Plant Disease • 2018 • 102:334-340 • https://doi.org/10.1094/PDIS-04-17-0537-RE

Critical Fungicide Spray Period for Citrus Black Spot Control in São Paulo State, Brazil

Fabrício Eustáquio Lanza, Tadeu Germano Metzker, Thiago Vinhas, Franklin Behlau, and **Geraldo José Silva Junior,**† Fundo de Defesa da Citricultura–Fundecitrus, Departamento de Pesquisa e Desenvolvimento, 14.807-040, Araraquara, São Paulo, Brazil

Abstract

The period of citrus black spot (CBS) control used in South Africa (SA) and Australia, from October to January or February, has not been as effective in São Paulo (SP), Brazil. This study aimed to evaluate different periods of protection and determine the critical period for CBS control in SP. A field trial was carried out for two seasons in a mature Valencia sweet orange orchard located in Mogi Guaçu, SP. Spray programs with a total of 60, 100, 140, 180, and 220 days of fruit protection (DFP) were evaluated. CBS symptoms and fruit drop decreased exponentially as the length of the period of protection increased. The reductions in CBS intensity and crop loss with these programs ranged from 34 to 96 and 50 to 77%, respectively. The programs with 180 and 220 DFP, which protected the fruit from September to March and May, showed the highest cost benefit. The critical period needed for CBS control in SP is longer than that in SA and Australia. The results obtained with the present study are helpful for scheduling a more efficient and rational program for CBS control not only in SP but also in other tropical and subtropical regions with similar weather conditions.

Citrus black spot (CBS), caused by *Phyllosticta citricarpa* (teleomorph *Guignardia citricarpa*), was first reported in Australia in the 1890s (Kiely 1948). Currently, the disease is one of the most important in tropical and subtropical citrus-growing regions of Africa, Asia, Oceania, and the Americas (EFSA 2014; Kotzé 1981, 2000). In Brazil, CBS has been reported since 1980 almost exclusively as a fruit disease (Kotzé 1981; Robbs et al. 1980). CBS affects the main commercial citrus species, causing lesions on fruit and leaves. CBS-triggered crop loss tends to be more severe on late-maturing sweet orange cultivars because fruit remain longer in the tree (Kotzé 1981, 2000). In some Mediterranean climatic regions, including Spain, Greece, and Italy in Europe and California in the United States, the disease is absent (EFSA 2014). However, due to the risk of introduction and the threat that CBS represents to the citrus industry, the disease is also important in CBS-free regions where the climatic conditions are suitable for disease establishment (EFSA 2014; Yonow et al. 2013).

The symptoms on fruit are diverse and commonly categorized into six types (i.e., hard spot, freckle spot, virulent spot, false melanose, lacy spot, and cracked spot) (Goes et al. 2000; Kotzé 1981; Silva Junior et al. 2016a). CBS lesions form within 40 to 340 days after inoculation, depending on the type of symptom, inoculum concentration, and fruit development stage at infection (Aguiar et al. 2012; Frare 2015). The lesions do not affect the pulp and are limited to the fruit epicarp (Marques et al. 2012). Nonetheless, CBS symptoms affect the quality of the fruit peel and reduce the value for the fresh market (Kotzé 1981; Spósito et al. 2011). Moreover, some countries restrict the import of symptomatic fruit from countries where the CBS occurs (Yonow et al. 2013).

P. citricarpa produces infective propagules in both sexual and asexual phases. In the sexual phase, pseudothecia containing ascospores are formed on the citrus leaf litter within 40 to 180 days after leaf fall. The production and release of ascospores depend on the alternation of wet and dry periods. These spores are released during or after rainfall, and are spread mainly by wind (Fourie et al. 2013; Kiely 1948; Kotzé 1981, 2000; McOnie 1964). The ascospores are most important for the primary cycle of CBS (i.e., the introduction of the disease into new areas) (Kiely 1948; Kotzé 1981; McOnie 1964). In the asexual phase, dark brown or black pycnidia, which produce conidia, are formed in hard, freckle, or virulent spots on fruit, dead twigs, and leaves in the tree well as on leaf litter (Kotzé 2000). During rainfall or irrigation, the conidia are washed down to the adjacent fruit, twigs, and leaves, where new infections occur (Spósito et al. 2011). The conidia are important for the secondary cycles of the disease (i.e., for autoinfecting the trees). These spores, along with the ascospores, may play a more significant role in the CBS epidemics in regions with higher rainfall such as Brazil (Reis et al. 2006; Spósito et al. 2011) and Ghana (Brentu et al. 2012).

In CBS endemic areas worldwide, the control of the disease is mainly based on fungicide sprays (Kotzé 2000; Miles et al. 2004; Schutte et al. 1997, 2003; Silva Junior et al. 2016a,b). In citrus orchards of South Africa (SA), where CBS management was first deployed, chemical control of the disease is restricted to the critical period to *P. citricarpa* infection, from October to January or February. The literature indicates that, in SA, infections do not occur after 120 to 150 days of petal fall, because of the weather conditions and the local pressure of inoculum, and the fungicide sprays for CBS control are not required after that period (Kotzé 1981; Schutte et al. 1997, 2003). A similar spray program is employed in Australia (Kiely 1948; Miles et al. 2004). In Brazil, following the first CBS detections, chemical control of the disease was initially based on the previous experience of SA; however, with lower effectiveness.

Failure to properly control the disease in São Paulo (SP), Brazil using the spray program from SA has encouraged growers over the years to empirically increase the number of fungicide sprays. The current standard CBS control program in SP has evolved to two copper sprays after petal fall, followed by different numbers of strobilurin (quinone-outside inhibitor) sprays (Scaloppi et al. 2012; Silva Junior et al. 2016a). The first sprays are performed with copper not only to protect fruit against CBS but also to control other citrus diseases such as citrus scab (caused by *Elsinoë* spp.) and melanose (caused by *Diaporthe citri*) (Silva Junior et al. 2016a). Despite the local advances in CBS management since the 1990s and based on recent findings regarding the favorability and fruit susceptibility period to infection by *P. citricarpa* (Aguiar et al. 2012; Frare 2015), further investigations became necessary to establish a suitable spray program for CBS control in SP. Thus, this study aimed to determine the critical period for fruit protection with fungicides in a late-maturing sweet orange orchard under SP conditions.

