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# Relative Contribution of Windbreak, Copper Sprays, and Leafminer Control for Citrus Canker Management and Prevention of Crop Loss in Sweet Orange Trees

**Franklin Behlau,[1,†] José Belasque , Jr.,[2] Rui P. Leite , Jr.,[3] Armando Bergamin Filho,[2] Timothy R. Gottwald,[4] James H. Graham,[5] Luis H. M. Scandelai,[1] Isabela V. Primiano,[1] Renato B. Bassanezi,[1] and Antonio J. Ayres[1]**

[1] Departamento de Pesquisa e Desenvolvimento, Fundo de Defesa da Citricultura (Fundecitrus), Araraquara, São Paulo 14807-040, Brazil
[2] Departamento de Fitopatologia e Nematologia, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, Piracicaba, São Paulo 13416-382, Brazil
[3] Laboratório de Bacteriologia, Instituto de Desenvolvimento Rural do Paraná, Londrina, Paraná 86047-902, Brazil
[4] United States Department of Agriculture, Agricultural Research Service, Fort Pierce, FL 34945, U.S.A.
[5] Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, U.S.A.

## Abstract

The management of citrus canker, caused by *Xanthomonas citri* subsp. *citri*, has been widely studied in endemic areas because of the importance of the disease in several citrus-producing countries. A set of control measures is well established, but no study has investigated the efficiency of each measure individually and their combination for disease suppression. This study comprised a 3-year field study to assess the relative contribution of three measures for the control of citrus canker and reduction of crop losses. Windbreak (Wb), copper sprays (Cu), and leafminer control (Lc) were assessed in eight different combinations in a split–split plot design. The orchard was composed of 'Valencia' sweet orange trees grafted onto 'Rangpur' lime. *Casuarina cunninghamiana* trees were used as Wb. Cu and Lc sprays were performed every 21 days throughout the year. Individually, Cu showed the highest contribution for canker control, followed by Wb. Lc had no effect on reducing citrus canker. Wb+Cu showed the highest efficiency for control of the disease. This combination reduced the incidence of diseased trees by approximately 60%, and the incidence of diseased leaves and fruit by ≥90% and increased the yield in 2.0- to 2.6-fold in comparison with the unmanaged plots. Cu sprays were important for reducing disease incidence and crop losses, whereas Wb had an additional contribution in minimizing the incidence of cankered, non-marketable fruit. The results indicated that the adoption of these measures of control may depend on the characteristics of the orchard and destination of the production.

*Keywords*: *Casuarina cunninghamiana*, crop loss, integrated management, *Phyllocnistis citrella*, protection, *Xanthomonas citri* subsp. *citri*

Historically, the primary and compulsory strategy for control of citrus canker, caused by *Xanthomonas citri* subsp. *citri* (*X. citri*), in several countries, is the eradication of diseased trees (Behlau 2021; Dopson 1964; Gottwald et al. 2002; Rossetti 1977). The adoption of eradication as the main strategy for control of the disease was encouraged by the characteristics of the pathosystem, such as the restricted host range, the absence of an insect vector, the short interval between pathogen infection and symptom appearance under optimum weather conditions, the low capacity of the pathogen to survive for a long period outside the host, and the predominant dissemination of the bacteria over short and medium distances from the inoculum source (Belasque et al. 2005; Dalla Pria et al. 2006; Danós et al. 1984; Goto et al. 1975; Gottwald et al. 1993; Graham et al. 1987). Eradication programs have contributed to the elimination or suppression of citrus canker outbreaks worldwide in the 1900s. During most of that time, there were no efficient management measures available, and the disease was considered a threat for the citrus industry (Behlau et al. 2016; Dopson 1964; Hill 1918). However, despite the remarkable success in eliminating or containing the spread of citrus canker, most of the eradication programs were terminated for various reasons over the years in important citrus-growing regions of Argentina, Uruguay, the U.S.A., and in 2017, Brazil (Behlau 2021). Alternatively, the end of these eradication programs forced the development of management approaches to neutralize or minimize the impact of the disease on the fruit quality and crop production (Behlau 2021; Canteros et al. 2017; Gochez et al. 2020; Leite and Mohan 1990).

Since the 1980s, studies conducted in citrus-producing countries where citrus canker became endemic have demonstrated the efficiency of distinct measures to minimize the impact of the disease on yield and fruit quality. The results of these researches associated with field observations over the years resulted in a set of broadly used control measures such as planting resistant or less susceptible citrus cultivars or species, establishment of arboreal windbreaks in the perimeter of the orchards, spray of copper-based bactericides, and control of the citrus leafminer (*Phyllocnistis citrella*; Behlau et al. 2008; Canteros et al. 2017; Gottwald et al. 1993; Gottwald and Timmer 1995; Graham et al. 2011; Leite and Mohan 1990; Stein et al. 2007). Citrus genotypes show a broad spectrum of susceptibility to citrus canker, ranging from highly resistant to highly susceptible (Gottwald et al. 1993, 2002). Planting citrus varieties that are field resistant to citrus canker is the most efficient and inexpensive measure of control. Although it is an important aspect to be considered for disease management, the susceptibility of the cultivar is usually not critical or a limiting factor when selecting the citrus to be

[†]Corresponding author: F. Behlau; franklin.behlau@fundecitrus.com.br

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planted in canker endemic areas. Instead, the greatest concerns are the marketability or quality of the fresh fruit, juice, and by-products. Hence, canker-susceptible citrus genotypes are frequently grown in endemic areas. In this case, an integrated management approach with the adoption of several control measures is necessary to prevent or minimize significant crop losses caused by the disease (Canteros et al. 2017; Leite and Mohan 1990).

Windbreaks can play an important role in the control of citrus canker (Gottwald and Timmer 1995; Leite and Mohan 1990). Windbreak barriers are formed by planting fast-growing, conical-shaped trees in the perimeter of the orchard (Graham et al. 2016b; Gottwald and Timmer 1995). The species usually used as windbreaks are *Corymbia torelliana*, *Juniperus virginiana*, and *Casuarina* spp. (Graham et al. 2016b; Moschini et al. 2014; Tamang et al. 2010). By slowing the speed and reducing the frequency of gusty winds within the orchard, the windbreak retards the dissemination of the canker bacterium and protects the trees from wounding caused by friction of plant parts and abrasion of soil particles (Bock et al. 2005, 2010, 2013; Gottwald and Timmer 1995; Leite and Mohan 1990). Wind-driven rain splash is the main natural means of dispersal of *X. citri* from the inoculum source (Bock et al. 2005, 2010). Upon landing on the plant surface, the bacterium penetrates the host through stomatal openings or wounds (Bitancourt 1957). Additionally, gusty wind events with speed higher than 5 to 8 meters per second (m/s) can further enhance infection either by projecting the splash-carried bacteria directly through the substomatal cavity (Bock et al. 2010; Gottwald et al. 2002; Graham et al. 2016b) or by intensifying wounding of leaves and fruit, which facilitates the entry of the bacterium into the host (Bock et al. 2013).

