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# Tree performance and peach fruits yield and quality under compounds sprays to induce sprouting

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The sprouting induction is a fundamental cultural practice in peach orchards grown in subtropical regions around the world. The evaluation of alternatives compounds to induce sprouting is necessary mainly considering its effects on fruit quality. Two alternative compounds such as 2.5% nitrogen fertilizer (NF) + 4% calcium nitrate (CN) and 4% calcium nitrate (CN), besides the control were compared with 0.6% hydrogen cyanamide (HC), the most effectiveness chemical to induce budburst in peach crops grown in subtropical regions of Brazil. The field experiment was undertaken in São Paulo state, southeast Brazil, in two crop seasons. The hydrogen cyanamide increased crop performance of the 'Douradão' peach. Regardless, nitrogen fertilizer + calcium nitrate promoted an intermediate yield performance. Both treatments enhanced total phenolic compounds in fruits and their antioxidant activity. The efficacy of hydrogen cyanamide in a region with low chill accumulation was confirmed. Furthermore, nitrogen fertilizer + calcium nitrate are promising sprouting inducing and may be an alternative to replace hydrogen cyanamide, because of improved crop performance and peaches quality and considering the need for new products with less toxicity and characteristics of reducing environmental risk profiles and for the consumer market.

Key words: Prunus persica, hydrogen cyanamide, nitrogen fertilizer, calcium nitrate, bioactive compounds.

# INTRODUCTION

The peach tree (*Prunus persica* (L.) Batsch) is among the most important temperate fruits grown in the world and is no longer only produced in regions characterized by a

cold winter period. More recently, the cultivation of peach trees has been extended to non-traditional areas in the subtropical and tropical regions worldwide, where the

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> climate is different from their natural habitat, with mild and dry winters and hot and rainy summers (Pantelidis et al., 2021).

The success of peach orchards in subtropical regions is related to the minimal requirements for chilling accumulation (Corrêa et al., 2019) and the crop loading requires modified techniques to overcome dormancy and allow adequate flowering, growth and fruit set. The main approaches and updates taken were the development of cultivars with low chilling requirements and chemical induction of bud break (Pio et al., 2019).

The cultivar Douradão was selected by Campinas Agronomic Institute (IAC), because of the low chilling requirement (that is, 200 h), besides being the main cultivar grown in the subtropical region of São Paulo state, Brazil. The tree has a good crop performance, compact growth and medium vigour (Barbosa et al., 1999). The fruits when ripen have a yellow background colour, yellow pulp, reaching up to 160 g. The pulp had sweet and sour flavour with 13.2° Brix and pulp yield 93% (Leonel et al., 2014).

The use of cultural practices is necessary to minimize the effects of low accumulation of cold in winter to enable bud emergence in the branches of the plants with balance and vigorous. In order to do that, there are several chemicals to induce bud emergence, such as hydrogen cyanamide (HC), potassium nitrates, calcium nitrates (CN), mineral oils (Seif El-Yazal et al., 2014) and nitrogen fertilizer (NF) (Segantini et al., 2015).

The HC has stood out due to its expressive outcomes allowing better performance to overcome dormancy (Souza et al., 2021) and HC 0.6% is widely used by peaches growers in subtropical regions of São Paulo state, Brazil (Ferreira et al., 2019). However, despite these most evident results, this chemical has been shown a rather toxicity to the repeated exposure by applicators (Hernández and Craig, 2016).

The possible restriction and forbidden of HC for dormancy interruption in temperate fruit trees leads to a concern among many growers in some countries around the world about how long the product will be available in their market (Petri et al., 2014). This situation raised a necessity to find alternative chemicals to HC to be used in subtropical climate regions, such as NF (Erger®) (Hawerroth et al., 2009; Segantini et al., 2015; İmrak et al., 2016) and potassium and CN (Seif El-Yazal et al., 2014; Michailidis et al., 2021). Ferreira et al. (2019) in preliminaries studies with peach cultivars associated with different concentrations of NF and CN, concluded that NF (2.5%) + CN (4%) provided wider sprouting, flowering and fruit production. These compounds stimulate bud emergence and flowering in the branches, standardizes the phenological stages and increases production efficiency. However, it is also necessary to verify their effects on the qualitative attributes of the fruits, since there are few reports regarding this information.

Peach fruits intended for fresh consumption must have

a good proportion of red colour in the peel, white or yellow colour in the pulp, high content of soluble solids, low acidity and resistance to transport and storage (Alves et al., 2012). In addition, fruits also have functional properties that provide protection to human health, such as polyphenols, one of the main antioxidant compounds found in peaches (Zhao et al., 2015).

