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Fungal Species of Botryosphaeriaceae Associated With Avocado Dieback in Southern Spain

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ABSTRACT

Avocado (*Persea americana*) has become one of the most important subtropical crops in southern Spain. In recent years, the scarcity of irrigation water has made this crop susceptible to opportunistic diseases. This sensitivity has been associated with a series of symptoms characterised by the death of branches, mummification of fruits, cankers and reddening of the vascular bundles. To identify the aetiology of the disease, surveys have been carried out on avocado farms in the most affected areas, including Málaga and Granada (southern Spain). The surveys were conducted from 2019 to 2022. A total of 252 fungal isolates were recovered from trees with symptoms of dieback disease. Of these isolates, 172 were initially identified by sequencing the rDNA internal transcribed spacer (ITS) region to identify potential pathogens compatible with the symptoms. Based on the number of isolates, the most prevalent were *Neofusicoccum* spp. (110 isolates) and *Lasiodiplodia* sp. (15 isolates), although some isolates of *Diaporthe* sp. (four isolates) and *Colletotrichum* sp. (eight isolates) were also identified. Pathogenicity experiments in plants, branches and fruits confirmed these fungi as possible causal agents as they reproduced the symptoms found in diseased avocado crops. The frequency of isolates associated with dieback symptoms, along with greater severity in the reproduced symptoms, shows Botryosphaeriaceae species as the main cause of dieback in avocado in southern Spain. The main objective of the present study is to elucidate the aetiology of the disease to establish the infection time and thereby lay the groundwork for designing control studies.

1 | Introduction

Avocado crops were established in Spain in the 1950s on the tropical coasts of Málaga and Granada (Andalusia, southern Spain) and are one of the region's most important strategic crops. Currently, avocado cultivation extends throughout the easternmost region of the Mediterranean coast and northwest Spain. The Canary Islands are also important production areas for this fruit because of their temperate climate. In 2022, approximately 106,500 t of avocados were produced in Spain. Portugal, Greece and Spain are the most important countries producing avocados in Europe, allowing this precious fruit to reach European markets at optimal ripeness with the lowest carbon footprint. However, the increasing incidence of dieback is linked to inclement weather, endangering the production system that is already established in the avocado sector. Although the presence of occasional dry branches has been common in avocado crops in southern Spain for several years, the increase in these symptoms has become worrisome. Andalusian production, which represents 46% of the national

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total, was estimated at 55,682 t for the 2023/24 season, representing a 28.7% drop compared to the previous season, according to the Regional Government (Junta de Andalucía 2025). The most important symptoms related to avocado dieback include necrosis and death of buds, twigs and branches in the tree canopy. Symptomatic plants exhibit dry branches and external necrosis in twigs, leaves and inflorescences (Auger et al. 2013; Hernández et al. 2023), reducing production and undermining plant health. These same symptoms in avocado crops, identified as dieback associated with Botryosphaeria, have been located in other countries such as Chile (Auger et al. 2013) and California, United States (Avenot et al. 2023). Botryosphaeria have been located around the world, associated with other crops such as grapevine, citrus and even forestry (Batista et al. 2021).

In general, this group of fungi, known as the Botryosphaeriaceae, usually causes trunk damage to woody plants by necrotising parts of trunk tissues, including the vascular bundles. Specifically, avocado plants show dieback, cankers in branches and stem-end rot in fruits. The most common species that can cause avocado dieback are *Barriopsis* (*B. fusca*), *Botryosphaeria* (*B. dothidea* and *B. quercuum*), *Diplodia* (*D. seriata* and *D. mutila*), *Dothiorella* (*D. iberica*), *Lasiodiplodia* (*L. theobromae*) and *Neofusicoccum* (*N. australe*, *N. luteum*, *N. parvum*, *N. ribis* and *N. vitifusiforme*) (García et al. 2021).

This fungal family currently has over 20 genera (Batista et al. 2021) distributed worldwide, from Argentina to Alaska and from the United States to China. These fungi are ascomycetes with very similar appearances that can have different interactions with the host; they can be saprophytic, endophytic or pathogenic (Phillips et al. 2013; Slippers and Wingfield 2007). Genera such as *Neofusicoccum* and *Lasiodiplodia* have been described as endophytes, along with other genera of the Botryosphaeriaceae, such as *Guignardia*, *Botryosphaeria*, *Dothidotthia*, *Pseudofusicoccum* and *Diplodia* (Crous et al. 2006). In agricultural and forestry plant communities, woody plants often display high levels of endophyte Botryosphaeriaceae colonisation (Burgess et al. 2006). The Botryosphaeriaceae are particularly successful as opportunistic endophytic colonists, and this is more evident in hosts in disturbed or nonoptimal environments when the plants are under stress or when the host grows in a nonnative environment where the normal metabolite availability of horizontally acquired endophytes is not present (Avenot et al. 2023; Slippers and Wingfield 2007). Diseases caused by Botryosphaeriaceae species are almost exclusively associated with some form of stress, including drought stress, the most common, extensive physical damage, biological stress and frost or heavy snow. Not all species with broad host ranges cause diseases in all the hosts they infest in all areas. For example, *B. dothidea* is a very important pathogen of fruit and nut trees in the United States but seems to be absent or rare on these hosts in South Africa (Pavlic 2013; Slippers and Wingfield 2007). The main objective of the present study is to elucidate the aetiology of the disease and thereby lay the groundwork for designing control studies. To this end, this study determines the species of the agent(s) causing dieback in avocados in southern Spain. The study also confirms the pathogenicity of the most common species found, as well as their distribution in the study region. This work reinforces previous studies, establishing the dominant and causative species of avocado dieback in the Axarquía region, the main avocado-producing area in southern Spain.

2 | Materials and Methods

2.1 | Field Surveys and Fungal Collection

Field surveys were carried out in avocado orchards from March to October 2019, June to July 2020 and June 2022 in the Andalusia region, southern Spain. The samples were collected from avocado trees with clear symptoms of dieback. Twenty-eight different farms located in the Axarquía region (Málaga province), the Granada province coast and Huelva province were evaluated in the study. One branch sample per tree was taken from at least two trees per farm (Table 1).

The samples were transported to the laboratory in closed sterile bags maintained at 4°C and processed on the same day or the next day. The avocado branch samples were surface disinfected by submerging them in a mixture of ethanol, commercial bleach and distilled water (1:2:7; vol:vol:vol). The samples were immersed for 3 min, followed by two consecutive rinses of 3 min each in sterile distilled water and subsequently dried using sterile filter paper. After the surface-disinfection process, small pieces from cankers and/or pycnidia were obtained from the samples and deposited in potato dextrose agar (PDA; Oxoid Ltd) acidified with 1 mL/L lactic acid to prevent bacterial contamination (Beuchat 1979). The samples were incubated for 5–7 days at 25°C for fungal development. The fungal mycelia growing from the avocado samples were obtained from a portion of a single hypha and isolated in pure cultures for further identification. The fungal collection consisted of 252 isolates; fungal isolates with the same characteristics from the same sample were ignored for identification, and 172 isolates were selected for further identification.

2.2 | DNA Extraction, PCR Amplification and Sequencing

After 5 days of mycelial growth at 25°C on PDA plates, the cultures were subjected to DNA extraction via the alkaline lysis method. For this, a small number of hyphae were deposited in a 1.5-mL Eppendorf tube with 25 μL of 0.25 M KOH and heated at 90°C for 10 min. Then, 25 μL of 0.5 M Tris and 0.25 M HCl pH 8 was added, and the mixture was heated again at 90°C for another 10 min. Finally, 50 μL of chloroform:isoamyl alcohol (24:1; vol:vol) mixture was added. The mixture was subsequently mixed well, incubated at 4°C for 30 min and centrifuged at 15,100 *g* for 2 min, after which the supernatant was recovered. Two microlitres of this DNA extraction mixture were used for the PCR. The preliminary identification of the 172 selected isolates was carried out by amplification and sequencing of the rDNA internal transcribed spacer (ITS) region using ITS-1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′), as described by White (1990). The primers for *β-Tubulin* Bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and Bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′), described by Glass and Donaldson (1995), were used for species confirmation of the

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TABLE 1 | Farm and sampling dates of the isolates obtained during the study over 3 years.