Materials and Methods

The field trial was carried out for two seasons (2010–11 and 2011–12) in a commercial, irrigated orchard in the municipality of Mogi Guaçu, SP, Brazil (latitude 22°13′13.1″ S, longitude 47°12′56.7″ W,

†Corresponding author: G. J. Silva Junior;
E-mail: geraldo.silva@fundecitrus.com.br

Accepted for publication 23 September 2017.

altitude 615 m above sea level), where CBS frequently occurs. The orchard was planted in 1995 and consisted of late-maturing Valencia sweet orange (*Citrus sinensis*) trees on Rangpur lime rootstock (*C. limonia*), with 346 trees/ha (spacing 7.6 by 3.8 m). Rainfall was measured daily in millimeters with standard rain gauges for two seasons. This information was used to estimate the proportion (frequency) of rainy days (volume of rainfall higher than 0.2 mm recorded during 24 h of a specific day) during the different protection

Table 1. Copper (Cu) and strobilurin (QoI) spray programs evaluated for citrus black spot control in Valencia sweet orange during 2010–11 and 2011–12 seasons in Mogi Guaçu, SP, Brazil

Number of sprays			Spray dates[a]	
Cu	QoI	Period (days)[b]	Season 1 (2010/2011)	Season 2 (2011/2012)
2	4	220	Sep 30, Oct 30, Nov 30, Jan 10, Feb 18, Mar 30	Sep 23, Oct 27, Nov 28, Jan 10, Feb 20, Mar 30
2	3	180	Sep 30, Oct 30, Nov 30, Jan 10, Feb 18	Sep 23, Oct 27, Nov 28, Jan 10, Feb 20
2	2	140	Sep 30, Oct 30, Nov 30, Jan 10	Sep 23, Oct 27, Nov 28, Jan 10
2	1	100	Sep 30, Oct 30, Nov 30	Sep 23, Oct 27, Nov 28
2	0	60	Sep 30, Oct 30	Sep 23, Oct 27
0[c]	0	0	…	…

[a] Copper was used in the first two sprays beginning at 70% petal fall at 30-day intervals (copper oxychloride at 90 g of metallic copper per 100 liters of water) and was followed by different numbers of strobilurin sprays at 40-day intervals (trifloxystrobin at 3.75 g per 100 liters of water).
[b] Total period of protection, calculated using 30 and 40 days for copper and strobilurin sprays, respectively.
[c] Untreated control trees.

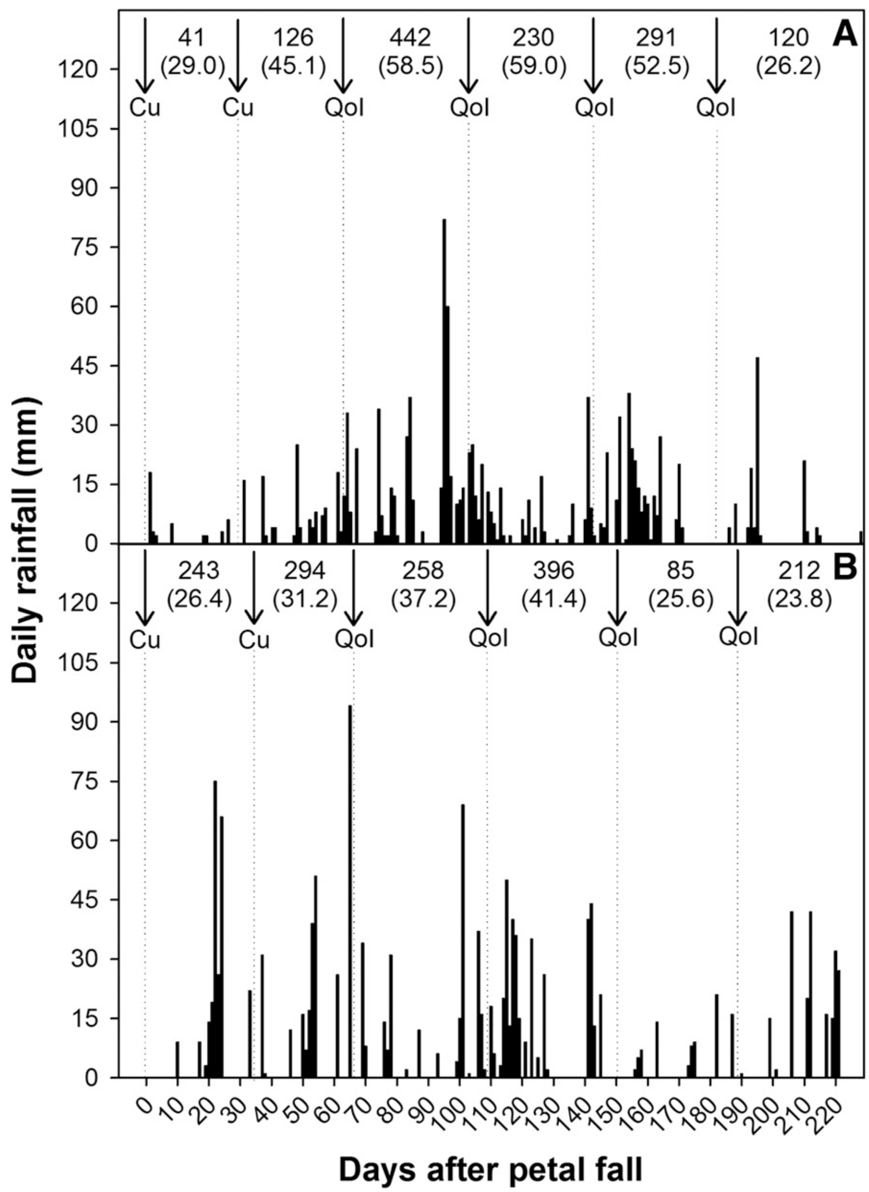

Fig. 1. Daily rainfall (in millimeters) during the spray periods for citrus black spot control on Valencia sweet orange orchard in the seasons **A**, 2010–11 and **B**, 2011–12 in Mogi Guaçu, SP, Brazil. Arrows followed by Cu and QoI represent the timing of copper and strobilurin applications, respectively. Numbers between arrows without and within parentheses indicate cumulative rainfall (in millimeters) and percentage of rainy days for each interval between sprays, respectively. After the last spray, a period of 40 days was considered.

periods assessed from the first spray (September) up to 40 days after the last spray (May).

