**Lenticel damage of avocado cv. Hass depends on spatiotemporal factors and influences the fungal structure community**

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 Grupo Cartama. Carrera 33 # 7 – 41, Medellín, Colombia. ORCID ID: 0000-0003-4809-5531*

*\*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, being Ascomycetes more abundant than Basydiomicetes. Among these Ascomycetes, taxa such as *Trichomerium*, *Pseudocercospora*, and *Colletotrichum* were enriched. 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.

*Trichomerium*, *Pseudocercospora*, and *Colletotrichum,*

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

**Introduction**

Colombia produced nearly 155.3 thousand t of avocado cv. Hass in 2021 (Procolombia 2021). However, the yield of Colombian crops (9.0 t/ha) is below the potential yield (30 t/ ha) estimated (Granados and Valencia 2018). Several factors are responsible for these low yields, including lenticel damage (Ramírez-Gil et al. 2019; Zamora-Magdaleno et al. 2001). This damage consists of 1 mm to 5 mm-long necrotic spots that develop on the exocarp of fruits around lenticels (Everett et al. 2001, 2008) and can affect fruits during harvest but is more limiting during the postharvest (Ramírez-Gil et al. 2019; Zamora-Magdaleno et al. 2001). The damage severity influences the fruits' value and destination (CODEX 2013) and is responsible for 5% to 30% of the rejected fruit (Everett et al. 2008).

The lenticel damage causes are controversial, but two hypotheses seem possible. The first one relates to mechanical stress suffered by fruits during harvest and postharvest (Zamora-Magdaleno et al. 2001; Milne 1997). According to this hypothesis, impact injury promotes polyphenols production leading to tissue oxidation and necrosis (Zamora-Magdaleno et al. 2001). 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 (Everett et al. 2008; Willingham et al. 2000; Reina Noreña et al. 2016; Molano 2007; Molina-Gayosso et al. 2012; Fuentes-Aragón et al. 2018), and fungicide application during the harvest have shown to reduce the damage severity (Reina Noreña et al. 2016; Molano 2007; Molina-Gayosso et al. 2012; Schoeman and Manicom 1998). Despite the attempts to clarify the damage causality, the results are inconclusive, as most studies reporting pathogenic fungi isolation have failed to replicate symptoms on healthy fruits (Willingham et al. 2000; Reina Noreña et al. 2016; Molina-Gayosso et al. 2012; Smith 1925).

The lenticel necrosis resulting from mechanical stress and fungal infections differ at the cellular level, but differences are not evident to the naked eye and can be mistaken (Zamora-Magdaleno et al. 2001; Kotzé and Darvas 1985). However, the type of damage resulting from non-living and living factors differ in their patterns; those caused by mechanical stress are uniform, while damages caused by fungal pathogens are non uniform and progressive (Green and Capizzi 1990). Furthermore, the structure of plant microbial communities has been shown to be influenced by several factors, including plant health (Gao et al. 2021; de Assis Costa et al. 2018; Yurgel et al. 2018; Diskin et al. 2017; Kusstatscher et al. 2020; Malacrinò et al. 2022) and geographical location (Abdelfattah et al. 2021).

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 this hypothesis. Then, we assessed the damage progression during the postharvest. 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**

These evaluations used fruits of avocado cv Hass collected between June 2019 and June 2021 from the harvests of two commercial avocado farms in Colombia, including two main harvests from December to January and two smaller mid-season harvests from June to July (from now on, referred to as traviesa harvests). The sampled farms, i.e., La Escondida and El Sinaí, were located in different Colombian avocado-growing regions and had distinct agroclimatic characteristics (Supplementary Table 1). Also, they differed in their lenticel damage affectations, with La Escondida presenting milder affectations than El Sinaí.

We labeled 30 trees with similar height, phenology, and age on each farm distributed in five plots in La Escondida and nine plots in El Sinai to ensure we were sampling the same trees through the study (Supplementary Table 2). On each harvest, we randomly sampled ten fruits per tree from these trees, resulting in samples of 300 fruits per farm per harvest which we used for the lenticel damage evaluations. On the traviesa harvest of 2020, we sample 12 other fruits from each farm, six with severe lenticel damage and six with mild lenticel damage, for the microbial ecology analysis. We selected these fruits from the 30 label trees maximizing the differences between severely and mildly damaged fruits.