The spray of copper-based bactericides is another important measure for citrus canker control (Behlau et al. 2007, 2010b, 2017; Graham et al. 2010, 2011, 2016a; McGuire 1988). Copper protects the citrus trees against the canker bacterium by forming a film on young expanding fruit and leaf flushes that prevents new infections and reduces inoculum build-up (Behlau et al. 2008, 2010a; McGuire 1988; Timmer et al. 1998). Copper compounds have no curative effect on the disease or systemic activity in the tree. Fixed forms of copper such as copper hydroxide, copper oxychloride, and copper oxide are the formulations most widely used (Behlau et al. 2017; Timmer and Zitko 1996). Applications are performed during the spring and summer months, when susceptible plant tissue is abundant and climatic conditions are most favorable for the pathogen dissemination and infection (Leite and Mohan 1990; Medina-Urrutia and Stapleton 1985). The optimal frequency, rate, and volume of the sprays, as well as the benefits of copper for canker control, have been comprehensively addressed in the literature (Behlau et al. 2010a, 2017; Ference et al. 2018; Graham et al. 2010; Scapin et al. 2015).

The citrus leafminer is also an important component of the *X. citri*-citrus pathosystem where the insect is present (Gottwald et al. 2007). The leafminer does not act as a vector, but instead facilitates the penetration of the bacterium into the host tissue (Belasque et al. 2005). The leafminer larvae feeds on young citrus leaves, stems, and fruit, causing wounds (Hall et al. 2010) that expose the mesophyll tissue to *X. citri* infection for a longer period than injuries caused by wind, thorns, or pruning (Gottwald et al. 2002; Jesus Jr. et al. 2006). Symptoms of citrus canker in leafminer-injured leaves appear more rapidly and are usually more severe than symptoms developed through stomatal penetration (Jesus Jr. et al. 2006). Thus, the control of the insect in the field is considered an essential strategy to avoid or reduce the severity of citrus canker outbreaks. Spray of abamectin-based insecticides has been the most used measure for that purpose (Canteros et al. 2017; Diepenbrock et al. 2019; Stein et al. 2007).

Although these measures of control have been proven efficient and are widely adopted where citrus canker is present, no study has weighed the efficiency of each measure individually or has measured the benefits of integrating them. Thus, the main objective of this study was to assess the relative contribution of the exclusive or combined use of arboreal windbreaks, spray of copper-based bactericide, and control of the leafminer for management of citrus canker and prevention of crop loss of sweet orange trees caused by the disease.

## Materials and Methods

**Experimental area.** The trial was carried out in a citrus orchard planted in May 2010 at the experimental station of the Rural Development Institute of Paraná, located in the municipality of Xambrê in Paraná state, Brazil (latitude 23°47′29.03″S, longitude 53°35′55.42″W, and altitude 363 m). The orchard was composed of 'Valencia' sweet orange (*Citrus sinensis* L. Osbeck) trees grafted onto 'Rangpur' lime (*Citrus limonia* Osbeck), planted at a spacing of 7.0 × 2.8 m (510 trees/ha) in an area of approximately 10 ha. As many trees had to be reset in the first years because of several causes, in July 2013, all citrus trees were pruned at 0.5 m in height to standardize their size. From 2015 to 2017, the trunk girth, tree height, and canopy volume were measured annually before harvest. Trunk girth was assessed with a measuring tape at 10 cm above the grafting line. Tree height was measured with a wood ruler. Tree width and depth were taken using a measuring tape. The canopy volume was determined by multiplying the height, width, and depth of each tree (Scapin et al. 2015).

The climate of the region where the experiment was conducted has a Cfa Köppen's climate classification type, i.e., humid subtropical characterized by hot and humid summer and mild winter with no defined dry season. The area was isolated from other commercial citrus plantings, which allowed the trees to grow for approximately 3.5 years before *X. citri* arrived naturally. Pathogen introduction probably occurred from scattered citrus trees in pastures and protected areas of native vegetation in farms nearby.

**Treatments and experimental design.** The measures for control of citrus canker assessed in the study were windbreak (Wb), copper sprays (Cu), and leafminer control (Lc). The treatments were comprised of one to three of these measures or none, totaling eight treatments: Wb+Cu+Lc, Wb+Cu, Wb+Lc, Wb, Cu+Lc, Cu, Lc, and no management (Nm). The experimental design was randomized blocks with subsubplots and three replicates (one per block), in which the main plots represented the presence or the absence of Wb, the subplots represented treatments with or without Cu sprays, and the subsubplots represented trees with or without Lc (Supplementary Fig. S1A). Each subsubplot (experimental plot) contained 112 trees arranged in seven rows with 16 trees per row.

Wb barriers were formed by *Casuarina cunninghamiana* trees planted in December 2011 around the main plots (approximately 1 ha) at 1-m spacing between trees (Supplementary Fig. S1A to C). Both the height and porosity of the Wb trees were characterized. The height was estimated annually from 2015 to 2017 before harvesting the citrus trees by triangulation using a digital laser distance meter (Supplementary Table S1). The porosity, i.e., the percentage of pore space to the space occupied by the Wb tree (Raine and Stevenson 1977; Heisler and Dewalle 1988), was estimated by analyzing pictures taken at 3 m from the Wb trees (three pictures from each side of each plot) in August 2017 with the software ImageJ (version 1.52a; Graham et al. 2016b). The average porosity measurements of the Wb barriers at the three plots were 16.7 (±3.4%), 14.7 (±3.1%), and 15.8% (±2.7%; Supplementary Table S1; Supplementary Fig. S1D to F). Wb plots were located at 60 m from the adjacent plots that did not receive this measure.

Cu-treated subplots were sprayed year-round, every 21 days using copper hydroxide (35% metallic copper, Kocide WDG Bioactive, Mitsui & Co). Sprays were performed until runoff point using a handgun sprayer during the first 3 years after planting. Thereafter, Cu was applied with a 4,000-liter capacity mounted tower air-blast sprayer (Arbus 4000, Jacto, Pompéia, Brazil). A total of 20 nozzles per side, model Disc & Core (Albuz, France) were used. The working speed was 5.5 km/h, with a tractor power outlet rotation of 540 rpm. The Cu rate and spray volume used were 40 mg of metallic copper/$m^3$ and 70 ml of spray mixture/$m^3$ of tree canopy, respectively (Behlau et al. 2017; Scapin et al. 2015). Based on the mean tree row volume per hectare, these parameters corresponded to 0.57, 0.78, and 0.97 kg metallic copper/ha and approximately 1,000, 1,350, and 1,700 liters/ha, applied in the three cropping seasons assessed (2015, 2016, and 2017), respectively (Behlau et al. 2021; Scapin et al. 2015).

The subsubplots that received Lc were sprayed year-round every 21 days with Abamex BR 18 (abamectin 18 g/liter) at 300 ml/2,000 liters using the same equipment, tractor speed, frequency, and spray volume used for Cu sprays. In Lc+Cu plots, the insecticide and the bactericide were co-applied. Complementary activities, such as fertilization and control of weeds, other diseases, and pests, were carried out using standard orchard management approaches, which did not interfere in assessment of the treatments.