The main factors that can determine and influence variables are the cultivar genotype. these the combination between rootstock and scion cultivars, environmental conditions and orchard management techniques (Minas et al., 2018), including compounds sprays for inducing budburst. These products have a direct action on the sprouting of the branches, but their use may also influence the physical and physicochemical attributes in fruits, such as size, soluble solids and acidity (Leonel et al., 2014; Ímrak et al., 2016), in addition to increase the contents of bioactive compounds and, consequently, their antioxidant activity (Asami et al., 2003). Chemical sprouting induction is a fundamental cultural practice for peach cultivation in warmer areas. However, there is a need for research to describe the influence of these compounds on fruit qualitative attributes. The goal of this study was to assess the effects of different compounds on the production and quality of fruits of 'Douradão' peach trees in two crop seasons, in a subtropical region of São Paulo state, Brazil.

#### MATERIALS AND METHODS

#### Site characterization and crop management

A replicated trial was carried out in two crop seasons, that is, 2017 and 2018, on an experimental orchard that belongs to the School of Agriculture (FCA/UNESP), São Paulo state, Brazil, located at 22°51'55"S, 48°27'22" and an altitude of 810 m a.s.l. The climate of the area is classified as Cfa according to Köeppen-Geiger, that is, a temperate warm climate (mesothermic) with an average air temperature of 19.3°C and annual precipitation of 1.374 mm (Cunha and Martins, 2009).

The number of hours of temperature below 7.2°C (Atkinson et al., 2013; Fádon et al., 2020) or 15°C (Putti et al., 2003) was recorded from January to December in 2017 and 2018 years as based on the methodology of Citadin et al. (2002). The area accumulated 4.2 chill hours (CH)  $\leq$  7.2°C from 1 March until 31 July in 2017 and 1.8 CH  $\leq$  7.2°C from 01 May until 31 August in 2018. While the accumulation of CH  $\leq$  15°C, during the same period, was 278.7 and 312.6  $\leq$  15°C in the first (2017) and in the second crop season (2018). The average rainfall recorded in 2017 was 1880.6 mm and in 2018 was 1310.89 mm.

The soil was classified as a sandy-textured dystroferric Red Latosol (EMBRAPA, 2013). Before the experiment implementation, soil samples (0 to 20 cm depth) were collected and the chemical characteristics were determined. Based on soil analysis and peach crop recommendations, experimental area was previously prepared with plowing, sorting and liming. The peach trees of Douradão cultivar were six years old and were planted at 6 m spacing between rows and 4 m between trees. The trees were conducted in an open vase design, grafted on to the 'Okinawa' rootstock and were managed according with the recommendations for commercial



Figure 1. Representative sequence of leaves fall, spraying of budburst compounds, bud emergence, tree flowering and fruit harvest.

peach orchards. The winter pruning was carried out on July month in both crop seasons evaluated. The hand thinning of the fruits was carried out 20 days after full flowering, keeping 3 to 5 fruits in each productive branch, depending on the length and thickness of the branches.

## **Products application**

The compounds used were HC (Domex®), NF (Erger®) and calcium nitrate (CN). The commercial product Dormex® contains 520 gL<sup>-1</sup> of hydrogen cyanamide and is classified as a systemic regulator of the chemical group of carbimide (hazard class I). Nitrogen fertilizer (Erger®) is composed of 6.1% of urea nitrogen, 5.8% of nitric nitrogen, 3.1% of ammoniacal nitrogen, and 4.7% of calcium oxide (CaO), mono-and-selected polysaccharides and diterpenes, it is available as soluble concentrate at low risk (VALAGRO, 2021). Calcium nitrate is a chemical compound with the molecular formula Ca(NO<sub>3</sub>)<sub>2</sub>, a white, solid and odourless inorganic salt, that contains 15.5% of nitrogen and 26.5% of calcium.

The compounds were sprayed immediately after winter pruning, as following treatments: control (100% water); 2.5% NF (Erger®) + 4.0% CN; 4.0% CN; 0.6% HC. All sprayings were performed in the morning of the same day, with adhesive spreader (Assist®) at 1L 100 L<sup>-1</sup> of water and each tree received 2.5 L of spray volume (Figure 1).

#### **Experimental design**

The experimental design was in randomized block, with five

replicates, in a split-plot arrangement (4 compounds  $\times$  2 crop seasons). Replicates consisted of tree useful trees per experimental plot.

#### Canopy volume, yield and production efficiency

The canopy volume  $(m^3)$  was determined by individual measurements of the tree height and by the sum of width in parallel and perpendicular directions to the tree row (crown) and by using the Equation 1 (Rufato et al., 2006).

$$CAV = \frac{2}{3} \pi \times C^2 \times H \tag{1}$$

Where: CAV = canopy volume; C = crown; H = height.

The yield (kg ha<sup>-1</sup>) was determined by the product of production per tree (kg) and planting density (that is, 417 trees ha<sup>-1</sup>) (Equation 2):

$$Y = PT \ x \ PD \tag{2}$$

Where: Y = yield; PT = production per tree; PD = planting density.

