

Validation of black spot [*Pseudocercospora purpurea* (Cooke) Deighton] management strategies in avocado crops in northern Tolima (Colombia)

Validación de estrategias de manejo de la peca [*Pseudocercospora purpurea* (Cooke) Deighton] en cultivos de aguacate en el norte del Tolima (Colombia)



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Black spot, caused by *Pseudocercospora purpurea*, symptoms in avocado fruits, variety Lorena, Fresno-Tolima.

Photo: E. Rodríguez-Polanco

ABSTRACT

Leaf spot, or black spot (BS), caused by the fungus *Pseudocercospora purpurea* (Cooke) Deighton, is one of the principal sanitary limitations in avocado production, particularly in the Antillean cultivars (Lorena, Choquette, Santana, and Semil 44) in the northern region of the Department of Tolima, Colombia. From 2014 to 2017, different experiments were carried out in the field to determine when higher fruit susceptibility occurs, the effect of pruning on inoculum reduction, and the chemical molecules with a high degree of control, adjusting their chronology and frequency of application. The results will facilitate the technical, economic, and environmental implementation and validation of a chemical strategy for management of this disease. The management strategy carried out with Agrosavia reported a 10% lower BS incidence in the fruits, as compared to the one carried out by producers, and a 23.75% return as a result of the increased fruit quality. This strategy used DMI fungicides (demethylation inhibitors), without resistance reports, and fungicides with a mixture of two active ingredients (DMI and QoI) and interspersed applications, sometimes in conjunction with CuOCl during periods of higher BS susceptibility in order to reduce the resistance induction risk in *P. purpurea*.

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Additional keywords: Lorena cultivar; chemical control; leaf spot; *Persea americana*.

RESUMEN

La peca o mancha negra del aguacate causada por el hongo *Pseudocercospora purpurea* (Cooke) Deighton, es una de las principales limitantes sanitarias para la producción de aguacate de los cultivares antillanos (Lorena, Choquette, Santana y Semil 44) en la región productora del norte del departamento del Tolima, Colombia. Durante 2014-2017, se realizaron diferentes experimentos en campo con el propósito de determinar la época de mayor susceptibilidad del fruto, el efecto de la poda en la disminución del inóculo, y la selección de moléculas químicas con mayor grado de control, ajuste de su cronología y frecuencia de aplicación. Estos resultados permitieron la implementación y validación técnica, económica y ambiental de una estrategia química de manejo de la enfermedad. La estrategia de manejo realizada por Agrosavia (MA) reportó una incidencia de peca en fruto menor en un 10% en comparación con la observada en el manejo realizado por los productores (MP), con una rentabilidad de 23,75% debida al incremento de frutos de primera calidad. En esta última se emplearon fungicidas DMI (inhibidores de la demetilación) aún sin reportes de resistencia, fungicidas con mezcla de dos ingredientes activos (DMI y QoI) en aplicación intercalada, y algunas veces en conjunto con CuOCl durante la época de mayor susceptibilidad de los frutos a la peca, encaminada a disminuir el riesgo de inducción de resistencia en *P. purpurea*.

Palabras clave adicionales: cultivar Lorena; mancha negra; control químico; *Persea americana*.

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INTRODUCTION

Leaf spot, or black spot (BS), caused by the fungus *Pseudocercospora purpurea* (Cooke) Deighton (Darvas *et al.*, 1987), causes significant losses (48%) in avocado production by decreasing fruit quality and making fruits unacceptable for export (Lonsdale, 1991; Reina-Noreña *et al.*, 2015). The fungus that causes this disease was first described by Cooke (1878), named *Cercospora purpurea*; however, in a revision by Deighton (1976), *C. purpurea* was renamed *Pseudocercospora purpurea* (Cke) Deighton. Currently, this fungus is classified within the phylum Ascomycota, subphylum Pezizomycotina, class Dothideomycetes, order Capnodiales, and family Mycosphaerellaceae (EPPO, 2020).

P. purpurea, its rapid spread and damage to avocados have made it one of the principal problems for the industry in Martinique, Cameroon, South Africa and in the French West Indies (Gustafson, 1976; Willis and Mavuso, 2007; Everett and Siebert, 2018). In Mexico, it is the second most common disease in avocados (Turu, 1969). In South Africa, black spot losses of up to 69% have been reported in fruits used for export from orchards without management in

a highly susceptible cultivar (Darvas *et al.*, 1987; Crous and Braun, 2003); it was reported for Australia in 1992 (Peterson and Grice, 1992).

The symptoms of this disease are observed in leaves, fruit, and stems during all growth stages, presenting small angular lesions, approximately 2.5 mm in diameter, with brown to purple coloration and surrounded by a yellow halo. As the disease progresses, the spots on the fruit sink, forming cracks without causing internal damage (Darvas, 1982; Dann *et al.*, 2013). These cracks, however, are frequent entry points for other pathogens, such as fungi (e.g., *Colletotrichum gloeosporioides*) that cause anthracnose (Tamayo, 2004).

Wind, rain, and insects play an essential role in the spread of *P. purpurea* spores. High relative humidity and high temperatures favor the development of the pathogen (Darvas *et al.*, 1987). Usually, the fungus remains dormant in the leaves for a period of approximately three months, becoming a source of inoculum for the infection of new fruits (Darvas, 1982).

Pruning crops during dry periods and removing residues are a useful strategy in the management of this disease (Mejía, 1999; Sánchez and Patricia, 2000; Tamayo, 2004). Complementary to pruning, chemical BS management initially used the spraying of chemical fungicides based on copper oxychloride, cupric hydroxide, benomyl, and carbendazim in rotation before harvest (Darvas, 1982; Lonsdale, 1991, 1992; Vermeulen *et al.*, 1992). However, disadvantages associated with their repeated use include costs of removing copper residues from fruits and fungal resistance to benomyl (Darvas *et al.*, 1987). Studies on fungicides with zinc, manganese, cyproconazole, flusilazole, or copper pentahydrates, applied when flower buds began to swell with monthly intervals until slightly before harvest, have demonstrated their efficiency in disease control (Darvas, 1982; Lonsdale, 1991; Sánchez and Patricia, 2000; Duvenhage, 2002; Willis and Mavuso, 2007).

In Colombia, BS is a frequent pathology on commercial avocado plantations in the Caldas, Risaralda, Quindío, Valle del Cauca, Antioquia, Cundinamarca, and Tolima Departments (Mejía, 1999; Tamayo, 2008; Reina-Noreña *et al.*, 2015). Since its emergence in 2012 in the producing areas in the north of the Tolima Department, BS has become the primary sanitary constraint on the production of Antillean- and Creole-type avocados. In these areas, BS causes considerable economic losses by decreasing fruit quality, 48% of production is cataloged as second- and third-quality fruits, and increasing production costs by approximately 4% because of an increase in the application of agrochemicals (Reina-Noreña *et al.*, 2015).