Sampling date	Farm name	No. of samples	No. of isolates	
			Botryosphaeriaceae family	Other fungi
18/03/2019	Molino las Monjas	7	4	4
	Nistrica	5	6	2
	Salsa Agrícola	3	4	2
	Flores	8	12	3
02/04/2019	La Señorita	8	9	0
	Río Seco	4	5	1
	Santiago Pardo	5	5	3
24/04/2019	Sarmiento	9	10	3
29/04/2019	La Mayora	3	4	0
	La Campiñuela	3	0	2
	Miguel Gutiérrez	4	0	2
	Vela	4	0	2
	Huerta Valle	3	0	1
11/06/2019	Las Coplas	4	2	5
	Provelpack	5	3	1
26/06/2019	Salsa Natura	4	4	0
	El Broker	3	1	0
	Los Cerros	2	0	1
18/07/2019	La Ventolera	3	2	2
	Agrojete	4	1	0
	Francisco Gallego	3	1	2
18/09/2019	Las Coplas	3	1	1
16/10/2019	Barranco	8	5	5
	La Señorita	4	4	3
	Mirallanos	3	0	2
10/06/2020	Vela	3	2	2
	La Arboleda	2	2	0
	María Alex	2	3	0
	La Campiñuela	6	4	1
	Antonio Mesa	2	5	0
	Los Arcos	2	4	0
06/07/2020	Huelva farm	4	2	4
	Barranco	4	9	0
	La Alegría	3	3	1
27/06/2022	Molino las Monjas	46	43	37

111 isolates pre-identified as *Neofusicoccum* spp. Finally, the *EF1* partial fragment was sequenced using primers EF1-688F (5′-CGGTCACTTGATCTACAAGTGC-3′) and EF1-1251R (5′-CCTCGAACTCACCAGTACCG-3′), described by Alves et al. (2008), for species confirmation of the isolates selected for the pathogenicity assay.

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TABLE 2 | Isolates selected for pathogenicity evaluation and their NCBI accession numbers.

Fungal strain	Species	Genetic region	Accession number
UMAF A1901	*Neofusicoccum australe*	ITS	OR960502
		β-Tubulin	PV357241
		EF1	PV925859
UMAF A1909	*Diaporthe foeniculina*	ITS	OR960510
		β-Tubulin	PV357242
		EF1	PV925860
UMAF A1911	*Neofusicoccum luteum*	ITS	OR960512
		β-Tubulin	PV357243
		EF1	PV925861
UMAF A1916	*Neofusicoccum parvum*	ITS	OR960517
		β-Tubulin	PV357244
		EF1	PV925862
UMAF A1929[a]	*Lasiodiplodia theobromae*	ITS	OR963361
		β-Tubulin	PV357245
	Lasiodiplodia citricola	*EF1*	PV925863
UMAF A1953	*Lasiodiplodia theobromae*	ITS	OR963563
		β-Tubulin	PV357246
		EF1	PV925864
UMAF A1960	*Diaporthe foeniculina*	ITS	OR963570
		β-Tubulin	PV357247
		EF1	PV925865
UMAF A1961	*Neofusicoccum parvum*	ITS	OR963571
		β-Tubulin	PV357248
		EF1	PV925866
UMAF A1963	*Neofusicoccum luteum*	ITS	OR963573
		β-Tubulin	PV357249
		EF1	PV925867
UMAF A1979	*Neofusicoccum australe*	ITS	OR963950
		β-Tubulin	PV357250
		EF1	PV925868

[a]Strain UMAF A1929 is identified as *Lasiodiplodia theobromae* by the rDNA internal transcribed spacer (ITS) and *β-Tubulin* sequences, while the *EF1* sequence gave the highest homology with *Lasiodiplodia citricola*.

The PCR for ITS and *β-Tubulin* amplification was performed in a 50-μL reaction mixture with 2 μL of fungal DNA extraction mixture. The PCR conditions were 94°C for 2 min; 34 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min 30 s; and 72°C for 10 min for the final extension. The PCR conditions for *EF1* amplification were obtained from Alves et al. (2004). The amplification was confirmed with a 1% agarose gel. An Illustra GFX PCR DNA and gel band purification kit (GE Healthcare UK Limited) was used to purify the amplified DNA. These DNA samples were sent to StabVida S.L. (Caparica, Portugal) for Sanger sequencing. The sequences were analysed by comparison against the BLAST database of the National Center for Biotechnology Information (NCBI). The new sequences generated in this study have been deposited in the NCBI database for public access (Table 2).

Genotyping was performed via Universal Primer (UP-PCR) as a complementary system to confirm the *Neofusicoccum* spp. in isolates selected for pathogenicity testing using the primer AA2M2 (5′-CTGCGACCCAGAGCGG-3′; Lübeck et al. 1998). The PCR amplification was conducted according to the specifications of Baskarathevan et al. (2013) and was run in 1.5% agarose at 50 V for 3 h. Ten fungal isolates identified at the species

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level and referred fungal strains from the Spanish Collection of Type Cultures (*Neofusicoccum mediterraneum* CECT20788, *N. luteum* CECT20790, *N. parvum* CECT20791, *N. australe* CECT20989 and *L. theobromae* GIHF272), which were used as controls, were included in the genotyping tests.

2.3 | Phylogenetic Analysis of *Neofusicoccum* spp. Isolates

Concatenated sequences of ITS and *β-Tubulin* were used for *Neofusicoccum* spp. phylogeny. The evolutionary history was inferred by using the maximum-likelihood method and the Tamura–Nei model (Tamura and Nei 1993). The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a neighbour-joining (NJ) tree (Saitou and Nei 1987) and a maximum-parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura–Nei model. The MP tree had the shortest length among 10 MP tree searches, each performed with a randomly generated starting tree. The analytical procedure encompassed 111 nucleotide sequences. The complete deletion option was applied to eliminate positions containing gaps and missing data, resulting in a final data set comprising 663 positions. Evolutionary analyses were conducted using MEGA 12 (Kumar et al. 2024), which uses up to four parallel computing threads.

2.4 | Effect of Temperature on Mycelial Growth Development

Cardinal temperatures for growth rates were determined for each of the 10 fungal isolates analysed in the pathogenicity tests (Table 2) and fungal isolates from public collections used as controls. The analysis was performed based on the method reported by Bellée et al. (2017). Briefly, 2 mm-diameter plugs from the margins of actively growing 5-day-old cultures at 25°C were transferred to PDA and incubated in the dark at 5°C, 15°C, 25°C, 35°C and 40°C. Colony radius was measured at daily intervals for 10 days or until the mycelium covered the entire surface of the agar plates. The growth rates (cm/day) for each strain were determined at each temperature by linear regression model. A second-order polynomial regression curve was used to determine the optimum growth temperature and maximum growth rate for each strain, based on relationships described by Pitt et al. (2013). One-way ANOVA was used to compare the significant differences between the obtained parameters. The experiment had three replicates and was repeated once.

2.5 | Pathogenicity Test of Representative Isolates on Avocado

A representative group of 10 pathogenic isolates was selected from the total isolates obtained and tested in three different systems: (i) avocado plants of the 1-year-old Hass cultivar, (ii) Hass detached stems 10 cm in length and maintained in a humidity-saturated environment, and (iii) Hass avocado fruit (Figure 1). The evaluated isolates were UMAF A1901 and UMAF A1979 (*N. australe*), UMAF A1909 and UMAF A1960 (*Diaporthe foeniculina*), UMAF A1929 (*Lasiodiplodia* sp.) and UMAF A1953 (*L. theobromae*), UMAF A1911 and UMAF A1963 (*N. luteum*), and UMAF A1916 and UMAF A1961 (*N. parvum*).

For in planta assessments, the fungi were grown on PDA plates for 5–7 days. When the mycelium covered the entire plate surface, plugs from the medium edge were extracted and applied to the branch wounds. The avocado (cv. Hass) branch wounds were created with the same diameter as the fungal inoculum. PDA plugs without fungal mycelia were used as a negative control. Three inoculations per tree and five trees per isolate were used for every experiment. The experiments were performed twice. Hence, the average data per tree (using three data/tree) resulted in 10 data points per fungal isolate, which were used for statistical analysis. The results were obtained after 6 months under greenhouse conditions and are expressed as the average lesion extents in centimetres from the inoculum point (Figure 1A–D).

For the in vivo assessment, detached avocado stems 10 cm in length were used; the same inoculum procedure was used as for the in planta experiment. PDA discs with fungal mycelia were obtained and applied to the stem surface wounds. Five detached stems inoculated once per isolate and incubated in one humidity-saturated chamber were used in every experiment. The results were obtained after incubation in humidity-saturated chambers for 4 weeks at 25°C and a light cycle of 16 h light and 8 h dark. The experiment was repeated three times per fungal isolate. Therefore, three average lesion extension lengths expressed in centimetres from five inoculated stems were used for statistical analysis and the total average was used as the resulting data (Figure 1E,F).

For the in fruit assessment, three avocado fruits (cv. Hass) were used per isolate tested. The fruits were superficially disinfected by immersion in a mixture of ethanol, commercial bleach and distilled water (1:2:7; vol:vol:vol) for 5 min, followed by two successive washes in sterile distilled water for 5 min each. The inoculations were performed as described for the previous pathogenicity assays; a PDA disc with mycelia was applied to the stem end of each fruit (Figure 1G–I). The inoculated fruits were incubated at 25°C for 8 days. The results were obtained as the disease incidence, distinguishing rotted fruit from non-rotted fruit and severity levels as categories of rot symptoms. The rotten fruits were classified into four categories: 0, with 0% rot symptoms; 1, with $< 25\%$ of fruit affected; 2, with 25% of fruit affected; and 3, with $> 25\%$ of fruit affected. The experiment was performed twice; hence, six values in total were obtained for statistical analysis (Figure 1J–M).

