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Etiology of the peduncle ringing of avocado (*Persea americana*) fruits cv. Hass

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ABSTRACT

Background/Objective. In recent years, a phytopathological problem has manifested itself in avocado cultivation whose etiology is unknown, this disease is known as peduncle ringing, mainly affects fruits of the cv. Hass. The objective of this research was to determine the etiological agent involved in the development of the disease.

Materials and Methods. A collection of avocado fruits was carried out with characteristic symptoms of peduncle ringing: brown peduncle and reddish to purple coloration in the pericarp, the collections were made in the municipalities of Coatepec Harinas, Donato Guerra and Malinalco State of Mexico. In the laboratory, fungi were isolated in PDA medium. Three species of fungi were identified by morphological analysis and confirmed at the DNA level by PCR. Pathogenicity tests were performed on the three species identified individually and in combinations. The variables evaluated were incidence, severity, days at symptom manifestation and percentage of fruits with damaged mesocarp.

Results. Fungi associated with peduncle ringing damage in avocado fruits cv. Hass were identified as *Alternaria tenuissima, Cladosporium cladosporioides* and *Epicoccum nigrum. A. tenuissima* showed its high pathogenic capacity to induce peduncle ringing in avocado fruits with 100% incidence and 40% severity. It should be noted that the data on incidence, severity and mesocarp damage are higher in treatments when *A. tenuissima, C. cladosporioides* and *E. nigrum* act independently than in consortia.

Conclusion. *A. tenuissima, C. cladosporioides* and *E. nigrum* inoculated independently and in consortia were shown to replicate the symptoms of ringing: peduncle in brown-colored fruits and reddish to purple coloration on the pericarp and the mummification of fruits.

Keywords: Pathogenicity, Avocado, Severity, Etiology

INTRODUCTION

Mexico is the world's largest producer of avocado (*Persea americana*), with a total volume of 2,973,344.42 t in 2023 (Food and Agriculture Organization of the United Nations, 2025), and has led global production of this fruit since 1961, contributing 15% of the world's avocado output at that time and 31% in 2019 (Díaz, 2021). The three main avocado-producing states are Michoacan (1,831,622.33 t), Jalisco (256,002.36 t), and the State of Mexico (123,446.04 t) (SIAP, 2022). However, as the cultivated area expands, this crop is increasingly affected by a complex of phytopathological problems throughout all stages of development. Among the most recently reported issues is fruit drop, especially in the Hass variety. This condition is linked to a disease known as peduncle ringing, which occurs at high incidence rates ranging from 5% to 100% fruit drop, causing significant economic losses for growers. Despite this, Hass remains the most widely planted variety (Valencia and Téliz, 2007). Although this disease is not reported globally, it is highly significant for Mexico's avocado sector.

The symptoms of this disease include the formation of a brown ring around the peduncle, approximately 1 cm from its base, which causes the pericarp to turn reddish to purple; fruit mummification; and eventually, fruit drop at various developmental stages. Longitudinal cuts of affected fruit reveal pulp necrosis and cavities with mycelial growth (Valencia and Téliz, 2007). High relative humidity (80%) and temperatures above 26 °C favor the appearance of the disease, with the most severe damage occurring during the rainy season, especially from June to July. For this reason, the phenomenon is known as the June drop, which coincides with fruit development following the winter bloom, when avocado trees bear the heaviest fruit load (Pineda-Fonseca *et al.*, 2006). However, according to García (2023; personal communication), fruit drop can reach up to 80% in young fruit from the off-season bloom. Therefore, peduncle ring-related fruit drop is not limited to a single season or bloom (Ramírez-Mendoza, 2023).

Despite the significant economic losses caused by this disease, its etiology remains unknown. Various authors have linked it to nutrient deficiencies (Finazzo *et al.*, 1994), water stress (Wolstenholme *et al.*, 1998), competition for carbohydrates and hormones (Salazar García *et al.*, 2005), as well as to phytopathogenic bacteria and fungi (Valencia and Téliz, 2007). Therefore, the aim of this study was to determine the etiology of peduncle ringing cv. Hass avocado fruit from three municipalities in the State of Mexico.

MATERIALS AND METHODS

Sample collection. cv. Hass avocado fruits showing early signs of peduncle ringing and visible symptoms were collected from three municipalities in the State of Mexico: Coatepec Harinas (18º57'03.43" N, 99º47'45.72" W, at 2,460 masl), Donato Guerra (19º18'43.18" N, 100º15'16.49" W, at 2,052 masl), and Malinalco (18º59'21.74" N, 99º28'48.03" W, at 2,085 masl). One eight-year-old orchard was selected in each municipality for sampling. Thirty fruits per orchard per municipality were harvested during the last week of May and the first week of June 2021. The samples were labeled, stored, and transported under refrigeration until processing at the Phytopathology Laboratory of the UAEMex University Center in Tenancingo, located at (18º58'06'' N, 99º36'48'' W, at 2,065 masl) in Tenancingo, State of Mexico.

Isolation. Fungal colony isolation was carried out following the methodology proposed by Trinidad-Ángel *et al.* (2017). The peduncle of each collected fruit was cut into pieces approximately 0.5 cm long, surface-sterilized in a 2% sodium hypochlorite solution for one minute, and then rinsed three times with sterile distilled water. Excess moisture was removed using sterile blotting paper. Five peduncle pieces were then placed in each Petri dish containing potato dextrose agar (PDA-BD Bioxon) culture medium (39 g L^{-1}). A total of 30 Petri dishes were prepared per municipality, resulting in 450 individual samples. The isolates were incubated at 25 °C and monitored every 24 hours. Once colonies appeared, transfers were made by placing a fragment of the culture medium containing the growing hyphal tips onto fresh PDA plates, which were incubated under the same conditions. Finally, single-spore cultures were obtained from pure colonies according to the methodology of Crous *et al.* (2009).