Different periods of fruit protection with fungicide after petal fall were evaluated for CBS control. All treated trees received two copper applications, the first at 70% petal fall, in September, and the second 30 days later, in October, resulting in approximately 60 days of fruit protection (DFP) with this fungicide. Treatments varied in the number and timing of strobilurin sprays, which started 30 days after the second copper application. Zero to four strobilurin sprays were performed at 40-day intervals, which added 0 to 160 days of protection. These sprays resulted in five total periods of protection with fungicide. Untreated control trees (UTC) were used as references (Table 1).

The spray volume used was 3,800 liters/ha or 11 liters/tree, which corresponded to approximately 100 ml/m^3 of tree canopy (Scapin et al. 2015; Silva Junior et al. 2016b). The fungicides used were copper oxychloride (Recop 840 WP, 50% metallic copper; Atanor) at a standard rate of 90 g of metallic copper per 100 liters of water, which corresponded to 90 mg/m^3 of tree canopy; and strobilurin (Flint 500 WG, 50% trifloxystrobin; Bayer CropScience) at a standard rate of 3.8 g of trifloxystrobin per 100 liters of water, which corresponded to 3.8 mg/m^3 of tree canopy. Regardless of the fungicide used, mineral oil (Agefix; Packblend) was added to the tank mixture at a final concentration of 0.25% (vol/vol). Applications were performed with a 4,000-liter capacity high-profile sprayer (Alfa 4000 Bilateral; Natali) at 2.7 km/h.

The treatments were arranged in a randomized complete block design with four replicates and 30 trees per plot divided into three rows of 10 trees. A guard row between treated rows was left unsprayed. All assessments were performed on the four innermost trees of each plot.

In the 2010–11 and 2011–12 seasons, disease assessments were performed from April to November 2011 and from May to December 2012, respectively. CBS incidence and severity were evaluated monthly on 200 fruit (50 per tree). CBS incidence was measured as the percentage of fruit with CBS symptoms at each assessment. CBS severity was measured as the percentage of diseased area on the outer canopy-facing portion of the fruit. The severity was estimated using a six-level scale taking into account all types of CBS symptoms on the assessed portion of the fruit (Spósito et al. 2004). The standardized area under incidence progress curves (AUIPC) and area under severity progress curves (AUSPC) were calculated using data from all assessments in each season (Madden et al. 2007).

The percentage of premature fruit drop (crop loss) was calculated by dividing the estimated weight of dropped fruit by the combined weight of dropped and harvested fruit. The weight of dropped fruit was estimated by multiplying the cumulative number of dropped fruit counted per tree from September to November 2011 (season 1) and from September to December 2012 (season 2) by the average fruit weight estimated at harvest in a 100-fruit sample (Silva Junior et al. 2016b).

The variables studied were subjected to analysis of variance using Statistica 7.0 (StatSoft Inc.). The AUIPC, AUSPC, and percentage of premature fruit drop with CBS for each treatment were compared using Tukey's test ($P \leq 0.05$).

The two-season average spray costs and financial return of CBS control were estimated for each treatment tested based on the price