We treated all the sampled fruits the same, trying to imitate the postharvest conditions of commercial fruits. Specifically, we manually collected the fruits and packed them in punnets with the help of trained farm personnel. We carried the punnets in a car to the laboratory, unpacked the fruits, and made the first lenticel damage evaluations. The time between fruit sampling and the first measurement was below one day for la Escondida and two days for El Sinai. The time differences were related to the distance between the farm to the laboratory. During this time, we kept the fruits at the environmental temperature. Once in the laboratory, we immersed the fruits in Timorex Gold Ⓡ (STK Bio-AG Technologies, Petach Tikva, Israel) at two ml/l and stored them at six °C for 21 days. We made the second lenticel damage evaluation after 21 days of cold storage. The fruits for the microbial ecology analysis were processed upon arrival at the laboratory, meaning that they did not undergo Timorex Gold Ⓡ immersion or cold storage

 Lenticel damage estimation

We developed a macro including FIJI (Schindelin 2012) and python scripts that used fruit photographs to estimate the extent of the lenticel damage and are publicly available in the GitHub repository <https://github.com/drunita/FIJI_macro_lenticel_damage_estimation>. This macro used the ImageJ adjustThresholdOnOpenImage function to differentiate between necrotic spots and healthy epicarp. Then, it used the ImageJ Analyze Particles functionality and a python script to estimate the lenticel damage's incidence (number of necrotic spots) and severity (necrotic area percentage: area of necrotic spots/(area of the fruit surface area)). We used two photos per fruit representing each fruit's face. Then, the macro averaged the data of both faces to get a single severity and incidence value per fruit.

We measured the lenticel damage incidence and severity upon fruit arrival to the lab (0 days postharvest; 0 dph) and after the 21 days (21 days postharvest; 21 dph) of cold storage at 6°C. Then, we used mixed-effect analyses to assess differences in the lenticel damage (0 dph) and damage progression (from 0 dph to 21 dph) between farm and harvest for both variables (incidence and severity). The models assessing differences in the lenticel damage (0 dph) between farms and harvest included farm (La Escondida and El Sinai) and harvest (2019\_m, 2020\_t, 2020\_m, 2021\_t) as fixed effects and, as random effects, farm (1 | farm), plots nested in farms (1 | fp) and trees nested in plots nested in farms (1 | fpt) intercepts (Supplementary Table 3). The models evaluating the progression of the damage included farm, harvest, and evaluation time (0 dph and 21 dph) as fixed effects and the fruits intercept (1|f) as the random effect (Supplementary Table 4). We also independently evaluated differences between the 0 dph and 21 dph severity and incidence damage for each farm's harvest. Here, the models included evaluation time (0 dph and 21 dph) as the fixed effect and the fruits intercept (1|f) as the random effect.

For the severity, we fitted the data into zero-inflated models with the negative binomial distribution (beta (logit)) and zero-inflated models with the beta distribution for the incidence (nbinom2 (log)) using the glmmTMB function of the R package glmmTMB (version 1.1.4) (Brooks et al. 2017). We employed AIC model selection to choose the best-fitted model among possible models describing the association between fixed effects and the lenticel damage severity and incidence, using the AICtab function of the R package bbmle (version 1.0.25) (Bolker 2022). Then, we assessed the goodness-of-fit of the best-fitted model using the R package DHARMa (version 0.4.5) (Harting 2022). We used the R library *ggplo2* (version 3.3.3) for visualizing the data (Wickham 2016).

**DNA extraction and sequencing**

For analyzing the microbial communities, we extracted DNA from avocados exocarp with mild and severe lenticel damage using a modified protocol (Inglis et al. 2018). We intended to use healthy fruits instead of mildly damaged in this analysis, but we failed to find fruits lacking lenticel damage in the field. Therefore, we tried to maximize the difference in the damages between the groups, with fruits in El Sinai having more pronounced differences than La Escondida (Supplementary Figure 1).