Production efficiency (kg  $m^{-3}$ ) was determined by the relationship between production per tree (kg) and the canopy volume ( $m^{-3}$ ) (Equation 3).

$$PE = \frac{PT}{CAV}$$
(3)

Where: PE = production efficiency.

#### Harvest and the physical analysis of the fruits

The fruits were harvested when reached physiological maturity by considering the change of the background colour from green to light yellow and reached the minimum value of 10°Brix (Figure 1). Each replicate consisted of twenty fruits per treatment. The following analysis were performed: average fruit mass, obtained by weighing the fruits on a semi-analytical balance (OWLABOR, 2000g Maximum Load Capacity  $\times$  0.01 g Readability); length and diameter, obtained by measuring the longitudinal and equatorial diameters of the fruits; longitudinal diameter/equatorial diameter ratio (LD/ED), through the relationship between fruit length and diameter.

#### The chemical analysis of the fruits

After measuring the physical properties, the same fruits from each plot were assessed and evaluated for chemical analysis. For evaluation, samples were crushed with the aid of a fruit mixer (Philips Walita Viva Collection - RI1364) to form a homogeneous extract of the peach. Except for the samples used to determine the total phenolic compounds, in which the peaches were sliced and frozen in liquid nitrogen and then manually ground with the aid of a mortar and pestle.

The potential for hydrogen (pH) was measured in homogenized pulp of the fruits (50 g) by using a digital potentiometer (Digimed model DMPH-2). Titratable acidity (TA) (g of citric acid 100 g-1) was performed by tritation with 0.1 N sodium hydroxide (NaOH) in a solution of 1 g of the homogenized fruit extract, 50 ml of distilled water and 0.3 ml of phenolphthalein (IAL, 2008), expressed as a percentage of citric acid. Soluble solids (SS) were measured with a digital refractometer (Atago 3405 PR-32a Digital Refractometer, PALETTE Series) using 1g of the homogenized fruit extract and expressed as °Brix. The maturation index was obtained by the relationship between soluble solids and titratable acidity (SS/TA).

The total phenolic content was determined using the Folin-Ciocalteau colorimetric method (Swain and Hills, 1959). Samples of 100 mg macerated and frozen in liquid nitrogen were diluted in 3 ml of pure methanol and incubated for 30 min in an ultrasonic bath. Subsequently, the samples were centrifuged for 15 min at 14,000 rpm and 4°C and the supernatant collected for analysis. The absorbance value at 765 nm was obtained in tests on an UV–Vis SP 2000 BEL Photonics® spectrometer and compared with a calibration curve obtained for gallic acid, expressed as mg 100 g<sup>-1</sup> gallic acid equivalents (GAE) in fresh weight (FW). All steps were performed in the dark.

The antioxidant activity (% 2,2-diphenyl-1-pycrylhydrazyl reduced DPPH) was assessed with 100 mg of the sample that was diluted in 3 mL of acetone, taken to a shaker for 40 min. After that, the samples were centrifuged for 15 min at 4°C and 15.000 rpm, from which 150  $\mu$ l were collected to react with 2850  $\mu$ l of the DPPH solution for 40 min, with spectrophotometer reading at 515 nm (Brand-Willians et al., 1995).

The total flavonoid content (mg of routine 100 g<sup>-1</sup> sample) was obtained by using 300 mg of fresh sample that was initially weighed and 4 ml of the 15% acidified methanol (MeOH) solution was added. The samples were taken to the ultrasonic bath at 30°C for 30 min. Thereafter, they were centrifuged for 30 min under rotation at 6000 rpm at 5°C. The supernatants were collected and 1 ml of the aluminium chloride solution was added. After 50 min of reaction in the dark, the readings were carried out at 425 nm (Awad et al., 2000).

#### Statistical analysis

The data were submitted to analysis of variance (ANOVA) at the 1 and 5% of probability levels and means were compared using Tukey test. For all statistical analyses, the computer program for analysis of variance, AgroEstat® software was used.

## RESULTS

## Canopy volume, yield and production efficiency

There was no significant interaction effect between compounds and crop seasons by canopy volume, yield and production efficiency (Table 1). However, these variables individually affected yield and production efficiency. HC presented higher productive performance than the other treatments (Table 2), increasing the yield by 53.47% (4.320 kg ha<sup>-1</sup>) and by 45.72% in production efficiency when compared to the control.

NF + CN presented an intermediate performance with 2.910 kg ha<sup>-1</sup> increasing the yield in 30.92% in relation to the control. NF + NC thereby had a lower outcome than HC but presented a positive outcome, in terms of vield. when compared to the control. The highest canopy volume, yield and production efficiency were observed in the 2017 crop season (Table 2). During this period, peach trees reached 4.56 m<sup>3</sup> of canopy volume, yield of 4.320 t ha<sup>-1</sup> and production efficiency of 2.65 kg m<sup>-3</sup>. The reduction in the crop season of 2018 occurred due to higher precipitations and winds that affected the plants after pruning and compounds spraying. These climate adverse weather conditions induced changes in trees phenology, with a negative impact, reducing sprouting and consequently canopy volume, flowering and fruit set rates and peach tree yield.