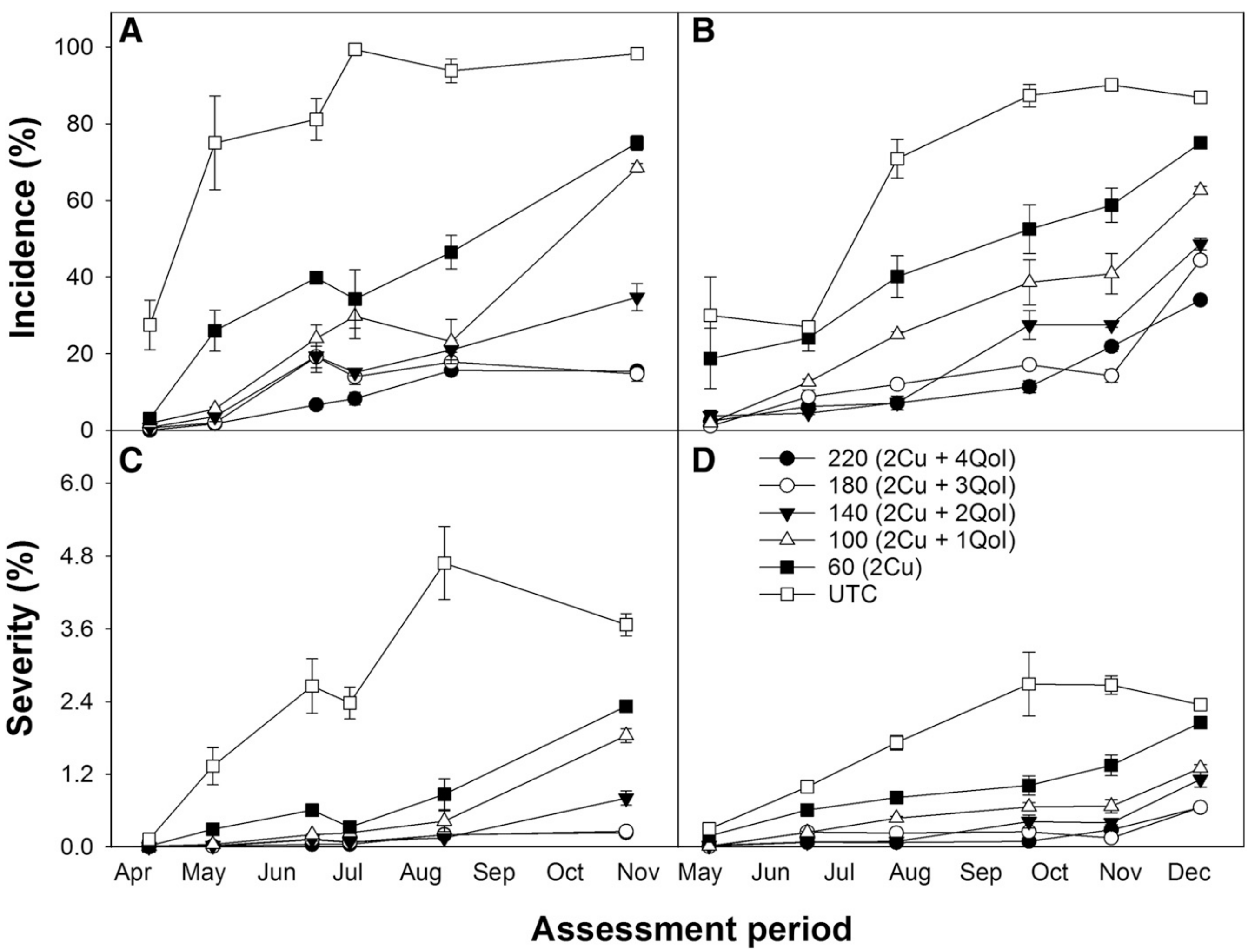

Fig. 2. Progress curves of the **A** and **B,** incidence and **C** and **D,** severity of citrus black spot on Valencia sweet orange fruit from trees subjected to different periods of protection (60 to 220 days) after petal fall with two copper (Cu) sprays and zero to four strobilurin (QoI) sprays, in season 1 (A and C, April to November 2011) and; season 2 (B and D, May to December 2012), in Mogi Guaçu, SP, Brazil. UTC = untreated control trees. Bars indicate the standard error of the mean.

in SP as follows: metallic copper (US$12.73/kg), trifloxystrobin (US $95.38/kg), mineral oil (US$2.51/liter), labor (US$2.37/h), and machinery (US$24.03/h). The financial return was estimated as the value of the surplus yield of a given treatment compared with the UTC subtracted from the CBS-related control costs of that treatment. The fruit price used was US$4.65/box of 40.8 kg. The currency conversion rate used was US$1.00 = R$2.30 (Brazilian real).

Nonlinear regression analysis, $y = a \times \exp(-b \times x)$, was used to estimate the relationship between the number of days of fruit protection (x) and the variables assessed (y), including the AUIPC, AUSPC, and percentage of premature fruit drop. The best-fit model was selected based on the coefficient of determination (R^2) and distribution of residuals (Madden et al. 2007). Statistical analysis was performed with Statistica software.

Results

Rain events occurred during all periods covered by the spray programs assessed, beginning in early spring (September) and ending in midfall (May) (Fig. 1). In the first and second seasons, the total rainfall during the control period was 1,250 and 1,495 mm, respectively, and the annual precipitation in 2011 and 2012 was 1,611 and 1,641 mm, respectively. The highest rainfall was recorded in season 1 between 60 and 100 DFP (November to January) and in season 2 from 100 and 140 DFP (January to February) (Fig. 1). Precipitation occurred in 26.2 to 59.0 and 23.8 to 41.4% of the days between sprays during the 220 days of the fruit protection period in seasons 1 and 2, respectively (Fig. 1). In both seasons, the highest frequencies of rainy days were observed between 60 and 140 DFP, from November to February (Fig. 1).

Despite the variations in precipitation, the weather conditions in both seasons were similarly conducive for CBS occurrence because incidence of diseased fruit exceeded 90% in the UTC trees in the two seasons (Fig. 2A and B). CBS incidence and severity on fruit increased in all treatments and were higher for UTC trees than treated trees in both seasons, regardless of the duration of protection period (Fig. 2). In the first season, the incidence of fruit with CBS on the UTC trees peaked at approximately 100%. In contrast, trees under 180 DFP (five sprays) and 220 DFP (six sprays) showed peaks of CBS incidence on fruit lower than 15% (Fig. 2A). In the second season, the incidence of fruit with CBS on the UTC trees reached 90%, as opposed to 44 and 38% with 180 and 220 DFP, respectively (Fig. 2B). The maximum CBS severity on fruit in UTC trees was 4.7 and 2.6% in the first and second seasons, respectively. Conversely, the severity on fruit from trees protected for 180 and 220 days did not exceed 0.6% in either season (Fig. 2C and D).

All treatments with fungicide sprays significantly reduced ($P <$ 0.001) the AUIPC and AUSPC in comparison with UTC trees in both seasons (Fig. 3A and B). Regardless of the season, the treatments with 180 DFP (five sprays) and 220 DFP (six sprays) resulted in the lowest AUIPC and did not differ significantly ($P < 0.001$) from each other. These treatments reduced the AUIPC by 78 to 88% in comparison with the UTC (Fig. 3A). Taking into account the results of the two seasons, the most effective treatments for reducing AUSPC were 140, 180, and 220 DFP. These treatments did not differ significantly ($P < 0.001$) and reduced the AUSPC from 84 to 96% in comparison with the UTC (Fig. 3B).

The different periods of protection evaluated significantly ($P <$ 0.001) reduced premature fruit drop when compared with the UTC, except 60 DFP in the second season. In the first year, significant differences were not detected among the periods of protection assessed. The percentage of premature fruit drop for the UTC was 16.8, which is approximately two- to fourfold higher than that observed for all the periods of protection (Fig. 3C). In the second season, the treatments with 140, 180, and 220 DFP did not differ from each other, showed the lowest percentage of premature fruit drop, and reduced the crop loss approximately three- to fivefold compared with the UTC (Fig. 3C). The percentage of premature fruit drop with 100 DFP was also lower than the UTC but significantly higher than the longer periods of protection.

The negative exponential model best described the response of CBS control to the number of protected days for all variables assessed in both seasons ($P < 0.001$). Coefficients of determination (R^2) were in the range of 0.83 to 0.96 in season 1 and 0.82 to 0.93 in season 2 (Fig. 4). This nonlinear regression analysis showed that the number of DFP accounted for the variability in incidence and severity of CBS and premature fruit drop due to the disease.

