

1 **SOURCES AND CONCENTRATIONS OF CUPRIC FUNGICIDES FOR THE**
2 **CONTROL OF CITRUS BLACK SPOT¹**
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4

5 **ABSTRACT** – Citrus black spot (CBS) is a severe disease for citriculture in the São Paulo
6 State, Brazil. Part of its management is focused on chemical control using cupric fungicides
7 and strobilurins. The objective of the present work was to evaluate the efficacy of three
8 sources and three concentrations of cupric fungicides (copper hydroxide, copper oxychloride
9 and cuprous oxide). Orange fruits of the Pera cultivar were bagged in the plants and the
10 treatment with cupric fungicide was applied. The fruits were inoculated (by spray) with
11 *Phyllosticta citricarpa* (1×10^4 conidia mL⁻¹) after 0, 7, 14, 21, and 28 days, and bagged again.
12 The evaluation of incidence and severity was did at the harvest time of fruits. A second
13 experiment was conducted under natural infection with the same treatments, consisting of
14 application of fungicides at the stages F1 (petal fall) and F2 (fruits with diameter of 1 cm),
15 using mechanized sprayer with mean flow of 7.35 L solution plant⁻¹. Subsequently, all
16 treatments received four applications of azoxystrobin (30 g ha⁻¹), plus mineral oil at 0.25%.
17 Four monthly evaluations were done to determine the CBS incidence and severity. The initial
18 applications with cupric fungicides are essential for the control of CBS; the fungicide copper
19 hydroxide showed the best control of CBS with the lowest rate of metallic copper (43.7 g of
20 Cu⁺⁺ 100 L⁻¹) in both experiments, regardless of the conduction conditions.

21
22 **Keywords:** *Citrus sinensis*. *Phyllosticta citricarpa*. Metallic copper.
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25 **FONTES E CONCENTRAÇÕES DE FUNGICIDAS CÚPRICOS NO CONTROLE DA**
26 **MANCHA PRETA DOS CITROS**
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29 **RESUMO** – A mancha preta dos citros é uma doença severa para a citricultura do Estado de
30 São Paulo. Parte do seu manejo é focado no controle químico usando fungicidas cúpricos e
31 estrobilurinas. Este trabalho teve o objetivo de avaliar a eficácia de três fontes e três
32 concentrações de fungicidas cúpricos (hidróxido de cobre, oxiclreto de cobre e óxido
33 cuproso). Frutos de laranja ‘Pera’ foram ensacados nas plantas seguido de tratamento com
34 fungicida cúprico. Posteriormente, após zero; sete; 14; 21 e 28 dias tais frutos foram

35 inoculados com *Phyllosticta citricarpa* (1x10⁴ conídios mL⁻¹), por pulverização, seguido de
36 novo ensacamento. A avaliação da incidência e severidade da doença ocorreu na colheita dos
37 frutos. Um segundo experimento, sob infecção natural, foi constituído pelos mesmos
38 tratamentos, sendo os fungicidas aplicados nos estádios F1 (queda de pétalas) e F2 (frutos
39 com 1cm de diâmetro) mediante pulverização tratorizada e vazão média 7,35 L calda planta⁻¹.
40 Subsequentemente, e de forma semelhante para todos os tratamentos, foram realizadas quatro
41 aplicações de azoxistrobina (30 g de i.a./ha) acrescida de óleo mineral a 0,25%. Foram
42 realizadas quatro avaliações mensais para determinação da incidência e severidade dos
43 sintomas. Concluiu-se que: as pulverizações iniciais com fungicidas cúpricos são
44 fundamentais para o controle da mancha preta dos citros; independente das condições de
45 condução, para ambos os ensaios o fungicida hidróxido de cobre propiciou a melhor resposta
46 de controle da mancha preta dos citros com a menor dosagem de cobre metálico (43,7 g de
47 Cu⁺⁺100L⁻¹).

48

49 **Palavras-chave:** *Citrus sinensis*. *Phyllosticta citricarpa*. Cobre metálico.

