

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

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5 DANILO FRANCO^{2*}, ANTONIO DE GOES², FERNANDA DIAS PEREIRA²

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

24
25 **Keywords:** *Citrus sinensis*. *Phyllosticta citricarpa*. Metallic copper.

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28 **FONTES E CONCENTRAÇÕES DE FUNGICIDAS CÚPRICOS NO CONTROLE DA**
29 **MANCHA PRETA DOS CITROS**

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32 **RESUMO** – A mancha preta dos citros é uma doença severa para a citricultura do Estado de
33 São Paulo. Parte do seu manejo é focado no controle químico usando fungicidas cúpricos e
34 estrobilurinas. Este trabalho teve o objetivo de avaliar a eficácia de três fontes e três

35 concentrações de fungicidas cúpricos (hidróxido de cobre, oxiclreto de cobre e óxido
36 cuproso). Frutos de laranja 'Pera' foram ensacados nas plantas seguido de tratamento com
37 fungicida cúprico. Posteriormente, após zero; sete; 14; 21 e 28 dias tais frutos foram
38 inoculados com *Phyllosticta citricarpa* (1x10⁴ conídios mL⁻¹), por pulverização, seguido de
39 novo ensacamento. A avaliação da incidência e severidade da doença ocorreu na colheita dos
40 frutos. Um segundo experimento, sob infecção natural, foi constituído pelos mesmos
41 tratamentos, sendo os fungicidas aplicados nos estádios F1 (queda de pétalas) e F2 (frutos
42 com 1cm de diâmetro) mediante pulverização tratorizada e vazão média 7,35 L calda planta⁻¹.
43 Subsequentemente, e de forma semelhante para todos os tratamentos, foram realizadas quatro
44 aplicações de azoxistrobina (30 g de i.a./ha) acrescida de óleo mineral a 0,25%. Foram
45 realizadas quatro avaliações mensais para determinação da incidência e severidade dos
46 sintomas. Concluiu-se que: as pulverizações iniciais com fungicidas cúpricos são
47 fundamentais para o controle da mancha preta dos citros; independente das condições de
48 condução, para ambos os ensaios o fungicida hidróxido de cobre propiciou a melhor resposta
49 de controle da mancha preta dos citros com a menor dosagem de cobre metálico (43,7 g de
50 Cu⁺⁺100L⁻¹).

51

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

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54 *Corresponding author

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57 ²Department of Phytossanitary, Universidade Estadual Paulista, Jaboticabal, SP, Brazil;
58 franco.danilo@gmail.com - ORCID: 0000-0003-3331-974X, adgoes@yahoo.com.br -
59 ORCID: 0000-0002-7749-3523, fe.eng.agronomica@gmail.com - ORCID: 0000-0002-6280-
60 1562.

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62 INTRODUCTION

63

64 Citrus black spot (CBS) is caused by the fungus *Phyllosticta citricarpa* McAlp.
65 (teleomorph: *Guignardia citricarpa* Kiely) (BALDASSARI; WICKERT; GOES, 2008), and
66 is associated with citrus plants in several countries in Africa, Asia, Oceania (KOTZÉ, 2000;
67 EPPO, 2017), South America and North America, and in Caribe (TIMMER et al., 2000;
68 SCHUBERT et al., 2010; HIDALGO and PÉREZ, 2010). All citrus varieties of economic

69 importance are susceptible to this fungus, with losses that can reach 40% of the production
70 (SILVA JÚNIOR et al., 2016).

71 The control of CBS is done usually by using fungicides; in Brazil, it is controlled
72 mainly with use of cupric and strobilurin fungicides (MOTTA, 2009; VINHAS, 2011; SILVA
73 JÚNIOR et al., 2016). Cupric fungicides are commonly applied after petal fall, corresponding
74 to the stages F1 and F2, until the fruits reach diameter of 1 cm (STOLLER, 2010;), followed
75 by two to five applications of strobilurin fungicides (SCALLOPPI et al., 2012), covering the
76 more susceptible period for fruits (AGUIAR et al., 2012), from petal fall until the end of the
77 summer, when rainfall periods of more than 8 hours still occur. Ikeda (2011) reported
78 satisfactory responses of control of CBS to the use of five or six applications of cupric
79 fungicides combined or alternated with strobilurins.

