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# Postharvest diseases and disorders in avocado cv. Hass and their relationship to preharvest management practices



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#### ABSTRACT

Postharvest diseases and disorders are two of the most important parameters associated with the quality of avocado fruit. The aim of this study was to identify postharvest diseases and disorders in Hass avocado plots and to evaluate their relationships with different preharvest agronomical practices. This work was developed in 20 commercial plots of Hass avocado dedicated to production for national and export markets. The first part of this work was associated with the identification and characterization of diseases and disorders related with post-harvest of avocado. In addition, it was determined the incidence of each disease and disorder based on simulation of postharvest scenarios for the national and export markets. Using a multinomial logistic regression model, it was possible to determine that the presence of each disease and disorder were related to crop management practices, soil and leaf and fruit nutrients levels. Most relevant postharvest disease and disorders were anthracnose, stem-end rot, chilling injury, and lenticel damage. Additionally, variables such as dry matter, plant pruning, and tissue concentration of Ca<sup>+2</sup> were related with some pathologies and disorders. This work presents an advance in the recognition of postharvest diseases and disorders in avocado under tropical conditions, besides determining the main edaphic and anthropogenic associated factors.

#### 1. Introduction

The avocado production system is developed in a large number of countries, however, crops for export are located mainly in the tropical and sub-tropical regions around the world (FAO, 2020). In recent years in Colombia, the cultivated area with avocado has been expanded very fast becoming one of the five most productive countries especially in commercial varieties for export such as Hass. Crop expansion has been driven mainly by an increased demand in national and international markets (Ramírez-Gil et al., 2018).

Quality of avocado fruits depends on aspects associated with preharvest and postharvest agronomical practices (Hernández et al., 2016; Pedreschi et al., 2016; Ramírez-Gil et al., 2019). Diseases and disorders are considered the most important factor for fruit damage at cosmetic, organoleptic and nutritional levels inducing a reduction in the perception of multifunctional quality (Ramírez-Gil et al., 2019). Diseases may be of biotic origin such as fruit rot usually associated with microbial pathogens, or abiotic origin such as those caused by physiological disorders (Burdon et al., 2013; Hernández et al., 2016; Ramírez-Gil et al., 2020; Sharma et al., 2017; White et al., 2009). Many of these problems are associated with excess of nitrogen in the last periods of fruit filling (Willingham et al., 2006), and imbalances that undermine Ca and Mg uptake (Everett et al., 2007). Additionally, good management of nutrients such as boron and zinc is recommended as it improves fruit quality (Crowley et al., 1996).

Storage conditions are critical to the onset of postharvest diseases and disorders. They include time length of postharvest handling and the temperature and gas composition as well as the degree of fruit maturity at harvest time (Burdon et al., 2013; Hernández et al., 2016; Pedreschi et al., 2016). Development of fruit diseases that are identified at post-harvest stages usually begin as preharvest quiescent processes. This condition poses a challenge for their management because disease presence is identified when fruits begin to soften or rot, symptoms that usually occur at the market stage when it is not possible to control the problem (Hartill and Everett, 2002; Sharma et al., 2017).

Most important postharvest disease of biotic origin of avocado fruits is anthracnose for which the best studied causal agent is the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. However, it is

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known that other *Colletotrichum* species difficult to identify at the taxonomic level may be involved, which may affect integrated disease management (Giblin et al., 2018; Hartill, 1991; Sharma et al., 2017; Weir et al., 2012). Anthracnose causes losses close to 80% in the absence of appropriate management measures, which include fungicide applications (Bosse et al., 2013). Another disease of economic importance is the stem-end rot complex, which is associated to a group of microorganisms being *Rhizopus stolonifer* (Ehrenb. Fr) Vuill, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (= *Diplodia natalensis* Pole-Evans), *C. gloeosporioides* and *Dothiorella gragaria* Sacc., the more frequently identified (Darvas and Kotze, 1979; Hartill and Everett, 2002; Ramírez-Gil et al., 2020).

The main cause of disorders of abiotic origin in avocado fruits is associated to sudden changes of temperature and storage at low temperatures (Burdon et al., 2013; Everett et al, 2007, 2008; Hartill, 1991; White et al., 2009). Main symptoms of abiotic postharvest disorders include physical defects that change fruit appearance such as irregular ripening, lenticel damage, and others (Burdon et al., 2013; Everett et al, 2007, 2008; Hartill, 1991; White et al., 2009).

Research on identification of diseases and disorders at postharvest stage in avocado cv. Hass fruits in Colombia are very limited. They may present a high economic impact at farm, packing and marketing levels affecting negatively the industry sustainability (Ramírez-Gil et al., 2020; Analdex, 2017). Knowledge about origin and preharvest factors that could be related or that determine postharvest diseases and disorders development is very poor. Based on previous information and current needs of production of avocado fruits of excellent quality in Colombia this work had three aims: (i) to identify and characterize postharvest diseases and disorders, (ii) to determine the incidence of postharvest diseases and disorders in avocado crops under national and export markets conditions and (iii) to identify preharvest variables and nutritional status of avocado plants potentially associated with the incidence of aforementioned postharvest diseases and disorders.

#### 2. Materials and methods

#### 2.1. Location and study area

Postharvest avocado cv. Hass diseases and disorders were surveyed in 20 plots planted with Hass avocado including orchards grown for export and for national markets. Plots were located in the Department of Antioquia, Colombia (Figure 1.). Sampled avocado trees were all of Hass variety grafted on West Indian rootstock of more than 5 years old, and planted at a distance of  $5 \times 6$ ,  $5 \times 7$ ,  $6 \times 6$ , and  $6 \times 7$  m. During this work, crop management practices were performed according to specific indications of farmers without additional intervention of researchers. Research was developed during years 2014, 2015 and 2016, corresponding to six cycles of fruit harvest. Each cycle was analyzed in duplicate through time. Sample analyses were performed in Laboratory of Fitotecnia Tropical and Laboratory of Biogeochemistry at Universidad Nacional de Colombia branch Medellín.