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51

52 INTRODUCTION

53

54 Citrus black spot (CBS) is caused by the fungus *Phyllosticta citricarpa* McAlp.
55 (teleomorph: *Guignardia citricarpa* Kiely) (BALDASSARI; WICKERT; GOES, 2008), and
56 is associated with citrus plants in several countries in Africa, Asia, Oceania (KOTZÉ, 2000;
57 EPPO, 2017), South America and North America, and in Caribe (TIMMER et al., 2000;
58 SCHUBERT et al., 2010; HIDALGO and PÉREZ, 2010). All citrus varieties of economic
59 importance are susceptible to this fungus, with losses that can reach 40% of the production
60 (SILVA JÚNIOR et al., 2016).

61 The control of CBS is done usually by using fungicides; in Brazil, it is controlled
62 mainly with use of cupric and strobilurin fungicides (MOTTA, 2009; VINHAS, 2011; SILVA
63 JÚNIOR et al., 2016). Cupric fungicides are commonly applied after petal fall, corresponding
64 to the stages F1 and F2, until the fruits reach diameter of 1 cm (STOLLER, 2010;), followed
65 by two to five applications of strobilurin fungicides (SCALLOPPI et al., 2012), covering the
66 more susceptible period for fruits (AGUIAR et al., 2012), from petal fall until the end of the
67 summer, when rainfall periods of more than 8 hours still occur. Ikeda (2011) reported

68 satisfactory responses of control of CBS to the use of five or six applications of cupric
69 fungicides combined or alternated with strobilurins.

70 Cupric fungicides are approved, for citrus plants, only for the control of citrus scab and
71 melanosis, except copper hydroxide (Kocide WDG Bioactive, Mitsui & Co. Brasil, S.A.)
72 (MAPA, 2017); however, they are used isolate or in combination with strobilurins for the
73 control of *P. citricarpa* (VINHAS, 2011). These fungicides are applied with four-week
74 intervals, beginning after $\frac{3}{4}$ of petals felled, and subsequently with 28-day intervals,
75 combined with application of strobilurins at 35-day to 42-day intervals (MOTTA, 2009). The
76 recommended rates of cupric fungicides are 0.75 to 1.25 g L⁻¹ for copper hydroxide, 2.5 g L⁻¹
77 for copper oxychloride, and 1.0 g L⁻¹ for cuprous oxide (MAPA, 2017). However, the rates
78 used at the stages F1 and F2, and in subsequent applications in combination with strobilurins
79 for the control of CBS under field conditions vary, and are usually based on the fungicide
80 metallic copper contents. These recommendations are from studies with copper oxychloride;
81 no studies comparing sources and concentrations of cupric fungicides are found.

82 In this context, the objective of the present work was to evaluate the efficacy of three
83 sources and three concentrations of cupric fungicides under artificial conditions of inoculation
84 with *P. citricarpa*, and under natural conditions, to determine the equivalence between the
85 different compounds in relation to metallic copper concentration in the solution.

86

87

88 MATERIAL AND METHODS

89

90 Experiment I – Protector effect for different sources and rates of cupric fungicides 91 against *Phyllosticta citricarpa* infection in orange fruits of the Pera cultivar

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93 The experiment was conducted in 2012 in an orchard with orange plants of the Pera
94 cultivar (plant spacing of 5.5 × 2 m), at a private property in Olímpia, state of São Paulo (SP),
95 Brazil (20°41'57.23"S and 48°59'35.33"W).

96 The isolated pathogen was from Conchal, SP, Brazil. It was multiplied in Petri dishes
97 containing BDA medium and maintained in BOD at temperature of 25°C.

