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Genetic and Phytopathogenic Characterization of Endemic *Colletotrichum* Isolates in Major Olive Cultivars of Greece

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Abstract: Olive anthracnose outbreaks caused by the *Colletotrichum* species complex in the Mediterranean region decrease both fruit yield and olive oil production while also drastically degrading olive oil quality. The presence of various *Colletotrichum* species able to produce disease symptoms in olive fruits significantly deteriorates the efforts for an efficient crop protection strategy. In this report, the major olive productive area of Peloponnese was screened for *Colletotrichum* species capable of generating anthracnose symptoms. Olive fruits of 12 different olive cultivars were collected from 60 groves distributed analogously in the Peloponnese. Thirty-two fungal strains isolated from asymptomatic olive drupes were identified morphologically as *Colletotrichum* spp. and were multilocus genetically analyzed. The 32 isolates were grouped into two primary lineages resembling the previously characterized *Colletotrichum acutatum* and *Colletotrichum nymphaeae* based on the conducted genetic analysis for five genetic loci. The virulence of 16 *Colletotrichum* spp. strains were evaluated in a detached fruit assay of 10 Greek olive cultivars. The results clearly suggested that fungal isolates belonging to both *C. acutatum* and *C. nymphaeae* exhibited different levels of pathogenicity in a cultivar-dependent manner. Thus, cultivars examined in terms of the % Disease Index (%DI) were divided into highly tolerant, tolerant, and susceptible, and those analyzed regarding the % Disease Severity Index (%DSI) were divided into tolerant and susceptible. Our results suggest that the Greek cultivars of Athinolia and Megaritiki are highly tolerant to the vast majority of *Colletotrichum* strains isolated from Peloponnesian groves and consist of a significant genetic material for the future design of crop protection programs against anthracnose breakouts.

Keywords: *Olea europaea*; Anthracnose (*Colletotrichum*); Greek olive cultivars; *Colletotrichum* species complex diversity; cultivar resistance

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1. Introduction

The olive tree (*Olea europaea* L.) is one of the most commonly cultivated species in the Mediterranean basin and a vital source of revenue for local societies. Greece is the third olive oil-producing country in the world, after Spain and Italy [1]. The prefecture of Peloponnese represents approximately 25% of olive oil and olive production in Greece [2]. Olive anthracnose, caused by the *Colletotrichum* species complex, is the most important fungal disease in Mediterranean olive-producing countries [3]. However, *Colletotrichum* caused anthracnose has been also reported in all continents, where olive trees are cultivated [3]. The first report of *Colletotrichum* in Greece dates back to 1920, at Corfu Island in the Ionian Sea [4], and until recently, it was considered a less significant disease with limited

economic importance. A notable outbreak of anthracnose has recently occurred, mainly in West-Central Greece, Peloponnese, and the island of Crete, which collectively represent 80% of Greek production [2] (ELSTAT, 2021), resulting in a significant deterioration of both olive fruits and olive oil yield [5,6]. This outbreak of anthracnose was caused by the extensive rainfalls in autumn and winter [5]. Moreover, other studies reported anthracnose outbreaks simultaneously in Italy and Spain [7].

While *Colletotrichum* spp. mainly infect drupes, they also produce considerable symptoms in various plant structures of the host [8]. In olive trees, *Colletotrichum* spp. typically impact flowers and olive fruit as a primary target, with olive leaves and branches being less frequently affected. Plant organs such as flowers, green shoots, stems, pedicels, and petioles may also be affected to a lesser degree [8]. Therefore, these various infected structures serve as the main potential source of infection for future disease outbreaks [3,9].

The autumn season in Greece as well as in other Mediterranean countries is typically characterized by elevated humidity and increased rainfall levels, which provides ideal conditions for anthracnose development, as it facilitates the germination of fungal conidia [10]. Indeed, symptoms of olive anthracnose are mainly manifested in autumn, which coincides with the time of fruit ripening processes [4]. Moral et al. [11] pointed out that the olive fruit maturity index plays an important role in the progression of the fungal infection. Mature drupes are preferentially affected by the pathogen, although green immature drupes are also susceptible under favorable environmental conditions [11,12]. Moreover, it has been suggested that the persistence of the infection may vary across seasons, which is mainly influenced by factors such as the type of olive cultivar, environmental conditions, agricultural practices, and the virulence of the pathogen [13].

The initial signs of disease on olive fruit appear as tiny brown spots or indentations, which develop rapidly and eventually cover a significant area of the fruit [10]. This progression often leads to premature fruit shedding or under certain environmental conditions to fruit mummification, with the fruit occasionally remaining attached to the trees and serving as the inocula for the next cycle coinciding with spring [4,14]. These outcomes lead to compromised fruit quality and subsequently reduced yield of both fruit and olive oil, as the olive oil of the infected fruit exhibits a reddish color and contains a high concentration of free fatty acids [15–17].

Managing anthracnose in plants has become increasingly challenging due to the existence of various and cosmopolitan *Colletotrichum* species, namely *Colletotrichum acutatum, Colletotrichum gloeosporioides, Colletotrichum simmondsii, Colletotrichum fioriniae, Colletotrichum clavatum*, and *Colletotrichum theobromicola*, which in many plant species are considered to be severe pathogens [18]. Similarly, at least three *Colletotrichum* complex species that are able to produce severe pathogenic symptoms in olives have been characterized so far. These are *C. acutatum sensu lato* and *C. gloeosporioides sensu lato*, whereas *Colletotrichum boninence sensu lato* is less capable of producing phytopathological symptoms (e.g., *C. kastri*) [7,17,19]. Considerable progress has been achieved in comprehending the diversity within the *Colletotrichum* genus by examining an extensive global assortment of isolates, focusing on phylogenetic, morphological, and pathological characteristics [7,20]. Several *Colletotrichum* species have been classified under the *C. acutatum* species complex, including *C. acutatum sensu stricto*, *C. fioriniae*, *C godetiae*, *C nymphaeae*, *C rhombiforme*, and *C simmpndsii*. *C. acutatum sensu lato* is generally considered the most aggressive, which is linked to recent outbreaks in the Mediterranean basin [7]. Accordingly, several studies indicated that *C. nymphaeae* and *C. acutatum sensu stricto* consist of the most aggressive species, thus playing a pivotal role in spreading olive anthracnose disease [3,21]. However, *C. acutatum* species exhibited altered levels of virulence in olives in a cultivar-dependent manner [3,22,23]. In the Mediterranean region, *C. godetiae* is commonly found, although there are indications that it is being supplanted by the more aggressive *C. acutatum sensu stricto* [3,20]. *C. gloeosporioides sensu lato*, on the other hand, comprises two genera able to develop anthracnose symptoms in olives, namely *C. gloeosporioides* and *C. theobromicola* [3]. The recent identification of various species within the species complex has shed light on their role in olive anthracnose disease and the latest outbreak in Greece [8]. Several lines of evidence suggest that the pre-

cise fungal species identification is crucial for the analysis of each pair of pathogen–cultivar interactions [7,23]. Taking into account the available data, it is suggested that fungal species characterization is essential for predicting losses and effective disease management [17,24].

The aim of the present study was the isolation and identification of *Colletotrichum* spp. strains from the Peloponnese region based on morphologically and molecular technics and further detect the adaptability and pathogenicity of the endemic *Colletotrichum* spp. isolates. A final goal was to evaluate ten olive cultivars from the Peloponnese region for their susceptibility against the two dominant Colletotrichum species, referred as *C. acutatum* and *C. nymphaea*.

