**Characterization of the lenticel damage of avocado cv. Hass and its associated fungal communities**

Sandra Mosquera1, Carolina Cataño1, Susan Saavedra2, Valeska Villegas-Escobar1\*

1 *CIBIOP Research Group, Biological Sciences Department, Universidad EAFIT, Carrera 49 No. 7 Sur - 50, Medellin, Colombia*

*2 Cartama….*

*\*Corresponding author, [vvilleg2@eafit.edu.co](mailto:vvilleg2@eafit.edu.co), ORCID ID: 0000-0002-9636-3644*

**Abstract**

The lenticel damage reduces the quality of avocado cv. Hass, leading to high fruit rejection rates and losses for the growers. The causes of this affectation are not fully understood, but mechanical stress and phytopathogens have been associated with the damage, suggesting that abiotic and biotic factors are involved. This study evaluated several aspects of the lenticel damage of avocado cv. Hass to unravel the nature of its causality. We followed the lenticel damage in two Colombian farms for two years and found that spatiotemporal factors influence the damage as it is non-uniform and progressive. Then, we used NGS to characterize fungal communities of fruits with severe and mild injuries and found that these communities varied between farms. We also found that the damage severity influenced the composition of the fungal communities as some taxa were more common in avocados with severe damage. Finally, we isolated different fungi from necrotic lenticel to see whether we found some of these enriched taxa. Still, the isolated fungi did not coincide, showing that standard culturable techniques fail to capture the complexity of the fungal communities associated with the lenticel damage.

**Keywords:** Persea americana, Next-generation sequencing, lenticelosis, *Pseudocercospora*, *Colletotrichum*

**Introduction**

Colombia produced nearly 155.3 thousand t of avocado cv. Hass in 2021 [1]. However, the yield of Colombian crops (9.0 t/ha) is below the potential yield (30 t/ ha) estimated for these crops [2]. Several factors are responsible for these low yields, including the lenticel damage [3, 4]. This damage consists of 1 mm to 5 mm-long necrotic spots that develop on the exocarp of fruits around lenticels [5, 6] and can affect fruits during harvest but is more limiting during the post-harvest [3, 4]. The damage severity influences the fruits' value and destination [7] and is responsible for 5% to 30% of the rejected fruit [6].

The lenticel damage causes are controversial, but two hypotheses seem possible. The first one relates to mechanical stress suffered by fruits during harvest and post-harvest [4, 8]. According to this hypothesis, impact injury promotes polyphenols production leading to tissue oxidation and necrosis [4]. The other hypothesis argues that pathogenic fungi are responsible for the damage. Accordingly, Fungi such as *Pseudocercospora purpurea*, *Colletotrichum* spp., *Neofusicoccum parvum, Phomopsis* spp.,and *Dothiorella* spp. have been isolated from necrotic lenticels of avocados [6, 9–13], and fungicide application during the harvest reduces the damage severity [10, 14]. Despite the attempts to clarify the damage causality, the results are inconclusive. The necrosis resulting from mechanical stress and fungal infections differ at the cellular level, but differences are not pronounced and can be mistaken [4, 15]. Also, most studies reporting pathogenic fungi isolation have failed to replicate symptoms on healthy fruits [9, 10, 12, 16].

All the above show that lenticel damage of avocado cv. Hass is poorly understood. This lack of understanding complicates the design of strategies to mitigate losses. Here, we evaluated some of the unknown aspects of the lenticel damage. We hypothesized that plant pathogens have a role and expected the damage to be non-uniform and progressive, with spatiotemporal components determining its occurrence. We also anticipated that fungal communities associated with fruits with lenticel damage would differ from those of healthy fruits. These communities would be enriched in plant pathogenic taxon, which could be isolated. We first characterized the damage behavior across harvest in two farms with distinctive agroclimatic characteristics to test these hypotheses. Then, we assessed the damage progression during the post-harvest. Finally, we used next-generation sequencing to characterize the fungal communities associated with mild and severe lenticel damages and contrasted the findings with fungal isolations.

**Material and Methods**

**Study area and sampling**

Lenticel damage was evaluated in two farms with different levels of affection in Colombia from June 2019 to June 2021. La Escondida farm has low levels of lenticel damage, while El Sinaí farm has high (Supplementary Table 1).

Thirty trees with similar height, phenology, and age, were selected from five and nine plots in La Escondida and El Sinai, respectively (Supplementary Table 2). Ten fruits per tree were collected to assess the lenticel damage in each harvest (300 fruits/farm). Twenty additional fruits were sampled from each farm during the main harvest of 2019 and traviesa harvest of 2021 for fungal isolations, and 12 in the traviesa harvest of 2020 for the microbial ecology analysis. Six of these 12 fruits had severe damage and the others mild damage. All the sampled fruits were packed in punnets and carried to the laboratory for analysis.

 Lenticel damage estimation

The lenticel damage was evaluated by analyzing fruits' photographs in a macro developed in FIJI [17]. Briefly, fruits were photographed on each face (two photos/fruit). The macro used photographs to estimate the incidence (number of necrotic spots) and severity (necrotic area percentage (area of necrosis/fruit surface area)) of the damage. Then, the macro averaged the data of both faces for each of the variables.

