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CROP PRODUCTION

'Navelina' oranges submitted to pre-harvest resistance inducers

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ABSTRACT. The objective of this study was to evaluate the physical-chemical characteristics, rot index and bioactive compounds of 'Navelina' oranges under postharvest refrigerated storage conditions after pre-harvest resistance induction in crops from 2015 and 2016. The field experimental design was completely randomized blocks. The treatment factors were composed of the following resistance inducers: noresistance inducer (control), selenium (Se), silicon (Si), acibenzolar-s-methyl (ASM), methyl jasmonate (MeJa), thiamethoxam (TMT) and imidacloprid (IMI). In the laboratory, the experimental design was the same as that in the field, but it used a two-factor scheme instead of a unifactorial scheme. In the two-factor scheme, factor A was composed of the abovementioned resistance inducers, and factor B was composed of the refrigerated storage periods (zero and 30 days). The analyses investigated the coloration, fresh mass loss, rot index, soluble solids, pH, titratable acidity, SS/TA ratio, ascorbic acid, total phenolic compounds and antioxidant capacity of the oranges. The application of pre-harvest resistance inducers was efficient in maintaining the physical-chemical characteristics of the 'Navelina' oranges in postharvest, increasing their bioactive compounds in comparison to the control. The resistance inducers Se, Si, MeJa, and IMI reduced rot rates, while ASM and MeJa prevented fresh fruit mass loss.

Keywords: *Citrus sinensis*; navel orange; elicitors; refrigerated storage; rotting.

Laranjas 'Navelina' submetidas a indutores de resistência na pré-colheita

RESUMO. Objetivou-se avaliar as características físico-químicas, o índice de podridões e os compostos bioativos dos frutos da laranjeira 'Navelina' na pós-colheita sob armazenamento refrigerado, após indução de resistência na pré-colheita, nas safras de 2015 e 2016. O delineamento experimental a campo foi em blocos completos casualizados. Os fatores de tratamento foram compostos pelos indutores de resistência sem indutor (controle), selênio (Se), silício (Si), acibenzolar-s-metil (ASM), metil jasmonato (MeJa), tiametoxam (TMT) e imidacloprido (IMI). No laboratório, o delineamento experimental utilizado foi o mesmo a campo, porém em esquema bifatorial, onde o fator A foi composto pelos indutores supramencionados e o fator B, pelos períodos de armazenamento refrigerado (zero e 30 dias). As análises realizadas foram coloração, perda de massa fresca, índice de podridões, sólidos solúveis, pH, acidez titulável, razão SS/AT, ácido ascórbico, compostos fenólicos totais e capacidade antioxidante das laranjas. A aplicação dos indutores de resistência na pré-colheita foi eficiente na manutenção das características físico-químicas das laranjas de umbigo 'Navelina' na pós-colheita, proporcionando aumento dos compostos bioativos, em comparação ao controle. Os indutores Se, Si, MeJa e IMI reduzem os índices de podridões, enquanto, o ASM e MeJa preveniram a perda de massa fresca dos frutos.

Palavras chave: *Citrus sinensis*; laranja umbigo; elicitores; armazenamento refrigerado; podridões.

Introduction

The orange production area of Brazil is the largest in the world; however, compared to other countries, it has the 10th highest productivity. The orange sector is highly organized and competitive, accounting for 30% of the global production and comprising one of the largest centers of orange juice production; in addition, over 19 million tons of oranges are harvested per productive cycle (FAO, 2016). Orange trees are susceptible to various diseases, primarily citrus canker. These diseases cause economic damage to production, increasing costs. The most frequent procedure for disease control consists of the use of agrochemicals and resistant cultivars and the encouragement of positive cultural practices and crop management.

