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1Control and yield loss modelling of circular leaf spot of persimmon caused by

2*Mycosphaerella nawae*

3

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12

13Abstract

14

15Symptoms of circular leaf spot of persimmon (CLSP), caused by *Mycosphaerella nawae*,
16consisted of necrotic spots on leaves, chlorosis and premature defoliation. Although CLSP
17is a foliar disease, early fruit maturation and abscission are frequently associated with the
18presence of lesions on leaves and defoliation, resulting in severe economic losses. Despite
19their importance for the design of efficient disease management programs, quantitative
20relationships between CLSP incidence and yield loss are unknown. Therefore, fungicide
21efficacy trials were conducted during two consecutive years in Spain to induce different
22levels of disease severity, defoliation, and yield loss. The effects of fungicide treatments on
23CLSP severity was analyzed by ordinal logistic regression models. Relative yield loss
24values were regressed against the percentage of affected leaves or defoliated obtained at
25different evaluation dates. The disease had high negative impact and complete yield loss

26was observed in the absence of effective fungicide treatments. Preventive applications of
27pyraclostrobin, trifloxystrobin, and mancozeb provided the best disease control and highest
28yields, up to 95.77 kg tree⁻¹. An exponential relationship of CLSP incidence and defoliation
29with yield loss was found. In general, model fit and predictive ability was superior when
30defoliation, rather than incidence, was used as explanatory variable. The impact of
31defoliation on yield loss was higher in earliest evaluation dates, suggesting that early leaf
32abscission may be the main factor contributing to premature fruit drop and subsequent
33yield loss. Substantial yield losses were observed even with relatively low levels of CLSP
34incidence and defoliation. Therefore, it was not possible to define a critical action
35threshold for CLSP management based on foliar symptoms.

36

37**Keywords** *Diospyros kaki*; crop loss; disease management; fungicides

38

39Introduction

40

41The oriental persimmon (*Diospyros kaki* L. f.) is a deciduous fruit tree believed to have
42originated in China (Badenes *et al.*, 2003). This country, together with Japan and Korea
43represent 95% of the 785,000 ha cultivated worldwide (FAOSTAT, 2009). In Spain,
44virtually all the production is based on the astringent cultivar ‘Rojo Brillante’. Persimmon
45fruits are harvested in October-November and treated in a controlled atmosphere to remove
46the astringency while avoiding softening (Arnal & Del Rio, 2003). As a result of the
47implementation of this postharvest treatment, cultivation and exports in Spain increased
48considerably during recent years (Perucho, 2015).

49 Circular leaf spot of persimmon (CLSP), caused by *Mycosphaerella nawae* Hiura &
50Ikata, was first observed in Spain in 2008 (Vicent, 2008; Berbegal *et al.*, 2010). In Spain,

51symptoms of the disease consisting of necrotic spots on leaves, leaf chlorosis and
52premature defoliation are usually observed in September and October. Although the fungus
53only infects the leaves, early fruit maturation and subsequent softening and abscission are
54associated with the foliar symptoms and defoliation, resulting in severe economic losses
55(Berbegal *et al.*, 2010). This disease was geographically restricted to persimmon-growing
56areas located in Japan and Korea, characterised by a summer-rainfall pattern (Ikata &
57Hitomi, 1929; Kang *et al.*, 1993). Detection of CLSP in Spain was the first report of the
58disease in a semi-arid area (Vicent *et al.*, 2012).

59 The fungus forms pseudothecia in leaf litter during winter and ascospores are
60formed as temperatures increase in spring (Kang *et al.*, 1993; Kwon *et al.*, 1997c). Field
61studies conducted in Korea indicated that temperatures over 15°C and rainfall were the
62most important factors inducing the release of ascospores (Kang *et al.*, 1993; Kwon *et al.*,
631995; 1997a). In laboratory studies, the discharge of *M. nawae* ascospores began at
64temperatures about 10°C and at least 1 mm of precipitation (Vicent *et al.*, 2011).
65Ascospores are wind-dispersed and infect persimmon leaves in spring in the presence of a
66film of water and adequate temperatures (Kang *et al.*, 1993; Kwon & Park, 2004). The
67asexual stage of *M. nawae* was identified in Korea as belonging to the genus *Ramularia*,
68but its role in field epidemics is not fully understood (Kwon *et al.*, 1998; Kwon & Park,
692004). In Spain, this secondary inoculum has not been observed (Vicent *et al.*, 2012). The
70disease is characterised by a particularly long incubation period of about four months, from
71infection in spring to symptom expression in late summer or early autumn (Vicent *et al.*,
722012).

73 Standard fungicide schedules for the control of CLSP in Korea consist of three to
74four foliar applications between mid-July to early August to protect the leaves during the
75infection period. Although the efficacy of fungicide schedules may differ depending on the

76year, nearly complete control was obtained under experimental conditions in Korea (Kwon
77*et al.*, 1997b; Kwon & Park, 2004). In Spain, preventive applications in spring with
78protectant and systemic fungicides showed also good efficacy for the control of CLSP,
79whereas postinfection sprays were ineffective (Vicent, 2008; Berbegal *et al.*, 2011).
80Cultural practices such as leaf litter removal and moving from flood to drip irrigation
81systems are also recommended to growers, although no quantitative evaluations of their
82efficacy have been conducted so far (Vicent *et al.*, 2011; 2012).

83 Early maturation and severe fruit drop are associated with leaf spots and defoliation
84caused by *M. nawae*, but quantitative relationships between the intensity of foliar
85symptoms and yield loss are not available. Disease-yield loss relationships have been
86determined for many pathosystems, mostly on field crops (Savary *et al.*, 2006). Most yield
87loss models are intended for one specific disease, but others may integrate several diseases
88simultaneously and also simulate crop growth (Rouse, 1988; Milne *et al.*, 2007; Colbach,
892010). Yield loss damage functions can be classified as single-point, or multiple-point, or
90area under the disease progress curve models depending whether disease intensity is
91measured at one or more times (Zadoks & Schein, 1979). Empirical models are often based
92on field experiments and integrate overall yield loss relationships, although they do not
93necessarily identify the underlying causal effects. Mechanistic or process-based models are
94usually derived from controlled experiments designed to investigate the physiological basis
95of yield loss. Both linear and nonlinear statistical procedures can be applied to fit disease
96intensity-yield loss data (Gaunt, 1995; Savary *et al.*, 2006). A yield loss model for CLSP
97would allow identifying potential action thresholds of disease incidence on leaves without
98compromising fruit integrity and would help to reduce the number preventive fungicide
99sprays applied in spring.

