

2 **STRATEGIES FOR INCREASING THE APPLE**  
3 **EPIDERMIS RED COLORED WITH**  
4 **PHYSIOGROW® COLOR**

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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 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 and replicated five replications.**Place and Duration of Study:**The experiment was carried out in the municipality of Fraiburgo-SC, Brazil (latitude 27°01'S, longitude 50°77' 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<sup>-1</sup>) 7 days before harvest (DAC); Physiogrow® color 2 L ha<sup>-1</sup> (30 DAC) + 2 L ha<sup>-1</sup> 7 DAC; Physiogrow® color 4 L ha<sup>-1</sup> (30 DAC) + 4 L ha<sup>-1</sup> 7 DAC; and Physiogrow® color 6 L ha<sup>-1</sup> (30 DAC) + 6 L ha<sup>-1</sup> 7 DAC. Production (kg plant<sup>-1</sup> and plant<sup>-1</sup> fruits) were evaluated; average fresh fruit mass (g); Classification of fruits by percentage of red coloration of the epidermis (<50%, 50-80%, > 80%); firmness of the pulp (lbpol<sup>-2</sup>) and soluble solids (°Brix). The harvest was carried out on 1/25/2018 and 2/16/2019, first and second harvest, respectively. Plant production, as well as pulp firmness of fruits, were not affected by treatments, in both crops. 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 coloration inferior to 50%, although a different

behavior among the harvests was observed. The application of Physiogrow<sup>®</sup> Color 8 L ha<sup>-1</sup> 7 DAC contributes to the improvement of the coloration of 'Royal Gala' apples.

9

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

11

## 12 **1. INTRODUCTION**

13

14 In Brazil, apple tree cultivation has evolved a lot in the last years, mainly with the  
15 development of management techniques that have increased productivity and quality of  
16 fruit produced [1]. The visual quality and size of the fruits play an important role in the  
17 national and international markets, since they establish the price of the apple when  
18 classified for packaging and export [2,3,4].

19

20 According to Iglesias and Alegre [5], the most important visual quality attribute to  
21 determine the market value of red or bicolor apples is the amount and intensity of the red  
22 epidermis. Considering that at the time of purchase, consumers first analyze the visual  
23 appearance of the fruits, only later to consider the internal or nutritional characteristics of  
24 the fruits [6,7].

25

26 The red color formation in the apple epidermis is linked to a complex series of  
27 interactions between environmental factors, orchard management, genetic  
28 characteristics of the cultivar and the stage of development of the fruit. The accumulation  
29 of anthocyanins corresponds to the genetic potential of the cultivar, which, in turn, is  
30 dependent on balanced nutrition and environmental factors such as light and  
31 temperature [8].

32

33 For Iglesias et al. [9], the easiest and most economical way to improve the color  
34 of apples is to plant new cultivars or mutations of cultivars with greater potential to  
35 synthesize anthocyanins in the region of interest. The most planted clones of the 'Gala'

36 apple tree, have a tendency to develop greater coloring of the fruits, however, they are  
37 still mostly bicolor.

38 In addition to orchard management techniques that aim to increase fruit  
39 exposure to light, commercial products (chemical or natural), fertilizers and growth  
40 regulators with biostimulant action, may promote increased red coloration of fruits. The  
41 growth regulators based on ethephon release ethylene, a hormone bound to maturation,  
42 increasing and intensifying the red coloration of the epidermis of apples [10]. However,  
43 ethephon may increase the risk of fruit drop losses in preharvest and reduce storage  
44 potential [11]. Some studies have shown that ethephon application may adversely affect  
45 the starch hydrolysis rate, reducing pulp firmness, titratable acidity and increasing  
46 respiratory rate, carotenoid biosynthesis and chlorophyll degradation [12,13].

47  
48 Blanke [14] reports that there are always new products on the market with high  
49 expectations to increase the anthocyanin synthesis and red color of the apple epidermis,  
50 especially leaf fertilizers and biostimulants. Fenili [8] reports the increase in red staining  
51 of apple fruits treated with foliar fertilizers based on potassium and micronutrients;  
52 however, their effects vary greatly from one year to the next, as well as changes in the  
53 quality and storage capacity of the fruits.