Reductions of premature fruit drop in treated trees compared with the UTC paid off the incurred costs of CBS control regardless of the number of sprays (Table 2). CBS control costs represented 25 to 38% of the financial return values among the different spray programs

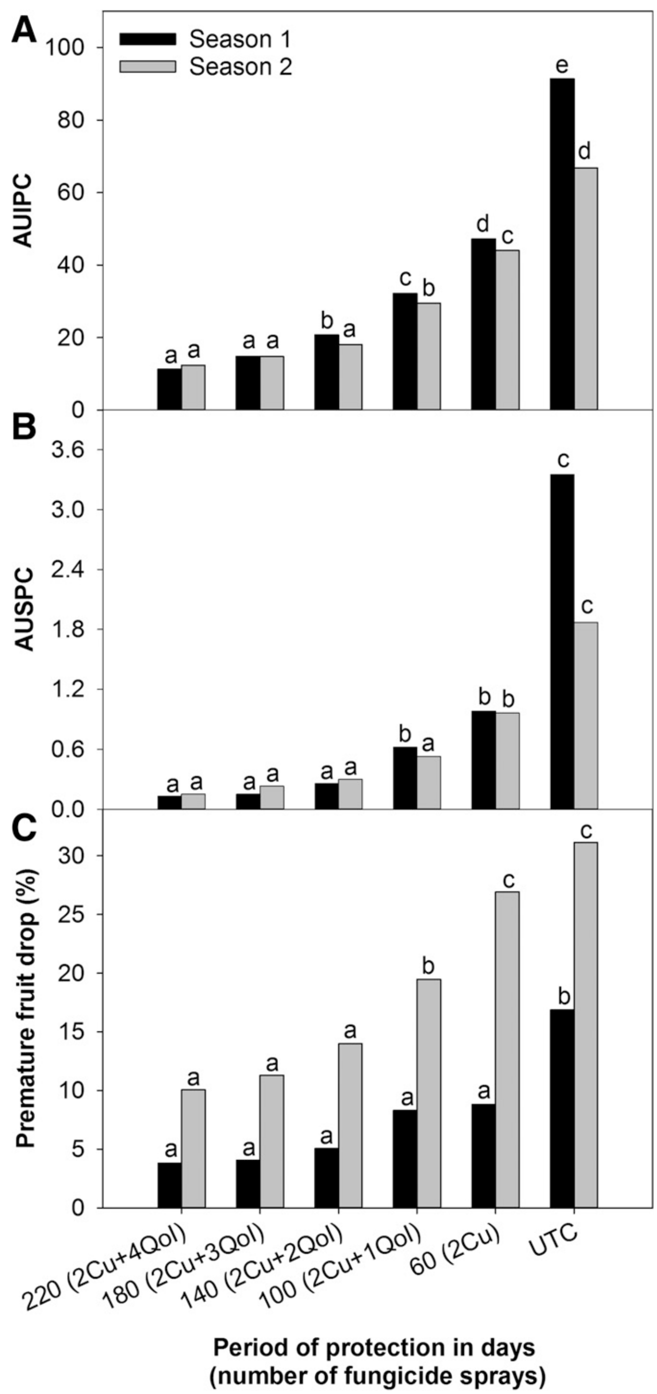

Fig. 3. A, Standardized area under incidence progress curve (AUIPC) and **B,** standardized area under severity progress curve (AUSPC) of citrus black spot on Valencia sweet orange fruit; and **C,** percentage of premature fruit drop from trees subjected to different periods of protection (60 to 220 days) after petal fall with two copper (Cu) sprays and zero to four strobilurin (QoI) sprays in season 1 (2010–11) and season 2 (2011–12) in Mogi Guaçu, SP, Brazil. UTC = untreated control trees. Columns with same shade and followed by same letter do not differ by Tukey's test ($P < 0.05$).

evaluated. The financial return increased with the number of sprays and ranged from US$282.29 (60 DFP, two sprays) to US$1,033.41 (220 DFP, six sprays). The return from six sprays was 73% greater than that obtained with two sprays and did not differ significantly from the return from five or four sprays (Table 2).

Discussion

The weather was conducive for CBS occurrence in both seasons of the study. The highest amounts of rainfall and frequency of rainy days were observed from October to January or February, which is considered the critical period for fruit infection by *P. citricarpa* in SA (Kotzé 1981, 2000; McOnie 1964; Schutte et al. 1997, 2003). However, a substantial amount of rainfall was also recorded beyond January, until April or May. This extended rainy period is commonly observed in the region of Mogi Guaçu, SP, where the climate is classified as Cwa (i.e., humid subtropical, with dry winters and hot summers) (Alvares et al. 2013). Under these conditions, the reduction of CBS intensity (incidence and severity) and CBS-triggered crop loss in a late-maturing cultivar of sweet orange conferred by the spray programs tested ranged from 34 to 96 and 50 to 77%, respectively. The differences in CBS control observed among the treatments reflected the duration of the control program (i.e., the number of sprays) and revealed the need of a longer period of protection in SP compared with SA and Australia.