The induction of systemic acquired resistance (SAR) is a promising alternative for disease control, as it exploits a natural defense mechanism of plants. After application, SAR can confer long-term protection against a broad spectrum of microorganisms (David, Yinong, Cassiana, & Monica, 2010). Sensitive plants can acquire a greater ability to defend against

pathogen attacks from primary infection. This process involves a series of biochemical and physiological reactions that trigger the production of several secondary metabolites (Hall, Kim, & DeLuca, 2011).

The substances that promote SAR induction that are most commonly reported in the literatureare: acibenzolar-s-methyl, methyl jasmonate, selenium, silicon, and neonicotinoids. Acibenzolar-s-methyl (ASM), a functional analog of salicylic acid that can activate plant defenses, such as pathogenesis-related proteins, is largely used in apples (Quaglia, Ederli, Pasqualini, & Zazzerini, 2011) and citrus (Graham & Myers, 2011; Neto, Maraschin, & DiPiero, 2015). Methyl jasmonate (MeJa) is the methyl ester of the phytohormone jasmonic acidand shows promising results in SAR induction, interfering in the physiological and/or biochemical processes, a sign of the endogenous molecules of loquats (Cai, Cao, Yang, & Zheng, 2011; Cao, Cai, Yang, Joyce, & Zheng, 2014), pomegranates(Sayyari et al., 2011)and bananas(Zhao et al., 2012). Silicon plays multiple roles in cell growth and development, combining physical and chemical barriers such as cell wall lignification and the induction of defense proteins against diseases (French-Monar, Rodrigues, Kordorfer, & Datnoff, 2010), as observed in avocados (Tesfay, Bertling, & Bower, 2011), cotton (Oliveira et al., 2012) and tomatoes (Andrade et al., 2013). Selenium is absorbed and transported by plants in the form of selenite, presenting a high antioxidant capacity and the induction of plant defense systems (Hasanuzzaman, Nahar, & Fujita, 2014). Recently, substances such as imidacloprid neonicotinoids (IMI) and thiamethoxam (TMT) have been used with success in inducing SAR in pomegranates (Graham & Myers, 2011; Bagio, Canteri, Barreto, & Júnior-Leite, 2016).

Therefore, the occurrence of diseases is one of the main factors of losses in orange production in all regions of Brazil, particularly in the southern region, where the amount and frequency of rainfall are high. Studies are necessary to investigate the application of resistance inducers during the pre-harvest period to promote fruit conservation, reduce pesticide applications and increase the levels of beneficial bioactive compounds in fruit to humans. This study aimed to evaluate the physical-chemical characteristics, rot indexes and bioactive compounds of 'Navelina' oranges under postharvest refrigerated storage after resistance induction in the pre-harvest 2015 and 2016 crops.

Material and method

Resistance inducers were applied in the 2015 and 2016 crops of a commercial orchard of 'Navelina' oranges (*Citrus sinensis* (L.) Osbeck) in Santa Silvana, the 6th district of the municipality of Pelotas, Rio Grande do Sul State, Brazil (31°25'58"S and 52°16'58" and 193 meters). The soil in the region, which is classified as red-yellow argisoil, is moderately deep, with medium texture in the A horizon and a clayey texture in the B horizon (Santos et al., 2006). The climate features a Cfa classification (Köppen & Geiger, 1928), i.e., a temperate or humid subtropical climate with hot summers and an average annual rainfall of 1,582 mm, average annual temperature of 17.7°C and average annual relative humidity of 78.8% (INMET, 2016).

The plants (4 years old) were installed under trifoliate rootstock (*Poncirus trifoliate* (L.) Raf.) with 6 m spacing between rows and 4 m between plants. The experimental field was handled in accordance with the requirements of integrated production for citrus (Marodin & Schafer, 2009). To the orchard, the fungicide Nativo® (trifloxystrobin and tebuconazole) was applied three times at an interval of 30 days, with the first application in the phenological stage of the newly formed fruit lets. In addition, the Bordeaux mixture (copper sulfate and lime) was used with six applications spaced 45 days, beginning during the flowering and finishing 60 days before harvest.