Accordingly, in order to validate different BS management strategies in Antillean avocados, experiments were carried out on commercial plantations for three years to establish when higher fruit susceptibility occurs, the effect of pruning on inoculum decreases, the chemical molecules with the highest degree of control, their chronology according to the time of highest fruit susceptibility, and the frequency of applications.

MATERIALS AND METHODS

Bioassay to evaluate the effect of fungicides and insecticides

For the fungicide and insecticide evaluation bioassays, three 'Lorena' avocado-producing farms located in the northern region of Tolima were selected (Tab. 1), each with a different BS severity degree according to a logarithmic diagrammatic scale (LDS) designed to measure severity in leaves ($r^2 = 0.74-0.92$; Fig. 1).

Each farm had a commercial Lorena cultivar plot in a productive stage, in which 25 trees were selected, and five fungicide and insecticide application treatments were established (fungicides = F; insecticides = I; fungicides and insecticides = F + I; control without application = T; and farmer = A; Tab. 2). The experiments were established with a completely randomized design, with five repetitions (trees) per treatment.

The chemical treatments were applied biweekly during 2014. Each fungicide or insecticide application treatment consisted of two molecules or mixtures of products. The treatments were applied using a Maruyama MS073D motor sprayer; on average, 2 L of the solution were sprayed per tree. Silwet® L-77 Ag adjuvant was used at a dose of 0.25 mL L⁻¹ in all applications.

The leaf severity was rated biweekly by monitoring two terminal branches from each cardinal point in the lower stratum of the trees. Each selected branch was divided and marked with a ribbon in two sections: from the internode where mature leaves and new (growing) leaves were differentiated. In each section, four leaves were evaluated, selected from each side of the ribbon. Each leaf was rated according to the LDS designed for that purpose.

The leaf severity was calculated according to the number of leaves counted for each scale class (0 to 4),

Table 1. Experiment field trials where the effect of chemical fungicides and insecticides in the management of black spot (*Pseudocercospora purpurea*) in 'Lorena' avocados was evaluated during semester B of 2014.

Municipality	Locality	Altitude (m a.s.l.)	Coordinates	ARH (%)	AT (°C)	BS severity degree	LDS class in leaves*
Mariquita	El Encanto	370	N 05°13'33.4" W 74°49'47.6"	83.684	26.397	Low	0 and 1
Fresno	El Ancla	1,150	N 05°11'23.6" W 75°00'07.7"	85.899	21.956	Medium	1 and 2
Fresno	Parcela 12	1,283	N 05°10'15.8" W 75°01'36.4"	90.297	20.601	High	3 and 4

*ARH: average relative humidity. **AT: average temperature. ***LDS: logarithmic diagrammatic scale to measure black spot severity in leaves.

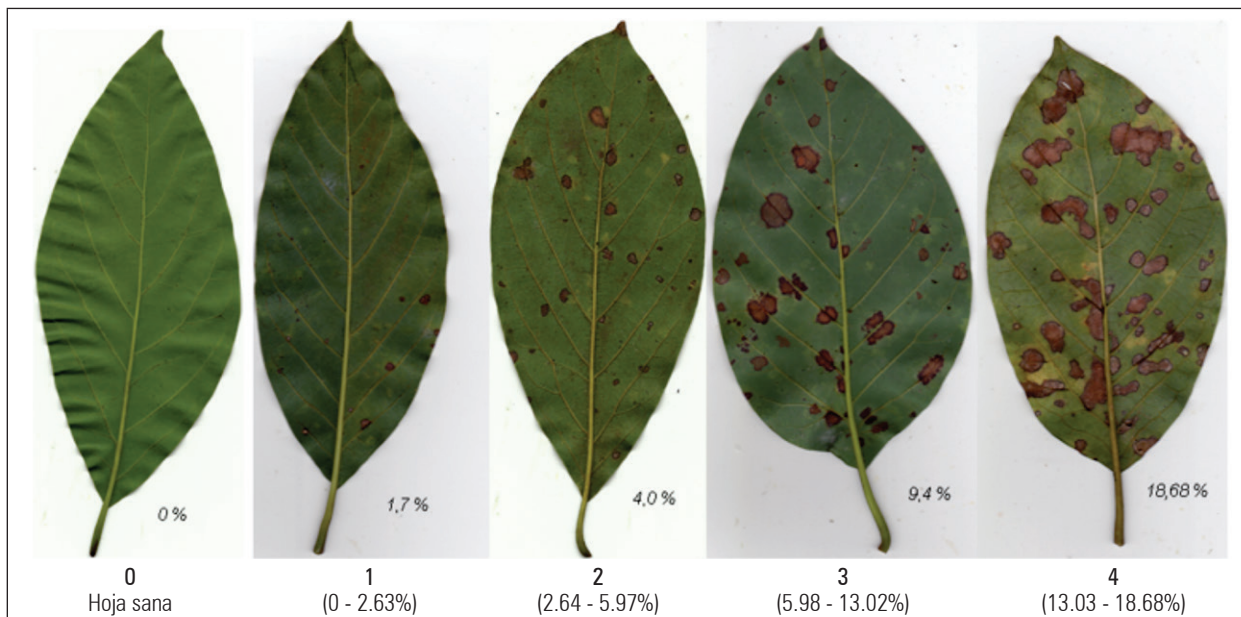


Figure 1. Logarithmic diagrammatic scale (LDS) to estimate black spot severity levels in 'Lorena' cultivar avocado leaves, defined using QUANT v.1.0.1 (Vale et al., 2001), with class limits established with DOSLOG (Osada-Velázquez et al., 1997). LDS with severity values in the range of 0-18.68%, where 0 = 0%, 1 = 1.7%, 2 = 4.0%, 3 = 9.4%, and 4 = 18.68%.

chosen based on the percentage of damage for each class; this result was divided by the number of leaves evaluated. The value of the percentages of each class was added to obtain the severity percentage of the branch section. The BS severity in the fruits was established using the diagrammatic scale (DS) adapted from Lonsdale (1991), with severity values in the range of 0–20%, where 0 = 0%, 1 = 5%, 2 = 10%, and 3 = 20%, in 10 fruits per repetition (tree) that were collected in a ripening state and stored separately in plastic boxes under normal environmental conditions (average temperature 21°C and average relative humidity 85%) for one week.