Fewer chill hours  $\leq$  15°C occurred during 2017 crop season. There were 4.2 CH below  $\leq$  7.2°C and 278.7  $\leq$  $15^{\circ}$ C in 2017 versus 1.8 CH  $\leq$  7.2°C and 312.6  $\leq$  15°C in 2018. The occurrence of cold temperatures, as well as more cold hours and relatively high daytime temperatures in 2018 determined the beginning of budding and flowering. This fact may have been a positive factor for the induction of sprouting, favouring the branches growth and consequently the canopy volume, yield and production efficiency, as the effective temperature for chill accumulation varies according to the cultivar, reaching 15°C in cultivars with less chill demand, such as the Douradão peach. Nevertheless, during August 2018, a period after high cumulative chilling hours  $\leq$  15°C (114.8 h) had intense rainfall (113.1 mm) and high winds, impairing negatively all the growth and yield variables evaluated.

## The physical analysis of the fruits

There was a significant interaction between compounds and crop seasons for the fresh mass and fruit diameter.

		F-values						
Variable	DF	Production and physical variables					L/D	
		Y	CAV	PE	FW	L	D	
Block	4	5.57**	0.45 <sup>NS</sup>	0.48 <sup>NS</sup>	0.2 <sup>NS</sup>	0.40 <sup>NS</sup>	1.78 <sup>NS</sup>	8.97**
Compounds (C)	3	18.99**	1.12 <sup>NS</sup>	6.77**	3.79 <sup>*</sup>	1.50 <sup>NS</sup>	2.05 <sup>NS</sup>	1.71 <sup>NS</sup>
Year (Y)	1	17.42**	14.65**	17.42**	1.81 <sup>NS</sup>	5.17**	1.14 <sup>NS</sup>	32.83**
CxY	3	0.14 <sup>NS</sup>	0.96 <sup>NS</sup>	0.30 <sup>NS</sup>	3.85 <sup>*</sup>	2.92 <sup>NS</sup>	3.93 <sup>*</sup>	1.68 <sup>NS</sup>
Mean		3.01	3.80	1.31	102.11	64.2	56.75	1.13
CV 1		9.09	12.48	18.69	10.44	4.55	3.93	2.17
CV 2		24.41	30.67	32.66	9.2	4.81	3.95	2.38
		Chemical variables						
	DF	SS	рН	ТА	МІ	FLA	AA	PHE
Block	4	1.23 <sup>NS</sup>	0.34 <sup>NS</sup>	2.27 <sup>NS</sup>	0.84 <sup>NS</sup>	0.51 <sup>NS</sup>	0.18 <sup>NS</sup>	2.27 <sup>NS</sup>
Compounds (C)	3	3.19 <sup>NS</sup>	6.79**	4.31*	2.57 <sup>NS</sup>	0.65 <sup>NS</sup>	15.90 <sup>**</sup>	4.31 <sup>*</sup>
Year (Y)	1	10.99**	0.83 <sup>NS</sup>	0.34 <sup>NS</sup>	2.09 <sup>NS</sup>	0.42 <sup>NS</sup>	0.83 <sup>NS</sup>	0.34 <sup>NS</sup>
CxY	3	6.19**	1.70 <sup>NS</sup>	1.89 <sup>NS</sup>	1.63 <sup>NS</sup>	1.25 <sup>NS</sup>	1.70 <sup>NS</sup>	1.89 <sup>NS</sup>
Mean		14.03	4.08	0.42	33.75	14.03	4.08	0.42
CV 1		5.54	6.35	15.25	12.85	5.54	6.35	15.25
CV 2		5.49	6.14	17.77	13.2	5.49	6.14	17.77

**Table 1.** F-values, degrees of freedom (DF), coefficients of variation (CV) and means of evaluated variables of 'Douradão' peach trees after compounds sprays to induce sprouting.

 $^{NS}$ = Not significant; \*= significant at 5%; \*\*= significant at 1%. Y = Yield; CAV = Canopy volume; PE = Production efficiency; FM = Fruit mass; FD = Fruit diameter; L = Fruit length; D = Fruit diameter; L/D = Ratio of fruit length and diameter; SS = Soluble solids; TA = Titratable acidity; MI = Maturation index; FLA = Total flavonoids; AA = Antioxidanty activity and PHE = Total phenolic compounds.

**Table 2.** Canopy volume (m<sup>3</sup>), yield (kg ha<sup>-1</sup>) and production efficiency (kg m<sup>3</sup>) in 'Douradão' peach trees as a function of compounds sprays to induce sprouting and crop seasons.