For the application of resistance inducers, the experimental design of the field used completely randomized blocks, with five replicates three plants per plot, and the evaluation of the central plant with a unifactorial scheme. The treatment was composed of resistance inducers [no resistance inducer (control, water), selenium (Se, 10 mg L^{-1}), silicon (Si, 400 mg L^{-1}), acibenzolar-s-methyl (ASM, 100 mg L^{-1}), methyl jasmonate (MeJa, 10 mg L^{-1}), thiamethoxam (TMT, 2000 mg L^{-1}) and imidacloprid (IMI, 714 mg L^{-1})].

The resistance inducers were applied during three applications in the orchard ata 15-day interval, with applications occurring 45, 30, and 15 days before harvest using the total dosage. The Si, ASM and MeJa products were applied by spraying with Coastal Spray (Guarani®) with a flat fan nozzle and fine droplet size (101-200 μ) in the entire plant canopy, avoiding run-off. A total of 0.1% non-ionic adhesive spreader Silwet L-77® was added. For the Se, TMT and IMI resistance inducers, syrups were prepared with water for each product and applied to the soil around the plant canopy.

When they reached commercial maturation, the oranges were collected randomly in four quadrants of the tree canopy, placed in plastic boxes, cleaned and sanitized, and transported to the Agronomy Laboratory, Department of Plant Science at the Universidade Federal de Pelotas (UFPel), where they underwent a standardized pre-screening by removing the damaged fruit. The fruit were then submitted to pre-cooling (15 ± 2°C) for 24 hours.

In the lab, the design used was the same established in the field but in a two-factorial scheme, where factor A was composed of the same resistance inducers described previously and factor B was composed by the storage periods(zero and 30 days). Time zero corresponded to the fruit that were not subjected to storage, and the 30-days to rage corresponded to refrigerator storage at 5 ± 1°C, under 85-95% relative humidity. After removal from the chamber, the fruit were submitted to a simulation of commercialization time, 7 days at 20±1°C. For each treatment, three replicates were used with 20 oranges each, and the same number of repetitions was used in the refrigerated storage, totaling 840 fruits.

The coloration was measured with a Minolta colorimeter CR-300, with the reading system CIE L⋆ a⋆ b⋆ and the chromatic tone represented by the hue angle (h^o) through the arctangent formula b⋆/a⋆. The result of this equation, expressed in radians, was then converted to degrees (Minolta, 1994). The fresh fruit loss was obtained by the difference between the initial and final mass of fruit in the cold storage period, and the values were expressed in percentages (%). The rot index was established by the percentage of fruit attacked by pathogens through the visual inspection of fruit, where fruit with lesions greater than or equal to 5 mm were considered to have rot. Both evaluations were conducted after 30 days of refrigerated storage. Soluble solids (SS) were quantified with a digital Refractometer (Atago®) model PAL-1, and the results were expressed in °Brix. The hydrogen potential (pH) was measured with a digital pH meter (Digimed®). For titra table acidity (TA), 10 mL of orange juice was added to 90 mL of distilled water. The sample titration was made with the aid of a digital burette (Brand®) containing a sodium hydroxide solution (0.1 N) up to pH 8.1. The titra table acidity was expressed as the percentage of citric acid. The SS/TA ratio of oranges was expressed by the relationship between the soluble solids and titra table acidity (SS/TA) (Zenebon, Pascuet, & Tiglea, 2008). The ascorbic acid content was quantified through the official AOAC (1997) method by oxidative titration with 2.6-dichlorophenol in dophenol (DCFI), in which the titration point is detected by the appearance of pink coloration, and the result is expressed in mg ascorbic acid per 100 g of the sample (Jacobs, 1958; Leme & Malavolta, 1950).