100 The objectives of this study were to: (i) study the effect of different fungicides on
101 the control of CLSP, and based on these results (ii) quantify the relationships between
102 CLSP incidence and premature defoliation to relative yield loss.

103

104 **Materials and methods**

105

106 Experiments 1 and 2 were conducted in 2009 in commercial ‘Rojo Brillante’ persimmon
107 orchards grafted on *D. virginiana* L. rootstock at Benimodo in Valencia Province, Spain.
108 Full flowering (BBCH 65, 50% of flowers open) was observed on 30 April (García-
109 Carbonell *et al.*, 2002). Trees were 8-yr-old in experiment 1 and 4 yr old in experiment 2.
110 Rows were oriented east to west with a 4 × 5-m tree spacing. Each treatment was
111 replicated four times on two-tree plots arranged in a randomised block design. Four non-
112 sprayed two-tree plots were included as a control and guard trees were located between
113 plots within rows. Trees were sprayed to runoff (~10 L tree⁻¹) with a hydraulic sprayer
114 (Maruyama, Japan) at 3,000 kPa with a hand-held spray gun.

115 Experiments 3 and 4 were conducted in 2010 in two 5-yr-old commercial ‘Rojo
116 Brillante’ persimmon orchards grafted on *D. virginiana* at Benimodo (experiment 3) and
117 11-yr-old trees grafted on *D. lotus* L. at L'Alcúdia (experiment 4) in Valencia Province,
118 Spain. Full flowering was observed on 13 May. Orchard characteristics, experimental
119 design, and fungicide application were the same described above, but spray volume rate
120 was reduced to ~3.5 L tree⁻¹.

121 The fungicides and application rates used in experiment 1 included the following:
122 copper oxychloride 0.5 and 1.0 g metallic copper (Cu) L⁻¹ (Quorum 70% wt/vol SC; IQV
123 S.A., Spain) applied on 14 May and 4 June, fosetyl-Al 2.0 g a.i. L⁻¹ (Aliette 80% wt/wt
124 WG; Bayer CropScience, S.L., Spain) applied on 14 May and 23 July, and tebuconazole

1250.2 g a.i. L⁻¹ (Folicur 25% wt/wt WG; Bayer CropScience, S.L., Spain) applied on 23 July.
 126Treatments evaluated in experiment 2 were the same including thiophanate-methyl 0.7 g
 127a.i. L⁻¹ (Topsin 70% wt/wt WG; Bayer CropScience, S.L., Spain) applied on 23 July.
 128Applications in May and June coincided with the infection period and the application in
 129July was scheduled as a postinfection spray (Vicent *et al.*, 2012).

130 Treatments evaluated in experiment 3 were: copper oxychloride 1.0 g Cu L⁻¹
 131(Cuproflow 38% wt/vol SC; Isagro S.P.A, Italy), mancozeb 1.6 g a.i. L⁻¹ (Dithane Neotec
 13275% wt/wt WG; Dow Agrosiences Ibérica, S.A., Spain), fenbuconazole 0.1 g a.i. L⁻¹
 133(Impala 5% wt/vol EW; Dow Agrosiences Ibérica, S.A., Spain), tebuconazole 0.1 g a.i. L⁻¹
 134¹ (Folicur 25% wt/wt WG; Bayer CropScience, S.L., Spain), thiophanate-methyl 0.7 g a.i.
 135L⁻¹ (Topsin 70% wt/wt WG; Bayer CropScience, S.L., Spain) mixed with 2.5 g L⁻¹ of non-
 136ionic surfactant (LI700; Kenogard S.A., Spain), trifloxystrobin 0.075 g a.i. L⁻¹ (Flint 50%
 137wt/wt WG; Bayer CropScience, S.L., Spain), and pyraclostrobin 0.075 g a.i. L⁻¹ (Cabrio
 13825% wt/vol EC; BASF Española, S.L.). Fungicide sprays were scheduled in April and May
 139to coincide with the infection period (Vicent *et al.*, 2012). All treatments were applied on
 14020 April, 5 May and 24 May, except for thiophanate-methyl which was applied only on 24
 141May.

142 Treatments evaluated in experiment 4 were: copper oxychloride 0.5 and 1.0 g Cu L⁻¹
 143¹ (Cuproflow 38% wt/vol SC; Isagro S.P.A, Italy), copper oxychloride 0.5 and 1.0 g Cu L⁻¹
 144(Quorum 70% wt/vol SC; IQV S.A., Spain), fosetyl-Al 2.0 g a.i. L⁻¹ (Aliette 80% wt/wt
 145WG; Bayer CropScience, S.L., Spain), fenbuconazole 0.1 g a.i. L⁻¹ (Impala 5% wt/vol EW;
 146Dow Agrosiences Ibérica, S.A., Spain), tebuconazole 0.1 g a.i. L⁻¹ (Folicur 25% wt/wt
 147WG; Bayer CropScience, S.L., Spain), thiophanate-methyl 0.7 g a.i. L⁻¹ (Topsin 70% wt/wt
 148WG; Bayer CropScience, S.L., Spain) mixed with 2.5 g L⁻¹ of non-ionic surfactant (LI700;
 149Kenogard S.A., Spain), and trifloxystrobin 0.075 g a.i. L⁻¹ (Flint 50% wt/wt WG; Bayer

150CropScience, S.L., Spain). Fungicide sprays were scheduled in April and May to coincide
151with the infection period (Vicent *et al.*, 2012). All treatments were applied on 21 April, 6
152May and 25 May, except for thiophanate-methyl which was applied only on 25 May.

153 Disease severity was assessed periodically, from 120 to 145 days after full
154flowering (DAFF) in 2009 and from 125 to 155 DAFF in 2010, on all leaves of 10 shoots
155arbitrarily selected in each tree (~ 70 leaves tree⁻¹) according to the following rating scale:
1560 = no lesions observed; 1 = less than 10 leaf spots; 2 = 10 to 20 leaf spots; 3 = more than
15720 leaf spots; 4 = defoliated (Vicent *et al.*, 2012). Defoliation was assessed counting the
158number of nodes in each shoot from which leaves had abscised. Marketable fruit from each
159tree were harvested in November and weighed. For each two-tree plot, average yield per
160tree was obtained and relative yield loss was calculated considering the highest yield for
161each experiment, which was considered as the attainable yield (Zadoks & Schein, 1979).
162The R version 3.1.0 was used in all statistical and graphical analyses (R Development Core
163Team, 2014).