Compounds	Canopy volume	Yield	Production efficiency
Control	3.30	2.010 <sup>c</sup>	1.46 <sup>b</sup>
NF + CN	4.29	2.910 <sup>b</sup>	1.66 <sup>b</sup>
CN	3.65	2.540 <sup>bc</sup>	1.89 <sup>b</sup>
HC	3.95	4.320 <sup>a</sup>	2.69 <sup>a</sup>
MSD	0.35	0.600	0.51
Crop seasons			
2017	4.56 <sup>a</sup>	4.320 <sup>a</sup>	2.65 <sup>a</sup>
2018	3.03 <sup>b</sup>	1.710 <sup>b</sup>	1.20 <sup>b</sup>
MSD	3.36	5.730	0.77

The same letter in the columns indicates that the results do not differ by Tukey test at 5% probability. MSD = Minimum significant difference.

Also, there was an isolated effect of crop season on length and length/diameter ratio (Table 3). All treatments performed similar values to the fruits' fresh mass during the first evaluation cycle (2017), varying from 94.85 to 108.65 g. In the second cycle (2018), the highest values of fresh mass were observed in the control (115.75 g) and HC (100.10 g) treatments.

The variable fruits diameter presented the significant

interaction between compounds and evaluation cycle (Table 1). The highest values were observed with HC (58.34 mm), CN (58.78 mm) and control (56.93 mm) in 2017, while in 2018 all compounds had the same fruit diameter, varying between 55.23 to 57.07 mm (Table 3). HC provided higher productive performance to the peach trees without compromising the size of the fruits, which presented high values for fresh mass and diameter in

Compounds	Fruit	mass	Fruit diameter			
	2017	2018	2017	2018		
Control	104.04 <sup>Aa</sup>	115.75 <sup>Aa</sup>	56.93 <sup>ABa</sup>	57.03 <sup>Aa</sup>		
NF + CN	94.85 <sup>Aa</sup>	93.28 <sup>Ba</sup>	54.23 <sup>Ba</sup>	56.39 <sup>Aa</sup>		
CN	108.65 <sup>Aa</sup>	93.00 <sup>Bb</sup>	58.78 <sup>Aa</sup>	55.23 <sup>Ab</sup>		
HC	106.32 <sup>Aa</sup>	100.10 <sup>ABa</sup>	58.34 <sup>Aa</sup>	57.07 <sup>Aa</sup>		
MSD 1	3.	60	17.51			
MSD 2	17	17.39		12.72		
	Fruit	lenght	Lenght/Diameter			
Years	2017	2018	2017	2018		
	63.09 <sup>b</sup>	65.31 <sup>a</sup>	1.11 <sup>b</sup>	1.16 <sup>a</sup>		
MSD	2.	07	0.	02		

**Table 3.** Fruit mass (g), diameter (mm), length (mm) and length/diameter in 'Douradão' peach as a function of different compounds sprays to induce sprouting and crop seasons.

The same letter in the columns and lines indicates that the results do not differ by Tukey test at 5% probability.

**Table 4**. Soluble solids (°Brix), titratable acidity (g citric acid 100  $g^{-1}$ ), pH and maturation index in 'Douradão' peach fruits as a function of compounds sprays to induce sprouting during two crop seasons.

Compounds —	Soluble	solids			Moturation Index
	2017 2018		I itratable acidity	рн	waturation index
Control	12.73 <sup>Bb</sup>	15.27 <sup>Aa</sup>	0.38 <sup>b</sup>	4.38 <sup>a</sup>	36.85
NF + CN	13.63 <sup>ABa</sup>	14.23 <sup>Aa</sup>	0.45 <sup>ab</sup>	3.88 <sup>b</sup>	32.16
CN	13.40 <sup>ABa</sup>	13.77 <sup>Aa</sup>	0.41 <sup>ab</sup>	4.06 <sup>ab</sup>	33.80
HC	14.78 <sup>Aa</sup>	14.50 <sup>Aa</sup>	0.48 <sup>a</sup>	3.99 <sup>b</sup>	32.20
MSD 1	1.7	1.76		0.34	7.09
MSD 2	1.31		0.08		

The same letter, uppercase in the columns and lowercase in the rows, indicates that the results do not differ by Tukey test at 5% probability. MSD 1 - minimum significant difference for the treatment means in the columns. MSD 2 - minimum significant difference for the means of treatments in the rows.

both productive cycles. The fruits had a greater average length of 65.31 mm (2018) when compared to 63.09 mm in the first cycle (2017), regardless of the treatment used. This performance also appeared in the LD/ED ratio, where the highest data were also observed during the second evaluation cycle. The same result was presented in the LD/ED ratio, where the highest results were also detected during the second evaluation cycle.