## 2.2. Polyphasic characterization of postharvest diseases and disorders in avocado cv. Hass

Polyphasic characterization is a multi-step process to correct diagnosis of plants diseases and disorders based on field symptomatology, pathogen isolation, macroscopic and microscopic microbial morphology, pathogenicity tests, Koch postulates, and molecular sequencing (Ramírez-Gil and Morales-Osorio, 2019). To perform the polyphasic characterization of postharvest diseases and disorders in avocado, a preliminary sampling of fruits was carried out. In each plot, the number of fruits to be sampled and measured were selected based on a simple random sampling, using the formula of maximum variance (Cochran, 1977). Subsequently, in each plot a sub-sample composed of 30 fruits ready for harvest (based on each farm multi-criterion: dry matter between 22 and 25%; size and fruit growth time, fruit color (opaque or dark green), pedice yellowish and swollen, and firmness measured by touch) were collected and transported to the laboratory in expanded polystyrene foam boxes at room temperature ( $20 \pm 2$  °C), whose duration was less than 5 h. Causal agents of diseases and origin of disorders were determined based on polyphasic characterization multi-step process that includes: (i) symptoms, (ii) isolation, (iii) use of taxonomic keys, (iv), pathogenicity tests for microorganisms and (v) molecular sequencing (Ramírez-Gil and Morales-Osorio, 2019). In addition, disorders were identified based on (i) symptoms, (ii) reproduction of symptoms according to the possible origin or cause of each disorder, and (iii) determination of each disorder name based on previous steps (i and ii), and comparison with available literature (Everett et al., 2008; Ginsberg, 1985; Kassim et al., 2013; Ochoa, 2012; White et al., 2009). Detailed procedures are described below.

In laboratory, the fruits were packed in cardboard boxes (22 cm wide x 30 cm long x 15 cm height). Later, fruits were ripened in climatic incubators (Memmert HPP-110 at  $20 \pm 1$  °C and  $90 \pm 2\%$  relative humidity). Fruit ripening was monitored based on scale of skin colour with values from 1 to 7, calibrated based on fruit firmness and ripening process (White et al., 2009). Color 1 (emerged green, hard) and a level 7 color (dark black, very overripe) (White et al., 2009). In addition, firmness was measured by touch technique (White et al., 2009). Based on this scale, ripening process was divided into three phases: (i) hard to softening (scale 0 to 3); (ii) firm-ripe to soft-ripe (scale 4 to 5); and (iii) overripe to very overripe (scale 6 to 7) (White et al., 2009).

In each phase destructive samplings were made. For identification of pathologies of biotic origin, small portions ( $\sim$ 1–3 mm<sup>2</sup>) of diseased tissues (epicarp, mesocarp, testa and cotyledons) were placed in sterile V8-agar (180 mL L<sup>-1</sup> of V8 juice, 24 g L<sup>-1</sup> of agar, Difco, USA), ampicillin (200 µg L<sup>-1</sup>), chloramphenicol (20 µg L<sup>-1</sup>) and benlate (100 µg L<sup>-1</sup>, Benomil ®) (V8-AACB), as semi-selective medium for the isolation of *Phytophthora* spp. Potato dextrose agar medium acidified with lactic acid (PDA-A) (Difco, USA) and vegetable juice agar (V8-AE) (Difco, USA), supplemented with streptomycin (100 µg L<sup>-1</sup>) were used for fungi isolation. Nutrient agar (Difco, USA), supplemented with Benomyl® (50 µg L<sup>-1</sup>) (AN-B) and yeast, dextrose, calcium carbonate (Difco, USA) supplemented with Benomyl® (50 µg L<sup>-1</sup>) (YDC-B) as media for bacteria. All media containing plant tissues were incubated at 25 °C for 15 days, with a photoperiod of 12 h of light and 12 h of darkness (Ramírez-Gil and Morales-Osorio, 2019).

Fruits used in pathogenicity tests were collected in crop fields from visually healthy plants, i.e., without any type of symptoms. In addition, fruits have to have adequate care during postharvest handling to avoid damage caused by blows or friction. Inoculation of fungal and bacterial isolates in healthy fruits was performed using two methods. The first was using an aqueous suspension at a final concentration of  $1 \times 10^{-3}$  to  $1 \times 10^{-6}$  of infective propagules x mL<sup>-1</sup>, prepared by resuspension of microorganisms cultured in PDA, AN, or YDC in sterile distilled water (50 mL). The suspension was sprayed directly on the fruit in a final volume of 10 mL. The second method of inoculation consisted of a fragment of tissue and culture medium (PDA, AN, or YDC) of 0.5 cm<sup>2</sup>, which was placed directly on fruits and adhered with micropore adhesive tape.

In both methods, fruits were incubated at three different temperatures (20, 15, and 5 °C). Re-isolation of microbial causal agents was performed in corresponding culture media described above. For pathogenicity tests, fruits were stored in plastic boxes (24 cm wide x 30 cm long x 6 cm high) at >85% of relative humidity to promote disease development. All pathogenicity tests were performed for a period of 40 days and the variable response evaluated was the incubation period. Each experimental unit was composed of five fruits and three replicates were implemented for a total of 15 individual fruits.

Microbial structures of isolates that were positive in pathogenicity tests were observed in the light microscope. Micro-assemblages were made using sporulated fruits and/or isolates grown in synthetic media and observed under light microscopy coupled with DIC technology (Differential Interference Contrast, Nikon Eclipse E200). Taxonomic keys for fungi (Barnett and Hunter, 1972; Seifert et al., 2011) and *Phytophthora* spp. (Erwin and Ribeiro, 1996) were used for morphological analysis of positive isolates. In addition, identification of isolates was supported by sequence analysis of the ITS region PCR-amplified using primers ITS'5-ITS'4 and ITS'1-ITS'4 (White et al., 1990). Purified PCR products were sent for sequencing following the company guidelines (Macrogen, Republic of Korea). Obtained sequences were manually cleaned, edited and aligned using the software BioEdit 6.0.6 and Chromas 1.45. Consensus sequences were compared with publicly available databases using the BLAST algorithm implemented at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi). Molecular sequencing was performed as support for isolates identification, but other analysis such as phylogenetic relationships were out of the scope of our research.

For identification of postharvest disorders, stemmatological characterization was performed, from which a name was assigned based on scientific literature. Additionally, different factors that may induce a disorder were evaluated trying to reproduce symptoms (Everett et al., 2008; Ginsberg, 1985; Kassim et al., 2013; Ochoa, 2012; White et al., 2009). For abiotic disorder diffuse flesh discolouration (associated with

chilling injury), symptom reproduction was carried out by incubating fruits with different dry matter contents (20, 25, and 30 %) at 20, 15, and 5 °C for 40 days. For necrosis in epicarp and flesh bruising of fruits, symptom reproduction was made by simulating free fall of fruits from a height between 1.5 and 3 m from soil surface, which corresponds to the average altitude of fruits in evaluated trees, and then, fruits were incubated at 20, 15, and 5 °C. Reproduction of skin spotting symptoms was performed by rubbing the fruit epidermis with a mechanical stirrer in a vessel (Struers Labopol-5) at 150 rpm per 1 min followed by incubation at 20, 15, and 5 °C. Dehydration was reproduced by incubating fruits to daily temperature changes of 5, 20, and 30 °C for 30 s each during 1 h, using a commercial fridge (Haceb ®, Medellín, Colombia) and a programmable temperature chamber (Binder ®). For irregular ripening, fruits with different dry matter contents (20, 25, and 30 %) were harvested and incubated up to ripening at different temperatures (5, 20, and 30 °C). For the disorder named seed malformation and necrosis, no controlled pathogenicity test was performed since this disorder is highly dependent on environmental conditions present during the period of fruit growth, which were especially the severe drought observed in ENSO



Figure 1. Localization of the avocado plots evaluated. The evaluated plots were located between 1800 and 2500 m of elevation, with annual temperature averages between 14 and 20 °C, precipitation of 1800–2600 mm and relative humidity between 75-99%.

phenomena, El Niño. Each experimental unit was composed of five fruits and three replicates were used for a total of 15 individual fruits.

