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Distribution of stem-end rot on the canopy in 'Hass' avocado trees in two coastal areas in Peru

Distribución de la pudrición peduncular en la canopia de arboles de palto cv. 'Hass' en dos áreas costeras del Perú

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Abstract

Stem-end rot (SER) of avocado is caused by several fungal species, and it is presented worldwide. This plant disease currently affects several avocado producer regions in Peru, causing fruit rot, impacting the industry negatively. Research about SER distribution in the canopy of avocado trees is limited. Thus, the present study aimed to compare which areas in the canopy are prone to have more SER in 'Hass' avocado harvested fruit in two different coastal areas in Peru. The experiment was conducted in the northern (Barranca) and southern (Cañete) of Lima. 'Hass'Avocado fruits from both producer areas were collected to identify the causal agent; *Lasiodiplodia theobromae* was isolated from infected fruits. Identification was conducted based on morphological features and a partial DNA sequence of the translation elongation factor 1-α gene (*tef1-α*). The results showed that fruits inside the tree canopy were prone to have a higher disease incidence than the fruits located in the external site ($P<0.001$). Besides, internal-site fruits displayed a higher percentage of infected fruit for each grade disease ($P<0.001$) than external-site fruits, except for grade 0 (fruits without symptoms) and grade 1. Finally, the results suggested that the altitude where the fruit is positioned on the canopy could influence the incidence of SER, where fruits located in the high part revealed less incidence than the low section. The results are valuable for enhancing management strategies and avoiding postharvest loss of avocado fruits in our region.

Keywords: *Lasiodiplodia theobromae, stem-end rot, avocado, canopy, SER*

Resumen

La pudrición peduncular del palto (SER por sus siglas en inglés) es causada por varias especies de hongos, y se presenta a nivel mundial. Esta enfermedad afecta actualmente a varias regiones productoras de palta en el Perú, causando la pudrición de la fruta, impactando negativamente a la industria. La investigación sobre la distribución del SER en la copa de los árboles de palta es escasa. Por ello, el presente estudio tuvo como objetivo comparar qué zonas de la copa son propensas a tener más SER en la fruta cosechada de palta 'Hass' en dos zonas costeras diferentes del Perú. El experimento se realizó en el norte (Barranca) y en el sur (Cañete) de Lima. Se recolectaron frutos de palta 'Hass' de ambas zonas productoras para identificar el agente causal; se aisló *Lasiodiplodia theobromae* de los frutos infectados. La identificación se realizó en base a las características morfológicas y a una secuencia parcial de ADN del gen del factor de elongación de traducción 1-α (*tef1-α*). Los resultados mostraron que los frutos dentro de la copa del árbol fueron propensos a tener una mayor incidencia de la enfermedad que los frutos situados en la parte externa ($P<0,001$). Además,

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los frutos situados en el interior mostraron un mayor porcentaje de frutos infectados para cada grado de la enfermedad ($P<0,001$) que los frutos situados en el exterior, excepto para el grado 0 (frutos sin síntomas) y el grado 1. Finalmente, los resultados sugirieron que la altitud en la que se encuentra el fruto en la canopia podría influir en la incidencia del SER, donde los frutos situados en la parte alta revelaron menor incidencia que la sección baja. Estos resultados son valiosos para mejorar las estrategias de manejo y evitar la pérdida poscosecha de los frutos de aguacate en nuestra región.

Palabras clave: *Lasiodiplodia theobromae, pudrición peduncular, aguacate, canopia, SER*

Introduction

Avocado (*Persea americana* Mill.) is a fruit-tree crop cultivated merely in tropical and subtropical areas worldwide because of climatic requirements such as temperature and rainfall (Food and agriculture organization of the United Nations [FAO], 2020; Ministerio de Agricultura y Riego [MINAGRI], 2008). This crop has experienced the fastest production growth, and currently, its global production climbed to 6.3 million tons in 2018 (Altendorf, 2019). The Americas are the biggest producers globally, where more than 70.0 % of the production came from this area (FAO, 2020). In Peru, avocado is an economically significant fruit, and it is considered one of the biggest producers worldwide. Peru is ranked as the third leading producer, followed by Mexico and the Dominican Republic, and the second avocado exporter worldwide (FAO, 2020; Altendorf, 2019; Centre De Cooperation International En Recherche Agronomique Pour Le Développement [CIRAD], 2019). In 2019, Peru registered more than 500 thousand tons over an expansion of 31 000 ha with a wholesale of 720 million USD (CIRAD, 2019).