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

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

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97

98 MATERIAL AND METHODS

99

100 Experiment I – Protector effect for different sources and rates of cupric fungicides 101 against *Phyllosticta citricarpa* infection in orange fruits of the Pera cultivar

102

103 The experiment was conducted in 2012 in an orchard with orange plants of the Pera
 104 cultivar (plant spacing of 5.5×2 m), at a private property in Olímpia, state of São Paulo (SP),
 105 Brazil ($20^{\circ}41'57.23''$ S and $48^{\circ}59'35.33''$ W).

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

108 The fungicides evaluated were copper hydroxide (Kocide WDG Bioactive[®] 462 g of
 109 copper hydroxide, Mitsui & Co. Brasil, S.A.), copper oxychloride (Recop[®] 840 g of copper
 110 oxychloride, Atanor do Brasil), and cuprous oxide (Redshield 750[®] 750 g of cuprous oxide,
 111 Agrovant Comércio de Produtos Agrícolas Ltda). The treatments and rates used are shown in
 112 Table 1.

113 Approximately 5,000 fruits at stage F2 (STOLLER, 2010) of 100 plants were bagged on
 114 02/23/2015 to avoid infections by *P. citricarpa* naturally presents in the area. When the plants
 115 were at stage F4, they were treated with cupric fungicide on 05/13/2016. Subsequently, the
 116 fruits were inoculated with *P. citricarpa* (1×10^4 conidia mL^{-1}) in intervals of 0, 7, 14, 21, and
 117 28 days. The inoculum was prepared and the inoculation was done according to the
 118 methodology described by Almeida et al. (2008). After inoculation, the fruits were bagged
 119 again, using double layer bags (crystal paper), according to the methodology adopted by
 120 Motta (2009). The fruits were kept in the bag until the harvest (10/31/2016).

121
 122 **Table 1.** Description of the cupric fungicide treatments for the control of citrus black spot in
 123 orange plants of the Pera cultivar, and their active ingredient concentrations, formulated
 124 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			

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

128
 129 The fungicides were applied according to an adaption of the method of Motta (2009);
 130 fruits with diameter of 40 to 50 mm were individually sprayed with fungicide, plus mineral oil
 131 at 0.20% (v v⁻¹).

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

136 The evaluations consisted of determination of the incidence (%) of symptomatic fruits
 137 for citrus black spot (CBS), and the disease severity. The CBS severity was estimated using a
 138 scale of grades, according to visual symptoms in percentage of lesion area—0 = fruits without
 139 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 =
 140 6.2% to 12.5%, 6 = more than 6.2% (SPÓSITO et al., 2004).

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

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

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

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

148
 149 **Experiment II - Evaluation of rates of cupric fungicides for the control of citrus black**
 150 **spot**

151

152 The experiment was conducted in 2007, under natural conditions of infection by *P.*
153 *citricarpa*, in an orchard with orange plants of the Pera cultivar (plant spacing of 7×3.5 m),
154 at a private property of commercial production in Olímpia, SP, Brazil ($20^{\circ}47'59.17''$ S,
155 $49^{\circ}2'34.94''$ W).

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

158 The fungicides were applied using a mechanized sprayer (FM Copling) with 54 nozzles
159 MagnoJet-DDC4, diffusers 25 with 758.42 kPa at 540 RPM, power takeoff with 1900 RPM,
160 and tractor speed of 3.4 km h^{-1} . The mean flowrate was $7.35 \text{ L plant}^{-1}$, equivalent to 134 mL
161 m^{-3} on the plants' canopies. The cupric fungicides were sprayed when the plants were at the
162 stages F1 and F2 (STOLLER, 2010), on 12/02/2015 and 12/23/2015. The spraying of
163 fungicides were resumed on 01/13/2016 with foliar applications of 500 g of azoxystrobin
164 (Vantigo[®], Syngenta Proteção de Cultivos Ltda, São Paulo) at concentration of 16 g per 100 L
165 of water plus mineral oil (Agefix[®], Packblend Indústria e Comércio de Lubrificantes Ltda) at
166 $0.25\% (\text{v v}^{-1})$ until 05/19/2016, totaling four applications with 42-day intervals.