## 2.3. Incidence of postharvest diseases and disorders in avocado cv. Hass crops for export and national markets

Fruits were collected from each avocado plot and for each period of evaluation (six periods) based on a simple random sampling using the formula of maximum variance (Cochran, 1977) and following the harvest criteria of each farm (national (size and fruit growth time, fruit color (opaque or dark green), and pedicel yellowish and swollen) or export markets (dry matter between 22 and 25%; size and fruit growth time, fruit color (opaque or dark green), pedicel yellowish and swollen, skin rough with notorious lenticels, seed separated from the pulp, and the pulp with a green-yellowish color and creamy texture with transparent and removable tegument and firmness (>25 lbs of pressure) (Ramírez-Gil et al., 2019)). The number of trees sampled were in the range of 90–120, being constant for each period. From each tree, 5 fruits with a commercial size ranging from fruit caliber 18–24 (weight >120 g; fruit with the most commercial caliber for Colombia) were harvested. In addition, a pre-selection of 90 fruits without apparent physical damage and with a weight higher than 120 g was performed. Samples were placed in polystyrene foam boxes (described before) previously disinfested (3 % sodium hypochlorite in sterile distilled water v-v) and sent to the laboratory of Fitotecnia Tropical for further processing. Time length and conditions for evaluation during postharvest stage were designed according to destination of fruits produced in 20 plots (10 from national and 10 from export markets) and according to product destination (i.e., national market for the city of Medellín (Antioquia, Colombia) or export for the European Union). The process was carried out under laboratory simulation, given the logistical, operational and regulatory difficulties under commercial pack house conditions.

For export, fruits were washed in tap water, sprayed with a fungicide (imidazole prochloraz<sup>™</sup>, as directed by the manufacturer) and were left to dry at room temperature. Then, three groups were selected by range of weight (group one: 120-180 g, group two: 181-220 g and group three >221 g). Each group of fruits was packed in cardboard boxes (22 cm wide x 30 cm long x 15 cm height) and were pre-cooled (from 20 °C to 5 °C), by lowering the temperature 5 °C every 10 min. Then, boxes were kept in darkness, at 5  $^\circ C$  and >90 % of relative humidity for a period of 15 days simulating time length and conditions between dispatch from packing to Europe. Later fruits were stored at 10  $^\circ$ C and 90% of relative humidity simulating conditions and average time length that fruits spend in a market chain and eat for consumers. For national market, fruits were processed similarly but without spraying with fungicide and were packed in disinfested reusable plastic boxes (24 cm wide x 30 cm long x 6 cm high). In addition two storage periods were tested, the first was carried out by 8 days at 20 °C of temperature and 80 % of relative humidity, simulating time length and conditions between dispatch from farm and the sale by wholesaler. In the second, fruits were stored at 10  $^{\circ}$ C and 90% of relative humidity simulating conditions and average time length that fruits spend in a market chain and eat by consumers. This procedure was carried out in a controlled chamber (Sanyo, Versatile Environmental Test Chamber, model MLR-351H).

In each simulated condition (national and export markets) the fruit ripening was monitored based on skin colour scales and firmness measured by touch technique (White et al., 2009). In addition, the ripening process was divided into three phases ((i) hard to softening (scale 0 to 3); (ii) firm-ripe to soft-ripe (scale 4 to 5); and (iii) overripe to very overripe (scale 6 to 7) (White et al., 2009)). In each phase thirty random fruits were selected. For identification of pathologies of biotic and abiotic origin, fruits were rinsed with tap water and non-ionic detergent for 1 min (30% of tween 20 in sterile distilled water (SDW) and dried in paper towels at room temperature (22–25 °C) (Ramírez-Gil and Morales-Osorio, 2019). The fruits were dissected and symptoms associated with abiotic disorders were determined by visual inspection. A portion of sample tissues was incubated in humid chambers at 20 °C and >90% relative humidity to corroborate if the microorganism found in the diseased tissue corresponded to the isolated on culture media described before. Additionally, the remaining of the tissues (epicarp, mesocarp, testa and cotyledons) was sections in small portions ( $\sim$ 1–3 mm<sup>2</sup>) and were paced on semiselective media culture following the methodology described before.

### 2.4. Relationship of incidence of postharvest diseases and disorders with pre and postharvest agronomical practices in avocado cv. Hass crops

When each plot was harvested following criteria of each farm, in each trees (described before) samples were collected at random from leaves and fruits (n: 3) from the middle third of each tree. Later for chemical characterization, ten random samples were selected from each plot. Dry matter of fruits was determined by taking a sample of mesocarp (50 g), which was weighted (initial weight) and incubated (Binder chamber ®) at 60 °C until constant weight was reached (final weight). The percentage of dry matter was obtained as the difference between initial and final weight of each sample multiplied by 100 (Lee, 1981). In addition, a sample of 2 g of wet weight was obtained from the fruit epicarp and leaves collected in fields. These samples were dried in an oven with a heating ramp of 2 °C every minute until 550 °C were reached followed by acid digestion with 96% sulfuric acid for quantification of potassium, calcium, and magnesium by atomic absorption. The same elements were determined from ten soil samples collected around a diameter of 2 m from the base of the plant stem, at a depth of 1 m from same numbers of trees tested ( $Ca^{+2}$ ,  $Mg^{+1}$ , and  $K^{+1}$ : 1M ammonium acetate).

Ratios of Ca/K, Ca/Mg, K/Mg and Ca + Mg/K, were calculated using data of these nutrients obtained from soil, leaves, and fruits. In addition, variables associated to crop management were obtained by a descriptive survey with structured and unstructured questions designed in such a way that answers were further transformed into a dichotomous value according to its compliance (1) or non-compliance (0).