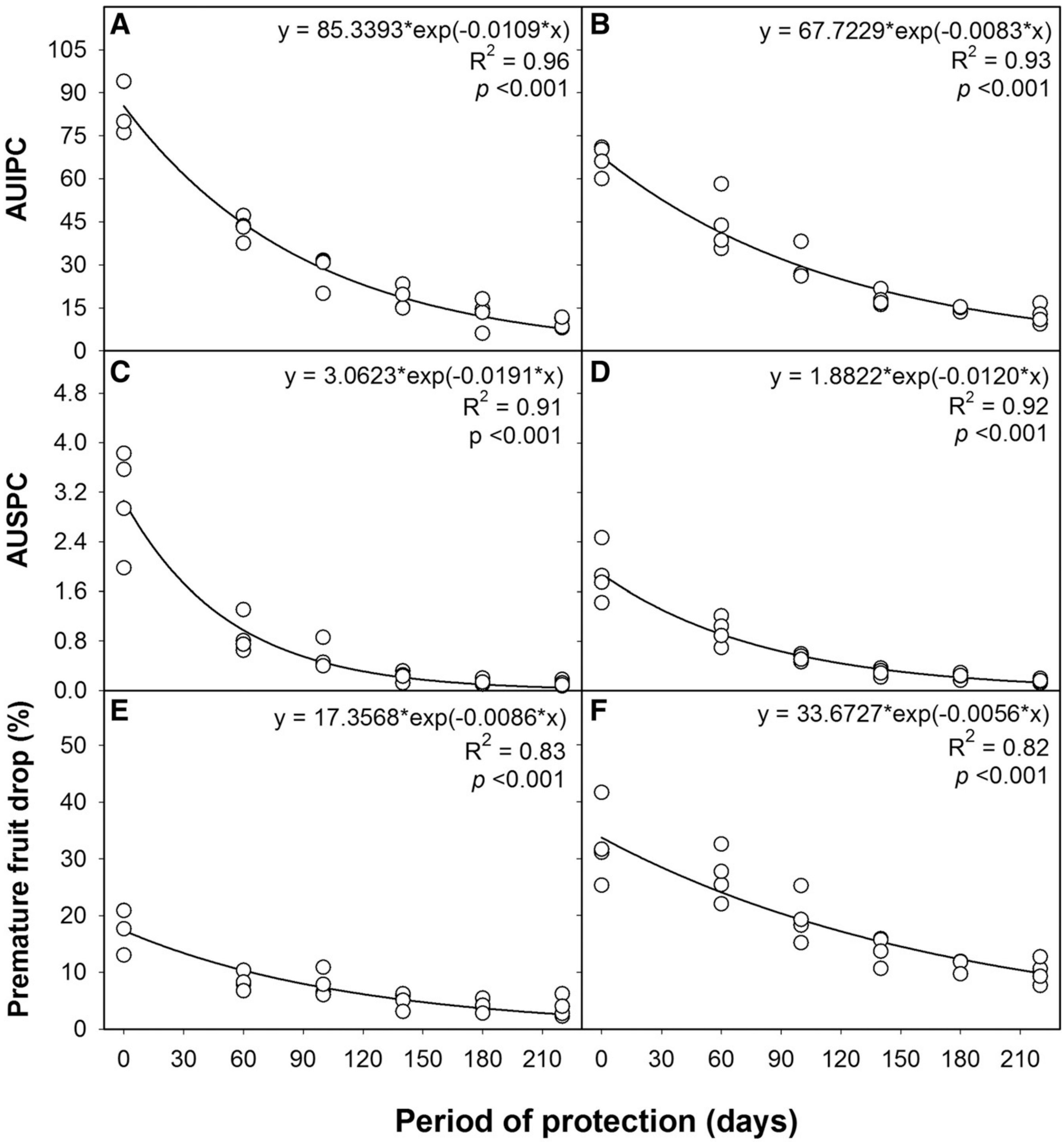

Fig. 4. Relationship between period of protection (days) on Valencia sweet orange trees and **A** and **B**, incidence (standardized area under incidence progress curve [AUIPC]) and **C** and **D**, severity (standardized area under severity progress curve [AUSPC]) of citrus black spot on fruit; and **E** and **F**, premature fruit drop (%) during the season 1 (first column) and season 2 (second column). Circles and continuous lines indicate the observed and estimated data, respectively, using a negative exponential model.

Table 2. Amount of chemicals (kg/ha or liters/ha), labor and machinery (h/ha), premature fruit drop (40.8-kg boxes/ha), citrus black spot (CBS) control cost (US$/ha), and financial return of the disease control (US$/ha) per season for each treatment tested in Valencia sweet orange orchard in Mogi Guaçu, SP, Brazil

Cu sprays[a]	QoI sprays[b]	Metallic Cu (kg/ha/season)	Trifloxystrobin (kg/ha/season)	Mineral oil (liters/ha/season)	Labor and machinery (h/ha/season)	CBS control cost (US$/ha/season)[c]	Premature fruit drop (box/ha/season)	Financial return (US$/ha)[d]
2	4	6.84	0.57	47.50	6.72	437.96	130.88 ± 12.99[c]	1,033.41 ± 60.40
2	3	6.84	0.43	38.00	5.60	370.97	156.83 ± 20.98	979.73 ± 97.59
2	2	6.84	0.29	28.50	4.48	303.99	184.74 ± 20.49	916.90 ± 95.30
2	1	6.84	0.14	19.00	3.36	237.01	273.26 ± 72.54	572.28 ± 337.30
2	0	6.84	…	9.50	2.24	170.03	350.03 ± 34.20	282.29 ± 159.00
0	0	…	…	…	…	…	447.41 ± 97.49	…

[a] Number of sprays of copper.
[b] Number of sprays of strobilurin.
[c] Estimate based on costs of metallic copper (US$12.73/kg), trifloxystrobin (US$95.38/kg), mineral oil (US$2.51/liter), labor (US$2.37/h), and machinery (US $24.03/h) practiced in SP during the two seasons (2010–11 and 2011–12).
[d] The financial return refers to the value of the difference in premature fruit drop between a given treatment and the untreated control trees subtracted by the CBS-related control costs of that treatment (US$4.65/fruit box of 40.8 kg). Conversion rate: US$1.00 = R$2.30 (Brazilian real). Mean of eight replicates/treatment (four replicates/season) ± standard error of the mean.