**Assessments.** Efficiency of the treatments for the control of citrus canker was measured by assessing the incidence of diseased trees and leaves, and harvested fruit, yield, and crop losses caused by the disease. Assessments began in January 2014, when the first trees with citrus canker were detected within the trial and ended in August 2017, when the third, and last, crop was harvested.

Incidence of diseased trees was assessed monthly for 30 consecutive months on all trees of the subsubplots from January 2014 until June 2016, when it reached 100% in all treatments. Trees with at least one leaf or fruit with symptoms were considered canker-positive. Assessments of leaves were performed monthly for 40 consecutive months, from January 2014 until April 2017 (end of the favorable period for canker development in the last season). The assessments were performed using a hierarchical-based sampling (Hughes and Gottwald 1998) on seven fixed groups of four marked trees randomly distributed in each subsubplot (28 trees/subsubplot). From January 2014 to July 2016, the assessments were performed over the entire canopy, by visually grading the trees from 0 to 100% symptomatic leaves. Because of the increasing size of the trees, from August 2016 to April 2017 the incidence of leaves with citrus canker was assessed on four half-a-meter branches (one per quadrant) marked at the middle height of the canopy. During this period, the incidence of affected leaves in each assessed tree was determined as the proportion of diseased leaves over the total number of leaves assessed per tree. Incidence of diseased leaves in each subsubplot was determined by averaging the incidences in all assessed trees.

The assessments of harvested fruit with canker, yield, and canker-related crop losses in 2015 were performed in the same groups of four trees in each subsubplot used to evaluate the incidence of diseased leaves (28 trees/subsubplot). In 2016 and 2017, the number of assessed trees per group was reduced to three (21 trees/subsubplot) and two (14 trees/subsubplot) trees, respectively. Incidence of fruit with citrus canker was assessed at harvest in August 2015, 2016 and 2017 from a 20-fruit sample (10 fruit from each side facing the row) arbitrarily drawn for each marked tree. Incidence of diseased fruit was determined over the total number of fruit assessed in the subsubplot (560, 420, and 280 fruit/subsub plot in the three seasons, respectively). Fruit yield was measured individually in the marked trees and the mean weight of harvested fruit per tree was calculated over all trees assessed in each subsubplot. Direct canker-related crop losses were assessed in 2016 and 2017 seasons as the percentage of the number of dropped fruit with citrus canker in relation to the total estimated fruit load per tree. Fruit load was composed of both the harvested fruit and the sum of the dropped fruit, with and without canker lesions. The number of harvested fruit per tree was estimated by dividing the fruit weight per tree by the average fruit weight, which was determined from the 20-fruit sample per tree used to assess the incidence of harvested fruit with citrus canker. Prematurely dropped fruit from marked trees were counted and assessed for the presence of citrus canker lesions every 15 days, from the beginning of the drop period in February or March until harvest in all assessed seasons. Abscised fruit were removed and discarded after each assessment.

In addition, leafminer infestation was monitored monthly by assessing the percentage of trees with leafminers and the percentage of leaves affected by the insect. Assessments of the incidence of leaf with leafminer injuries were performed initially over the entire canopy (percentage of injured leaves per tree) and later on marked branches (percentage of injured leaves per branch) as described for the assessments of citrus canker incidence on leaves.

**Data analysis.** The temporal progress of the incidence of leaves with leafminer injuries and the incidence of trees and leaves with citrus canker were compared by the area under leafminer (AULMPC) or disease (AUDPC) progress curves for each treatment, calculated by trapezoidal integration (Madden et al. 2007). AULMPC and AUDPC were divided by their respective duration time to obtain the standardized areas (AULMPC* and AUDPC*; Madden et al. 2007). AULMPC*, AUDPC* for diseased trees and leaves, the peak incidence of diseased leaves, yield, and citrus canker-related crop loss of each treatment were subjected to analysis of variance of a split−split plot design. Levene's test and Shapiro−Wilk test were performed to check homogeneity of variance and normality of residues, respectively. Residuals of yield data from 2017 were not normally distributed and Box−Cox transformation was performed using the 'boxcox' function from the MASS package (Venables and Ripley 2002), where $\lambda = -0.061$. Fixed-effect models were fitted for each variable with Wb, Cu, Lc, and blocks as fixed factors. The function "ssp.plot" from the R statistical agricolae package (De Mendiburu 2020) was used to obtain analysis of variance, in which Wb was considered the main-plot, Cu the subplot, and Lc the subsubplot. The main effects and their interaction were considered significant at 95% level of confidence. The statistical analyses and graphical visualizations were performed using the program RStudio v.1.2.5033.

**Weather data.** Two weather stations (Davis Vantage Pro 2, Hayward, CA) were set up at the beginning of the trial. Each station was centered in a plot with or without Wb and placed within-row between two trees. The variables measured were minimum, mean, and maximum temperatures, rainfall, and wind speed. Data were recorded at 30-s intervals and transformed to average temperatures, cumulative rainfall, and number of gusty wind events per month with speed of ≥8 m/s. This speed has been shown to enhance canker severity in sweet oranges (Serizawa and Inoue 1974).

## Results

The trunk girth, height, and canopy volume of the citrus trees were similar in all plots during the trial. Trunk girth increased, on average, 1.7 cm per year: from 12.1 cm (±0.4 cm) in 2015 to 15.5 cm (±0.9 cm) in 2017. In 2015, 2016, and 2017, the average height of the citrus trees was 3.2 m (±0.2 m), 3.5 m (±0.2 m), and 3.7 m (±0.1 m), respectively. The canopy volume was 28.1 $m^3$ (±2.3 $m^3$) in 2015, increasing 9.1 $m^3$ from 2015 to 2016 and 9.9 $m^3$ from 2016 to 2017. In August 2015, 2016, and 2017, the height of the Wb trees was 14.1 m (±0.8 m), 15.7 m (±1.1 m), and 16.8 m (±1.0 m), respectively. At that time, the height difference between the Wb and the orange trees was 10.9 m (±0.8 m), 12.2 m (±1.1 m), and 13.1 m (±1.0 m), respectively. Over the three cropping seasons, the Wb trees were 4.2- to 4.8-fold higher than the citrus trees (Supplementary Table S1).

The number of gusty wind events per month over the 3 years assessed with speed higher than 8 m/s ranged from 6 to 78 and from 1 to 16 outside and inside the Wb plots, respectively (Supplementary Fig. S2A). Overall, the Wb reduced the incidence of gusty winds within the protected plots by 80.7%. During the most favorable period for disease development (September through April), the average number of gusty wind events per month outside and inside the Wb were, respectively, 28.1 and 6.6 in 2014/2015, 24.9 and 3.8 in 2015/2016, and 27.9 and 3.5 in 2016/2017 (Supplementary Fig. S2A). The mean air temperature inside and outside the Wb varied similarly throughout the seasons from 16.6°C to 27.2°C (Supplementary Fig. S2B). The maximum and minimum air temperatures were, on average, 5.5°C higher and lower than the mean air temperature, respectively. The cumulative rainfall from September through April was 836 mm in 2014/2015, 1,292 mm in 2015/2016, and 890 mm in 2016/2017 (Supplementary Fig. S2B).

