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Mango anthracnose integrated management

Abstract

Mango anthracnose in mango in the tropical and subtropical regions of Mexico is a disease that induces serious economic losses, caused by *Colletotrichum gloesporioides*. The objective of this research was to determine the efficiency of integrated management for anthracnose control and to identify the critical moment for disease management. The work was carried out on cv. 'Ataúlfo', in Guerrero, Mexico, in three production cycles. The identification of the pathogen was confirmed and during a period of 11 months the abundance of spores in the canopy of the trees was examined. Three treatments were evaluated: integrated management with severe pruning (MIM-Pruning), integrated management without severe pruning (MIM) and Control treatment. The climate was conducive to the development of the disease (>80% incidence). The greatest increase in spores, with 65.9 to 84.1 spores/week, was observed during summer vegetative growth, in the phase of flowering and fruit set on the rachis, with its subsequent decrease in fruit growth. The MIM-Pruning and MIM treatment showed consistent results of less area under the disease curve progress (AUDCP), in foliage and flowering, causing a greater definitive fruit set per inflorescence and marketable fruits per tree with respect to the Control ($P \leq 0.05$). Field pathogen population management should begin with the spraying of fungicide products in the summer vegetative growth phase and before the full flowering stage. Pruning is a practice that should be included in anthracnose management.

Keywords: mango, anthracnose, integrated pest management

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Introduction

Mexico is the fifth mango producing country in the world and the second of an exporter with 20% (232,643 tons), after India with 24% (286,775 tons).[1] At the national level, the state of Guerrero contributes 19% (395,396 tons) of the national production, which places it as the second mango producer, after Sinaloa (409,572 tons), followed by Nayarit (304,819 tons), Chiapas (270,695 tons), Oaxaca (207,701 tons), Michoacán (170,286 tons) and Jalisco (110,913 tons).[2] In this value chain, damage caused by anthracnose is identified as a critical point in the production stage, the causal agent being *C. gloesporioides* (Penz.) Penz. and Sacc., which causes serious economic losses in the tropical and subtropical regions of Mexico and the world.[3-5] The fungus is reported as a pathogen that affects leaves, inflorescences and fruits in pre and post harvest. In the postharvest phase, anthracnose is reported to cause losses of 5 to 20%[6] and the use of synthetic chemical fungicides and treatment with hot water at 52±2 °C for 10 min have been suggested to minimize damage to mango fruits.[7,8] As well as the use of natural and biodegradable biofungicides, such as trans-cinnamaldehyde, citral and phenylacetaldehyde, for the control of anthracnose disease in mango after harvest.[9] Fitzell and Peak[10] determined that spores are the most important source of inoculum in Australia, which are produced on terminal branches, mummified inflorescences, floral bracts and leaves Under laboratory conditions spores are produced over a wide temperature range (10-30 °C) and relative humidity of 95-97%.

In mango orchards, the disease causes symptoms on leaves, branches, panicles and fruits. On leaves, the lesions are irregular, necrotic, frequently surrounded by chlorotic halos and not delimited by the veins. Lesions may coalesce and form large necrotic areas, especially along the margins. Under favorable conditions, salmon-colored to elongated fruiting bodies (acervuli) of the pathogen may form on lesions and expand and coalesce with time. In severe infections, the fungus can invade branches and cause dieback. In panicles, the flowers bear small black dots that gradually enlarge and coalesce to cause flower blight. The fruits can be affected at any stage of their development. Meanwhile, the severe infection of young fruits occurs as mummification. While the infections in larger fruits remain latent until the fruits ripen, and the disease is observed with the appearance of black, irregular and sunken lesions on the surface of the fruits.[11-15]

High relative humidity constitutes one of the primary factors in the spread and development of the disease.[8] The conidia produced in terminal branches, mummified inflorescences, floral bracts and leaves are the main source of inoculum.[10,11] and are produced abundantly where there are wet surfaces, although also at relative humidity greater than 90%. Conidia are dispersed via rainwater runoff onto other leaves and flowers, where they cause secondary infections.[16] In the case of postharvest symptoms, the developing fruits are infected in the field, but the infections remain quiescent until the onset of ripening, which occurs after harvest.[11,12,14] The appressoria become melanized and strengthened, facilitating infection in the cuticle through the penetrating hyphae. The presence and prevalence of melanized appressoria has been used to predict anthracnose infection and when control measures are needed. In general, the initial stages of infection (conidia germination and appressorium formation) of *Colletotrichum* in mango fruits are favored by temperatures ranging from 20 to 30°C and relative humidity (RH) > 95% for 12 h.[10,17]