We collected exocarp samples using sterile blades and homogenized them in a mortar using liquid nitrogen. Then, we washed 100 mg of homogenized material twice with 1 mL of prewash buffer**.** We added 1 mL of the lysis buffer to the washed samples and incubated them for 1 h at 65°C. We centrifuged the samples at 8000 x g for 5 min and added 1 mL of chloroform:isoamyl alcohol (24:1) (Sigma-Aldrich, St. Luis, United States) to the supernatant for purifying the DNA. We precipitated the DNA using 0.1 volumes of 3 M sodium acetate pH 5.2 (Amresco, Dallas, United States), 0.66 volumes of isopropanol (ITW Reagents, Chicago, United States), and overnight incubation at -20°C. We washed DNA twice with 70% ethanol (Sigma-Aldrich) and resuspended it in 50 µL of TE buffer (Bio Basic, New York, United States).

We quantified the DNA concentration using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, United States) with the Qubit dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific). Then, we sent the samples to the BaseClear facility (Leiden, Netherlands) for paired-end sequencing of fragments of fungal ribosomal DNA region's internal transcribed spacer (ITS) and bacterial16SrRNA in Illumina's Miseq platform. We targeted nearly 400 bp of the ITS for the fungal sequences using the ITS1-F forward (5'- CTTGGTCATTTAGAGGAAGTAA -3') and ITS2 reverse (5'- GCTGCGTTCTTCATCGATGC-3') primers in combination (Usyk et al. 2017). For the bacterial sequences, we targeted the nearly 460 bp of the variable V3-V4 regions with the S-D-Bact-0341-b-S-17 forward (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-a-A-21 reverse (5′-GACTACHVGGGTATCTAATCC-3′) primers. BaseClear performed the normalization and library preparation.

**ITS and 16S amplicon analysis**

We used the opensource program DADA2 (version 1.18.0) for filtering, de-replicating, removing chimeras, pairing forward and reverse sequences, and constructing the amplicon sequence variants (ASVs) tables (Callahan et al. 2016). During these processes, we used Cutadap (version 3.1) (Martin 2011) to remove the primers and assured a minimum of 10 bp overlapping between sequences and a quality score above 30 (Martin 2011; Callahan et al. 2016). We also retained unique sequences occurring in several samples.

We assigned a taxonomic identity to the ASVs in Qiime2 (version 2020.11). Specifically, we used the fungal Unite databases with 97% dynamic grouping and 99% (version 8.3, https://dx.doi.org/10.15156/BIO/1264708) and the bacterial Silva database (release 132) (<https://www.arb-silva.de/no_cache/download/archive/qiime/>) for training the Naive Bayes classifier with the *q2-feature-classifier.* Then, we used the *classify-sklearn* method of the *q2-feature-classifier* to assign a taxonomic identity to the ASVs (Callahan et al. 2016; Abarenkov et al. 2021; Bolyen et al. 2019; Pérez-Jaramillo et al. 2017). During this process, we used the Qiime2 functionality *filter-table* to remove mitochondrial, chloroplast, and arcuate archaeal sequences (Bolyen et al. 2019). We lost most of the bacterial reads after the mitochondrial, chloroplast, and arcuate archaeal removal suggesting that most of the sequences belonged to avocado chloroplast or mitochondrial. Therefore, we were not able to analyze the bacterial communities. From now on, we focus on the fungal community analysis using the resulting ASV tables and taxonomic classification.

**Composition and diversity of fungal communities**

We constructed rarefaction curves using the ASV tables and the rarecurve function of the R package vegan (version 2.5-7) (Oksanen et al. 2012) to determine whether our sampling depth was sufficient for analyzing the fungal communities. Then, We calculated the alpha diversity metrics (Richness, Shannon, Chao, Pielou's evenness, and Faith's phylogenetic diversity indexes) to compare the diversity of the fungal communities of avocado cv Hass with severe and mild lenticel damages. For these analyses, we used the *core-metrics-phylogenetic* and alpha diversity methods of Qiime2 (version 2020.11) with a rarefication depth of 18145 sequences which was our minimal sample depth (Supplementary Figure 2) (Bolyen et al. 2019). We used the t-student test with the Welch approximation (Shannon, evenness, and Faith) or general linearized models (glm) with the Poisson distribution (Poisson(link = "log")) (Richness and Chao) to compare these metrics between the mildly and severely damaged fruits from each farm. These analyses used the t.test and glm functions of the R library *stats* (version 4.0.4) (R Development Core Team 2021).