#### 2.5. Data statistical analysis

Incidence of each postharvest pathology and disorder was determined as the number of affected fruits divided by the total number of fruits evaluated multiplied by 100. For variables incidence and incubation period, data homoscedasticity and normality were determined using criteria of Levene and Kolmogorov-Smirnov, respectively. Analysis of variance of one factor (Anova) was performed followed by means comparison by the Tukey test (P < 0.05). Relationships between incidence of each disease and corresponding variables associated with crop management, dry matter content, nutrient concentration and relationships between calcium, potassium, and magnesium in soil, fruits, and leaves, were analyzed using a multinomial logistic regression model based on nominal (Dummy) and quantitative variables. Selection of informative variables within the model was based on the log-likelihood-ratio test of models, p-value using Wald tests (z-tests) and akaike information criterion. All Analyses were performed using the computing program R (R Development Core Team, 2020).

#### 3. Results

#### 3.1. Identification of postharvest diseases in avocado cv. Hass

Fruits showing anthracnose symptoms presented circular lesions of different diameter (2–10 mm) in the epidermis, of light brown color in the surface, becoming depressed and changing its tone to black through time. On these lesions a mass of conidia of pink or salmon color was observed (Figure 2 A). Inside fruits, mesocarp soft rot of light color was present (Figure 2 B) which, with disease advance turned dark brown and finally extended to all mesocarp (Figure 2 C). Morphological characteristics of isolated microorganism and DNA sequences (Tables 1 and 2.)

corresponded to *Colletotrichum gloeosporioides sensu lato* (Barnett and Hunter, 1972; Giblin et al., 2018; Seifert et al., 2011; Sharma et al., 2017). Pathogenicity tests were positive resulting in an incubation period of 7.2 days at a temperature of 20 °C, which increased as temperature decreased (Table 1).

Stem-end rot was characterized by tissue weakening in the zone of fruit union with the peduncle leading to fruit detachment with abundant mycelia appearing later on (Figure 2 D). External and internal areas of this zone presented darkening of vascular bundles throughout mesocarp (Figure 2 E) and incipient soft rot with light tones, which extended over mesocarp through time. This symptomatology advanced towards dark brown lesions with defined borders (Figure 2 F). Microbiological analysis of symptomatic fruits revealed the presence of five different microorganisms (Barnett and Hunter, 1972; Seifert et al., 2011) (Table 1 and Figures 2 I-P) which gave positive results in corresponding pathogenicity tests. Incubation periods ranged from 10.5 to 29.0 days (Table 1). Based on morphological characteristics and gene sequences these microorganisms were identified as *L. theobromae, Colletorrichum gloeosporioides sensu lato; Rhizopus stolonifer; Pestalotiopsis oxyanthi* and *Phomopsis* sp. (Tables 1 and 2).

#### 3.2. Identification of postharvest disorders in avocado cv. Hass

Chilling injury was associated with epidermal necrosis without a spatial pattern and or defined size. Inside the fruit, it was characterized by tissue softening, changes in coloration, which ranged from light to dark black without a specific size or spatial pattern. In some cases darkening of basal bundles was observed throughout mesocarp (Figure 3 A, B, C, D). The shortest (P < 0.05) reproduction of symptoms of this

disorder (15.5 days) was observed at 5  $^{\circ}$ C and at a dry matter content of 20 %, followed by a dry matter values of 30 and 25 %, respectively (Table 1).

Fruit damage associated with flesh bruising was characterized by epidermal necrosis (Figure 3 D). In the inner part of mesocarp, light or dark lesions were observed with or without defined borders (Figures 3 F, G, and H). Affected tissue did not show softening when compared with healthy tissue. Reproduction of this disorder at incubation temperatures of 20, 15 and 5 °C lasted 17.4, 18.6 and 19.2 days on average, respectively (Table 1).

Irregular ripening disorder was characterized by the presence of zones inside the mesocarp with different degrees of hardness and maturation. In addition, when fruits were cut, the pulp remained attached to seeds (Figure 3I). The shortest time to show corresponding symptoms (P < 0.05) was of 10.2 days at a dry matter content of 20 % at a temperature of 30 °C, followed by low (20 %) and medium (24 %) dry matter content at 30 °C. At a dry matter content of 25 % and medium and low temperature (20–5 °C), symptoms were not observed. However, at 30 °C and similar dry matter content symptoms were evident. For a dry matter content of 30 %, symptoms were observed at the three temperatures tested, but with a shorter time of onset at 30 °C than those observed at 20 and 5 °C (P < 0.05) (Table 1).

Fruits with lenticel damage exhibited spots with porous appearance, erupting and scraped of brown color, which evolve towards necrotic areas of small size (<2 mm) with random epidermal distribution (Figure 3 J). This damage was more evident in green fruits because during maturation and normal color change towards dark brown or purple, lenticel damage became less visible. In reproducibility tests, the temperature did not significantly affect the time of apparition of this



Figure 2. Symptomatology and microorganisms associated with biotic causal agents of postharvest diseases in avocado cv. Hass. A to C: anthracnose. A: external symptomatology, B: mesocarp soft rot, and C: mesocarp necrosis. D to F: stem-end rot. D: external symptomatology, E: darkening of vascular bundles in mesocarp, and F: mesocarp soft rot and necrosis. G: Colony of Colletotrichum gloeosporioides sensu lato, H: Conidia of Colletotrichum gloeosporioides sensu lato. I: Colony of Lasiodiplodia theobromae, K: Conidia of Lasiodiplodia theobromae. L: Colony of Rhizopus stolonifer, M: Sporangia and sporangiophores of Rhizopus stolonifer, N: Colony of Pestalotiopsis oxyanthi, O: Conidia of Pestalotiopsis oxyanthi, P: Colony of Pho*mopsis* sp., J:  $\alpha$  and  $\beta$  conidia of *Phomopsis* sp.

