis (Silva et al., 2013). The increase of leaf area after the application of Si through various sources was also observed in sugarcane (*Saccharum officinarum*) (Sobral et al., 2011), physalis (*Physalis angulata*) (Assis et al., 2013) and passion fruit (*Passiflora edulis*) (Costa et al., 2018).

Higher values of F_0 indicate low efficiency in the transfer of excitation energy between pigment molecules of photosystem II light collectors (PSII) (Goltsev et al., 2016). They can also indicate lower energy transfer efficiency to the PSII reaction center after the dissociation of the antenna complex from PSII and damage to the thylakoids (Goltsev et al., 2016). Si treatment reduced F_O significantly for one of the cultivars in this study (Beckblue), meaning that the mineral improved energy use compared to the control. In rice (*Oryza sativa*) plants, it was also reported that the application of Si reduced fluorescence emission (F_O) (Tatagiba et al., 2016).

The FV parameter is related to the maximum quantum yield of the PSII. When FV is low, it indicates the lower activity of the PS_{II} and the dissipation of the excitation energy as heat (Goltsev et al., 2016). Thus, it improved PS_{II} performance and lowered the dissipation of energy. Another study showed that the application of Si increased F_V in cocoa (*Theobroma cacao*) (Souza et al., 2012).

When F_M values are low, it indicates that the PS_{II} electron acceptors cannot be fully reduced (Goltsev et al., 2016). Therefore, the lowest level of F_M indicates that the photosynthetic apparatus of plants without Si application was under more significant stress than those with Si application. Another study reported that in cocoa, higher maximum fluorescence (F_M) values were found with the application of 7.15 mL L^{-1} of Si (Souza et al., 2012).

The F_V/F_M ratio represents the maximum primary photochemical efficiency of PS_{II}, and for most developed plants under stress-free conditions, the maximum value is 0.83 (Pokorska & Romanowska, 2007; Goltsev et al., 2016). In our study, the mean for cultivars with Si application was 0.76, while for plants without Si application, it was 0.74 (Figure 2E). The lower F_V/F_M evidence that the plant may be under stress, which impairs PS_{II} functions and decreases electron transfer efficiency (Goltsev et al., 2016). Through stress reduction, Si may have allowed greater photosynthetic efficiency and electron transfer in the evaluated cultivars. Other studies also reported that the application of Si increased the F_V/F_M ratio (Tatagiba et al., 2016; Souza et al., 2012), especially at the dose of 6.5 mL L^{-1} of potassium silicate (Souza et al., 2012).

In blueberry plants submitted to foliar application of Si, the F_V/F_M ratio was slightly higher (Figure 2E), the range considered ideal for healthy plants, which is around 0.86 (Goltsev et al., 2016). Plants without Si showed an even lower F_V/F_M ratio, which shows that Si allowed a greater efficiency of the photosynthetic apparatus and in the transfer of electrons to the Climax and Brightwell cultivars through the reduction of stress.

The F_V/F_O ratio indicates the maximum efficiency of the photochemical process in PSII and/or the potential photosynthetic activity, and its decrease indicates a decline in the photochemical rate (Goltsev et al., 2016). Therefore, Si promoted an increase in the photochemical rate for all of the cultivars in our study. Another study also reported that the application of salicylic acid to tomato plants increased the F_V/F_O ratio (Favaro et al., 2019).

The results of better photosynthesis parameters for plants with Si application can be related to the more significant leaf area for all cultivars caused by the Si deposition below the cuticle. This deposition can change the architecture of the plant, making them more upright, capturing more sunlight, and therefore increasing the photosynthetic rate (Silva et al., 2013). Also, it is speculated that plants treated with Si have a greater capacity to transfer the excitation of energy from the pigments to the formation of reducing power (NADPH and ATP), which increases the capacity of assimilation of CO_2 in the biochemical phase of photosynthesis (Baker, 2008; Favaro et al., 2019).

The increase in PAL enzyme activity in response to Si application in blueberry is promising. PAL is involved in some biosynthetic routes of secondary metabolism, such as the synthesis of phenolic compounds as phenylpropanoids, coumarins, flavonoids, condensed tannins and lignin (Taiz et al., 2017). Another study reported that the application of Si in rice also increased the activity of the PAL enzyme, as well as peroxidase and polyphenol oxidase (Cai et al., 2008). The increase in PAL activity may be associated with the ability of Si to promote greater expression of genes encoding this enzyme (Rodrigues & Datnoff, 2015).