To analyze the phenolic compounds and antioxidant capacity of the fruit, the exocarp or epicarp (peel) was separated from the endocarp (pulp) and evaluated separately to monitor translocation in the fruit (Chitarra & Chitarra, 2005). Total phenolic compounds were quantified using the Folin-Ciocalteau reagent, as described by Swain and Hillis (1959), and expressed in mg of chlorogenic acid equivalent (CAE) per 100 g^{-1}. The antioxidant capacity was determined by spectrophotometry, according to a method adapted from Brand-Williams, Cuvelier, and Berset (1995), and the results were expressed as μg of Trolox equivalent antioxidant capacity (TEAC) g^{-1}.

The 2015 and 2016 crops were used as replicates. The data were analyzed for normality by the Shapiro-Wilk test and homoscedasticity by the Hartley test. Subsequently, the data were submitted to an analysis of variance ($p \leq 0.05$). To determine significance, the effects of the resistance inducers were analyzed by the Tukey test ($p \leq 0.05$), and the effects of the storage period were analyzed by the t test ($p \leq 0.05$). To compare the control with the resistance inducers, the Dunnett test ($p \leq 0.05$) was carried out. The presence of correlations between the dependent variables of this study was analyzed with the Pearson correlation coefficient (r) ($p < 0.0001$).

Result and discussion

For color variables (L⋆ and b⋆), soluble solids (SS) and ascorbic acid, there were interactions with the treatment factors tested (Tables 1 and 2), while the color expressed by a⋆ and the hue angle, the pH, the titratable acidity (TA) and the ratio of SS/TA only had significant interactions with the main effects of the storage period (Table 3). The applications of resistance inducers did not change the luminosity coloration of 'Navelina' oranges, as expressed in L⋆ coordinates, in either assessment period; however, L⋆ values decreased over the storage period for degradative processes in all but the TMT resistance inducer treatments. Compared to the control, all resistance inducers maintained the fruit luminosity levels (L⋆), except for the MeJa treatment at day zero, which had a higher level (Table 1). As observed in this study, the storage effect also reduced the luminosity parameters of

'Valencia Delta' fruit submitted to resistance inducers (Pereira, Machado, & Costa, 2014). An investigation on the effectiveness of the MeJa resistance inducer applied during pre-harvest in mangoes (*Mangifera indica* L.) showed uniform development of the red color in the peel after harvesting, with an increase in the L⋆ and a⋆ values (Muengkaew, Chaiprasart, & Warrington, 2016). This increase is possibly due to MeJa resistance inducer performance in accumulating certain proteins related to pathogenesis, thus promoting metabolic changes that keep color strength in oranges (Brinceño et al., 2012).

Regarding the coloration values of the b⋆ coordinate, the highest values determining the intensity of yellow-orange in oranges were produced by the resistance inducers Se, MeJa and IMI at day zero, with no significant resistance inducer effects observed at 30 days of refrigerated storage (Table 1). For storage purposes, ASM and TMT resistance inducers increased the b⋆ intensity in the fruit. When compared to the control, differences were found for the ASM, TMT and IMI treatments only at the end of the storage period.

Table 1. Coloration of 'Navelina' orange fruit with different resistance inducers applied during pre-harvest. Coloration is expressed by luminosity level (L⋆) and intensity of yellow-orange (b⋆). Storage period data represents refrigerated storage with a subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Resistance Inducers	L⋆			b⋆		
	Storage period					
	0	30		0	30	
Control	69.32	64.51		69.32	64.51	
Selenium	71.42	aA[1]^{ns}	66.48 aB^{ns}	71.42	aA[1]^{ns}	66.48 aB^{ns}
Silicon	71.19	aA^{ns}	66.88 aB^{ns}	71.19	aA^{ns}	66.88 aB^{ns}
Acibenzolar-s-methyl	70.74	aA^{ns}	67.17 aB^{ns}	70.74	aA^{ns}	67.17 aB^{ns}
Methyl Jasmonate	72.67	aA⋆	67.40 aB^{ns}	72.67	aA⋆	67.40 aB^{ns}
Thiamethoxam	70.30	aA^{ns}	68.41 aA^{ns}	70.30	aA^{ns}	68.41 aA^{ns}
Imidacloprid	71.39	aA^{ns}	67.62 aB^{ns}	71.39	aA^{ns}	67.62 aB^{ns}
C.V. (%)	3.0			3.0		