Since most of the early infected fruits fell during physiological fall,[11] it was thought that the pathogen in mature fruit penetrated the fruit after physiological fall. However, Chang et al.,[18] determined, at the field level by inoculating *C. gloeosporioides* conidia, that the invasion of panicles in the full flowering stage resulted in 67.2% of panicles without setting any fruit and 61.1% of fallen fruits, with the highest latent infection rate in immature fruits, and the largest infection rate and infected area in mature fruits during the postharvest stage, thus indicating that the full bloom stage is the critical stage and suggesting the control of the pathogen before the full flowering stage. In an epidemiological study, Noriega et al.,[15] determined that the greatest increase in *Colletotrichum* sp. spores occurred in the phenological state of pre-flowering, flower emission, fruit set until harvest, with

dew point temperatures between 18.2 at 23.7 °C, which favored the condensation of water on leaves, flowers and fruits, favoring infection and dispersion by dragging spores and splashing. Therefore, the objective of the research was to determine the efficiency of integrated management for the control of the anthracnose of the 'Ataulfo' mango and to identify the critical moment for the management of the disease.

Material and methods

The work was carried out on cv. 'Ataúlfo', with a high incidence of anthracnose (>80%), in the municipality of Atoyac de Álvarez in the state of Guerrero, Mexico, during the 2019-2020, 2020-2021 and 2021-2022 cycles. The orchard was located at the geographic coordinates 17° 07' 48.39''N, 100° 26' 56.23''W, at 05 meters above sea level, with a warm sub-humid climate (Aw1).[19] A real framework planting system, with a spacing of 10 x 10 m and 19-year-old trees with a micro-sprinkler irrigation system.

Meteorology

The daily records of precipitation, temperature, relative humidity and dew point were made using a Vantage PRO2® weather station, Davis, USA, Hayward, CA, located at the level of the tree canopy, the sensors located at a height of 1.8 to 2.5 m from the floor. These records were organized from the resting state of the buds to the physiological maturity of the fruit. The range and mean values of the meteorological records recorded during the production stage for two cycles are listed in Table 1.

Table 1 Range and daily average of climatic variables 2020 and 2021. Atoyac de Álvarez, Gro., Mexico

Climatic variables	2020*		2021	
	Range	Accumulate**/ Average***	Range	Accumulate**/ Average***
Precipitation (mm)**	0.0 - 134.4	1,812.1	0.0 - 143.7	1,195.3
Maximum temperature (°C)***	24.8 - 38.0	34.2	23.8 - 39.3	32.4
Minimum temperature (°C)	15.2 - 27.5	21.7	12.3 - 27.4	21.2
Medium temperature (°C)	22.2 - 36.7	27.2	20.8 - 34.9	26.5
RH (%)	63.0 - 95.0	80.0	60.3 - 97.5	81.8
Dew point (°C)	15.3 - 31.0	23.2	17.3 - 31.1	22.0

*Period. February 05 to December 31, 2020; 01 January 01 to December 31, 2021.

Fungal isolation

From December 2019 to March 2020, five trees were randomly selected per hectare, taking into account size, age and uniform appearance, in which four branches were selected, oriented in each cardinal point, at a height of 1.0 to 1.8 m. On a monthly basis, an inflorescence with blight symptoms was sampled per branch, which were transferred to the Phytopathology Laboratory of the Guerrero Autonomous University, for the isolation of fungi present in the rachis and petals of the affected inflorescences. Isolations were made on Petri dishes containing papadextrose-agar (PDA) and subcultures were obtained from the margins of the growing colonies. Monoconidial cultures were made by the scratch technique on an agar plate. The morphometric identification of the obtained isolates was carried out with the keys of Ellis,[20] Barnett and Huntter[21] and Heuchert et al.[22]

Spore dispersal

The abundance of spores in the air, at the level of the tree canopy, was recorded over a period of 11 months using a Burckard-type volumetric trap with 7-day monitoring.[23] The spore trap was placed in the experimental plot with the control treatment, at a height of 2 m in the first third of the tree canopy. The spores were impacted on a cylindrical drum, covered by a transparent tape where they were deposited, the tape was cut into 39.5 mm sections corresponding to each 24-hour period and mounted on a slide. The spore count was performed in three transects at 400x magnification, calculating the average observed per day. When less than five spores were observed, another three transects were counted and the average was calculated; to report the concentration of spores accumulated in seven days. The trap was operated daily during vegetative development, flowering, setting, and fruit growth.