The occurrence of the citrus leafminer was uniform throughout the experimental area, as most of the trees (>90%) in all plots had citrus leafminer-damaged leaves during the trial. Despite the fact that all trees were affected by the insect, the incidence of mined leaves was significantly higher (approximately 50%) in plots with no Lc in comparison with plots that did not receive this measure (Fig. 1). The change in the evaluation methodology during the experimental period better captured the differences between plots with and without Lc, which was more evident when the assessments were performed using marked branches rather than the entire canopy (Fig. 1A).

Citrus canker was first detected in the experimental area in January 2014. By that time, the initial average incidence of diseased trees ranged from 0.3 to 2.1% among the treatments (Fig. 2A). Although the disease had been present in all trees of the trial by May 2016, the treatments resulted in different disease progression curves. In Nm and Lc plots, it took approximately 14 months for the incidence of trees with citrus canker to reach approximately 100% (Fig. 2A). By contrast, in Wb+Cu plots, irrespective of Lc treatment, it took 28 months from the beginning of the epidemic for disease incidence to reach 100%. In the other treatments, the time to detect the disease in all trees was intermediate, being 16 months for Lc and Wb plots, 25 months for Cu+Lc and Wb+Lc plots, and 27 months for Cu-treated plots (Fig. 2A).

The incidence of diseased leaves fluctuated seasonally throughout the years and the annual peak of citrus canker incidence on leaves was observed at the end of summer or beginning of fall (February to April; Fig. 2B). The main effect of Cu sprays on the reduction of the maximum incidence of diseased leaves was significant in all seasons ($P_{2015} = 0.003$, $P_{2016} = 0.002$, and $P_{2017} < 0.001$; Table 1). In 2015, 2016, and 2017 the highest incidence of affected leaves in Cu-treated plots was 3.0-, 7.4-, and 3.8-fold lower than in plots without Cu applications, respectively. The main effect of the Wb on reducing the peak of diseased leaves was significant in 2016 and 2017 ($P_{2016}$ = 0.001 and $P_{2017}$ = 0.040; Table 1), and almost significant in 2015 ($P_{2015}$ = 0.086; Table 1). In addition, there was a significant interaction between Wb and Cu on the peak of diseased leaves in 2015 ($P$ = 0.019) and 2016 ($P$ = 0.008). In 2017, this interaction

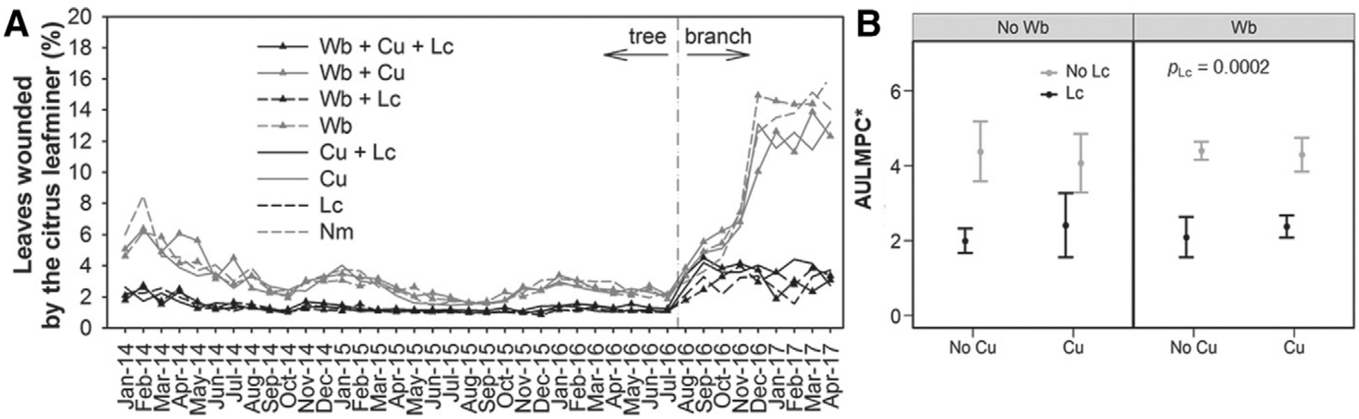


**Fig. 1. A,** Progress curves of the incidence of Valencia sweet orange leaves injured by the citrus leafminer, and **B,** the respective standardized area under the leafminer progress curves (AULMPC*) in plots under windbreak protection (Wb), copper sprays (Cu), and/or leafminer control (Lc), and no management (Nm). Assessments of the incidence of leaves injured by the citrus leafminer were performed over the entire canopy from January 2014 until July 2016, and over marked branches from August 16 to April 2017. The $P$ value of Lc indicates the significance of the leaf miner control on reducing the incidence of mined leaves in comparison with plots that did not receive the control of the insect. Whiskers indicate the standard error of the mean.