Due to a high-tech production system and climatic conditions, the coastal region is the most important area in Peru (CIRAD, 2019; MINAGRI, 2008). As a result, the planted area in Peru has been increasing rapidly, becoming avocado a valuable crop. Among the existing cultivars are Hass, Fuerte, Ettinger, Zutano, and Bacon. However, the cultivar Hass is the most extended one produced for the international market (CIRAD, 2019; MINAGRI, 2008).

Like other big-extended crops, avocado faces limitation factors in its production; the phytosanitary aspect is considered one of the main ones. One of the plant diseases that are infecting avocados is stem-end rot (SER), affecting these fruits after harvest. SER is a postharvest disease that infects this fruit and others, including mango and citrus. (Diskin et al., 2017; Zhang, 2014; Zhang & Swingle, 2005). This plant disease lives endophytically until favorable conditions occur with fruit ripening (Johnson et al., 1992). This plant disease starts in the stem end, a section attached to the fruit, showing a shriveling. After that, a decay that appears in this zone produces a dark discoloration and softening of the pulp. This disease advances through vascular bounds, sometimes showing from dark to brown color. As the avocado ripens, these lesions expand to the whole pulp, eventually showing a complete decayed fruit (Guarnaccia et al., 2016; Madhupani & Adikaram, 2017; Twizeyimana et al., 2013).

Many species of the Botryosphaeria family cause SER. Among the species reported is *Lasiodiplodia theobromae*, a worldwide-distributed plant pathogen infecting more tropical and subtropical areas (Punithalingam, 1976; Voorhees, 1942). *L. theobromae* has been identified as infecting over 500 host plants, including fruit trees, vegetables, and ornamental plants (Punithalingam, 1980).

This fungus has been reported infecting other fruit trees such as peach, mango, and grapevine (Li et al., 1995; Khanzada et al., 2004; Úrbez-Torres et al., 2008). Also, among the symptoms described in fruit-crop diseases are sunken necrotic lesions, gummosis, earlier defoliation, twig dieback, reduced vigor, and as a consequence, a lower production (Li et al., 1995; Khanzada et al., 2004). Due to its infection features, *L. theobromae* rarely takes place when the fruits remain in the tree. This pathogen remains latent in the fruit tissue, and symptoms are expressed until harvest; at this point, the plant pathogen develops the infection in this plant tissue.

Some management approaches to control SER have been described to reduce the presence of this plant disease during the postharvest time. Among them are using ripening inhibitors, harvest practice, Pre and postharvest chemical and biological control, application of plant extracts, and physical control (Galsurker et al., 2018). Even though several articles have focused on the biology and management of this plant disease, information about the distribution of SER on avocado trees in the canopy is still scarce. Thus, this study aimed to understand which canopy areas in the 'Hass' avocado tree are more prevalent to this plant disease. The results will help enhance the management of this pathogen and select which areas in the canopy must be fully and well protected when strategy management measures are applied on the field.

Materials and methods

Location and data collection

The experiments were conducted in two different 'Hass' avocado commercial plots, located in the northern (Barranca) and southern (Cañete) regions of Lima. These places were located in the coastal area of Peru. The maximum and minimum were recorded; Barranca showed values from 14 °C to 28 °C, and Cañete from 15 °C to 29 °C. In addition, relative humidity (HR) for Barranca and Cañete displayed values between 85 % to 100 % and 80 % to 98 %, respectively. The locations were chosen because of historical disease presence.

Plant and fruit materials

The evaluated plant materials were 5-years-old 'Hass' avocado trees cultivar Hass on Zutano rootstock with a height between 6 m to 7 m and natural leaf mulch in both areas. In addition, both sites were conducted under exportable conditions, with mechanical (Barranca) and manual (Cañete) pruning conducted annually after harvest in August, 'Hass' avocado fruits were usually harvested with short pedicel using secateurs. No fungicides applications were applied to managed SER directly; however, fungicides to control Lasidioplodia were applied during the season (Thiabendazole and Copper sulfate pentahydrate). In the northern-studied area (Barranca), the planted distance was 2 m x 6 m with a density of 1000 tree per ha with a drip irrigation system. The experimental plot in Cañete had a planted distance of 7 m x 3.5 m with a density of 400 tree per ha irrigated by micro-sprinkler. 'Hass' avocado trees were selected arbitrarily on the plot assigned. The Avocado fruits were collected during a commercial harvest season from June to August.