2. Materials and Methods

2.1. Selection of Experimental Olives Groves and Plant Material

The olive drupes were collected from a total of 60 groves from the Peloponnese region, where 12 different cultivars were systematically cultivated as previously reported [25]. The 12 collected cultivars, namely Asprolia, Arbequina, Athinolia, Kalamon, Koroneiki, Koutsoyrelia, Manaki, Mavrolia, Megaritiki, Myrtolia, Nemoutiana, and Picual, were proportionally divided into in 60 groves, which depended upon the total cultivated area of each cultivar in the Peloponnese region. From the 60 groves, 27 olive groves were selected from the regional unit of Messinia, 18 from the regional unit of Lakonia (Sparta), 7 from the regional unit of Argolis, 5 from the regional unit of Korinthos, and 3 from the regional unit of Arcadia (Figure 1). The experimental olive groves were selected based on their geographical location, variety, and the criteria associated with cultivation practices. A detailed profile of cultural practices for each producer had been created to facilitate the selection of the groves. Additionally, highly productive regions were represented proportionally in the experiment. From each olive grove, 6 representative olive trees were marked and olive fruit was collected at the stage of commercial ripening according to farmers' practices, which depended on the cultivar, microclimatic conditions, and agricultural practices. From each marked tree, at least 300 gr of olive fruit was collected. Then, the samples were placed in paper bags and transported to the laboratory on the same day for further experiments.

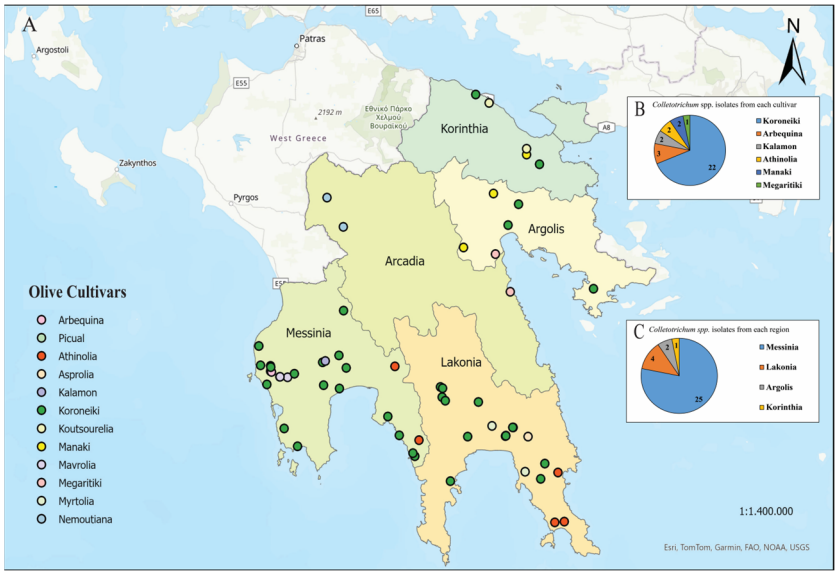

Figure 1. (**A**) Geographical distribution of 12 olive cultivars collected. Each cultivar is represented by a different color circle in the map; (**B**) the number of *Colletotrichum* spp. strains isolated from each cultivar; (**C**) abundance of *Colletotrichum* isolates in every Peloponnesian region.

2.2. Isolation of Colletotrichum spp. from Olive Drupes

Colletotrichum spp. strains were isolated from ostensibly healthy olive drupes. The collected olive fruits were initially washed thoroughly with tap water and then surface-

sterilized using 70% (*w*/*v*) ethanol for 5 min, 0.5% (*w*/*v*) NaOCl containing 0.2% (*w*/*v*) Tween20 for 5 min, and 70% (*w*/*v*) ethanol for 30 s. The drupes were finally rinsed at least four times with sterilized double distilled water [26]. The surface-sterilized olive drupes were then cut into small pieces using a sterile scalpel and placed in Petri dishes containing a PDA medium. Plates were incubated at 25 °C and macroscopically observed for fungal colony growth for 7 days. The isolated pure strains were classified based on their conidial microscopic observation and mycelium morphology and color (white–grey and pinkish) prior to further experimentation.

2.3. DNA Extraction of Colletotrichum spp. Isolates

DNA extractions from pure fungal cultures of *Colletotrichum* spp. strains were prepared using a Pure Link Plant Total DNA Purification Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentration and the quality of the samples were estimated using NanoDrop One (Thermo Scientific, Carlsbad, CA, USA). Then, the samples were normalized and used for PCR amplification.

2.4. Genetic Characterization of Colletotrichum spp. Isolates

Primers were selected against five genetic loci previously used for *Colletotrichum* spp. genetic characterization [27–31] (Table S1). In more detail, primers targeted *KLAP1* (*Key Lime Anthracnose* Pathogenicity*1*), *5.8S-ITS*, *B-TUBULIN2* (*TUB2*), *HISTONE-H3*, and *Internal Transcribed Spacer* (*ITS*) genes, as described in Table S1. The PCR mixture used for each reaction contained 1 μL of each primer (Table S1), 4 μL 5× PCR buffer, 0.4 μL dNTPs, 0.2 μL Phusion DNA polymerase (Thermo Fisher Scientific), and 5 μg DNA template. PCR was carried out in a Verity Thermal Cycler (Applied Biosystems, Foster City, CA, USA) using the following cycle: initial denaturation at 98 °C for 30 sec, followed by 35 cycles of 98 °C/10 s (denaturation), 45–72 °C/30 s (annealing), 72 °C/30 s (extension), and a final step of 72 °C for 10 min. A total volume of 5 μL of each PCR product was loaded and visualized after electrophoresis in 1.5% (*w*/*v*) agarose gel. The PCR products were purified using a GeneJet PCR Purification Kit (Thermo Fisher Scientific, Carlsbad, CA, USA) following the instructions provided by the manufacturer.

2.5. Analyses of Sequences and Species Identification

The phylogenetic analysis of the *Colletotrichum* spp. strains was performed based on the nucleotide sequences of the five genetic loci that were compared to previously characterized sequences of *Colletotrichum* spp. available at NCBI (US National Center for Biotechnological Information) (http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 6 August 2024), which are summarized in Table S2, as well as between the different isolates using NCBI BLAST (Basic Local Alignment Tool) and blastn algorithms. A multiple alignment of the sequenced amplicons was then performed using the ClustalW multiple alignment algorithm with the MEGA 11.0 (Molecular Evolutionary Genetics Analysis) program. Phylogenetic trees were constructed using the neighbor-joining method, using the MEGA 11.0 program. The reliability and stability of the dendrogram relationships were assessed through 1000 bootstrap samples.

2.6. Ex Vivo Virulence Evaluation

Sixteen selected fungal strains were inoculated on small-sized detached olive drupes to evaluate their virulence under ex vivo conditions, as previously described in Tsalgatidou et al. [32], with the appropriate modifications. Briefly, sterilized olive drupes were first artificially wounded and then inoculated with an aliquot of 10 μL of conidia suspension (10^5 spores/mL), using a hemocytometer. Inoculated olive drupes from ten different cultivars (Asprolia, Athinolia, Kalamon, Koroneiki, Koutsoyrelia, Manaki, Mavrolia, Megaritiki, Myrtolia, and Nemoutiana) were transferred to plastic boxes, maintaining high humidity, and were incubated for 7 days in a growth chamber at 25 °C in the dark.

After seven days of incubation, Disease Incidence (DI%) was calculated as the percentage of infected olive drupes. The infected area of each olive drupe by *Colletotrichum* was measured using the ImageJ software v.1.8.0 analysis tool, and Disease Severity (DS%) was determined as the percentage of the infected area. The Disease Severity Index (DSI%) was scored on a 0 to 9 rating scale, with 0 = healthy fruits, 1 = 1–10%, 3 = 11–25%, 5 = 26–50%, 7 = 51–75% and 9 = >75% infected fruit area and was calculated based on the following formula:

$$\% \text{ Disease Severity Index, (DSI)} = [\Sigma(n \times i)/(N \times Z)] \times 100,$$

where n stands for the number of fruits in a specific value of the disease rating scale, i stands for the corresponding value of the scale, N stands for the total number of fruits, and Z stands for the highest value of the disease rating scale.

Colletotrichum strains were reisolated from the symptomatic olive tissues, confirming their identity and Koch's postulates.

2.7. Statistical Analysis

The experiments of detached fruit assay were arranged as a completely randomized design with three replications of at least ten fruits per strain.