164 Disease severity categories were treated as an order factor, which was modelled with a
165proportional odds logistic regression model using the polr function of the MASS package
166for R (Venables and Ripley, 2002)

$$167 \quad \text{logit} [P (Y_i \leq j)] = \alpha_{j0} - \beta_1 X_{1i} - \dots - \beta_k X_{ki} \quad i=1, \dots, n \quad (1)$$

168where $P (Y_i \leq j)$ is the cumulative probability between 0-1 for category j of Y_i , $j = 1, \dots, 4$
169are the categories of CLSP severity, α_{j0} are the thresholds for CLSP severity categories, β_p ,
170 $p = 1, \dots, k$ are the coefficients for the k fungicide treatments evaluated ($X_1 \dots X_k$), and n is
171the total number of two-tree plots. The proportional odds model assumes that the *logit* of
172the cumulative probabilities changes linearly as the explanatory variables change, and also
173that the slope of this relationship is the same regardless of the disease severity category
174(Bilder & Loughin, 2015). In our case, the non-treated control was used as the reference

175level and the odds ratio for each fungicide treatment was calculated as $e^{-\beta}$ based on the
 176cumulative probabilities. Because of the proportional odds assumption, the odds ratio for
 177each fungicide treatment stays the same no matter how disease severity is dichotomized
 178into two levels. This proportional odds assumption was evaluated by the Likelihood Ratio
 179Test (LRT) between the proportional odds model and the non proportional odds model
 180(Tutz, 2011) using the VGAM package for R (Yee, 2010). The goodness of fit was
 181assessed by a χ^2 test of the residual deviance against the null model (i.e. including only the
 182intercept). A significant effect of a fungicide treatment was considered when the 95%
 183confidence interval of the odds ratio did not overlap the null value of 1 (Bilder & Loughin,
 1842015).

185 The effects of fungicides on yield were analyzed only descriptively because
 186untreatable problems of heterocedasticity were detected due to the presence of zero yield in
 187several treatments. After some preliminary assessments, relative yield loss values were
 188regressed against the percentage of affected leaves (i.e. 1-4 in the rating scale) or defoliated
 189(i.e. 4 in the rating scale) obtained 125, 140, 147 or 155 DAFF in each two-tree plot of
 190experiments 3 and 4 with the exponential function (Kutner *et al.*, 2004)

$$191 \quad Yl_i = 1 - \gamma_1 \exp^{(-\gamma_2 D_i)} \quad i=1, \dots, n \quad (2)$$

192where Yl_i is the relative yield loss at plot i , D_i is the percent of affected or defoliated leaves
 193at plot i in each evaluation date (DAFF), γ_1 and γ_2 are the parameters of the model, and n is
 194the total number of two-tree plots. A model was fitted with nonlinear regression separately
 195for each evaluation date (DAFF) using the nls function for R with the Gauss-Newton
 196algorithm. Adequate mean structure was evaluated by the residual plot. Hypothesis of
 197equal variances was examined by the Levene's test and the normality assumption of the
 198residuals with a Q-Q plot. Possible correlation was check graphically with a lag-one plot of
 199the residuals (Ritz & Streibig, 2008). The root mean squared error (RMSE) of the models

200was calculated. Independent data including CLSP incidence and defoliation at 155 DAFF
201and associated yield losses published by Berbegal *et al.* (2011) were used to evaluate the
202prediction quality of the models by the root mean squared error of prediction (RMSEP).

203

204Results

205

206 In experiment 1 conducted at Benimodo in 2009, the percentage of leaves in the
207severity category 0 (no lesions) ranged from 0.18% in the non-treated control 145 DAFF to
20899.57% with copper oxychloride at 0.5 g a.i. L⁻¹ (Fig. 1). Leaves in the severity category 4
209(defoliated) ranged from 0% with copper oxychloride and fosetyl-Al at 120 and 133 DAFF
210to 64.16% in the non-treated control at 145 DAFF. In experiment 2 conducted at Benimodo
211in 2009, leaves with no lesions ranged from 0% with tebuconazole 145 DAFF to 100%
212with copper oxychloride at 1 g a.i. L⁻¹ (Fig. 1). Defoliated leaves ranged from 0% with
213copper oxychloride and fosetyl-Al at 120 DAFF to 73.99% in the non-treated control at
214145 DAFF. In experiment 3 conducted at Benimodo in 2010, leaves with no lesions ranged
215from 0.17% in the non-treated control 147 DAFF to 99.82% with trifloxystrobin at 125
216DAFF (Fig. 2). Defoliated leaves ranged from 0% with trifloxystrobin at 125 DAFF to
21797.19% in the non-treated control at 147 DAFF. In experiment 4 conducted at L'Alcúdia in
2182010, leaves with no lesions ranged from 0% with copper oxychloride (0.5 g a.i. L⁻¹),
219fosetyl-Al and tebuconazole at 147 DAFF to 92.97% with trifloxystrobin at 125 DAFF
220(Fig. 2). Defoliated leaves ranged from 0.76% with trifloxystrobin at 125 DAFF to 90.06%
221in the non-treated control at 147 DAFF.

222 The proportional odds assumption was not rejected by χ^2 test ($P > 0.05$) in the ordinal
223logistic regression of CLSP severity in either experiment and evaluation dates. In both
224cases, models including the fungicide treatments significantly reduced the residual

deviance ($P < 0.05$) compared with the null model. The factor block was not significant ($P > 0.05$) and so it was not included in the models.

In experiment 1, coppers and fosetyl-Al significantly reduced ($P < 0.05$) CLSP severity compared with the non-treated control in all evaluation dates, and tebuconazole only at 145 DAFF (Table 1). The estimated odds of CLSP severity being below a particular category changed from 1.3 times with tebuconazole up to 62.92 times with copper oxychloride (1 g a.i. L⁻¹) at 145 DAFF. The predicted probabilities for the severity category in the non-treated control ranged from 0.014 at 145 DAFF to 0.925 at 120 DAFF. With coppers, the probability for the same severity category ranged from 0.361 at 145 DAFF to 0.996 at 120 DAFF, and from 0.089 to 0.990 with fosetyl-Al. No substantial differences were observed between the two copper concentrations evaluated.

In experiment 2, a significant reduction of CLSP severity was detected with coppers and fosetyl-Al ($P < 0.05$) in all evaluation dates (Table 2). The odds ratio for copper oxychloride (1 g a.i. L⁻¹) at 120 DAFF was not calculated because all leaves were of severity category 0. The effect of tebuconazole was not significant and thiophanate-methyl reduced CLSP severity only at 145 DAFF. The estimated odds of CLSP severity being below a particular category changed from 2.16 times with thiophanate-methyl up to 69.92 times with copper oxychloride (1 g a.i. L⁻¹) at 145 DAFF. The predicted probability for the severity category 0 ranged from 0.006 to 0.923 in the non-treated control, from 0.241 to 1 with coppers, and from 0.092 to 0.989 with fosetyl-Al at 145 and 120 DAFF, respectively.