Orchards management

The treatments were: integrated management with severe pruning (MIM-Pruning), integrated management without severe pruning (MIM) and the Control. A MIM-Pruning treatment was applied to 50 trees, which included severe pruning in May 2019 and application of paclobutrazol 20 cm3/tree to the soil, after the first vegetative flow in June 2019; In the 2019-2020, 2020-2021 and 2021-2022 cycles, the following was applied: N, P, K fertilizer with the formula 120-60-60 divided into three applications; dolomite (Ca 53% and Mg 44%) 500 kg ha-1 applied in the first and third cycle; compost (bovine) 1,000 kg ha-1; foliar fertilizer, 2 L ha-1 of chelates (Mg 1.0%, S 4.0%, B 0.04%, Co 0.002%, Cu 0.04%, Fe 3.0%, Mn 3.0%, Mo 0.25%, Zn 0.005%); foliar induction in September with phosphonitrate, 04-31-00, with two applications every 8 days; disease management with eight fungicide applications. The MIM treatment in 50 trees also included paclobutrazol 20 cm3/tree in June 2019 and the same previous activities were applied in the 2019-2020, 2020-2021 and 2021-2022 cycles: fertilization, soil improvers and foliage spraying for diseases control. In the Control without pruning, five trees were selected, the activities included: soil application of paclobutrazol 20 cm3/tree in June 2019, incorporation of dolomite (Ca 53% and Mg 44%) 250 kg ha-1, compost (bovine) 500 kg ha^{-1} and pest control for the three study cycles. The trees with the treatments included: periodic irrigation management, every 15 days, from November-March, humidifying the dripping area at field capacity and for fruit fly control Ceratrap® with 25 traps per hectare.

Evaluated variables

For the study variables 1) severity of anthracnose in foliage, 2) severity of disease in flowering and 3) fruit set, five trees were selected per treatment based on size and uniform appearance. Severity in foliage was monitored every 15 days, from September to November, in four branches per tree, oriented by cardinal point, the penultimate vegetative flow of two leaves from the middle part were marked, with a total of eight leaves per tree per sampling. The scale used was the one proposed by Sundravadana et al.,[3] with a range from 0 to 5, on leaves 0=no spots (0% damage) and 5=more than 25 spots per leaf (>80% damage). The severity of inflorescences was monitored every eight days, from November to January, from the emergence of inflorescences, to the beginning of fruit set, in four branches per

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tree oriented by cardinal point, with a total of four panicles per tree per sampling. The scale used was the one proposed by Sundravadana et al.,[3] with a range from 0 to 9, in the inflorescence 0=0% damage (infection not observed) and 9=more than 50% of the inflorescence damaged. The setting and/or fruit set was monitored in the selected branches to monitor the severity of foliage and flowering. The evaluations of tied fruit set were of a fruit size of 6 to 7 cm in length. Yield was evaluated through two cuts, one per week in the period from February to March 2020, 2021 and 2022, counting the number and weight of fruits per tree.

The area under the disease progress curve (AUDPC) was calculated for each leaf and/or panicle using the Campbell & Madden[24] formula. AUDPC values were used in the analysis of variance to compare the amount of disease between trees with different treatments, with the formula:

$$AUDPC = \sum_{i=1}^{n-1} 0.5\left[\left(X_i + X_{i+1}\right)\left(t_{i+1} - t_i\right)\right]$$

Where Xi = the cumulative severity of disease expressed as a proportion at the i-th observation, ti = time to the i-th evaluation in days, n = the total number of observations.