2.6 | Statistical Analysis

Data distributions were tested via one-way analysis of variance (ANOVA) with the Kolmogorov–Smirnov normality test. In the case of a failed test, Kruskal–Wallis one-way analysis of variance on ranks was applied ($p \leq 0.001$). All

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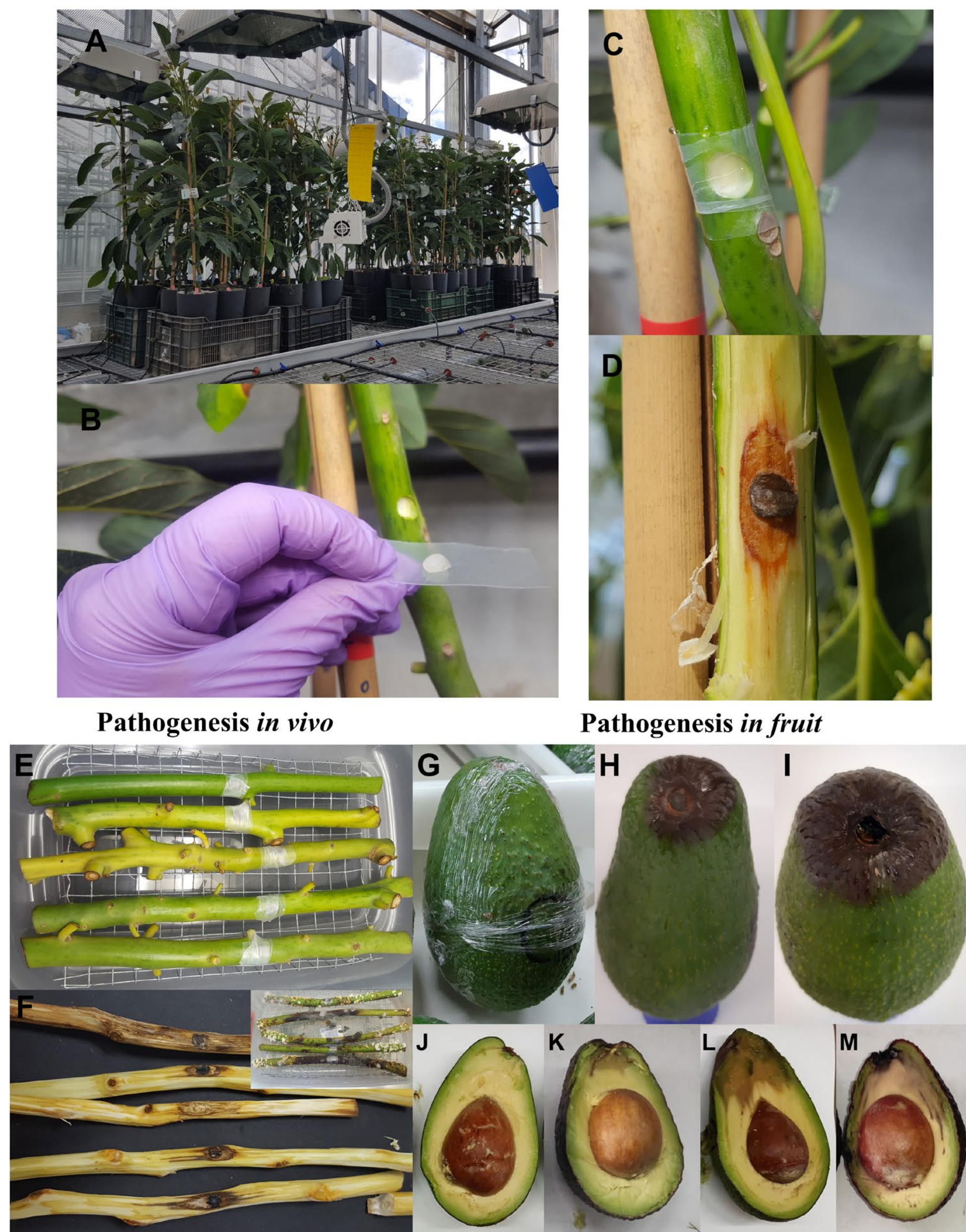

FIGURE 1 | Inoculation process for pathogenicity analysis of selected fungal isolates. (A) Avocado trees used for pathogenicity testing in planta. The plants were maintained in pots under controlled conditions. (B) Branch wounding was performed by punching, and the inoculum, a circular piece of agar with fungal mycelium, was fixed on the wound with Parafilm. (C) Details of the inoculation system. (D) Necrosis damage caused by fungal growth after 6 months of incubation. (E) Detached stems used as grafting rods were inoculated via the same technique used for in planta pathogenicity testing and were maintained in a humidity-saturated chamber. (F) Necrosis damage caused by inoculated fungal isolates in detached stems. (G) Inoculation system used on avocado fruits. The agar piece with mycelial growth was placed in the stem. (H, I) Rot symptoms with different severities produced by fungal activity.(J–M) Different rot severities used for the virulence categories: 0, with no rot symptoms; 1, with < 25% rot area; 2, with 25% rot area; and 3, with > 25% rot area, respectively.

pairwise multiple comparison procedures were performed via the Student–Newman–Keuls method.

The data analyses were performed with SigmaPlot v. 12.0 for Windows (Grafiti LLC). The 99% confidence interval for each response variable was obtained based on the standard error, and the significant differences between the data were estimated.

3 | Results

3.1 | Fungal Isolation From Dieback Disease in Avocado Trees

The main areas that were considered for sampling were the avocado crop region in Málaga (Axarquía region), located east of Málaga Province, close to the Alboran Sea and the coast

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FIGURE 2 | The main dieback symptoms were observed in avocado crops in the Axarquía region of Málaga Province in southern Spain. (A) General overview of avocado crops in the Axarquía region. (B) Avocado panicles affected by dieback disease. (C) Dead branches of an avocado tree showing dieback symptoms. (D) Typical symptoms of dieback on an avocado tree. (E) Dead and mummified avocado fruit due to dieback attack. (F–H) Reddish vascular bundles located in trees affected by dieback; these reddish bundles were found in young branches (F), 2 cm-diameter wood (G) and wood from old branches (H). (I) Perseitol deposits (red arrow) on an avocado tree branch with dieback symptoms. (J) Conidia development on dry and old wood. (K) A few days of fungal mycelial growth on culture media from avocado samples. (L) Spore observation by microscopic magnification of two species of Botryosphaeriaceae located during sampling in Axarquía.

of Granada Province, where some samples were also taken (Table S1). A total of 186 samples from 28 farms were analysed, and a few samples from the Huelva farm (Andalusia West) were also analysed, resulting in a total of 252 isolates (Table 1). The sampling was conducted mainly in 2019 and was conducted again in 2020 in those farms where the highest incidences were observed. In 2022, samples were again taken from the most representative farm for dieback incidence in the Axarquía region.

Avocado tree samples with dieback symptoms were collected from orchards with trees at least 5 years old (Figure 2A). The principal symptoms observed consisted of dry and necrotic tissues that started from floral buds and extended to leaves, stems and branches towards the trunk (Figure 2B–D). In later symptoms, the infection could affect the first fruits, drying and mummifying them while they remained attached to the branches (Figure 2E). At the same time, the vascular bundles changed to a reddish colour as they moved deeper into the branches (Figure 2F–H), which weakened

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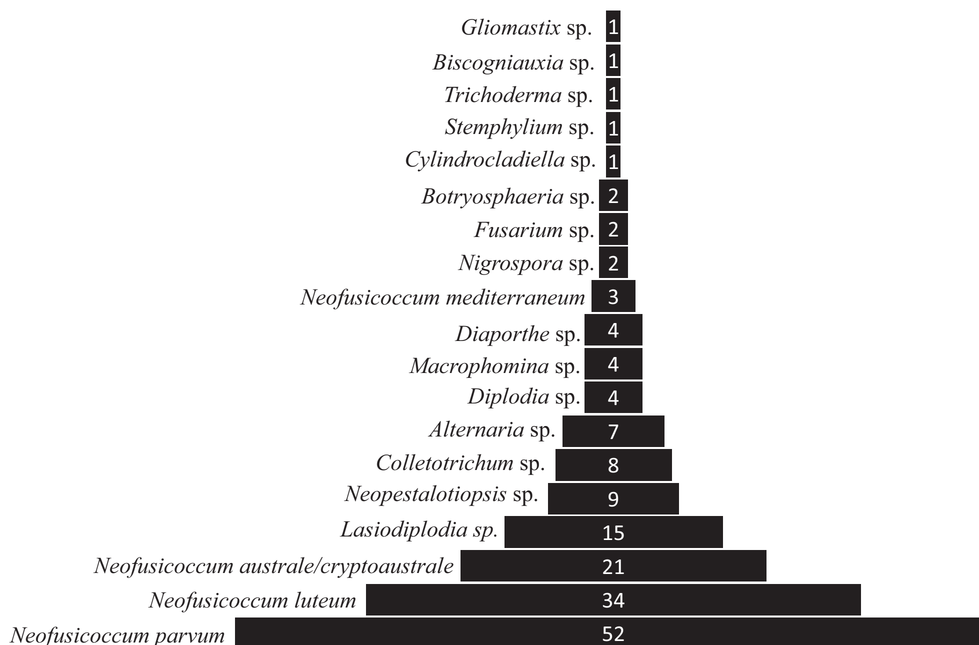

FIGURE 3 | Schematic representation of taxon abundances in the 172 isolates selected for identification. The number of isolates identified in each taxon is indicated.

and finally resulted in the complete drying of the branches. In addition, cankers could appear at branch intersections, sometimes accompanied by perseitol deposition, which could also affect the vascular bundles of that zone (Figure 2I).