	Table 1.	Variables evaluated in	n pathogenicit	y tests and re	production of s	symptoms of	postharvest	diseases and	disorders	in fruits of avo	cado cv. Hass.
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Causal agent	Disease	Incubation period <sup>1</sup>	Incubation period <sup>2</sup>	Incubation period <sup>3</sup>	Re-isolation
Colletotrichum gloeosporioides sensu lato	Anthracnose	7.2 c	15.0 b	25.1a	+
Colletotrichum gloeosporioides sensu lato	Stem-end Rot	10.5 b	13.0 b	28.3 a	+
Lasiodiplodia theobromae	Stem-end Rot	12.9 b	16.2 b	29.0 a	+
Pestalotiopsis oxyanthi	Stem-end Rot	14.4 b	16.0 b	27.1 a	+
Rhizopus stolonifer.	Stem-end Rot	12.2 b	14.2 b	27.3 a	+
Phomopsis sp.	Stem-end Rot	14.3 b	18.3 b	26.3 a	+
Chilling injury <sup>4</sup>	Chilling injury	<sup>3</sup> n.a <sup>4</sup> n.a <sup>5</sup> n.a	n.a n.a n.a	15.5 b 38.4 a 18.6 b	n.a
Necrosis in the epicarp and mesocarp <sup>5</sup>	Mesocarp coloration change	17.4 a	18.6 a	19.2 a	n.a
Lenticel damage <sup>6</sup>	Lenticel damage	10.2 a	9.4 a	10.6 a	n.a
Irregular ripening <sup>7</sup>	Irregular ripening	<sup>3</sup> 15.1 c <sup>3</sup> 20.1 b <sup>3</sup> 10.3 ca	20.2 b - 29.4 b	35.3 с - -28.3 с	n.a
Dehydration <sup>8</sup>	Dehydration	10,0	23,1	21,3	n.a

<sup>1, 2, 3</sup> For pathologies associated with biotic causal agents and abiotic disorders such as cold damage, lenticel damage and epicarp and mesocarp necrosis, temperatures were 20, 15 and 5 °C; while for irregular ripening and dehydration temperatures were 30, 20, and 5 °C. <sup>3, 4, 5</sup> Represent dry matter content values associated with 20, 25 and 30%, respectively. Different letters in columns indicate significant differences identified by the Duncan test P < 0.01. <sup>4, 5, 6, 7,</sup> and <sup>8</sup> Is not incubation period, this value is associated with time length of symptom reproduction.

Table 2. Descriptions of morphological structures of microorganisms associated with postharvest diseases in avocado cv. Hass.

Disease	Characteristics of microorganism isolated	Structure	Length (µm)	Width (µm)	Microorganism
Anthracnose and Stem-end rot.	White-gray mycelia, hyaline, unicellular conidia, with a shape varying from cylindrical, oval to ellipsoid-fusiform.	Conidia	$\textbf{9.8.2}\pm\textbf{2.9}$	2.6 ± 1.2	Colletotrichum gloeosporoides sensu lato
Stem-end rot	Dark gray mycelium without sporulation on PDA, but when inoculated on fruits, septate hyaline and pigmented conidia were observed, globular or ellipsoid, with truncated basal portion, thick cell wall, structures surrounded by a septal paraphysis.	Conidia	$20.8\pm2.11$	$17.8\pm1.22$	Lasidiplodia theobromae
Stem-end rot	Fast-growing microorganism in PDA, showing	Sporangia	$\textbf{45.8} \pm \textbf{9.1}$	$40.4\pm8.1$	Rhizopus stolonifer.
	abundant aerial and spongy mycelia, thick and prominent hyphae, abundant and prominent brown-colored rhizoids, on which non- branched sporangiophores originated supporting sporangia in their upper part.	sporangiophore	$5.4 \pm 1.1$	$4.8\pm0.9$	
Stem-end rot,	Fast growing white colony in PDA media on which intense black oily protuberances appeared, abundant conidia of fusoid type with 2 or 3 septa, brown in the center and hyaline at the ends, with one or more appendages at the apexes.	Conidia	7.9 1.4	2.9 0.4	Pestalotiopsis oxyanthi
Stem-end rot	Grayish-white mycelia in PDA media, from	Conidia alpha (α)	$5.9 \pm 1.2$	$2.2\pm0.3$	Phomopsis sp.
	which globe shaped and unilocular dark- colored pycnidia were observed in solitary or aggregated form, immersed in a stromal mass; within them there was also evidence of unicellular, hyaline, alpha ( $\alpha$ ) type conidia, constricted in the center and with a blunt base, and beta ( $\beta$ ) conidia, longer, filiform, curved or flexuous, without septa.	Conidia beta (β)	16.9 ± 3.2	$3.1\pm0.7$	

Structure length and width are the mean and their respective standard deviation based on the quantification of 20 individual structures.

disorder (P > 0.05), with values of 10.2, 9.4 and 10.6 days at 15, 10 and 5 °C, respectively (Table 1).

Dehydrated fruits showed decreased firmness due to water loss on epidermis located with preference around the insertion of peduncle in the fruit body (Figure 3 K). This damage was only external and did not affect the fruit mesocarp. Reproduction of symptoms was positive when fruits were subjected to changes in temperature around 30 °C (Table 1).

Malformation and necrosis of seeds were only observed during the harvest season of September–October of 2016, which coincided with a severe drought during the first stages of fruit growth and filling (December, January, and February) associated with El Niño climatic phenomenon (Ramírez-Gil and Morales-Osorio, 2018). Symptoms observed included small fruits, malformation of seeds with testa detachment and intense black color. In most cases, the mesocarp was unaffected in its physical and organoleptic characteristics except when the testa remained adhered to pulp (Figure 3 L).

The timeline associated with presence of post-harvest pathologies and disorders in Hass avocado showed that in the initial phases of ripening process (hard to softening), the number of phytosanitary problems is minor with respect to the next stage (firm-ripe to soft-ripe), while the overripe and very overripe fruit was the most susceptible phase (Figure 4).



**Figure 3.** Symptoms associated with postharvest disorders in avocado cv. Hass. A to E: chilling injury; A: external damage, B; internal tissue softening with purple coloration, C: Mesocarp internal necrosis, D: darkening of vascular bundles in mesocarp, E: advanced internal necrosis of mesocarp, and darkening of mesocarp and basal bundles. F to H: flesh bruising; F: specific necrotic area, G: advanced necrotic areas, and H: changes in mesocarp coloration. I: Irregular ripening. J: Lenticel damage. K: Dehydration. L: Malformation and necrosis of seeds.



Figure 4. Postharvest diseases and disorders timeline in cv. Hass avocado associated with ripening process. The fruit ripening was monitored based on skin color and firmness scales (White et al., 2009).

### 3.3. Incidence of postharvest diseases and disorders in plots for export and national markets

According to product destination (i.e., national or export) or within each region (North, East, and Southwest), incidence of anthracnose and stem-end rot did not show significant differences (P > 0.05) (Figure 5 A). Highest incidences (P < 0.05) of all diseases and disorders were quantified in plots producing avocados for national markets. Incidence values for all problems of 16.8, 14.10 and 13.4% in the Northern, Eastern and Southwestern regions, respectively, were recorded in plots intended for national markets. In contrast, general incidence values of 10.3, 8.10 and 9.4% were found in export plots, respectively, for the same regions (Figure 5 B).