Collection and isolation

'Hass' avocado fruits were collected from the evaluated area (Barranca). They were transported immediately to Universidad Nacional Agraria La Molina (UNALM). Isolation procedures were carried out in the plant disease clinic of the Plant Pathology department at UNALM. The pathogen's isolation was performed by washing with clean tap water and immersing the fruits with sodium hypochlorite at 1 % for one minute and air-dried. Then they were rinsed in distilled water for two minutes and air-dried to avoid contamination. Fruits were placed in a moist chamber at 25 °C. Fruits with initial typical SER symptoms were selected for isolation. Pieces of the fruit stem-end were cut into small sections (from 2 mm to 3 mm) with a sterile scalpel. The disinfected pieces were placed in Potato Dextrose Agar with Oxitetraciclina (PDA+) and stored at 25 °C for four days in dark conditions. Once the mycelium was visible, they were transferred to PDA+ again to get pure culture.

Morphological and molecular characterization

Morphological identification was made by taxonomical fungi keys elaborated by Barnett & Hunter (2006). Also, morphological structures such as pycnidia and conidia were examined using a compound microscope (DL1000 LED LEICA, Wetzlar, Germany).

For molecular characterization, DNA extraction was performed following the method described by Saitoh et al. (2006) and Balogun et al. (2008). Three-day older cultures were used. A 5-mm diameter plug of active mycelia growth extracted from the culture was placed in a 1.5-mL sterile Eppendorf tube. A total of

500 µL of lysis buffer (100 mM Tris-HCl, 50 mM ethylenediaminetetraacetic acid [EDTA], 1M KCl; pH 8.0) was added to the tube, and the mycelium was dispersed with a sterile toothpick. After 10 minutes at room temperature, 300 µL of phenol: chloroform: isoamyl alcohol in the following volume rate 25:24:1 was added. The mixture was centrifuged for 10 minutes at 1200 rpm. A supernatant of 300 µL was transferred to a new 1.5-mL Eppendorf tube and was stored at 37 °C for 30 minutes (Thermo Mixer Eppendorf). A 300 µL of isopropanol was added, and it was stored at -20 °C for 15 minutes. Finally, this was centrifuged at 1200 rpm for 10 minutes at room temperature, and the supernatant was discarded. DNA was washed with 1 mL of ethanol at 70 % by centrifugation at 1200 rpm for 5 minutes, and the ethanol was discarded. DNA pellets were air-dried, and 30 µL of nuclease-free water was added. The mixture was stored at -30 °C.

For identification at the species level, a couple of primers previously developed (EF1-728F and EF1- 986R) were used to amplify Translation Elongation Factor 1-alpha (*tef1-α*) (Carbone & Kohn, 1999) (Table 1). The amplification of the *tef1-α* was carried out in a 25.0 µL reaction, using 15.4 µL of HPLC-grade water, 5 µL of Buffer (5X), 2 µL of MgCl2, 0.5 µL of dNTP, 0.5 µL for each primer (forward and reverse), 0.1 µL Taq-Polymerase and 1 µL of gDNA. The amplification reactions were conducted in a SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific, Singapore) with the following protocol: 5 min at 94 °C; 40 cycles of 1 min at 94 °C, 1 min at 58.1 °C, 1 min at 74 °C, and a final extension of 7 min at 74 °C. PCR products were sent for sequencing to the University of California Riverside (UCR). The *tef1-α* gene sequences of isolates of *L. theobromae* from previous studies were used to compare homology by using BLAST (Altschup et al., 1990). This procedure was used for each isolate to identify the percentage of homology with *L. theobromae*. Finally, sequences of *tef1*-α gen of *Lasiodiplodia* spp. and *Diplodia seriata* (Table 2) were obtained from GenBank, and they were used for phylogenetic analysis. Alignment and phylogenetic analyses were performed by Molecular Evolutionary Genetics Analysis (MEGA-X v 10.2,2) using five isolates (Table 3). Maximum likelihood analysis and Bootstrap values were calculated using 1000 replicates. The evolution Model for the analysis was T92: Tamura 3-Parameter.