Lenticel damage was evaluated at 0 days post-harvest (0 dph). Then, fruits were immersed in Timorex Gold Ⓡ (Stockton) at two ml/l and stored at 6°C, simulating post-harvest conditions of commercial fruits. A second measurement was performed after 21 days (21 dph). Differences in damages severities and incidences between farms and between over-time measurements (0 dph and 21 dph) were evaluated using mixed-effect analyzes. The models assessing differences between farms included the interaction between farm and harvest as the fixed effect and, as random effects, intercepts for plot and the nested effect of trees in plots (Supplementary Table 3). The models evaluating the difference between measurements included the interaction between measurement and farm as the fixed effect and intercepts for harvest and fruit as random effects (Supplementary Table 4).

 Linear mixed-effects models (lmer) were used for analyzing the severity, and the data were transformed with the logarithm of the severity plus one. Generalized linear mixed models with the Poisson family (glmer (family = Poisson)) were used for analyzing the incidence with no data transformation. Visual inspection of models showed no deviation from linearity, homogeneity of variance, or normality. Complex models were compared with simpler models to assess the contribution of fixed effects, using the *likelihood* *ratio* test with a confidence level of 95% (p-value: 0.05). These analyses used *lmer* and *glmer* functions of the R library *lme4* (version 1.1-26) [18] and the *anova* function of the R library *stats* (version 4.0.4) [19]. The results were visualized using the R library *ggplo2* (version 3.3.3) [20].

**DNA extraction and sequencing**

 DNA was extracted from avocados exocarp with mild and severe lenticel damage to characterize the fungal communities using a modified protocol [21]. Exocarp samples were homogenized, and 100 mg of homogenized material was washed twice with one ml of the prewash buffer**.** Samples were lysed using one ml of the lysis buffer and one h of incubation at 65°C. The DNA was separated with one ml of chloroform:isoamyl alcohol (24:1) (Sigma-Aldrich), and precipitated with 0.1 volumes of 3 M sodium acetate pH 5.2 (Amresco), 0.66 volumes of isopropanol (ITW Reagents) and overnight incubation at -20°C. The DNA was washed twice with 70% ethanol (Sigma-Aldrich), and the DNA was resuspended in 50 µl of TE buffer (Biobasic). DNA concentration was quantified using a Qubit fluorometer (Thermo Fisher Scientific) with the Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific). The DNA quality and integrity were verified by spectrophotometry and electrophoresis.

DNA suspensions were sent to BaseClear (Holland) for paired-end sequencing of fragments of the internal transcribed spacer (ITS) of the ribosomal DNA region in the Ilumina's Miseq platform. Chloroplast and mitochondrial blocking primers were employed during the sequencing, and the sequences demultiplexed in the FASTAQ format were obtained from the company. The company performed the quality controls and normalization of the samples and prepared the Illumina libraries.

**ITS amplicon analysis**

Filtering, de-replication, removal of chimeras, and pairing of forward and reverse ITS sequences were done using the opensource program DADA2 (version 1.18.0) [22]. These processes ensured a minimum of 10 bp overlapping between sequences and used a quality score above 30. Primers sequences were removed using Cutadap (version 3.1) [23]. Sequences were assigned to amplicon sequence variants (ASVs), retaining unique sequences occurring in several samples. The taxonomic identity was assigned to the ASVs in Qiime2 (version 2020.11) using the *q2-feature-classifier* with the *classify-sklearn* method and the Unite databases with 97% dynamic grouping and 99% (version 8.3) [22, 24, 25]. The dataset was trained with the Qiime2 (version 2020.11) *q2-feature-classifier* using the Naive Bayes classifier method [25]. The ASVs tables were filtered to exclude mitochondrial, chloroplast, and arcuate archaeal sequences with the *filter-table* functionality of Qiime2 (version 2020.11) [25]. The resulting tables were used for the analyses.

**Composition and diversity of fungal communities**

Alpha diversity metrics (Richness, Shannon, and Faith's phylogenetic diversity indexes) were calculated using the *core-metrics-phylogenetic* and alpha diversity methods of Qiime2 (version 2020.11) with a rarefication depth of 18145 sequences considering rarefaction curves [25]. A unidirectional anova was used to compare the alpha diversities between fruits with mild and severe damages using the *anova* function of the R library *stats* (version 4.0.4) [19].

A principal coordinate analysis (PCoA) and a principal coordinate canonical analysis (CAP) analysis constrained to the damage strength (mild and severe) were used to compare the fungal communities (beta diversity). The weighted-UniFrac-distance metric and the ordinate function of the R library *phyloseq* (version 1.34.0) were used for these analyses [26]. ASVs tables were normalized using the *cumNorm* function of the R library *metagenomeSeq* (version 3.4) with the CSS (cumulative-sum scaling) method [27]. Differences between fungal communities were evaluated with permutational multivariate analysis of variance with the *adonis* and *anova.cca* functions of the R library *vegan* (version 2.5-7) [28]. Visualization of principal component biplanes was done using the *plot\_ordination* function of the R library *phyloseq* (version 1.34.0)[26].