98 The fungicides evaluated were copper hydroxide (Kocide WDG Bioactive[®] 462 g of
99 copper hydroxide, Mitsui & Co. Brasil, S.A.), copper oxychloride (Recop[®] 840 g of copper
100 oxychloride, Atanor do Brasil), and cuprous oxide (Redshield 750[®] 750 g of cuprous oxide,

101 Agrovant Comércio de Produtos Agrícolas Ltda). The treatments and rates used are shown in
 102 Table 1.

103 Approximately 5,000 fruits at stage F2 (STOLLER, 2010) of 100 plants were bagged on
 104 02/23/2015 to avoid infections by *P. citricarpa* naturally presents in the area. When the plants
 105 were at stage F4, they were treated with cupric fungicide on 05/13/2016. Subsequently, the
 106 fruits were inoculated with *P. citricarpa* (1×10^4 conidia mL⁻¹) in intervals of 0, 7, 14, 21, and
 107 28 days. The inoculum was prepared and the inoculation was done according to the
 108 methodology described by Almeida et al. (2008). After inoculation, the fruits were bagged
 109 again, using double layer bags (crystal paper), according to the methodology adopted by
 110 Motta (2009). The fruits were kept in the bag until the harvest (10/31/2016).

111
 112 **Table 1.** Description of the cupric fungicide treatments for the control of citrus black spot in
 113 orange plants of the Pera cultivar, and their active ingredient concentrations, formulated
 114 product rates, and metallic copper equivalent rates.

Treatments	Concentration g kg ⁻¹	Rate per 100 liters of solution	
		Formulated Product	Metallic Copper
Copper hydroxide (538 g kg ⁻¹)	538	125.0	43.7
Copper hydroxide (538 g kg ⁻¹)	538	100.0	35.0
Copper hydroxide (538 g kg ⁻¹)	538	75.0	26.2
Copper oxychloride (840 g kg ⁻¹)	840	200.0	100.8
Copper oxychloride (840 g kg ⁻¹)	840	180.0	90.7
Copper oxychloride (840 g kg ⁻¹)	840	135.0	68.0
Cuprous oxide (860 g kg ⁻¹)	860	120.0	90.0
Cuprous oxide (860 g kg ⁻¹)	860	75.0	56.3
Cuprous oxide (860 g kg ⁻¹)	860	56.3	42.2
Control			

115 Copper hydroxide = Kocide WDG Bioactive[®], Mitsui & Co. Brasil, S.A.; Copper oxychloride
 116 = Recop[®], Atanor do Brasil; and Cuprous oxide = Redshield 750[®], Agrovant Comércio de
 117 Produtos Agrícolas Ltda.

118

119 The fungicides were applied according to an adaption of the method of Motta (2009);
120 fruits with diameter of 40 to 50 mm were individually sprayed with fungicide, plus mineral oil
121 at 0.20% (v v⁻¹).

122 A completely randomized experimental design was used, with a 3×3×5 factorial
123 arrangement consisted of three fungicides, three rates, and 5 inoculation times, with four
124 replications. The plots consisted of 20 fruits. The control treatment consisted of fruits without
125 application of cupric fungicide and with artificial inoculation with *P. citricarpa*.

126 The evaluations consisted of determination of the incidence (%) of symptomatic fruits
127 for citrus black spot (CBS), and the disease severity. The CBS severity was estimated using a
128 scale of grades, according to visual symptoms in percentage of lesion area—0 = fruits without
129 visual symptoms, 1= up to 0.8%, 2 = 0.8% to 1.6%, 3 = 1.6% to 3.1%, 4 = 3.1% to 6.2%, 5 =
130 6.2% to 12.5%, 6 = more than 6.2% (SPÓSITO et al., 2004).

131 The grades attributed to fruits were used to determine the disease index (DI), according
132 to Wheeler (1969):

$$DI = \frac{1}{N} \sum_{i=0}^m i \cdot n_i$$

133 where N is the number of total fruits evaluated, i is the grade of the fruit, n_i is the number of
134 fruits with grade I , and m is the maximum grade.

135 The grades attributed, disease index, and the other parameters were subjected to
136 analysis of variance, and the means were compared by the Scott-Knott test at 5% probability
137 of error.