In order to assess the effect of cultivars and strains on %DSI and %DI parameters, the data were analyzed as two-way ANOVA with the factors being the cultivar (Asprolia, Athinolia, Kalamon, Koroneiki, Koutsoyrelia, Manaki, Mavrolia, Megaritiki, Myrtolia, and Nemoutiana) and strain (FAth9.1, FAth24.1, FArb3.4, FKor1.4, FKor4.2, FKor5.1, FKor7.2, FKor10.1, FKor18.2, FKor34.1, FKor37.2, FKor44.3, FKor47.1, FKal19.1, FKal46.1, and FMeg61.1). The significant differences were determined by Tukey's HSD test ($\alpha = 0.05$).

One-way ANOVA was used to assess significant differences among strains for each cultivar separately. The significant differences were determined by Tukey's HSD test ($\alpha = 0.05$).

Statistical analysis was carried out using JMP 10.0 (SAS, USA). A cluster heatmap for cultivars and strains was constructed based on the Euclidean distance using a publicly available online tool (https://www.bioinformatics.com.cn/en, accessed on 6 August 2024) [33].

3. Results

3.1. Morphology and Geographical Distribution of Colletotrichum Isolates

Colletotrichum isolates were detected in 6 out of the 12 cultivars selected for the present study across the Peloponnese region (Figure 1A), whereas the vast majority, 22 out of the 32 isolates in total, were isolated from the Koroneiki cultivar (Figure 1B). Furthermore, 25 out of the 32 *Colletotrichum* strains were isolated from the Messinia region, whereas only 5 strains were isolated from Lakonia (Figure 1C), despite the fact that the latter exhibited comparable number of olive trees (ELSTAT 2021). In more detail, *Colletotrichum* strains were isolated from ostensibly healthy olive drupes, which were derived from groves located in four different geographical areas (Messinia, Lakonia, Argolis, and Korinthos) of the Peloponnese region (Figure 1C).

A total of 32 *Colletotrichum* spp. isolates were morphologically classified into two main categories (Figure 2). The first included isolates showing pale grey-to-white aerial mycelia, while the Petri dish bottom view of the PDA culture was pinkish (Figure 2A). The second category comprised fungal colonies with a grey center and white peripheral aerial mycelia, and the bottom view of the PDA culture was white with grey spore masses (Figure 2B). The first group encompassed fungal strains classified as the *C. acutatum* species complex and the second as *C. nymphaeae*. Conidia morphology examination revealed that *C. acutatum* were cylindrical, fusiform, and unicellular, while the *C. nymphaeae* samples were hyaline and appeared slightly translucent under the microscope and unicellular, but some became septate as they matured (Figure 2C). The conidium morphology for all isolates is illustrated in Figure S1, while the representative conidia for *C. acutatum* and *C. nymphaeae* are presented in Figure 2C.

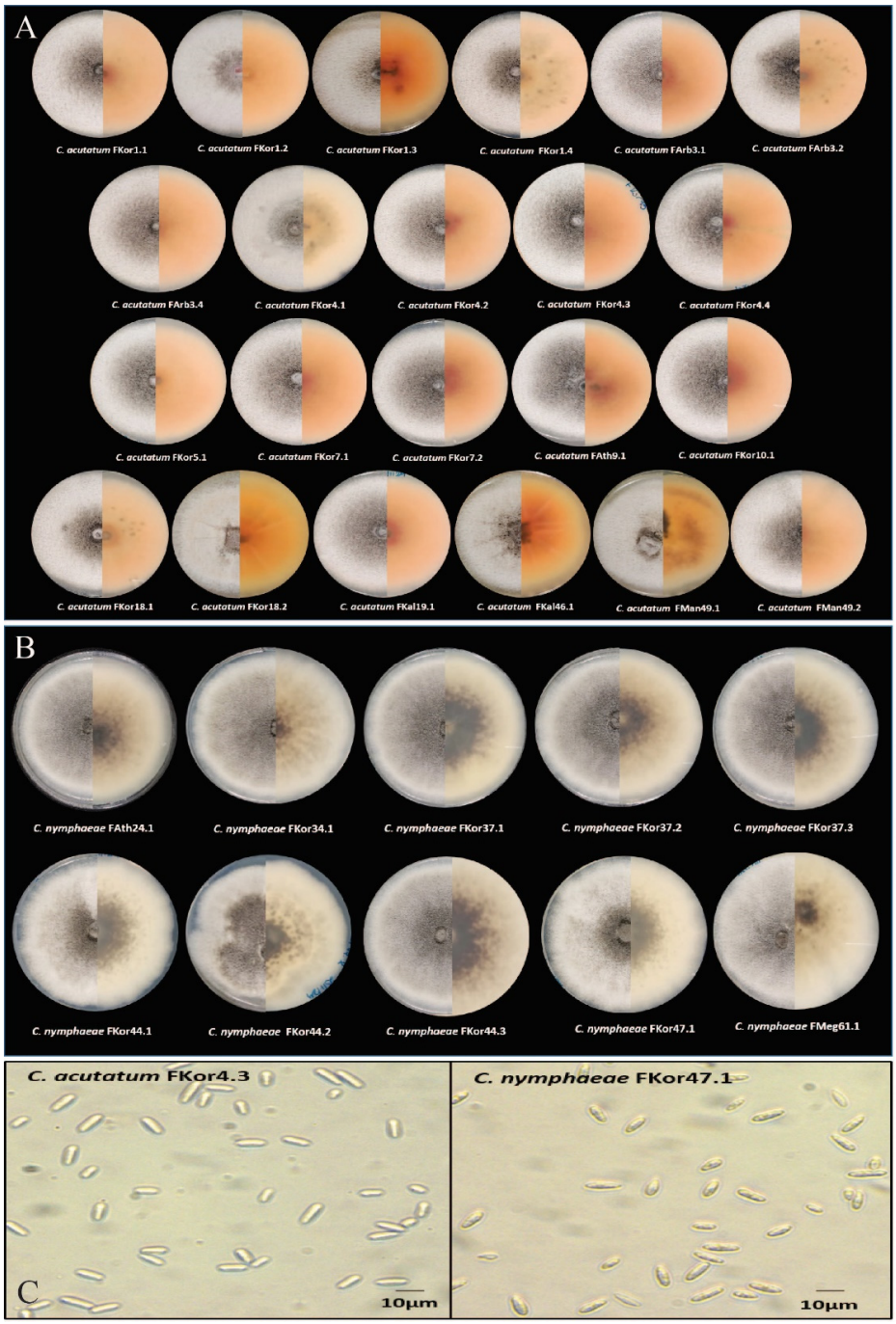

Figure 2. Top and bottom views of colony morphology of PDA cultures of (**A**) 22 isolates classified as *C. acutatum* and (**B**) 10 isolates classified as *C. nymphaeae*; (**C**) representative conidia for the isolated strains of *C. acutatum* FKor4.3 on the left and *C. nymphaeae* FKor47.1. on the right.

3.2. Phylogenetic Analysis

Thirty-two isolates exhibiting morphological similarities to previously characterized *Colletotrichum* spp. were subjected to multilocus analysis for five genetic loci, namely *KLAP1*, *5.8S-ITS*, *TUB2*, *HISTONE-H3*, and *ITS* genes. The genetic investigation of the sequences revealed that the 32 isolates were categorized into *Colletotrichum* spp. and were subjected to further phylogenetic analysis. The concatenated complete sequences of the 32 strains for the five genetic loci examined in this study were aligned with previously characterized *Colletotrichum* species (Table S2). All the studied isolates belong to the *C. acutatum* species complex, forming two major lineages and exhibiting extensive similarity to the characterized *C. acutatum* and

C. nymphaeae (Figure 3). Interestingly, isolates belonging to the *C. gloeosporioides* species complex were not detected among the studied samples. The majority of the isolates (22 samples) were grouped under the *C. acutatum* species complex, while 10 strains were similar to *C. nymphaeae*. Intriguingly, FKor7.1 and FAth24.1 exhibited less genetic similarity to the other isolates grouped in the same lineage. The accession numbers of all isolates are presented in Table S3.