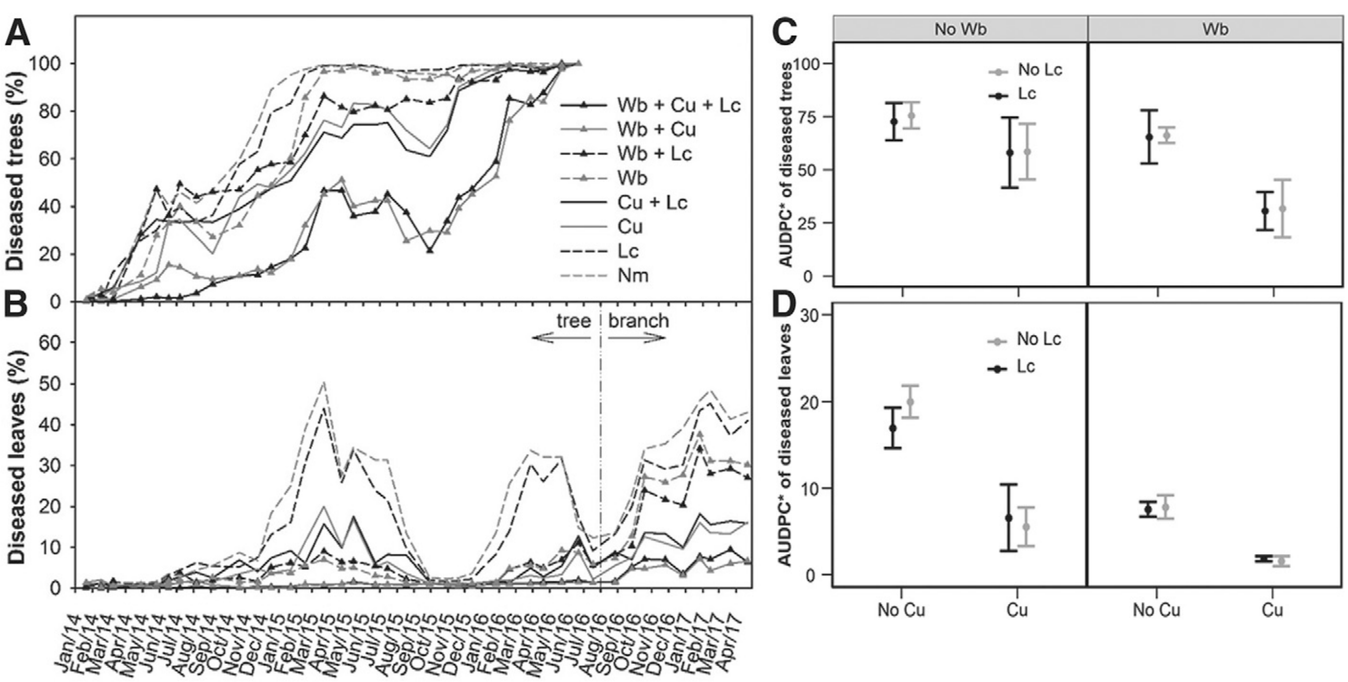


**Fig. 2.** Progress curves of the incidence of **A,** trees and **B,** leaves of Valencia sweet orange with citrus canker and the respective standardized areas under the disease progress curves (AUDPC*), **C** and **D,** in plots under windbreak protection (Wb), copper sprays (Cu), and/or leafminer control (Lc), and no management (Nm). Assessments of the incidence of diseased trees began in January 2014 and ended in June 2016, when incidence in all treatments reached 100%. Assessments of the incidence of diseased leaves were performed over the entire canopy from January 2014 until July 2016, and over marked branches from August 2016 to April 2017. Whiskers indicate the standard error of the mean.

followed the same trend of the previous seasons but was not as significant ($P$ = 0.090). In these years, Wb led to peaks of disease incidence even lower in plots with Cu. The Lc had no significant main effect ($P_{2015}$ = 0.485, $P_{2016}$ = 0.915, and $P_{2017}$ = 0.838) or interaction with other measures ($P$ = 0.222 to 0.996) on the occurrence of cankered leaves in any of the seasons (Table 1). The combination of Wb and Cu, irrespective of Lc, provided a reduction of 85.5 to 98.7% in the peak incidence of diseased leaves in comparison with trees under Nm (Table 2).

The main effect of Cu sprays was significant on both AUDPC* of diseased trees ($P$ = 0.013; Table 1) and AUDPC* of diseased leaves ($P$ < 0.001; Table 1). In contrast, the main effect of Wb or Lc was not significant on the AUDPC* of diseased trees ($P_{wb}$ = 0.219 and $P_{Lc}$ = 0.724, Table 1; Fig. 2C) or leaves ($P_{wb}$ = 0.091 and $P_{Lc}$ = 0.390; Table 1; Fig. 2D). There was a significant interaction between Wb and Cu on the AUDPC* of diseased leaves ($P$ = 0.009; Table 1). Wb had an additional effect in reducing the AUDPC* of diseased leaves when combined with Cu sprays, from 6.1 to 1.7 (Fig. 2D). Both Wb+Cu+Lc and Wb+Cu treatments reduced the AUDPC* of diseased trees and leaves by approximately 60 and 90%, respectively, in comparison with the Nm treatment (Table 2). The AUDPC* values of diseased trees for the Wb+Cu+Lc and Wb+Cu treatments were 30.6 and 31.7, respectively (Fig. 2C), as opposed to 75.6, observed for the Nm treatment (Fig. 2C). Congruent with that, the AUDPC* values of diseased leaves for Wb+Cu+Lc, Wb+Cu, and Nm were 1.9, 1.6, and 20.0, respectively (Fig. 2D).

Cu applications had a significant effect on the incidence of harvested fruit with citrus canker across all seasons ($P_{2015}$ = 0.003, $P_{2016}$ < 0.001, and $P_{2017}$ < 0.001; Fig. 3; Table 3). Irrespective of Wb and Lc, Cu sprays reduced the diseased fruit from 41.1 to 13.7% in 2015, from 52.3 to 18.8% in 2016, and from 36.6 to 9.6% in 2017. The main effect of Wb was significant on the incidence of harvested fruit with citrus canker in 2016 and 2017 ($P_{2016}$ = 0.045 and $P_{2017}$ = 0.003; Table 3), but not in 2015 ($P_{2015}$ = 0.105; Table 3). There was a significant interaction between Wb and Cu on the percentage of harvested fruit with citrus canker in 2017 ($P$ < 0.001; Table 3). In this season, the combination of Cu and Wb provided an additional reduction in the incidence of harvested fruit with citrus canker, from 16.9 (Cu) to 2.1% (Wb+Cu; Fig. 3). Conversely, Lc did not affect the percentage of harvested fruit with citrus canker in any of the seasons ($P_{2015}$ = 0.729, $P_{2016}$ = 0.815, and $P_{2017}$ = 0.116; Table 3). The incidence of cankered fruit produced by Wb+Cu trees, regardless of Lc treatment, was >90% lower in comparison with the Nm trees (Table 2).

Cu applications had a significant effect on yield across all seasons ($P_{2015}$ = 0.005, $P_{2016}$ = 0.002, and $P_{2017}$ < 0.001; Table 3). This measure reduced crop losses caused by citrus canker regardless of the combination with Wb or Lc in the three seasons. On average, Cu applications increased the yield from 23.3 to 34.3 kg/tree in 2015, from 51.7 to 80.0 kg/tree in 2016, and from 66.6 to 121.6 kg/tree in 2017 (Fig. 4). Wb only had a significant effect on yield in 2017 ($P_{2017}$ = 0.042; Table 3), increasing yield from 85.3 kg/tree to 102.8 kg/tree (Fig. 4). Likewise, the interaction between Wb and Cu on

**Table 1.** Significance ($P$ value) of the windbreak (Wb), copper sprays (Cu), leafminer control (Cu), and the interaction (*) of these measures on reducing the standardized area under disease progress curve (AUDPC*) of the incidence trees and leaves of Valencia sweet orange with symptoms of citrus canker and the incidence peak of diseased leaves (%) per season

| Factor | Diseased trees AUDPC*[a] | Diseased leaves AUDPC*[b] | Peak of diseased leaves (%)[c] | | |
|---|---|---|---|---|---|
| | | | 2015 | 2016 | 2017 |
| Wb | 0.219[d] | 0.091 | 0.086 | **0.001** | **0.040** |
| Cu | **0.013** | **<0.001** | **0.003** | **0.002** | **<0.001** |
| Lc | 0.724 | 0.390 | 0.485 | 0.915 | 0.838 |
| Wb*Cu | 0.184 | **0.009** | **0.019** | **0.008** | 0.090 |
| Wb*Lc | 0.913 | 0.390 | 0.341 | 0.640 | 0.905 |
| Cu*Lc | 0.884 | 0.070 | 0.996 | 0.408 | 0.222 |
| Wb*Cu*Lc | 0.849 | 0.147 | 0.728 | 0.306 | 0.948 |

[a] AUDPC* of the incidence of canker-affected trees assessed monthly for 30 consecutive months on all trees from January 2014 until June 2016, when the incidence reached 100% in all treatments.

[b] AUDPC* of the incidence of canker-affected leaves assessed monthly for 40 consecutive months on marked trees from January 2014 until April 2017 (end of the favorable period for canker development in the last season).

[c] Maximum incidence of diseased leaves per season.

[d] Calculated $P$ values obtained from the analysis of variance. Treatments were analyzed as split−split plot randomized complete block design. Significant $P$ values (≤0.05) are in bold, which indicates that the use of an individual measure or an interaction between measures decreased the disease parameter assessed.