54  
55 Physiogrow® Color, which is an organic liquid leaf fertilizer based on free L-  
56 amino acids and organic acids, with potential to be used to improve the coloring of fruits  
57 in the apple tree. Since it contains, in its formulation, precursor amino acids of ethylene  
58 biosynthesis and anthocyanins, chlorophyllase and monosaccharide regulators.

59  
60 The objective of the present study was to evaluate different dosages and number  
61 of applications of the commercial product Physiogrow ® Color on the percentage of red  
62 coloration of the epidermis and fruit quality in 'Royal Gala' apple trees, in the Midwest  
63 region of the state of Santa Catarina.

64

## 65 2. MATERIAL AND METHODS

66

67 The experiment was conducted in an experimental orchard in the Midwest  
68 Region of the state of Santa Catarina, Fraiburgo (latitude 27°01'S, longitude 50°77 'W,  
69 altitude 950 meters).

70

71 'Royal Gala' apple trees grafted on the Marubakaido rootstock with 15-year-old  
72 M-9 grafts were harvested in the 2017/2018 and 2018/2019 seasons. The planting  
73 density of the orchard used was 2,500 plants ha<sup>-1</sup>, with a spacing of 4 m between rows  
74 and 1 m between plants. The plants were managed in the central leader's system,  
75 according to the recommendations of the apple production system and practices  
76 recommended in the integrated system of production of apple trees [15].

77

78 The experimental design was randomized blocks, with seven treatments and five  
79 replications. The treatments were: Control (No application); Physiogrow® color (4, 8 and  
80 12 L ha<sup>-1</sup>) 7 days before harvest (DAC); Physiogrow® color 2 L ha<sup>-1</sup> (30 DAC) + 2 L ha<sup>-1</sup> 7  
81 DAC; Physiogrow® color 4 L ha<sup>-1</sup> (30 DAC) + 4 L ha<sup>-1</sup> 7 DAC; and Physiogrow® color 6 L  
82 ha<sup>-1</sup> (30 DAC) + 6 L ha<sup>-1</sup> 7 DAC. The application of the treatments was performed with a  
83 motorized costal spray, containing a tip with three spray tips D-S fan type, using a  
84 volume of syrup equivalent to 1000 L ha<sup>-1</sup>, until the point of drip.

85 The harvest was carried out on 1/25/2018 and 2/16/2019, first and second  
86 harvest, respectively. Production (kg plant<sup>-1</sup> and plant<sup>-1</sup> fruits) were evaluated; average  
87 fresh fruit mass (g); Classification of fruits by percentage of red coloration of the  
88 epidermis (<50%, 50-80%, > 80%); firmness of the pulp (Lib) and soluble solids (° Brix)  
89 according to Scolaro et al [16].

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

93 The statistical analysis of the data was performed through analysis of variance, the  
 94 variables whose results revealed significance ( $p < 0.05$ ), were submitted to the  
 95 comparison of means by the Scott-Knott test, and or regression analysis at 5%  
 96 probability. Statistical analyzes were performed by Sisvar<sup>®</sup>, version 5.6.

97

### 98 3.1 RESULTS AND DISCUSSION

99 In both crops, the application of Physiogrow<sup>®</sup> color did not promote alteration or  
 100 reduction in the production (kg and fruits plant<sup>-1</sup>) and in the average fresh mass of the  
 101 fruits of the treated plants compared to the control treatment (Table 1). This fact, of great  
 102 importance, as it shows that the product did not cause too much acceleration of fruit  
 103 maturation, which could cause pre-harvesting of fruits, as reported by Sun et al. [11],  
 104 who identified this problem in apple trees treated with etephon.