[1]Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$) that compared the effects of the resistance inducers in each storage period. Means followed by the same uppercase letter in the row do not differ by the t test ($p \leq 0.05$) comparing the storage of each resistance inducer. ⋆ and ^{ns} mean significant and not significant, respectively, in relation to the control (no resistance inducer) by the Dunnett test ($p \leq 0.05$). C.V: coefficient of variation.

Regarding the soluble solids (SS) of the 'Navelina' orange fruit, there were no differences among the resistance inducers in either assessment period (Table 2). However, the oranges treated by the ASM resistance inducer showed an increase in sugar contents during the storage period. In addition, there were no differences between the treatments and control in either assessment period (zero and 30 days) and no effects on sugar metabolism throughout the storage period.

The applied resistance inducers did not affect the ascorbic acid levels in each period. However, for the ASM treatment, the reduction of these levels during refrigerated storage caused degradation during fruit ripening. Fruit treated with the resistance inducers Se and IMI showed higher levels of ascorbic acid compared to the control in both assessment times. In comparison, the ascorbic acid levels of the MeJa treated fruit were higher only at 30+7 days (Table 2). Other studies have shown that storage of 'Pera Bianchi' oranges was linked with increases in the fruit ascorbic acid levels from 48.89 mg 100 mL^{-1} at 15 days to 56.76 mg 100 mL^{-1} at 45 days of storage (Rosa, Clemente, Oliveira, Todisco, & Costa, 2016).

Table 2. Soluble solids (°Brix) and ascorbic acid (mg 100 g^{-1}) of 'Navelina' orange fruit treated by resistance inducers during pre-harvest. Data are shown for periods of refrigerated storage with a subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Resistance Inducers	Soluble Solids (°Brix)			Ascorbic Acid(mg 100 g^{-1})		
	Storage period					
	0	30		0	30	
Control	10.15	11.23		44.69	43.49	
Selenium	10.91	aA^{ns}	11.58 aA^{ns}	51.78	aA⋆	49.44 aA⋆
Silicon	10.75	aA^{ns}	11.06 aA^{ns}	48.21	aA^{ns}	46.72 aA^{ns}
Acibenzolar-s-methyl	10.16	aA^{ns}	10.48 aA^{ns}	48.36	aA^{ns}	45.72 aB^{ns}
Methyl Jasmonate	10.41	aA^{ns}	10.46 aA^{ns}	49.10	aA^{ns}	47.69 aA⋆
Thiamethoxam	10.40	aA^{ns}	11.33 aA^{ns}	47.91	aA^{ns}	46.48 aA^{ns}
Imidacloprid	10.73	aA^{ns}	11.18 aA^{ns}	52.23	aA⋆	48.86 aA⋆
C.V. (%)	5.5			6.0		

[1]Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$) that compared the effects of the resistance inducers in each storage period. Means followed by the same uppercase letter in the row do not differ by the t test ($p \leq 0.05$) comparing the storage of each resistance inducer. ⋆ and ^{ns} mean significant and not significant, respectively, in relation to the control (noresistance inducer) by the Dunnett test ($p \leq 0.05$). C.V: coefficient of variation.