We used a principal coordinate canonical analysis (CAP) analysis constrained to the origin of the fruits and the damage strength (Sinai-Mild, Sinai-Severe, Escondida-Mild, and Escondida-Severe) to visualize the fungal community structures (beta diversity). These analyses used the weighted-UniFrac-distance metric and the ordinate function of the R library *phyloseq* (version 1.34.0) (McMurdie and Holmes 2013). ASVs tables were normalized using the *cumNorm* function of the R library *metagenomeSeq* (version 3.4) with the CSS (cumulative-sum scaling) method (Pérez-Espinoza and Brambila 2005). Differences between fungal communities were evaluated with permutational multivariate analysis of variance (PERMANOVA) with the *adonis* and *anova.cca* functions of the R library *vegan* (version 2.5-7) (Oksanen et al. 2012). Visualization of principal component biplanes was done using the *plot\_ordination* function of the R library *phyloseq* (version 1.34.0) (McMurdie and Holmes 2013).

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) (Pérez-Espinoza and Brambila 2005). 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) (Wickham 2016; McMurdie and Holmes 2013).

**Results**

**Lenticel damage varies between farms and harvest and increases during cold storage**

The literature about the causalities of the lenticel damage of avocado cv Hass is controversial. Physical damage suffered by fruits during harvest and postharvest and fungal pathogens are the most accepted causes (Everett et al. 2008; Willingham et al. 2000; Reina Noreña et al. 2016; Molano 2007; Molina-Gayosso et al. 2012; Fuentes-Aragón et al. 2018; Zamora-Magdaleno et al. 2001). However, physical damages differ in nature from those caused by pathogens. The formers are mostly uniform, while the laters are mostly non-uniform and progressive (Green and Capizzi 1990). Therefore, we collected avocado cv Hass during the main and traviesa harvests between June 2019 and June 2021 of two commercial farms to characterize the lenticel damage behavior.

The severity and incidence of the lenticel damage at 0 dph varied depending on the harvest and farm (p-value < 0.001). However, La Escondida's fruits had lower severities and incidences than El Sinai's in most harvests (Fig. 1, Supplementary Table 3, Supplementary Table 4). The exception was the main harvest of 2020, in which El Sinai fruits had slightly lower severities than those of La Escondida (Fig. 1, Supplementary Table 3). Similarly, fruits in the main harvests had lower damage severities and incidences than the traviesa harvests on both farms (Fig. 1 and Supplementary Table 3). These results show that the lenticel damage is not uniform and spatiotemporal components influence its development.

The lenticel damage also intensified after 21 days of cold storage (21 dph). Still, the extent of the aggravation varied widely between farms and harvests (Fig. 1 and Supplementary Table 3). We saw increases in damage severities between 1.9 and 23.8 times and damage incidences between 1.5 to 7.1 during this period, depending on the farm and the harvest (Supplementary Table 3). Whether the fruits came from main or traviesa harvests did not seem to influence the damage increases since, for La Escondida, the hights increments occurred in the traviesa harvest of 2021, and for El Sinai, they occurred in the main harvest of 2020 (Fig. 1, Supplementary Table3, Supplementary Table 5). These results show that the lenticel damage is progressive and aggravates during postharvest, even when fruits do not undergo additional mechanical stress.

The not uniform and progressive nature of the lenticel damage suggests that pathogens participate in the damage formation and progression. Therefore, we decided to characterize the microbial communities of avocados cv Hass to find out whether damaged fruits had distinctive communities.

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

To characterize microbial communities associated with lenticel damage, we collected from the traviesa harvest of 2020 of each farm fruit with severe and mild lenticel damage. We used mildly damaged instead of healthy fruits since we found no fruits without lenticel damage, with even the healthiest fruits having necrotic lenticels. Therefore, we picked the fruits to maximize the differences between the mild and severe damages, with fruits in El Sinai having more pronounced differences than La Escondida (Supplementary Figure 1). We intended to analyze fungal and bacterial communities but lost most of our bacterial reads while cleaning our sequences. Therefore, we focused our analysis on the fungal communities.