**Table 2.** Relative contribution (%) of windbreak (Wb), copper sprays (Cu), and leafminer control (Lc) applied alone or in combination compared with the non-managed plots on the incidence of trees, leaves, and harvested fruit of Valencia sweet orange with symptoms of citrus canker, yield, and crop losses caused by the disease; standardized area under disease progress curve (AUDPC*)

| Treatment | Diseased trees AUDPC*[a] | Diseased leaves AUDPC*[b] | Peak of diseased leaves (%)[c] | | | Harvested fruit with citrus canker (%) | | | Yield (kg/tree) | | | Citrus canker-related crop loss (%)[d] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2015 | 2016 | 2017 | 2015 | 2016 | 2017 | 2015 | 2016 | 2017 | 2016 | 2017 |
| Wb + Cu + Lc | −59.5[e] | −90.7 | −98.7 | −96.3 | −85.5 | −93.5 | −89.2 | −95.7 | +181.3 | +83.9 | +133.0 | −85.4 | −89.4 |
| Wb + Cu | −58.1 | −92.1 | −98.3 | −96.9 | −91.1 | −95.0 | −90.7 | −96.5 | +138.2 | +90.8 | +125.0 | −88.8 | −93.1 |
| Wb + Lc | −13.4 | −62.2 | −82.2 | −81.2 | −42.2 | −44.6 | −43.3 | −59.8 | +104.2 | +17.0 | +52.5 | −43.9 | −57.2 |
| Wb | −12.4 | −60.8 | −86.0 | −83.4 | −35.9 | −60.2 | −47.0 | −69.0 | +112.4 | +35.3 | +26.2 | −49.7 | −66.4 |
| Cu + Lc | −23.2 | −67.1 | −68.9 | −85.4 | −68.1 | −64.7 | −55.3 | −64.7 | +127.6 | +59.4 | +105.5 | −65.0 | −67.7 |
| Cu | −22.6 | −72.2 | −60.3 | −90.7 | −72.0 | −53.3 | −56.6 | −72.9 | +113.3 | +62.1 | +107.6 | −61.2 | −71.1 |
| Lc | −3.9 | −15.2 | −12.9 | −9.9 | −6.9 | −13.9 | −8.8 | −2.3 | +35.8 | −2.5 | −1.7 | −9.6 | +3.7 |

[a] AUDPC* of the incidence of canker-affected trees assessed monthly for 30 consecutive months on all trees from January 2014 until June 2016, when the incidence reached 100% in all treatments.

[b] AUDPC* of the incidence of canker-affected leaves assessed monthly for 40 consecutive months on marked trees from January 2014 until April 2017 (end of the favorable period for canker development in the last season).

[c] Maximum incidence of diseased leaves per season.

[d] Citrus canker-related crop losses refer to the percentage of the number of dropped fruit with citrus canker in relation to the total estimated fruit load per tree.

[e] (−) and (+) indicate the reduction or increase in %, respectively, in comparison with the non-managed plots.

yield was significant only in 2017 ($P < 0.001$; Table 3). On the contrary, the effect of Lc on yield was not significant across seasons ($P_{2015} = 0.175$, $P_{2016} = 0.190$, and $P_{2017} = 0.240$; Table 3). In the three seasons, yield increase in Wb+Cu plots, irrespective of Lc, ranged from 83.9 to 181.3% in comparison with the Nm plots (Table 2).

Both Wb and Cu had a significant effect on the citrus canker-related crop losses, i.e., the percentage of the number of dropped fruit with citrus canker in relation to the total estimated fruit load per tree, in both 2016 ($P_{Wb} = 0.041$, $P_{Cu} = 0.004$; Table 3) and 2017 ($P_{Wb} < 0.001$, $P_{Cu} > 0.001$; Table 3), but, as observed for the incidence of cankered fruit and yield, the interaction of these two control measures was significant only in 2017 ($P_{2017} = 0.001$; Table 3). In these seasons, Wb reduced crop losses by 2.0- and 2.8-fold, respectively, in comparison with plots with no Wb. Likewise, crop losses in plots with Cu sprays was 2.9 and 3.6 times lower in 2016 and 2017, respectively, when compared with plots with no Cu application. In 2017, plots with Wb+Cu, regardless of Lc, had the lowest percentage of citrus canker-related crop losses (3.2%) among all treatments (Fig. 5). Conversely, in that year, the crop losses caused by the disease reached 37.2% in the plots without Cu and/or Wb. The reduction in crop losses when Wb and Cu were combined ranged from 88.8 to 93.1%, in comparison with Nm plots (Table 2).

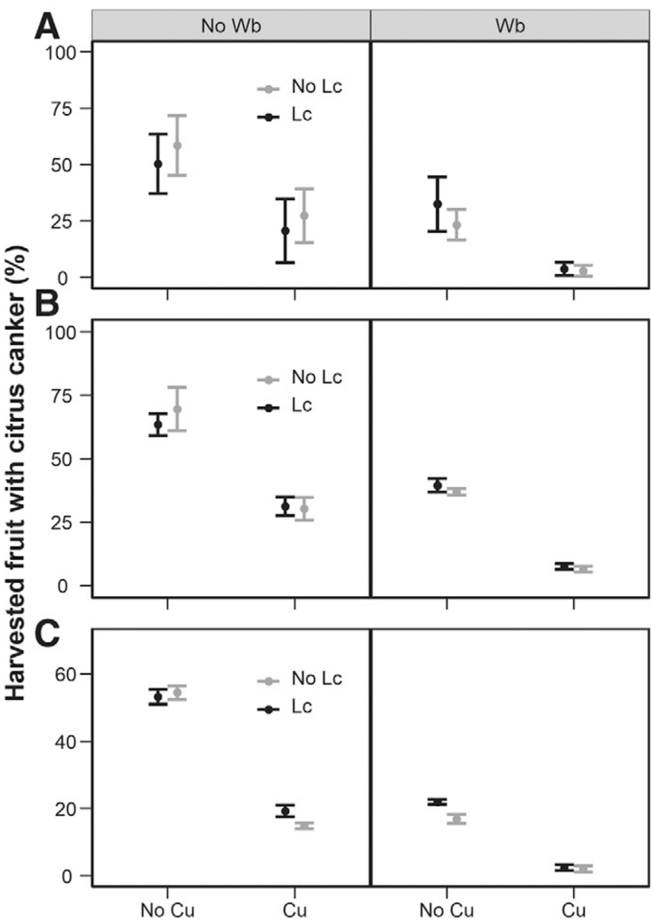


**Fig. 3.** Incidence of fruit with citrus canker harvested from Valencia sweet orange trees in plots under windbreak protection (Wb), copper sprays (Cu), and/or leafminer control (Lc), and no management (Nm) in **A,** 2015; **B,** 2016; and **C,** 2017. Whiskers indicate the standard error of the mean.