Morphological identification. Based on their growth and characteristics, colonies obtained from single-spore cultures were transferred to specific media to promote sporulation. *Alternaria* sp. was cultured on potato carrot agar (PCA) and incubated at 25 ± 2 °C under white light (17 W) with a photoperiod of 16 hours of light and 8 hours of darkness for 5 to 7 days. *Cladosporium* sp. and *Epicoccum* sp. were grown on PDA in Petri dishes; *Cladosporium* sp. did not require special conditions, while *Epicoccum* sp. was exposed to continuous white light (17 W) for 12 days. Both were maintained at approximately 25 °C. For genus and species identification, morphocultural traits were recorded, including colony color, growth pattern, mycelium characteristics, and sporulation under specific media and conditions. Conidial size was also measured 30 conidia per genus were measured for length and width using an ocular micrometer under an Axiostar Plus microscope. The average conidial size was calculated from the total analyzed. Taxonomic keys by Barnett and Hunter (1998) were used, along with genus-specific keys: Simmons (2006) for *Alternaria* sp., Aveskamp *et al.* (2010) for *Epicoccum* sp., and Bensch *et al.* (2012) for *Cladosporium* sp.

Molecular identification. From each morphologically identified fungus, a single colony was used to confirm its identification through genomic analysis. DNA was extracted following the protocol provided in the E.Z.N.A. commercial extraction kit (Product Manual); specifically, seven-day-old monospore cultures were used. The presence of DNA fragments was verified by electrophoresis on 2% agarose gels; staining was performed with ethidium bromide (5 mg L^{-1}), and the gels were documented using the Gel Doc photo documentation system (Bio-Rad, USA). Sequences were obtained from two regions amplified by PCR: the ITS (internal transcribed spacer) ITS4 18S-5.8S (TCCTCCGCTTATTGATATGC) and ITS5 5.8S-28S (GGAAGTAAAAGTCGTAACAAGG) (White *et al.*, 1990). Amplification products were 566 bp for the fungus *A. tenuissima*, and 528 bp for both *C. cladosporioides* and *E. nigrum*. Partial sequences were also obtained from the β-tubulin gene using primers β-T2a (GGTAACCAAATCGGTGCTGCTTTC) and β-T2b (ACCCTCAGTGTAGTGACCCTTGGC), resulting in 261 bp for *A. tenuissima*, 347 bp for *C. cladosporioides*, and 302 bp for *E. nigrum*. Both genes were amplified under standard PCR conditions using a professional thermal cycler (Biometra, Germany) in a final volume of 25 µL containing 1X buffer, 0.75 mM $MgCl_2$, 0.2 mM dNTPs, 10 µM primers, 1 unit of Platinum Taq DNA polymerase, and 80 ng of DNA. The amplification

cycles were as follows: one cycle at 96 °C for 3 minutes; 35 cycles at 95 °C for 30 seconds; one cycle at 55 °C for 30 seconds; one cycle at 72 °C for 45 seconds; and a final cycle at 72 °C for 4 minutes.

The amplified products from each fungus were sequenced in both directions (5'–3' and 3'–5') by the Instituto Potosino de Investigación Científica y Tecnológica A.C. The resulting sequences were edited using BioEdit version 7.0.0. (Hall, 1999) and aligned with the GenBank database of the National Center for Biotechnology Information (NCBI, USA).

Pathogenicity tests. To increase the inoculum, monospore colonies of the three genera under study were used. *Cladosporium* sp. was cultured on PDA medium for 10 days, *Epicoccum* sp. on PDA for 20 days, and *Alternaria* sp. on PCA for 13 days, all under the specific light and temperature conditions previously described.

To obtain the inoculum (conidia) for each fungal genus, 10 mL of sterile distilled water were added to each plate with sporulated growth, and the colonies were scraped using a mycological-bacteriological loop to release the conidia. This process was repeated on as many colonies as necessary to obtain a sufficient amount of inoculum. The suspension was collected using a micropipette (Boeco®) and transferred into beakers, which were agitated for one minute. Spore counts were performed using a Neubauer chamber (Marienfeld®) under a compound microscope (Carl Zeiss®). The number of spores was determined using the formula proposed by Troya and Vaca (2014): C = Spores counted in two fields / Recounted surface mm² × Depth mm × Dilution. Using these data, a suspension of 1×10^5 conidia mL^{-1} was prepared for in vitro pathogenicity tests on avocado fruits.

Avocado fruits cv. Hass were collected from a commercial orchard in Malinalco, State of Mexico. Fruits with healthy-looking pedicels, known locally as “huevo de paloma” due to their size, were selected, stored under refrigeration, and transported to the laboratory for pathogenicity testing. The fruits were surface-disinfested with 2% sodium hypochlorite, followed by three rinses with sterile distilled water. Moisture was removed using sterile blotting paper, and immediately afterward, the pedicels were placed in floral water tubes with plastic suction caps (vials used for floral arrangements), each containing 10 mL of sterile distilled water.

The vials containing the peduncles and fruits were secured on the bases of rectangular cake domes, and the peduncles were wounded using disinfected dissection needles. A 2 oz spray bottle was used to apply the treatments (fungal inoculation) by spraying. The inoculation process was carried out under aseptic conditions in a laminar flow hood. Temperature and relative humidity were recorded inside one of the domes using a Hobo® data logger.