In experiments 3 and 4, all fungicide treatments significantly reduced ($P < 0.05$) CLSP severity compared with the non-treated control, except of fosetyl-Al and tebuconazole at 147 DAFF in experiment 4 (Tables 3 and 4). In experiment 3, the estimated odds of CLSP severity being below a particular category changed from 1.69 times with thiophanate-methyl at 125 DAFF up to 1765.32 times with pyraclostrobin at 147 DAFF. The predicted

250probabilities for the severity category 0 ranged from 0.003 to 0.432 in the non-treated
251control, from 0.169 to 0.935 with copper, and from 0.442 to 0.981 with mancozeb at 147
252and 125 DAFF, respectively (Table 3). With the triazoles, the probabilities ranged from
2530.020 with tebuconazole at 147 DAFF to 0.870 with fenbuconazole at 125 DAFF. In the
254case of thiophanate-methyl, the probabilities ranged from 0.032 to 0.564. With the QoI
255fungicides, the probabilities ranged from 0.667 at 147 DAFF to 0.998 at 125 DAFF with
256trifloxystrobin.

257 In experiment 4, the estimated odds of CLSP severity being below a particular
258category changed from 1.5 times with tebuconazole at 140 DAFF up to 52.15 times with
259trifloxystrobin at 147 DAFF. The predicted probabilities for the severity category 0 in the
260non-treated control were 0.010 and 0.225 at 147 and 125 DAFF, respectively (Table 4).
261With coppers the probabilities ranged from 0.019 to 0.807 at 147 and 125 DAFF,
262respectively. The copper concentration of 1 g a.i. L⁻¹ showed higher efficacy at 140 and
263147 DAFF compared with 0.5 g a.i. L⁻¹. Fosetyl-Al had probabilities from 0.013 147 DAFF
264to 0.400 at 125 DAFF. With the triazoles, the probabilities ranged from 0.013 with
265tebuconazole at 147 DAFF to 0.776 with fenbuconazole at 125 DAFF. In the case of
266thiophanate-methyl, the probability ranged from 0.028 to 0.520 at 147 and 125 DAFF,
267respectively. Trifloxystrobin had a probability for the severity category 0 of 0.339 at 147
268DAFF and 0.931 at 125 DAFF.

269 Complete yield loss was obtained in all treatments evaluated in 2009. In 2010, no fruit
270were harvested in the non-treated control plots and trees treated with fenbuconazole,
271tebuconazole, thiophanate-methyl in experiment 3 at Benimodo and copper oxychloride
272(0.5 g a.i. L⁻¹), tebuconazole and thiophanate-methyl in experiment 4 at L'Alcúdia (Table
2735). The highest average yields were obtained with the QoI fungicides, pyraclostrobin and
274trifloxystrobin. Relative high average yields of 33.74 kg tree⁻¹ were obtained with

275mancozeb in experiment 3. Mean yield obtained with copper oxychloride (1 g a.i. L⁻¹) was
27630.44-35.68 kg tree⁻¹ in experiment 4 but only 2.58 kg tree⁻¹ in experiment 3.

277Fenbuconazole had a mean yield of 19.67 kg tree⁻¹ in experiment 4 whereas only 9.36 kg
278tree⁻¹ were harvested with fosetyl-Al.

279 Due to the lack of yield data in 2009, only the data from experiments 3 and 4 in 2010
280were used to relate CLSP incidence and defoliation with yield losses. The estimated
281parameter values ($P < 0.01$) of the exponential function describing the relationship between
282CLSP incidence and relative yield loss were: $\hat{a} \hat{Y}_1 = 0.63 \pm 0.05 \text{ SE}$, $\hat{b} \hat{Y}_2 = 9.61 \pm 2.22 \text{ SE}$
283for 125 DAFF, $\hat{a} \hat{Y}_1 = 1.06 \pm 0.09 \text{ SE}$, $\hat{b} \hat{Y}_2 = 2.77 \pm 0.30 \text{ SE}$ for 140 DAFF, $\hat{a} \hat{Y}_1 = 1.24 \pm 0.15$
284SE, $\hat{b} \hat{Y}_2 = 2.26 \pm 0.26 \text{ SE}$ for 147 DAFF, and $\hat{a} \hat{Y}_1 = 1.31 \pm 0.16 \text{ SE}$, $\hat{b} \hat{Y}_2 = 2.39 \pm 0.27 \text{ SE}$
285for 155 DAFF (Fig. 3). The estimators ($P < 0.01$) for the exponential function between
286defoliation and relative yield loss were: $\hat{a} \hat{Y}_1 = 0.44 \pm 0.04 \text{ SE}$, $\hat{b} \hat{Y}_2 = 95.38 \pm 36.03 \text{ SE}$ for
287125 DAFF, $\hat{a} \hat{Y}_1 = 0.75 \pm 0.04 \text{ SE}$, $\hat{b} \hat{Y}_2 = 8.02 \pm 1.13 \text{ SE}$ for 140 DAFF, $\hat{a} \hat{Y}_1 = 0.80 \pm 0.04$
288SE, $\hat{b} \hat{Y}_2 = 3.72 \pm 0.43 \text{ SE}$ for 147 DAFF, and $\hat{a} \hat{Y}_1 = 0.84 \pm 0.04 \text{ SE}$, $\hat{b} \hat{Y}_2 = 2.91 \pm 0.29 \text{ SE}$
289for 155 DAFF (Fig. 4).

290 No systematic pattern was observed in any of the residual plots so mean functions were
291adequately capturing the average trend in the data. The assumption of variance
292homogeneity was not satisfied ($P < 0.01$) for CLSP incidence at 147 DAFF and defoliation
293at 125, 147 and 155 DAFF. The Q-Q plots showed a relative good agreement between the
294standardized residuals and the normal distribution, except for CLSP incidence at 140, 147
295and 155 DAFF. No linear trend was observed in the lag-one plot of the residuals. Values of
296RMSE for CLSP incidence ranged from 0.1834 at 140 DAFF to 0.2217 at 147 DAFF, and

2970.1393 at 155 DAFF to 0.2491 at 125 DAFF for defoliation. The value of RMSEP at 155
298DAFF was 0.3703 for CLSP incidence and 0.1852 for defoliation.