To assess whether some fungal taxa were differentially abundant in the fungal communities of fruits with mild and severe damages. ASVs tables were filtered using the function *calculateEffectiveSamples* from the R library *metagenomeSeq* (version 3.4) and normalized with the CSS method. A Zero-Inflated Gaussian Distribution Mixture Model was applied using the *fitZig* function from the R library *metagenomeSeq*. The model coefficients were compared with moderated t-tests using the functions *makeContrasts* and *eBayes* from the R library *Limma* (v.3.46.0)[27]. P-values were adjusted with the Benjamini–Hochberg correction method, and taxa with adjusted *p-values* below 0.05 were considered differentially abundant. The taxonomic relation and relative abundance of enriched ASVs were visualized using the *plot\_tree* of the R library *phyloseq* (version 1.16.2) and the *ggplo2* library of R (version 3.3.3) [20, 26].

**Isolation of fungal strains from healthy and necrotic lenticel**

Samples of nearly 25 mm2 containing healthy or necrotic lenticels were taken from avocados exocarp for fungal isolation. Samples' surface was disinfected with 2% sodium hypochlorite (ProtoKimica) and 70% ethanol (ProtoKimica). Samples were seeded in 50% potato dextrose agar (PDA) (Alpha Bioscience) plates and incubated at 30°C. Growing mycelium was subjected to multiple passages in 50% PDA to obtain pure colonies, and isolates were stored in 20% glycerol (ITW reagents) at –80°C and activated in 50% PDA.

**Molecular identification of fungal isolates**

 DNA of fungal isolates was extracted from 48 h-Sabouraud (Merck) cultures using DNeasy Powersoil kit following manufacturer's indications (Qiagen). The extracted DNA was used to amplify 550 bp fragments of the ITS region using ITS1 (5'-TCCGTAGGTGAACCCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3') primers and 25 µl PCR reactions of GoTaq® Green Master (Promega) according to manufacturer's instructions. The PCR products were sequenced with Sanger sequencing using the same primers (ITS1 and ITS4). Sequences were processed, and their taxonomic identity was assigned using the Basic Local Alignment Search Tool (BLAST) and the NCBI database in the platform *Geneious Prime* (version 2020.2.4) (https://www.geneious.com).

**Results**

**Lenticel damage varies between farms and harvest**

To characterize the behavior of lenticel damage, avocados cv. Hass were collected during main and traviesa harvests between June 2019 through June 2021 in two commercial farms. The severity and incidence of the damage at 0 dph depended on the farm and harvest (p-value < 0.001), with La Escondida fruits having overall lower (< 0.6-fold) incidences and severities than El Sinai fruits in most harvests (Fig. 1, Supplementary Table 3). The exception was the main harvest of 2020, in which El Sinai fruits had slightly lower severities and incidences than those of La Escondida f(Fig. 1, Supplementary Table 3). At 0 dph, La Escondida fruits had severities of 0.5% on average, with a maximum of 5.3%. El Sinai fruits had severities around 1.0 %, with a maximum of 8.1%. Regarding the incidence, La Escondida fruits had on average 46 necrotic spots, with the most affected fruit having 300, while El Sinai fruits had on average 85 necrotic spots and a maximum of 520.

The lenticel damage was most damaging during both traviesa harvests in El Sinai (Fig. 1 and Supplementary Table 3). These harvests also had the highest daily precipitation and relative humidity (Supplementary Table 1). In La Escondida, the traviesa harvest of 2020 also had fruits with high affections, high daily precipitation, and relative humidity. However, this farm's traviesa harvest of 2021 had mild lenticel damage despite having high daily precipitation and relative humidity. These results show that the lenticel damage varies between harvest and farms, suggesting a spatiotemporal component affected by climatic variables. This spatiotemporal component was also present at the tree and plot level, with 5 % and 7 % of the damage incidence and severity variation not explained by the farm or harvest but resulting from differences between fruits of different trees and plots (Supplementary Table 1, Fig. 1).

**Lenticel damage increases during cold storage**

Commercial avocados cv. Hass undergo cold storage (6⁰C) during the post-harvest. Some evidence shows that the damage increases during this storage as marketers report receiving fruits with damages more severe than those evidenced by packing plants [8].

The lenticel damage severity and incidence increased after 21 d at 6⁰C, regardless of the farm and harvest. The increments were higher for El Sinai fruits than La Escondida fruits (Fig. 2, Supplementary Table 4). The harvest influenced the damage to some extent, as 20% of the lenticel damage differences unexplained by the farm and cold storage came from differences between harvests (Supplementary Table 4).

**Fruits from different farms have distinct fungal communities, and the lenticel damage influences their structure**

Lenticel damage has been associated with mechanical stress suffered by fruits during harvest and post-harvest [4]. However, the spatiotemporal component of the lenticel damage and its increases during cold storage suggest that other factors are also involved. Fungal pathogens might be among those factors[4, 10, 11]. To assess this association, we characterized the fungal communities of fruits with mild and severe damages in both farms. Although fruits were selected attempting to maximize differences between severe and mild damages, the differences were more pronounced in El Sinai fruits (Supplementary Fig. 1).