Our samples had different sizes. Therefore, we used the minimal sample depth (18145 sequences) as the refraction depth to avoid bias in our alpha diversity analysis (Supplementary Figure 2). Fungal communities of avocado cv. Hass had between 370 and 583 AVSs, with a mean of nearly 450 AVSs (Supplementary Table 6). They also had Shannon diversities between 4.4 and 6.5, Chao1 between 434 and 691, evenness between 0.51 and 0.73, and Fath's PD between 43.3 and 67.1 (Fig 2, Supplementary Table 6). The fungal communities of mildly and severely damaged fruits had similar (p-value > 0.05) alfa diversities (Shanon), evenness, and composition (Faith\_pd) on both farms. These communities also had a similar (p-value > 0.05) richness (Chao 1) in La Escondida. However, the fungal communities of severely damaged fruits had a higher richness (p-value = 0.013) than those of mildly damaged fruits in El Sinai (Fig 2, Supplementary Table 6)

The fungal communities formed distinct groups in the CAP biplane, with the axis explaining 69.5 % of the community's variation (Figure 3). The lenticel damage alone was insufficient to explain the differences in these populations' composition (*p-value*: 0.1) since the effect of the lenticel damage on the fungal populations depended on the farm (*p-value*: 0.001) (Figure 3, Supplementary Table 7). Therefore, we evaluated the effect of the lenticel damage on the structure of these fungal communities on each farm. Fruits with severe and mild lenticel damage had distinctive fungal communities in both farms according to the permutational multivariate analysis of variance (p-value < 0.05) (Supplementary Table 7). Despite fruits from La Escondida showing an extent of overlapping in the CAP biplane (Figure 3).

**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 avocado 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 extent. Some taxa were unique to fruits from El Sinai (1 class, 4 orders, 22 families, and 55 genera) or La Escondida (11 orders, 29 families, and 67 genera). However, only a few were unique to severely damaged (3 classes, 3 orders, 2 families, and 5 genera) or mildly damaged fruits (7 families and 8 genera) (Supplementary Fig. 3).

Ascomycetes and Basidiomycetes were the dominant phyla in these fungal communities. These phyla had comparable relative abundances in mildly damaged fruits, near 30 %. However, in severely damaged fruits, Ascomycetes were more abundant. Nearly half of the fungi belonged to this phylum, compared with 15 % belonging to Basidiomycetes (Fig 4 and Supplementary Table 8). Regarding the classes, Dothideomycetes was the most common, having abundances close to 13 % or higher in both farms' fruits. However, they were more abundant (20 % or higher) in fruits with severe lenticel damage.

We found no classes that were exclusively present in fruits with severe or mild lenticel damage. However, some classes were restricted to one farm's fruits. For example, Lecanoromycetes and Taphrinomycetes were only present in La Escondida fruits, and Spiculogloeomycetes was present in El Sinai fruits. On the contrary, other classes were common to both farms, including Cystobasidiomycetes, Eurotiomycetes, Exobasidiomycetes, Leotiomycetes, Sordariomycetes, and Tremellomycetes. Among these classes, Leotiomycetes was particularly abundant in fruits from La Escondida (abundances close to 12 % or higher) and only present in mildly damaged fruits from El Sinai at relatively low abundances (7 %) (Fig 4 and Supplementary Table 8).

Focusing on the ASVs, we found that both farms had ASVs enriched in fruits with severe lenticel damage ( *p-value* < 0.05, Fig. 5 and 6, Supplementary Tables 9 and 10). In particular, we found seven ASVs that had increased abundance in La Escondida severely damaged fruits, including two Basidiomycetes and five Ascomycetes (Fig. 5, Supplementary Table 9). Both Basidiomycetes belonged to the class *Cystobasidiomycetes*, with one identified as *Cystobasidium sp*. (ASV30)and the other laking of taxonomic assignment at the genus, family, or order levels (ASV31). TheAscomycetesincluded a *Lecanoromycetes* identified as Bacidina sp. (ASV18) and a Dothideomycete identified as Septhoma sp. (ASV24), which had a particularly increased abundance in these severely damaged fruits. The remaining three Ascomycetes (ASV1, ASV42, ASV41) had no taxonomic assignment (Not classified) but clustered with other Dothideomycetes (Fig. 5, Supplementary Table 9).