The CBS control programs with 60 and 100 DFP (from late September until early January) were not as effective as with longer protection periods (from late September until late March and early May). These results support the 1990s findings following initial CBS detection in Brazil (Goes et al. 1990). At that time, the spray program commonly used by citrus growers from November to early February did not effectively control the disease as in SA and Australia (Calavan 1960; Kotzé 1981; Miles et al. 2004). Although some regions in the CBS endemic areas of SP, SA, and Australia are classified as the same Koppen-Geiger climate types (Cwa, Cfa, and Cfb), the precipitation in SP during fruit development is approximately twofold higher, not only from October to January but also from February to April (Alvares et al. 2013; Martínez-Minaya et al. 2015). That is, the average accumulated rainfall recorded in the field trial from October to January and from February to April was 910 and 410 mm, respectively. In contrast, the historical average precipitation for the same periods in CBS endemic areas of both SA and Australia is less than 440 and 240 mm, respectively (https://en.climate-data.org). These differences in precipitation associated with fruit overlap commonly observed in late-maturing cultivars may contribute to increase the inoculum build-up as the orchards age in SP. In addition to the ascospores, the conidia produced on symptomatic fruit as well as in dead twigs and washed down to adjacent fruit, are considered an additional source of inoculum to exacerbate the CBS epidemics in SP (Reis et al. 2006; Spósito et al. 2011). This scenario may explain the lower effectiveness of the spray programs with up to 100 DFP observed both in the present study and following CBS appearance in SP. Moreover, it also suggests that the CBS intensity and required control programs may differ in regions under the same Koppen-Geiger climate classification.

The greatest reductions of CBS impact were obtained with 140, 180, and 220 DFP, which rendered protection to the crop from September to late February, late March, and early May, respectively. These programs had no significant difference in CBS severity in either season. However, in the first season, the incidence of fruit with CBS in trees with 140 DFP was significantly higher than in trees treated with the extended spray programs. Moreover, although the premature fruit drop for these three treatments did not differ seasonally from each other, the estimated average crop loss for trees under 140 DFP was approximately 30 to 50 boxes/ha higher than that estimated for longer spray programs. These differences may, depending on the fruit price, affect the financial return and justify the use of a prolonged protection period. Alternatively, to reduce spray costs, 140 DFP may be used in seasons with late bloom (petal fall after October), in orchards of early-maturing cultivars, and in areas with shorter rainfall periods.

Our results indicated a negative and strong relationship between the variables assessed and the period of fruit protection. Nevertheless, as demonstrated by nonlinear regression, protecting the fruit for more than 220 days may not consistently improve disease control or reduce the premature fruit drop. This is probably due to weather conditions less favorable to *P. citricarpa* infection in the autumn, as rainfall becomes scarce. In addition, even if late infection occurs, most of the crop is usually harvested (November to December) before the CBS symptoms are expressed, which may take up to 340 days (Baldassari et al. 2006; Brentu et al. 2012; Frare 2015).

In addition, this study also showed that the period of fruit susceptibility to *P. citricarpa* infection needs to be further investigated. For instance, in SA, the fruit is considered susceptible for 120 to 150 days after petal fall (October to January or February) (Kotzé 1981, 2000). In this location, fungicide sprays are not required after January, regardless of the rainfall or the abundance of ascospores (Fourie et al. 2013; Kotzé 1981; McOnie 1964). In Australia, the fruit susceptibility period is predominantly similar to SA but may eventually last up to 180 days (Kiely 1948). However, our results indicated that, in SP, the fruit need to be protected for a longer period than previously reported. This is in agreement with recent studies that showed the development of CBS symptoms on sweet orange fruit artificially inoculated from green stage (1.5 cm in diameter) until ripe stage after color break (7 cm) (Aguiar et al. 2012; Frare 2015). The annual precipitation in SP may contribute to accumulate more inoculum on the canopy over the years, and the weather conditions, particularly after February, seem to be more favorable for an increase in the intensity of CBS outbreaks compared with SA and Australia. Likewise, in Ghana, where the annual volume of rainfall is similar to SP, an extended period of protection (210 DFP, from May to November) was demonstrated to be necessary to control CBS during the main bloom season (Brentu et al. 2012).

Taking into account the consistency and reproducibility of the results obtained during the two seasons, this study showed that, in SP, the fruit needs to be protected against CBS for 180 to 220 days. Thus, the spray program for the main bloom should start in September (petal fall stage) and end in February or March, which will confer protection to the fruit until April or May. Shorter or longer protection periods may be used depending on the number of blooms per season, the duration of rainy period, the maturation of the cultivar, and the destination of the crop (fresh market or processing). Although the risk for development of *P. citricarpa* strains resistant to strobilurin fungicides is low (Hincapie et al. 2014; Stammler et al. 2013), no more than four sprays are advised to be used in each season for all citrus diseases (Dewdney et al. 2016). This study provides growers an efficient and rational program to use for CBS control in most parts of the SP citrus belt and in other regions with similar climate conditions and rainfall.

Acknowledgments

We thank M. S. Scapin, W. A. Ferreira, G. O. Santos, and D. R. Marin for technical assistance; P. Fourie for sharing information regarding CBS in SA and for critical reading of this manuscript; and the Brazilian Council for Scientific and Technological Development–CNPq (process number 458052/2014-0) for financial support.

Literature Cited

Aguiar, R. L., Scaloppi, E. M. T., Goes, A., and Spósito, M. B. 2012. Período de incubação de *Guignardia citricarpa* em diferentes estádios fenológicos de frutos de laranjeira 'Valência'. Trop. Plant Pathol. 37:155-158.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., and Sparovek, G. 2013. Koppen's climate classification map for Brazil. Meteorol. Z. 22:711-728.

Baldassari, R. B., Reis, R. F., and Goes, A. 2006. Susceptibility of fruits of the 'Valencia' and 'natal' sweet orange varieties to *Guignardia citricarpa* and the influence of the coexistence of healthy and symptomatic fruits. Fitopatol. Bras. 31:337-341.

Brentu, F. C., Oduro, K. A., Offei, S. K., Odamtten, G. T., Vicent, A., Peres, N. A., and Timmer, L. 2012. Crop loss, aetiology, and epidemiology of citrus black spot in Ghana. Eur. J. Plant Pathol. 133:657-670.

Calavan, E. C. 1960. Black spot of citrus. Calif. Citrogr. 46:4-24.

Dewdney, M. M., Schubert, T. S., Estes, M. R., Roberts, P. D., and Peres, N. A. 2016. Citrus black spot. Pages 73-77 in: 2016 Florida Citrus Pest Management Guide. M. E. Rogers and M. M. Dewdney, eds. University of Florida, Institute of Food and Agricultural Services, Gainesville.