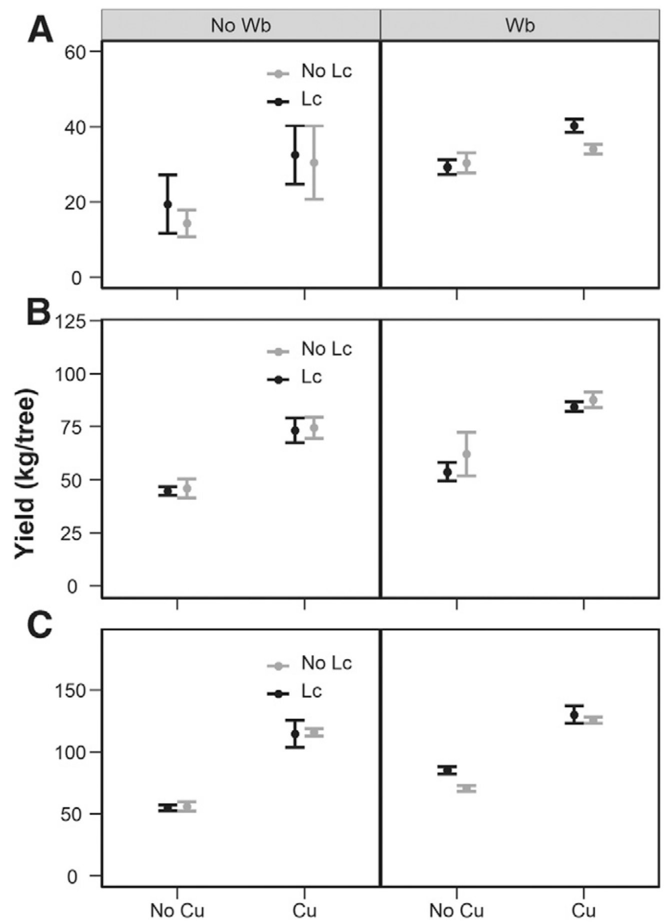


**Fig. 4.** Yield of Valencia sweet orange trees in plots under windbreak protection (Wb), copper sprays (Cu), and/or leafminer control (Lc), and no management (Nm) in **A,** 2015; **B,** 2016; and **C,** 2017. Whiskers indicate the standard error of the mean.

**Table 3.** Significance (*P* value) of the windbreak (Wb), copper sprays (Cu), leafminer control (Cu), and the interaction (*) of these measures on reducing the incidence of fruit with symptoms of citrus canker and related crop losses and on increasing yield of Valencia sweet orange for three consecutive seasons

| | Harvested fruit with citrus canker (%) | | | Yield (kg/tree) | | | Citrus canker-related crop loss (%)[b] | |
|---|---|---|---|---|---|---|---|---|
| Factor[a] | 2015 | 2016 | 2017 | 2015 | 2016 | 2017 | 2016 | 2017 |
| Wb | 0.105[a] | **0.045** | **0.003** | 0.252 | 0.050 | **0.045** | **0.041** | **<0.001** |
| Cu | **0.003** | **<0.001** | **<0.001** | **0.005** | **0.002** | **<0.001** | **0.004** | **<0.001** |
| Lc | 0.729 | 0.815 | 0.116 | 0.175 | 0.190 | 0.167 | 0.834 | 0.094 |
| Wb*Cu | 0.516 | 0.298 | **<0.001** | 0.143 | 0.964 | **<0.001** | 0.337 | **0.001** |
| Wb*Lc | 0.098 | 0.815 | 0.662 | 0.797 | 0.370 | 0.075 | 0.289 | 0.522 |
| Cu*Lc | 0.623 | 0.218 | 0.829 | 0.615 | 0.610 | 0.224 | 0.869 | 0.534 |
| Wb*Cu*Lc | 0.485 | 0.227 | 0.070 | 0.238 | 0.599 | 0.251 | 0.696 | 0.573 |

[a] Calculated *P* values obtained from the analysis of variance. Treatments were analyzed as split–split plot randomized complete block design. Significant *P* values (≤0.05) are in bold, which indicates that the use of an individual measure or an interaction between measures significantly decreased disease incidence and citrus canker-related crop losses or increased yield.

[b] Citrus canker-related crop losses refer to the percentage of the number of dropped fruit with citrus canker in relation to the total estimated fruit load per tree.

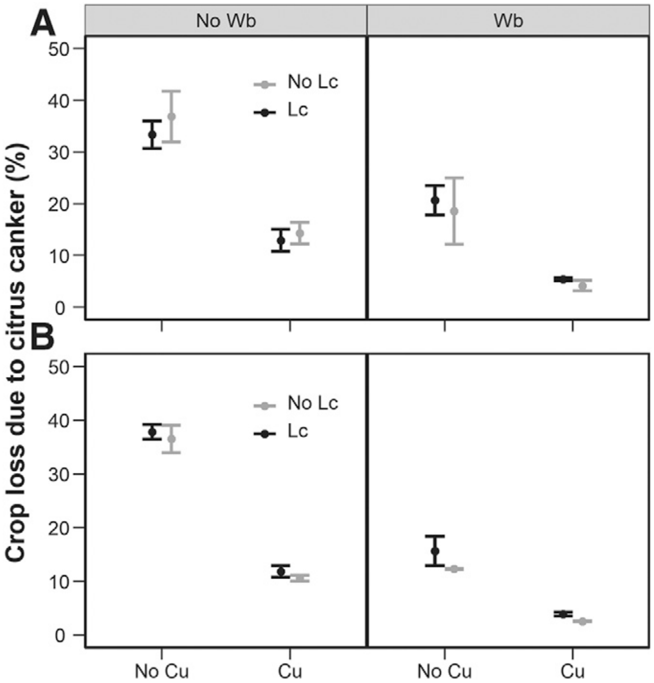


**Fig. 5.** Direct citrus canker-related crop losses, in percentage, as a relation between the number of dropped fruit with citrus canker lesions and the total estimated number of fruit load per Valencia sweet orange tree in plots under windbreak protection (Wb), copper sprays (Cu), and/or leafminer control (Lc), and no management (Nm) in **A,** 2016 and **B,** 2017. Whiskers indicate the standard error of the mean.

## Discussion

This field study unraveled the relative contribution of windbreak, copper sprays, and leafminer control in the management of citrus canker. As a single measure, copper sprays had the highest contribution in reducing citrus canker incidence and crop losses. The main effect of copper was significant for all parameters assessed. The windbreak was also important. However, this measure was not as efficient as copper, as the main effect of the windbreak on disease control was not significant in all assessments. The leafminer control had no effect in reducing citrus canker incidence. Besides the main effects, there was also a significant interaction between copper sprays and windbreak in some assessments. The combination of these two measures had the highest contribution not only in slowing the temporal progress of the disease but also in reducing its impact on fruit quality and yield of sweet orange trees. Our results corroborate earlier reports demonstrating that windbreak and copper sprays are very efficient for controlling citrus canker (Behlau et al. 2007, 2008, 2017; Gottwald and Timmer 1995; Graham et al. 2010, 2011, 2016a; Leite and Mohan 1990), but contrast with previous studies inferring that leafminer control could make a significant contribution for citrus canker management (Hall et al. 2010; Jesus Jr. et al. 2006).