Preliminary validation of chemical and cultural management practices for BS control

During 2015, two experiments were established in commercial Lorena cultivar lots located in the

municipality of Fresno (Tab. 3). In each commercial lot, three experiment plots with approximately 20 trees each were identified: i) the integrated management plot (IM), ii) the conventional producer management plot (CPM), and iii) the unmanaged or control plot (UCM). In the IM, formative pruning and biweekly applications of the fungicides selected in the previous experiment (i.e., azoxystrobin, difenoconazole, and copper oxychloride) in a rotating scheme were carried out. In the CPM, the fieldwork considered necessary by producers for controlling BS was implemented. In the unmanaged plot (UCM), no intervention was carried out.

The experimental trial was established with a completely randomized design with five repetitions (trees) per treatment that were in the center of each plot. The severity in the leaves and fruits was recorded in the same manner as with the fungicide and insecticide evaluation bioassay. In each observation,

Table 3. Location of the field experiments for the preliminary validation of chemical and cultural management practices for black spot (*Pseudocercospora purpurea*) control during 2015.

Municipality	Farm	Coordinates	Altitude (m a.s.l.)
Fresno	La Ceibita	05°09'23.4" N and 74°59'58.3" W	1,235
Fresno	El Ancla	05°11'23.6" N and 75°00'07.7" W	1,150

the diameter of five fruits was measured at random, representing the average development status.

Establishment of the highest fruit BS susceptibility period

An experiment field trial was established in two commercial lots (La Ceibita and El Ancla) with the Lorena variety in the productive stage to identify the fruit stage with the highest vulnerability to *P. purpurea* (Tab. 3) infections. Five treatments with various applications of the fungicide azoxystrobin were established at different fruit development times (Tab. 4). Azoxystrobin was applied biweekly in doses of 0.2 g L⁻¹. The BS severity was determined in 50 ripe fruits collected per treatment, using the DS adapted from Lonsdale (1991). The trial was established with a completely randomized block design with three repetitions; the experiment unit was one tree, where 10 branches with set fruits and those in early development stages were marked.

Selection of the active ingredients for BS control

Starting in the second half of 2016, the efficiency of different active ingredients (AI) for controlling BS was evaluated in two commercial lots with 20 Lorena variety trees in Fresno, Tolima (El Ancla and La

Ceibita; Tab. 3). Four treatments were established (Tab. 5): QoI + DMI = rotation of the two a.i. pre-selected in the initial tests, DMI + QoI + DMI = rotation of a commercial product with two a.i., and a product with another a.i. (this treatment included last-generation fungal molecules); inorganic fungicides = low toxicity inorganic fungicides, an ecological or environmental option for controlling BS, and control = without application.

The application of fungicides began in October when the fruits had an average development of one month. In total, seven applications were made; the products were applied twice a week. In January of 2017, fruits from six trees located in the center of each trial were harvested. A sample of 100 fruits per treatment was used to calculate the BS incidence; the incidence was estimated as the percentage of fruits with symptoms / total fruits per treatment x 100.

Technical, economic, and environmental evaluation of the implemented management practices

During the first production cycle of the crop in 2017, three trials were carried out on commercial lots of 'Lorena' avocados in the municipality of Fresno (Vereda Palenque – Santo Tomás farm; Vereda La Sierra – El

Table 4. Description of the treatments used with azoxystrobin to establish the highest fruit susceptibility period for black spot (*Pseudocercospora purpurea*) infection during the first semester of 2016.

Treatment	Description of the fungicide application *	Application periods (2016)	Number of applications
1MAF	Applications from flowering to 1 month after flowering (AF)	February 15 to March 15	3
2MAF	Applications from flowering to 2 months AF	February 15 to April 15	5
3MAF	Applications from flowering to 3 months AF	February 15 to May 15	7
4MAF	Applications from flowering to 4 months AF	February 15 to June 15	9
Control	Controls (without applications)	-	0

*50% Azoxystrobin was applied in doses of 0.2 g L⁻¹ on a biweekly application schedule.

Table 5. Active ingredients (a.i.), dose, and number of applications per treatment used in the a.i. selection test for controlling black spot (*Pseudocercospora purpurea*) in 'Lorena' avocados during the second semester of 2016.

Treatment **	Active ingredients*	Dose of a.i./application	Applications
QoI (metoxi-acrilato) + DMI (triazol)	Azoxystrobin + Diphenconazole	0.1 g L ⁻¹ + 0.25 g L ⁻¹	4 + 3
DMI (triazol), QoI (oximino-acetatos) + DMI (triazol)	Tebuconazole, Trifloxystrobin + Triadimenol	0.2 g L ⁻¹ , 0.1 g L ⁻¹ + 0.25 g L ⁻¹	4 + 3
Inorganic fungicides	Sulphocalcic broth + Bordeaux + Copper oxychloride	100 cm ³ L ⁻¹ + 500 cm ³ L ⁻¹ + 1.2 g L ⁻¹	2 + 2 + 3
Control	Control (without applications)	-	0

*Biweekly application frequency. **Fungicides: QoI, quinone outside inhibitors; DMI, demethylase inhibitor.

Palmito farm; Vereda La Sierra – Las Mirlas farm) to identify the technical, economic, and environmental differences between the management proposed by Agrosavia (MA) and the traditional management carried out by the producer (MP) for controlling BS in avocados. Each trial had a completely randomized design with 10 repetitions (trees) in their productive stage.

In the experiment MP lot, the applications that the producer considered necessary were made, and, in the MA lot, a rotation with AIs was used with an application frequency of 21 d (Tab. 6). A Maruyama MS073D sprayer was used; on average, 2 L of the agrochemical solution and water were sprayed per tree. Furthermore, Silwet® L-77 Ag adjuvant was used at a dose of 0.25 mL L⁻¹ in all applications.

The number of applications, products, and dose were recorded to perform a cost analysis of each management treatment. The traceability of the applied agrochemicals was established in a sample of five ripe fruits per treatment with gas chromatography coupled to a tandem mass spectrometer (scanning 169 molecules), with liquid chromatography coupled to a tandem mass spectrometer (scanning 190 molecules), and with ultraviolet spectrophotometry; these analyses (CS2_01_B – low-level dithiocarbamates – UV; GMS_02_B - GC-MSMS; and LMS_02_B - LC-MSMS) were carried out in the external laboratory of PRIMORIS – Colombia, under the authority and accreditation (BELAC 057-TEST/ISO 17025) of PRIMORIS Holding, Technologiepark 2/3, B-9052 Zwijnaarde, Ghent, Belgium.