EFSA. 2014. Scientific opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. EFSA J. 12:3557.

Fourie, P., Schutte, T., Serfontein, S., and Swart, F. 2013. Modeling the effect of temperature and wetness on *Guignardia* pseudothecium maturation and ascospore release in citrus orchards. Phytopathology 103:281-92.

Frare, G. F. 2015. Influência da idade do fruto no período de incubação e na expressão de diferentes tipos de sintomas da mancha preta dos citros. Ph.D. thesis, USP, Escola Superior de Agricultura Luiz de Queiroz, São Paulo, Brazil.

Goes, A., Baldassari, R. B., Fichtenberger, E., Aguilar-Vildoso, C. I., and Spósito, M. B. 2000. Cracked spot, a new symptom of citrus black spot in Brazil. Page 145 in: Proc. Int. Soc. Citriculture, Orlando, FL.

Goes, A., Graça, J., Barros, J. C. S. M., and Pinheiro, J. E. 1990. Controle da pinta preta em frutos de tangerina 'Rio' (*Citrus deliciosa*) ocasionada por *Phyllosticta citricarpa* (*Guignardia citricarpa*). Fitopatol. Bras. 15:73-75.

Hincapie, M., Wang, N. Y., Peres, N. A., and Dewdney, M. M. 2014. Baseline sensitivity of *Guignardia citricarpa* isolates from Florida to azoxystrobin and pyraclostrobin. Plant Dis. 98:780-789.

Kiely, T. B. 1948. Preliminary studies on *Guignardia citricarpa* spp.: The ascigerous stage of *Phoma citricarpa* and its relation to black spot of citrus. Proc. Linn. Soc. N. S. W. 73:17-20.

Kotzé, J. M. 1981. Epidemiology and control of citrus black spot in South Africa. Plant Dis. 65:945-950.

Kotzé, J. M. 2000. Black spot. Pages 23-25 in: Compendium of Citrus Diseases. J. H. Timmer, L. W. Garnsey, and S. M. Graham, eds. American Phytopathological Society, St. Paul, MN.

Madden, L. V., Hughes, G., and van den Bosch, F. 2007. The Study of Plant Disease Epidemics. American Phytopathological Society Press, St. Paul, MN.

Marques, J. P. R., Spósito, M. B., Mello, A. F. S., Amorim, L., Mondin, M., and Appezzato-da-Glória, B. 2012. Histopathology of black spot symptoms in sweet oranges. Eur. J. Plant Pathol. 133:439-448.

Martínez-Minaya, J., Conesa, D., López-Quílez, A., and Vicent, A. 2015. Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*. A historical analysis of disease spread in South Africa. Eur. J. Plant Pathol. 143:69-83.

McOnie, K. C. 1964. Latent occurrence in citrus and other hosts of *Guignardia* easily confused with *G. citricarpa*, the citrus black spot pathogen. Phytopathology 54:40-43.

Miles, A. K., Willingham, S. L., and Cooke, A. W. 2004. Field evaluation of strobilurins and a plant activator for the control of citrus black spot. Australas. Plant Pathol. 33:371-378.

Reis, R. F., Goes, A., and Timmer, L. W. 2006. Effect of temperature, leaf wetness, and rainfall on the production of *Guignardia citricarpa* ascospores and on black spot severity on sweet orange. Fitopatol. Bras. 31:29-34.

Robbs, C. F., Pimentel, J. P., and Ribeiro, R. L. D. 1980. A mancha preta dos frutos cítricos causada por *Phoma citricarpa*. Fitopatol. Bras. 5:455.

Scaloppi, E. M. T., Aguiar, R. L., Goes, A., and Spósito, M. B. 2012. Efeito do manejo cultural e químico na incidência e severidade da mancha-preta dos citros. Rev. Bras. Frutic. 34:102-108.

Scapin, M. S., Behlau, F., Scandelai, L. H. M., Fernandes, R. S., Silva Junior, G. J., and Ramos, H. H. 2015. Tree-row-volume-based sprays of copper bactericide for control of citrus canker. Crop Prot. 77:119-126.

Schutte, G. C., Beeton, K. V., and Kotzé, J. M. 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Dis. 81:851-854.

Schutte, G. C., Mansfield, R. I., Smith, H., and Beeton, K. V. 2003. Application of azoxystrobin for control of benomyl-resistant *Guignardia citricarpa* on 'Valencia' oranges in South Africa. Plant Dis. 87:784-788.

Silva Junior, G. J., Feichtenberger, E., Spósito, M. B., Amorim, L., Bassanezi, R. B., and Goes, A., eds. 2016a. Pinta preta dos citros: A doença e seu manejo, first ed. Fundecitrus, Araraquara, São Paulo, Brazil.

Silva Junior, G. J., Scapin, M. S., Silva, F. P., Silva, A. R. P., Behlau, F., and Ramos, H. H. 2016b. Spray volume and fungicide rates for citrus black spot control based on tree canopy volume. Crop Prot. 85:38-45.

Spósito, M. B., Amorim, L., Bassanezi, R. B., Yamamoto, P. T., Felippe, M. R., and Czermainski, A. B. C. 2011. Relative importance of inoculum sources of *Guignardia citricarpa* on the citrus black spot epidemic in Brazil. Crop Prot. 30:1546-1552.

Spósito, M. B., Bassanezi, R. B., and Amorim, L. 2004. Resistência à mancha preta dos citros avaliada por curvas de progresso da doença. Fitopatol. Bras. 29: 532-537.

Stammler, G., Schutte, G. C., Speakman, J., Miessner, S., and Crous, P. W. 2013. *Phyllosticta* species on citrus: Risk estimation of resistance to QoI fungicides and identification of species with cytochrome *b* gene sequences. Crop Prot. 48:6-12.

Yonow, T., Hattingh, V., and Villiers, M. 2013. CLIMEX modelling of the potential global distribution of the citrus black spot disease caused by *Guignardia citricarpa* and the risk posed to Europe. Crop Prot. 44:18-28.