Pycnidia formation was also found in avocado orchards affected by dieback, in infected, dry and dead branches and plant remains, which could be easily seen as blackish dots on the surfaces of dead branches (Figure 2J). Samples with these characteristics were obtained from fungal isolates (Table 1), which were largely compatible with the Botryosphaeriaceae fungi (Figure 2K,L).

3.2 | Molecular Identification of Fungal Isolates

The 172 selected fungal isolates represented the variability detected during sample analysis. The initial genus determination was performed by ITS sequence comparison (Table S2). The *Neofusicoccum* genus was assigned to 111 isolates, *Lasiodiplodia* sp. was assigned to 14 isolates, *Diplodia* sp. to four isolates and *Botryosphaeria* sp. to two isolates, all of them belonging to the Botryosphaeriaceae group. Further, other pathogens related to dieback symptoms were detected, such as eight isolates of *Colletotrichum* sp. and four isolates of *Diaporthe* sp. Amplification and sequencing of *β-Tubulin* (Table S3) readjusted the number of isolates belonging to each species of *Neofusicoccum* isolated in this study (Figure 3). For complementation, multilocus phylogenetic analyses with the highest log likelihood (−75,646.88) were performed, using ITS and *β-Tubulin* sequences, in order to confirm the identification of the *Neofusicoccum* spp. isolates obtained (Figure S1).

Regarding the isolates selected for the pathogenicity experiments, *TEF1* was used as the third gene in the species identification (Table 2, Table S3). A genotyping test was used as a complementary method to confirm the fungal species of the isolates assayed in the pathogenicity test (Figure 4). The band-code of Np (*Neofusicoccum parvum* CECT20791) was highly similar to those of UMAF A1916 and UMAF A1961, which were previously identified as *N. parvum*, except for an additional band that appears to be the isolate UMAF A1916. Similarly, the Nl (*N. luteum* CECT20790) band-code was similar to UMAF A1911 and UMAF A1963, previously identified as *N. luteum* and Na (*N. australe* CECT20989) was similar to UMAF A1901 and UMAF A1979, previously identified as *N. australe*. The band codes of UMAF A1909 and UMAF A1960, identified as *D. foeniculina*, did not match those of any of the controls used and Nm (*N. mediterraneum* CECT20788) did not match any of the isolates analysed. Finally, Lt (*L. theobromae* GIHF272), UMAF A1929 and UMAF A1953, identified as *L. theobromae*, did not show a sufficient number of bands to create a code, so additional isolates of the genus *Lasiodiplodia* did not show sufficient confidence to clarify the species. Due to the phylogenetic closeness between *N. australe* and *N. cryptoaustrale*, more molecular analysis is required to distinguish them than was carried out here, so the isolates identified in this group are named *N. australe/cryptoaustrale*.

The identification analysis revealed that *N. parvum* was the dominant species with 52 isolates (30.2%), followed by *N. luteum* with 34 isolates (19.8%), *N. australe/cryptoaustrale* with 21 isolates (12.2%) and *Lasiodiplodia* sp. with 15 isolates (8.7%). The species *N. mediterraneum* was represented by only 1.7% of the isolates (Figure 3). The fungal species were not homogeneously distributed across all farms (Figure 5). *N. parvum* was present in almost all the farms except four, namely, Salsa Agrícola, Las Coplas, Provelpack and Salsa Natura, where *N. luteum* was the dominant fungus isolated. *N. parvum* was the only pathogen isolated from the Río Seco, El Broker and La Arboleda farms in Málaga Province and from the La Ventolera and Francisco Gallego farms in Granada Province. The remaining farms had at least two or more Botryosphaeriaceae species recovered. The

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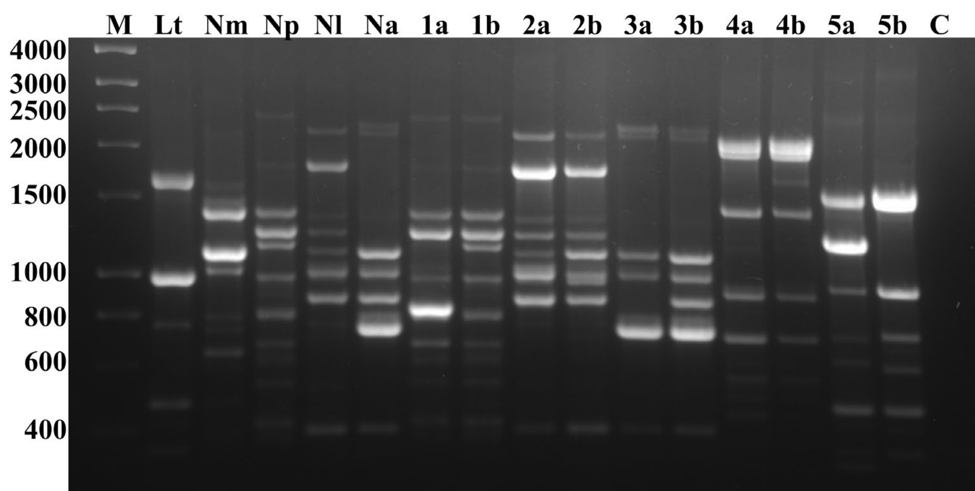

FIGURE 4 | The identification of Botryosphaeriaceae species was confirmed via UP–PCR using the primer AA2M2. Lines, M: HyperLadder1 kb (Meridian Bioscience), Lt: *Lasiodiplodia theobromae* GIHF272, Nm: *Neofusicoccum mediterraneum* CECT20788, Np: *Neofusicoccum parvum* CECT20791, Nl: *Neofusicoccum luteum* CECT20790, Na: *Neofusicoccum australe* CECT20989, 1a: UMAF A1916, 1b: UMAF A1961, 2a: UMAF A1911, 2b: UMAF A1963, 3a: UMAF A1901, 3b: UMAF A1979, 4a: UMAF A1909, 4b: UMAF A1960, 5a: UMAF A1929, 5b: UMAF A1953, C: negative control for PCR.

other two species of *Neofusicoccum*, *N. australe/cryptoaustrale*, were isolated from 50% of the farms visited in La Axarquía, and *N. mediterraneum* was isolated from only two farms, Barranco in Vélez-Málaga and Sarmiento in Benamocarra, very close to Vélez-Málaga.

3.3 | Growth Rate of Fungal Isolates Selected for Pathogenicity Analysis

Fungal isolates selected for pathogenicity analysis grew over a range of temperatures from 15°C to 35°C (Table 3). No growth was obtained at the most extreme temperatures of 5°C and 40°C in any of the isolates tested. Only the isolates corresponding to *Lasiodiplodia* sp. managed to achieve some growth at 35°C without reaching the maximum rate during the 10 days of the test. At 15°C, growth was obtained in all analysed isolates, with a slower growth rate in those corresponding to *D. foeniculina*, which did not reach maximum growth within the 10 days of the test. The remaining isolates reached maximum growth after 6–7 days of incubation. A higher growth rate was obtained at 25°C, where *Neofusicoccum* spp. species reached maximum growth rate on the fifth day of incubation, *Lasiodiplodia* sp. isolates reached it on the third day and *Diaporthe* sp. presented the lowest growth rate. Regarding optimal growth temperature, *Lasiodiplodia* sp. showed the highest temperature and *N. luteum* the lowest, with all the strains having an optimum between 21°C and 24°C. The optimal temperature for *N. australe* isolates was the most variable, ranging from 20.6°C (UMAF A1979) to 23.3°C (UMAF A1901), with the CECT20989 collection isolate at 21.9°C (Table 3).

3.4 | Pathogenicity Analysis in Avocado Plants and Fruits

The results obtained from inoculated plants revealed that the isolates produced different levels of virulence (Figure 6A). The most virulent isolate was UMAF A1929 (*Lasiodiplodia* sp.), followed by UMA A1963 (*N. luteum*). On the other hand, the least virulent isolate was UMAF A1901 (*N. australe/cryptoaustrale*), followed by UMAF A1911 (*N. luteum*) and UMAF A1960 (*Diaporthe* sp.). The rest of the tested isolates presented intermediate virulence, ranging from 6 to 8 cm lesion length, with high variability.