Fungal communities of avocado cv. Hass consisted of 370 to 583 AVSs with an average of nearly 450 AVSs (Fig. 3A). These communities had Shannon diversities between 4.4 and 6.5 (Fig. 3B), and Fath's PD between 43.3 and 67.1 (Fig 3C). Farm or severity of the damage did not affect communities richness, Shannon diversities, or Fath's PD (*p-value* > 0.05), except for El Sinai, in which severe damages associated with richer fungal communities (Fig. 3).

Fungal communities were separated into distinct groups in the PCoA and CAP biplanes (Fig. 4). 70.1% of the community's variation was related to the combined effect of farm and damage severity according to the permutational multivariate analysis of variance (*p-value*: 0.001). Nonetheless, the farm was the main separation driver, explaining 56.6% of the overall variation between populations (*p-value*: 0.001). The extent of the damage, on the other hand, was barely significant (*p-value*: 0.097) and less pronounced. It explained only 9.0% of the community's variation.

**Some fungal taxa are common in fruits with severe lenticel damage**

Two phyla, 23 classes, 67 orders, 168 families, and 278 genera composed the fungal communities of avocados cv. Hass. Many of these taxa (17 classes, 34 orders, 74 families, and 97 genera) were shared between fruits regardless of the farm or damage severity (Supplementary Fig. 2). On the other hand, few taxa were unique to the fungal communities of severely damaged fruits (Supplementary Fig. 2). One commonality between these fruits was their increased relative abundances of Ascomycetes (between 36.2 % and 73.7 %), compared with mildly damaged fruits (between 17.8 % and 59.9 %) (*p-value*: 0.001, Fig. 5A). The opposite occurred for Basidiomycetes. Basidiomycetes relative abundances were higher in mildly damage fruits (between15.4 % and 49.6 %), compared with fruits with severe damages (between 2.6 % to 21.5 %) (*p-value*: 0.004, Fig. 5A).

Genera in these Ascomycetes and Basidiomycetes communities depended on the farm and damage extent (Fig. 5B and 5C). However, some trends occurred between farms. The Ascomycete genera *Trichomerium*, *Pseudocercospora*, and *Colletotrichum,* had increased relative abundances in fruits with severe damages, while *Hyphozyma* and *Cladosporium* in mildly damaged fruits (Fig. 5B). Considering each farm, the Ascomycetes *Pestalotiopsis*, *Geastrumia*, *Cyphellophora* and *Chaetothyrina*, and Basidiomycetes *Saitozyma*, *Meira*, *Ceraceosorus*, and *Bulleribasidium* were increased in El Siani fruits with severe damage (Fig. 5B and 5C). Whereas Ascomycetes *Setophoma*, *Pleurophoma*, *Meyerrozyma* and *Diaporthe*, and Basidiomycetes *Moniliopthora*, *Kockovaella*, *Itersonilia*, *Gjaerumia*, *Derxomyces*, and *Cystobasidium* were prevalent in La Escondida (Fig. 5B and 5C).

Seven ASVs were enriched severely damaged fruits in La Escondida and 21 in El Sinai ( *p-value* < 0.05, Fig. 6 and 7, Supplementary Table 5 and 6). La Escondida enriched ASVs included one Basidiomycete of the genus Cystobasidium (ASV\_30) and two ascomycetes of the genera Setophoma (ASV\_24) and Bacidina (ASV\_18). The remaining four were not classified, but three of them (ASV\_1, ASV\_42, ASV\_41) clustered with ASVs of the class Dothideomycetes (Cluster III) and one (ASV\_31) with the ASVs classified as Cystobasidium sp. (Cluster XII) (Fig 6 and Supplementary Table 5). The Ascomycota classes Eurotiomycetes (Cluster II), Dothideomycetes (clusters III and IV), and Sordariomycetes (Cluster V) were common among enriched ASVs severely damaged fruits in El Sinai. Enriched ASVs in these classes belonged to the genera Trichomerium (ASV\_129, ASV\_92, ASV\_110), Pseudocercospora (ASV\_14), Geastrumia (ASV\_40) and Colletotrichum (ASV\_124; ASV\_65, ASV\_114). The genus of the other Ascomycetes was unidentified. Two of the remaining enriched ASVs in El Sinai (ASV\_179, ASV\_145) cluster with Basidiomycetes in Cluster VIII, but their genus was undefined. Only one enriched ASVs (ASV\_96) in El Siani belonged to a phylum other than Ascomycota and Basidiomycota. However, the taxonomy of this ASV is unknown (Fig 7 and Supplementary Table 6).

**The communities of cultivable fungi are poor predictors of the fungal communities associated with the lenticel damage**

Forty-nine fungal strains were isolated from healthy and necrotic lenticels of avocado cv. Hass and 39 were identified. All isolates were *Ascomycetes*, and the most common genera were *Colletotrichum*, with 19 isolates, and *Cytospora*, with ten. These most common genera were isolated from both farms. *Alternaria*, *Diaporthe*, *Neofusicoccum*, *Neurospora*, and *Phyllosticta* were also among the genera isolated, and they had one to three representatives. These genera were restricted to one farm (Table 1; Supplementary Table 7). We found no clear trend between the isolated taxon and the health status of the lenticel. Also, no isolated taxon besides *Colletotrichum* coincided with the ASVs enriched in severely damaged fruits. However, *Colletotrichum* spp. were isolated from healthy and necrotic lenticel.