The experimental design was completely randomized with seven treatments, consisting of each fungus individually and in combination: T1 = (*Alternaria tenuissima* + *Epicoccum nigrum* + *Cladosporium cladosporioides*), T2 = (*A. tenuissima* + *E. nigrum*), T3 = (*A. tenuissima* + *C. cladosporioides*), T4 = (*E. nigrum* + *C. cladosporioides*), T5 = (*E. nigrum*), T6 = (*A. tenuissima*), T7 = (*C. cladosporioides*), plus a control (sterile distilled water) = T8. Each treatment included 15 replicates (fruits), and the experiment was conducted under conditions of 23 ºC and 86% relative humidity. The variables evaluated were: incidence, severity, days to symptom appearance, and days to the appearance of signs (mycelium) on the mesocarp of the fruits. Incidence and severity

were assessed every 24 hours over a 15-day period after inoculation, during which the fruits began to show signs of dehydration.

Incidence was recorded by counting the number of fruits that developed typical ring symptoms. The calculation was performed using the following formula: $I = ((N \text{ of diseased fruits})/(\text{Total number of fruits})) \times 100$, as proposed by Anculle and Álvarez (2006), where I is the percentage of incidence; *N of diseased fruits* represents the fruits that became diseased out of the total inoculated, while *Total number of fruits* is the total number of fruits inoculated in the treatment.

Severity was evaluated using a class scale developed by the arbitrary method, based on the assumption that the human eye can perceive diseased tissue as less than 50% and healthy tissue as greater than this value (Horsfall and Barratt, 1945). Fruits with varying degrees of disease symptoms, as well as healthy fruits, were collected, leading to the creation of a six-class scale ranging from 0 to 5, where 0 = healthy fruit and 5 = fruits with 100% severity (Figure 1). Based on this scale, damage was assessed on avocado fruits subjected to the different treatments. The data obtained were converted to percentage of severity using the formula by Townsed and Heuberger (1943): $\mathbf{P = [(\Sigma (n \times v)) / (N \times C)] \times 100}$, where **P** is the severity level in percentage; **n**, number of samples per category; **v** represents the numerical value of each category; **N** is the total number of samples; and **C** represents the highest category. The variable *days to symptom appearance* on peduncle or fruit was recorded every 24 h from inoculation over a period of 15 days; in this final evaluation, the fruits were dissected and mesocarp damage was assessed as 1 = presence or 0 = absence.

Re-isolation of inoculated fungi. From the avocado fruits that received the different treatments (15 per treatment), as well as the controls, 50% per treatment were selected to recover the initially inoculated fungi. The same methodology used for the original fungal isolations was applied. Pure colonies were obtained on specific media, their morphological and cultural characteristics were recorded, and they were compared with the inoculated isolates, thus fulfilling the final step of Koch's postulates (López, 2021).

Statistical analysis. The data for the variable *severity* were transformed using *Ln* and subjected to tests for normality, homoscedasticity, and independence, and then analyzed using ANOVA and mean separation with Tukey's test ($p \leq 0.05$), and DGC ($p \leq 0.05$) for *incidence*, with the support of the statistical package InfoStat student version (Di Rienzo *et al*., 2008).

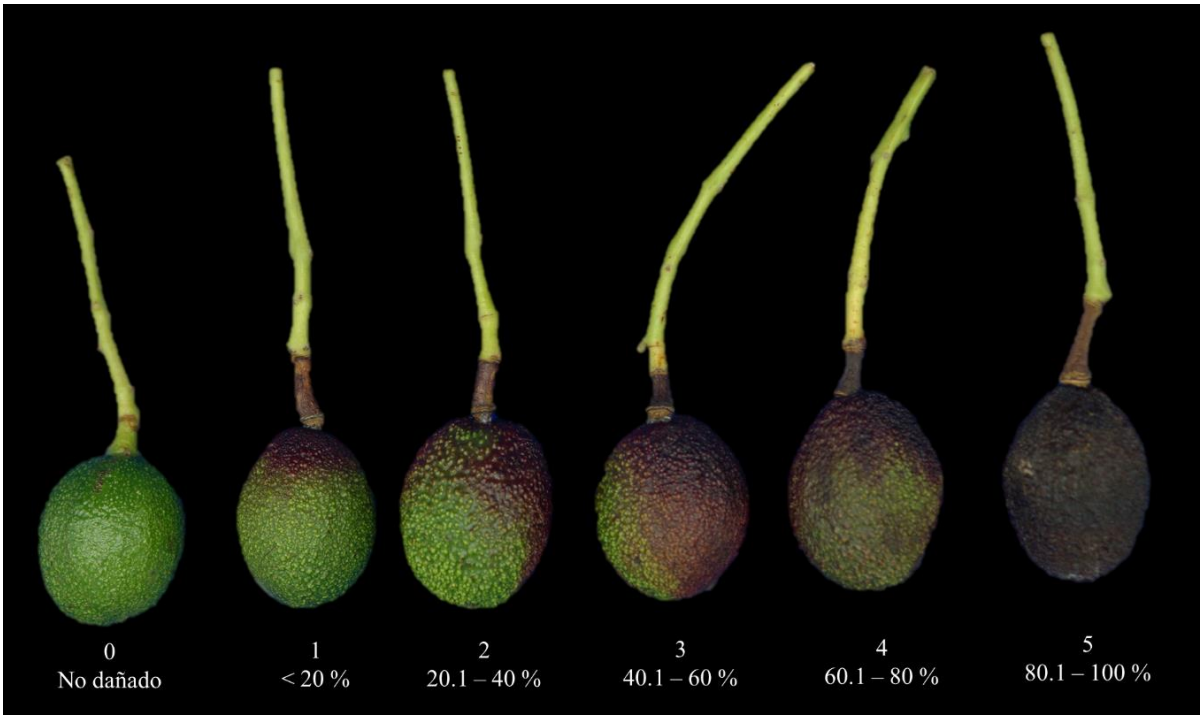

Figure 1. Severity scale for peduncle ringing symptoms in avocado fruits cv. Hass, proposed for assessing lesions in inoculated fruits.