The higher production of lignin gives more mechanical strength to the plant, especially in vascular tissues, and favors the conduction of water and minerals through the xylem (Taiz et al., 2017). Thus, the higher fruit weight, yield and leaf area may have been indirectly benefited as a result of the higher PAL activity. Another study reported that fertilization with Si, applied both via soil and foliar improved the number of strawberry fruits (Silva et al., 2013). The application of Si was also reported to increase the diameter of fruits in sapodilla trees (*Manilkara achras*) (Lalithya et al., 2014).

Studies on the effect of foliar application of Si on strawberries have divergent results. One study reported an increase in strawberry mass (Silva et al., 2013). Meanwhile, another study reported no difference (Munaretto et al., 2018). Other studies also reported that the application of Si increased fruit yield in strawberry (Silva et al., 2013) and tomato (Marodin et al., 2014). In sapodilla plants, the application of Si modified leaf angle, resulting in a greater number, diameter, and mass of fruits (Lalithya et al., 2014). The change in leaf angle improves light interception and, thus, more significant photosynthetic activity, with a consequent increase in metabolite translocation and plant performance (Das et al., 2019). Si increases carbon fluxes and enzyme activity in the Krebs cycle, which also leads to greater photosynthetic efficiency (Das et al., 2019).

The content of organic acids in tissue plants can increase by Si application, which influences the titratable acidity of the fruits (Silva et al., 2013). It was reported in rice that foliar application of Si could increase the content of organic acids such as citric, succinic, and malic due to increased carbon fluxes and enzyme activity in the Krebs cycle and, consequently, higher production of their metabolite intermediates (Das et al., 2019). Another study also reported that soil and foliar application of Si increased the titratable acidity in strawberries (Silva et al., 2013). In contrast, another study reported no effect of the foliar application of Si on the titratable acidity in strawberries (Munaretto et al., 2018).

Si can increase the activity of enzymes such as PAL in wheat (*Triticum aestivum*), which is related to the synthesis of phenolic compounds (Gomes et al., 2005). Si catalyzes the deamination of phenylalanine to form phenylpropanoids, which are precursors of phenolic compounds (Gunes et al., 2007; Gomes et al., 2008). In addition, Si is related to the formation of bioactive compounds in plants and the improvement of the photosynthetic process and activates secondary metabolic pathways (Gunes et al., 2007; Munaretto et al., 2018). Studies reported that Si application did not affect the levels of phenolic compounds in strawberry (Munaretto et al., 2018) and soybean (Ferreira et al., 2006) plants.

Anthocyanins are flavonoids from the compound phenolics group responsible for the blue, violet, and shades of red (Singleton, 1987). Therefore, the increase of anthocyanins is related to the increase of Phenolic compounds. Other studies also reported that foliar application of Si increased anthocyanin content in strawberry fruits (Munaretto et al., 2018; Silva et al., 2013).

The POD enzyme is related to cell protection and disease resistance through the oxidation of phenolic substances to quinones in the tissue aging process (Mohammadi & Kazemi, 2002). POD is an enzyme with an essential role in enhancing antioxidant capacity, promoting the oxidation of phenolics, forming ethylene, and improving respiratory strength (Chittoor et al., 1999). POD levels increase with fruit ripening as a defensive response to neutralize free radicals (Chittoor et al., 1999). The decrease in POD activity in blueberries found in this study could be related to the lower stress conditions in fruits treated with Si. Si application was also reported to improve POD activity in wheat (Gomes et al., 2005) and potato (Gomes et al., 2008).

Conclusions

The performance of the evaluated blueberry cultivars was better through Silicon application, with greater performance of vegetative growth, fruit yield, and fruit quality, especially in cultivar Climax, which was the most suitable in terms in of fruit yield in this study. Photosynthetic attributes, fruit yield, fluorescence attributes, and phenylalanine activity were benefited with the application of Si.

In the post-harvest aspects, foliar application of Si improved the quality of blueberry fruits, with greater mass and diameter in cultivars Beckblue and Climax, and greater number of fruits in cultivar Brightwell. In the three cultivars evaluated, the application of Si increased the titratable acidity content, the content of phenolic compounds and anthocyanins. The application of Si reduced peroxidase activity for all cultivars.

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