299 The models predicted yield losses of 0.85, 0.29, 0.11 and 0.08 for a CLSP incidence of
3000.15 at 125, 140, 147 and 155 DAFF, respectively (Fig. 3). Yield losses higher than 0.85
301were predicted by the models with CLSP incidences of 0.75, 0.93 and 0.91 at 140, 147 and
302155 DAFF, respectively. A yield loss of 0.39 was predicted by the model with as little as
3030.005 CLSP incidence at 125 DAFF. The models predicted yield losses of 0.72, 0.27, 0.21
304and 0.16 with a defoliation of 0.005 at 125, 140, 147 and 155 DAFF, respectively (Fig. 4).
305Yield losses higher that 0.85 were predicted by the models with defoliations of 0.015,
3060.205, 0.455 and 0.595 at 125, 140, 147 and 155 DAFF, respectively.

307

308Discussion

309

310In the absence of effective fungicide programs, high disease severity and complete yield
311loss were recorded in the experimental plots (Table 5). Similar losses were observed in
312commercial orchards in Spain where the disease was poorly managed. Due to the climatic
313differences between Spain and the production areas in Japan and Korea, where the disease
314was initially restricted, such a severe impact in a semi-arid area was not foreseen.
315Nevertheless, modelling studies indicated that the epidemic potential of the disease under
316Mediterranean conditions was found to be higher than previously thought (Makowski *et*
317*al.*, 2011), showing the limitations of correlative modelling techniques commonly used in
318pest risk analysis.

319 No commercial fruit were harvested from trees with only one spray of tebuconazole
320or thiophanate-methyl (Table 5). In the case of thiophanate-methyl, disease severity was
321significantly lower than in the non-treated control in all evaluation dates only in 2010

322(Tables 3 and 4). Epidemiological studies on CLSP conducted in Spain indicated that the
323infection periods in the area of study occurred from March to June (Vicent *et al.*, 2012),
324and that applications of thiophanate-methyl in April and May were effective for CLSP
325control (Bebegal *et al.*, 2011). Nevertheless, in our study thiophanate-methyl was applied
326in 2009 as a postinfection treatment in July, whereas in 2010 it was applied in late May,
327possibly preventing subsequent infections in June. In Korea, Kwon *et al.* (1997b) obtained
328high levels of disease control by applying one or two sprays of benomyl, carbendazim or
329myclobutanil at the end of the infection period. However, these experiments were
330conducted in young plants and yields were not quantified.

331 In general, the severity of foliar symptoms was reduced compared to the non-
332treated control when two or three fungicide sprays were applied through the infection
333period in spring (Tables 1, 2, 3 and 4). Likewise, Bebegal *et al.* (2011) reported good
334disease control with 2-4 fungicide sprays scheduled from April to June. In our study, two
335sprays of copper oxychloride at 1g Cu L⁻¹ and three sprays at 0.5g Cu L⁻¹ did not have any
336substantial effect on yield (Table 5). The amount of marketable fruit increased with three
337sprays of copper oxychloride at 1g Cu L⁻¹, but values were somewhat below the attainable
338yield in each orchard. In addition, all copper treatments induced some phytotoxicity on
339leaves.

340 Triazole fungicides are widely used for the control of *Mycosphaerella* diseases
341(Oliver and Hewitt, 2014) and have been reported to reduce the intensity of foliar
342symptoms caused by *M. nawae* (Kwon *et al.*, 1997b; Bebegal *et al.*, 2011). However,
343disease severity was not substantially reduced with the two triazoles evaluated in our study,
344fenbuconazole and tebuconazole (Tables 1, 2, 3 and 4), and no appreciable effects on yield
345were observed (Table 5). No marketable fruit were harvested from the plots treated with
346fosetyl-Al in 2009 and only 9.36 kg tree⁻¹ in 2010 (Table 5), though a significant reduction

in CLSP severity was detected in several evaluations (Tables 1, 2 and 4). This fungicide was reported also to give some control of *Septoria albopunctata* Cooke in blueberry (*Vaccinium corymbosum* L.) (Brannen *et al.*, 2004). Nevertheless, no fungitoxic effects of fosetyl-Al on *Mycosphaerellaceae* are known, so its activity might be associated with host defense mechanisms.

As in other studies on CLSP management (Berbegal *et al.*, 2011), the QoI fungicides evaluated, pyraclostrobin and trifloxystrobin, and the dithiocarbamate mancozeb provided the best disease control and highest yields (Table 5). Marketable fruit harvested from these treatments were in the range of the attainable yield expected for each orchard. In 2010, pyraclostrobin and mancozeb were temporarily authorised in Spain for the control of CLSP. In the present study, fungicides were evaluated alone to determine their efficacy, but persimmon growers in Spain are advised to alternate groups with different modes of action to avoid build-up of resistance (Vicent *et al.*, 2015).

Previous studies on the control of CLSP used only incidence data as the total percentage of leaves with at least one lesion and defoliated (Berbegal *et al.*, 2011) or disease severity measured using an ordinal scale, but analyzed as a continuous variable (Kwon *et al.*, 1997b). In our study, CLSP severity was analyzed by an ordinal logistic regression model, which is most adequate for disease rating scales and integrates more information than previous analysis using disease incidence alone (Tutz, 2011). It would be interesting to explore also possible relationships among CLSP severity, time of symptom appearance, leaf duration and fruit drop. However, these types of analyses requires the use of statistical methods for censored data, which are difficult to apply to this experimental design (Hosmer *et al.*, 2008).

The application of fungicides is perhaps the most common method used to obtain different levels of disease severity in yield loss studies (James, 1974; Sah & MacKenzie,

3721987). It is considered particularly appropriate when no other fungal diseases may
 373interfere, as was the case of CLSP in Spain. Problems of interplot interference among
 374treatments were not suspected in our study because orchards were severely affected by the
 375disease, so infected leaf litter was homogenously distributed prior to the experiments. In
 3762010, it was possible to obtain a broad range of disease severities and relative yield loss
 377values, obtaining a full representation of the disease-yield loss relationship. In contrast,
 378disease severities in 2009 were very high and complete yield loss was obtained in all
 379fungicide schedules.

380 The exponential function used in our study described the relationships of CLSP
 381incidence and defoliation to relative yield loss, and was similar to the general loss model
 382for crops by Madden *et al.* (1981). With the use of receiver operating characteristic (ROC)
 383curve analysis (Fabre *et al.*, 2003) it would be possible to define a damage threshold for
 384CLSP, but due to the strong effect of disease incidence and defoliation on yield (Figs. 1
 385and 2), it seems that foliar symptoms should be kept at very low levels for the economic
 386control of the disease. Moreover, in addition to the direct primary losses evaluated, long
 387term secondary losses such as tree weakening and negative effects on subsequent fruit set
 388should be also considered (Zadoks & Schein, 1979; Ojiambo *et al.*, 2006). Nevertheless,
 389fungicide trials were conducted following the explosive emergence of the disease in 2008,
 390which resulted in exceptionally high inoculum levels in the orchards. Higher fungicide
 391efficacies are common nowadays in Spain because inoculum levels were progressively
 392reduced after the implementation of disease control programs (Vicent *et al.*, 2012). During
 393last years, CLSP was effectively managed with 3-4 sprays with QoIs, triazoles and
 394mancozeb covering the infection period in spring (Vicent *et al.*, 2015).