138

139 **Experiment II - Evaluation of rates of cupric fungicides for the control of citrus black** 140 **spot**

141

142 The experiment was conducted in 2007, under natural conditions of infection by *P.*
143 *citricarpa*, in an orchard with orange plants of the Pera cultivar (plant spacing of 7 × 3.5 m),
144 at a private property of commercial production in Olímpia, SP, Brazil (20°47'59.17"S,
145 49°2'34.94"W).

146 The treatments evaluated in this experiment, representing the cupric fungicide
147 variations, were the same used in Experiment I (Table 1).

148 The fungicides were applied using a mechanized sprayer (FM Copling) with 54 nozzles
149 MagnoJet-DDC4, diffusers 25 with 758.42 kPa at 540 RPM, power takeoff with 1900 RPM,

150 and tractor speed of 3.4 km h⁻¹. The mean flowrate was 7.35 L plant⁻¹, equivalent to 134 mL
151 m⁻³ on the plants' canopies. The cupric fungicides were sprayed when the plants were at the
152 stages F1 and F2 (STOLLER, 2010), on 12/02/2015 and 12/23/2015. The spraying of
153 fungicides were resumed on 01/13/2016 with foliar applications of 500 g of azoxystrobin
154 (Vantigo[®], Syngenta Proteção de Cultivos Ltda, São Paulo) at concentration of 16 g per 100 L
155 of water plus mineral oil (Agefix[®], Packblend Indústria e Comércio de Lubrificantes Ltda) at
156 0.25% (v v⁻¹) until 05/19/2016, totaling four applications with 42-day intervals.

157 A randomized block experimental design was used, consisting of 10 treatments and four
158 replications. The plots consisted of three rows of 11 plants, totaling 33 plants or 808.5 m².

159 The evaluations were carried out on 08/10/2016, 09/08/2016, 10/06/2016, and
160 11/14/2016 to determine the CBS incidence and severity in 100 random fruits collected from
161 the five central plants of each plot. The CBS severity was estimated using a scale of grades
162 (SPÓSITO et al., 2004), and the data were used to determine the disease index (DI),
163 according to the same equation used in Experiment I.

164 The DI data were used to determine the area under the disease progress curve (AUDPC)
165 by the trapezoidal method $(DI1 + DI2)/2 * (Day2 - Day1)$. The fruits were harvested on
166 11/30/2016, when the fruit yield per plant (kg) was determined. The data were subjected to
167 analysis of variance and the means were compared by the Scott-Knott test at 5% probability
168 of error. The data were also subjected to regression analysis to determine the rates of each
169 fungicide based on linear and quadratic responses of the AUDPC.

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171

172 **RESULTS AND DISCUSSION**

173

174 **Experiment I**

175

176 All treatments with the highest rates of cupric fungicides presented on average lower
177 incidence and severity of citrus black spot (CBS) (Table 2). The results showed direct and
178 significant responses between fungicide rates and CBS incidence and severity, except for the
179 treatment with copper oxychloride at 90.7 g 100 L⁻¹. However, high rates on equivalent
180 metallic copper do not necessarily mean high concentrations of fungicide (SILVA JÚNIOR et
181 al., 2016).

182 The number of days after the application of fungicides had a significant and negative
183 effect, with lower CBS incidence and severity when the inoculation with *Phyllosticta*

184 *citricarpa* and the treatment was carried out on the same day. The inoculations between 7 and
 185 28 days after the fungicide application resulted in similar protections. This result is related to
 186 those reported by Motta (2009), who found incidence of 30% and 80% when the inoculation
 187 was at 1 and 28 days after application of copper oxychloride, respectively. Thus, the
 188 methodology used is efficient to evaluate and replicate results, and can be used to evaluate
 189 new formulations or fungicides.

190 The interaction between the fungicide rates, fungicide sources, and days after treatment
 191 of fruits was not significant, denoting that the protection tends to decrease over time at the
 192 same proportion for all the treatments, even when using different fungicide rates and sources.
 193 These results showed that the best interval between applications of cupric fungicides depends
 194 on the control level desired, in terms of CBS incidence and severity. Thus, it can be weekly
 195 for production of fruits with lower incidence, and up to 28 days for production of fruits with
 196 incidence and severity within limits that avoid the fall of fruits. Motta (2009) found similar
 197 results, with increases in CBS incidence and severity as a function of increases in the intervals
 198 between the application of copper oxychloride and artificial inoculation with *P. citricarpa*;
 199 they reported satisfactory protection up to 21 days after the application.