Table 1. Primers used to amplify gDNA of EF- α gene of *Lasiodiplodia* spp.

Target gene	Primer	Direction	Sequence (5'-3')	Cite
EF-α	EF1- 728F	Forward	CATCGAGAAGTTCGAGAAGG	(Carbone & Kohn, 1999)
	EF1- 986R	Reverse	TACTTGAAGGAACCCTTACC	

Table 2. Isolates used in the present study

Isolate	Species	Host	Origin	Collector	GenBank accession no. (EF1-α)
CMW9074	*Lasiodiplodia theobromae*	*Pinus sp*	Mexico	B. Slippers	AY236901
CMW10130	*L. theobromae*	*Vitis donniana*	Uganda	J. Roux	AY236900
CBS115812	*L. gonubiensis*	*Syzygium cordatum*	South Africa	D. Pavlic	DQ458877
CF/UENF427	*L. theobromae*	*Persea americana*	Brazil	P. Santos	KY223707
CBS 164-96	*L. theobromae*	Fruit on coral reef coast	New Guinea	A. Aptroot	AY640258
CMW8230	*Diplodia seriata*	*Picea glauca*	Canada	J. Reid	DQ280418
CMW8230	*D. seriata*	*Malus domestica*	South Africa	W. A. Smith	DQ280419

Table 3. List of *Lasiodiplodia theobromae* isolates from 'Hass' avocado fruit

Isolate	Specie[a]	Host (*Persea americana* Mill.)	Origin (Dep-Prov)[b]
1BAR_EF	*L. theobromae*	'Hass'	Lima - Barranca
2BAR_EF	*L. theobromae*	'Hass'	Lima - Barranca
3BAR_EF	*L. theobromae*	'Hass'	Lima - Barranca
4BAR_EF	*L. theobromae*	'Hass'	Lima - Barranca
5BAR_EF	*L. theobromae*	'Hass'	Lima - Barranca

[a] *L. theobromae* from avocado tree were determined based on morphology and phylogenetic analyses.

[b] Dep = Department and Prov = Province

Distribution of stem-end rot in the canopy of 'Hass'avocado fruit

Two experiments were conducted in Barranca to analyze the distribution of SER in the internal and external part of the canopy in 'Hass' avocado fruits after being harvested. A total of 400 avocado fruits were collected from 40 trees; for each tree, ten avocados were selected, five from each part of the canopy. The collected avocado fruits had an exportable fruit size, categorized as number 16, whose dry matter value was between 23 % and 24 %. Avocado fruits were placed in a plastic container (40 cm x 30 cm x 40 cm) as a moist chamber with a humid paper towel at the bottom, and each fruit was placed above a Petri dish to avoid contact with the paper towel. The avocado fruits were stored at 22 °C in dark conditions, and after 14 days, they were analyzed by cutting the fruit down the middle lengthwise. Typical symptoms in the avocado fruit were counted as infected ones. Evaluation of the severity of the plant disease in avocado fruit was classified in five grades (G) depending on the percentage of damage: G0= 0 %, G1 = 1 % to 5 %, G2 = 6 % to 25 %, G3 = 26 % to 50 % and G4 = >50 %.

Additionally, three experiments were conducted to understand the influence of the fruit position where the canopy was divided in three sections in the presence of SER in Cañete. The avocado tree canopy was divided into three sections: high (H), middle (M), and low (L). Four avocado trees were selected arbitrarily for the experiment, and four avocado fruits were collected for each section of each tree. The size and storage of the collected fruit and the evaluation of the incidence of SER were as it was described previously.

Statistical Analysis

The data obtained from the evaluation of SER on 'Hass' avocado fruit were recorded and tabulated in an Excell spreadsheet document. This data was analyzed by SAS (Statistical Analysis System version 9.4, Cary, NC). The percentage of avocado stem-end rot data obtained in the experiment was tested by one-way analysis (ANOVA) with the PROC GLM command. In addition, the means were compared with Tukey analysis with a significant level of 0.05. Homogeneity and normality were assessed and satisfied.