The incidence, severity, and quality variables of the fruits were estimated in 50 fruits per tree. The incidence was estimated as a percentage (number of fruits with symptoms / total number of fruits per

treatment × 100). The severity was established in ripe fruits according to the DS adapted from Lonsdale (1991), and the fruit quality index was calculated based on the commercial quality categories of avocados in the region. The severity values up to 5% were first-quality fruits, values from 5.1 to 10% corresponded to second quality, and values from 10.1 to 20% were third-quality fruits. In the local market, for first-quality fruits, 100% of the price/kg was stipulated on the date paid, along with 50% for second-quality fruits, and 25% for third-quality fruits. With this in mind, the percentage of first-quality fruits produced and the production value of each treatment were estimated.

RESULTS

Chemical fungicide evaluation bioassay

El Encanto (Low severity degree). The severity values in growing leaves and growing fruits were not adjusted to the normal distribution; therefore, a non-parametric ANOVA test (NPAR1WAY) was performed, which established a statistical difference ($P < 0.0038$) between the treatments evaluated for severity in growing leaves. The F + I treatment had the lowest value, with $0.2085 \pm 0.045\%$, and the remaining treatments showed a similar behavior (Fig. 2A). The growing fruit severity did not have statistical differences between the treatments ($P = 0.6410$; Fig. 2B). The severity values in the harvested fruits were lower than 5%.

El Ancla (Medium severity degree). The severity values in the growing leaves and growing fruits (approximately 4 to 6 cm in diameter) were not adjusted to the normal distribution; therefore, a

Table 6. Rotation order of the active ingredients (a.i.) used for the control of black spot (*Pseudocercospora purpurea*) in avocado. The rotation of a.i. started from flowering to harvest in the first half of 2017.

Application order	Fungicide *
1	Difenoconazole (0.18 g L ⁻¹)
2	Tebuconazole (0.20 g L ⁻¹), Trifloxystrobin (0.10 g L ⁻¹)
3	Triadimenol (0.25 g L ⁻¹) + Copper oxychloride (0.59 g L ⁻¹)
4	Tebuconazole (0.20 g L ⁻¹), Trifloxystrobin (0.10 g L ⁻¹)
5	Azoxystrobin (0.1 g L ⁻¹) + Copper oxychloride (2.35 g L ⁻¹)
6	Tebuconazole (0.20 g L ⁻¹), Trifloxystrobin (0.10 g L ⁻¹)
7	Difenoconazole (0.18 g L ⁻¹)

* A rotation cycle of seven applications in the order established in the table was performed.

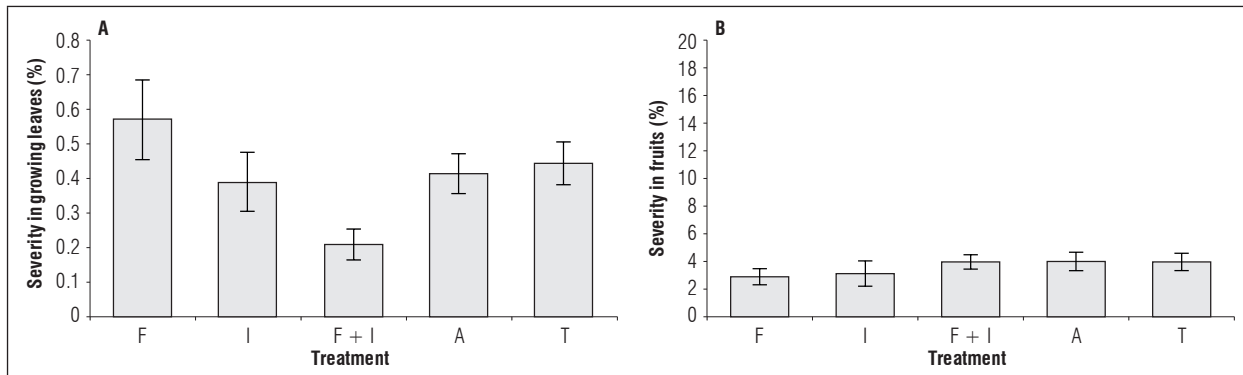


Figure 2. Severity of black spot (*Pseudocercospora purpurea*) in growing leaves (a) and growing fruits (b) of 'Lorena' avocados according to the logarithmic diagrammatic scale designed for leaves and the diagrammatic scale designed for fruits, adapted from Lonsdale (1991), during week 42 of 2014 in El Encanto (Mariquita). F = fungicide, I = insecticide, F + I = fungicide + insecticide, A = farmer, and T = control. The vertical bars reflect the standard error of the mean in growing leaves ($n = 10$), and fruits ($n = 50$).

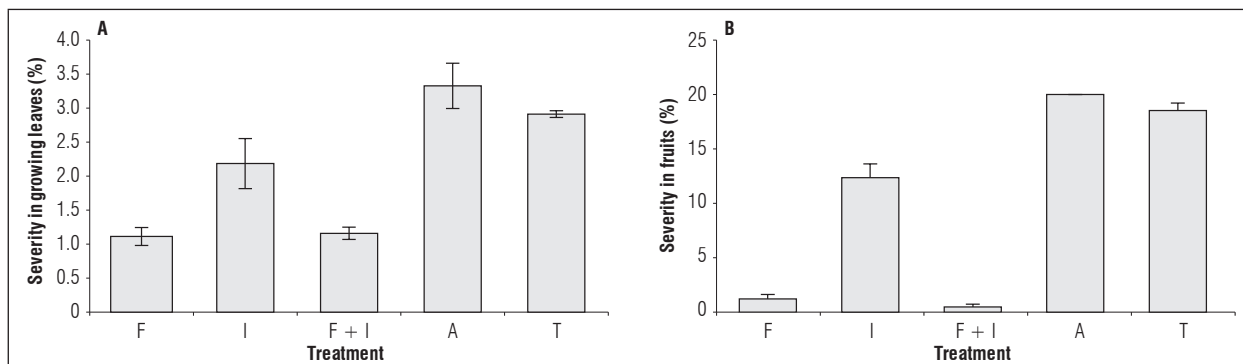


Figure 3. Severity of black spot (*Pseudocercospora purpurea*) in growing leaves (A) and growing fruits (B) of 'Lorena' avocados according to the logarithmic diagrammatic scale designed for leaves and the diagrammatic scale designed for fruits, adapted from Lonsdale (1991), during week 51 of 2014 in El Ancla (Fresno). F = fungicide, I = insecticide, F + I = fungicide + insecticide, A = farmer, and T = control. The vertical bars reflect the standard error of the mean in growing leaves ($n = 10$) and fruits ($n = 50$).