RESULTS

The symptoms observed in the field initially included the presence of a brown ring and spot on the peduncle and a purple discoloration on the exocarp (Figures 2A–B), followed by a cork-like appearance of the peduncle and a reddish to purple coloration of the fruit's exocarp (Figure 2C). In more advanced stages, peduncle strangulation and fruit mummification were observed, with the fruit either remaining attached to the tree or detaching from it (Figure 2D). When the damage was severe, branches with fruits showing different stages of the disease were observed (Figure 2E).

Figure 2. Typical symptoms of peduncle ringing in avocado fruits cv. Hass. A and B) Fruits with dark brown ringing on the peduncle; C) Corky peduncle and fruit with purple coloration on the exocarp; D) Final stage of the ringing symptom, mummified avocado fruit with necrotic peduncle; E) Branch with fruits in different stages of ringing development.

From the 450 samples cultured from 90 avocado fruits collected across the three municipalities, a total of 220 isolates were obtained, grouped into eight fungal genera. The most frequently isolated were *Alternaria* sp., *Cladosporium* sp., and *Epicoccum* sp., with average frequencies of 35.6%, 21.2%, and 20.5%, respectively (Table 1).

Data recorded for the fungi found in each collection municipality indicate that *Epicoccum*, *Cladosporium*, and *Alternaria* sp. were predominant in all three locations. *Alternaria* sp. was the most frequent, with an average of 52.6% of isolates in Coatepec Harinas, 27.9% in Donato Guerra, and 26.3% in Malinalco. *Cladosporium* sp. accounted for 13.1%, 27.9%, and 22.4% of isolates, respectively, while *Epicoccum* sp. was recorded at 18.4%, 23.5%, and 19.7% for Coatepec Harinas, Donato Guerra, and Malinalco, respectively (Table 1).

Table 1. Frequency of fungi isolated from peduncles of avocado fruits cv. Hass collected from commercial orchards in the municipalities of Coatepec Harinas, Donato Guerra, and Malinalco, State of Mexico, Mexico.

Genera of fungi	**% of isolated colonies by municipality**		
	Coatepec Harinas	**Donato Guerra**	**Malinalco**
Alternaria sp.	52.63	27.94	26.31
Epicoccum sp.	18.42	23.52	19.73
Cladosporium sp.	13.15	27.94	22.36
Colletotrichum sp.	6.57	0	0
Rhizopus sp.	5.26	0	3.94
Aspergillus sp.	0	0	7.89
Verticillium sp.	0	1.47	1.31
Pestalotia sp.	0	2.94	1.31
Unidentified	3.94	16.17	17.10

Morphometric identification. Based on cultural, morphological, and micrometric characteristics, three deuteromycete fungi were identified.

Alternaria tenuissima. On PCA medium (potato carrot agar), the colony reached a diameter of 5 cm between days 5 and 7 at 25 ± 2 ºC, under white light and darkness alternating 16 h light and 8 h dark. The growth was flat with entire margins and an olive green coloration that darkened to blackish as it matured, showing a pair of concentric rings. Conidial chains consisted of 4 to 13 conidia on PCA medium, where the presence of primary conidiophores with upright growth was observed, averaging 104.6 × 5 µm in length × width. The conidia were muriform in shape, with 4–7 transverse septa and 2–3 longitudinal septa, and an average size of 15–37.5 × 7.5–17.5 µm in length × width; they were light to dark brown with a punctate ornamentation surrounding them (Figures 3A–C). Based on cultural and morphometric characteristics, the species corresponds to *Alternaria tenuissima* (Simmons, 2007).

***Cladosporium cladosporioides*.** On PDA medium, the colony showed slow growth, ranging from a minimum of 11.06 mm to a maximum of 23.83 mm in diameter between days 18 and 20 at 22 ± 2 ºC. Growth was circular with feathery white margins, olive green to dark coloration on the surface, and black on the reverse side. The surface was flat and velvety, with mycelium firmly attached to the culture medium, appearing fluffy or woolly. Sporulation on PDA was abundant (++). It exhibited septate brown mycelium and conidia in long, branched chains. The conidia were small, subglobose to ovoid, with intercalary conidia ranging from ellipsoid to ovoid and occasionally subcylindrical, aseptate, and olive brown to dark brown in color. They were sometimes grouped into ramoconidia. Spores measured 2.5–7.5 × 2.5–5 µm in length × width. Conidiophores were short and straight, septate, with olive to brown tips (Figures 3D–F). Based on morphometric and cultural characteristics, the species was identified as *Cladosporium cladosporioides* (Bensch *et al.*, 2012).

***Epicoccum nigrum*.** On PDA medium, the colony exhibited slow growth, reaching 2.38 cm in diameter between days 12 and 15 under continuous white light at 22 ºC for the formation of conidia and chlamydospores. Under the microscope, chlamydospores were observed as multicellular, globose structures, dark brown to brown in color, measuring 12.5 to 22.5 µm. The mycelium was septate and hyaline when young, turning brown as it matured. Aerial mycelium was scarce but tufted or clustered at the center of the colony, white in color (Figures 3G–I). Based on morphometric and cultural characteristics, the fungus was identified as *Epicoccum nigrum*, in accordance with the description by Aveskamp *et al.* (2010).