In El Sinai, the ASVs with increased abundance in severely damaged fruits comprised eight Ascomycetes and one ASV with no phylum assigned (ASV96) (Fig. 6, Supplementary Table 10). Among the Ascomycetes, we found two Dothideomycetes identified as *Geastrumia sp.* (ASV40) *and Pseudocercospora sp.* (ASV 14)*;* one Sordariomycetes identified as *Colletotrichum* sp.(ASV65); and one Eurotiomycetes laking of family and genus assignment (Not classified) but belonging to the *Chaetothyriales* order (ASV10)*.* The remaining four Ascomycetes had no class assigned (Not classified), but three of them were related to other *Dotidiomyectes* (ASV70, ASV7, and ASV3) and the other (ASV40) to the enriched Eurotiomycete (ASV10) (Fig. 6, Supplementary Table 10).

We also found that fruits with mild lenticel damage had enriched ASVs in both farms ( *p-value* < 0.05, Fig. 5 and 6, Supplementary Tables 9 and 10). Mildly damaged fruits in La Escondida had 18 enriched ASVs, including 11 Ascomycetes, six Bacidiomycets, and one ASV with no phylum assigned (ASV22). While these fruits in el Sinai had nine enriched ASVs in El Sinai, including three Ascomycetes, five Bacidiomycets, and one ASV with no phylum assigned (ASV37). Among these enriched ASVs, two had increased abundance in both farms, including an Ascomycete in the Dothideomycetes class identified as *Cladosporium sp.* (ASV4) *and a Basidiomycetes* in the *Tremellomycetes* class identified as *Vishniacozyma* sp. (ASV11).

The other ASVs enriched in fruits with mild lenticel damage in *La Escondida* includedfive Basidiomycetes; two *Tremellomycetes* identified as *Vishniacozyma* spp. (ASV75 and ASV15); two *Exobasidiomycetes* identified as *Meira* sp. (ASV25) and *Golubevia* sp. (ASV53); and a *Cystobasidiomycetes* identified as *Erythrobasidium sp.* (ASV57)*.* Also,they included 10 Ascomycetes; a *Lecanoromycetes* identified as *Bacidina* sp. (ASV34)*;* a *Leotiomycetes* identified as *Hyphozyma sp.* (ASV6)*; a Eurotiomycetes* laking of taxonomic assignment at the genus, family, but belonging to the *Chaetothyriales order* (ASV10 )*;* and a Dothideomycetes identified as *Neodevriesia sp*  (ASV60)*. The* remaining six Ascomycetes had no class assigned (Not classified) but were related to other *Dotidiomyectes* (ASV2,ASV7, ASV3, ASV9, and ASV12) and the enriched *Eurotiomycete* (ASV10) (Fig. 5, Supplementary Table 10).

In El Sinai, the other ASVs enriched in fruits with mild lenticel damage included four Basidiomycetes in the *Cystobasidiomycetes class, two* identified as *Symmetrospora spp. (ASV20 and ASV33) and two* laking of order, family, and genus assignment (Not classified) (ASV54 and ASV31). Also, they included *two Ascomycetes,* one Dothideomycetes identified as *Aureobasidium* sp. (ASV26) and one without class assignment (Not classified) but related to other *Dotidiomyectes* (ASV2) (Fig. 6, Supplementary Table 10).

**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 (Green and Capizzi 1990). 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 (Turechek and McRoberts 2013). 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 (Turechek and McRoberts 2013). Other evaluations have also evidenced the temporal variation of the lenticel damage and its association with high humidity and precipitation (Reina Noreña et al. 2016; Schoeman and Manicom 2000). These evaluations attribute this association to climatic conditions that favor pathogen proliferation. The association between high humidity, rain, and *Colletotrichum* proliferation is well documented (Pandey et al. n.d.; Mouen Bedimo et al. 2010; Mekonnen et al. 2015). However, other evaluations have also demonstrated that high humidity makes avocado fruits more vulnerable to lenticel damage by mechanical injury (Everett et al. 2008).

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 (Milne 1997). Damage caused by a living organism is progressive, while those caused by non-living factors are not (Green and Capizzi 1990). 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 (Green and Capizzi 1990).