395 In general, model performance was better with defoliation as explanatory variable
 396(Fig. 4). For a same level of defoliation, the impact on yield loss was higher in earliest

397evaluation dates. These results suggests that early leaf abscission was probably the main
398factor contributing to premature fruit drop and subsequent yield loss as it was indicated for
399other diseases (Backman & Crawford, 1984; Waggoner & Berger, 1987; McGovern *et al.*,
4002003; Ojiambo & Scherm, 2006). As with other fungal pathogens, leaf chlorosis and
401subsequent defoliation may be induced by ethylene produced as a consequence of fungal
402infection (Broekaert *et al.*, 2006). Since fruits are not directly infected by the pathogen,
403early ripening and abscission might be associated to ethylene formed in response to water
404stress caused by premature defoliation (Nakano *et al.*, 2002). In any case, the physiological
405processes behind CLSP symptom expression still need to be elucidated.

406 Foliar symptoms caused by *M. nawae* are characterised by necrotic spots
407accompanied by extensive chlorosis of the leaf surface. Although leaf chlorosis can affect
408yield by reducing the photosynthetic potential of the tree canopy, it was not included in the
409disease rating scale used in the present study. Alternative disease-related variables such as
410healthy leaf area index (HLAI) or healthy leaf area absorption (HAA), which take into
411account leaf spot severity, defoliation as well as leaf chlorosis, may improve the
412performance of yield loss models for CLSP (Waggoner & Berger, 1987; Aquino *et al.*,
4131992; Bergamin-Filho *et al.*, 1997; Leite *et al.*, 2006). Optical coherence tomography has
414been recently used by Wijesinghe *et al.* (2016) to identify morphological differences
415between healthy persimmon leaves and those infected by *M. nawae*. Likewise, the
416potential of spectroscopy, imaging and volatile profiling techniques for CLSP monitoring
417may be explored, also for infected but asymptomatic leaves during the incubation period
418(Sankaran *et al.*, 2010).

419 Yield loss models have been used for short- and long-term disease control decisions
420in several crops. The main goal of these kind of models is to predict losses as early as
421possible in order to provide treatments, so they usually include parameters such as the time

422of disease onset or critical disease thresholds (Zadoks & Schein, 1979; Savary *et al.*, 2006).
423Due to the long incubation period of circular leaf spot, symptom expression nearly
424coincides with harvest and postinfection treatments are ineffective. Therefore, preventive
425fungicide sprays should be applied in spring, months before the first symptoms are visually
426perceptible. Nevertheless, the models of defoliation could be used to predict yield losses
427few weeks prior to harvest, which may be of practical value to adjust harvesting and
428storage logistics as well as to estimate potential market supply.

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581**Table 1** Probabilities and odds ratios of the proportional odds logistic regression model for circular leaf spot severity on ‘Rojo Brillante’

582persimmon shoots with the fungicide treatments evaluated at Benimodo in 2009 (experiment 1).

Evaluation dates and fungicides (g a.i. L ⁻¹)	Probabilities ^a					Odds ratio
	Sev. 0	Sev. 1	Sev. 2	Sev. 3	Sev. 4	
120 DAFF ^b						
Copper oxychloride 0.5	0.996	0.003	0.001	0.000	0.000	19.03 (6.90-78.80) ^c
Copper oxychloride 1.0	0.989	0.008	0.001	0.001	0.001	7.16 (3.41-17.53)
Fosetyl-Al 2.0	0.990	0.008	0.001	0.001	0.001	7.72 (3.68-18.90)
Tebuconazole 0.2	0.945	0.041	0.007	0.003	0.004	1.39 (0.08-2.21)
Non-treated	0.925	0.056	0.010	0.005	0.006	
133 DAFF						
Copper oxychloride 0.5	0.985	0.013	0.001	0.000	0.000	33.49 (17.97-71.25)
Copper oxychloride 1.0	0.963	0.033	0.003	0.001	0.001	13.03 (8.47-20.97)
Fosetyl-Al 2.0	0.941	0.051	0.005	0.001	0.001	8.05 (5.54-12.04)
Tebuconazole 0.2	0.634	0.303	0.043	0.011	0.009	0.86 (0.67-1.10)
Non-treated	0.666	0.279	0.038	0.010	0.008	
145 DAFF						
Copper oxychloride 0.5	0.469	0.264	0.132	0.106	0.029	62.92 (48.66-81.68)
Copper oxychloride 1.0	0.361	0.276	0.167	0.152	0.045	40.20 (31.02-52.27)
Fosetyl-Al 2.0	0.089	0.144	0.182	0.372	0.213	6.95 (5.53-8.77)
Tebuconazole 0.2	0.018	0.036	0.063	0.292	0.591	1.30 (1.03-1.65)
Non-treated	0.014	0.028	0.051	0.254	0.653	

583^a 0 = no lesions observed; 1 = less than 10 leaf spots; 2 = 10 to 20 leaf spots; 3 = more than 20 leaf spots; 4 = defoliated.584^b DAFF = Days after full flowering (BBCH 65) on 30 April 2009.585^c In brackets 95% confidence interval.

586**Table 2** Probabilities and odds ratios of the proportional odds logistic regression model for circular leaf spot severity on ‘Rojo Brillante’
 587persimmon shoots with the fungicide treatments evaluated at Benimodo in 2009 (experiment 2).

