STRATEGIES FOR INCREASING THE APPLE EPIDERMIS RED COLORED WITH PHYSIOGROW[®] COLOR

7 ABSTRACT

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> Aims: Evaluate different dosages and number of applications of the commercial product Physiogrow[®] Color on the percentage of red coloration of the epidermis, productivity and fruit quality in 'Royal Gala' apple trees, in the Midwest region of the state of Santa Catarina, Brazil. Study design: The experiment was arranged in a randomized block design with five replications. Place and Duration of Study: The experiment was carried out in the municipality of Fraiburgo-SC, Brazil (latitude 27º01'S, longitude 50º77' W, altitude 950 meters), during the growing seasons of 2017/2018 and 2018/2019. **Methodology:** The treatments were: Control (No application), Physiogrow[®] color (4, 8 and 12 L ha⁻¹) 7 days before harvest (DBH), Physiogrow[®] Color 2 L ha⁻¹ (30 DAC) + 2 L ha⁻¹7 DBH, Physiogrow[®] Color 4 L ha⁻¹ (30 DBH) + 4 L ha⁻¹7 DBH, and Physiogrow[®] Color 6 L ha⁻¹ (30 DBH) + 6 L ha⁻¹ 7 DBH. The variables evaluated were: Production (kg plant¹ and fruits plant¹), average fresh fruit mass (g), classification of fruits by percentage of red coloration of the epidermis (<50%, 50-80% and > 80%), firmness of the pulp (Ib in⁻²) and soluble solids ($^{\circ}$ Brix). The harvest was carried out on January 25, 2018 and February 16, 2019, first and second year, respectively. Plant production, as well as pulp firmness of fruits, were not affected by treatments, in both years. Physiogrow® Color promoted better distribution of fruits in categories of greater red coloration of the epidermis and reduction of the percentage of fruits in the category with

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coloration inferior to 50%, although a different behavior among the harvests was observed. The application of Physiogrow[®] Color 8 L ha⁻¹ 7 DBH contributes to the improvement of the coloration of 'Royal Gala' apples.

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10 Keywords: Malus domestica L., Maturation, Post-harvest.

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1. INTRODUCTION

In Brazil, apple tree cultivation has evolved a lot in the last years, mainly with the development of management techniques that have increased productivity and quality of fruit produced [1]. In 2017, Brazilian production was about 1.3 million tons, in a planted area of 33,138 hectares [2]. Being this production concentrated in the South Region of the country, with more than 2,300 producers involved [3]. The domestic market is the main destination of the fruits produced, mainly in fresh form, and only 7% for the exportation [4].

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The visual quality and size of the fruits play an important role in the national and international markets, since they establish the price of the apple when classified for packaging and export [5,6,7].

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According to Iglesias and Alegre [8], the most important visual quality attribute to determine the market value of red or bicolor apples is the amount and intensity of the red epidermis. Considering that at the time of purchase, consumers first analyze the visual appearance of the fruits, only later to consider the internal or nutritional characteristics of the fruits [9,10].

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The red color formation in the apple epidermis is linked to a complex series of interactions between environmental factors, orchard management, genetic characteristics of the cultivar and the stage of development of the fruit. The accumulation of anthocyanins corresponds to the genetic potential of the cultivar, which, in turn, is dependent on balanced nutrition and environmental factors such as light and temperature [11].

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For Iglesias et al. [12], the easiest and most economical way to improve the color of apples is to plant new cultivars or mutations of cultivars with greater potential to synthesize anthocyanins in the region of interest. The most planted clones of the 'Gala' apple tree, have a tendency to develop greater coloring of the fruits, however, they are still mostly bicolor.

44 In addition to orchard management techniques that aim to increase fruit 45 exposure to light, commercial products (chemical or natural), fertilizers and growth 46 regulators with biostimulant action, may promote increased red coloration of fruits. The 47 growth regulators based on ethephon release ethylene, a hormone bound to maturation, 48 increasing and intensifying the red coloration of the epidermis of apples [13]. However, 49 ethephon may increase the risk of fruit drop losses in preharvest and reduce storage 50 potential [14]. Some studies have shown that ethephon application may adversely affect 51 the starch hydrolysis rate, reducing pulp firmness, titratable acidity and increasing 52 respiratory rate, carotenoid biosynthesis and chlorophyll degradation [15,16].

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Blanke [17] reports that there are always new products on the market with high expectations to increase the anthocyanin synthesis and red color of the apple epidermis, especially leaf fertilizers and biostimulants. Fenili [8] reports the increase in red staining of apple fruits treated with foliar fertilizers based on potassium and micronutrients; however, their effects vary greatly from one year to the next, as well as changes in the quality and storage capacity of the fruits.