Differences were found between incidence of each postharvest disease or disorder in plots for export, and within each region (P < 0.05) (Figure 6 A). The most important disease was stem-end rot followed by anthracnose with incidences in the range of 6.67–12.47 %. Problems with incidences of less to 7 % and in decreasing order were: chilling injury, irregular ripening, fresh bruising, lenticel damage and dehydration (Figure 6 A). Plots dedicated to domestic markets (Figure 6 B) presented higher disease incidences (P < 0.05) when compared to those

observed in plots dedicated to export markets (Figure 6 A). In addition, changes in frequency and importance of each disease or disorder were observed in the regions tested. In the Northern zone, anthracnose had a higher (P < 0.05) incidence compared to stem-end rot (Figure 6 B). Incidence of chilling injury decreased (P < 0.05) and incidence of irregular ripening increased in the same region (P < 0.05) (Figure 6 B). Lenticel damage, fresh bruising and dehydration showed similar incidence values in plots evaluated (Figure 6 A and B).

Seed malformation and necrosis were only identified during the harvest period of 2016 and predominantly in the Southwestern region of Antioquia (Figure 6 A and B). In some fruits, several pathologies or disorders were found simultaneously such as chilling injury damage, fresh bruising and mixed infections of anthracnose and stem-end rot as well as anthracnose together with lenticel damage.

### 3.4. Relationship of postharvest diseases and disorders with preharvest and harvest agronomical practices

Incidence of anthracnose and stem-end rot were negatively correlated with different preharvest agronomical practices such as appropriate



**Figure 5.** Causal agents and incidence of postharvest diseases and disorders in avocado cv. Hass in plots for national and export markets. A: Microorganisms associated and incidence of stem-end rot disease in plots aimed for national (light blue) and export (dark blue) markets. B: Incidence of postharvest pathologies in avocado for each plot evaluated for national or export markets. Error bars represent the confidence intervals of the mean. Equal letters indicates that there are no significant differences (P > 0.05) based on Tukey mean separation test. Incidence was calculated as the number of particular cases divided by the total evaluated multiplied by 100.



Figure 6. Incidence of each postharvest diseases and disorder in avocado cv. Hass plots in each region and for national or export markets. A: Incidence of each postharvest disease or disorder in plots aimed for export markets. B: Incidence of each postharvest disease or disorder in plots aimed for national markets. Error bars represent the confidence interval of the mean. Equal letters indicates that there are no significant differences (P > 0.05) based on Tukey mean separation test.

pruning, foliar application of copper oxychloride, disinfestation of harvesting tools and fruit transport baskets, integrated pest management and foliar spray of calcium. In addition, calcium concentration and Ca/K relationship in the epidermis presented a significant inverse relationship (P < 0.05) with disease incidence Plant age and high plant densities in

crop fields presented a significant (P < 0.05) and positive relationship with the same already mentioned pathologies (Table 3).

High (>30%) or low (<22%) content of dry matter at harvest were associated with higher incidence of chilling injury. A low value of dry matter (<23%) was related with an increase of irregular ripening.

Type of variable	Variable -values			Coefficients <sup>3</sup>	Significance <sup>4</sup>	Plots employing	this practice (%)
Nutrients concentrations in leaves	K <sup>1</sup>	$0.4\pm0.001^{5,a}$	ns	P > 0.05	na		
	Ca <sup>1</sup>		$1\pm0.03$	ns	P > 0.05		
	Mg <sup>1</sup>		$0.5\pm0.02$	ns	P > 0.05		
	Ca/K		$2.4\pm0.27$	ns	P > 0.05		
	Ca/Mg         2           K/Mg         0           Ca + Mg/K         2		$2.5\pm0.45$	ns	P > 0.05		
			$\textbf{0.9} \pm \textbf{0.06}$	ns	P > 0.05		
			$2.2\pm0.27$	ns	P > 0.05		
Nutrients concentrations in fruits	K <sup>1</sup>	$0.5\pm0.003^6$	$1.1\pm0.01^{c}$	ns	P > 0.05	na	
	Ca <sup>1</sup>	$0.3\pm0.10$	$0.4\pm0.12$	-0.65 <sup>8</sup>	0.052 <sup>8</sup>		
	Mg <sup>1</sup>	$0.09\pm0.08$	$0.055\pm0.04$	ns	ns		
	Ca/K	$0.6\pm0.01$	$0.81 \pm 0.02$	-0.75 <sup>9</sup> -0.76 <sup>10</sup>	$00.0480^9 0.0471^{10}$		
	Ca/Mg	$3.3\pm0.9$	$\textbf{2.5} \pm \textbf{0.4}$	ns	P > 0.05		
	K/Mg	$5.5\pm0.05$	$\textbf{3.6} \pm \textbf{0.9}$	ns	P > 0.05		
	Ca + Mg/K	$0.78\pm0.27$	$1.0 \pm 0.01$	-0.78 <sup>11</sup>	$0.0377^{11}$		
Nutrients concentrations in soil	K <sup>2</sup>		$0.45 \pm 0.005^{7}$	ns	P > 0.05	na	
	Ca <sup>2</sup> Mg <sup>2</sup>		$1\pm0.09$	ns	P > 0.05		
			$\textbf{0.7} \pm \textbf{0.07}$	ns	P > 0.05		
	Ca/K		$2\pm0.62$	ns	P > 0.05		
	Ca/Mg K/Mg		$1.8\pm0.6$	ns	P > 0.05		
			$0.6\pm0.02$	ns	P > 0.05		
	Ca + Mg/K		$2.8\pm0.61$	ns	P > 0.05		
Nutrients concentrations in fruits Nutrients concentrations in soil Management practices	Pruning		Anthracnose <sup>12</sup>	-0.188	0.006	80 <sup>13</sup>	50 <sup>14</sup>
	Foliar application of copper oxychloride		Anthracnose	-0.012	0.005	92.4	45.4
	Disinfestation of harve	esting tools and fruit transport baskets	Stem-end rot	-4.3	0.003	78.4	0
	Foliar spray of calcium	n	Anthracnose	-0.14	0.001	80.4	45.7
	Plant age		Stem-end rot	8.5	0.004	na	na
	High plant densities (>300 trees $ha^{-1}$ )		Anthracnose	3.2	0.0001	58.7	25.7
	High (>30%) content	Chilling injury	0.8478	0.0160	25.4	68.7	
	Low (<22%) content of dry matter at harvest		Irregular ripening	-0.978	0.0060	14.4	35.4
	Soils analysis of nutrients		na	ns	P > 0.05	100	62.4
	Foliar analysis of nutri	na	ns	P > 0.05	65.4	25.1	
	Hass grafted on Indian or Hass rootstock		na	ns	P > 0.05	na	na
	Certified nurseries		na	ns	P > 0.05	70.5	35.9
	Integrated pest management		Anthracnose	-0.41	0.001	82.5	31.8
			Stem-end rot	-0.15	0.0002		
	Drainage infrastructur	e	na	ns	P > 0.05	65.4	25.8
	Girdling		na	ns	P > 0.05	35.4	24.1

Table 3. Relationships between variables and incidence of diseases and disorders in postharvest in avocado cv. Hass and disorder in analyzed using a multinomial logistic regression model.