Evaluation dates and fungicides (g a.i. L ⁻¹)	Probabilities ^a					Odds ratio
	Sev. 0	Sev. 1	Sev. 2	Sev. 3	Sev. 4	
120 DAFF ^b						
Copper oxychloride 0.5	0.988	0.007	0.002	0.001	0.001	6.78 (3.24-16.57) ^c
Copper oxychloride 1.0	1.000	0.000	0.000	0.000	0.000	^{-d}
Fosetyl-Al 2.0	0.989	0.006	0.002	0.001	0.001	7.78 (3.71-18.99)
Tebuconazole 0.2	0.901	0.058	0.021	0.009	0.012	0.75 (0.50-1.14)
Thiophanate-methyl 0.7	0.891	0.063	0.023	0.010	0.013	0.68 (0.45- 1.02)
Non-treated	0.923	0.045	0.016	0.007	0.009	
133 DAFF						
Copper oxychloride 0.5	0.928	0.047	0.010	0.008	0.006	5.03 (3.53-7.32)
Copper oxychloride 1.0	0.967	0.022	0.004	0.004	0.003	11.58 (7.18-19.82)
Fosetyl-Al 2.0	0.979	0.014	0.003	0.002	0.002	18.61 (10.84-34.87)
Tebuconazole 0.2	0.665	0.194	0.053	0.049	0.038	0.77 (0.60-0.99)
Thiophanate-methyl 0.7	0.736	0.159	0.041	0.036	0.027	1.09 (0.84-1.42)
Non-treated	0.719	0.168	0.044	0.039	0.030	
145 DAFF						
Copper oxychloride 0.5	0.241	0.299	0.182	0.228	0.051	55.34 (42.08-73.15)
Copper oxychloride 1.0	0.286	0.311	0.169	0.193	0.040	69.92 (53.37-92.08)
Fosetyl-Al 2.0	0.092	0.181	0.181	0.404	0.142	17.73 (13.87-22.79)
Tebuconazole 0.2	0.007	0.018	0.028	0.232	0.716	1.17 (0.91-1.50)
Thiophanate-methyl 0.7	0.012	0.032	0.048	0.331	0.577	2.16 (1.68-2.78)
Non-treated	0.006	0.015	0.024	0.209	0.746	

^a 0 = no lesions observed; 1 = less than 10 leaf spots; 2 = 10 to 20 leaf spots; 3 = more than 20 leaf spots; 4 = defoliated.

^b DAFF = Days after full flowering (BBCH 65) on 30 April 2009.

^c In brackets 95% confidence interval.

^d Odds ratio was not calculated because all leaves were of severity category 0.

593**Table 3** Probabilities and odds ratios of the proportional odds logistic regression model for circular leaf spot severity on ‘Rojo Brillante’
 594persimmon shoots with the fungicide treatments evaluated at Benimodo in 2010 (experiment 3).

Evaluation dates and fungicides (g a.i. L ⁻¹)	Probabilities ^a					Odds ratio
	Sev. 0	Sev. 1	Sev. 2	Sev. 3	Sev. 4	
125 DAFF ^b						
Copper oxychloride 1.0	0.935	0.033	0.012	0.014	0.005	19.00 (13.29-27.94) ^c
Mancozeb 1.6	0.981	0.010	0.004	0.004	0.002	67.10 (38.02-131.92)
Fenbuconazole 0.1	0.870	0.064	0.025	0.029	0.012	8.79 (6.59-11.85)
Tebuconazole 0.1	0.565	0.168	0.087	0.122	0.057	1.70 (1.37-2.12)
Thiophanate-methyl 0.7	0.564	0.168	0.087	0.123	0.058	1.69 (1.35-2.12)
Trifloxystrobin 0.075	0.998	0.001	0.000	0.000	0.000	727.30 (163.17-12805.34)
Pyraclostrobin 0.075	0.990	0.005	0.002	0.002	0.001	124.80 (60.12-318.39)
Non-treated	0.432	0.185	0.111	0.178	0.094	
140 DAFF						
Copper oxychloride 1.0	0.516	0.231	0.079	0.078	0.096	94.91 (68.06-134.54)
Mancozeb 1.6	0.675	0.177	0.050	0.046	0.051	184.78 (132.16-262.69)
Fenbuconazole 0.1	0.142	0.173	0.110	0.170	0.405	14.72 (10.71-20.58)
Tebuconazole 0.1	0.082	0.117	0.086	0.157	0.558	7.95 (5.78-11.12)
Thiophanate-methyl 0.7	0.109	0.144	0.099	0.167	0.481	10.82 (7.91-15.06)
Trifloxystrobin 0.075	0.835	0.098	0.024	0.021	0.022	450.87 (313.54-659.41)
Pyraclostrobin 0.075	0.848	0.091	0.022	0.019	0.020	496.84 (341.53-735.51)
Non-treated	0.011	0.019	0.017	0.043	0.909	
147 DAFF						
Copper oxychloride 1.0	0.169	0.211	0.164	0.138	0.318	74.37 (45.98-128.43)
Mancozeb 1.6	0.442	0.262	0.118	0.071	0.107	288.62 (177.61-500.36)
Fenbuconazole 0.1	0.035	0.064	0.077	0.101	0.723	13.31 (8.19-23.04)
Tebuconazole 0.1	0.020	0.038	0.049	0.070	0.823	7.48 (4.52-13.13)
Thiophanate-methyl 0.7	0.032	0.058	0.071	0.096	0.744	11.96 (7.34-20.74)
Trifloxystrobin 0.075	0.667	0.191	0.064	0.033	0.045	729.80 (445.25-1274.10)
Pyraclostrobin 0.075	0.829	0.107	0.030	0.015	0.019	1765.32 (1053.18-3141.01)
Non-treated	0.003	0.005	0.008	0.012	0.972	

^a 0 = no lesions observed; 1 = less than 10 leaf spots; 2 = 10 to 20 leaf spots; 3 = more than 20 leaf spots; 4 = defoliated.

^b DAFF = Days after full flowering (BBCH 65) on 13 May 2010.

^c In brackets 95% confidence interval.

599**Table 4** Probabilities and odds ratios of the proportional odds logistic regression models for circular leaf spot severity on ‘Rojo Brillante’
 600persimmon shoots with the fungicide treatments evaluated at L’Alcúdia in 2010 (experiment 4).