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61 Physiogrow[®] Color, which is an organic liquid leaf fertilizer based on free L-62 amino acids and organic acids, with potential to be used to improve the coloring of fruits 63 in the apple tree. Since it contains, in its formulation, precursor amino acids of ethylene

64 biosynthesis and anthocyanins, chlorophyllase and monosaccharide regulators.

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The objective of the present study was to evaluate different dosages and number of applications of the commercial product Physiogrow ® Color on the percentage of red coloration of the epidermis and fruit quality in 'Royal Gala' apple trees, in the Midwest region of the state of Santa Catarina.

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71 2. MATERIAL AND METHODS

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The experiment was conducted in an experimental orchard in the Midwest
Region of the state of Santa Catarina, Fraiburgo, Brazil (latitude 27º01'S, longitude
50º77 'W, altitude 950 meters).

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77 'Royal Gala' apple trees grafted on the Marubakaido rootstock with 15-year-old 78 M-9 grafts were harvested in the 2017/2018 and 2018/2019 seasons. The planting 79 density of the orchard used was 2,500 plants ha⁻¹, with a spacing of 4 m between rows 80 and 1 m between plants. The plants were managed in the central leader's system, 81 according to the recommendations of the apple production system and practices 82 recommended in the integrated system of production of apple trees [18, 19].

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The experimental design was randomized blocks, with seven treatments and five replications. The treatments were: Control (No application), Physiogrow® Color (4, 8 and $12 L ha^{-1}$) 7 days before harvest (DBH), Physiogrow® color 2 L ha^{-1} (30 DBH) + 2 L ha^{-1}7 DBH, Physiogrow® Color 4 L ha^{-1} (30 DBH) + 4 L ha^{-1}7 DBH, and Physiogrow® Color 6 L ha^{-1} (30 DBH) + 6 L ha^{-1}7 DBH. The application of the treatments was performed with a motorized costal spray, containing a tip with three spray tips D-S fan type, using a volume of syrup equivalent to 1000 L ha^{-1}, until the point of drip. 91 The harvest was carried out on January 25, 2018 $\frac{1}{25}$, 2018 and February 16, 92 2019, first and second year, respectively. The variables evaluated were: Production (kg 93 plant⁻¹ and fruits plant⁻¹), average fresh fruit mass (g), classification of fruits by 94 percentage of red coloration of the epidermis (<50%, 50-80% and > 80%); firmness of 95 the pulp (Lb in⁻²) and soluble solids (°Brix) according to Scolaro et al [20].

The firmness of the pulp was determined with the aid of an analogue penetrometer (GÜSS), equipped with a 11 mm diameter tip, in two opposite regions, in the equatorial portion of the fruits, after removal of a thin layer of the bark.

The statistical analysis of the data was performed through analysis of variance, the variables whose results revealed significance (P < 0.05), were submitted to the comparison of means by the Scott-Knott test, and or regression analysis at 5% probability Statistical analyzes were performed by Sisvar[®], version 5.6. [21]

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104 **3.1 RESULTS AND DISCUSSION**

105 In both years, the application of Physiogrow® Color did not promote alteration or 106 reduction in the production (kg and fruits plant¹) and in the average fresh mass of the 107 fruits of the treated plants compared to the control treatment (Table 1). This fact, of great 108 importance, as it shows that the product did not cause too much acceleration of fruit 109 maturation, which could cause pre-harvesting of fruits, as reported by Sun et al. [14], 110 who identified this problem in apple trees treated with etephon.

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Table 1. Production (kg and fruits plant⁻¹) and average fresh mass of the fruits (g)
of 'Royal Gala' apple trees submitted to different treatments. 2017/2018 and
2018/2019. Fraiburgo-SC, Brazil, 2019.

	Production	
Treatments	kg fruits plant ⁻¹	Fresh mass of the fruits (g)
	Season 2017/2018	

Control (No application)	17,2 ^{ns}	169,3 ^{ns}	102,0 ^{ns}
Physiogrow Color 4L ha ⁻¹	19,4	209,3	93,7
Physiogrow <mark>Color</mark> 8L ha ⁻¹	20,9	196,3	106,9
Physiogrow Color 12L ha ⁻¹	17,6	175,3	101,0
Physiogrow <mark>Color</mark> 2 + 2L ha ⁻¹	26,8	277,3	99,5
Physiogrow <mark>Color</mark> 4 + 4L ha ⁻¹	24,2	240,5	101,5
Physiogrow <mark>Color</mark> 6 + 6L ha ⁻¹	22,8	215,5	107,8
Average	21,2	211,9	101,8
CV (%)	31,5	35,7	9,9
	Se	ason 2018/2019	9
Control (No application)	18,7 ^{ns}	151,6 ^{ns}	128,5 ^{ns}
Physiogrow <mark>Color</mark> 4L ha⁻¹	25,6	206,2	127,2
Physiogrow <mark>Color</mark> 8L ha⁻¹	23,8	196,2	120,7
Physiogrow Color 12L ha ⁻¹	22,0	175,8	124,5
Physiogrow <mark>Color</mark> 2 + 2L ha ⁻¹	28,4	218,4	132,5
Physiogrow C <mark>olor</mark> 4 + 4L ha ⁻¹	25,4	182,2	138,1
Physiogrow Color 6 + 6L ha ⁻¹	16,9	126,6	133,7
Average	23,0	179,6	129,3
CV (%)	33,6	17,8	12,7