Results and Discussion

Development of avocado stem-end rot on 'Hass'avocado fruits

Dark necrosis in the peduncle area developed once the plant disease started developing in the 'Hass' avocado fruit. The fungus colonization was usually initiated from the stem-end of the fruit to the whole fruit, and it was faster in the core than the rind. SER was capable of developing soft brown to black decay symptoms in the entire fruit (Figure 1).

Morphological and molecular characterization

A total of 5 isolates were obtained from Barranca. These isolates were used for morphological characterization. *Lasiodiplodia* was identified following a taxonomical key made by Barnett &

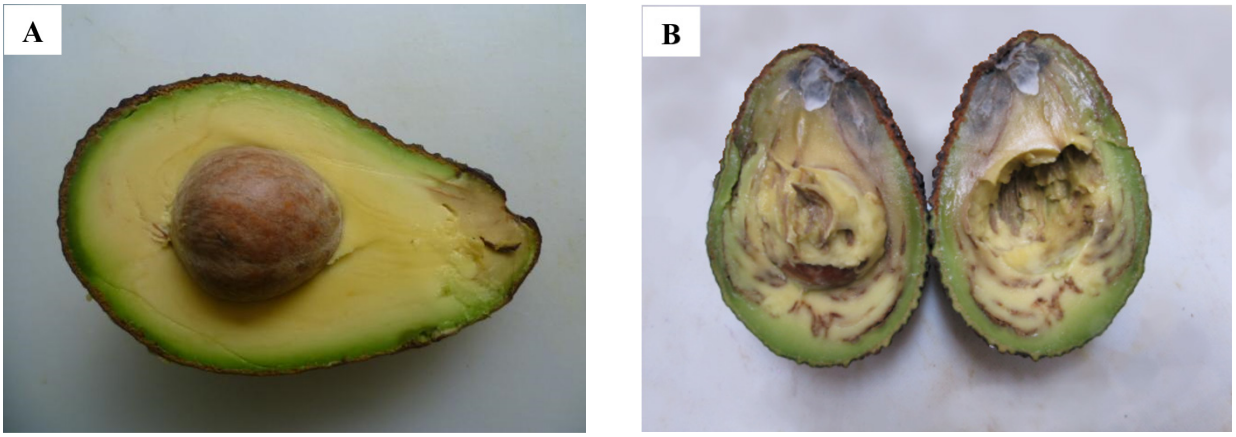

Figure 1. A. Initial symptoms and **B.** advanced infection of SER in 'Hass' avocado fruits.