200

201 **Table 2.** Incidence and disease index of citrus black spot (*Phyllosticta citricarpa*) in orange
 202 fruits of the Pera cultivar as a function of cupric fungicides rates and sources and days after
 203 the application, in the 2015/2016 crop season.

Sources of variation		Incidence (%)	Disease index
Treatments	Rate (g of metallic copper 100 L ⁻¹)		
1. Copper hydroxide (538 g kg ⁻¹)	43.7	16.9 A	0.22 a
2. Copper hydroxide (538 g kg ⁻¹)	35.0	24.5 b	0.31 b
3. Copper hydroxide (538 g kg ⁻¹)	26.2	26.5 b	0.34 b
4. Copper oxychloride (840 g kg ⁻¹)	100.8	14.7 a	0.19 a
5. Copper oxychloride (840 g kg ⁻¹)	90.7	13.0 a	0.17 a
6. Copper oxychloride (840 g kg ⁻¹)	68.0	32.5 c	0.41 c
7. Cuprous oxide (860 g kg ⁻¹)	90.0	17.9 a	0.24 a
8. Cuprous oxide (860 g kg ⁻¹)	56.3	20.8 b	0.27 b

9. Cuprous oxide (860 g kg ⁻¹)	42.2	38.9 c	0.49 c
10. Control		54.0 d	0.68 d
F Test		13.612 **	18.058 **
Days after application			
0		18.7 a	0.25 a
7		25.7 b	0.33 b
14		26.5 b	0.34 b
21		31.2 b	0.40 b
28		27.8 b	0.35 b
F Test		5.047 **	4.996 **
Interaction (Treatment × Days after application)			
F Test		1.052 ns	1.103 ns
CV (%)		32.88	10.87

204 The data of incidence and severity of citrus black spot were transformed according to the
205 equations $x' = \arcsen\sqrt{x/100}$, and $x' = \sqrt{x + 0.5}$, respectively. Means followed by the
206 same letter in the columns do not differ by the Scott-Knott test at 5% probability of error. **
207 = significant by the analysis of variance at 1% probability of error; ns = not significant by the
208 analysis of variance at 5% probability of error.

209

210 Experiment II

211

212 According to the first evaluations, in August, the highest cupric fungicide rates were
213 significantly more efficient to reduce the CBS incidence (Table 3). However, they did not
214 necessarily represent the same metallic copper rate (Table 1), contradicting the
215 recommendation by equivalence (SILVA JÚNIOR et al., 2016b). These data showed that the
216 rate should be specific for each cupric fungicide source, and cannot be generalized or
217 equalized by the equivalent metallic copper. These differences were less pronounced over the
218 evaluations, although a high effectiveness had been maintained. The CBS incidence in the
219 control treatment varied from 44% to 94% from the first (10/08) to the last evaluation (14/11),
220 while in the most efficient chemical treatment it varied from 28% to 77%. These are similar
221 results to those found by Scaloppi et al. (2012), who found that better responses are
222 dependent on the simultaneous use of different alternatives, including fungicide with different

223 properties from protectors and cultural practices that reduce the inoculum, thus, reducing the
 224 CBS incidence and severity.

225
 226 **Table 3.** Incidence of citrus black spot (*Phyllosticta citricarpa*) in orange fruits of the Pera
 227 cultivar treated with different cupric fungicides rates and sources in the 2015/2016 crop
 228 season.