NPAR1WAY was performed, establishing highly significant differences ($P < 0.001$) between the evaluated treatments (Fig. 3A). Treatments F + I and F showed the lowest values, $1.138 \pm 0.089\%$ and $1.092 \pm 0.130\%$, respectively (Fig. 3A). For the severity in growing fruits, highly significant differences ($P < 0.0001$) were found between the treatments (Fig. 3B). The lowest severity values corresponded to treatments F + I and F, with values close to 1%, i.e., healthy fruits. Treatments A and T exhibited the highest values, close to 20%.

Parcela 12 [Plot 12] (High severity degree): The severity values in the growing leaves were adjusted to the normal distribution. The ANOVA analysis

established significant differences ($P < 0.0001$) between the evaluated treatments (Fig. 4A). The BS severity in the growing leaves exhibited the lowest and statistically equal values for the F + I and F treatments, 3.17 ± 0.67 and 2.72 ± 0.22 , respectively; the highest values corresponded to treatments A and T, 9.6 ± 0.98 and 8.08 ± 0.61 , respectively. The data for growing fruits did not show a normal distribution. The NPAR1WAY determined significant differences ($P < 0.0001$) between the treatments (Fig. 4B). The BS severity in the growing fruits exhibited the lowest and statistically equal values for the F + I and F treatments, with values close to 5%; the highest values corresponded to treatments A and T, with values close to 20%.

Preliminary validation of chemical and cultural management practices for BS control

La Ceibita. At the end of the observations, the lowest severity values in the growing leaves were found in the IM treatment, $1.714 \pm 0.108\%$, followed by UCM and CPM, with values of $2.860 \pm 0.304\%$ and $6.132 \pm 0.890\%$, respectively (Fig. 5A). The final observation in the growing fruits when they had an average diameter of 78.7 mm showed the lowest severity values for the IM treatment, 6.35%, followed by UCM and CPM, with 12.0 and 18.0%, respectively (Fig. 5B).

El Ancla. At the end of the observations, the lowest severity values in new leaves were found in the IM treatment, $1.692 \pm 0.151\%$, followed by UCM and CPM, with values of $2.633 \pm 0.303\%$ and $3.204 \pm 0.483\%$, respectively (Fig. 6A). The final observation in the growing fruits when they had an average diameter of 75.9 mm indicated that the lowest severity values were found in the IM treatment, 7.8%, with the same value in UCM and followed by CPM, 19.86% (Fig. 6B).

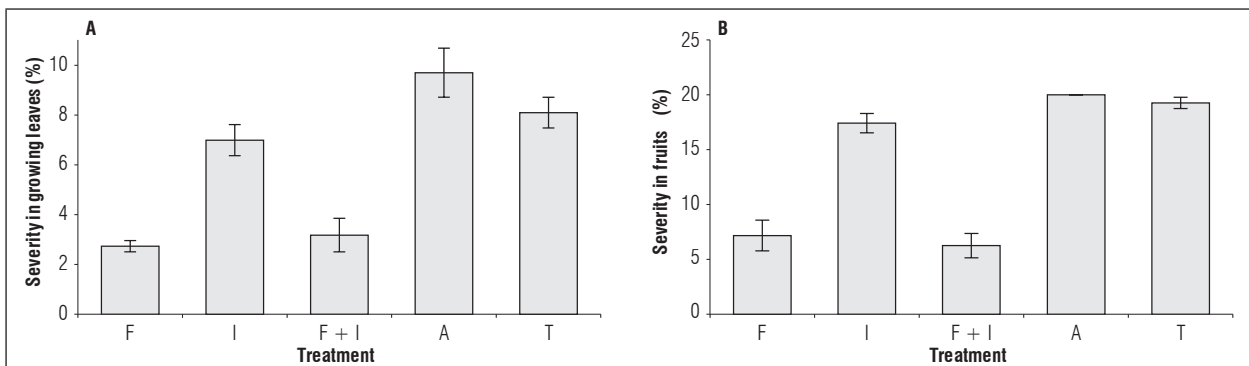


Figure 4. Severity of black spot (*Pseudocercospora purpurea*) in growing leaves (A) and growing fruits (B) of 'Lorena' avocados according to the logarithmic diagrammatic scale designed for leaves and the diagrammatic scale designed for fruits, adapted from Lonsdale (1991), during week 52 of 2014 in Parcela [Plot] 12 (Fresno). F = fungicide, I = insecticide, F + I = fungicide + insecticide, A = farmer, and T = control. The vertical bars reflect the standard error of the mean in growing leaves ($n = 10$) and fruits ($n = 50$).

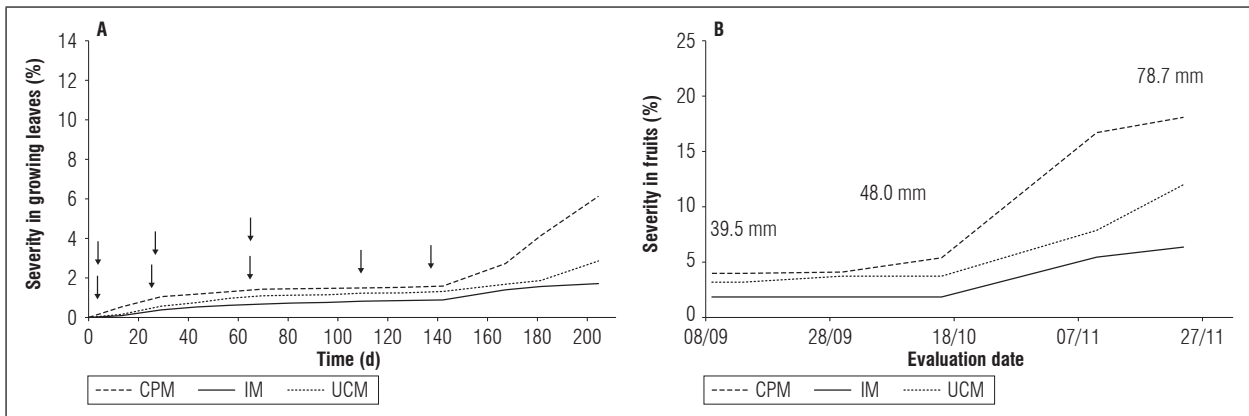


Figure 5. Severity of black spot (*Pseudocercospora purpurea*) in growing leaves (A) and growing fruits (B) of 'Lorena' avocados according to the logarithmic diagrammatic scale designed for leaves and the diagrammatic scale designed for fruits, adapted from Lonsdale (1991), during weeks 24 to 46 of 2015 on the La Ceibita farm. CPM: conventional producer management plot, IM: integrated management plot, and UCM: unmanaged or control plot. Each point represents the mean in growing leaves ($n = 30$) and in growing fruits ($n = 50$). CPM = 3 and IM = 5 fungicide applications. The solid arrows indicate the applications of fungicides for controlling BS in the IM; the dotted arrows indicate the applications of fungicides for controlling black spot in the CPM.

