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Boric acid in germination of pollen grains and fruit set of peach cultivars in subtropical region[1]

Ácido bórico na germinação de grãos de pólen e fixação de fruto em pessegueiros em região subtropical

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ABSTRACT - The pollination and fertilization process directly affects the crop yield of peach. Previous studies have shown that some cultivars lack consistent cropping (yield variation) in subtropical regions. The application of boric acid at flowering can minimize this problem. This study quantified the germination of pollen grains and fruit set on 18 cultivars of peach in a subtropical region when treated with boric acid at bud. The initial spray treatments were three concentrations of boric acid (400 mgL^{-1}, 800 mgL^{-1} and 1,200 mgL^{-1}) plus a control treatment of water. Treatments were sprayed on the shoots when the flower buds were flower bud swelling on early maturing cultivars Aurora 2 and Doçura 2. There is a cultivar difference where 'Aurora 2' had a negative effect from the application of boric acid on the percentage of germinated pollen grains and fruit set. 'Doçura 2' has a positive results at the concentration of 400 mgL^{-1} of boric acid. In a follow-up experiment with 18 cultivars, using only the water control and 400 mgL^{-1} boric acid treatment, boron increases pollen grain germination and fruit set for some cultivars. The increase in germination and fruit set varies significantly among the cultivars. The concentration of 400 mgL^{-1} of boric acid increases pollen germination and fruit set, but not in all cultivars.

Key words: *Prunus persica.* Pollination. Fertilization. Boron.

RESUMO - Os processos de polinização e fecundação estão diretamente relacionados a capacidade reprodutiva do pessegueiro. Estudos anteriores demonstram que existem cultivares que apresentam alternância de safra em regiões subtropicais. A aplicação de ácido bórico no florescimento pode minimizar este problema. Objetivou-sequantificar a germinação de grãos de pólen e fixação de frutos em 18cultivares de pessegueiros, em região subtropical, sob aplicação de ácido bórico. Para a definição dos tratamentos houve uma fase preliminar onde foram aplicadas três concentrações de ácido bórico (400 mgL^{-1}, 800 mgL^{-1} e 1.200 mgL^{-1}), utilizando-se como testemunha a água. Os tratamentos foram aplicados nos ramos mistos, onde as gemas encontravam-se no estádio de ponta prateada nas cultivares Aurora 2 e Doçura 2, consideradas mais precoces e assim utilizadas como pré-teste. De acordo com os resultados, a cultivar 'Aurora 2' apresenta efeito negativo à aplicação de ácido bórico na porcentagem de germinação de grãos de pólen e fixação de frutos. 'Doçura 2' apresenta resultado positivo à concentração de 400 mgL^{-1} de ácido bórico. Posteriormente, definiu-se a testemunha e o tratamento com a 400 mgL^{-1} para as demais cultivares, pois apresentaram os melhores resultados em relação a germinação de grãos de pólen e fixação de frutos. O aumento da germinação dos grãos de pólen e a fixação de frutos de pessegueiro é variante entre as cultivares e a concentração de 400 mgL^{-1} de ácido bórico aumenta a germinação do pólen e fixação de frutos, mas não em todas as cultivares.

Palavras-chave: *Prunus persica.* Polinização. Fecundação. Boro.

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INTRODUCTION

Pollination and fertilization are the most important processes of sexual reproduction in plants (NAVA *et al.*, 2009). Thus, knowledge of the factors that affect them provides an understanding of the causes of poor fruiting and subsequently, aids in the implementation of new management practices in orchards to enhance fruit yields (SILVA *et al.*, 2016).

The adoption of low chill peach cultivars in subtropical and tropical regions with mild winters enable the harvest of fruits in times of less supply (BARBOSA *et al.*, 2010). The start of peach harvest occurs earlier in relation to the traditional peach-growing regions in Rio Grande do Sul and Santa Catarina state, in the Brazilian Southeast (ARAÚJO *et al.*, 2008). That earlier maturity is due to the warmer hibernal climate, which enables pruning and the shoot induction of buds with chemicals to begin in the winter as there is no risk of late frosts (BETTIOL NETO *et al.*, 2011).