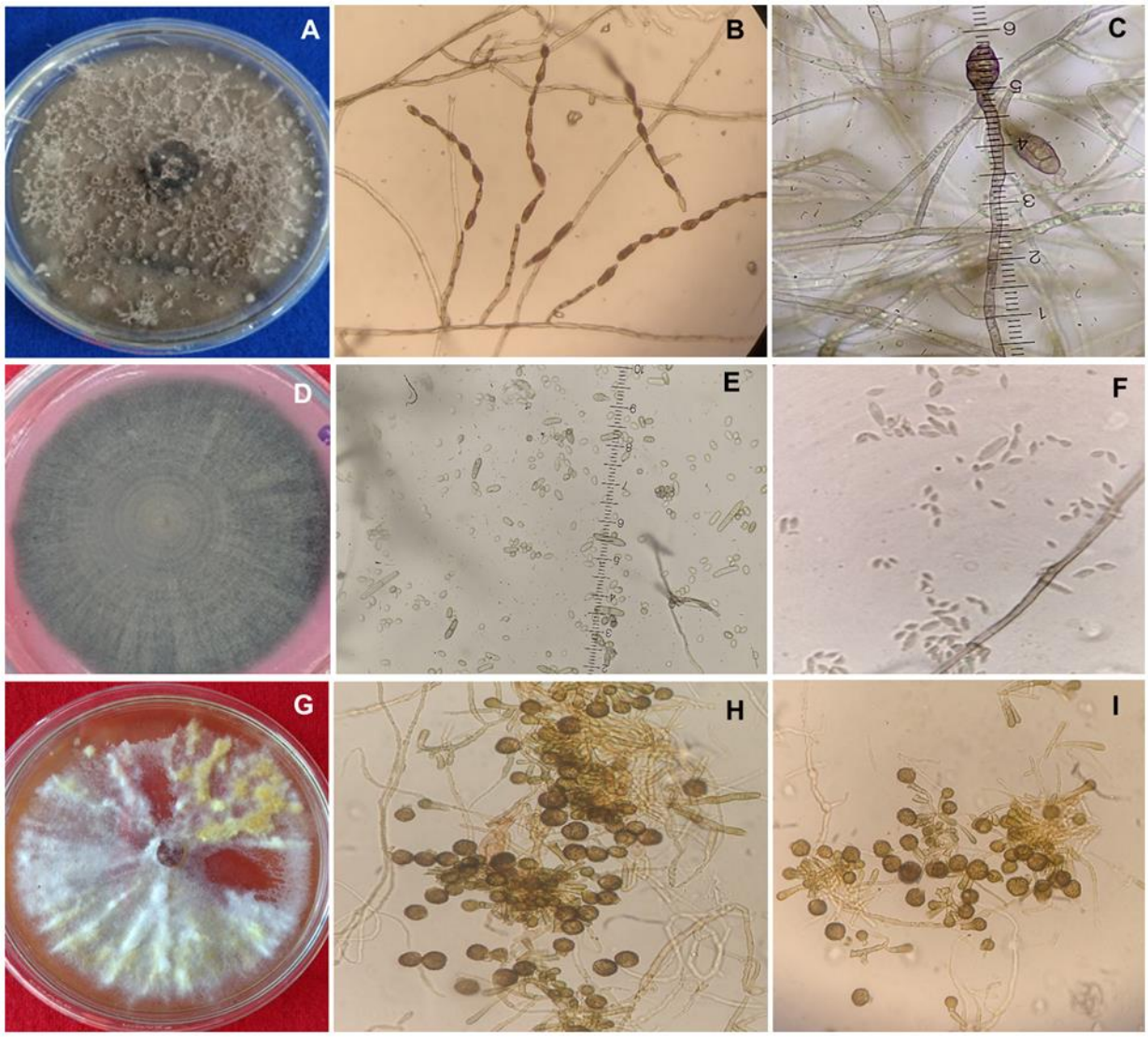

Figure 3. Cultural and morphometric characteristics of *Alternaria tenuissima, C. cladosporioides* and *E. nigrum.* A) Colony of *A. tenuissima* flat growth grown in PCA medium. B) Chain conidia of 4-13 originating from primary conidiophores of erect growth. C) Measurement of muriform conidia with micrometric ruler. D) Colony of *C. cladosporioides* with flocy micelium grown in PDA medium, front. E) Measurement of unicellular-cell conidia. F) Solitary conidia detached from the conidiophore. G) Colony of *Epicoccum nigrum* flat growth and development of cottony mycelium in PDA medium. H) Formation of multicelular chlamydospores. I) Septate mycelium and globose chlamydospores of *E. nigrum.*

Identification by PCR. The three fungal genera: *Alternaria* sp., *Cladosporium* sp., and *Epicoccum* sp. were analyzed by PCR amplification. The *Alternaria* sp. isolate, using ITS sequences, was aligned through multiple sequence alignment with *Alternaria tenuissima*, showing 100% identity with accession MT573466. Similarly, the region amplified with the β-tubulin gene aligned with sequences of *A. tenuissima*, showing 100% identity corresponding to accession MN445975.

For the *Epicoccum* sp. isolate, the sequences were aligned with *Epicoccum nigrum*, accession MG602540 (100% identity). The region amplified from the β-tubulin gene also aligned with *E. nigrum*, accession MK051182 (98% identity). In the case of *Cladosporium* sp., the sequences aligned with *Cladosporium cladosporioides*, accession

MT573472 (100% identity). The region amplified from the β-tubulin gene aligned with *C. cladosporioides*, showing 98.22% identity with accession MH780075.

Symptoms in the pathogenicity test. The symptoms were replicated in avocado fruits cv. Hass inoculated with the fungi under evaluation, applied either individually or in combination. In general, a brown, cork-like spot initially appeared on the fruit peduncle; the lesion progressed until the peduncle collapsed, and the fruits showed reddish to purplish spots.