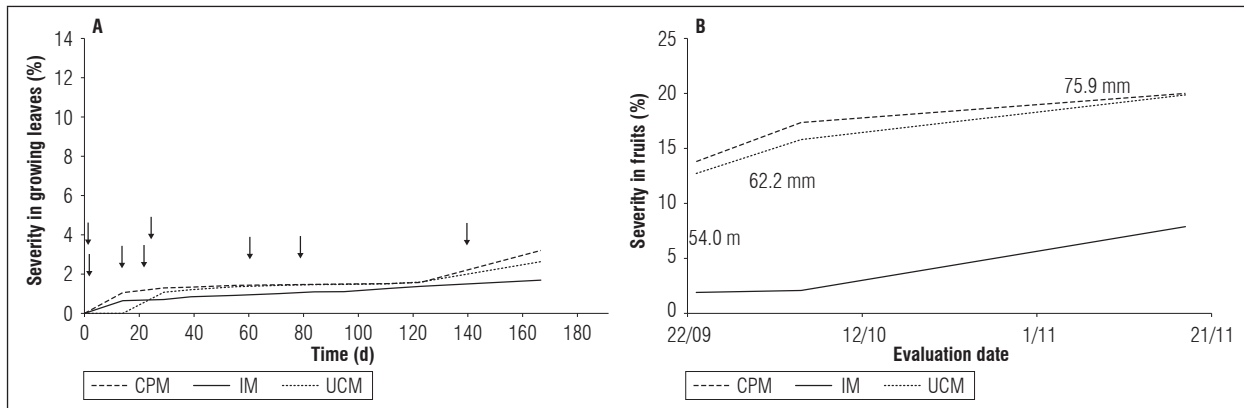


Figure 6. Severity of black spot (*Pseudocercospora purpurea*) in growing leaves (A) and growing fruits (B) of 'Lorena' avocados according to the logarithmic diagrammatic scale designed for leaves and the diagrammatic scale designed for fruits adapted from Lonsdale (1991), during weeks 26 to 46 of 2015 on the El Ancla farm. CPM: conventional producer management plot, IM: integrated management plot, and UCM: unmanaged or control plot. Each point represents the average in growing leaves ($n = 30$) and fruits ($n = 50$). CPM = 2 and IM = 6 fungicide applications. Solid arrows indicate the applications of fungicides for BS control in the IM, and the dotted arrows indicate the applications of fungicides for black spot control in the CPM.

Establishment of the highest BS fruit susceptibility period

The incidence and severity values in ripe fruits did not show significant differences between the farms ($P \geq 0.06$). The lowest final incidence value in the ripe fruits was 38.416 ± 9.334 for 4MAF and was statistically different from the control treatment, which had the highest value of 100. Moreover, the control

had a significantly similar behavior to that of 1MAF (92.663 ± 2.835 ; Fig. 7A).

The lowest final fruit severity values were statistically similar in 4MAF (3.933 ± 1.486) and 4MAF (4.9 ± 1.34), with values lower than 5%. Treatments 4MAF (12.40 ± 1.51) and 4MAF (8.10 ± 1.773) showed values higher than 5% (Fig. 7B).

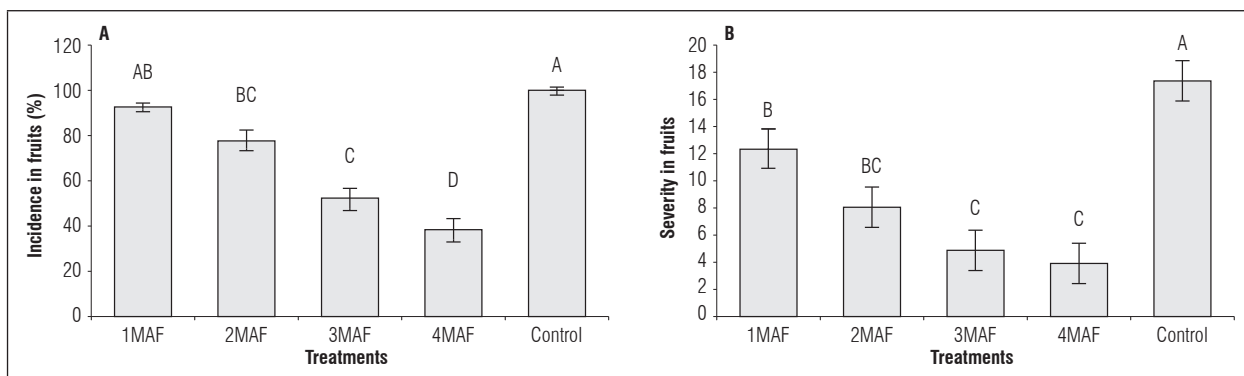


Figure 7. Incidence of black spot (*Pseudocercospora purpurea*) (A) and its severity (B) in ripe 'Lorena' avocado fruits according to the diagrammatic scale for fruits adapted from Lonsdale (1991), during weeks 27 to 48 of 2016 on the La Ceibita and El Ancla farms. 1MAF: flowering to one month after flowering (AF), 2MAF: flowering to two months AF, 3MAF: flowering to three months AF, 4MAF: flowering to four months AF, and Control: without applications. The bars indicate the standard error ($n = 50$). Different letters indicate significant differences, according to Tukey's test ($P \leq 0.05$).

Selection of the active ingredients for BS control

There were significant statistical differences between the treatments; the lowest incidence in the ripe avocado fruits was in treatment DMI + QoI + DMI, $43.122 \pm 4.775\%$, which was reduced by more than 50% compared to the control (without application), $89.773 \pm 8.549\%$. The inorganic fungicides and the control treatment were statistically equal (Fig. 8).

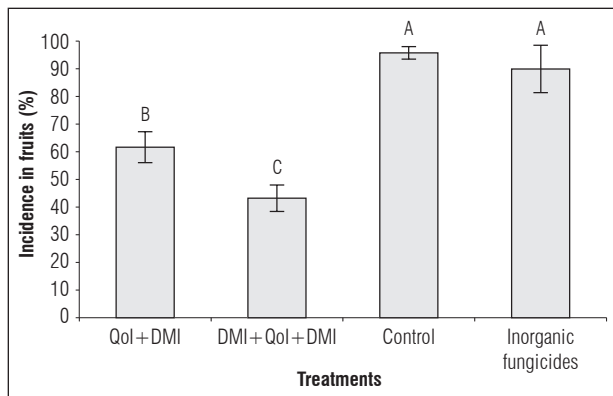


Figure 8. Incidence of black spot (*Pseudocercospora purpurea*) in ripe avocado fruits during the second semester of 2016. QoI+DMI: azoxystrobin + difenoconazole, DMI+QoI+DMI: tebuconazole, trifloxystrobin + triadimenol, Control: without application and Inorganic fungicides: sulphocalcic broth + bordeaux + copper oxychloride. The bars indicate the standard error ($n = 10$). Different letters indicate significant differences, according to Tukey's test ($P < 0.05$).