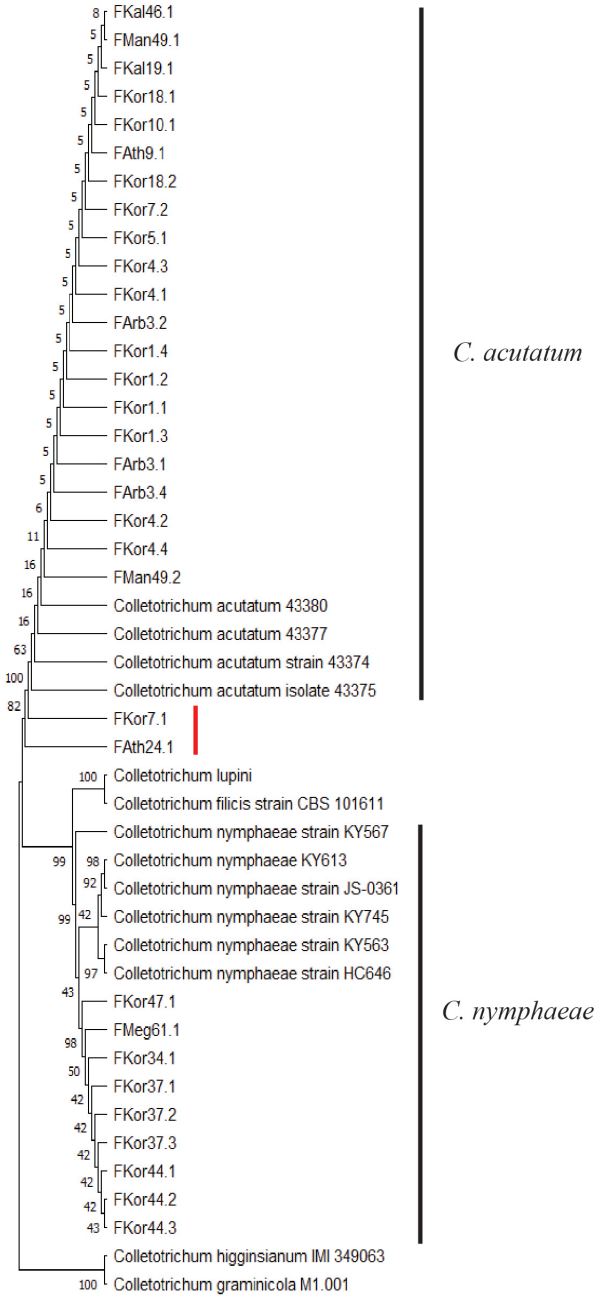

Figure 3. Phylogenetic tree constructed by the neighbor-joining method with 1000 bootstrap replicates using concatenated sequences of *KLAP1*, *5.8S-ITS*, *TUB2*, *HISTONE-H3*, and *ITS* genes of 32 *Colletotrichum* strains isolated in Peloponnese and previously characterized *Colletotrichum* strains listed in Table S2. The strains *C. higginsianum*, *C. graminicola, C. filisis*, and *C. lupini* were used as outgroup sequences. The outgroup strains FKor7.1 and FAth24.1 in the *C. acutatum* lineage are marked with the red line.

3.3. The Pathogenicity of Ex Vivo *Colletotrichum* spp. Isolates

After the initial screening of the 32 isolated *Colletotrichum* spp. strains, 16 isolates that exhibited high virulence were systematically evaluated for their ability to cause anthracnose in olive fruit of the 10 cultivars mainly cultured in the Peloponnese region, specifically Athinolia, Koroneiki, Asprolia, Kalamon, Koutsourelia, Manaki, Mavrolia, Megaritiki, Myrtolia, and Nemoutiana. Among the representative strains selected for analysis, 10 were classified as *C. acutatum* (FArb3.4, FAth9.1, FKal19.1, FKor1.4, FKor4.2, FKor5.1, FKor7.1, FKor10.1, FKor18.2, and FKal46.1), and 6 (FAth24.1, FKor34.1, FKor37.2, FKor44.3, FKor47.1, and FMeg61.1) were identified as *C. nymphaeae* (Figure 4). The pathogenicity of the fungal strains regardless of the species showed significant fluctuation among the olive varieties (Figure 4). Additionally, for strains that exhibited high %DSI, the %DI was mitigated and vice versa (e.g., FKor5.1 and Fkal19.1 both belonging to *C. acutatum* and FAth24.1 belonging to *C. nymphaeae*). Indeed, strains with low rates of fruit infection were capable of producing severe symptoms, while strains exhibiting a high number of infected fruits generated mild symptoms (Table S5). These results are more obvious in Figure 5A,B, where the heatmap for the %DI value of classified olive cultivars is presented for (i) susceptible cvs (Koroneiki, Mavrolia, Nemoutiana, Asprolia, and Kalamon), (ii) tolerant cvs (Manaki and Koutsourelia), and (iii) highly tolerant cvs (Athinolia, Myrtolia, and Megaritiki) On the other hand, the heatmap for the %DSI value is shown for cultivars categorized as tolerant (Athinolia, Myrtolia and Megaritiki, Manaki, and Koutsourelia) and susceptible (Koroneiki, Mavrolia, Nemoutiana, Asprolia, and Kalamon). However, both heatmaps (Figure 5) clearly suggest that Koroneiki, Mavrolia, Nemoutiana, Asprolia, and Kalamon are susceptible varieties for the majority of the examined strains, excluding FArb3.4 and FKor1.4. These particular strains were less efficient in infecting olive fruit and simultaneously produced less severe symptoms in all the tested cultivars. The most vulnerable cultivar to anthracnose, regarding both %DI and %DSI values, was Kalamon; conversely, the most tolerant olive cultivar was Athinolia (Figure 5C,D). Interestingly, the strains FKor7.1 and FAth24.1 showed limited similarity to other congener *C. acutatum* isolates and were extremely virulent since they demonstrated high values for both %DI and %DSI (Tables S4 and S5). Paradoxically, Koutsourelia cv. was tolerant to the most virulent strains and susceptible to isolates that are generally non-virulent even for the susceptible cultivars. Among the most virulent strains of *Colletotrichum* spp. was FAth24.1, isolated from Athinolia, which according to the results was the most tolerant cultivar.