In the mesocarp of the fruits, brown to black spots appeared, forming speckled patterns and ring-shaped lesions around the pulp, eventually leading to its decomposition. In some fruits, cavities with white mycelial growth were observed (Figures 4B-C). No marked differences in symptoms were observed among the treatments; in other words, there was no clear pattern distinguishing specific symptoms.

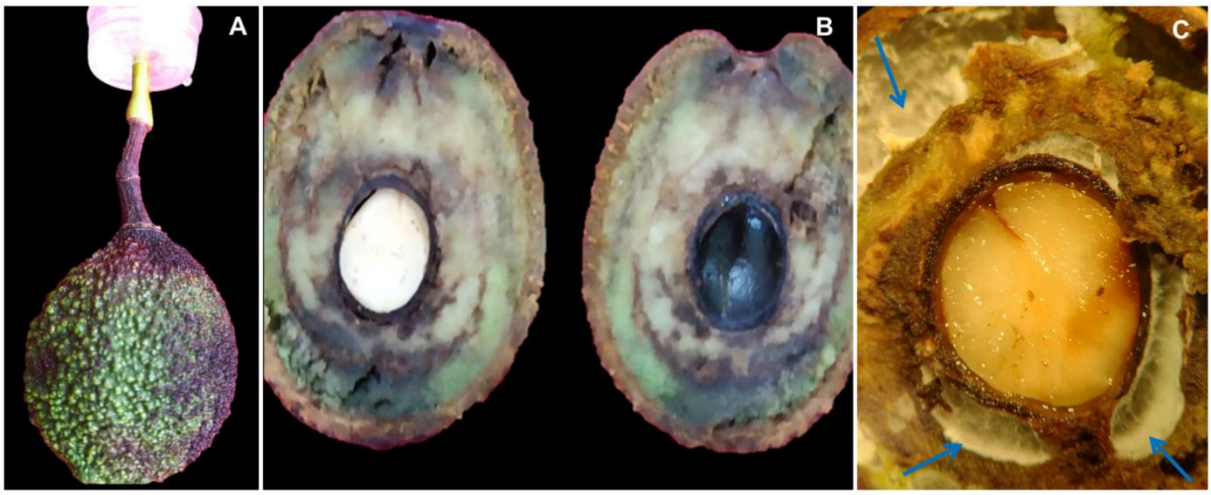

Figure 4. Symptomatology developed in fruits inoculated with *Epicoccum nigrum*. A) Fruit with peduncle damage and purplish discoloration on the exocarp. B) Longitudinal section of the fruit showing brown to dark lesions in the mesocarp. C) Mycelial growth in the cavities of the fruit mesocarp, observed under a stereoscopic microscope.

Fruits inoculated with *A. tenuissima* + *E. nigrum* + *C. cladosporioides* (T1) showed small brown spots on the peduncles, leading to their constriction; reddish spots appeared on the exocarp, and irregular dark spots were observed in the mesocarp. Fruits inoculated with *A. tenuissima* + *E. nigrum* (T2) exhibited brown spots on the peduncles, which eventually led to their mummification; the fruit exocarp showed reddish discoloration and mesocarp decomposition. The treatment with *A. tenuissima* + *C. cladosporioides* (T3) was characterized by brown to reddish spots on the peduncle and pedicel of the fruits, with no symptoms on the exocarp and only black dots recorded in the mesocarp. In the inoculation with *E. nigrum* + *C. cladosporioides* (T4), the fruits had brown spots on the peduncles, which later caused their mummification; reddish discoloration appeared on the exocarp.

In fruits individually inoculated with the fungi, the symptoms were similar. With *E. nigrum* (T5), symptoms appeared mainly in the fruit mesocarp, where brown spots and decomposition were observed; small orange spots could be seen on the exocarp. Inoculation with *A. tenuissima* (T6) resulted in brown spots and mummification of the

peduncle, and the fruits showed brown spots and mesocarp decomposition. In the treatment with *C. cladosporioides* (T7), brown spots and mummification were observed on the peduncles, while the fruits showed a brown to reddish discoloration on the exocarp at the junction with the peduncle; only brown spots were present in the mesocarp. Fruits that were not treated with the fungi (Control) showed no symptoms, and both peduncles and fruits remained healthy (Figures 5T1-T8).

Figure 5. Symptoms observed in fruits inoculated with the different fungi, both in combination and individually: T1) *A. tenuissima* + *E. nigrum* + *C. cladosporioides*. T2) *A. tenuissima* + *E. nigrum*. T3) *A. tenuissima* + *C. cladosporioides*. T4) *E. nigrum* + *C. cladosporioides*. T5) *E. nigrum*. T6) *A. tenuissima*. T7) *C. cladosporioides*. T8) Control.

The response of the severity and incidence variables, as well as the number of days until symptom appearance in avocado fruits cv. Hass inoculated with the fungi *A. tenuissima*, *C. cladosporioides*, and *E. nigrum*, both individually and in combination, (Table 2). The table shows that the presence of the fungi, whether applied individually or in combination, formed a single statistical group, which differed significantly from the control (T8), which was treated only with sterile distilled water (Table 2). Although no statistical differences were found among the inoculated treatments, the highest severity of damaged fruits was recorded in T2, with 50.7%, corresponding to the combination of *A. tenuissima* + *E. nigrum*, followed by T7 with 46.7%, and T6 with 40.0%, using *C. cladosporioides*. The control group showed a severity of 1.3%, representing one fruit in class 1 (< 20%) on the severity scale; this may have been due to the fruit being infected in the field (Table 2). Regarding incidence, the data showed a statistically significant difference among treatments. The highest incidence (100%) occurred in fruits inoculated only with *A. tenuissima* (T6), followed by the rest of the treatments (T4, T5, T7, T2, and T1), which formed a statistically similar group. A third group was formed by the treatment with *A. tenuissima* and *C. cladosporioides* (T3) and the control (T8) (Table 2). The latter

showed one symptomatic fruit with damaged mesocarp, which may be attributed to field infection.