The results obtained from inoculated detached stems differed from those obtained from inoculated plants (Figure 6B). The lesions produced were generally smaller than those produced in plants, highlighting UMAF A1911 (*N. luteum*) as the most virulent isolate. The least virulent isolate in this assay was UMAF A1963 (*N. luteum*), followed by UMAF A1901 (*N. australe/cryptoaustrale*). The remaining isolates presented lesions ranging from 2.5 to 4 cm in length with high variability. Finally, the inoculation of avocado fruits indicated that UMAF A1916 (*N. parvum*) and UMAF A1929 (*Lasiodiplodia* sp.) were the most virulent isolates, followed by UMAF A1953 (*L. theobromae*) and UMAF A1911 (*N. luteum*). The least virulent strains were UMAF A1961 (*N. parvum*), followed by UMAF A1901 (*N. australe/cryptoaustrale*) and UMAF A1909 (*Diaporthe* sp.) (Figure 6C).

To fulfil Koch's postulates, the inoculated fungal isolates were recovered from infected plants, and their species were confirmed (Table 4). Furthermore, fungal infection development in inoculated avocado plants was also evaluated by fungal isolation from the upper and lower sections of the inoculated points. The results revealed that the inoculated fungus was recovered from at least 70% of the evaluated samples, as was the case for the *Diaporthe* sp. isolates, UMAF A1909 and UMAF A1960 and *N. australe/cryptoaustrale* UMAF A1979. Only UMAF A1901 (*N. australe/cryptoaustrale*) was detected below the section of the inoculation point in 33% of the analysed samples; all the rest were detected above the section of the inoculation point, except UMAF A1909 (*Diaporthe* sp.), UMAF A1961 (*N. parvum*) and UMAF A1963 (*N. luteum*) which were detected only at the infection point.

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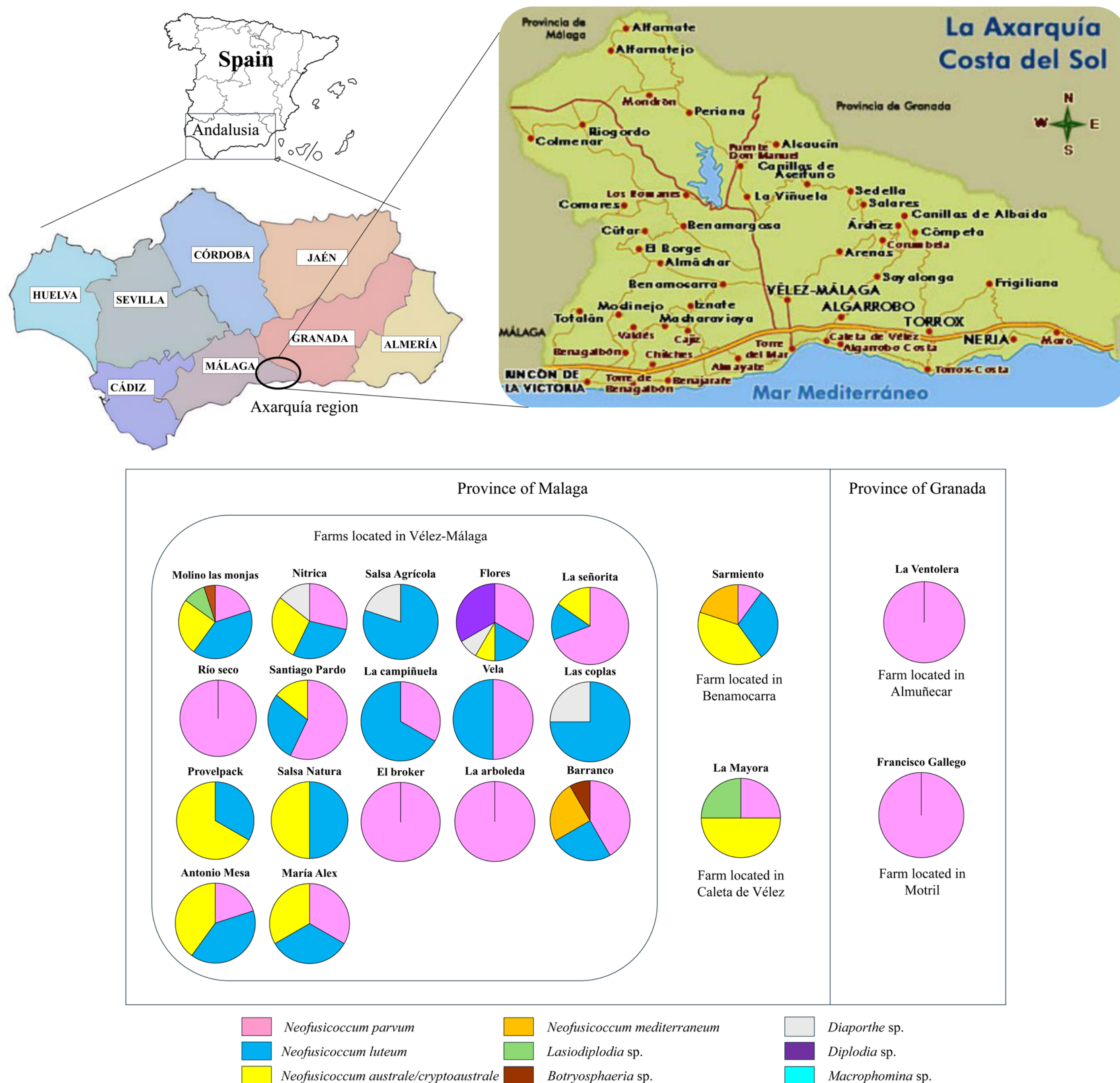

FIGURE 5 | Geographical location of the avocado farm regions from which the samples were taken and the relative abundances of pathogens, genera and species of the potential causal agents of dieback disease in the avocado plants of each farm sampled in the current study in the Axarquía region.

4 | Discussion

Fungal pathogens in the Botryosphaeriaceae have been reported as the causal agents of severe losses in a wide range of woody hosts, including agricultural, horticultural and forest plants (Palavouzis et al. 2023). As examples, *Botryosphaeria* spp. have been associated with grapevine in Morocco (Baiz et al. 2024) and *Neofusicoccum* spp. have been isolated from wood symptoms in weeping cypress in China (Li et al. 2010) and from avocado in California (McDonald et al. 2009).

The avocado crops in Spain are restricted to specific locations around the country, such as the Canary Islands, Valencia Community, some northern localities and Andalusia. The regions with more intense production in Andalusia are Málaga and Granada, where the climate conditions are considered subtropical (Rodríguez-Pleguezuelo et al. 2012). The characteristics of this location in Spain are favourable winters with mild temperatures and hot and dry summers. Although the temperatures are acceptable for growing avocados, the water availability can be problematic, as there is no regular annual rainfall. The close relationship between dieback disease produced by the Botryosphaeriaceae and stressed hosts is known (Slippers and Wingfield 2007). The temperature and rainfall records collected in the Axarquía region from 2013 to 2023 show that since 2018, when there was greater water availability, rain has not been able to cover the needs for irrigation water and its availability decreased every year, with 2023 having the greatest degree of

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TABLE 3 | Cardinal temperatures of fungal isolates used in pathogenesis.

Species and strain	Growth rate[a] 15°C	25°C	35°C	Maximum growth rate[b]	Optimal growth temperature (°C)	R^{2}[c]
Neofusicoccum australe						
UMAF A1901	0.54	1.06	0.0	1.09d	23.3cd	0.971
UMAF A1979	0.79	0.84	0.0	0.94d	20.6g	0.991
CECT20989	0.94	1.22	0.0	1.30cd	21.9f	0.971
Diaporthe foeniculina						
UMAF A1909	0.42	0.65	0.0	0.68e	22.6e	0.986
UMAF A1960	0.36	0.53	0.0	0.54e	22.3e	0.966
Neofusicoccum luteum						
UMAF A1911	0.79	1.05	0.0	1.09d	21.9f	0.961
UMAF A1963	0.77	1.00	0.0	1.06d	21.9f	0.998
CECT20790	0.80	0.99	0.0	1.06d	21.7f	0.970
Neofusicoccum parvum						
UMAF A1916	0.81	1.68	0.0	1.70b	23.4bc	0.988
UMAF A1961	0.64	1.23	0.0	1.40c	23.3cd	0.959
CECT20791	1.21	1.88	0.0	1.94b	22.6e	0.994
Lasiodiplodia sp.						
UMAF A1929	0.87	1.4	0.21	1.42c	23.0d	0.965
UMAF A1953	0.96	2.8	0.18	2.80a	24.1a	0.998
GIHF 272	1.20	2.73	0.09	2.70a	23.6b	0.991

Note: The different letters displayed for the maximum growth rate and optimal growth temperature indicate statistically significant differences between the values obtained from the species and strains used in the analysis.
[a]Growth rate values (cm/day) from linear regression equations obtained by radial mycelial growth along 10 days for each temperature.
[b]Combines data from duplicate trials at temperatures 15°C, 25°C and 35°C.
[c]R^2 values of polynomial regression equations used to determine optimal temperatures for maximum growth rate (cm/day).

drought (Figure S2). This period from 2019 to 2023 coincides with the further development of dieback in avocado crops in La Axarquía.