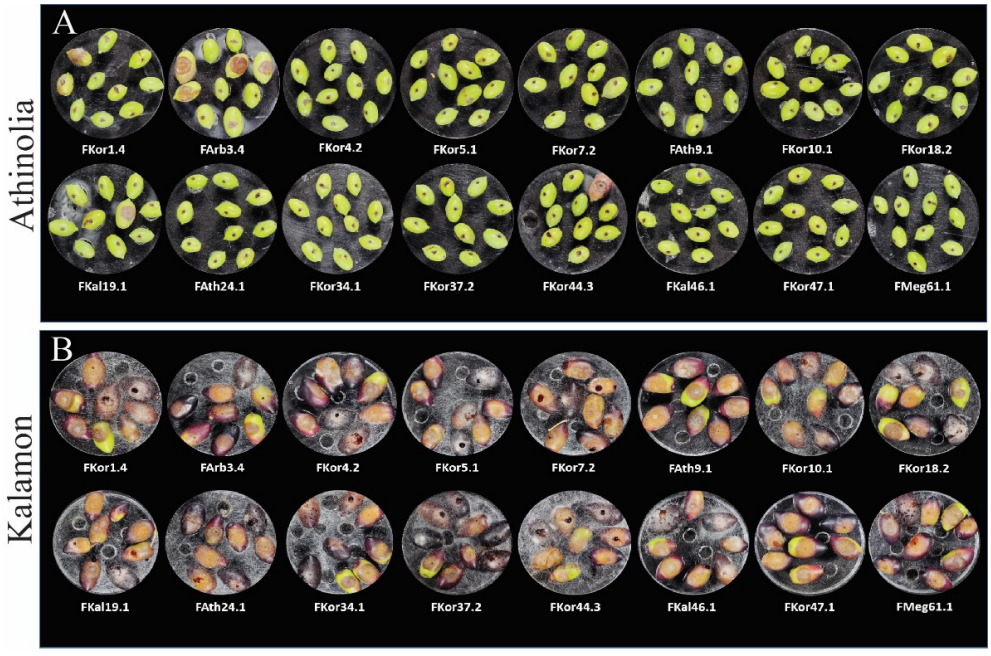

Figure 4. *Cont.*

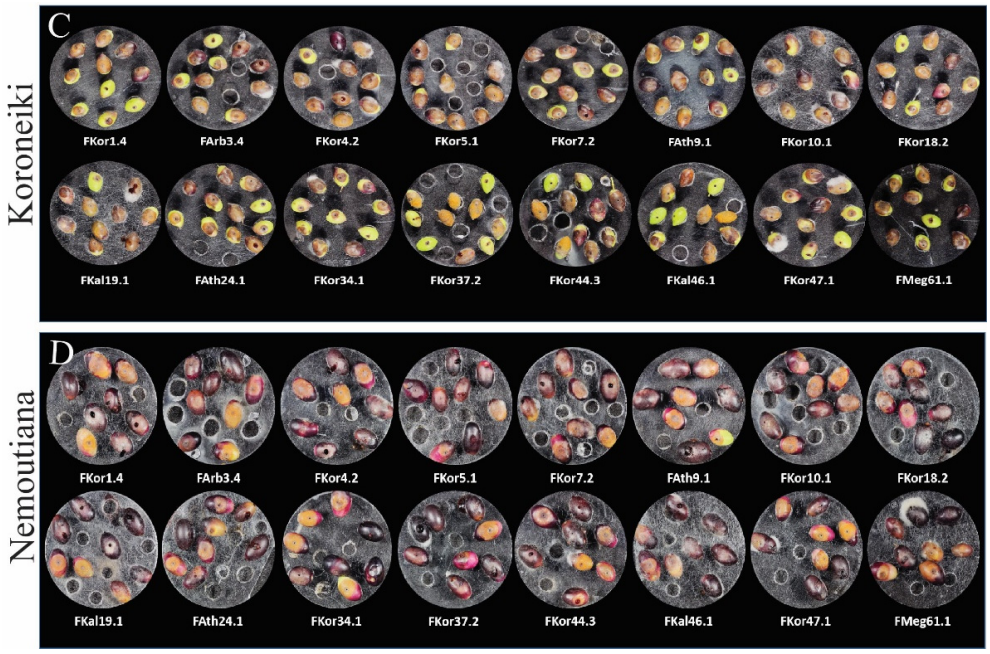

Figure 4. Representative figures of ex vivo pathogenicity assays of 16 fungal strains in different olive cultivars" (**A**) Athinolia (characterized as the most tolerant cultivar); (**B**) Kalamon (characterized as the most susceptible cultivar); (**C**) Koroneiki; and (**D**) Nemoutiana (relatively susceptible to anthracnose).

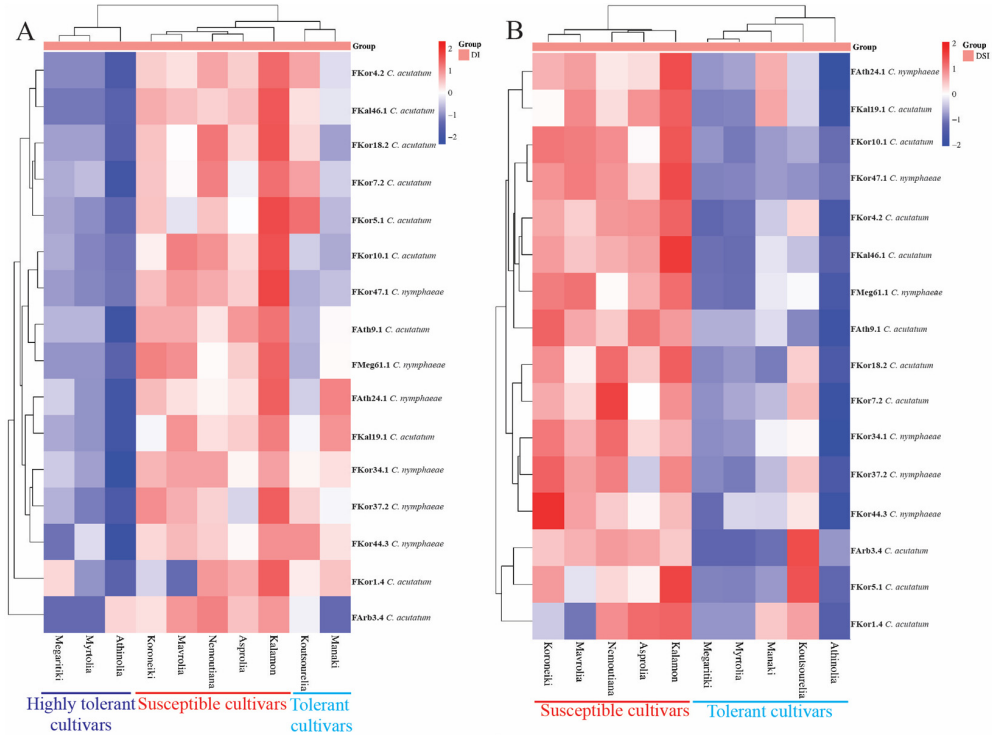

Figure 5. *Cont.*

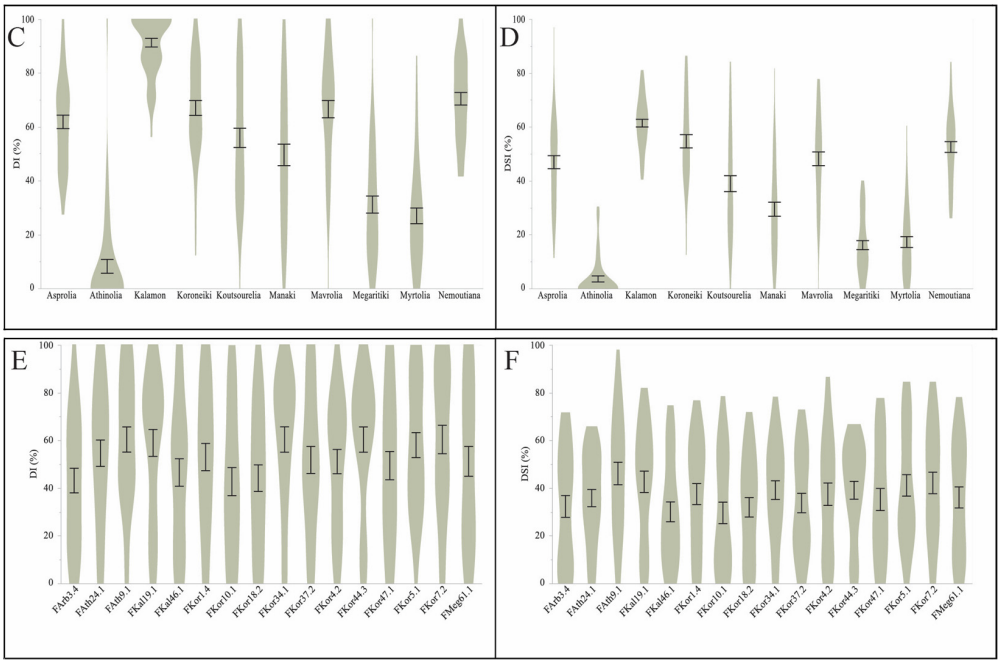

Figure 5. Heatmap and hierarchical clustering of 16 strains evaluated for their ability to cause anthracnose in olive fruit of 10 major cultivars cultured in Peloponnese: (**A**) Disease Severity Index (DSI (%)) was considered the dependent variable to determine the similarity between olive cultivars or Colletotrichum strains. After clustering analysis, two groups were formed, which separated the cultivars into susceptible and tolerant; (**B**) Disease Incidence (DI (%)) was considered the dependent variable to determine the similarity between olive cultivars or Colletotrichum strains. Clustering analysis using the %DI factor highlighted three groups, which were the susceptible, tolerant, and highly tolerant cultivars. Violin plots of (**C**) DI (%) and (**D**) DSI (%) for the different cultivars irrespective of the strain are presented, as well as violin plots of (**E**) DSI (%) and (**F**) DI (%) for the different strains irrespective of the cultivar. Vertical bars indicate the SE of the mean.