167 A randomized block experimental design was used, consisting of 10 treatments and four
168 replications. The plots consisted of three rows of 11 plants, totaling 33 plants or 808.5 m^2 .

169 The evaluations were carried out on 08/10/2016, 09/08/2016, 10/06/2016, and
170 11/14/2016 to determine the CBS incidence and severity in 100 random fruits collected from
171 the five central plants of each plot. The CBS severity was estimated using a scale of grades
172 (SPÓSITO et al., 2004), and the data were used to determine the disease index (DI),
173 according to the same equation used in Experiment I.

174 The DI data were used to determine the area under the disease progress curve (AUDPC)
175 by the trapezoidal method $(DI1 + DI2)/2 * (\text{Day2} - \text{Day1})$. The fruits were harvested on
176 11/30/2016, when the fruit yield per plant (kg) was determined. The data were subjected to
177 analysis of variance and the means were compared by the Scott-Knott test at 5% probability
178 of error. The data were also subjected to regression analysis to determine the rates of each
179 fungicide based on linear and quadratic responses of the AUDPC.

180

181

182 **RESULTS AND DISCUSSION**

183

184 **Experiment I**

185

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

192 The number of days after the application of fungicides had a significant and negative
 193 effect, with lower CBS incidence and severity when the inoculation with *Phyllosticta*
 194 *citricarpa* and the treatment was carried out on the same day. The inoculations between 7 and
 195 28 days after the fungicide application resulted in similar protections. This result is related to
 196 those reported by Motta (2009), who found incidence of 30% and 80% when the inoculation
 197 was at 1 and 28 days after application of copper oxychloride, respectively. Thus, the
 198 methodology used is efficient to evaluate and replicate results, and can be used to evaluate
 199 new formulations or fungicides.

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

210
 211 **Table 2.** Incidence and disease index of citrus black spot (*Phyllosticta citricarpa*) in orange
 212 fruits of the Pera cultivar as a function of cupric fungicides rates and sources and days after
 213 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	

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

219

220 Experiment II

221

222 According to the first evaluations, in August, the highest cupric fungicide rates were
223 significantly more efficient to reduce the CBS incidence (Table 3). However, they did not
224 necessarily represent the same metallic copper rate (Table 1), contradicting the

225 recommendation by equivalence (SILVA JÚNIOR et al., 2016b). These data showed that the
 226 rate should be specific for each cupric fungicide source, and cannot be generalized or
 227 equalized by the equivalent metallic copper. These differences were less pronounced over the
 228 evaluations, although a high effectiveness had been maintained. The CBS incidence in the
 229 control treatment varied from 44% to 94% from the first (10/08) to the last evaluation (14/11),
 230 while in the most efficient chemical treatment it varied from 28% to 77%. These are similar
 231 results to those found by Scaloppi et al. (2012), who found that better responses are
 232 dependent on the simultaneous use of different alternatives, including fungicide with different
 233 properties from protectors and cultural practices that reduce the inoculum, thus, reducing the
 234 CBS incidence and severity.

235
 236 **Table 3.** Incidence of citrus black spot (*Phyllosticta citricarpa*) in orange fruits of the Pera
 237 cultivar treated with different cupric fungicides rates and sources in the 2015/2016 crop
 238 season.