Evaluation dates and fungicides (g a.i. L ⁻¹)	Probabilities ^a					Odds ratio
	Sev. 0	Sev. 1	Sev. 2	Sev. 3	Sev. 4	
125 DAFF ^b						
Copper oxychloride 0.5 ^c	0.619	0.203	0.060	0.063	0.055	5.61 (4.40-7.15) ^c
Copper oxychloride 1.0 ^c	0.807	0.116	0.028	0.027	0.022	14.39 (10.89-19.14)
Copper oxychloride 0.5 ^d	0.601	0.210	0.063	0.067	0.059	5.19 (4.05-6.66)
Copper oxychloride 1.0 ^d	0.662	0.186	0.053	0.054	0.046	6.74 (5.28-8.63)
Fosetyl-Al 2.0	0.400	0.255	0.100	0.121	0.123	2.30 (1.82-2.90)
Fenbuconazole 0.1	0.776	0.132	0.033	0.032	0.026	11.97 (9.25-15.56)
Tebuconazole 0.1	0.539	0.230	0.075	0.082	0.075	4.03 (3.19-5.09)
Thiophanate-methyl 0.7	0.520	0.235	0.078	0.086	0.080	3.74 (2.95-4.74)
Trifloxystrobin 0.075	0.931	0.044	0.010	0.009	0.007	46.39 (32.30-68.38)
Non-treated	0.225	0.228	0.120	0.182	0.245	
140 DAFF						
Copper oxychloride 0.5 ^c	0.091	0.134	0.087	0.185	0.503	2.35 (1.83-3.02)
Copper oxychloride 1.0 ^c	0.304	0.254	0.105	0.148	0.189	10.21 (7.90-13.25)
Copper oxychloride 0.5	0.118	0.161	0.097	0.192	0.432	3.13 (2.44- 4.02)
Copper oxychloride 1.0	0.268	0.246	0.108	0.160	0.218	8.54 (6.63-11.03)
Fosetyl-Al 2.0	0.083	0.125	0.083	0.181	0.528	2.12 (1.65-2.73)
Fenbuconazole 0.1	0.258	0.243	0.109	0.163	0.226	8.11 (6.35-10.40)
Tebuconazole 0.1	0.061	0.097	0.068	0.163	0.612	1.50 (1.16-1.95)
Thiophanate-methyl 0.7	0.115	0.158	0.096	0.192	0.438	3.04 (2.37-3.91)
Trifloxystrobin 0.075	0.615	0.207	0.056	0.062	0.060	37.23 (28.57-48.72)
Non-treated	0.041	0.069	0.052	0.134	0.704	
147 DAFF						
Copper oxychloride 0.5 ^c	0.019	0.064	0.048	0.040	0.830	1.93 (1.33-2.83)
Copper oxychloride 1.0 ^c	0.111	0.261	0.125	0.079	0.424	12.77 (9.09-18.24)
Copper oxychloride 0.5	0.027	0.088	0.063	0.051	0.770	2.80 (1.94-4.08)
Copper oxychloride 1.0	0.110	0.258	0.124	0.079	0.429	12.53 (8.94-17.88)
Fosetyl-Al 2.0	0.013	0.045	0.035	0.030	0.877	1.31 (0.88-1.96)
Fenbuconazole 0.1	0.066	0.185	0.107	0.076	0.566	7.22 (5.18-10.24)
Tebuconazole 0.1	0.013	0.044	0.035	0.030	0.878	1.30 (0.86-1.96)
Thiophanate-methyl 0.7	0.028	0.093	0.066	0.053	0.759	2.98 (2.08-4.32)
Trifloxystrobin 0.075	0.339	0.369	0.094	0.046	0.153	52.15 (37.11-74.62)
Non-treated	0.010	0.035	0.027	0.024	0.904	

^a 0 = no lesions observed; 1 = less than 10 leaf spots; 2 = 10 to 20 leaf spots; 3 = more than 20 leaf spots; 4 = defoliated.

^b DAFF = Days after full flowering (BBCH 65) on 13 May 2010.

^c Cuproflow 38%.

^d Quorum 70%.

^e In brackets 95% confidence interval.

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Table 5 Effects of fungicide applications on yield of ‘Rojo Brillante’ persimmon at Benimodo (experiment 3) and L’Alcúdia (experiment 4) in 2010.

Benimodo		L’Alcúdia	
Fungicides (g a.i. L ⁻¹)	Yield (kg tree ⁻¹)	Fungicides (g a.i. L ⁻¹)	Yield (kg tree ⁻¹)
Copper oxychloride 1.0	2.58 ± 1.82	Copper oxychloride 0.5 ^a	0.00 ± 0.00
Mancozeb 1.6	33.74 ± 5.37	Copper oxychloride 1.0 ^a	30.44 ± 21.52
Fenbuconazole 0.1	0.00 ± 0.00	Copper oxychloride 0.5 ^b	3.20 ± 2.26
Tebuconazole 0.1	0.00 ± 0.00	Copper oxychloride 1.0 ^b	35.68 ± 13.59
Thiophanate-methyl 0.7	0.00 ± 0.00	Fosetyl-Al 2.0	9.36 ± 6.62
Trifloxystrobin 0.075	47.64 ± 7.89	Fenbuconazole 0.1	19.67 ± 13.91
Pyraclostrobin 0.075	53.39 ± 2.09	Tebuconazole 0.1	0.00 ± 0.00
Non-treated	0.00 ± 0.00	Thiophanate-methyl 0.7	0.00 ± 0.00
		Trifloxystrobin 0.075	95.77 ± 0.34
		Non-treated	0.00 ± 0.00

^aCuproflow 38%.

^bQuorum 70%.

Figure 1 Circular leaf spot severity in persimmon ‘Rojo Brillante’ at 120, 133 and 145 days after full flowering (DAFF) on 30 April in experiment 1 (top) and experiment 2 (down) conducted at Benimodo in 2009 (C5, copper oxychloride 0.5 g a.i. L⁻¹; C1, copper oxychloride 1 g a.i. L⁻¹; FA, fosetyl-Al; TB, tebuconazole; TM, thiophanate-methyl; NT, non-treated).

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654 **Figure 2** Circular leaf spot severity in persimmon ‘Rojo Brillante’ at 125, 140 and 147 days after full
655 flowering (DAFF) on 13 May in experiment 2 (top) conducted at Benimodo and experiment 4
656 (down) conducted at L’Alcúdia in 2010 (C5, copper oxychloride 0.5 g a.i. L⁻¹; C1, copper
657 oxychloride 1 g a.i. L⁻¹; Q5, copper oxychloride 0.5 g a.i. L⁻¹; Q1, copper oxychloride 1 g a.i. L⁻¹;
658 MA, mancozeb; FA, fosetyl-Al; FB, fenbuconazole; TB, tebuconazole; TM, thiophanate-
659 methyl; TS, trifloxystrobin; PS, pyraclostrobin; NT, non-treated).
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673 **Figure 3** Relationship between circular leaf spot incidence on ‘Rojo Brillante’ persimmon and relative
674 yield loss. Evaluations at 125, 140, 147 and 155 days after full flowering (DAFF) on 13 May
675 2010 in two experiments conducted at Benimodo and L’Alcúdia, Spain.
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690**Figure 4** Relationship between defoliation due to circular leaf spot on ‘Rojo Brillante’ persimmon and
691 relative yield loss. Evaluations at 125, 140, 147 and 155 days after full flowering (DAFF) on
692 13 May 2010 in two experiments conducted at Benimodo and L’Alcúdia, Spain.
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