Technical, economic and environmental evaluation of BS management

The BS incidence and severity in the ripe fruits showed significant statistical differences ($P < 0.05$) between the treatments (Fig. 9). The lowest incidence was observed in the MA, with 10.22% less BS than in the CPM. The BS severity in the ripe fruits was significantly lower in the MA, 13.34% less than in the CPM.

The amount invested in each treatment was calculated in Colombian pesos (COP) (Tab. 8). The average BS control cost (COP\$1,369.23) was 60.7% lower than the cost for the MA (COP\$3,484.42). The average production cost per semester, regardless of the BS control, was COP\$18,243.61/tree.

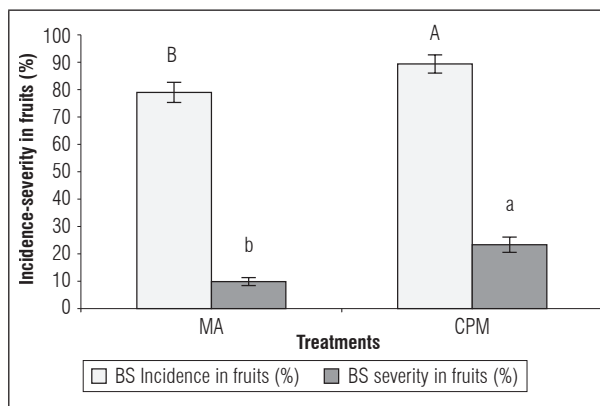


Figure 9. Incidence and severity of black spot (*Pseudocercospora purpurea*) in ripe avocado fruits during 2017. MA: management carried out by Agrosavia, CPM: conventional producer management. The bars indicate the standard error ($n = 10$). Different letters indicate significant differences, according to Tukey's test ($P < 0.05$).

For an economic analysis, it is necessary to know the income that the producer received when selling the product and to compare that amount with costs and expenses to establish profitability. When weighting the quality index, the price per kilogram of fruit the producer sells can be established according to the average fruit quality. For the economic analysis, an approximate yield of 40 kg per tree was used for the Lorena cultivar (Bernal *et al.*, 2013). The average quality index (as a percentage) was calculated for each management treatment (Fig. 10).

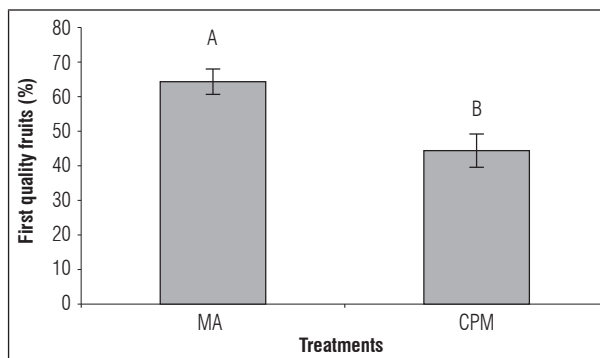


Figure 10. Quality index for black spot (*Pseudocercospora purpurea*) in harvested 'Lorena' avocado fruits during 2017. MA: management carried out by Agrosavia, CPM: conventional producer management. The bars indicate the standard error ($n = 10$). Different letters indicate significant differences, according to Fisher's test ($P < 0.05$).

Table 7. Summary of fungicide applications and their respective costs (in thousands of COP) for each black spot (*Pseudocercospora purpurea*) management test plot.

Farm (management)	MA*	Las Mirlas (CPM)**	El Palmito (CPM)	Santo Tomas (CPM)
Number of applications	7	6	1	4
Commercial products (amount/tree)	1) Diphenconazole (2.1 cm ³) 2) Tebuconazole, trifloxystrobin (3 cm ³) 3) Bayfidan (2.5 cm ³) and Cu oxychloride (2.5 g) 4) Tebuconazole, trifloxystrobin (3 cm ³) 5) Azoxystrobin (0.4 g) and Cu oxychloride (8 g) 6) Tebuconazole, trifloxystrobin (2 cm ³) 7) Diphenconazole (1.5 cm ³)	1) Tebuconazole, trifloxystrobin (2 cm ³) 2) Mancozeb M45 (3.4 g) 3) Metalaxil (2.4 g) 4) Cu oxychloride (8 g) 5) Cu oxychloride (8 g) 6) Cu oxychloride (4 g) and Dithane (4 g)	1) Benomyl (4 g)	1) Tebuconazole, Trifloxystrobin (3 cm ³) 2) Tebuconazole, Trifloxystrobin (2 cm ³) 3) Mancozeb (8 g) 4) Mancozeb (4 g) and Febuconazole (2.5 cm ³)
Black spot management cost per tree and semester (in COP)	\$3,484.42	\$1,913.10	\$390.67	\$1,803.92
Average black spot management cost per tree and semester (in COP)	\$3,484.42	\$1,369.23		

*MA: management carried out by Agrosavia, **CPM: conventional producer management.

The first-quality fruit index obtained in the MA was 65.17 ± 3.72 and was $44.97 \pm 4.88\%$ in the CPM. With the value of the first-quality fruit index, the income of the producer and the benefit/cost (B/C) ratio of each management treatment were established. According to the B/C ratio, the profitability of the MA was 23.75% higher than the CPM. This was due to the sale of better-quality fruits, even with higher production costs (Tab. 8).

The traceability results of the applied agrochemicals did not yield traces higher than or equal to the limits established for each molecule. This result indicated that the MA and the CPM had the same environmental impact (Tab. 9).

Table 8. Income for the management carried out by Agrosavia (MA), and the conventional management carried out by the producers (CPM), estimated according to the quality of the fruit and the benefit/cost ratio of each management treatment during 2017.

Estimated price for first-quality fruit/kg	\$2,000	
Quality index (%)	*CPM = 44.97	MA = 65.17
Price/kg	\$899.4	\$1,303.4
Income/tree	\$35.976	\$52,136
Production costs/tree	\$19,612.84	\$21,728.03
B/C ratio	1.83	2.40

Table 9. Traceability analysis results of pesticides for each black spot (*Pseudocercospora purpurea*) management test plots.