Treatments	Rate g 100 L ⁻¹	Incidence			
		Evaluation 1	Evaluation 2	Evaluation 3	Evaluation 4
		08/10/2016	09/08/2016	10/06/2016	11/14/2016
Copper hydroxide (538 g kg ⁻¹)	125.0	22.0 a	33.1 a	46.0 a	67.3 a
Copper hydroxide (538 g kg ⁻¹)	100.0	32.8 b	42.0 a	52.3 a	81.5 b
Copper hydroxide (538 g kg ⁻¹)	75.0	42.3 b	43.8 a	46.3 a	77.0 a
Copper oxychloride (840 g kg ⁻¹)	200.0	23.0 a	36.5 a	51.0 a	73.0 a
Copper oxychloride (840 g kg ⁻¹)	180.0	22.0 a	36.7 a	52.8 a	73.0 a
Copper oxychloride (840 g kg ⁻¹)	135.0	40.3 b	48.0 b	56.5 a	88.8 b
Cuprous oxide (860 g kg ⁻¹)	120.0	33.3 b	36.5 a	40.8 a	72.5 a
Cuprous oxide (860 g kg ⁻¹)	75.0	38.8 b	41.3 a	44.5 a	74.5 a
Cuprous oxide (860 g kg ⁻¹)	56.3	46.6 b	49.6 b	53.3 a	87.0 b
Control		44.4 b	58.4 c	72.8 b	94.3 b
F Test		6.563 **	8.11 **	3.964 **	2.92 *
CV (%)		8.40	6.40	12.31	39.10

229 The data of incidence of citrus black spot were transformed according to the equation
 230 $x' = \arcsen\sqrt{x/100}$. Means followed by the same letter in the columns do not differ by the
 231 Scott-Knott test at 5% probability of error. ** = significant by the analysis of variance at 1%
 232 probability of error; * = significant by the analysis of variance at 5% probability of error.

233

234 The treatments had no effect on the CBS severity (Table 4); the CBS severity increased
 235 even in plots treated with fungicide. No treatment completely controlled the CBS. The
 236 fungicides and rates presented no differences for the control of CBS; they were different only
 237 from the control treatment.

238

Treatments	Rate g 100 L ⁻¹	Severity			
		Evaluation	Evaluation	Evaluation	Evaluation
		1 08/10/2016	2 09/08/2016	3 10/06/2016	4 11/14/2016
Copper hydroxide (538 g kg ⁻¹)	125.0	0.3 a	0.4 a	0.6 a	0.9 a
Copper hydroxide (538 g kg ⁻¹)	100.0	0.4 b	0.6 b	0.8 a	1.1 a
Copper hydroxide (538 g kg ⁻¹)	75.0	0.6 d	0.6 b	0.7 a	1.2 a
Copper oxychloride (840 g kg ⁻¹)	200.0	0.2 a	0.4 a	0.6 a	0.9 a
Copper oxychloride (840 g kg ⁻¹)	180.0	0.3 a	0.5 a	0.7 a	1.0 a
Copper oxychloride (840 g kg ⁻¹)	135.0	0.4 b	0.6 b	0.8 a	1.1 a
Cuprous oxide (860 g kg ⁻¹)	120.0	0.4 b	0.4 a	0.5 a	0.9 a
Cuprous oxide (860 g kg ⁻¹)	75.0	0.5 c	0.5 b	0.6 a	1.0 a

Cuprous oxide (860 g kg ⁻¹)	56.3	0.6 d	0.6 b	0.7 a	1.1 a
Control		0.7 d	0.9 c	1.1 b	1.9 b
F Test		16.21 **	11.11 **	4.08 **	8.15 **
CV (%)		4.01	3.98	6.55	6.12

239 **Table 4.** Severity of citrus black spot (*Phyllosticta citricarpa*) in orange fruits of the Pera
240 cultivar treated with different cupric fungicides rates and sources in the 2015/2016 crop
241 season.

242 The data of severity of citrus black spot were transformed according to the equation $x' =$
243 $\sqrt{x/0.5}$. Means followed by the same letter in the columns do not differ by the Scott-Knott
244 test at 5% probability of error. ** = significant by the analysis of variance at 1% probability
245 of error.

246
247

248 The initial cupric fungicide applications by spraying were essential to control CBS,
249 since the control treatment without application of cupric fungicides presented higher CBS
250 incidence and severity (Table 5).