<sup>1</sup> Percentage in dry base (g/100g). <sup>2</sup> Interchangeable bases (cmolc kg<sup>-1</sup>). <sup>3</sup> log-likelihood-ratio test of models. <sup>4</sup> p-value using Wald tests (z-tests). <sup>5, 6 and 7</sup> Mean and standard deviation values of concentration in leaves, epicarp and mesocarp respectively. <sup>8 and 9</sup> Relationship with anthracnose. <sup>10</sup> Relationship with stem-end rot. <sup>11</sup> Relationship with lenticel damage. <sup>12</sup> Named of diseases or disorders with statistical significance. <sup>13</sup> Export marker. <sup>14</sup> National market. Does it not applied: na. No significant differences (P > 0.05): ns.

<sup>a</sup> Standard of avocado leaf analysis (deficient: <0.5, <0.35 and <0.15; adequate: 1–3, 0.75–2 and 0.25–0.80; excessive: >4, >3 and >1 to Ca, K and Mg respectability) (Lahav and Kadman, 1980).

Similarly, a high relationship of Ca/K in the epidermis was associated with a lower incidence of chilling injury. In addition, lenticel damage was inversely correlated with Ca + Mg/K relationship for epicarp and mesocarp necrosis, dehydration, and seed malformation and necrosis, no relationship was found with any variable tested (Table 3).

#### 4. Discussion

### 4.1. Identification of postharvest diseases and disorders in avocado cv. Hass

*Colletotrichum gloeosporioides* and to a lesser extent *Colletotrichum acutatum* and *Colletotrichum boninense* have been usually associated with avocado anthracnose (Giblin et al., 2018; Hartill, 1991; Sharma et al., 2017). In the present work, symptoms and morphological and molecular

characterization, coincided with those reported for *C. gloeosporioides* (Giblin et al., 2018; Hartill, 1991; Sharma et al., 2017). Its recent denomination as *Colletotrichum gloeosporioides sensu lato* was implemented since the identification of a particular species within this complex group has been the subject of an intense debate beyond the scope of this work (Giblin et al., 2018; Hartill, 1991; Sharma et al., 2017; Weir et al., 2012). Each of the causal agents associated with stem-end rot, developed disease symptoms with variable incubation periods when inoculated either individually or in combination. Results suggest that this disease is caused by several microorganisms in agreement with previous results (Darvas and Kotze, 1979; Hartill, 1991; Hartill and Everett, 2002; Ochoa, 2012).

Symptomatology associated with chilling injury observed in this work, coincided with worldwide reports (Burdon et al., 2013; Eksteen, 1995; Ochoa, 2012; Swarts, 1984; White et al., 2009). Chilling injury

symptoms have been attributed to several factors including storage at low temperature, high concentration of ethylene, harvest of over-ripened fruits and specific conditions present in each producing region (Eksteen, 1995; Ochoa, 2012; White et al., 2009). Symptoms of fresh bruising coincided with previous reports when fruits were exposed to preharvest or postharvest blows, vibrations and chilling injury (White et al., 2009).

Irregular ripening was associated with high or low dry matter content, in addition to high ripening temperature. It is argued that these two factors are the more common etiology of this disease (Gamble et al., 2010; Ochoa, 2012; Pedreschi et al., 2016; White et al., 2009). This pathology was considered different to chilling injury because symptoms reproduction was positive with high temperatures and low values of dry matter content (Table 1).

The etiology of lenticel damage has not yet been clearly established. It has been suggested that lesions generated during lenticel damage may lead to secondary infections by microorganisms (Everett et al., 2008). Symptoms observed in the present work, when fruits were rubbed between them were similar to those reported by Everett et al. (2008). Under field conditions, a similar effect may happen as a consequence of wind. During postharvest, improper handling during fruits selection and packing processes may exert a friction that eventually induces disease symptoms. Symptomatology of dehydration, which may be induced by multiple causes associated with water loss events, such as high temperatures during postharvest handling, long storage periods and early harvest (Ochoa, 2012; White et al., 2009).

We failed to reproduce symptoms of seed malformation and necrosis under laboratory conditions. Fruits exhibiting this disorder came from plots where the stage of fruit initial growth and filling coincided with low levels of precipitation (<30 mm month-1) and high temperatures (>25 °C month). Such conditions happened during the first three months of 2016 due to El Niño climatic phenomenon (Ramírez-Gil and Morales-Osorio, 2018). As ENSO phenomenon is increasing in frequency and intensity, it is tempting to speculate that incidence of this pathology will increase in coming years, therefore, more research is needed to prevent and manage this disorder.

### 4.2. Incidence of postharvest diseases and disorders in plots for export and national markets

Stem-end rot showed the highest incidence in export plots. In plots whose production is destined to local markets, stem-end rot was only lower than anthracnose in the Northern zone. These results also differed to reports from countries like Israel and Australia, where stem-end rot and anthracnose causes losses of up to 20 % (Darvas and Kotze, 1979; Giblin et al., 2018; Hartill, 1991; Hartill and Everett, 2002; Peterson, 1978; Sharma et al., 2017). The presence of a particular postharvest pathology or disorder depends on many variables, which may be related to preharvest or postharvest activities, including local edaphoclimatic conditions, plant genotype, pathogen populations and agronomical practices. Conditions in our work may be drastically different to those observed in other countries were research has been reported. In Colombia, strong influence is performed by the ENSO phenomenon that may cause excessive precipitations (La Niña) or severe drought (El Niño) (Ramírez-Gil and Morales-Osorio, 2018). Incidence for most diseases may be reduced during dry conditions because humidity favors pathogen development. In addition, different agronomical practices are performed and adapted to local conditions. Some crop labors may induce or suppress disease development (Hartill and Everett, 2002).

A lower incidence of anthracnose may be associated with postharvest applications of Prochloraz, which is reported as the most used disease management strategy (Bosse et al., 2013). However, pesticide residue limits may hamper fruit export especially for the European Union market. For national markets, postharvest application of fungicides is not performed, what may be a likely explanation of why anthracnose incidence increased in fruits coming from these plots. At the worldwide level, relevance and importance of stem-end rot have been highly associated with inadequate harvest procedures and storage conditions (Menge and Ploetz, 2003).