Molecular identification, phylogenetic analysis, genotyping and cardinal growth temperature were performed to determine the causal agents of dieback. Molecular identification and phylogenetic analysis are powerful techniques used to classify organisms and understand their evolutionary relationships. In this study, *Neofusicoccum* is shown to be the most common genus associated with avocado dieback in southern Spain, with the species *N. parvum*, followed by *N. luteum*, being the most isolated. However, other species, such as *N. australe*, have also been identified. *N. australe* has been found only in temperate and Mediterranean ecosystems, while others have a wider range of dispersion (Batista et al. 2021). *N. luteum* has a wider range, including tropical and subtropical zones and *N. parvum* is the best adapted as it has been detected from the north to the south, except the boreal forest and montane grasslands (Batista et al. 2021).

The phylogenetic analysis used to group and confirm the species we found in this study was not strong enough. The genus *Neofusicoccum*, as defined by Crous et al. (2006), is part of Clade 6, which includes *Botryosphaeria*-like species with anamorphs similar to *Fusicoccum* and synanamorphs similar to *Dichomera*. The name *Neofusicoccum* was created for this group. When using sequences from public databases, a careful selection process is needed. Even after using BlastN to analyse and organise them, some species like *N. terminaliae* and *N. ursorum*, or *N. stellenboschiana* and *N. cryptoaustrale*, still group together, as shown by Choi et al. (2021).

Something similar occurs with the species of *Lasiodiplodia*, where Madhu et al. (2025) report that the phylogenetic trees derived from individual ITS and *β-Tubulin* sequences and concatenated sequences did not offer sufficient resolution to accurately place the *Lasiodiplodia* species. This limitation was further reflected in Blast search results of these genes, which also failed to differentiate species clearly, complicating accurate identification and differentiation (Madhu et al. 2025).

Genotyping and growth temperatures were performed as additional analyses in the isolates selected for pathogenicity assays. Genotyping is a useful tool for classification (Lübeck et al. 1998) and has been applied to isolate specific markers in *N. parvum* and *N. luteum* (Baskarathevan et al. 2013). In the present study,

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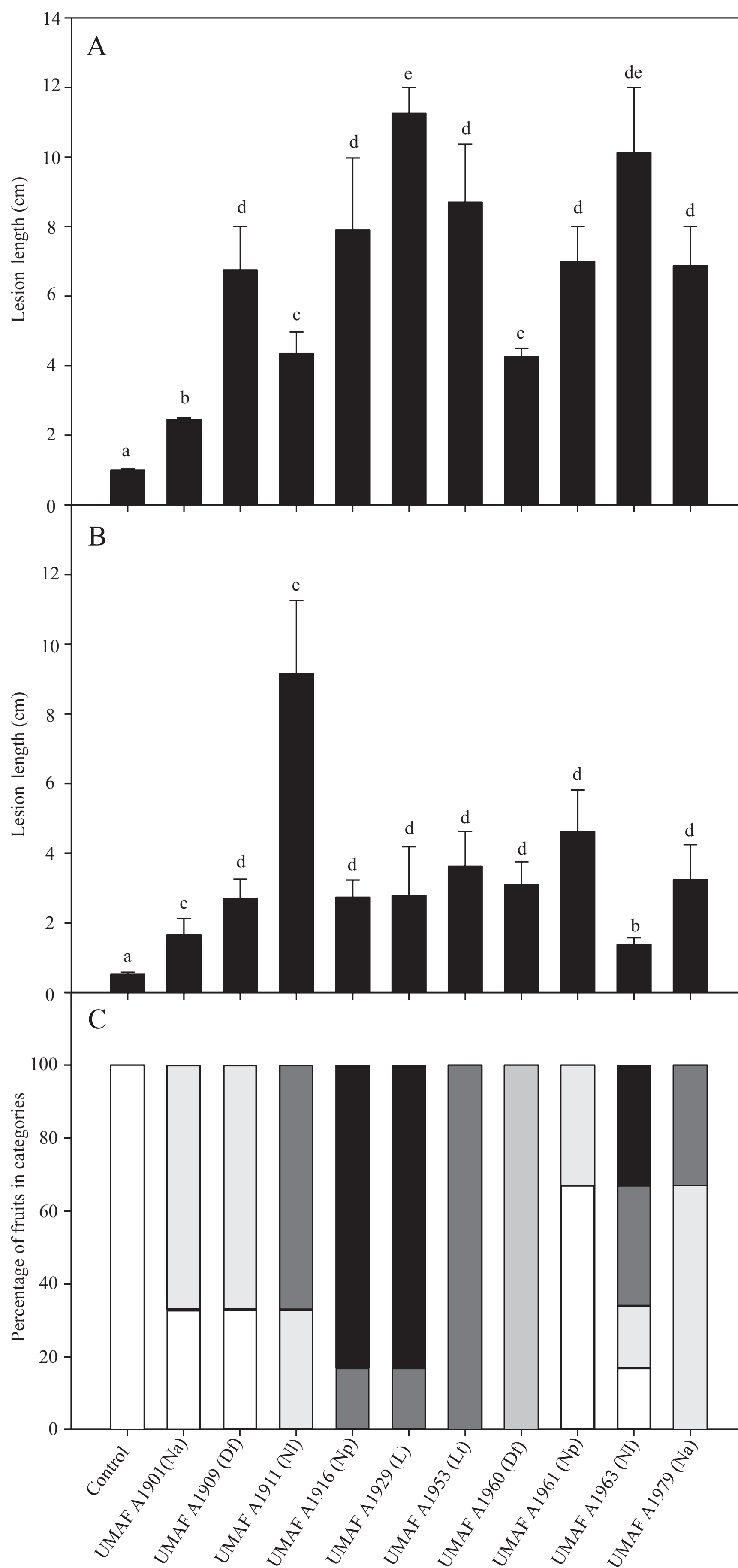

FIGURE 6 | Legend on next page.

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FIGURE 6 | Virulence of the fungal isolates analysed for pathogenicity. (A) Pathogenicity in planta; virulence is expressed as the lesion size in branches of plants maintained in pots under greenhouse conditions. (B) Pathogenicity in vivo, with virulence expressed as the lesion size in branches obtained from avocado plants older than 5 years. The detached stems were inoculated and maintained in humidity-saturated chambers. (C) Pathogenicity in fruits; virulence is expressed as categories. Category 0 (in white), with 0% rot symptoms; 1 (light grey), with < 25% affected fruit; 2 (dark grey), with 25% affected fruit; 3 (black), with > 25% affected fruit.

TABLE 4 | Incidence percentage of inoculated fungi in avocado plants of the 1-year-old Hass cultivar.

Inoculated fungus	Above inoculation point (%)	Inoculation point (%)	Below inoculation point (%)	Re-identification
Negative control	0	0	0	—
Neofusicoccum australe UMAF A1901	28	100	33	*N. australe*[a]
Diaporthe foeniculina UMAF A1909	0	70	0	*D. foeniculina*
Neofusicoccum luteum UMAF A1911	58	100	0	*N. luteum*
Neofusicoccum parvum UMAF A1916	20	100	0	*N. parvum*
Lasiodiplodia theobromae UMAF A1929	50	100	0	*L. theobromae*
L. theobromae UMAFA1953	40	100	0	*L. theobromae*
D. foeniculina UMAF A1960	33	70	0	*D. foeniculina*
N. parvum UMAF A1961	0	90	0	*N. parvum*
N. luteum UMAF A1963	0	100	0	*N. luteum*
N. australe UMAF A1979	10	70	0	*N. australe*

Note: Isolation and re-identification of every inoculated fungus from inoculation points and sections above and below them. The test was performed in each inoculation.
[a]Indefinite identification between *Neofusicoccum australe* and *N. cryptoaustrale*.

the application range was slightly expanded, and UP-PCR was used to confirm the species previously identified by ITS and *β-Tubulin* sequencing, using specific band codes. The polymorphism between isolates of the same species from this study was shown as the presence/absence of genomic bands in the band codes, which may correspond to markers specific to those particular isolates (Baskarathevan et al. 2013). These results support further population monitoring and studies on the ecology and location of genetic markers of the different strains detected in the field as the main causal agents of dieback in the southern area of Spain. Growth studies at different temperatures have confirmed that *Neofusicoccum* species have a growth range between 15°C and 25°C, their optimal temperature ranging between 21°C and 24°C; only *Lasiodiplodia* sp. was able to withstand temperatures above 30°C (Bellée et al. 2017; Pitt et al. 2013). This range of 15°C–25°C coincides with the average daytime temperatures in winter and spring in the southern region of Spain, where this study was conducted.

The early detection of avocado dieback disease in Andalusia was previously described (Arjona-Girona et al. 2019; Zea-Bonilla et al. 2007). From these studies, the incidence and damage caused by dieback increased, probably due to an increase in water stress caused by the gradual reduction in rainfall (Figure S2). In the present study, the number of isolates obtained and the number of isolates analysed has increased, extending the study both in sampling points and years. The results obtained partially confirm what was reported in 2014, with *N. parvum*, *N. luteum*, *N. australe/cryptoaustrale* and *L. theobromae* continuing to be the pathogens most frequently isolated from samples with dieback symptoms. However, *Colletotrichum gloeosporioides* and *N. mediterraneum* have lost their importance as the main causes of the disease because their isolation frequency was not comparable to that of *N. parvum* or *N. luteum*. This study suggests that the Botryosphaeriaceae are principally responsible for dieback in avocado trees in southern Spain.