251 According to the area under the disease progress curve (AUDPC) for CBS incidence,
252 only the lowest rates of copper oxychloride (135 g 100 L⁻¹) and cuprous oxide (75 g 100 L⁻¹)
253 presented significant lower efficiency, increasing the CBS incidence (Table 5). The AUDPC
254 for CBS severity showed that all the treatments were significantly different, and that they
255 were different from the control treatment. Regarding the copper hydroxide, only the highest
256 rate (125 g 100 L⁻¹) was equivalent to the best treatments; the intermediate and highest rates
257 of copper oxychloride and cuprous oxide presented the best results of control.

258

259 **Table 5.** Area under the disease progress curve (AUDPC) for incidence and severity of citrus
260 black spot (*Phyllosticta citricarpa*) and yield of orange plants of the Pera cultivar as a
261 function of cupric fungicides rates and sources, in the 2015/2016 crop season.

Treatments	Rate g 100 L ⁻¹	AUDPC		Orange yield
		Incidence (%)	Disease index	Kg plant ⁻¹ 11/30/2016

Copper hydroxide (538 g kg ⁻¹)	125.0	4114.6 a	52.3 a	70.9 a
Copper hydroxide (538 g kg ⁻¹)	100.0	5011.8 a	68.6 b	68.6 a
Copper hydroxide (538 g kg ⁻¹)	75.0	4911.7 a	74.1 b	49.9 a
Copper oxychloride (840 g kg ⁻¹)	200.0	4504.4 a	53.7 a	75.4 a
Copper oxychloride (840 g kg ⁻¹)	180.0	4555.4 a	60.1 a	77.0 a
Copper oxychloride (840 g kg ⁻¹)	135.0	5574.4 b	71.7 b	62.3 a
Cuprous oxide (860 g kg ⁻¹)	120.0	4301.6 a	51.5 a	68.7 a
Cuprous oxide (860 g kg ⁻¹)	75.0	4682.4 a	60.8 a	61.7 a
Cuprous oxide (860 g kg ⁻¹)	56.3	5571.1 b	69.2 b	69.4 a
Control		6584.1 c	108.8 c	58.2 a
F Test		5.70 **	9.02 **	0.40 ns
CV (%)		6.17	7.88	39.46

262 The data of incidence and severity of citrus black spot were transformed according to the
 263 equations $x' = \arcsen\sqrt{x/100}$, and $x' = \sqrt{x + 0.5}$, respectively. Means followed by the
 264 same letter in the columns do not differ by the Scott-Knott test at 5% probability of error. **
 265 = significant by the analysis of variance at 1% probability of error; ns = not significant by the
 266 analysis of variance at 5% probability of error.

267
 268 As in Experiment I, the results of Experiment II showed no equivalence in metallic
 269 copper rate in the treatments with the best control of CBS. The treatment 1 (copper hydroxide
 270 at 125 g 100 L⁻¹) had the lower metallic copper concentration in the solution (43.7 g 100 L⁻¹).
 271 When using 42.2 g 100 L⁻¹ with cuprous oxide, the results were significantly lower. These
 272 were similar results to those found by Feichtenberger et al. (2001), who found equivalence of
 273 control between cupric fungicides using a high rate of metallic copper (90 g of Cu⁺⁺ 100 L⁻¹).

274 The orange yield presented no significant differences (Table 5). The sequence of the
 275 experiment with applications of azoxystrobin maintained the CBS severity at lower levels in
 276 the control treatment, presenting no yield losses, but losses in the fruit aesthetical quality,
 277 which were not suited for marketing as fresh fruits.