Analysis of data

The statistical design was randomized blocks, with five replications per treatment. Data on severity of the disease, AUDPC, set and/or fruit set per panicle and yield were subjected to analysis of variance with the PROCGLM procedure: the comparison of means by Tukey's multiple test ($P<0.05$), using the SAS statistical analysis system.[25]

Results and discussion

Environmental conditions

During the mango production cycles, the environmental conditions were favorable for the anthracnose development in leaves and panicles (Table 1). The accumulated rainfall had values between 1,812.1 to 1,195.3 mm per year, which was above normal, with relative humidity between 60.3 to 97.5%, with a dew point between 15.3 and 31.1°C, conditions for the condensation of water on the organs of culture and facilitate the infection and spread of the pathogen. In addition, at average temperatures of 27.2°C, maximum of 34.2°C and minimum of 21.7°C favorable for both the crop and the pathogen. Warm, sunny conditions extended through spring, summer, and early fall provide optimal conditions for the growth of mango vegetative and floral flushes. The month of September is when the highest rainfall of the season occurs and it is when the producers of the Coast of Guerrero begin with the spraying of nitrates to advance flowering, which occurs during October to November.

Isolates

The fungi *Colletrotrichum* sp. and *Lasiodiplodia* sp. showed the highest frequency of isolations with 69 and 25 % in rachis respectively and 79 and 21 % in flowers for both fungi. On the other hand, *Cladosporium* sp. showed a low rachis isolation frequency (4%) and it was not isolated from the flowers. The average per panicle isolation was 74, 23 and 2 percent for *Colletrotrichum* sp, *Lasiodiplodia* sp. and *Cladosporium* sp. respectively (Table 2). The isolates were identified morphometrically, the *Colletotrichum* conidia were straight, cylindrical, with an obtuse apex and a truncated base; the average size was 13.2 x 4.2 µm, which correspond to the values indicated by Sutton[26] for *Colletrotrichum gloesporioides*. In the case of *Lasiodiplodia* conidia, the average size was 22.82 x 11.06 µm, located in the range indicated by Punithalingam[27] for *Lasiodiplodia theobromae*. *Cladosporium* conidia were measured using digital images from a scanning electron microscope (Jeol® model 5800 LV), measuring an average of 4.8 x 1.78 µm, with a variation of 1.5 - 1.6 x 0.28 - 0.62 µm. These values indicate a reduced variation, but they are 1.05 and 1.15 µm less than the spore size reported by Guillén-Sánchez et al.[28] for *Cladosporium tenuissimum*, who made measurements in a photomicroscope and therefore with different treatment of the study material, which explains the numerical differences recorded.

Table 2 Frequency of fungi in blighted inflorescences of 'Ataulfo' mango on the Coast of Guerrero, Mexico, 2019-2020

Fungi	Rachis isolation frequency (%)	Flower Isolation Frequency (%)	Panicle isolation frequency (%)
Colletotrichum sp.	69	79	74
Lasiodiplodia sp.	25	21	23
Cladosporium sp.	4	0	2

Spore dispersal

The fluctuation of spores of *Colletotrichum* sp. at the plant canopy level is shown in Figure 1, where its presence is observed throughout the cycle, from spring and summer vegetative growth, the flowering phase, the setting and growth of fruits, with a clear monthly variation. At the end of the summer vegetative growth stage and beginning of flower bud emergence, the highest populations are recorded with 65.9 to 84.1 spores/week from early to late August. Subsequently, when the first panicles sprout, full flowering occurs and the mooring begins and the physiological fall of fruits, spore populations between 47.9 and 60.6 spores/week from October to November were detected. During fruit growth, airborne spores decrease rapidly from 47.0 to 12.4 spores/week. These observations suggest an important and short-lived role of mango flowers in the anthracnose epidemic. In this regard, in a study of blueberries with *C. floriniae*, they found a strong bioactivity of flowers in the disease cycle, indicating that floral extracts, rich in sucrose, play an important role in the epidemiology of fruit rot, which decreases as the fruits develop.[29] The studies by Chang et al.,[18] in Taiwan on cv. Irwin concluded that the full bloom stage was the critical stage for control of *C. gloeosporioides* and suggest that control of the pathogen population at the field level be prior to the full bloom stage. The increases in the spore populations of *Colletotrichum* sp. during the summer vegetative growth phase, as well as the beginning of the emergence of the flowering phase, settling and fruit growth confirm that the concentration of the pathogen must be controlled in the field with the elimination of plant residues and spraying chemicals before the full bloom stage.