The number of days until symptom appearance after inoculation with the treatments averaged between 4.5 and 6.4 days. Notably, in all treatments, the first symptoms appeared starting on the third day after fungal inoculation. In the case of the single symptomatic fruit in the control treatment, symptoms also appeared on the third day (Table 2).

Table 2. Incidence, severity, and time to symptom appearance in avocado fruits cv. Hass inoculated with *A. tenuissima*, *C. cladosporioides*, *E. nigrum*, and their combinations.

Treat	Inoculated pathogens	Percentages		Time to manifestation of symptoms		Damaged fruits in mesocarp
		Severity	Incidence	$\bar{x}$ on days*	Rank in days+	%
1	*A. tenuissima* + *E. nigrum* + *C. cladosporioides*	32.0a	53b	6.4	3 - 9	33.3
2	*A. tenuissima* + *E. nigrum*	50.7a	60b	5.3	3 - 9	46.7
3	*A. tenuissima* + *C. cladosporioides*	25.3a	27c	5.0	3 - 6	26.7
4	*E. nigrum* + *C. cladosporioides*	30.7a	80b	4.5	3 - 6	66.7
5	*E. nigrum*	38.7a	73b	5.5	3 - 12	66.7
6	*A. tenuissima*	40.0a	100a	7.4	3 - 12	53.3
7	*C. cladosporioides*	46.7a	73b	4.5	3 - 9	60
8	Control	1.3b	6.7c	--	3	6.7

Different letters indicate significant differences according to Tukey's test ($p<0.05$) for severity and DGC ($p<0.05$) for incidence.
*Average number of days to symptom appearance after inoculation with the treatments.
+Day range refers to the first and last day symptoms were observed.

On the other hand, the data for the variable "fruits with mesocarp damage" show that the treatments with the highest percentages were those inoculated with *E. nigrum* + *C. cladosporioides* and with *E. nigrum* alone, both recording 66.7% of fruits with mesocarp damage, followed by the treatment inoculated with *C. cladosporioides* alone, at 60%. The treatments with the lowest percentages of mesocarp damage were *E. nigrum* + *C. cladosporioides* at 26.7% and *A. tenuissima* + *E. nigrum* + *C. cladosporioides* at 33.3%.

Reisolation of fungi from inoculated fruits. All three inoculated fungi were recovered. A total of 115 colonies of *A. tenuissima*, 92 of *E. nigrum*, and 60 of *C. cladosporioides* were obtained. All isolates corresponded to the fungi and treatments originally applied to the peduncles and fruits of avocado cv. Hass, although colonies were obtained in the control, it is assumed that it was contamination that came from the field fruits (Table 3). The most frequently recovered fungus was *Alternaria tenuissima* at 43.07%, followed by *Epicoccum nigrum* at 34.45%, and *Cladosporium cladosporioides* at 22.38%.

Table 3. Number of colonies and fungal species recovered from *in vitro* pathogenicity tests on avocado fruits cv. Hass.

	Treatments	Number of colonies recovered per treatment		
		A. tenuissima	*E. nigrum*	*C. cladosporioides*
1	*Alternaria tenuissima* + *Epicoccum nigrum* + *Cladosporium cladosporioides*	26	15	9
2	*Alternaria tenuissima* + *Epicoccum nigrum*	30	16	---
3	*Alternaria tenuissima* + *Cladosporium cladosporioides*	20	---	11
4	*Epicoccum nigrum* + *Cladosporium cladosporioides*	--	31	21
5	*Epicoccum nigrum*		30	---
6	*Alternaria tenuissima*	39	---	---
7	*Cladosporium cladosporioides*	---	---	19
8	Control	1	2	5

DISCUSSION

The fungi isolated from avocado fruits with symptoms of peduncle ringing rot were *Alternaria tenuissima*, *Cladosporium cladosporioides*, and *Epicoccum nigrum*, identified through morphological and molecular analyses. The lesions, symptoms, and signs were reproduced in pathogenicity tests on avocado fruits cv. Hass: peduncle ringing rot, reddish to purplish discoloration of the exocarp, brown to black spots on the mesocarp, and, in more advanced stages, decomposition with cavities showing white mycelial growth that led to fruit mummification. These findings indicate that the fungi act individually or in consortia and support the hypothesis that the disease is caused by a fungal complex.

Valencia and Téliz (2007) observed this issue in avocado production and associated it with *Diplodia* sp., *Alternaria* sp., *Helminthosporium* sp., *Dothiorella* sp., *Colletotrichum* sp., *Pestalotia* sp., *Hyalodendron* sp., *Sthemphyllum* sp., *Penicillium* sp., and *Glomerella* sp., although they did not report pathogenicity tests. Salgado (1993) isolated fungi from tissue with peduncle ringing lesions in avocado fruits collected in the municipality of Coatepec Harinas and reported the presence of *Colletotrichum* sp. and *Alternaria* sp., as well as *Fusarium* sp. and *Verticillium* sp.; thus, he suggested the involvement of a fungal complex in this disease and proposed subjecting the isolates to pathogenicity tests.