The a⋆ coordinate for orange fruit coloration intensified throughout the storage period, with the orange color becoming more reddish. Based on the hue angle, the fruit lost its typical yellowish coloring. Similarly, the pH of oranges increased throughout the storage period (Table 3), atypical feature of the cultivar studied (Koller, 2013). With ripening, oranges lost acidity, as shown by a rapid increase of the pH, the inverse of the hydrogen ion concentration used in respiration and ripening. There was a reduction in the levels of citric acid and the ratio of SS/TA with storage time (Table 3), which consequently reduced the fruit flavor. In studies conducted on 'Valencia Delta' oranges during storage at room temperature, the application of postharvest coating was associated with increased acidity (citric acid) and the SS/TA ratio in oranges, while the coloration tone (hue angle) decreased over the storage period (Pereira et al., 2014).

Table 3. Coloration(a⋆ and hue angle), pH, titra table acidity (% citric acid) and SS/TA ratio in 'Navelina' oranges over a refrigerated storage period with the subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Variables	Storage period		C.V.
	0	30	(%)
a⋆	13.85 b[1]	21.12 a	29.29
Hue angle	78.35 a	73.31 b	5.18
pH	3.46 b	3.60 a	3.90
Titratable acidity (% citric acid)	1.02 a	0.93 b	12.48
SS/TA ratio	11.40 a	10.86 b	10.43

[1]Means followed by the same lowercase letter in the row do not differ by the t test ($p \leq 0.05$) comparing the storage periods. C.V: coefficient of variation

The ASM and MeJa resistance inducer treatments differed from the control after 30 days of refrigerated storage with subsequent simulation of commercialization time (7 days at 20 ± 1°C) (Table 4). In another study, the application of salicylic acid activated the synthesis of secondary metabolites, promoters of systemic resistance; however, it did not affect the biomass loss of fresh fruit (Borsatti, Mazaro, Danner, Nava, & Dalacosta, 2015), which corroborates the results of this work.

Regarding rot rate after 30 days of refrigerated storage, the ASM and TMT resistance inducer treatments did not differ from the control (Table 4). However, the treatments with other resistance inducers were efficient in rot control in the studied period, signaling defense responses and inducing biosynthesis substances generating physical and chemical barriers. In another study investigating 'Satsumas' tangerines stored at 14 ± 2°C, the application of resistance inducers in the postharvest period reduced rot significantly during the first six days of storage (Zhu et al., 2015).

The total phenolic compounds and antioxidant capacity of both peel and pulps howed interactions with the treatment factors tested (Tables 5 and 6). At day zero, the Si and ASM resistance inducer treatments showed higher levels of total content of phenolic compounds in the pulp than the others (Table 5). However, at 30 days, there was no significant difference between the resistance inducer treatments. When comparing the resistance inducers with control, only the IMI treatment did not differ in either assessment period evaluated. Previous studies have shown that resistance inducers increase the demand of enzymes for the biosynthesis of phenolic compounds needed to fight pathogens (Oliveira, Varanda, & Félix, 2016).

Table 4. Fresh mass loss (%) and rot index (%) of 'Navelina' oranges treated with resistance inducers in the pre-harvestperiod of the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Resistance inducers	Fresh mass loss (%)	Rot index (%)
Control	8.43 ab[1]	6.66a
Selenium	9.75 A	1.66b
Silicon	8.45 Ab	0.83b
Acibenzolar-s-methyl	6.71 B	5.03ab
Methyl Jasmonate	6.46 B	0.83b
Thiamethoxam	7.91 ab	5.03ab
Imidacloprid	7.01 ab	0.83b
C.V. (%)	30.2	124.7

[1]Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$). C.V: coefficient of variation.

In the case of the phenolic compounds in the pulp, there was no difference between the resistance inducer treatments at both zero and 30 days after cold storage (Table 5). Similarly, storage period had no effect on the phenolic compounds in the pulp. However, when compared with control, fruit from the Se and Si resistance inducer treatments showed higher values in the two assessment periods. The application of these resistance inducers in postharvest raises levels of phenolic compounds in plant tissues, which usually have antioxidant properties that are highly beneficial to humans (Romanazzi et al., 2016). The Se and Si resistance inducers confer tolerance to oxidative stress by strengthening the defense system in plants through increased antioxidant capacity (Hasanuzzaman, Nahar, & Fujita, 2014).