Nevertheless, some cultivars in subtropical regions develop a form of alternate bearing, where crop yields vary between consecutive years (SOUZA *et al.*, 2013). Therefore, research of methods that increase fruit set may provide yield increases in peach cultivars grown in tropical and subtropical regions.

According to Chagas *et al.* (2009), pollen grains should have approximately 50% germination for good fruit set to occur and subsequent high yields. A number of organic and inorganic compounds affect in vitro germination, and boron is one of the most important elements (RAMOS *et al.*, 2008; CHAGAS *et al.*, 2010; NOGUEIRA *et al.*, 2016). Nogueira *et al.* (2015) found that the application of 900 mgL^{-1} of boric acid on panicles of the loquat 'Mizauto' increased by 24.3% pollen grain germination. Boron mechanism of action is to interact with sugar and forming an ionizable complex sugar-borate, which reacts more rapidly with the cell membranes (PFAHLER, 1967). Moreover, Nava *et al.* (2009) found that the application of boron in the form of boric acid in the bloom period increased peach fruit set though boron is a nutrient that is rarely found at low levels or deficient in orchards and thus is not added annually in managed orchards.

The objective in this study was to determine ifthe application of boric acid increases pollen grain germination and fruit set in peach cultivars cultivatedin a subtropical region.

MATERIALS AND METHODS

The experiment was conducted in an experimental orchard in subtropical area in Brazil between the months of May and August of 2015. The Koppen climate classification is Cwa - Subtropical climate (21º14'S, 45º00'W and 918 m of average altitude). The peach cultivars were grafted on the rootstock 'Okinawa' and were four-years old.

It was an pre-test experiment in beginning of June 2015, the production pruning was performed followed by application of 0.25% hydrogen cyanamide (a.i) on two early cultivars ('Aurora-2' and 'Doçura-2') to synchronize the bloom to test the concentration of boric acid and measure any improvements in pollen grain germination and fruit set. For this purpose, 64 shoots approximately 25 cm in length were demarcated per cultivar. The following concentrations of boric acid were applied with the aid of a spray bottle with a capacity of 500 mL (volume of the liquid about 2.0 to 2.5 L plant^{-1}) when the flower buds were flower bud swelling: 400, 800, 1,200 mgL^{-1} plus a control treatment of water. The experimental design was a randomized block 4 x 2 factorial with the first factor the cultivars and the second factor the application or not of boric acid.There were four blocks (replications) and four shoots (samples) per experimental unit.

After the flowers reached the balloon stage, four flower buds per plot per treatment were collected late afternoon to quantify the germination of pollen grains. The anthers were removed from the flower buds with aid of pair of tweezers. The anthers were stored on Petri dishes at a controlled temperature (27 °C) for 12 hours for anthesis, full dehiscence and release of pollen grains, according to the methodology of Zambon *et al.* (2014) and Silva *et al.* (2016). Pollen was distributed with a fine bristle brush onto the surface of Petri dishes containing 20 ml of the previously established culture medium containing 90 gL^{-1} sucrose, 400 mgL^{-1} of boric acid, 370 mgL^{-1} of calcium nitrate, pH buffered at 6.5 and 10 gL^{-1} agar, as determined by Chagas *et al.* (2009). Subsequently, the dishes were capped and kept in the absence of light for 24 h environment temperature. Each block consisted of four Petri dishes.

The pollen grains were viewed under a monocular microscope with a 10x objective to quantify the germinated pollen grains. It was considered a germinated pollen grain when the length of the pollen tube exceeded twice the grain's diameter (FIGUEIREDO *et al.*, 2013).

Fruit set was measured 30 days from full bloom by counting the fruits that grew (i.e., fruitset) on the shoots and was determined by the formula: Fruit Set (%) = (number of fruits/number of open flowers) x 100.

On 18 peach cultivars: 'Aurora-2', 'Bonão', 'Centenário', 'Delicioso Precoce', 'Doçura-2', 'Douradão', 'Dourado-2', 'Flordaprince', 'Jóia 3', 'Kampai', 'Libra', 'Maciel', 'Maravilha', 'Okinawa', 'Ouro Mel-4', 'Premier', 'Régis' and 'Tropical', in Cwa - Subtropical climate (21°14' S, 45°00' W and 918 m of average altitude), pruning was done in late June, followed by the application of hydrogen cyanamide at 0.25%. Based on the pre-test results, the best concentrations were 400 mgL^{-1} boric acid and the water control; therefore, an experiment was conducted using the 400 mgL^{-1} dose and the control on these 18 cultivars using the same application protocol. The experimental design was a randomized complete block in a 18 x 2 factorial with the first factor the cultivars and the second factor the application or not of boric acid with four blocks (replications) and four shoots (samples) per experimental unit.