Treatments	Rate g 100 L ⁻¹	Incidence			
		Evaluation	Evaluatio	Evaluation	Evaluation
		1	n 2	3	4
		08/10/2016	09/08/2016	10/06/2016	11/14/2016
			6		
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	120.0	33.3 b	36.5 a	40.8 a	72.5 a

kg ⁻¹)					
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

239 The data of incidence of citrus black spot were transformed according to the equation
 240 $x' = \arcsen\sqrt{x/100}$. Means followed by the same letter in the columns do not differ by the
 241 Scott-Knott test at 5% probability of error. ** = significant by the analysis of variance at 1%
 242 probability of error; * = significant by the analysis of variance at 5% probability of error.

243

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

248

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

Treatments	Rate g 100 L ⁻¹	Severity			
		Evaluation	Evaluation	Evaluation	Evaluation
		1	2	3	4
		08/10/2016	09/08/2016	10/06/2016	11/14/2016
1. Copper hydroxide (538 g kg ⁻¹)	125.0	0.3 a	0.4 a	0.6 a	0.9 a
2. Copper hydroxide (538 g kg ⁻¹)	100.0	0.4 b	0.6 b	0.8 a	1.1 a
3. Copper hydroxide (538 g kg ⁻¹)	75.0	0.6 d	0.6 b	0.7 a	1.2 a

4. Copper oxychloride (840 g kg ⁻¹)	200.0	0.2 a	0.4 a	0.6 a	0.9 a
5. Copper oxychloride (840 g kg ⁻¹)	180.0	0.3 a	0.5 a	0.7 a	1.0 a
6. Copper oxychloride (840 g kg ⁻¹)	135.0	0.4 b	0.6 b	0.8 a	1.1 a
7. Cuprous oxide (860 g kg ⁻¹)	120.0	0.4 b	0.4 a	0.5 a	0.9 a
8. Cuprous oxide (860 g kg ⁻¹)	75.0	0.5 c	0.5 b	0.6 a	1.0 a
9. Cuprous oxide (860 g kg ⁻¹)	56.3	0.6 d	0.6 b	0.7 a	1.1 a
10. Control		0.7 d	0.9 c	1.1 b	1.9 b
16.2					
F Test		1 **	11.11 **	4.08 **	8.15 **
CV (%)		4.01	3.98	6.55	6.12

252 The data of severity of citrus black spot were transformed according to the equation $x' =$
253 $\sqrt{x/0.5}$. Means followed by the same letter in the columns do not differ by the Scott-Knott
254 test at 5% probability of error. ** = significant by the analysis of variance at 1% probability
255 of error.

256

257

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

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

268

269

270 **Table 5.** Area under the disease progress curve (AUDPC) for incidence and severity of citrus
 271 black spot (*Phyllosticta citricarpa*) and yield of orange plants of the Pera cultivar as a
 272 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

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

278
 279 As in Experiment I, the results of Experiment II showed no equivalence in metallic
 280 copper rate in the treatments with the best control of CBS. The treatment 1 (copper hydroxide
 281 at 125 g 100 L⁻¹) had the lower metallic copper concentration in the solution (43.7 g 100 L⁻¹).
 282 When using 42.2 g 100 L⁻¹ with cuprous oxide, the results were significantly lower. These
 283 were similar results to those found by Feichtenberger et al. (2001), who found equivalence of
 284 control between cupric fungicides using a high rate of metallic copper (90 g of Cu⁺⁺ 100 L⁻¹).

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

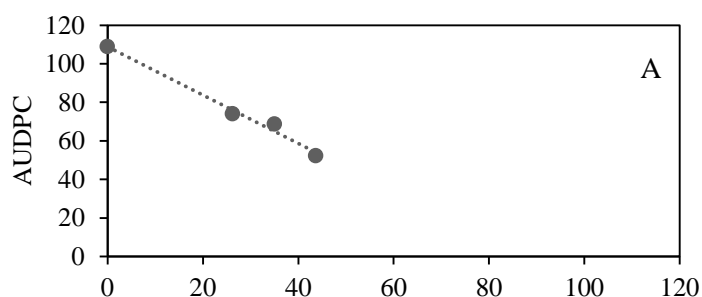
289 Significant linear responses of the CBS were found for all fungicide treatments,
 290 indicating that the better control levels of CBS are achieved with the use of increasing rates of
 291 Cu^{++} (Table 6 and Figure 1). However, this result makes unfeasible the determination of the
 292 threshold for control of CBS and maximum fungicide rate, within the range of rates
 293 established for this experiment.