Table 5. Total phenolics (mg CAE 100 g^{-1}) in the pulp and peel of 'Navelina' oranges treated by resistance inducers applied in pre-harvest. The storage period represents a period of refrigerated storage with the subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Resistance inducers	Total phenolics in pulp		Total phenolics in peel	
	(mg CAE 100 g^{-1})			
	Storage period			
	0	30	0	30
Control	108.94	85.82	367.74	350.58
Selenium	129.02 dA[1] ⋆	106.26 aB ⋆	425.44 aA ⋆	409.61 aA ⋆
Silicon	161.38 aA ⋆	103.95 aB ⋆	424.32 aA ⋆	411.98 aA ⋆
Acibenzolar-s-methyl	147.22 abA ⋆	104.37 aB ⋆	403.28 aA [ns]	386.44 aA [ns]
Methyl Jasmonate	134.18 bcA ⋆	102.57 aB ⋆	395.01 aA [ns]	380.34 aA [ns]
Thiamethoxam	135.59 bcA ⋆	113.02 aB ⋆	389.13 aA [ns]	371.29 aA [ns]
Imidacloprid	117.89 dA [ns]	98.03 aB [ns]	375.56 aA [ns]	359.56 aA [ns]
C.V. (%)	7.2		7.4	

[1]Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$) that compared the effects of the resistance inducers in each storage period. Means followed by the same uppercase letter in the row do not differ by the t test ($p \leq 0.05$) comparing the storage of each resistance inducer. ⋆ and [ns] mean significant and not significant, respectively, in relation to the control (noresistance inducer) by the Dunnett test ($p \leq 0.05$). C.V: coefficient of variation.

Table 6. Antioxidant capacity (DPPH, μg TEAC g^{-1}) in the pulp and peel of 'Navelina' oranges treated by resistance inducers applied in pre-harvest. The storage period represents a period of refrigerated storage with the subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops. Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

Resistance inducers	DPPH in pulp		DPPH in peel	
	(μg TEAC g^{-1})			
	Storage period			
	0	30	0	30
Control	241.64	127.31	351.08	232.41
Selenium	389.23abA ⋆	153.00aB ^{ns}	455.54aA ⋆	281.54abB ⋆
Silicon	453.18aA ⋆	150.08aB ^{ns}	472.54aA ⋆	303.19aB ⋆
Acibenzolar-s-methyl	363.39bA ⋆	138.14aB ^{ns}	421.29aA ^{ns}	247.29bB ^{ns}
Methyl Jasmonate	397.58abA⋆	159.06aB ^{ns}	440.82aA⋆	268.49abB ^{ns}
Thiamethoxam	327.29bA ⋆	173.64aB ⋆	438.20aA ⋆	272.69abB ^{ns}
Imidacloprid	410.65abA ⋆	173.69aB ⋆	441.85aA ⋆	274.20abB ⋆
C.V. (%)	13.8		11.1	

[1]Means followed by the same lowercase letter in the column do not differ by the Tukey test ($p \leq 0.05$) that compared the effects of the resistance inducers in each storage period. Means followed by the same uppercase letter in the row do not differ by the t test ($p \leq 0.05$) comparing the storage of each resistance inducer. ⋆ and ^{ns} mean significant and not significant, respectively, in relation to the control (noresistance inducer) by the Dunnett test ($p \leq 0.05$). C.V: coefficient of variation.

The antioxidant capacity in the pulp was higher for the treatments with the resistance inducers Se, Si, MeJa, and IMI, differing from the others at day zero. At 30 days, there were no differences between the resistance inducer treatments (Table 6). A reduction in the antioxidant capacity of the oranges was observed during storage for all resistance inducers. However, the antioxidant capacity of the oranges treated with TMT and IMI differed from the control at day zero and at 30 days. Neonicotinoids induce a defense with increased bioactive compounds through the increased biosynthesis of enzymes primarily in young citrus plants, which keep this induction for a long period, (Graham & Myres, 2013).