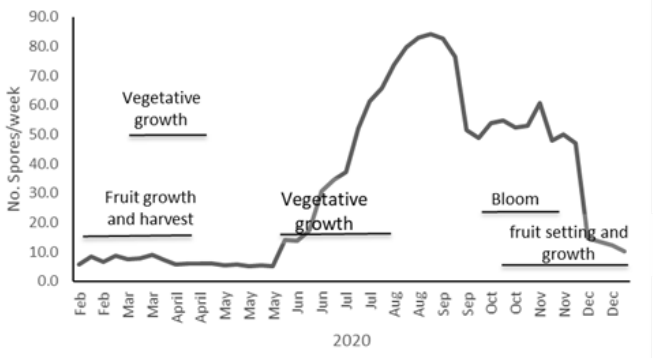

Figure 1 Capture of *Colletotrichum* sp. spores, from February to December 2020. Atoyac de A. Gro., Mexico.

Anthracnose in leaves, panicles and fruits

The progress of the severity of the disease in leaves, AUDPC, during 15 weeks in the 2019 and 2020 cycles and 09 weeks in 2021 is shown in Figure 2. In the first 2019 cycle, the MIM-Pruning treatment significantly reduced the severity of anthracnose compared to the MIM and Control treatments, respectively (P≤ 0.05; Figure 2). In 2020 and 2021, the MIM-Pruning and MIM treatments generally significantly reduced the severity of anthracnose, AUDCP, on the leaves compared to the Control treatment in both production cycles (P≤ 0.05; Figure 2). However, there were no statistically significant differences between these treatments.

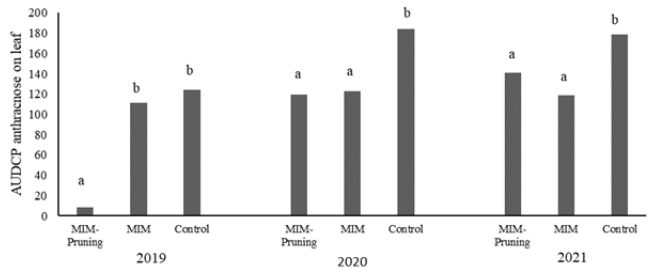

Figure 2 Value of the area under the disease progress curve (AUDPC) of anthracnose in mango leaves. Bars with the same letter are statistically equal according to Tukey's test (P ≤ 0.05).

When analyzing the effect of the MIM-Pruning and MIM treatments during the 2019 sampling, the severity was reduced by 93 and 10%, respectively, compared to the Control treatment without pruning and without chemical protection. In contrast, during 2020 both treatments reduced the severity of anthracnose by 35 and 34% compared to the Control. However, in 2021, the MIM-Pruning treatment decreased by only 21% and the MIM maintained a 34% reduction in the progress of the severity of the disease in leaves.

The progress of the disease severity in the panicles, AUDPC, during six weeks in the three production cycles is shown in Figure 3. In the first cycle 2019, the MIM-Pruning treatment significantly reduced the severity of anthracnose compared with the Control treatment, and there were no differences with the MIM treatment (P≤ 0.05; Figure 3). In the second cycle 2020 none of the treatments showed statistical differences. In contrast, in the third cycle, 2021, the MIM-Pruning and MIM treatments showed significant differences in the progress of anthracnose severity, AUDPC, compared to the Control treatment (P≤ 0.05; Figure 3). However, there were no statistically significant differences between the MIM-Pruning and MIM treatments.

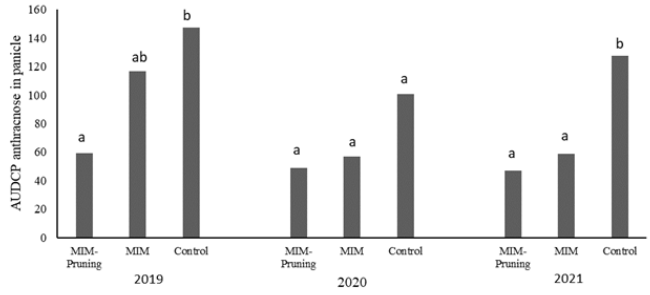

Figure 3 Value of the area under the disease progress curve (AUDPC) of anthracnose in mango panicles.

When considering the effect of the MIM-Pruning and MIM treatments during the sampling of the panicles in 2019, the severity was reduced by 60 and 21%, respectively, compared to the Control treatment without pruning and without chemical protection. In 2020, both treatments reduced the severity of anthracnose by 51 and 44% compared to the Control, however, no significant differences were detected. In contrast, in 2021, the MIM-Pruning treatment decreased the AUDPC of the panicles by 64% and the MIM maintained a 42% reduction in the progress of the severity of the disease in mango panicles.