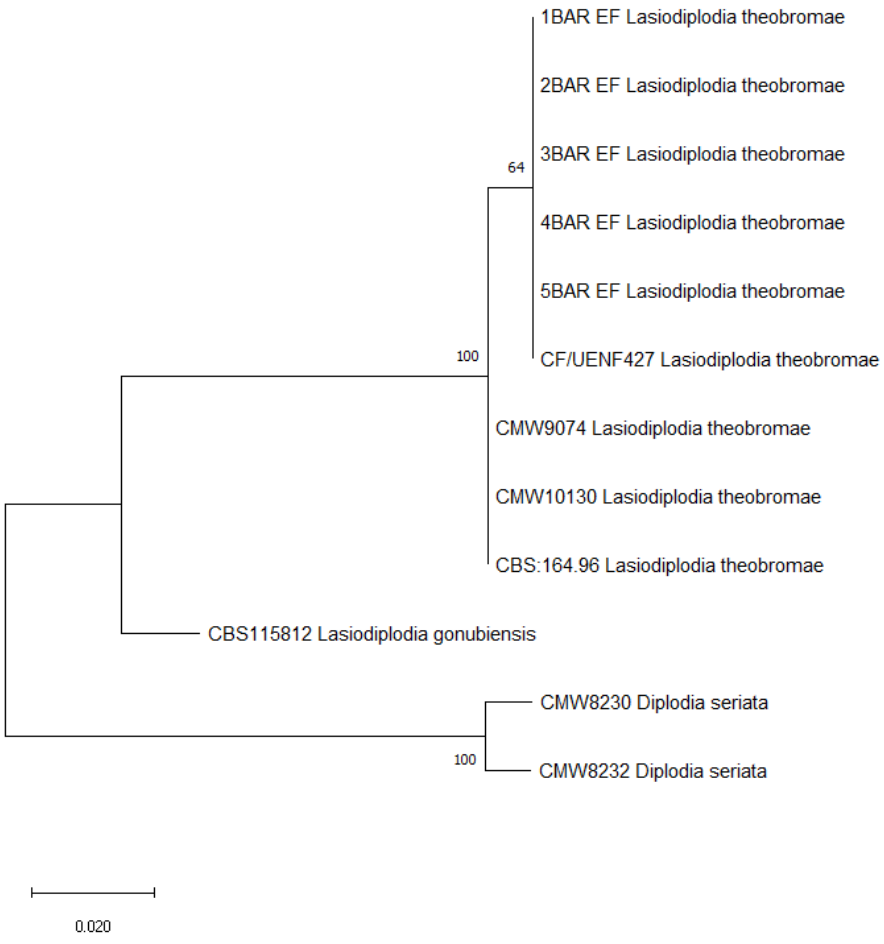

Figure 2. Phylogeny of *Lasiodiplodia theobromae* based on analysis of partial sequences of Translation Elongation Factor 1-alpha (EF-1α). Support bootstrap values were obtained by using 1000 replicates generated in MEGA-X v.10.2.2. The phylogeny was constructed using the genus *Diplodia* as the outgroup.

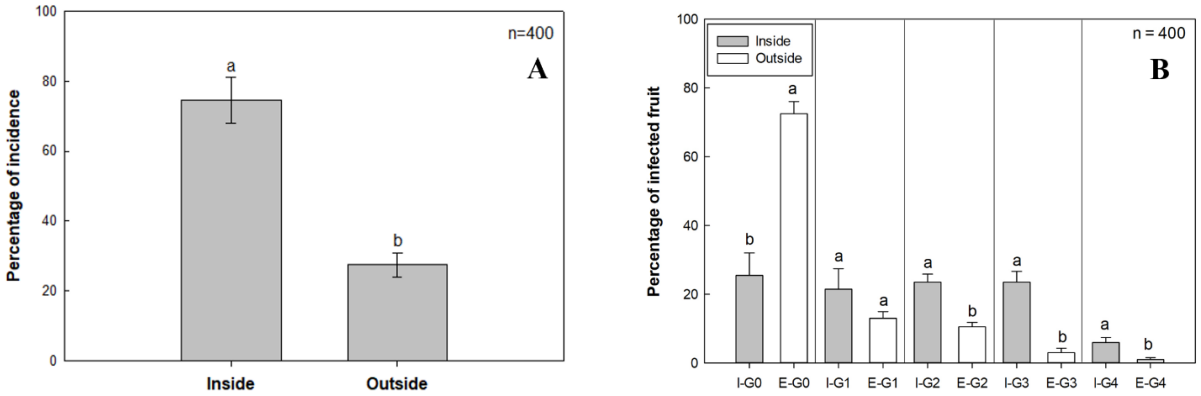

Figure 3. **A.** Effect of the position of 'Hass'avocado fruit in the incidence and **B.** severity grade of SER on the canopy of 'Hass' avocado fruit. I = Internal, E = External, G = Severity grade of SER. The experiments were conducted in the northern region of Lima (Barranca). Lines in the graphs show standard deviations.

Hunter (2006). Besides, the isolate had similar features to the description that was made by Punithalingam (1976) in terms of the appearance of the colony and the conidia. In addition, we compared the results with other previous studies (Pereira et al., 2009; Úrbez-Torres et al., 2006). Furthermore, in this study, five genomic DNA from five isolates were examined. The sequence was compared in GenBank, and these isolates were assigned to *Lasiodiplodia theobromae*, matching morphological features. The phylogenetic tree included two clades notoriously different (Figure 2). *L. theobromae* isolates showed a bootstrap support value of 100%. *Lasiodiplodia* isolates collected in Barranca were put together in the same group with isolates that were collected in Mexico, Uganda, Brazil, and New Guinea. *L. gonubiensis* and *Diplodia seriata* were classified in a different group.