During export, fruits are exposed to low temperature conditions (5 °C), which are conducive to chilling injury onset (Eksteen, 1995; Ochoa, 2012; White et al., 2009). In clear contrast, usually fruits for national market are not exposed to temperatures lower than 10 °C, which may explain why the incidence of this disorder is reduced. Previous research suggested that low temperatures increase the accumulation of phenols that serve as substrate for the polyphenoloxidase enzyme (PPO), which increases darkening of avocado pulp (Lelyveld and Bower, 1984). Similarly, an early or late harvest as well as pre-harvest stresses such as water deficit and the nutritional content of the fruit, are considered as the main causes of postharvest pathologies of abiotic origin (Chaplin et al., 1982; Ochoa, 2012).

As mentioned, fresh bruising has been associated with shock and vibration (Ochoa, 2012; White et al., 2009). Operators in most plots dedicated to national markets frequently handle fruits with less care than they do in export farms. Workers behavior may explain higher incidence of this disorder measured in fruits destined to national markets. Occurrence of irregular ripening has been associated with harvest of fruits outside of the appropriate degree of maturation (Ochoa, 2012; White et al., 2009). In local markets, this pathology presents a high incidence maybe because workers are not properly trained to follow clear parameters and usually the harvest time is based more on each farmer criteria. Usually, the dry matter content is used in companies dedicated to export markets, as an indirect parameter of fruit maturation (Kassim et al., 2013; Lee, 1981; Ochoa, 2012; White et al., 2009). However, given the presence of this disease in plots for export other causes or inappropriate practices cannot be ruled out. There were no differences between plots evaluated for lenticel damage and dehydration. It is argued that lenticel damage may be worsened when fruits are harvested during the rainy season, therefore, appropriate measures for prevention should be taken during this season (Everett et al, 2007, 2008).

### 4.3. Relationship of postharvest diseases and disorders with preharcest and harvest agronomical practices in avocado cv. Hass plots

Dry matter content has been related to different post-harvest diseases in Hass avocados such as chilling injury (Burdon et al., 2013), irregular ripening, watery texture, loss of taste, wrinkling and coloration changes (Gamble et al., 2010). We found similar results indicating that measuring this variable is an important factor to increase fruit quality and decrease postharvest losses. In the present study, significant correlations were identified between incidence of pathologies of biotic and abiotic origin with Ca/K and Ca + Mg/K relationships in soil and epicarp and foliar calcium content. Evidence suggests that calcium is a nutritional element highly related to avocado fruit quality. Low calcium levels have been related to chilling injury, short post-harvest life periods and darkening of vascular bundles (Chaplin et al., 1982; Everett et al., 2007; Hofman et al., 2002). Calcium assimilation is related to soil levels of magnesium (Mg) and potassium (K) since an antagonism between them has been observed during plant uptake (Martin-Prevel et al., 1987). In contrast, high levels of K have been associated to higher incidence of these diseases (Everett et al., 2007), which may be due to the mentioned antagonism with calcium.Overall, results have allowed to recommend growers applications to increase the (calcium + magnesium)/potassium ratio above 0.065 in fruit for a better postharvest quality and storage shelf life (Everett et al., 2007).

Calcium is fundamental part of cell walls and middle lamella and associates with a large number of proteins involved in cell signaling (Buchanan et al., 2013; White and Broadley, 2003). Besides a number of physiological responses, calcium has been studied for the role it plays in the plant response and adaptation to biotic and abiotic stresses and its importance in fruit pre and postharvest quality and shelf life span (Sugimoto et al., 2010).

As in most fruits, avocado requires calcium for proper development, firmness and adequate storage life. Any influence of calcium concentration on avocado fruit quality would seem to occur early in fruit development rather than just before harvest (Bower, 1985; Penter and Stassen, 2000). In several investigations, preharvest and postharvest applications of calcium have shown increased postharvest quality of avocado fruits (Barrientos-Priego et al., 2016; Penter and Stassen, 2000). Beneficial effects are dependent of appropriate water availability during fruit development (Bower, 1985), fruit calcium levels, canopy density (Everett et al., 2007), tree vigour (Willingham et al., 2006), and other factors. It was observed that application of Ca(NO<sub>3</sub>)<sub>2</sub> decreased the respiratory rate and ethylene production during storage at room temperature and under chilling. In addition, increase the calcium concentration in the exocarp and mesocarp, decreased weight loss, polyphenol oxidase activity and chilling injury (Barrientos-Priego et al., 2016). The increased resistance of avocado tree to diseases was related to a 40% increase in the concentration of  $Ca^{+2}$  in the fruit flesh (Willingham et al., 2006).

Other preharvest agronomical practices that were significantly related to postharvest pathologies were the foliar application of copper oxychloride, pruning, plant age, crop density and disinfestation of tools. Copper oxychloride application is usually carried out during preharvest stages for avocado disease management (Everett et al., 2008; Hartill, 1991; Hartill and Everett, 2002; Peterson, 1978). As mentioned for other fungicides, careful attention should be paid to residue limits to avoid export limitations. Another relationship that has been found correlated with postharvest pathologies is the canopy index. It has been observed that a greater leaf area increases incidence of postharvest diseases possibly associated with microclimates with higher humidity and aged tissues (Everett et al., 2008). Tree density may be related to pruning that would lead to canopy index decrease and tree age and high density that eventually would lead to canopy index increase.

Results suggest that disease and disorders development not may be attributed to one only factor but to multiple conditions that get together and determine the extent of disease incidence and severity. Further exploration of multiple environmental and biotic conditions that determine disease appearance and development is required for implementation of integrated disease management programs in sustainable avocado crops.

#### 5. Conclusions

Most results associated with disorders were related to inadequate harvest criteria, fruit handling mismanagement (bumps, frictions, among others), and storage problems. As mentioned in this work, these situations indicate the need for adequate harvesting criteria, handling protocols and trained personnel. It is also necessary to carry out a logistic analysis for the whole chain value, in order to identify optimum harvest times, the smallest number of steps in handling processes and design of transport routes as efficient as possible, which in turn would allow for a lower cost in transportation and shorter post-harvest processing times with minimum damage.

#### Declarations

#### Author contribution statement

Joaquín Guillermo Ramírez-Gil: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Juan Gonzalo MoralesOsorio: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Juan Camilo Henao-Rojas: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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#### Data availability statement

Data will be made available on request.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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#### J.G. Ramírez-Gil et al.

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