The data obtained in all the experiments were submitted to variance analysis, the quantitative means being submitted to quadratic regression at 5% of probability and the qualitative media evaluated by the mean grouping test of Scott & Knott at the level of 5%.

RESULTS AND DISCUSSION

According to the second-degree equations for the variables of percent pollen grain germination and fruit set, there was a distinct relationship to both cultivars. For 'Aurora-2', the absence of boric acid provided 90.4% germination of pollen grains, while the concentration of 400 mgL^{-1} yielded 87.7% and 1,200 mgL^{-1} 63.2% germination (Figure 1A). A distinct pattern was observed in 'Doçura-2' where the absence of boric acid gave a 57.9% germination of the pollen grains compared to the concentration of 400 mgL^{-1} which had 77.3%, while the 1,200 mgL^{-1} decreased germination (Figure 1B).

The same pattern occurred for the percentage of fruit set. In 'Aurora-2', the absence (i.e., water control) of boric acid had 74.8% fruit set, while 400 mgL^{-1} yielded 70.7% and 1,200 mgL^{-1} 60.2% (Figure 1A). For 'Doçura-2', the absence of boric acid gave 70.6% fruit set, and the 400 mgL^{-1} yielded a 78.1% set, but the 1,200 mgL^{-1} had poor set of only 9.7% (Figure 1B).

Variable boron effects on pollen germination were reported by Nogueira *et al.* (2015), who found that the application of boric acid onto the flowers and panicles of loquats increased the germination of pollen grains, but higherboron concentrations reduced germination.

There were significant differences in the germination values of pollen grains and fruit set, both within cultivars and the treatment with or without boric acid (Table 1). The application of 400 mgL^{-1} of boric acid on the shoot increased the pollen grain germination percentage for cultivars 'Delicioso Precoce', 'Jóia-3' and 'Maravilha' (increase of 30.8%, 29.3% and 26.8%, respectively). According to Nava *et al.* (2009), boron increases germination of pollen grains *in vitro*. Furthermore, Nyomora *et al.* (2000) found that the application of boron also increased *in vivo* germination of pollen as well as the pollen tube growth. Therefore, the decrease in germination of pollen grains of cultivars 'Bonão', 'Flordaprince', 'Libra', 'Premier' and 'Régis' may have been due to the toxicity of boric acid (400 mgL^{-1}). However, high natural germination of pollen grains was observed in some cultivars, such as 'Aurora 2' and 'Ouro Mel-4', which had statistically higher germination than the other cultivars, whether or not boric acid was applied. The lowest germination percentage was for 'Centenário' with only 19.5%.

Figure 1 - Percentage of pollen grain germination and fruit set of peach cultivars 'Aurora-2' (a) and 'Doçura-2' (b) sprayed with different concentrations of boric acid