## The chemical analysis of the fruits

A significant interaction of compounds and evaluation cycles was observed in the soluble solids content of the fruits and the compounds individually affected the pH and titratable acidity. The compounds did not influence the maturation index of the fruits, with values from 32.16 to 36.85 (Table 4). HC presented the highest contents of soluble solids (14.78° Brix) during 2017 evaluation cycle

and there were no significant differences between compounds in 2018, since the contents of soluble solids stayed from 13.77 and 15.27 °Brix (Table 4).

NF + CN and CN treatments showed peach fruits with 0.45 g of citric acid/ 100 g of pulp and 0.41 g of citric acid/ 100 g of pulp, respectively. These values not differed from HC and control treatments. The control presented less titratable acidity (0.38 g of citric acid/ 100 g of pulp) and consequently, higher pH, in comparison with HC. There was no significant interaction between compounds and crop seasons for the bioactive compounds. However, the compounds to induce sprouting had effect in the antioxidant activity and the content of total phenolic compounds in fruits. In all treatments, the content of total flavonoids varied from 9.15 to 11.07 mg 100 g<sup>-1</sup> (Table 5).

NF + CN (126.77 mg 100 g<sup>-1</sup>) and HC (106.08 mg 100 g<sup>-1</sup>) increased the antioxidant activity and the total phenolic compounds (532.74 and 505.35 mg 100 g<sup>-1</sup>, respectively) contents of the fruits, in relation to the

Compounds	Total flavonoids	Antioxidant activity	Total phenolic compounds
Control	9.15 <sup>ª</sup>	82.68 <sup>c</sup>	288.98 <sup>c</sup>
NF + CN	9.90 <sup>a</sup>	126.77 <sup>a</sup>	532.74 <sup>a</sup>
CN	10.17 <sup>a</sup>	91.80 <sup>bc</sup>	340.84 <sup>bc</sup>
HC	11.07 <sup>a</sup>	106.08 <sup>ab</sup>	505.35 <sup>ab</sup>
MSD	4.09	20.23	179.29

**Table 5.** Total flavonoids (mg 100 g<sup>-1</sup>), antioxidant activity (mg 100g<sup>-1</sup>) and total phenolic compounds (mg 100g<sup>-1</sup>) in 'Douradão' peach fruits as a function of compounds sprays to induce sprouting during two crop seasons.

The same letter in the columns indicates that the results do not differ by Tukey test at 5% probability.

control. These favourable data represent an improvement of about 34.8% in antioxidant activity and 45.7% in total phenolic compounds with HC + CN sprays and 22.06% in antioxidant activity and about 40.6% in total phenolic compounds in peach fruits through HC sprays to induce sprouting.

# DISCUSSION

The 2017 crop season presented better climatic conditions to the tree performance and production of the fruits in comparison with 2018. Such outcome that compromised the trees performance could be explained by climate changes that happened during the second evaluation crop season and was related by the higher precipitations and winds that occurred in August 2018, exactly during the period of resumption of plant growth, when they were in the sprouting and flowering stages, impairing the branches growth and led to intense flower fall, decreasing the fruit set and fruit yield.

According to Pio et al. (2019) the cultivation of peach trees has been extended to non-traditional areas in the subtropical and tropical regions, which not have well defined climatic seasons and sometimes are subject to sudden and adverse weather changes, compromising the crop loading. Besides that unpredictable weather changes, a major problem in the cultivation of most deciduous fruit trees in subtropical regions is the lack of chilling hours to break bud dormancy, unless the trees were exposed to sufficient chilling in winter (Ímrak et al., 2016) affecting sprouting and flowering of the branches (Ghrab et al., 2014; Fadón et al., 2020) and consequently, reducing the crop performance (Atkinson et al., 2013) and the fruit quality (Di Vaio et al., 2014).

The lowest yield observed in the control treatment lead a hypothesis that the trees did not have their chill requirements completely satisfied. Uniform bud break and flowering is essential to improve tree productivity and growth as well as to reduce the length of time for harvest to occur (Coletti et al., 2011).

Hydrogen cyanamide presented the best yield and production efficiency performance reinforced the reason because of it has been widely used to break bud dormancy in peach orchards and its application response is generally associated with the action of amino acids and plant hormones (Seif El-Yazal et al., 2014). Amino acids are the exchange currency of nitrogen between sources and absorbent tissues in plants and an important source of components used for cell growth differentiation (Couturier et al., 2010). Nitrogen compounds, including amino acids, were constituted at low levels in the buds during the dormancy stage and reached maximum levels just before bud opening (Imrak et al., 2016).