We found variation in fungal communities associated with fruits' exocarp between farms. The observation of geographical location affecting fruits' microbial communities is not novel (Malacrinò et al. 2022; Abdelfattah et al. 2021; Zhang et al. 2021). A study evaluating apples' microbial communities at different spatial scales demonstrated that communities were similar across tissues but varied between orchids and growing regions (Abdelfattah et al. 2021). Therefore, 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 (Gao et al. 2021; de Assis Costa et al. 2018; Yurgel et al. 2018; Diskin et al. 2017; Kusstatscher et al. 2020). Avocado fruits with severe damages had Ascomycota and Basidiomycota relative abundances close to 53% and 12 %, respectively. These observations align with those of other evaluation reporting relative abundances between 50 to 100 % for Ascomycota and below 10 % for Basidiomycota (de Assis Costa et al. 2018; Diskin et al. 2017; Abdelfattah et al. 2021). 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 respectively. 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 (Everett et al. 2008; Willingham et al. 2000; Reina Noreña et al. 2016; Fuentes-Aragón et al. 2018). Studies about *Trichomerium* are less common, but this genus was recently included in the fungal complex responsible for the sooty blotch and flyspeck (Chen 2016). This complex comprises over 100 fungal species and produces symptoms like lenticel damage in several crops (Gleason et al. 2019). Avocado might be among these crops, but more evidence is necessary(Perez Martinez et al. 2009).

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 (Gao et al. 2021; Kusstatscher et al. 2020). 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 (Zamora-Magdaleno et al. 2001; Reina Noreña et al. 2016). 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 (Gao et al. 2021; Kusstatscher et al. 2020). 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 (Everett et al. 2008; Willingham et al. 2000; Reina Noreña et al. 2016; Molano 2007; Molina-Gayosso et al. 2012; Fuentes-Aragón et al. 2018; Guarnaccia et al. 2016; Smilanick and Margosan 2001; Mathioudakis et al. 2020; McDonald and Eskalen 2011). 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 (Willingham et al. 2000; Reina Noreña et al. 2016; Molina-Gayosso et al. 2012). 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 postharvest. 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.

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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. and Saavedra, S.; Project administration: Villegas – Escobar, V.; Supervision: Villegas – Escobar, V.

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**Conflict of Interest**

The authors declare no competing interests

**Data availability**

Raw sequence data have been deposited at the Genebank Database under accession number PRJNA817593. 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 of avocado cv. Hass at 0 days and 21 days postharvest (0dph and 21dpc) for fruits collected from the La Escondida and El Sinai farms between 2019 and 2021. Bars and error bars represent the severity (A) and incidence (B) means and standard deviations of the damage. The \* show differences between the 0dph and 21dpc incidences and severities for each farm harvest according to the generalized linear mixed analysis at a 95 % confidence level (p-value < 0.05), and the letter after the year denotes main (m) and traviesa (t) harvest. The data for this figure is in Supplementary Table 3.

**Fig 2**. Alfa diversity for the fungal communities of the avocado cv. Hass fruits with different severities of lenticel damage (Mild and Severe) from La Escondida and El Sinai farms during the traviesa harvest of 2020. Bars and error bars represent the mean and standard deviation for the alfa diversity index Shannon (A), Chao (B), Evenness (C) and Faith pd (D) alpha-diversity (n: 6). Points show the alfa diversity metrics for each the fruits. The asterisk denotes statistical differences at the 95.0 % confidence level according to the general linearized models (glm) with the Poisson distribution (Poisson(link = "log")). The data for this figure is in Supplementary Table 6.

**Fig 3**. Fungal communities' structure for fruits of avocado cv. Hass fruits with mild and severe lenticel damages 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 is the bidimensional plane of the canonical analysis of principal coordinates (CAP) constrained by the farm and the strenght of the damage (69.5 % of the overall variance; p-value: 0.001 acording to the permanova test). Each point represents a fruit's fungal community, the shapes show the farm (La Escondida (circles) and El Sinai (triangles)), and the colors the damage strength (Severe (dark gray) and Mild (light gray)) of the fruit. The ellipses 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 Basidiomycota (B) and Ascomycota (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**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **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 |

**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.