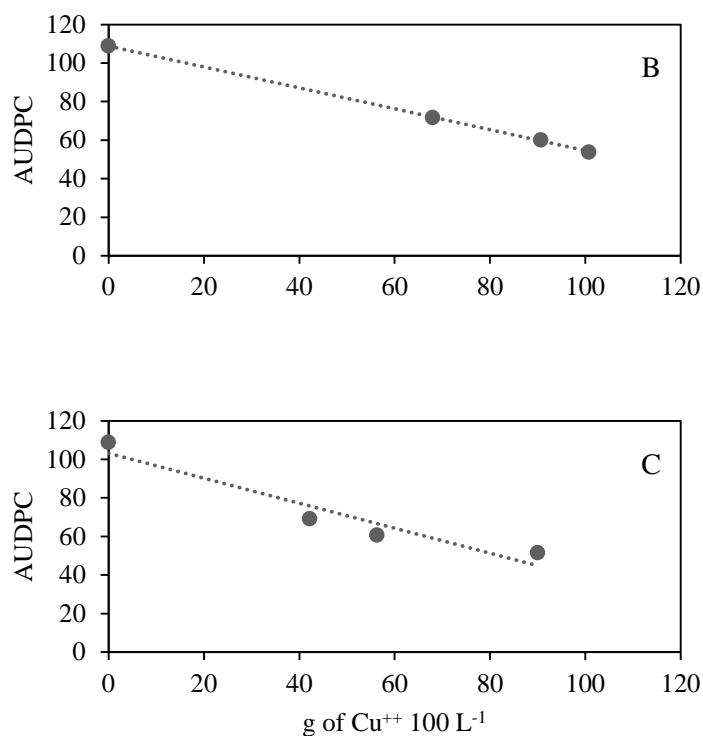
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295 **Table 6.** Regression by analysis of variance of the area under the disease progress curve
 296 (AUDPC) for citrus black spot (*Phyllosticta citricarpa*) in orange plants of the Pera cultivar,
 297 for each cupric fungicide as a function of the 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%

298





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

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

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308

309 CONCLUSION

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311 The fungicides copper hydroxide, copper oxychloride, and cuprous oxide at rates of
 312 43.7, 90.7, and 56.3 g of Cu⁺⁺ 100 L⁻¹, respectively, are efficient and present similar results
 313 for the control of citrus black spot when applied with 21-day intervals and before applications
 314 of strobilurin fungicides.

315 The fungicides copper hydroxide, copper oxychloride, and cuprous oxide at rates of
316 43.7, 90.7 and 90 g of Cu⁺⁺ 100 L⁻¹, respectively, can control protectively citrus fruits from
317 infections with *Phyllosticta citricarpa* up to 28 days.

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319

320 REFERENCES

321

322 AGUIAR, R. L. et al. Período de incubação de *Guignardia citricarpa* em diferentes estádios
323 fenológicos de frutos de laranjeira 'Valência'. **Tropical Plant Pathology**, 37: 155-158, 2012.

324

325 ALMEIDA, T. F.; REIS, R. F.; GOES, A. Method of inoculation of *Guignardia citricarpa*
326 (*Phyllosticta citricarpa*) on 'Pêra Rio' sweet orange fruit. In: IX INTERNATIONAL
327 CONGRESS OF PLANT PATHOLOGY, 2008, Torino. **Proceedings...** 2008. International
328 Society for Plant Pathology, 2008. 1: 158-159.

329

330 BALDASSARI, R. B.; WICKERT, E.; GOES, A. Pathogenicity, colony morphology and
331 diversity of isolates of *Guignardia citricarpa* and *G. mangiferae* isolated from *Citrus* spp.
332 **Europe Journal Plant Pathology**, 120: 103-110, 2008.