116 * ns: not significant (P>.05).

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118 The pulp firmness of the harvested fruits did not change, in both evaluated 119 harvests. However, differences were observed in soluble solids contents between 120 seasons (Table 2). In the 2017/2018 year, the plants treated with the split application of Physiogrow® Color, regardless of the dose, presented lower levels of soluble solids. In 121 the 2018/2019 year, the treatments Physiogrow[®] Color 8 L ha⁻¹ and Physiogrow[®] Color 122 123 12 L ha⁻¹ applied to 7 DBH did not differ from the control treatment, which presented the 124 lowest concentrations of soluble solids in comparison to the other treatments. The 125 increase of soluble solids concentration in the fruits can be attributed to higher ethylene 126 production, which in turn increases starch hydrolysis, respiration and soluble solids 127 content, as well as increasing the yellowing of the fruit, leading to maturation. or not, to 128 reduce the flesh firmness of the fruits [16, 22,23].

- 130 **Table 2.** Firmness of pulp (Lb in⁻²) and soluble solids (^eBrix) of the fruits of 'Royal
- 131 Gala' apple trees submitted to different treatments. 2017/2018 and 2018/2019.
- 132 Fraiburgo-SC, Brazil, 2019.
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Treatments	Firmness of pulp <mark>(Lb in⁻²)</mark>	Soluble solids (ºBrix)
	Season 20	17/2018
Control (No application)	21,2 ^{ns}	13,7a 🔨
Physiogrow Color 4L ha ⁻¹	21,5	11,5a
Physiogrow Color 8L ha ⁻¹	21,7	11,6a
Physiogrow Color 12L ha ⁻¹	22,8	11,4a
Physiogrow Color 2 + 2L ha ⁻¹	22,6	9,0b
Physiogrow Color 4 + 4L ha ⁻¹	21,2	9,7b
Physiogrow Color 6 + 6L ha ⁻¹	20,8	10,1b
Average	21,7	11,0
CV (%)	5,7	13,8
	Season 20	18/2019
Control (No application)	17,7 ^{ns}	12,1 b
Physiogrow Color 4L ha ⁻¹	16,9	13,4 a
Physiogrow Color 8L ha ⁻¹	16,9	12,0 b
Physiogrow Color 12L ha ⁻¹	17,0	11,9 b
Physiogrow Color 2 + 2L ha ⁻¹	18,1	13,9 a
Physiogrow Color4 + 4L ha ⁻¹	16,8	13,7 a
Physiogrow Color 6 + 6L ha ⁻¹	16,4	13,2 a
Average	17,1	12,9
CV (%)	11,8	4,9

Means followed by the same letter in the column do not differ from one another by the Scott-Knott test (P=.05). ns: not significant (P>.05).

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137 The application of Physiogrow[®] Color promoted a better fruit distribution in 138 categories with higher commercial value. That is, reduction of the percentage of fruits 139 with epidermal coloration inferior to 50% and increase of the percentage of fruits with red 140 color of the epidermis superior to 80%. Differential behavior was observed between the 141 harvests studied (Table 3). In season 2017/2018, the plants were treated with Physiogrow[®] Color 8 L ha⁻¹ 7DBH, which reduced the production of fruits with red 142 143 epidermis color by 50% and 51%, and increased 187, 6% of fruits allocated in the 144 category of greater coloration of the epidermis, compared to untreated plants (Control). Physiogrow[®] Color 12 L ha⁻¹ 7 DBH and Physiogrow[®] Color 6 L ha⁻¹ 30DBH + 6 L ha⁻¹ 7 145 146 DBH treatments also increased the percentage of fruits in the higher staining category. 147 In the second harvest, 2018/2019, there was greater variation among treatments, mainly in the percentage of fruits in the category of less staining that was quite irregular. However, the gain in the percentage of fruits in the highest color category observed in the previous crop, was repeated again in this harvest with the treatments Physiogrow[®] Color 8 L ha⁻¹ 7 DBH, but with less intensity, only 24,9%, not differing (4 L ha⁻¹ 30 DBH + 4 L ha⁻¹ 7 DBH) and (6 L ha⁻¹ 30 DBH + 6 L ha⁻¹ 7 DBH)], which increased by 13.2% and 25,2% the fruits of greater red coloration of the epidermis, respectively.