Table 3 shows the results of the fruits tied and/or set by the rachis, approximately 08-09 cm in size, a month and a half before harvest for the three cycles. It is observed that the MIM-Pruning treatment with 7.8 and 6.4 fruits/rachis for the 2021 and 2022 cycles, respectively, showed significant statistical differences with the Witness treatment with 2.1 and 1.2 fruits/rachis in these same production cycles (P≤ 0.05). However, no statistical differences were detected between the MIM-Pruning and MIM treatments in any of the three cycles. These data indicate that with respect to fruit set up to the second cycle, significant differences were observed with greater efficiency of integrated management with pruning compared to the Control for the management of the anthracnose in vegetative growth and flowering, causing a greater definitive fruit set by inflorescence.

Table 3 Effect of integrated management treatments on the average number of rachis with fruit set in 'Ataulfo' mango

Treatments	Rachis with fruits (No.)					
	Cycle 2019		Cycle 2020		Cycle 2021	
Integrated management with pruning (MIM-Pruning)	7.6	a	7.8	a	6.4	a
Integrated management without pruning (MIM)	7.8	a	5.3	ab	5.4	a
Control	4.4	a	2.1	b	1.2	b

[1]Values with the same letter are statistically equal according to Tukey's test (P<0.05).

In the Control treatment, the invasion of the panicles resulted in 56, 27 and 19% of the panicles with fruit set for the 2019, 2020 and 2021 cycles, respectively. While the MIM-Pruning and MIM treatment, fruit set was above 68% of panicles.

Table 4 shows the yield results per tree of the three cycles. In the second cycle 2020-2021, it is observed that the MIM-Pruning treatment with 135.8 kg/tree and MIM 108.8 kg/tree showed statistical differences with the Control treatment with 58.3 kg/tree (P≤ 0.05); likewise, statistical differences were detected between the MIM-Pruning and MIM treatments in this cycle. In the third cycle 2021-2022, the MIM-Pruning treatment with 156.4 kg/tree and the MIM treatment with 148.1 kg/tree showed statistical differences with the Control treatment with 53.5 kg/tree (P≤ 0.05); however, no statistical differences were detected between the MIM-Pruning and MIM treatments.

Table 4 'Ataulfo' mango yield in three production cycles

Treatments	Yield per tree/kg					
	Cycle 2019		Cycle 2020		Cycle 2021	
Integrated management with pruning (MIM-Pruning)	128.2	a	135.8	a	156.4	a
Integrated management without pruning (MIM)	131.6	a	108.8	b	147.1	a
Control	74.2	a	58.3	c	53.5	b

[1]Values with the same letter are statistically equal according to Tukey's test (P<0.05).

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Anthracnose infection was higher in the Control treatment and there was no protection against diseases, which indicates that the applications of fungicides in the aerial part were effective in managing anthracnose in the field. The best results were achieved in the MIM-Pruning and MIM treatments, which substantially increased production per tree. However, since the results of these two treatments only differed in the second cycle, with an increase of 18 kg/tree in favor of MIM-Pruning, and in both the first and third cycles, no statistical differences were found between them. Severe pruning should be included in disease management programs for trees that have not received pruning. Asrey et al.,[30] indicate that an unpruned tree becomes very large, which inhibits the penetration of light within the canopy, the sprouting of new leaves decreases, the photosynthetic activity remains low and there is a high incidence of pests and diseases due to at high relative humidity.

In plantations where the environmental conditions favor the development of the disease, of up to 25 sprays have been reported,[31] which shows the importance of this study results for the management of the infection in the field, from the economic and environmental aspects by reducing the number of sprays in the management of anthracnose. On the other hand, the proposed anthracnose integrated management includes protection from the emergence of the first vegetative shoots of summer, in floral induction until harvest, which is proposed in this study, and justifies a plan control with eight applications. Likewise, the effect of the MIM-Pruning and MIM treatments on the retention of the fruits to the rachis and on the final production is specified.

Conclusion

Integrated management with and without severe pruning showed consistent results in controlling the severity of anthracnose in foliage and flowering, increasing the final fruit set per inflorescence and yield per tree. The management of the pathogen concentration in the field begins with chemical products spraying in the summer vegetative growth phase and before the full flowering stage. Pruning is a practice that should be included in disease management.

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Conflicts of interest

All the authors made significant contributions to the document, agree with its publication and state that there are no conflicts of interest in this regard.

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