333

334 BRASIL. Ministério da Agricultura e do Abastecimento. **AGROFIT - Sistema de**
335 **Agrotóxicos Fitosanitários.** Disponível em:
336 <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 24 mai.
337 2017.

338

339 European and Mediterranean Plant Protection Organization - EPPO. **PQR-EPPO data base**
340 **on quarantine pests.** 2017. Disponível em:
341 <<http://www.eppo.int/DATABASES/pqr/pqr.htm>>. Acesso em: 30 ago. 2017.

342

343 FEICHTENBERGER, E.; et al. Competição de fungicidas à base de cobre no controle da
344 mancha preta (*Guignardia citricarpa*) em laranjeiras 'Folha Murcha'. **Fitopatologia**
345 **Brasileira**, 26: 444-449, 2001.

346

347 HIDALGO G.; PÉREZ V. L. Diferenciación morfológica, cultural y biológica de *Guignardia*
348 *citricarpa* y *Guignardia mangiferae* en frutos cítricos de Cuba. **Fitosanidad**, 14: 141-152,

349 2010.
350
351 IKEDA, M. **Efeito de fungicidas do grupo químico das estrobilurinas no controle da**
352 **mancha preta dos citros, na produção e na qualidade tecnológica dos frutos.** 2011. 35 f.
353 Dissertação (Mestrado em Agronomia: Área de Concentração em Produção Vegetal) -
354 Faculdade de Ciências Agrárias e Veterinárias, Unesp, Jaboticabal, 2011.
355
356 KOTZÉ, J. M. Citrus black spot. In: TIMMER, L.W.; GARNSEY, S. M.; GRAHAM, J. H.
357 (Eds.). **Compendium of Citrus Diseases.** St. Paul, MN: The American Phytopathological
358 Society, 2000. v 2, cap.6, p.595-634.
359
360 MOTTA, R. R. **Determinação do período residual de fungicidas protetor e sistêmico para**
361 **o controle de *Guignardia citricarpa* em frutos cítricos.** 2009. 84 f. Dissertação (Mestrado
362 em Agronomia: Área de Concentração em Produção Vegetal) – Faculdade de Ciências
363 Agrárias e Veterinárias, Unesp, Jaboticabal, 2009.
364
365 SCALOPPI, E. M. T. et al. Efeito do manejo cultural e químico na incidência e severidade da
366 mancha-preta dos citros. **Revista Brasileira de Fruticultura**, 34: 102-108, 2012.
367
368 SCHUBERT, T. et al. **Citrus Black Spot (*Guignardia citricarpa*) Discovered in Florida.**
369 Disponível em: <www.doacs.state.fl.us/pi/pest_alerts/pdf/citrus-black-spotpest-alert.pdf>.
370 Acesso em: 22 mai. 2017.
371
372 SILVA JUNIOR, G. J. et al. **Pinta preta: a doença e seu manejo.** Araraquara, SP:
373 Fundecitrus, 2016. 208 p.
374
375 SPÓSITO, M. B. et al. Elaboração e validação de escala diagramática para avaliação da
376 severidade da mancha preta em frutos cítricos. **Fitopatologia Brasileira**, 29: 81-85, 2004.
377
378 STOLLER DO BRASIL LTDA. **Guia de fases de desenvolvimento: Citros.** 1. ed.
379 Cosmópolis, SP, 2010. 4 p.
380
381 TIMMER, L. W.; GARNSEY, S. M.; GRAHAM, J. H. **Compendium of Citrus Diseases.** 2.
382 ed. Lake Alfred, FL: IFLA, 2000, 92 p.

383

384 VINHAS, T. **Controle químico da *Guignardia citricarpa*, agente causal da mancha preta**
385 **dos citros em frutos de laranja ‘Valência’**. 2011. 41 f. Dissertação (Mestrado em
386 Agronomia: Área de Concentração em Master Citrus) - Fundo de Defesa da Citricultura,
387 Araraquara, 2011.

388

389 WHEELER, B. E. J. **An introduction to plant disease**. London: John Wiley & Sons, 1969.
390 374 p.