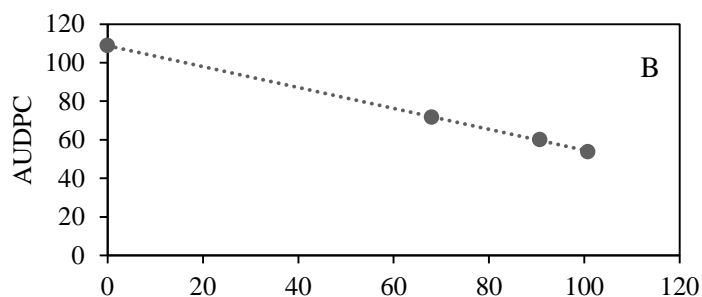
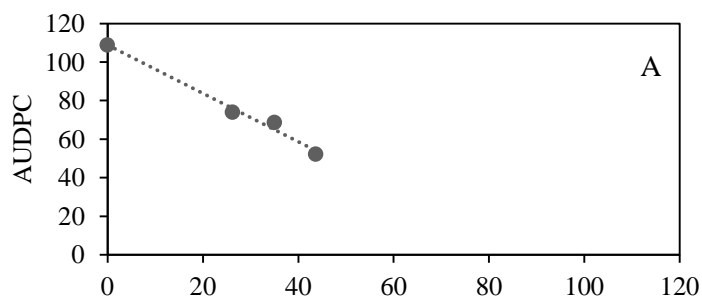
278 Significant linear responses of the CBS were found for all fungicide treatments,
 279 indicating that the better control levels of CBS are achieved with the use of increasing rates of
 280 Cu⁺⁺ (Table 6 and Figure 1). However, this result makes unfeasible the determination of the

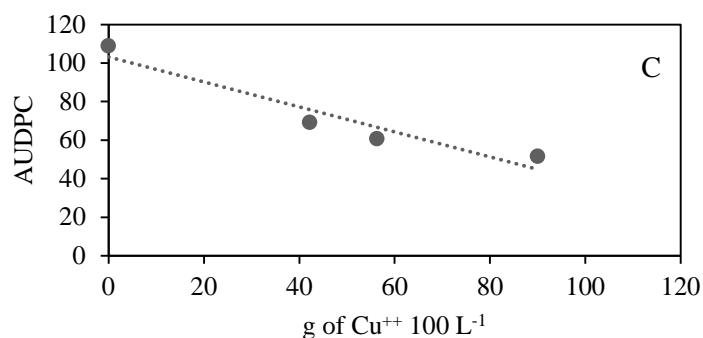
281 threshold for control of CBS and maximum fungicide rate, within the range of rates
 282 established for this experiment.

283
 284 **Table 6.** Regression by analysis of variance of the area under the disease progress curve
 285 (AUDPC) for citrus black spot (*Phyllosticta citricarpa*) in orange plants of the Pera cultivar,
 286 for each cupric fungicide as a function of the rate used, in the 2015/2016 crop season.

Cupric fungicide	Equation model	Equation	CV (%)	F Test	R ²
Copper hydroxide	Linear	$y = -1.2523x + 108.79$	20.69	27.178**	98.80%
Copper oxychloride	Linear	$y = -0.5431x + 108.82$	15.38	56.837**	99.98%
Cuprous oxide	Linear	$y = -0.6483x + 103.14$	14.84	60.386**	91.85%

287





288 A = Copper hydroxide; B = Copper oxychloride; C = cuprous oxide

289 **Figure 1.** Graph of the linear regression model for the area under the disease progress curve
 290 (AUDPC) for citrus black spot (*Phyllosticta citricarpa*) in orange of the Pera cultivar, for
 291 each cupric fungicide as a function of the metal rate copper used, in the 2015/2016 crop
 292 season.

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295 CONCLUSION

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297 The fungicides copper hydroxide, copper oxychloride, and cuprous oxide at rates of
 298 43.7, 90.7, and 56.3 g of Cu⁺⁺ 100 L⁻¹, respectively, are efficient and present similar results
 299 for the control of citrus black spot when applied with 21-day intervals and before applications
 300 of strobilurin fungicides.

301 The fungicides copper hydroxide, copper oxychloride, and cuprous oxide at rates of
 302 43.7, 90.7 and 90 g of Cu⁺⁺ 100 L⁻¹, respectively, can control protectively citrus fruits from
 303 infections with *Phyllosticta citricarpa* up to 28 days.

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