SER of avocado constitutes a severe problem in the industry because it restricts its commercialization after harvesting. This disease was reported caused by several fungal species, mainly of the Botryosphaeriaceae family (Twizeyimana et al., 2013; Wanjiku et al., 2020). Fungal species population studies of this plant disease in our region is scarce, and to our knowledge, this is the first attempt to identify the causal agent. The isolation of *L. theobromae* from avocado fruits in the study with SER symptoms coincides with other reports where this pathogen was reported (Darvas & Kotze, 1987; Menge & Ploetz, 2003). *L. theobromae*, an ascomycete, is prevalent in tropical and subtropical zones, infecting more than 500 species worldwide (Punithalingam, 1976, 1980). Besides, the presence of this fungus on fruits concurs with other reports of *L. theobromae* such as citrus fruit (Zhang, 2014), blueberry (Xu et al., 2015), Mamey Zapote (Tovar-Pedraza et al., 2012), and Mango (Munirah, 2017). The explanation for identifying *L. theobromae* in the isolates studied could be that this fungal pathogen could be prevalent in our country, as reported in others such as Israel (Menge & Ploetz, 2003). Nevertheless, *L. theobromae* could be absent in other regions, including the US (Menge & Ploetz, 2003; Twizeyimana et al., 2013). Thus, further analysis with a significant number of isolates needs to be done to identify and understand the fungal population species related to stem-end rot in avocado fruits in Peruvian conditions.

Distribution of stem-end rot in the canopy of 'Hass' avocado trees

Evaluation of the incidence of SER in the canopy of 'Hass' avocado fruit showed a statistical difference between internal and external positions ($P = <0.001$). The highest incidence was shown in the interior area, where the mean incidence of SER was 37.25 % ± 6.5 %; a lower value was obtained in the external site whose mean value was 13.75 % ± 3.5 % (Figure 3A). These results

showed that the position of the fruit on the canopy influences the incidence of this pathogen. An additional analysis was conducted where the grade of the damage of this pathogen in the fruit was measured (Figure 3B). The percentage of fruit with Grade 0 (Fruits without symptoms) was higher in the external than the internal part of the canopy ($P = <0.001$). Conversely, evaluation of the severity of SER showed that mean frequency where higher in all the grades evaluated (G1, G2, G3, G4), showing a statistical difference in all of them except for G1.

Additionally, three experiments were conducted to study the influence of fruit-position height on the percentage of incidence of SER. In the first and second experiments, the highest incidence value of SER was obtained at the low section of the canopy (Figure 4A and 4B). Nevertheless, only one experiment showed a statistical difference between the low and high sections ($P = 0.03$). Besides, in neither of the cases, the middle and low positions showed a statistical difference. The third experiment was performed to evaluate the grade of severity of this pathogen at different sections (Figure 4C). The percentage of avocado fruits without symptoms (G0) decreased from high to low section. No difference was found evaluating the grade (G1, G2, G3, and G4) of the severity of SER in different sections. Interestingly avocado fruit at middle and low section only showed a grade of severity G3 and G4 for each section respectively.

In the study, fruits on the internal part of the canopy showed a statistical difference with fruits that are located on the external site. In addition, the severity grade of SER was higher in the internal part than the external for all the categories, except grade 0 (G0). These results could be supported by a previous study where it was found that the firmness in some cultivars was higher in avocado fruits exposed to the sun than the shaded fruit (Woolf et al., 2000). Also, the concentration of antifungal compounds diene after harvesting could affect the infection of SER in the flesh fruit. It was reported that, even though the initial content of diene was similar in shade and sun fruit, the concentration of diene in the fruit flesh decreased faster in shade fruit