Seif El-Yazal et al. (2014) reported an increase in the amino acids contents, such as proline and arginine and of growth promoting hormones, such as auxins and gibberellins, in the buds of 'Anna' apple with HC 6% sprays. The biochemical changes observed in HC were associated with an increase in flowering and fruit production when compared to the control. The mechanisms of action in HC are not yet clear. Some studies demonstrated its inhibitory property on catalase activity, which led to increased levels of hydrogen peroxide  $(H_2O_2)$  in buds (Pérez and Lira, 2005); therefore, promoting oxidative stress that overcomes dormancy (Pinto et al., 2021). The product application inhibited the enzymes activity that is involved in the reactive oxygen species formation. Catalase, within these enzymes, is responsible to decompose hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into molecular oxygen and water. Such inhibition may provide a transient increase in the content of H<sub>2</sub>O<sub>2</sub> in the HC-sprouted buds. Therefore, there is a reorientation of the carbon flow towards the pentose cycle and a high cellular relationship of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) (Pinto et al., 2021).

The greatest HC efficiency is associated with the presence of -C≡N radicals in its composition, which is considered more reactive than the other compounds sprouting inducers (Segantini et al., 2015; Pinto et al., 2021). This way of HC action may possibly explain its best responses in breaking dormancy, induction and standardization of sprouts in 'Douradão' peach trees, when compared with other compounds and the control. The best efficiency of HC in 'Anna' apple trees was also observed by Seif El-Yazal et al. (2014), who concluded that HC presented higher plants production than

potassium nitrate, CN, thiourea and control treatments.

NF + CN are also considered as an alternative to induce budburst in peaches in mild winter regions. NF + NC stand out as a new generation of products to induce the bud emergence. These compounds also offer less harm than toxic chemicals in the environment (Petri et al., 2014). Erger® is a mixed mineral fertilizer composed of inorganic nitrogen, carbohydrates (mono and polysaccharides), calcium and diterpenes (Valagro, 2021). These components can increase sprouting in the branches by stimulating cellular respiration, reactivating nitrogen metabolism, cell division and elongation (White and Broadley, 2003; Cantón et al., 2005; Ímrak et al., 2016). These processes occur intensely during the resumption of plant growth. The activation of these metabolic pathways is associated with the genes expression that induce sprouting (Vergara and Perez, 2010) from the synthesis of growth promoting hormones (gibberellins and auxins), amino acids and biogenic amines (Mohamed et al., 2010; Seif El-Yazal and Rady, 2013).

NF could be used alone or combined with calcium nitrate (CN). The latter is used to induce bud emergence in peach trees that are grown in mild winter regions (Ferreira et al., 2019) and during cherry tree dormancy improving fruit quality at harvest (Michailidis et al., 2021).

El-Sabagh et al. (2012) stated that dormancy breaking treatments were found to be effective by changing the polyamines and nitrogen in the buds. Several studies observed physiological changes in fruit trees when chilling requirements were satisfied or compounds to induce sprouting was applied. These changes are related to respiratory activity, synthesis of plant growth hormones, carbohydrate dynamics and biogenic amines, since nitrogen compounds are mainly formed through decarboxylation of amino acids (Mohamed et al., 2010; Seif El-Yazal and Rady, 2013). The data obtained for all compounds evaluated are also interesting considering the anticipation and concentration of the harvest period. This is because of the concentration of the harvest occurred on days with less rainfall, leading to improvements in the soluble solids content and in the maturation index, in addition to the phytossanitary aspects. Producers often emphasize fruit quality characteristics that provide high yield, large fruit size, disease resistance and ease of harvest in minimum picking intervals (Palmer, 2011; Matias et al., 2013; Raseira et al., 2018).

The use of chemical compounds to induce bud break and their effects are lower explained on fruit quality. Thus, there are few reports and sometimes can be divergent. Pio et al. (2019) mentioned that the products can negatively impact fruit quality. According to Hauagge (2000), although it is possible to break dormancy with chemical substances, growth production and fruit quality are generally lower than those obtained in adapted cultivars (Luedeling, 2012). The author emphasize that the factor that determine the adaptation of peach trees in the tropics and subtropics is the ability to produce quality fruits at temperatures that are often warmer than in their region of origin and the fruit quality is higher in orchards that are not treated with bud break inducers compared to treated orchards.

A hypothesis for these reports is that the compounds to induce sprouting can increase competition between vegetative and reproductive structures of trees (Segantini et al., 2015). The vegetative development can compromise the availability of carbohydrates reserves during the fruit growth period, reducing fresh matter accumulation, consequently reducing the fruit mass and fruit diameter. Nevertheless, this effect can be contradictory, as observed in first evaluation cycle when there were no significant differences between the treatments evaluated and in the second evaluation cycle these differences occurred, when compounds were applied.

Sprouting inducing compounds effects on fruit quality would be indirect, due to the uniformity and concentration of sprouting, flowering and fruiting, reducing the harvest period (Bettiol Neto et al., 2014). Differences in chemical variables of fruit quality, such as pH, titratable acidity, soluble solids and maturation index, are probably associated with the concentration of the harvest season, which can have a positive or negative effect, depending on the climatic conditions in which the fruits' ripening occurs and can also vary annually. The highest levels of soluble solids are associated with climatic conditions of the growing regions, since high temperature leads to sweeter peach fruits (Ímrak et al., 2016; Leonel et al., 2014).