The pathogenicity test performed in the present study revealed that the severity of symptoms depends on the species and the specific isolates within the species. *D. foeniculina* belongs to a different family than Botryosphaeriaceae; however, it is related to trunk disease in grapevines (Makris et al. 2022), and in many other woody crops. In Andalusia it has also been described associated with branch dieback and shoot blight of walnut, in coinfections with Botryosphaeriaceae (Jash et al. 2025), and was isolated from a few samples, so its pathogenicity in avocado was also tested. This ascomycete fungal species, belonging to the Diaporthaceae family, could induce necrotic symptoms; however,

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it belongs to the less virulent group along with *N. australe/crypto-australe*. However, unlike the latter, *Diaporthe* sp. can interact the *Neofusicoccum* spp., influencing the severity of symptoms (López-Moral et al. 2023). The severities of the other species tested, namely, *N. parvum*, *N. luteum* and *L. theobromae*, depended on the tissues evaluated. When the pathogens were inoculated into live plants, the most virulent isolate (UMAF A1929) was identified as *L. theobromae*, followed by UMAF A1963, which was identified as *N. luteum*. The *N. parvum* isolates were also virulent but were significantly less so than the other two isolates. These results differ from those of previous studies in which *N. parvum* was shown to be the most virulent isolate tested for pathogenicity (Arjona-Girona et al. 2019). However, because the species *N. parvum*, *N. luteum* and *L. theobromae* are described as causing dieback (Biju et al. 2021; Fernández et al. 2021; Otoya-Martinez et al. 2023), their virulence is linked to the isolate, and the degree of virulence can vary among these three species depending on the isolates evaluated. The difference in aggressiveness is linked to the capacity and variety of the set of effectors produced, such as cell wall-degrading enzymes, secondary metabolites and peptidases. *Neofusicoccum* and *Lasiodiplodia* are among those with the highest number of genes encoding CAZymes involved in cell wall degradation (Belair et al. 2023). Finally, *L. theobromae* and *N. parvum* were the principal pathogens identified in the fruit test. *L. theobromae* has been described as a postharvest pathogen that causes stem end rot and fruit rot in many varieties of fruits, including guava, mango and avocado (Garibaldi et al. 2012; Ma et al. 2021; Zee et al. 2021). Although *N. parvum* is not a common postharvest pathogen, it was identified as the causal agent of mango stem end rot and could produce rot in inoculated avocados (Guirado-Manzano et al. 2024).

The conclusion of this study is that the two main genera currently causing dieback in avocado crops in southern Spain are *Neofusicoccum* spp., which have been present in the region for at least 10 years. These opportunistic pathogens are common members of the microbiota of the plant phyllosphere, so their control becomes very difficult (Slippers and Wingfield 2007). Much of this difficulty is because different species produce dieback symptoms, so other interactions can lead to the same result in the host. The incidence of these symptoms depends on the characteristics of the host, such as the plant variety, location, type of tissue and/or stress response (Batista et al. 2021). These opportunistic pathogens are distributed worldwide, although there could be variability among isolates of the same species, which could be related to better adaptation to local conditions.

Author Contributions

María Crespo: investigation, methodology. **Lucía Guirado-Manzano:** investigation, methodology. **David Sarmiento:** conceptualization, investigation. **Emilio Guirado:** conceptualization, investigation. **Antonio de Vicente:** funding acquisition. **Dolores Fernández-Ortuño:** conceptualization. **Francisco M. Cazorla:** funding acquisition, supervision, writing – review and editing. **Eva Arrebola:** writing – original draft, writing – review and editing, conceptualization, formal analysis, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in RIUMA, the Institutional repository of the University of Malaga (https://riuma.uma.es/xmlui/) at https://doi.org/10.24310/riuma.38318.

References

Alves, A., A. Correia, J. Luque, and A. J. L. Phillips. 2004. "*Botryosphaeria corticola* sp. nov. on *Quercus* Species, With Notes and Description of *Botryosphaeria stevensii* and Its Anamorph *Diplodia mutila*." *Mycologia* 96: 598–613. https://doi.org/10.1080/15572536.2005.11832956.

Alves, A., P. W. Crous, A. Correia, and A. J. L. Phillips. 2008. "Morphological and Molecular Data Reveal Cryptic Speciation in *Lasiodiplodia theobromae*." *Fungal Diversity* 28: 1–13.

Arjona-Girona, I., D. Ruano-Rosa, and C. J. López-Herrera. 2019. "Identification, Pathogenicity and Distribution of the Causal Agent of Dieback in Avocado Orchards in Spain." *Spanish Journal of Agricultural Research* 17: e1003. https://doi.org/10.5424/sjar/2019171-13561.

Auger, J., F. Palma, I. Pérez, and M. Esterio. 2013. "First Report of *Neofusicoccum australe* (*Botryosphaeria australis*), as a Branch Dieback Pathogen of Avocado Trees in Chile." *Plant Disease* 97: 842. https://doi.org/10.1094/PDIS-10-12-0980-PDN.

Avenot, H. F., D. Vega, M. L. Arpaia, and T. J. Michailides. 2023. "Prevalence, Identity, Pathogenicity, and Infection Dynamics of Botryosphaeriaceae Causing Avocado Branch Canker in California." *Phytopathology* 113: 1034–1047. https://doi.org/10.1094/PHYTO-11-21-0459-R.

Baiz, Z., S. Dkour, and A. Sellami. 2024. "Management of a New Grapevine Dieback in Morocco: Botryosphaeria Dieback (Black Dead Arm) Caused by Botryosphaeriaceae." *Archives of Phytopathology and Plant Protection* 57: 75–94. https://doi.org/10.1080/03235408.2024.2336898.

Baskarathevan, J., M. V. Jasper, E. E. Jones, and H. J. Ridgway. 2013. "Development of Isolate-Specific Markers for *Neofusicoccum parvum* and *N. luteum* and Their Use to Study Rainwater Splash Dispersal in the Vineyard." *Plant Pathology* 62: 501–509. https://doi.org/10.1111/j.1365-3059.2012.02675.x.

Batista, E., A. Lopes, and A. Alves. 2021. "What Do We Know About Botryosphaeriaceae? An Overview of a Worldwide Cured Dataset." *Forests* 12: 313. https://doi.org/10.3390/f12030313.

Belair, M., J. D. Restrepo-Leal, C. Praz, et al. 2023. "Botryosphaeriaceae Gene Machinery: Correlation Between Diversity and Virulence." *Fungal Biology* 127: 1010–1031. https://doi.org/10.1016/j.funbio.2023.03.004.

Bellée, A., G. Comont, A. Nivault, et al. 2017. "Life Traits of Four Botryosphaeriaceae Species and Molecular Responses of Different Grapevine Cultivars or Hybrids." *Plant Pathology* 66: 763–776. https://doi.org/10.1111/ppa.12623.

Beuchat, L. R. 1979. "Comparison of Acidified and Antibiotic-Supplemented Potato Dextrose Agar From Three Manufacturers for Its Capacity to Recover Fungi From Foods." *Journal of Food Protection* 42: 427–428.

Biju, C. N., A. Jeevalatha, M. F. Peeran, et al. 2021. "Association of *Lasiodiplodia theobromae* With Die-Back and Decline of Nutmeg

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as Revealed Through Phenotypic, Pathogenicity and Phylogenetic Analyses." *Biotech* 9: 422. https://doi.org/10.1007/s13205-021-02961-y.

Burgess, T. I., M. Sakalidis, and G. E. St.J. Hardy. 2006. "Gene Flow of the Canker Pathogen *Botryosphaeria australis* Between *Eucalyptus globulus* Plantations and Native Eucalypt Forests in Western Australia." *Austral Ecology* 31: 559–566. https://doi.org/10.1111/j.1442-9993.2006.01596.x.

Choi, S., N. C. Paul, K. H. Lee, H. J. Kim, and H. Sang. 2021. "Morphology, Molecular Phylogeny, and Pathogenicity of *Neofusicoccum parvum*, Associated With Leaf Spot Disease of a New Host, the Japanese Bay Tree (*Machilus thunbergii*)." *Forests* 12: 440. https://doi.org/10.3390/f12040440.

Crous, P. W., B. Slippers, M. J. Wingfield, et al. 2006. "Phylogenetic Lineages in the Botryosphaeriaceae." *Studies in Mycology* 55: 235–253. https://doi.org/10.3114/sim.55.1.235.

Fernández, L., D. Paiva, I. Roxo, J. Trovão, H. Bragança, and A. Portugal. 2021. "First Report of *Neofusicoccum luteum* as the Causal Agent of Canker and Die-Back of *Cupressus sempervirens*." *Silva Lusitana* 29: 101–114. https://doi.org/10.1051/silu/20212902101.