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Table 3. Red coloration of the epidermis (%) of fruits produced by 'Royal Gala'
 apple trees submitted to different treatments. 2017/2018 and 2018/2019. Fraiburgo-

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- 157 **SC**, **Brazil**, 2019.
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Treatments	Red col	oration of the epidern	nis (%) of fruits
	<50%	<mark>50-80</mark> %	>80%
		Season 2017/20	18
Control (No application)	30,9 a	58,6 ^{ns}	10,5 b
Physiogrow <mark>Color</mark> 4L ha ⁻¹	32,5 a	57,4	10,0 b
Physiogrow <mark>Color</mark> 8L ha ⁻¹	15,9 b	53,9	30,2 a
Physiogrow Color 12L ha ⁻¹	30,7 a	45,2	24,1 a
Physiogrow Color 2 + 2L ha ⁻¹	38,5 a	49,6	11,9 b
Physiogrow Color 4 + 4L ha ⁻¹	49,0 a	46,4	4,6 b
Physiogrow Color 6 + 6L ha ⁻¹	₩35,1 a	44,7	20,2 a
Average	33,2	50,8	15,9
CV (%)	16,7	10,6	24,4
		Season 2018/201	19
Control (No application)	12,8 c	52,3 ^{ns}	34,9 b
Physiogrow <mark>Color</mark> 4L ha ⁻¹	14,2 c	48,3	37,6 b
Physiogrow Color 8L ha ⁻¹	14,1 c	42,3	43,6 a
Physiogrow Color 12L ha ⁻¹	20,4 b	46,4	33,2 b
Physiogrow <mark>Color</mark> 2 + 2L ha ⁻¹	27,6 a	41,5	30,9 b
Physiogrow Color 4 + 4L ha ⁻¹	13,1 c	47,3	39,5 a
Physiogrow Color 6 + 6L ha ⁻¹	18,6 b	37,7	43,7 a
Average	17,3	45,1	37,6
CV (%)	17,1	9,8	10,1

159 Means followed by the same letter in the column do not differ from one another by the

160 Scott-Knott test (P=.05). * ns: not significant (P>.05).

162 It is worth mentioning that there was a difference of 22 days in the date of 163 harvest between seasons. Since the 2017/2018 harvest was held in ideal harvesting 164 point for storage, while the crop 2018/2019 was held in later time, and there may be 165 impairment of the observed data, the natural ripening of the fruit.

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167 These results were evident in the data presented in tables 2 and 3, characterized 168 by the lower firmness of pulp, higher concentration of soluble solids and greater 169 allocation of fruits in the category of greater red coloration of the epidermis.

For Severino et al. [24], in the early harvest cultivars, where the light level is adequate, the main limiting factor for anthocyanins and red color accumulation in the apple epidermis is temperature, since during the maturation stage of these cultivars, thermal amplitude and especially the night temperature are generally not ideal for anthocyanin synthesis. On the other hand, in late cultivars, the factor that becomes limiting is light, since the temperature and its amplitude are favorable for anthocyanin synthesis and red color.

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178 In the second year of the study, it was observed an increase in the yellow 179 background color in the fruits harvested due to the later harvest season and climatic 180 conditions unfavorable to the development of the red color in the fruits, marked by rainy 181 weeks and cloudy days. The yellowing of the background color is another negative 182 attribute, since in addition to reducing the post-harvest life of the fruits, the interest of the 183 consumers also diminishes, since the yellow color conveys the sensation of a very 184 mature fruit [22]. The red color formation in the apple epidermis starts simultaneously 185 with the yellowing of the background color of the fruit, when chlorophyll degradation and 186 carotenoid synthesis is occurring. For Tijskens et al. [25], the most important process in 187 the formation of red color is related to the degradation of chlorophyll, rather than the 188 formation of any flavonoid.

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190 **4.1 CONCLUSION**

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192	Physiogrow [®]	Color does not	affect the p	production of	f the plants,	nor the firmness
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- 193 of the fruit pulp produced. However, it may influence the content of soluble solids (° Brix).
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- 195 Physiogrow[®] Color improves the distribution of fruits in categories of greater red
- 196 color of the epidermis and reduces the percentage of fruits in the category with
- 197 coloration inferior to 50%, with variable intensity between the years.
- 198
- 199 The application of Physiogrow[®] Color 8 L ha⁻¹ 7 DBH contributes to the
- 200 improvement of the coloration of 'Royal Gala' apples
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203 COMPETING INTERESTS DISCLAIMER:

- Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the
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