4. Discussion

A plethora of *Colletotrichum* species exhibiting high morphological and genetic diversity is capable of causing anthracnose symptoms in olive trees, which renders the strategic planning for effective disease management extremely challenging [3,13,23]. Our knowledge of the specific *Colletotrichum* species involved in a field can aid in understanding the epidemiology of the disease, including its spread, survival mechanisms, and infection cycles. This information is crucial for predicting disease outbreaks and implementing timely interventions. Furthermore, identifying the species of *Colletotrichum* can assist in breeding programs aimed at developing olive cultivars with resistance to specific pathogens. This is essential for long-term, sustainable control of the disease. Some *Colletotrichum* species might be subjected to regulatory and quarantine measures to prevent their spread to new regions. Accurate identification helps in complying with these regulations and protecting other olive-producing areas from potential outbreaks.

In the present study, the multilocus genetic analysis of *Colletotrichum* isolates linked the initial morphological classification to the strains' genetic background. Indeed, endemic Peloponnesian *Colletotrichum* strains were grouped into two major lineages very similar to *C. acutatum* and *C. nymphaeae* species. ITS single-locus genetic analysis is not a reliable marker for this fungal genus due to its failure to phylogenetically resolve cryptic species [34]. Thus, five genetic loci were analyzed, namely *KLAP1*, *5.8S-ITS*, *TUB2*, *HISTONE-H3* and *ITS* genes to effectively clarify the isolates' genetic diversity. Both lineages of isolated fungi were categorized into the *C. acutatum sensu lato* group, which is in accordance with previously published reports suggesting that the dominant phytopathogenic species is *C. acutatum*, whereas the important phytopathogen *C. gloeosporioides*, very common in the neighbor-

ing country Italy, has not been detected in Greece thus far [3,5,7,8]. Similarly, *C. godetiae* responsible for anthracnose outbreaks in several European countries was absent from our samples, confirming previously published reports from other Greek research groups [5–8]. Among the genetic loci examined in this study, the most significant was *KLAP1*, which is recognized as an important pathogenicity factor in *Colletotrichum acutatum* [35,36]. Specifically, its function in pathogenicity involves acting as an effector that manipulates host cellular processes to suppress plant immune responses and facilitate infection. *KLAP1* is often associated with the formation of appressoria, which are specialized structures that generate turgor pressure to breach the host epidermis. The formation and function of appressoria are critical for the successful invasion of the plant by *Colletotrichum* species. KLAP1 is involved in the early stages of infection, helping the fungus to colonize the host plant tissues. Also, KLAP1 might contribute to host specificity and the degree of virulence of *C. acutatum* [35]. In this study, single-locus phylogenetic analysis of *KLAP1* effectively classified the fungal isolates to *C. acutatum* and *C. nymphaeae* species, which renders this marker extremely important for *C. acutatum* genetic analysis.

The most crucial species for olive anthracnose are *C. acutatum* and *C. gloeosporioides* species complexes [10]. *C. acutatum* is characterized by its fusiform (spindle-shaped) conidia, while other similar species like *C. nymphaeae* are recognized by their distinct conidia shape and pathogenicity in olives [37]. By contrast, *C. gloeosporioides* produces more cylindrical conidia and has a wide distribution [23]. Several reports suggest that the most virulent *Colletotrichum* species belong to the *C. acutatum* species complex, including *C. acutatum*, *C. godetiae*, and *C. nymphaeae* [3,23]. Our results suggested that certain strains of *C. acutatum* and *C. nymphaeae* were comparably aggressive, exhibiting high %DI and %DSI values.

Identifying *Colletotrichum* species in an olive field is crucial as they impact agricultural productivity, disease management, and economic stability, and all these factors translate into economic benefits with the corresponding environmental sustainability. It is well documented that different species of *Colletotrichum* can vary in their pathogenicity, virulence, and the symptoms they cause [7,8,23]. Thus, species identification helps in developing targeted and effective disease management strategies, including the use of appropriate fungicides and cultural practices [38]. The 16 strains of *Colletotrichum* isolated in the present study exhibited different levels of virulence in a cultivar-dependent manner, which is in accordance with previous studies with Greek, Italian, Spanish, and Portuguese cultivars [3,7,8,23]. These data suggest that cultivar-specific metabolic regulation is implicated in plant–pathogen interactions, possibly via the biosynthesis of certain metabolites such as amino acids, signaling-related sugars, and secondary metabolism compounds such as phenolics [4,8].

The *Colletotrichum* strains were isolated from asymptomatic olive drupes and healthy olive fields, and they were collected from the main olive-producing areas of the Peloponnese region such as Messinia, Lakonia (Sparta), Argolis, Arcadia, and Korinthia, in which olive has been cultivated since ancient times. In half of the cultivars studied, the fungus was isolated in the laboratory, although the isolated samples were asymptomatic. Interestingly, the most important oil-producing variety in Greece (Koroneiki), which has been generally considered highly resistant to *C. acutatum* by researchers [4,39], was found to be a very susceptible phenotype in the present study; this was associated with high values for both %DI and %DSI after inoculation with the isolated endemic *C. acutatum* strains (Figure 5). It is also noteworthy that the vast majority (78.13%) of the *Colletotrichum* strains were isolated from Koroneiki (Figure 1B). This fact defines the variety Koroneiki as particularly sensitive to attacks by *Colletotrichum*. This sensitivity, if combined with favorable conditions, such as high humidity and rainfalls in autumn and abnormal temperature fluctuations [24], leads to great destruction and may partly explain the recent outbreaks in the Peloponnese region, where, until recently, anthracnose was considered an insignificant disease.

Two of our fungal isolates, FAth24.1 and FKor7.1, which were phylogenetically classified as *C. acutatum*, showed limited genetic similarity to other *C. acutatum* isolates. Additionally, FAth24.1 phenotypically resembled the *C. nymphaeae* strains, while FKor7.1 exhibited

a typical pinkish *C. acutatum* phenotype. It is well known that the same genus of fungi can have colonies of different phenotypes. Phenotypic switching in fungi is an in vitro reversible phenomenon that is defined as the spontaneous emergence of colonies with altered colony morphology [40]. Furthermore, changing environmental conditions can affect cellular morphology and lead to the formation of new morphotypes, with each morphotype having a potential impact on both pathogen survival and disease epidemiology [41]. More experiments for the precise genetic classification of FAth24.1 are needed since this strain was characterized as highly aggressive in ex vivo experiments. Interestingly, this strain was isolated from Athinolia, which included the most tolerant of the 10 varieties examined, suggesting an active plant–pathogen crosstalk.

The analysis of %DSI and %DI clarified the categorization of the studied cultivars into susceptible, tolerant, and highly tolerant. The most susceptible proved to be Koroneiki, Mavrolia, Nemoutiana, Asprolia, and Kalamon. The Koroneiki olive cv. is the cornerstone of Greek olive cultivation, celebrated for its high oil yield, superior oil quality, and adaptability to the Mediterranean climate. The cultivar's resilience and economic importance make it a favorite among Greek olive growers and a vital part of Greece's agricultural heritage [42–44]. Furthermore, the Kalamon olive cultivar is highly desirable for its excellent table olives [45]. This cultivar is well adapted to the Mediterranean climate and soils, demonstrating good drought tolerance and moderate resistance to pests and diseases. It is a cherished component of Greek cuisine and a valuable agricultural export. The susceptibility to *Colletotrichum* infections, for both Koroneiki and Kalamon, must be taken into account and dealt with in a timely manner since these are very important Greek products. Mavrolia, Nemoutiana, and Asprolia account for a smaller area of cultivation and are therefore less marketable products; nevertheless, it is important to take timely measures against anthracnose for cultivation to be effective. Understanding the relationship between these factors and disease development is crucial for effective management. By implementing integrated disease management practices, including cultural methods, chemical treatments, and the use of resistant varieties, the impact of this disease can be mitigated, ensuring healthier olive crops and better yields.