García, J. F., D. P. Lawrence, A. Morales-Cruz, et al. 2021. "Phylogenomics of Plant-Associated Botryosphaeriaceae Species." *Frontiers in Microbiology* 12: 652802. https://doi.org/10.3389/fmicb.2021.652802.

Garibaldi, A., D. Bertetti, M. T. Amatulli, J. Cardinale, and M. L. Gullino. 2012. "First Report of Postharvest Fruit Rot in Avocado (*Persea americana*) Caused by *Lasiodiplodia theobromae* in Italy." *Plant Disease* 96: 460. https://doi.org/10.1094/PDIS-10-11-0886.

Glass, N. L., and G. C. Donaldson. 1995. "Development of Primer Sets Designed for Use With the PCR to Amplify Conserved Genes From Filamentous Ascomycetes." *Applied and Environmental Microbiology* 61: 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995.

Guirado-Manzano, L., S. Tienda, J. A. Gutiérrez-Barranquero, A. de Vicente, F. M. Cazorla, and E. Arrebola. 2024. "Biological Control and Cross Infections of the *Neofusicoccum* spp. Causing Mango Postharvest Rots in Spain." *Horticulturae* 10: 166. https://doi.org/10.3390/horticulturae10020166.

Hernández, D., O. García-Pérez, S. Perera, M. A. González-Carracedo, A. Rodríguez-Pérez, and F. Siverio. 2023. "Fungal Pathogens Associated With Aerial Symptoms of Avocado (*Persea americana* Mill.) in Tenerife (Canary Islands, Spain) Focused on Species of the Family Botryosphaeriaceae." *Microorganisms* 11: 585. https://doi.org/10.3390/microorganisms11030585.

Jash, S., A. Sarkar, G. Moinuddin, and R. Kundu. 2025. "Phomopsis." In *Compendium of Phytopathogenic Microbe in Agro-Ecology*, edited by N. Amaresan and K. Kumar, 547–569. Springer.

Junta de Andalucía, J. 2025. "Síntesis de Campaña, Sector Subtropicales, Aguacate 2023/24." Observatorio de precios y mercado. https://ws142.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/FrontController?action=RecordContent&table=11031&element=4791464&subsector=.

Kumar, S., G. Stecher, M. Suleski, M. Sanderford, S. Sharma, and K. Tamura. 2024. "MEGA12: Molecular Evolutionary Genetics Analysis Version 12 for Adaptive and Green Computing." *Molecular Biology and Evolution* 41: msae263. https://doi.org/10.1093/molbev/msae263.

Li, S. B., J. Z. Li, S. C. Li, Z. H. Lu, J. H. Wang, and H. Zhang. 2010. "First Report of *Neofusicoccum parvum* Causing Dieback of Chinese Weeping Cypress in China." *Plant Disease* 94: 641. https://doi.org/10.1094/PDIS-94-5-0641C.

López-Moral, A., M. Lovera, B. I. Antón-Domínguez, et al. 2023. "Effect of Cultivar Susceptibility, Fruit Maturity, and Natural Wounds on the Infection of English Walnut (*Juglans regia* L.) Fruits by Botryosphaeriaceae and *Diaporthe* Fungi." *Journal of Plant Pathology* 105: 1391–1401. https://doi.org/10.1007/s42161-023-01492-0.

Lübeck, P. S., I. A. Alekhina, M. Lübeck, and S. A. Bulat. 1998. "UP-PCR Genotyping and rDNA Analysis of *Ascochyta pisi* Lib." *Journal of Phytopathology* 146: 51–55. https://doi.org/10.1111/j.1439-0434.1998.tb04749.x.

Ma, Y., T. Ahmad, Y. Zheng, C. Nie, and Y. Liu. 2021. "First Report of Postharvest Stem End Rot of Mango Fruit (*Mangifera indica*) Caused by *Lasiodiplodia theobromae* in China." *Plant Disease* 105: 2715. https://doi.org/10.1094/PDIS-01-21-0168-PDN.

Madhu, G. S., A. T. Rani, B. M. Muralidhara, et al. 2025. "Diversity, Phylogeny, and Pathogenicity of *Lasiodiplodia* spp. Infecting Avocado in India and Development of Sensitive Point-Of-Care LAMP Assay for Detection of *Lasiodiplodia pseudotheobromae*." *Physiological and Molecular Plant Pathology* 138: 102687. https://doi.org/10.1016/j.pmpp.2025.102687.

Makris, G., S. Solonos, M. Christodoulou, and L. Kanetis. 2022. "First Report of *Diaporthe foeniculina* Associated With Grapevine Trunk Diseases on *Vitis vinifera* in Cyprus." *Plant Disease* 106: 1294. https://doi.org/10.1094/PDIS-03-21-0503-PDN.

McDonald, V. M., S. Lynch, and A. Eskalen. 2009. "First Report of *Neofusicoccum australe*, *N. luteum*, and *N. parvum* Associated With Avocado Branch Canker in California." *Plant Disease* 93: 967. https://doi.org/10.1094/PDIS-93-9-0967B.

Otoya-Martinez, N., L. G. Leite, R. Harakava, et al. 2023. "Disease Caused by *Neofusicoccum parvum* in Pruning Wounds of Grapevine Shoots and Its Control by *Trichoderma* spp. and *Xenorhabdus szentirmaii*." *Fungal Biology* 127: 865–871. https://doi.org/10.1016/j.funbio.2022.12.002.

Palavouzis, S., A. Triantafyllopoulou, C. Tsoukas, A. K. Tzima, and E. Paplomatas. 2023. "Dual Labelled Probe Assays for Differentiation of *Botryosphaeria dothidea*, *Neofusicoccum mediterraneum* and *Neofusicoccum parvum*, Based on Polymorphisms in the *MAT1-2-1* Gene." *Phytopathologia Mediterranea* 60: 177–182. https://doi.org/10.36253/phyto-14375.

Pavlic, D. 2013. "Taxonomy and Population Diversity of Botryosphaeriaceae Associated With Woody Hosts in South Africa and Western Australia." [PhD thesis]. University of Pretoria.

Phillips, A. J. L., A. Alves, J. Abdollahzadeh, et al. 2013. "The Botryospaheriaceae Genera and Species Known From Culture." *Studies in Mycology* 76: 51–167. https://doi.org/10.3114/sim0021.

Pitt, W. M., R. Huang, C. C. Steel, and S. Savocchia. 2013. "Pathogenicity and Epidemiology of Botryosphaeriaceae Species Isolated From Grapevines in Australia." *Australasian Plant Pathology* 42: 573–582. https://doi.org/10.1007/s13313-013-0221-3.

Rodríguez-Pleguezuelo, C. R., V. H. Duran-Zuazo, J. L. Muriel-Fernández, and D. Franco-Tarifa. 2012. "Physico-Chemical Parameters of Mango (*Mangifera indica* L.) Fruits Grown in a Mediterranean Subtropical Climate (SE Spain)." *Journal of Agricultural Science and Technology* 14: 365–374.

Saitou, N., and M. Nei. 1987. "The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees." *Molecular Biology and Evolution* 4: 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.

Slippers, B., and M. J. Wingfield. 2007. "Botryosphaeriaceae as Endophytes and Latent Pathogens of Woody Plants: Diversity, Ecology and Impact." *Fungal Biology Reviews* 21: 90–106. https://doi.org/10.1016/j.fbr.2007.06.002.

Tamura, K., and M. Nei. 1993. "Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and Chimpanzees." *Molecular Biology and Evolution* 10: 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023.

White, T. J., T. D. Bruns, S. B. Lee, and J. W. Taylor. 1990. "Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for

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Phylogenetics." In *PCR Protocols: A Guide to Methods and Applications*, edited by M. A. Innis, D. H. Gelfand, and J. J. Sninsky, 315–322. Academic Press.

Zea-Bonilla, T., M. A. González-Sánchez, P. M. Martín-Sánchez, and R. M. Pérez-Jiménez. 2007. "Avocado Dieback Caused by *Neofusicoccum parvum* in the Andalucia Region, Spain." *Plant Disease* 91: 1052. https://doi.org/10.1094/PDIS-91-8-1052B.

Zee, K. Y., N. Asib, and S. I. Ismail. 2021. "First Report of *Lasiodiplodia theobromae* Causing Postharvest Fruit Rot on Guava (*Psidium guajava*) in Malaysia." *Plant Disease* 105: 2716. https://doi.org/10.1094/PDIS-12-20-2732-PDN.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Phylogenetic tree of *Neofusicoccum* spp. isolates obtained in the study. **Figure S2:** Climate records for the 10-year period from 2013 to 2023. **Table S1:** List of farms visited for dieback sampling in this study. **Table S2:** Information obtained from fungal isolate rDNA internal transcribed spacer (ITS) sequences search in the NCBI database. **Table S3:** Information obtained by β-tubulin sequencing from *Neofusicoccum* spp. Isolates and *TEF1* sequencing of fungal isolates used in pathogenicity analysis.

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