A microorganism common to both studies is *Alternaria* sp. In this study, *Alternaria tenuissima* was the most frequently isolated species in each of the sampled municipalities (Table 1); moreover, in the pathogenicity tests, both individually and in consortium (T1, T2, and T3), it demonstrated a high pathogenic capacity to induce peduncle ringing rot in avocado fruits. *A. tenuissima* alone showed 100% incidence, 40% fruit severity, and an average of 53.3% mesocarp damage, all within just 3 to 12 days after inoculation (Table 2). The data on incidence, severity, and mesocarp damage were higher in treatments where the fungi acted independently (*Alternaria tenuissima*, *Cladosporium cladosporioides* and *Epicoccum nigrum*) than in combination with other fungi (Table 2). These individual values were only surpassed by the treatment combining *Epicoccum nigrum* + *Cladosporium cladosporioides*, which showed 80% incidence and 66.7% of fruits with mesocarp damage. Symptom appearance occurred between three and twelve days after inoculation; notably, in all seven treatments with phytopathogenic fungi, symptoms began to manifest three days after contact.

The combination of *A. tenuissima* + *C. cladosporioides* showed the lowest incidence (27%), severity (25.3%), and mesocarp damage (26.7%) (Table 2). In one study, *C. cladosporioides*, *A. alternata* and *Fusarium chlamydosporum* were identified as pathogens causing fruit drop and necrosis in papaya. These findings indicate that at least two genera detected in this study (*Cladosporium cladosporioides* and *Alternaria tenuissima*) have a history of causing fruit drop during the early stages of fruit development (Vásquez *et al.*, 2012).

Pérez and Sánchez (2019) report *Cladosporium* as a fungus that can include both saprophytic and opportunistic species; the latter can cause disease when the host's defense mechanisms are weakened. They also note that *Cladosporium* species can be endophytes, which may become pathogenic under conditions of nutritional imbalances or water stress in the plant. In relation to this, Zhang *et al.* (2010) highlight the plasticity of the *Cladosporium* genus, which may largely explain the biological dynamics within the *C. cladosporioides*–Host–Environment–Human system. Along the same lines, Sánchez-Fernández *et al.* (2013) state that the relationship between endophytic fungi and the host plant can range from mutualistic to pathogenic. When the virulence factors of the fungus and the plant's defenses are balanced, an endophytic relationship is maintained; conversely, when the host is under stress, the imbalance favors the fungus, which then behaves as a pathogen and causes disease symptoms in the host. Additionally, *Cladosporium* is capable of releasing enzymes such as cellulases, pectinases, peptidases, hemicellulases, glycosidases, hydrolases, glycosyltransferases, carbohydrate esterases, polysaccharide lyases, and xylanases, which facilitate fungal entry and degradation of the plant tissue's cell wall (Yew *et al.*, 2016).

On the other hand, *Epicoccum nigrum* (synanamorph of *Phoma epicoccina*) (Aveskamp *et al.*, 2010) has been reported as a fungus used in biological control for disease management (Taguiam *et al.*, 2021). However, in this study, it was shown to be a

pathogenic fungus responsible for peduncle ringing rot in avocado fruits, acting either individually or in association with *A. tenuissima* and/or *C. cladosporioides* (Table 2).

According to Andersen *et al.* (2009), *Epicoccum* species are considered weak pathogens; however, fruits of various species infected by this pathogen show rot and a brown halo. Lin *et al.* (2015) reported *E. sorghinum* as the causal agent of twisted leaf disease in sugarcane (*Saccharum officinarum*), leaf spots in cabbage (*Brassica oleracea* var. *capitata*), and infections in species of no agricultural importance. In melon crops (*Cucumis melo*), *E. nigrum* causes necrosis and brown spots on small fruits, and in postharvest stages, it leads to fruit decomposition characterized by reddish discoloration (Lin *et al.*, 2018). This information is supported by Taguiam *et al.* (2021), who note that this pathogen causes postharvest fruit decay, manifesting as red-colored symptoms.

Hernández *et al.* (2023) identified *Neofusicoccum cryptoaustrale/stellenboschiana* as the causal agents of dieback symptoms and peduncle rot, although they were unable to determine which of the two species was responsible. Similarly, Dann *et al.* (2021) report that among the fungal species most frequently isolated from ongoing monitoring in avocado crops in Australia are *Alternaria* spp., *Colletotrichum* spp., and *Botryosphaeria* spp.—well-known avocado pathogens that cause postharvest fruit diseases such as anthracnose and peduncle rot.

CONCLUSIONS

A total of 220 fungal colonies were isolated from avocado fruit samples cv. Hass showing symptoms of peduncle ringing rot, including a distinct ring on the fruit peduncle or brown spots, peduncles with a cork-like appearance, fruits with reddish to purplish exocarp, and in some cases, mummified fruits.

The three fungal colonies present at the collection sites, in order of highest frequency, were *Alternaria tenuissima* (35.6%), *Epicoccum nigrum* (20.6%), and *Cladosporium cladosporioides* (21.2%).

Using Koch's postulates, it was demonstrated that these colonies, when inoculated individually and in combination, reproduced the symptoms of peduncle ringing rot in avocado fruits cv. Hass. No differential symptoms were observed among the inoculated fungi, making field identification difficult. The pathogenic role of the other isolated fungi is not ruled out, nor is the potential involvement of abiotic stress factors such as nutrition, hormonal imbalance, and water deficiency, among others. Therefore, continued research on this pathology is recommended.

LIMITATIONS

The limitations encountered during the development of the research included the lack of available information on the topic. Previous studies related to peduncle ring rot do not report pathogenicity tests, and therefore, there is no verifiable evidence that the pathogens isolated in earlier investigations are responsible for the ring damage observed on avocado fruit peduncles.

CONFLICT OF INTERESTS

The authors explicitly declare that there is no conflict of interest related to this research.

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