105

106 **Table 1–Production (kg and fruits plant<sup>-1</sup>) and average fresh mass of the**  
 107 **fruits (g) of 'Royal Gala' apple trees submitted to different treatments. 2017/2018**  
 108 **and 2018/2019. Fraiburgo-SC, Brazil, 2019.**

109

Treatments	Production		Average fresh mass of the fruits (g)
	kg plant <sup>-1</sup>	frutos plant <sup>-1</sup>	
Season 2017/2018			
Control (no application)	17,2 <sup>ns</sup>	169,3 <sup>ns</sup>	102,0 <sup>ns</sup>
Physiogrow 4L ha <sup>-1</sup>	19,4	209,3	93,7
Physiogrow 8L ha <sup>-1</sup>	20,9	196,3	106,9
Physiogrow 12L ha <sup>-1</sup>	17,6	175,3	101,0
Physiogrow 2 + 2L ha <sup>-1</sup>	26,8	277,3	99,5
Physiogrow 4 + 4L ha <sup>-1</sup>	24,2	240,5	101,5
Physiogrow 6 + 6L ha <sup>-1</sup>	22,8	215,5	107,8
Average	21,2	211,9	101,8
CV (%)	31,5	35,7	9,9
Season 2018/2019			
Control (no application)	18,7 <sup>ns</sup>	151,6 <sup>ns</sup>	128,5 <sup>ns</sup>

Physiogrow 4L ha <sup>-1</sup>	25,6	206,2	127,2
Physiogrow 8L ha <sup>-1</sup>	23,8	196,2	120,7
Physiogrow 12L ha <sup>-1</sup>	22,0	175,8	124,5
Physiogrow 2 + 2L ha <sup>-1</sup>	28,4	218,4	132,5
Physiogrow 4 + 4L ha <sup>-1</sup>	25,4	182,2	138,1
Physiogrow 6 + 6L ha <sup>-1</sup>	16,9	126,6	133,7
Average	23,0	179,6	129,3
CV (%)	33,6	17,8	12,7

110 \* ns: not significant (P>.05).

111

112 The pulp firmness of the harvested fruits did not change, in both evaluated  
 113 harvests. However, differences were observed in soluble solids contents between  
 114 seasons (Table 2). In the 2017/2017 crop, the plants treated with the split application of  
 115 Physiogrow® Color, regardless of the dose, presented lower levels of soluble solids. In  
 116 the 2018/2019 crop, the treatments Physiogrow® Color 8 L ha<sup>-1</sup> and Physiogrow® Color  
 117 12 L ha<sup>-1</sup> applied to 7DAC did not differ from the control treatment, which presented the  
 118 lowest concentrations of soluble solids in comparison to the other treatments. The  
 119 increase of soluble solids concentration in the fruits can be attributed to higher ethylene  
 120 production, which in turn increases starch hydrolysis, respiration and soluble solids  
 121 content, as well as increasing the yellowing of the fruit, leading to maturation. or not, to  
 122 reduce the flesh firmness of the fruits [18,13,19].

123

124 **Table 2 - Firmness of pulp (Pounds) and soluble solids (°Brix) of the fruits**  
 125 **of 'Royal Gala' apple trees submitted to different treatments. 2017/2018 and**  
 126 **2018/2019. Fraiburgo-SC, Brazil, 2019.**

127

Treatments	Firmness of pulp (Pounds)	Soluble solids (°Brix)
<b>Season 2017/2018</b>		
Control (No application)	21,2 <sup>ns</sup>	13,7a
Physiogrow 4L ha <sup>-1</sup>	21,5	11,5a
Physiogrow 8L ha <sup>-1</sup>	21,7	11,6a
Physiogrow 12L ha <sup>-1</sup>	22,8	11,4a
Physiogrow 2 + 2L ha <sup>-1</sup>	22,6	9,0b

Physiogrow 4 + 4L ha <sup>-1</sup>	21,2	9,7b
Physiogrow 6 + 6L ha <sup>-1</sup>	20,8	10,1b
Average	21,7	11,0
CV (%)	5,7	13,8
<b>Season 2018/2019</b>		
Control (No application)	17,7 <sup>ns</sup>	12,1 b
Physiogrow 4L ha <sup>-1</sup>	16,9	13,4 a
Physiogrow 8L ha <sup>-1</sup>	16,9	12,0 b
Physiogrow 12L ha <sup>-1</sup>	17,0	11,9 b
Physiogrow 2 + 2L ha <sup>-1</sup>	18,1	13,9 a
Physiogrow 4 + 4L ha <sup>-1</sup>	16,8	13,7 a
Physiogrow 6 + 6L ha <sup>-1</sup>	16,4	13,2 a
Average	17,1	12,9
CV (%)	11,8	4,9