The yield of well-cultivated trees depends on the total light interception by each part of the canopy structure (Palmer, 2011; Matias et al., 2013). This important effect arises because photosynthetic carbon fixation is a function of the sunlight captured by a tree or orchard. Inside the tree canopy, fruit quality changes in response to its architectural position. In general, fruit size and quality decrease from the top to the lower layers of tree canopy, but there is high variability in growth of individual fruits within tree canopies, even when fruit to fruit competition is minimized (Minas et al., 2018).

Peach is one of the most popular stone fruits suitable for direct consumption and an interesting material for food industry, being considered a rich source of bioactive compounds and therefore, capable of inducing some prohealth effects (Nowicka and Wodjdylo, 2019). Peach carotenoids act as pigments and are also involved in the protection of plant cells against oxidative stress (Asami et al., 2003).

The HC and NF + CN promoted an increase in total phenolic compounds and in antioxidant activity in both crop seasons. These results are due to the positive correlation that exists between these two variables, suggesting that total phenolic compounds are mostly responsible for the antioxidant activity in peach fruits (Zhao et al., 2015). Some studies also have already presented positive correlations between total phenolic contents and antioxidant activity in fresh figs (Solomon et al., 2006; Veberic et al., 2008).

These results are very interesting considering that the compounds from secondary metabolism play an important role in the interaction of plants with the environment. Polyphenols are secondary metabolites involved in various physiological functions, such as plant disease resistance from pathogens, protection against ultraviolet radiation and colouring several parts of plant structures, such as flowers and fruits (Oliveira et al., 2016).

Products of secondary metabolism also have protective action against abiotic stresses, such as those associated with changes in temperature, water content, levels of light, UV exposure and mineral nutrient deficiency (Perez, 2021). In food research, there is an ingoing popularity to the antioxidant capacity of these compounds, since they can scavenge free radicals, which are harmful to the health (Nascimento et al., 2011).

The phenolic compounds are substances that have at least one aromatic ring in which at least one hydrogen is replaced by a hydroxyl group. These compounds are synthesized from two routes major metabolic pathways: the shikimic acid pathway and the mevalonic acid pathway, which is less significant in superior plants (Perez, 2021). The composition of phenolic compounds results from the partial oxidation of sugars through glycolysis and tricarboxylic acid cycle (Soethe, et al. 2016) and varies among species and cultivars (Oliveira et al., 2016). The biosynthesis and accumulation of bioactive compounds depends on management practice, weather changes, ripening stages and storage conditions (Leong and Oey, 2012).

Though peach trees can be grown in regions with little winter cold, the development and bud emergence can be erratic, which hinders production, since there will be lower percentages of budding and flowering (Alves et al., 2012; Citadin et al., 2014), besides prolonging the phenological stages (Ghrab et al., 2014). Therefore, compromising both production and fruits development until their physiological maturation (Atkinson et al., 2013), which will later reflect on the phenols contents and antioxidant activity, as presented in our study.

The influence of induce sprouting compounds on the peaches biochemical variables still is unknown or not well explained. Nonetheless, it is a common knowledge that these attributes may be influenced by the management techniques adopted in the orchards, which can lead a concentration of the flowering and harvest period (Citadin et al., 2014; Pio et al., 2019). In this experiment, the harvest can be done earlier, in months with less rainfall and more sunny days allowing favourable results for the total phenolic compounds and antioxidant acitivity. The ultraviolet radiation significantly affects plant cells,

especially UV-B; thus, radiation increases in phenolic contents (Meyers et al., 2003), explaining why all fruits showed high values of polyphenols and flavonoids at high temperatures, as it is associated to radiation exposures (Lima et al., 2013; Di Vaio et al., 2014).

Ferreira et al. (2019) found increased antioxidant activity in peach fruits (200 mg 100 g<sup>-1</sup> TEAC) at 1.90% NF + 4% CN. According to the authors, this result may be associated with the fact that the applied concentration provided more adequate sprouting to the vegetative branches of the trees, allowing a greater balance between their reproductive and vegetative structures, enhancing some qualitative attributes of the fruits, during their growth and maturation stage.

# Conclusion

The results confirmed the efficiency of HC (0.6%), as well as evidenced the use of NF (2.5%) + CN (4%) as an alternative for inducing sprouting of the 'Douradão' peach tree in temperate warm climate. The quality of the fruits was enhanced by the sprays of sprouting inducers, so that the spraying of HC and NF + CN increased the contents of total phenolic compounds and, consequently, resulted in peaches with greater antioxidant activity.

# **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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