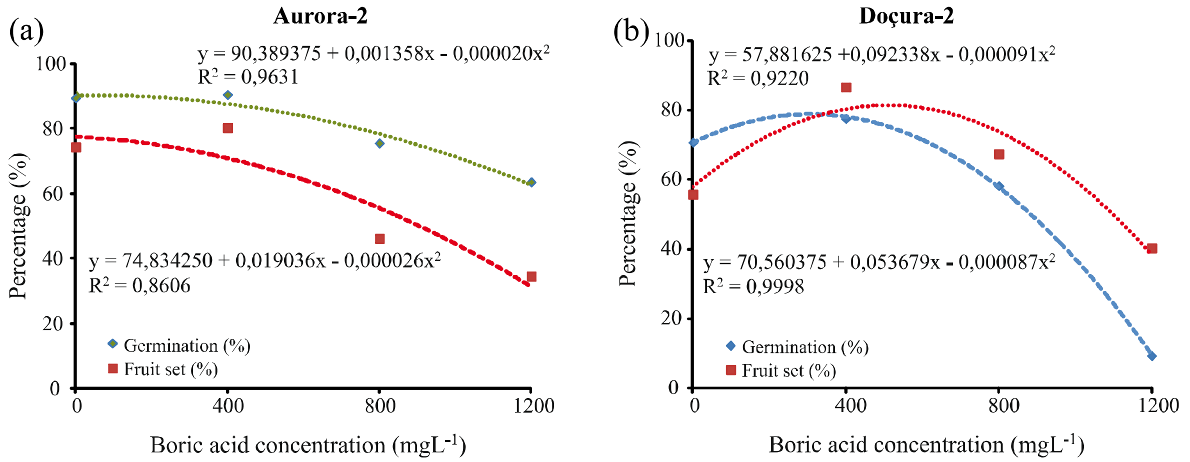

Table 1 - Germination percentage of pollen grains and percent fruit set of 18 peach cultivars treated with 0 or 400 mgL^{-1} of boric acid

Cultivar	Germination (%)*		Fruit set (%)*	
	0	400 mgL^{-1}	0	400 mgL^{-1}
Aurora-2	91.31 aA	89.33 aA	74.35 aA	80.28 bA
Bonão	76.08 bA	64.52 cB	55.69 bB	69.35 dA
Centenário	19.53 fA	24.99 eA	54.36 bB	100.00 aA
Del. Precoce	58.07 eB	76.83 bA	69.70 aA	75.00 dA
Dourado-2	58.75 eA	57.68 dA	69.05 aA	81.25 bA
Douradão	65.71 cA	61.38 cA	41.66 cB	68.48 dA
Doçura-2	70.74 cA	77.56 bA	55.77 bB	86.66 bA
Flordaprince	91.31 aA	57.56 dB	68.19 aB	82.50 bA
Jóia3	45.25 eB	58.50 dA	55.49 bB	90.91 bA
Kampai	48.84 eA	51.24 dA	70.00 aB	82.75 bA
Libra	77.23 bA	63.86 cB	29.16 dB	55.00 eA
Maciel	73.15 bA	75.50 bA	41.66 cA	51.05 eA
Maravilha	53.03 dB	67.25 cA	50.00 bB	84.72 bA
Okinawa	79.67 bA	76.87 bA	60.28 aB	78.55 cA
Ouro Mel-4	85.27 aA	86.35 aA	53.57 cB	68.94 dA
Premier	55.98 dA	18.51 eB	52.04 bB	73.21 dA
Régis	74.64 bA	61.99 cB	55.00 bB	75.12 dA
Tropical	60.32 dA	55.47 dA	51.14 bB	88.75 bA
CV (%)	9.30	10.70		

*Means followed by the same capital letter in the row and small letter in the column do not differ from one another by the Scott Knott test at 5% of probability

The application of boric acid provided a positive effect on fruit set in 14 cultivars, increasing by 88.6% (‘Libra’), 84.0% (‘Centenário’), 73.5% (‘Tropical’), 44% (‘Maravilha’), 64.4% (‘Douradão’), 63.8% (‘Jóia 3’), 56.0% (‘Doçura 2’), 40.7% (‘Premier’), 36.6% (‘Régis’), 30.3% (‘Okinawa’), 28.7% (‘Ouromel 4’), 24.5% (‘Bonão’), 21.0% (‘Flordaprince’) and 18.2% (‘Kampai’). During the fruit development process, boron has the important role of stimulating the germination of pollen grains and pollen tube growth Lee, Kim and Han (2009), which are necessary for fertilization and subsequent fruit growth. Barbosa *et al*. (1991) stressed the importance of high rates of fruit set, as they provide greater numbers of peaches on the shoots. In the case of cultivar ‘Tropical’ for example, with fruit setof 40%, trees had 40 fruits per meter of shoot (BARBOSA *et al*., 1989). High fruit set allows a homogeneous selection of fruits at the time of the cultural practice of fruit thinning.

CONCLUSION

Pollen grain germination and fruit set is variable among the peach cultivars commercially grown in subtropical Brazil. The application of 400 mgL^{-1} of boric acid increases fruit set on most of the cultivars tested, whereas pollen grain germination increases only in some but not all of the selected cultivars.

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