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

130

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

148

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

152

Treatments	Red coloration of the epidermis (%) of fruits		
	<50%	50_80 %	>80%
<b>Season 2017/2018</b>			
Control (No application)	30,9 a	58,6 <sup>ns</sup>	10,5 b
Physiogrow 4L ha <sup>-1</sup>	32,5 a	57,4	10,0 b
Physiogrow 8L ha <sup>-1</sup>	15,9 b	53,9	30,2 a
Physiogrow 12L ha <sup>-1</sup>	30,7 a	45,2	24,1 a
Physiogrow 2 + 2L ha <sup>-1</sup>	38,5 a	49,6	11,9 b
Physiogrow 4 + 4L ha <sup>-1</sup>	49,0 a	46,4	4,6 b
Physiogrow 6 + 6L ha <sup>-1</sup>	35,1 a	44,7	20,2 a
Average	33,2	50,8	15,9
CV (%)	16,7	10,6	24,4
<b>Season 2018/2019</b>			
Control (No application)	12,8 c	52,3 <sup>ns</sup>	34,9 b
Physiogrow 4L ha <sup>-1</sup>	14,2 c	48,3	37,6 b
Physiogrow 8L ha <sup>-1</sup>	14,1 c	42,3	43,6 a
Physiogrow 12L ha <sup>-1</sup>	20,4 b	46,4	33,2 b
Physiogrow 2 + 2L ha <sup>-1</sup>	27,6 a	41,5	30,9 b
Physiogrow 4 + 4L ha <sup>-1</sup>	13,1 c	47,3	39,5 a
Physiogrow 6 + 6L ha <sup>-1</sup>	18,6 b	37,7	43,7 a
Average	17,3	45,1	37,6
CV (%)	17,1	9,8	10,1

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

155

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

160



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

164            For Severino et al. [20], in the early harvest cultivars, where the light level is  
165 adequate, the main limiting factor for anthocyanins and red color accumulation in the  
166 apple epidermis is temperature, since during the maturation stage of these cultivars,  
167 thermal amplitude and especially the night temperature are generally not ideal for  
168 anthocyanin synthesis. On the other hand, in late cultivars, the factor that becomes  
169 limiting is light, since the temperature and its amplitude are favorable for anthocyanin  
170 synthesis and red color.

171  
172            In the second year of the study, it was observed an increase in the yellow  
173 background color in the fruits harvested due to the later harvest season and climatic  
174 conditions unfavorable to the development of the red color in the fruits, marked by rainy  
175 weeks and cloudy days. The yellowing of the background color is another negative  
176 attribute, since in addition to reducing the post-harvest life of the fruits, the interest of the  
177 consumers also diminishes, since the yellow color conveys the sensation of a very  
178 mature fruit [18]. The red color formation in the apple epidermis starts simultaneously  
179 with the yellowing of the background color of the fruit, when chlorophyll degradation and  
180 carotenoid synthesis is occurring. For Tijskens et al. [21], the most important process in  
181 the formation of red color is related to the degradation of chlorophyll, rather than the  
182 formation of any flavonoid.

183

#### 184 **4.1 CONCLUSION**

185

186            Physiogrow® Color does not affect the production of the plants, nor the firmness  
187 of the fruit pulp produced. However, it may influence the content of soluble solids (° Brix).

188

189 Physiogrow® Color improves the distribution of fruits in categories of greater red  
190 color of the epidermis and reduces the percentage of fruits in the category with  
191 coloration inferior to 50%, with variable intensity between the years.

192

193 The application of Physiogrow® Color 8 L ha<sup>-1</sup> 7 DAC contributes to the  
194 improvement of the coloration of Royal Gala apples

195

196

197 **COMPETING INTERESTS DISCLAIMER:**

198 **Authors have declared that no competing interests exist. The products**  
199 **used for this research are commonly and predominantly use products in**  
200 **our area of research and country. There is absolutely no conflict of interest**  
201 **between the authors and producers of the products because we do not**  
202 **intend to use these products as an avenue for any litigation but for the**  
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