**Discussion**

The lenticel damage of avocado cv. Hass is poorly understood, and its causality is unknown. Two hypotheses are considered the most likely, one related to mechanical stress suffered by the fruits and the other to plant pathogens. We found that the lenticel damage has a non-uniform pattern and is progressive. The severity and incidence of the damage changed across trees, plots, farms, and harvest and increased during cold storage. We also found that fungal communities associated with fruits varied from one farm to another, and the lenticel damage influenced these communities' composition to some extent. Some taxa were more prevalent in fruits with severe damages. However, most enriched taxa were not among the fungal strains isolated from necrotic lenticels.

Damages resulting from non-living and living factors differ in their patterns. Those caused by non-living factors such as impact injury have uniform patterns. On the contrary, damage caused by living factors such as fungal pathogens has non-uniform patterns [29]. A spatial-temporal component influenced the lenticel damage at different scales. The damage varied not only between farms but between plots and trees. This scale variability suggests that the physical environment restrains whatever is causing the damage [30]. The lenticel damage also varied across harvests, with the most severe damages occurring in harvests with the highest humidity and precipitation. These observations further suggest that the damage responds to the physical environment, supporting a plant pathogen involvement [30]. Other evaluations have also evidenced the temporal variation of the lenticel damage and its association with high humidity and precipitation [10, 31]. These evaluations attribute this association to climatic conditions that favor pathogen proliferation. The association between high humidity, rain, and *Colletotrichum* proliferation is well documented [32–34]. However, other evaluations have also demonstrated that high humidity makes avocado fruits more vulnerable to lenticel damage by mechanical injury [6].

We saw an increase in the lenticel damage during cold storage. The damage almost doubled during this period in which the fruits were not subjected to further mechanical stress. The damage progressive nature has been reported before and is another evidence suggesting plant pathogen or pathogen-complex involvement [8]. Damage caused by a living organism is progressive, while those caused by non-living factors are not [29]. The observed increments varied depending on the harvest and were more pronounced in El Sinai fruits. These observations are not consequent with the uniform pattern of damages caused by non-living factors [29].

We found variation in fungal communities associated with fruits' exocarp between farms. The observation of geographical location affecting fruits' microbial communities is not novel [35, 36]. A study evaluating apples’ communities at different spatial scales demonstrated that communities were similar across tissues but varied between orchids and growing regions [36]. Environmental conditions likely influence the fungal community's assembly.

We saw variation in fungal communities between mildly and severely damaged fruits. However, differences were less evident than those between farms. Several studies have evaluated the association between microbial communities assembling and plant health. These studies have shown that diseased fruits and other plant tissue have distinct communities [37–41]. relative abundances. These observations align with those of other evaluation reporting relative abundances between 50 to 100 % for Ascomycota and below 10 % for Basidiomycota [36, 38, 40]. An interesting finding was the low and high relative abundances of Ascomycetes (close to 29 %) and Basidiomycetes (close to 29 %) in avocados with mild damages. The dominance of Basidiomycota might indicate a low probability of lenticel damage development.

Some fungal genera such as *Trichomerium*, *Pseudocercospora*, and *Colletotrichum* were more common in severely damaged fruits regardless of the farm. These observations agree with several studies that have isolated *Pseudocercospora* and *Colletotrichum* from necrotic lenticels [6, 9, 10, 13]. Studies about *Trichomerium* are less common, but this genus was recently included in the fungal complex responsible for the sooty blotch and flyspeck [42]. This complex comprises over 100 fungal species and produces symptoms like lenticel damage in several crops [43]. Avocado might be among these crops, but more evidence is necessary [44].

The extent of the association between the lenticel damage and fungal community composition and diversity varied between farms and was most pronounced in El Sinai. Severely damaged fruits in this farm had distinct fungal communities, which were more diverse. This finding was unexpected as higher diversity is usually associated with healthy plants [37, 41]. The association between this increased diversity and the lenticel damage is unknown. However, it might come from saprophytes or other plant pathogenic fungi colonizing advanced damages [4, 10]. We found that these distinct communities were enriched in several Dothideomycetes, Eurotiomycetes, and Sordariomycetes, fungal classes that have been associated with diseased plants in other systems [37, 39]. Differences between mildly and severely damaged fruits' fungal communities were less pronounced in La Escondida. These communities were comparable in their alfa diversities, and some of them were indistinguishable between mildly and severely damaged fruits. However, differences between the mild and severe damages in this farm were not as remarkable as in El Sinai. More significant damages might relate to greater effects on the fungal communities, but more evaluations are necessary to test this hypothesis.

We isolated different fungi from healthy and necrotic lenticel to see whether some of the taxa enriched in the fungal communities of avocado with severe and mild lenticel damage could be isolated. Several of the isolated fungi coincided with those isolated by other authors from diseased avocados [6, 9–13, 45–48]. However, we found no clear trend between the lenticel health status and the isolated taxon. Also, isolated fungi did not coincide with the enriched taxa, with *Colletotrichum* being the exception*.* Our results showed that fungal communities associated with the lenticel damage are complex, and more than one species likely cause the damage. This scenario would explain the impossibility of several works to recreate the lenticel damage symptoms in healthy fruits [9, 10, 12]. Most studies have used culturable techniques like ours. Culturable methods in these evaluations might have failed to capture the complexity of fungal communities responsible for the damage. Our study presents evidence supporting the hypothesis that lenticel damage has biotic components. The damage cannot be fully explained by mechanical stress suffered by the fruit during the post-harvest. However, further evaluations, including comprehensive isolation techniques and pathogenicity tests, are still necessary to fully prove the participation of pathogenic fungi causing the lenticel damage of avocado cv. Hass.