Treatment	Substances analyzed ***	Communication limit (CL) ****
MA*	CS2_01_B - Low level dithiocarbamates - UV	Without compounds \geq CL
	GMS_02_B - GC-MSMS	Without compounds \geq CL
	LMS_02_B - LC-MSMS	Without compounds \geq CL
Las Mirlas (CPM)**	CS2_01_B - Low level dithiocarbamates - UV	Without compounds \geq CL
	GMS_02_B - GC-MSMS	Without compounds \geq CL
	LMS_02_B - LC-MSMS	Without compounds \geq CL
El Palmito (CPM)	CS2_01_B - Low level dithiocarbamates - UV	Without compounds \geq CL
	GMS_02_B - GC-MSMS	Without compounds \geq CL
	LMS_02_B - LC-MSMS	Without compounds \geq CL
Santo Tomás (CPM)	CS2_01_B - Low level dithiocarbamates - UV	Without compounds \geq CL
	GMS_02_B - GC-MSMS	Without compounds \geq CL
	LMS_02_B - LC-MSMS	Without compounds \geq CL

*MA: management carried out by Agrosavia, **CPM: conventional producer management.

***Authority and accreditation (BELAC 057-TEST/ISO 17025) of PRIMORIS Holding, Technologiepark 2/3, B-9052 Zwijnaarde, Ghent, Belgium.

****Communication limits of all compounds by method are available in the customer section of www.primoris-lab.com

DISCUSSION

The results indicated that the application of fungicides reduced the severity of BS in growing leaves and fruits, contrary to the hypothesis of the producers in the area that an arthropod was spreading BS. The insecticide treatment had the highest values and was statistically similar to the farmer and control treatments. The initial severity degree in the growing leaves (low, medium, and high) contributed directly to the final damage degree observed in the harvested fruits (Fig. 2, 3, and 4). These results are consistent with those reported by Pohronezny *et al.* (1994), who pointed out that most of the BS inoculum comes from infected leaves that remain on the tree until they are completely senescent.

In Parcela 12 [plot 12], which had a higher degree of initial severity (3 and 4 LDS), a higher fungicide effect was observed, with a severity reduction of 67 and 75% in the growing leaves and fruits, respectively, as compared to the farmer treatment. The application of fungicides maintained the severity value in the fruits at a maximum of 5%, classified according to the local market as top quality. The fruits from the farmer treatment had a value close to or higher than 10%, cataloged as second-quality fruits (Fig. 4A and B).

The pruning, carried out after the harvest in combination with the biweekly, rotating applications of fungicides (IM), decreased the BS severity in the growing leaves and fruits by 40%, as compared to the CPM. The IM treatment exhibited a fruit severity value of 5%, whereas the CPM treatment reached a value of 20%; this result indicated that the pruning practice reduced the initial inoculum, reducing the damage or staining of fruits in the next harvest. According to Vanderplank (1960), the balance between two opposite processes, infection and removal, establishes the amount of the disease in the field. New infections produce lesions that become infectious and later enable the appearance of new lesions. However, the infectious tissue decreases as the lesions ages and no longer forms spores. When the infection is much more intense, as compared to decreases in diseased tissue (aging or removal), the intensity of the disease will grow rapidly.

The highest fruit susceptibility period in the 'Lorena' avocado was two months after flowering until harvest (Fig. 7A and 7B), i.e. starting when the fruits had a diameter more than 4 cm, which in this producing

area generally corresponded to the month of April for the first harvest and October for the second harvest of the year. This result agrees with previous observations that cataloged very small fruits or those near maturity as almost immune, and fruits that measure between $\frac{1}{4}$ and $\frac{3}{4}$ of their final size as susceptible (Pohronezny *et al.*, 1994).

The biweekly rotation of fungicides (tebuconazole, trifloxystrobin, and triadimenol) reduced the incidence in the harvested fruits by 52%, as compared to the control treatment without application of fungicides (Fig. 8); furthermore, the rotation of the fungicides azoxystrobin and difenoconazole (selected during the first experiment) reduced the incidence of harvested fruits by 29.83%, as compared to the control treatment. A single spraying of azoxystrobin showed a lower BS control than with rotation ending with copper oxychloride (CuOCl); the latter decreased the percentage of staining, as compared to the control treatment; thus, it was considered the best treatment (Darvas, 1982).

According to Duvenhage (2002), a lower BS incidence was observed when applying azoxistrobin or chlorothalonil in combination with CuOCl, as well as a lower incidence of diseases and maturation disorders during the postharvest stage. This rotation allowed a 50% reduction of CuOCl, which is consistently applied at high volumes in commercial avocado crops in South Africa, thus reducing its accumulation in fruits and soil. Complementary to these results, a higher BS control was obtained with two applications of azoxistrobin (October and November), followed by two applications of CuOCl (December and January), with azoxistrobin as an alternative fungicide that could replace two applications of CuOCl (Willis and Duvenhage, 2003). Previous results have reported that, among triazoles, difenoconazole was better than propiconazole and nearly as good as CuOCl at controlling BS (Willis, 2005).

The sustained use of MBC fungicides (methyl benzimidazole carbamates), especially benzimidazoles, in the continuous control of BS in commercial crops, such as celery, peanuts, and sugar beets, has caused fungal resistance (Berger, 1973; Georgopoulos and Dovas, 1973; Clark *et al.*, 1974; Littrell, 1974; Budakov *et al.*, 2014).

The recent use of specific-site fungicides, such as QoI (external quinone inhibitors), in areas with particularly high pathogen pressure induced fungal resistance

in this group of fungicides in commercial sugar beet, soybean, and grape crops (Birla *et al.*, 2012; Kikuhara *et al.*, 2014; Price *et al.* 2015). Our research points to the use of DMI fungicides (demethylation inhibitors; as there are still no reports of resistance), fungicides with a mixture of active ingredients, and their rotation in joint applications with CuOCl reduce the risk of resistance induction in *P. purpurea*.

The MA reported a BS incidence in the fruits of less than 10%, as compared to that observed in the CPM, with a 23.75% return as the result of the increase in first-quality fruits. However, the use of DMI fungicides, even without resistance reports, with a mixture of two AIs (DMI and QoI) in interspersed applications, sometimes used jointly with CuOCl during the highest BS fruit susceptibility period, were aimed at reducing the risk of resistance induction in *P. purpurea*.

CONCLUSION

Fruit protection with the application of DMI fungicides without reports of resistance, fungicides with a mixture of active ingredients (DMI and QoI), and their rotation with CuOCl during the highest BS fruit susceptibility period (i.e., two months after flowering until harvest) decreased staining, increased the quality of the harvested fruits, and limited the appearance of resistance in *P. purpurea* to these fungicides.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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