This study sheds light on the gradation of the susceptibility of Greek olive cultivars and categorizes isolated species to the *Colletotrichum* species complex according to the cultivation area, findings that may be useful in strategic management programs against anthracnose.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10080847/s1, Figure S1: Morphology of conidia; Figure S2: Phylogenetic analysis based on KLP1 gene; Table S1: List of primers; Table S2: GenBank accession numbers of strains used for phylogenetic analysis; Table S3: GenBank accession numbers of fungi strains isolated; Table S4: Disease Severity Index (DSI%); Table S5: Disease Incidence (DI%).

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References

1. Aydin, M.; Tombuloglu, H.; Hernandez, P.; Dorado, G.; Unver, T. Olive-Tree Genome Sequencing: Towards a Better Understanding of Oil Biosynthesis. In *Oil Crop Genomics*; Tombuloglu, H., Unver, T., Tombuloglu, G., Hakeem, K.R., Eds.; Springer International Publishing: Cham, Switaerland, 2021; pp. 75–87.
2. ELSTAT. Agricultural Producrtion in Greece. 2021. Available online: https://www.statistics.gr/en/statistics/-/publication/SPG06 (accessed on 6 August 2024).
3. Talhinhas, P.; Loureiro, A.; Oliveira, H. Olive anthracnose: A yield- and oil quality-degrading disease caused by several species of *Colletotrichum* that differ in virulence, host preference and geographical distribution. *Mol. Plant Pathol.* **2018**, *19*, 1797–1807. [CrossRef]
4. Cacciola, S.O.; Faedda, R.; Sinatra, F.; Agosteo, G.E.; Schena, L.; Frisullo, S.; di San Lio, G.M. Olive anthracnose. *J. Plant Pathol.* **2012**, *94*, 29–44.
5. Iliadi, M.K.; Tjamos, E.C.; Antoniou, P.P.; Tsitsigiannis, D.I. First Report of *Colletotrichum acutatum* Causing Anthracnose on Olives in Greece. *Plant Dis.* **2017**, *102*, 820. [CrossRef]
6. Markakis, E.A.; Roditakis, E.N.; Kalantzakis, G.S.; Chatzaki, A.; Soultatos, S.K.; Stavrakaki, M.; Tavlaki, G.I.; Koubouris, G.C.; Bagkis, N.; Goumas, D.E. Characterization of Fungi Associated with Olive Fruit Rot and Olive Oil Degradation in Crete, Southern Greece. *Plant Dis.* **2021**, *105*, 3623–3635. [CrossRef] [PubMed]
7. Riolo, M.; Pane, A.; Santilli, E.; Moricca, S.; Cacciola, S.O. Susceptibility of Italian olive cultivars to various *Colletotrichum* species associated with fruit anthracnose. *Plant Pathol.* **2023**, *72*, 255–267. [CrossRef]
8. Kolainis, S.; Koletti, A.; Lykogianni, M.; Karamanou, D.; Gkizi, D.; Tjamos, S.E.; Paraskeuopoulos, A.; Aliferis, K.A. An integrated approach to improve plant protection against olive anthracnose caused by the *Colletotrichum acutatum* species complex. *PLoS ONE* **2020**, *15*, e0233916. [CrossRef] [PubMed]
9. Moral, J.; Trapero, A. Assessing the Susceptibility of Olive Cultivars to Anthracnose Caused by *Colletotrichum acutatum*. *Plant Dis.* **2009**, *93*, 1028–1036. [CrossRef]
10. Talhinhas, P.; Mota-Capitão, C.; Martins, S.; Ramos, A.P.; Neves-Martins, J.; Guerra-Guimarães, L.; Várzea, V.; Silva, M.C.; Sreenivasaprasad, S.; Oliveira, H. Epidemiology, histopathology and aetiology of olive anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. *Plant Pathol.* **2011**, *60*, 483–495. [CrossRef]
11. Moral, J.; Bouhmidi, K.; Trapero, A. Influence of Fruit Maturity, Cultivar Susceptibility, and Inoculation Method on Infection of Olive Fruit by *Colletotrichum acutatum*. *Plant Dis.* **2008**, *92*, 1421–1426. [CrossRef] [PubMed]
12. Leoni, C.; Bruzzone, J.; Villamil, J.J.; Martínez, C.; Montelongo, M.J.; Bentancur, O.; Conde-Innamorato, P. Percentage of anthracnose (*Colletotrichum acutatum* s.s.) acceptable in olives for the production of extra virgin olive oil. *Crop Prot.* **2018**, *108*, 47–53. [CrossRef]
13. Moral, J.; Agustí-Brisach, C.; Raya, M.C.; Jurado-Bello, J.; López-Moral, A.; Roca, L.F.; Chattaoui, M.; Rhouma, A.; Nigro, F.; Sergeeva, V.; et al. Diversity of *Colletotrichum* Species Associated with Olive Anthracnose Worldwide. *J. Fungi* **2021**, *7*, 741. [CrossRef] [PubMed]
14. Ferronato, B.; Ingold, A.; Moreira, V.; Bentancur, O.; Alaniz, S.; Mondino, P. Detection and quantification of *Colletotrichum* survival on olive tree (*Olea europaea* L.). *Eur. J. Plant Pathol.* **2023**, *167*, 77–87. [CrossRef]
15. Chattaoui, M.; Raya, M.C.; Bouri, M.; Moral, J.; Perez-Rodriguez, M.; Trapero, A.; Msallem, M.; Rhouma, A. Characterization of a *Colletotrichum* population causing anthracnose disease on Olive in northern Tunisia. *J. Appl. Microbiol.* **2016**, *120*, 1368–1381. [CrossRef] [PubMed]
16. Talhinhas, P.; Neves-Martins, J.; Oliveira, H.; Sreenivasaprasad, S. The distinctive population structure of *Colletotrichum* species associated with olive anthracnose in the Algarve region of Portugal reflects a host–pathogen diversity hot spot. *FEMS Microbiol. Lett.* **2009**, *296*, 31–38. [CrossRef] [PubMed]
17. Romero, J.; Santa-Bárbara, A.E.; Moral, J.; Agustí-Brisach, C.; Roca, L.F.; Trapero, A. Effect of latent and symptomatic infections by Colletotrichum godetiae on oil quality. *Eur. J. Plant Pathol.* **2022**, *163*, 545–556. [CrossRef]
18. Farr, D.F.; Aime, M.C.; Rossman, A.Y.; Palm, M.E. Species of *Colletotrichum* on Agavaceae. *Mycol. Res.* **2006**, *110*, 1395–1408. [CrossRef]
19. Baroncelli, R.; Talhinhas, P.; Pensec, F.; Sukno, S.A.; Le Floch, G.; Thon, M.R. The Colletotrichum acutatum Species Complex as a Model System to Study Evolution and Host Specialization in Plant Pathogens. *Front. Microbiol.* **2017**, *8*, 2001. [CrossRef] [PubMed]
20. Schena, L.; Abdelfattah, A.; Mosca, S.; Li Destri Nicosia, M.G.; Agosteo, G.E.; Cacciola, S.O. Quantitative detection of *Colletotrichum godetiae* and *C. acutatum* sensu stricto in the phyllosphere and carposphere of olive during four phenological phases. *Eur. J. Plant Pathol.* **2017**, *149*, 337–347. [CrossRef]
21. Schena, L.; Mosca, S.; Cacciola, S.O.; Faedda, R.; Sanzani, S.M.; Agosteo, G.E.; Sergeeva, V.; Magnano di San Lio, G. Species of the *Colletotrichum gloeosporioides* and *C. boninense* complexes associated with olive anthracnose. *Plant Pathol.* **2014**, *63*, 437–446. [CrossRef]