**Acknowledgments**

We want to acknowledge the Universidad EAFIT (Colombia), the avocado exporters CARTAMA, and the Department of Science, Technology, and Innovation (COLCIENCIAS) for funding the project qualified by the National Council of Tax Benefits CNBT (code 7968-869-76148). We also thank the supercomputing resources made available by the Centro de Computación Científica Apolo at Universidad EAFIT. This research was made possible by the "Permit for the Collection of Specimens of Wild Species of Biological Diversity for Non-Commercial Scientific Research Purposes (resolution 1566 of 2014)" given by the National Authority for Environmental Licenses - ANLA of Colombia.

References

1. Procolombia (2021) The Colombian Hass avocado will be present for The Big Game. In: Press Room | PROCOLOMBIA. https://procolombia.co/noticias/en/colombian-hass-avocado-will-be-present-big-game. Accessed 1 Feb 2022

2. Granados W, Valencia JC (2018) Cadena de aguacate. Indicadores e Instrumentos

3. Ramírez-Gil JG, López JH, Henao-Rojas JC (2019) Causes of Hass Avocado Fruit Rejection in Preharvest, Harvest, and Packinghouse: Economic Losses and Associated Variables. Agronomy 10:8. https://doi.org/10.3390/agronomy10010008

4. Zamora-Magdaleno T, Cárdenas-Soriano E, Cajuste-Bontemps JF, Colinas-León MT (2001) Anatomy of damage by friction and by <i>Colletotrichum gloeosporioides<i> penz. in avocado fruit “hass.” Agrociencia 35: 237-244 200 35:237–244

5. Everett KR, Hallett IC, Yearsley C, et al (2001) Lenticel damagae. Avocado Growers Association Annual Research Report, New Zealand

6. Everett KR, Hallett IC, Rees-George J, et al (2008) Avocado lenticel damage: The cause and the effect on fruit quality. Postharvest Biology and Technology 48:383–390. https://doi.org/10.1016/j.postharvbio.2007.09.008

7. CODEX (2013) Norma de CODEX para el agucate (CODEX ATAN 197-1995)

8. Milne DL (1997) Avocado Quality Assurance: Who? Where? When? How? In: Conference ’97: Searching for Quality. Australian Avocado Grower’s Federation, Inc. and NZ Avocado Growers Association, Inc., New Zealand, pp 14–37

9. Willingham SL, Cooke AW, Coates LM, Pegg KG (2000) Pepper spot: A new preharvest Colletotrichum disease of avocado cv. Hass. Australian Plant Pathology 1

10. Reina Noreña J, Mayorga Cobos MJ, Caldas Herrera SJ, et al (2016) El problema de la peca en cultivos de aguacate (Persea americana Mill.) del norte del Tolima, Colombia. Corpoica Ciencia y Tecnología Agropecuaria 16:265. https://doi.org/10.21930/rcta.vol16\_num2\_art:372

11. Molano PJT (2007) Enfermedades del Aguacate. 20

12. Molina-Gayosso E, Silva-Rojas HV, García-Morales S, Avila-Quezada G (2012) First Report of Black Spots on Avocado Fruit Caused by *Neofusicoccum parvum* in Mexico. Plant Disease 96:287–287. https://doi.org/10.1094/PDIS-08-11-0699

13. Fuentes-Aragón D, Juárez-Vázquez SB, Vargas-Hernández M, Silva-Rojas HV (2018) *Colletotrichum fructicola* , a Member of *Colletotrichum gloeosporioides sensu lato* , is the Causal Agent of Anthracnose and Soft Rot in Avocado Fruits cv. “Hass.” Mycobiology 46:92–100. https://doi.org/10.1080/12298093.2018.1454010

14. Schoeman MH, Manicom BQ Control of Colletotrichum Speckle of Hass Avocado. 4

15. Kotzé JM, Darvas JM Symptoms and Causes. 7

16. Smith CO (1925) Blast of Avocados - A Bacterial Disease

17. Schindelin J (2012) Fiji: an open-source platform for biological-image analysis

18. Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67:1–48. https://doi.org/10.18637/jss.v067.i01

19. R Development Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

20. Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

21. Inglis PW, Pappas M de CR, Resende LV, Grattapaglia D (2018) Fast and inexpensive protocols for consistent extraction of high quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. PLoS ONE 13:e0206085. https://doi.org/10.1371/journal.pone.0206085

22. Callahan BJ, McMurdie PJ, Rosen MJ, et al (2016) DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583. https://doi.org/10.1038/nmeth.3869

23. Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

24. Abarenkov K, Zirk A, Piirmann T, et al (2021) UNITE QIIME release for Fungi

25. Bolyen E, Rideout JR, Dillon MR, et al (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857. https://doi.org/10.1038/s41587-019-0209-9

26. McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217. https://doi.org/10.1371/journal.pone.0061217

27. Pérez-Jaramillo JE, Carrión VJ, Bosse M, et al (2017) Linking rhizosphere microbiome composition of wild and domesticated Phaseolus vulgaris to genotypic and root phenotypic traits. ISME J 11:2244–2257. https://doi.org/10.1038/ismej.2017.85

28. Oksanen J, Blanchet FG, Kindt R, et al (2012) vegan: Community Ecology Package

29. Green JL, Capizzi J A SYSTEMATIC APPROACH TO DIAGNOSING PLANT DAMAGE. 37

30. Turechek WW, McRoberts N (2013) Considerations of Scale in the Analysis of Spatial Pattern of Plant Disease Epidemics. Annu Rev Phytopathol 51:453–472. https://doi.org/10.1146/annurev-phyto-081211-173017

31. Schoeman MH, Manicom BQ (2000) Report on the epidemiology of pepper spot on Hass avocado. South African Avocado Growers’ Association Yearbook 23:95–97

32. Pandey RR, Arora DK, Dubey RC Effect of environmental conditions and inoculum density on infection of guava fruits by Colletotrichum glososporioides. 8

33. Mouen Bedimo JA, Bieysse D, Nyassé S, et al (2010) Role of rainfall in the development of coffee berry disease in Coffea arabica caused by Colletotrichum kahawae, in Cameroon. Plant Pathology 59:324–329. https://doi.org/10.1111/j.1365-3059.2009.02214.x

34. Mekonnen Y, Chala A, Alemayehu S (2015) Prevalence of Colletotrichum spp. Infecting Fruits in Southern Ethiopia. 11:34–41. https://doi.org/10.5829/idosi.wjas.2015.11.1.1842

35. Zhang Y, Cong W, Shi SY (2011) Repeated fed-batch lactic acid production in a packed bed-stirred fermentor system using a pH feedback feeding method. Bioprocess and Biosystems Engineering 34:67–73. https://doi.org/10.1007/s00449-010-0447-1

36. Abdelfattah A, Freilich S, Bartuv R, et al (2021) Global Analysis of the Apple Fruit Microbiome: Are All Apples the Same?

37. Gao M, Xiong C, Gao C, et al (2021) Disease-induced changes in plant microbiome assembly and functional adaptation. Microbiome 9:187. https://doi.org/10.1186/s40168-021-01138-2

38. de Assis Costa OY, Tupinambá DD, Bergmann JC, et al (2018) Fungal diversity in oil palm leaves showing symptoms of Fatal Yellowing disease. PLoS ONE 13:e0191884. https://doi.org/10.1371/journal.pone.0191884

39. Yurgel SN, Abbey, Lord, Loomer N, et al (2018) Microbial Communities Associated with Storage Onion. Phytobiomes Journal 2:35–41. https://doi.org/10.1094/PBIOMES-12-17-0052-R

40. Diskin S, Feygenberg O, Maurer D, et al (2017) Microbiome Alterations Are Correlated with Occurrence of Postharvest Stem-End Rot in Mango Fruit. Phytobiomes Journal 1:117–127. https://doi.org/10.1094/PBIOMES-05-17-0022-R

41. Kusstatscher P, Cernava T, Abdelfattah A, et al (2020) Microbiome approaches provide the key to biologically control postharvest pathogens and storability of fruits and vegetables. FEMS Microbiology Ecology 96:fiaa119. https://doi.org/10.1093/femsec/fiaa119

42. Chen C (2016) Taxonomy Of Peltaster, Trichomerium, And Related Genera Associated With Sooty Blotch And Flyspeck. PhD Thesis, Northwest A&F University

43. Gleason ML, Zhang R, Batzer JC, Sun G (2019) Stealth Pathogens: The Sooty Blotch and Flyspeck Fungal Complex. Annu Rev Phytopathol 57:135–164. https://doi.org/10.1146/annurev-phyto-082718-100237

44. Perez Martinez JM, Batzer J, Ploetz R, Gleason M (2009) Avocado, banana, carambola and mango are hosts of members of the sooty blotch and flyspeck complex

45. Guarnaccia V, Vitale A, Cirvilleri G, et al (2016) Characterisation and pathogenicity of fungal species associated with branch cankers and stem-end rot of avocado in Italy. Eur J Plant Pathol 146:963–976. https://doi.org/10.1007/s10658-016-0973-z

46. Smilanick JL, Margosan DA (2001) Management of Postharvest Decay of Avocado Fruit. In: California Avocado Research Symposium. Riverside, pp 105–112

47. Mathioudakis MM, Tziros GT, Kavroulakis N (2020) First Report of Diaporthe foeniculina Associated with Branch Canker of Avocado in Greece. Plant Disease 104:3057. https://doi.org/10.1094/PDIS-04-20-0900-PDN

48. McDonald V, Eskalen A (2011) Botryosphaeriaceae Species Associated with Avocado Branch Cankers in California. Plant Disease 95:1465–1473. https://doi.org/10.1094/PDIS-02-11-0136

Sta**tements & Declarations**

**Author contributions**

Conceptualization: Mosquera, S., Villegas-Escobar, V., and Saavedra, S.; Methodology: Mosquera, S., Villegas-Escobar, V., and Saavedra, S.; Investigation: Mosquera, S., CatañoC., and Saavedra, S.; Formal analysis: Mosquera, S., and Villegas-Escobar, V.; Writing-original draft preparation: Mosquera, S.; Review and editing: Mosquera, S., and Villegas-Escobar, V.; Funding acquisition: Villegas-Escobar, V., and Saavedra, S.; Resources: Villegas – Escobar, V.; Project administration: Villegas – Escobar, V.; Supervision: Villegas – Escobar, V.

