

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

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6
7 **ABSTRACT**
8

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 and replicated with 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⁻¹) 7 days before harvest (DBH), Physiogrow® Color 2 L ha⁻¹ (30 DAC) + 2 L ha⁻¹ DBH, Physiogrow® Color 4 L ha⁻¹ (30 DBH) + 4 L ha⁻¹ DBH, and Physiogrow® Color 6 L ha⁻¹ (30 DBH) + 6 L ha⁻¹ DBH. **The variables evaluated were:** Production (kg plant⁻¹ and fruits plant⁻¹) were evaluated, 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 (lb in²) and soluble solids (°Brix). The harvest was carried out on January 25, 2018 1/25/2018 and February 16, 2019. 2/16/2019, first and second year harvest, respectively. Plant production, as well as pulp firmness of fruits, were not affected by treatments, in both years Scrops. 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® Color 8 L ha⁻¹ 7 DBH 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. (write
59 reference)

60

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

65

66 2. MATERIAL AND METHODS

67

68 The experiment was conducted in an experimental orchard in the Midwest
69 Region of the state of Santa Catarina, Fraiburgo, Brazil (latitude 27°01'S, longitude
70 50°77'W, altitude 950 meters).

71

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

78

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

86 The harvest was carried out on January 25, 2018 ~~1/25/2018~~ and February 16,
87 2019 ~~2/16/2019~~, first and second year, respectively. The variables evaluated were:
88 Production (kg plant⁻¹ and fruits plant⁻¹) ~~were evaluated~~, average fresh fruit mass (g),
89 classification of fruits by percentage of red coloration of the epidermis (<50%, 50-80%

90 and > 80%); firmness of the pulp (Lb in⁻²) and soluble solids (°Brix) according to Scolaro
91 et al [16].

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

95 The statistical analysis of the data was performed through analysis of variance, the
96 variables whose results revealed significance ($P < 0.05$), were submitted to the
97 comparison of means by the Scott-Knott test, and or regression analysis at 5%
98 probability Statistical analyzes were performed by Sisvar[®], version 5.6. [17]

99

100 3.1 RESULTS AND DISCUSSION

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

107

108 **Table 1. Production (kg and fruits plant⁻¹) and average fresh mass of the fruits (g)**
109 **of 'Royal Gala' apple trees submitted to different treatments. 2017/2018 and**
110 **2018/2019. Fraiburgo-SC, Brazil, 2019.**

111

Treatments	Production		
	kg plant ⁻¹	fruits plant ⁻¹	Average 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 Color 8L ha ⁻¹	20,9	196,3	106,9
Physiogrow Color 12L ha ⁻¹	17,6	175,3	101,0

Physiogrow Color 2 + 2L ha ⁻¹	26,8	277,3	99,5
Physiogrow Color 4 + 4L ha ⁻¹	24,2	240,5	101,5
Physiogrow Color 6 + 6L ha ⁻¹	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 ^{ns}	151,6 ^{ns}	128,5 ^{ns}
Physiogrow Color 4L ha ⁻¹	25,6	206,2	127,2
Physiogrow Color 8L ha ⁻¹	23,8	196,2	120,7
Physiogrow Color 12L ha ⁻¹	22,0	175,8	124,5
Physiogrow Color 2 + 2L ha ⁻¹	28,4	218,4	132,5
Physiogrow Color 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

112 * ns: not significant ($P > .05$).

113

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

125

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

129

Treatments	Firmness of pulp (Lb in ⁻²)	Soluble solids (°Brix)
Season 2017/2018		
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 2018/2019		
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 Color 4 + 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

130 Means followed by the same letter in the column do not differ from one another by the
131 Scott-Knott test ($P=0.05$). ns: not significant ($P>0.05$).

132

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

148 + 4 L ha⁻¹ 7 DBH) and (6 L ha⁻¹ 30 DBH + 6 L ha⁻¹ 7 DBH)], which increased by 13.2%
 149 and 25,2% the fruits of greater red coloration of the epidermis, respectively.

150

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

154

Treatments	Red coloration of the epidermis (%) of fruits		
	<50%	50-80 %	>80%
Season 2017/2018			
Control (No application)	30,9 a	58,6 ^{ns}	10,5 b
Physiogrow Color 4L ha ⁻¹	32,5 a	57,4	10,0 b
Physiogrow Color 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 2018/2019			
Control (No application)	12,8 c	52,3 ^{ns}	34,9 b
Physiogrow Color 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 Color 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

155 Means followed by the same letter in the column do not differ from one another by the
 156 Scott-Knott test ($P=0.05$). * ns: not significant ($P>0.05$).

157

158 It is worth mentioning that there was a difference of 22 days in the date of
 159 harvest between seasons. Since the 2017/2018 harvest was held in ideal harvesting

160 point for storage, while the crop 2018/2019 was held in later time, and there may be
161 impairment of the observed data, the natural ripening of the fruit.

162

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

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

173

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

185

186 **4.1 CONCLUSION**

187

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

190

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

194

195 The application of Physiogrow® Color 8 L ha⁻¹ 7 DBH contributes to the
196 improvement of the coloration of 'Royal Gala' apples

197

198

199 **COMPETING INTERESTS DISCLAIMER:**

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