The antioxidant capacity in orange peels, at day zero, showed no difference s among the resistance inducers; however, at 30 days, there was a reduction in the capacity with the application of the ASM resistance inducer (Table 6). Similar to the pulp, the antioxidant capacity in the peel decreased with storage time for all resistance inducers. Higher levels in the resistance inducer treatments than in the control were observed, mainly for the resistance inducers Se, Si and IMI at day zero. Induced resistance raises the synthesis of phenolic compounds in plant tissues through the stress caused by the resistance inducers that lead to changes in phenolic metabolism, as these compounds exhibit antioxidant properties (Wu et al., 2014; Orabi, Dawood, & Salman, 2015).

Regarding correlations between the treatments and measured variables, significant results were found for the total phenolics and antioxidant capacity variables, which showed the highest positive correlation coefficients (Table 7) for all resistance inducers used. These compounds confer an increase in receptors in the cell membrane, thus mimicking the inevitable phenomenon of electron leakage of chloroplasts, mitochondria and plasma membrane (Bhattacharjee, 2012; Sharma, Jha, Dubey, & Pessarakli, 2012). When there was an increase in the levels of total phenolics, there was also an increase in the antioxidant capacity of oranges. In this context, regarding the association between phenolic compounds and antioxidant capacity of orange pulp, the Si and ASM resistance inducers showed correlation coefficients that were higher than those in the control. A previous study showed that the application of resistance inducers to 'Fortune' tangerines in pre-harvest provided an increase in the gene expression and synthesis of phenolic compounds (Llorens, Scalschi, Fernández-Crespo, Lapeña, & García-Agustín, 2015).

The Se and Si resistance inducer treatments obtained correlation coefficients between the antioxidant capacities in the peel and pulp that were higher than those in the control, demonstrating that Se and Si promoted an increased antioxidant capacity that was transported from the peel to the pulp of the oranges (Table 7). This behavior is due to the powerful antioxidant capacity of phenolic compounds. In 'Valencia' and 'Lane Late' oranges, the application of resistance inducers in postharvest as a curative activity showed the positive effect of increasing bioactive compounds in citrus plants (Moscoso-Ramírez & Palou, 2013).

Table 7. Pearson correlation coefficients (r, $p < 0.0001$) among the total phenolic compounds (phenols) and antioxidant capacity (DPPH)in 'Navelina' oranges treated with the resistance inducers selenium (Se), silicon(Si), acibenzolar-s-methyl (ASM), methyl jasmonate (MeJa), thiamethoxam (TMT), and imidacloprido (IMI) that were applied in the pre-harvest period. Oranges were submitted to refrigerated storage with a subsequent simulation of commercialization time (7 days at 20 ± 1°C) in the 2015 and 2016 crops.Ufpel, Pelotas, Rio Grande do Sul State, Brazil.

	Control	Selenium	Silicon	ASM	MeJa	TMT	IMI
			DPPH in pulp				
Phenols inpulp	0.91844	0.75758	0.98504	0.95590	0.85551	0.78681	0.81085
			Phenols in pulp				
DPPH in peel	0.91922	0.69533	0.92491	0.90886	0.72638	0.70688	0.86271
			DPPH in pulp				
	0.96059	0.97714	0.96091	0.90057	0.91894	0.89481	0.95486

Conclusion

The application of resistance inducers in the pre-harvest period is an efficient method to maintain the physical-chemical properties of 'Navelina' oranges during postharvest, providing increased bioactive compounds in both the peel and pulp when compared to the control. The resistance inducers Se, Si, MeJa, and IMI reduce the rot index, while ASM and MeJa prevent the loss of fruit fresh mass.

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