Copper sprays have been widely used in citrus-growing areas where citrus canker is endemic since the 1970s, when this management approach was first deployed in Argentina (Canteros et al. 2017). Copper is the main measure for control of citrus canker in several citrus-growing regions (Behlau et al. 2017; Ference et al. 2018; Gochez et al. 2020). Further, it has been demonstrated that fixed copper compounds are more efficient than soluble copper forms (Behlau et al. 2017; Graham et al. 2010, 2011). In addition, spray volume and copper rate based on the canopy volume have been established to minimize environmental impacts by reducing water consumption and waste of product (Behlau et al. 2021; Scapin et al. 2015). This study not only confirmed that copper is an important tool against citrus canker, but also demonstrated that it is the most efficient measure available to control the disease. The efficiency of the copper sprays in the control of citrus canker may vary with the age and cultivar of the citrus trees, the use of complementary measures, and the weather conditions in the season. Nevertheless, in most cases, as in this study, copper sprays provided a reduction of 50 to near 100% in the incidence of diseased trees, leaves, or fruit (Behlau et al. 2011). More importantly, as also demonstrated in this field trial, the copper sprays may minimize or neutralize the crop losses caused by the disease (Behlau et al. 2010b, 2017; Graham et al. 2010, 2016a).

In a previous study, windbreaks were more important for reducing the spread of citrus canker within the orchard than copper sprays (Gottwald and Timmer 1995). However, it is worth mentioning that in the referred study the 4-week copper spray interval may have reduced the efficiency of the measure in controlling the disease. It is known that copper needs to be applied at intervals no longer than 3 weeks apart for best performance (Behlau et al. 2010b). Longer spray intervals may favor bacterial infection in new growing susceptible tissues and increase disease severity. Copper has no curative activity, as it acts preventively and protectively. After application, fixed copper forms a protective film on the treated surface that slowly releases $Cu^{2+}$ that is toxic to the bacterium (Menkissoglu and Lindow 1991). Thus, leaf and fruit expansion, rather than rainfastness, is more important for the loss of efficiency of copper in citrus canker control (Graham et al. 2010).

Differently from the results observed by Gottwald and Timmer (1995) in Argentina, the windbreak did not delay the disease progress curve of affected trees. Instead, this measure had a significant contribution in reducing the incidence of leaves and fruit with citrus canker, as was also reported by Graham et al. (2016b) in Florida, U.S.A. The efficiency of the natural barrier was likely related to the height of the windbreak trees, which was 4.2 to 4.8 times higher than the citrus trees in this study. To effectively protect the orchard, it is critical that the windbreak barrier is higher than the crop trees (Heisler and Dewalle 1988; Tamang et al. 2010). When this condition was not achieved, the windbreak had no effect on suppressing the disease (Behlau et al. 2007, 2008). The windbreak contributed to the control of citrus canker by reducing the frequency of gusty wind events with speed higher than 8 m/s (Supplementary Fig. S2). This wind speed has been demonstrated to be a threshold for exacerbating the penetration of *X. citri* into the host (Serizawa and Inoue 1974). In addition, the windbreaks also prevent wounds made by thorns, twigs, and soil particle abrasion that serve as an efficient entry for the bacterium into the plant tissues (Bock et al. 2010).

Leafminer control did not contribute to the control of citrus canker or to the reduction of crop losses when applied alone or in combination with windbreak or copper sprays. This result contrasts with previous studies that have demonstrated the importance of the leafminer injuries on the severity of the disease and spatial distribution of the foci after an incursion. When feeding on flushes or young fruit, the larvae of the citrus leafminer facilitates the penetration of *X. citri* into the mesophile of the plant tissue by forming wounds that expose the plant tissues to infection (Hall et al. 2010; Jesus Jr. et al. 2006). Consequently, the injuries caused by leafminer increases not only the size of the cankered area but also the bacterial inoculum within the orchard (Belasque et al. 2005; Jesus Jr. et al. 2006). In addition, wounds formed by the leafminer remain exposed to infection by *X. citri* dispersed during rainstorms for a longer period than wounds caused by thorns or pruning (Jesus Jr. et al. 2006) and allow that lower concentration of bacterial inoculum causes disease than wounds or natural openings (Christiano et al. 2007; Gottwald et al. 2002). These conditions have increased the distance of secondary foci from the original focus and the distribution of the disease after arriving in the orchard became less aggregate (Danós et al. 1984; Gottwald et al. 2007).

A possible reason for the lack of efficiency of leafminer control for suppressing citrus canker in this study could be related to the infestation of the orchard by the insect during the trial. Although the incidence of mined leaves in the plots that received control of the leafminer was lower than in plots without the spray of insecticide, the incidence of affected leaves in untreated plots remained relatively low during the trial. This was more noticeable when the assessments were based on the entire tree canopy. However, when the assessments were changed to marked branches, the incidence of leafminer injured leaves increased and the difference between treated and untreated plots became more evident. Thus, the level of incidence of leafminer in the area did not seem to be the main or the only reason for not observing a significant contribution of the control of the insect in the control of citrus canker, but also the fact that windbreak and, mainly, the copper sprays, had higher contributions in the suppression of the disease as observed previously. In the only previous study that has assessed the efficiency of leafminer control on canker incidence under field conditions, the combination of insecticide sprays for leafminer control with copper sprays had also not improved the management of the disease (Stein et al. 2007). Stein et al. (2007) also showed that the use of insecticide against the leafminer as the only measure for citrus canker control showed a slight reduction on the severity of the disease. Likewise, although no significant differences were detected, it was possible to observe in our study that plots that received only leafminer control tended to have lower incidences of diseased trees, leaves, and fruit in comparison with plots with no management. Hence, the results indicate that the leafminer control has a greater importance in preventing the establishment of primary and secondary foci in areas under exclusion and eradication of citrus canker. In orchards where the disease is endemic, the contribution of leafminer control for canker management is marginal.

The combination of windbreak with copper sprays showed the greatest contribution in reducing the incidence of citrus canker and related crop losses. Thus, ideally, both measures are expected to be concomitantly applied for an efficient management of the disease. However, because of limitations related to shading, nutrient and water competition, maintenance, and displacement of area (Andreu et al. 2008; Cleugh 2003), arboreal windbreaks are not widely adopted in canker endemic areas (Gochez et al. 2020). This study showed that copper sprays played a major role in reducing disease incidence and crop losses, whereas the windbreak had an additional contribution in minimizing the incidence of cankered, non-marketable fruit. Thus, the results indicated that the use of windbreaks may be facultative in orchards for production of fruit for juice processing depending mainly on the susceptibility of the cultivar and topography (Gochez et al. 2020; Owen-Turner and Hardy 2006), but mandatory in orchards intended for fresh fruit production. This measure has been proven to be especially important for the canker endemic areas to meet the requirements for commercialization of fresh citrus fruit to other countries (Behlau 2021; Canteros et al. 2017).

We have demonstrated the weighted importance of windbreaks, copper sprays, and leafminer control for managing citrus canker. The selection of the correct measures, which are critical for minimizing crop losses caused by the disease, depends on the cost benefit of each measure, which varies based on the citrus cultivar, climatic favorability for disease development, topography of the area, wind incidence, and destination of the production.

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