22. Talhinhas, P.; Gonçalves, E.; Sreenivasaprasad, S.; Oliveira, H. Virulence diversity of anthracnose pathogens (*Colletotrichum acutatum* and *C. gloeosporioides* species complexes) on eight olive cultivars commonly grown in Portugal. *Eur. J. Plant Pathol.* **2015**, *142*, 73–83. [CrossRef]
23. Licciardello, G.; Moral, J.; Strano, M.C.; Caruso, P.; Sciara, M.; Bella, P.; Sorrentino, G.; Di Silvestro, S. Characterization of *Colletotrichum* strains associated with olive anthracnose in Sicily. *Phytopathol. Mediterr.* **2022**, *61*, 139–151. [CrossRef]
24. Peres, F.; Talhinhas, P.; Afonso, H.; Alegre, H.; Oliveira, H.; Ferreira-Dias, S. Olive Oils from Fruits Infected with Different Anthracnose Pathogens Show Sensory Defects Earlier Than Chemical Degradation. *Agronomy* **2021**, *11*, 1041. [CrossRef]
25. Kottaridi, K.; Milionis, A.; Demopoulos, V.; Nikolaidis, V.; Tsalgatidou, P.C.; Tsafouros, A.; Kotsiras, A.; Vithoulkas, A. Comparative analysis of machine learning classification algorithms for predicting olive anthracnose disease. *J. Auton. Intell.* **2024**, *7*, 1466. [CrossRef]
26. Sun, J.; Guo, L.; Zang, W.; Ping, W.; Chi, D. Diversity and ecological distribution of endophytic fungi associated with medicinal plants. *Sci. China Ser. C Life Sci.* **2008**, *51*, 751–759. [CrossRef]
27. Azevedo-Nogueira, F.; Gomes, S.; Carvalho, T.; Martins-Lopes, P. Development of high-throughput real-time PCR assays for the *Colletotrichum acutatum* detection on infected olive fruits and olive oils. *Food Chem.* **2020**, *317*, 126417. [CrossRef]
28. Azevedo-Nogueira, F.; Gomes, S.; Lino, A.; Carvalho, T.; Martins-Lopes, P. Real-time PCR assay for *Colletotrichum acutatum* sensu stricto quantification in olive fruit samples. *Food Chem.* **2021**, *339*, 127858. [CrossRef]
29. Garrido, C.; Carbú, M.; Fernández-Acero, F.J.; Boonham, N.; Colyer, A.; Cantoral, J.M.; Budge, G. Development of protocols for detection of *Colletotrichum acutatum* and monitoring of strawberry anthracnose using real-time PCR. *Plant Pathol.* **2009**, *58*, 43–51. [CrossRef]
30. Kang, E.C.; Hassan, O.; Kim, K.-M.; Chang, T. Molecular Characterization and Fungicide Sensitivity of Jujube Pathogens *Colletotrichum gloeosporioides* Sensu Stricto and *Colletotrichum nymphaeae* in South Korea. *Plant Dis.* **2022**, *107*, 861–869. [CrossRef]
31. Weir, B.S.; Johnston, P.R.; Damm, U. The *Colletotrichum gloeosporioides* species complex. *Stud. Mycol.* **2012**, *73*, 115–180. [CrossRef]
32. Tsalgatidou, P.C.; Thomloudi, E.-E.; Baira, E.; Papadimitriou, K.; Skagia, A.; Venieraki, A.; Katinakis, P. Integrated Genomic and Metabolomic Analysis Illuminates Key Secreted Metabolites Produced by the Novel Endophyte *Bacillus halotolerans* Cal.l.30 Involved in Diverse Biological Control Activities. *Microorganisms* **2022**, *10*, 399. [CrossRef]
33. Tang, D.; Chen, M.; Huang, X.; Zhang, G.; Zeng, L.; Zhang, G.; Wu, S.; Wang, Y. SRplot: A free online platform for data visualization and graphing. *PLoS ONE* **2023**, *18*, e0294236. [CrossRef] [PubMed]
34. Sharma, G.; Maymon, M.; Meshram, V.; Freeman, S. Characterization of *Colletotrichum* Isolates from Strawberry and Other Hosts with Reference to Cross-Inoculation Potential. *Plants* **2022**, *11*, 2373. [CrossRef]
35. Chen, H.-Q.; Cao, L.; Dekkers, K.L.; Rollins, J.A.; Ko, N.J.; Timmer, L.W.; Chung, K.-R. A gene with domains related to transcription regulation is required for pathogenicity in *Colletotrichum acutatum* causing Key lime anthracnose. *Mol. Plant Pathol.* **2005**, *6*, 513–525. [CrossRef] [PubMed]
36. You, B.-J.; Choquer, M.; Chung, K.-R. The *Colletotrichum acutatum* Gene Encoding a Putative pH-Responsive Transcription Regulator Is a Key Virulence Determinant During Fungal Pathogenesis on Citrus. *Mol. Plant-Microbe Interact.* **2007**, *20*, 1149–1160. [CrossRef] [PubMed]
37. Materatski, P.; Varanda, C.; Carvalho, T.; Dias, A.B.; Campos, M.D.; Rei, F.; Félix, M.D. Diversity of *Colletotrichum* Species Associated with Olive Anthracnose and New Perspectives on Controlling the Disease in Portugal. *Agronomy* **2018**, *8*, 301. [CrossRef]
38. Varveri, M.; Papageorgiou, A.G.; Tsitsigiannis, D.I. Evaluation of Biological Plant Protection Products for Their Ability to Induce Olive Innate Immune Mechanisms and Control *Colletotrichum acutatum*, the Causal Agent of Olive Anthracnose. *Plants* **2024**, *13*, 878. [CrossRef] [PubMed]
39. Moral, J.; Xaviér, C.J.; Viruega, J.R.; Roca, L.F.; Caballero, J.; Trapero, A. Variability in Susceptibility to Anthracnose in the World Collection of Olive Cultivars of Cordoba (Spain). *Front. Plant Sci.* **2017**, *8*, 1892. [CrossRef]
40. Jain, N.; Fries, B.C. Antigenic and phenotypic variations in fungi. *Cell. Microbiol.* **2009**, *11*, 1716–1723. [CrossRef]
41. Francisco, C.S.; Ma, X.; Zwyssig, M.M.; McDonald, B.A.; Palma-Guerrero, J. Morphological changes in response to environmental stresses in the fungal plant pathogen *Zymoseptoria tritici*. *Sci. Rep.* **2019**, *9*, 9642. [CrossRef]
42. Anastasopoulos, E.; Kalogeropoulos, N.; Kaliora, A.C.; Falirea, A.; Kamvissis, V.N.; Andrikopoulos, N.K. Quality Characteristics and Antioxidants of Mavrolia cv. Virgin Olive Oil. *J. Am. Oil Chem. Soc.* **2012**, *89*, 253–259. [CrossRef]
43. Katsoyannos, E.; Batrinou, A.; Chatzilazarou, A.; Bratakos, S.M.; Stamatopoulos, K.; Sinanoglou, V.J. Quality parameters of olive oil from stoned and nonstoned Koroneiki and Megaritiki Greek olive varieties at different maturity levels. *Grasas Y Aceites* **2015**, *66*, e067. [CrossRef]
44. Kosma, I.; Vavoura, M.; Kontakos, S.; Karabagias, I.; Kontominas, M.; Apostolos, K.; Badeka, A. Characterization and Classification of Extra Virgin Olive Oil from Five Less Well-Known Greek Olive Cultivars. *J. Am. Oil Chem. Soc.* **2016**, *93*, 837–848. [CrossRef]
45. Evangelou, E.; Kiritsakis, K.; Sakellaropoulos, N.; Kiritsakis, A. Table Olives Production, Postharvest Processing, and Nutritional Qualities. In *Handbook of Vegetables and Vegetable Processing*; John Wiley & Sons: Hoboken, NJ, USA, 2018; pp. 727–744.