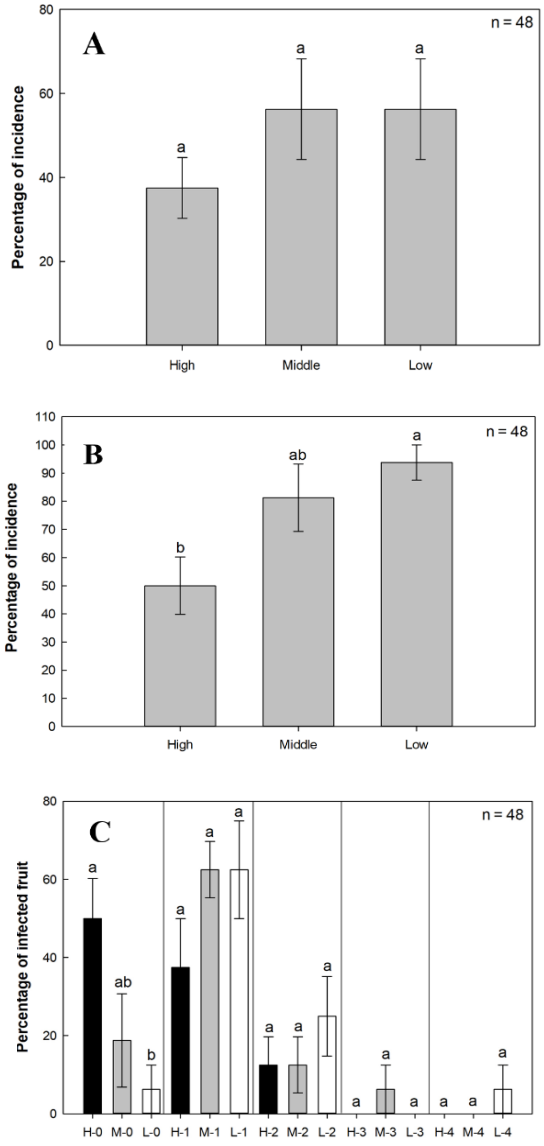

Figure 4: A. and B. Effect of the position of the 'Hass' avocado fruit in the incidence in two experiments and **C.** severity grade of 'Hass' avocado fruits on different fruit-position height of the canopy. H= High; M= Middle and L=Low. The experiment was conducted in the southern region of Lima (Cañete). Lines in the graphs show standard deviations.

than fruits exposed to the sun when they were stored at 20 °C. However, diene concentration in the harvested fruit peel does not vary seven days after being harvested. After this period of time, it follows a different pattern; this compound increases in sun fruits at a double rate than shaded fruit (Woolf et al., 2000). Additionally, similar findings concur with an experiment conducted in mango where fruits exposed to sunlight, whose

level of anthocyanin was higher, presented less fungal development than fruits located in the internal part of the canopy (Diskin et al., 2017; Sivankalyani et al., 2016).

Regarding the influence of the fruit-position fruit on SER incidence, there was no statistical difference between the middle and low section in the plant's canopy. Interestingly, fruits located at high section showed the lowest SER incidence compared to the low and middle section. However, the high section only did show statistical differences with the low section in one experiment. Because of logistics, we were not able to collect more fruits per plant. That is why for further research, we suggest increasing the number of avocado fruits in future experiments to get stronger statistical results for this evaluation. A popular explanation that could explain this is that conidia from branches cannot reach the high part of the canopy. An additional study evaluating conidia at different sections of the canopy should be performed to corroborate this hypothesis. As mentioned previously, the exposition of fruits to the sunlight could have been influenced the results; fruits located at the low section could have been receiving less sunlight than the middle and high sections, affecting the presence of SER. Pruning has been considered good practice to reduce the impact of this disease in harvest conditions (Galsurker et al., 2018). As described, the lack of adequate pruning practice on the avocado experiment areas could have reduced the exposition of fruits for internal and low sections of the canopy, triggering feasible conditions for SER. Consequently, inadequate pruning could negatively impact the effectiveness of chemical applications if this practice wants to be integrated to manage this pathogen because not a good application coverage could be reachable under these circumstances. A variation of the avocado tree could have influenced our data. It is reported that the age of the tree and holding time influence the prevalence of SER on other crops (Brown & Miller, 1999; Zhang, 2014).

Conclusions

Our study identifies *Lasiodiplodia theobromae* as potential pathogens causing SER in 'Hass' avocado fruit. Besides, our findings demonstrate that fruits located in the internal and low part of the canopy had less presence of SER than Hass avocado fruits positioned in the external, middle, and high parts, respectively. These findings help understand the distribution of SER on the canopy of avocado trees, giving valuable information to enhance strategies management by providing information about which canopy areas must be fully and well protected. These future strategies management could diminish the damage of this fungal pathogen whose infection affects the commercial product during postharvest, causing significant economic losses in agriculture.

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