**Funding**

This work was supported Universidad EAFIT (Colombia), the avocado exporters CARTAMA, and the Department of Science, Technology and Innovation (COLCIENCIAS) through the project qualified by the National Council of Tax Benefits CNBT (code 7968-869-76148).

**Conflict of Interest**

The authors declare no competing interests

**Data availability**

Raw sequence data have been deposited at the European Nucleotide Archive (ENA) under accession numbe XXX. Any additional datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval**

This study was performed in line with the approval of the Ethics Committee of Universidad EAFIT

**Figure Captions**

**Fig 1**. Lenticel damage for avocado cv. Hass fruits collected from the La Escondida and El Sinai farms between 2019 and 2021 at harvest time (0 days post-harvest, 0dph). Bars and error bars represent mean and standard deviation for the severity (A) and incidence (B) of the damage observed in the farms for each harvest at 0 dph. The points show the severity and incidence means for the fruits collected from the different plots, and the letter after the year denotes main (m) and traviesa (t) harvest..

**Fig 2**. Lenticel damage for avocado cv. Hass fruits collected from the La Escondida and El Sinai farms between 2019 and 2021 at harvest time (0 days post-harvest, 0 dph) and after 21 days of storage at 6⁰C (21 days post-harvest, 21 dph). Shown are the means and standard deviations for the severity (A) and incidence (B) of the damage for the two evaluatin points (0 dph and 21 dph). The points show the severity and incidence means for the fruits collected in the four harvest, and the letter after the year denotes main (m) and traviesa (t) harvest.

**Fig 3**. Alfa diversity for the fungal communities of the avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) collected from the La Escondida and El Sinai farms during the traviesa harvest of 2020. Bars and error bars represent mean and standard deviation for the alfa diversity index richness (A), Shannon (B), and Faith pd (C) alpha-diversity (n: 6). The points show the alfa diversity metrics the fruits and the asterisk denote statistic differences at the 95.0 % confidence level (\*) according to the t test.

**Fig 4**. Fungal communities' structure for fruits of avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) collected from the La Escondida and El Sinai farms during the traviesa harvest of 2020. Cumulative sum scaling (CSS) transformed reads were used to calculate weighted unifrac distance. Shown are the bidimensional planes of the principal coordinates analysis (PCoA) (72.6 % of the overall variance) (A) and canonical analysis of principal coordinates (CAP) constrained by the farm (La Escondida and El Sinai) (68.8 % of the overall variance; p-value: 0.001) (B). Each point represents the fungal community of a fruit, and the colors show the origin (La Escondida o El Sinai) and damage severity (Severe or Mild) of the fruit. The ellipsis are the 95 % confidence level ellipses.

**Fig 5.** Taxonomic composition of the fungal communities of the avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) collected from the La Escondida and El Sinai during the traviesa harvest of 2020. Shown are the relative abundances of the fungal families of the entire communities (A) and the genus for the communities of Ascomycota (B) and Basidiomycota (C).

**Fig 6.** Taxonomy and relative abundance of the amplicon sequence variants (ASVs) enriched (p-value < 0.05) in the fungal communities of avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) collected from the La Escondida during the traviesa harvest of 2020. Shown are the taxonomic relation (A) and the relative abundances (B) of the ASVs group by color according to the clade (Supplementary Table 5).

**Fig 7.** Taxonomy and relative abundance of the amplicon sequence variants (ASVs) enriched (p- value < 0.05) in the fungal communities of avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) collected from the La Sinai during the traviesa harvest of 2020. Shown are the taxonomic relation (A) and the relative abundances (B) of the ASVs group by color according to the clade (Supplementary Table 6).

**Tables**

**Table 1.** Fungal isolates originated from healthy and necrotic lenticels of avocado cv. Hass fruits collected from the La Escondida and El Sinai during the main harvest of 2019 and traviesa harvest of 2021.

**Table 1.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Isolates** | **La Escondida** | | **El Sinai** | | **Total** |
| **Necrotic** | **Healthy** | **Necrotic** | **Healthy** |
| *Alternaria* sp. | 1 | 1 | 0 | 0 | 2 |
| *Colletotrichum* sp. | 6 | 5 | 7 | 1 | 19 |
| *Cytospora* sp. | 6 | 1 | 0 | 3 | 10 |
| *Diaporthe* sp. | 0 | 0 | 1 | 1 | 2 |
| *Neofusicoccum* sp. | 0 | 1 | 0 | 0 | 1 |
| *Neurospora* sp. | 2 | 0 | 0 | 0 | 2 |
| *Phyllosticta* sp. | 0 | 0 | 3 | 0 | 3 |
| **Total** | 15 | 8 | 11 | 5 | 39 |