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Species of the Genera *Neopestalotiopsis* and *Alternaria* as Dominant Pathogen Species Attacking Mastic Trees (*Pistacia lentiscus* var. Chia)

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Abstract: Between 2018 and 2021, several mastic trees (*Pistacia lentiscus* var. Chia) sampled in the field and the nursery of the Chios Mastiha Growers Association (CMGA) were analyzed to determine the cause of dominant diseases. Symptoms included defoliation, leaf, and twig blight, wilting and/or apoplexy of trees and apoplexy of young hardwood cuttings. Moreover, brown discoloration had also been observed on older woody parts of the trees such as branches and tree trunks. Several pathogens have been isolated and identified as the causing agents. *Neopestalotiopsis* and *Alternaria* species were isolated consistently from necrotic tissues of mastic trees (branches, twigs, and leaves) in the field and the nursery. All fungal isolates' pathogenicity was confirmed by applying Koch's postulates on young mastic trees under glasshouse conditions. Fungal pathogens were identified by sequence analyses of the ITS, β-tubulin, and histone gene regions. *Alternaria* species were analyzed further by sequencing the endopolygalacturonase (*endoPG*) and the *Alternaria* major allergen (*Alta1*) genes. More specifically, the isolates were identified as *Neopestalotiopsis clavispora, Alternaria arborescens*, and *A. alternata* based on morphological features and sequence analyses. This is the first report of *N. clavispora*, *A. arborescens*, and *A. alternata* on *P. lentiscus* var. Chia.

Keywords: *Neopestalotiopsis clavispora*; *Alternaria arborescens*; *Alternaria alternata*; mastic production

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1. Introduction

The mastic tree (*Pistacia lentiscus* var. Chia) of the *Anacardiaceae* family, is an evergreen dioecious bush that produces mastic gum and belongs to the Mediterranean maquis vegetation. Interestingly, the production of mastic gum is restricted to the southern part of the island of Chios, a Greek island [1]. The beneficial properties of mastic were already known since antiquity, and information regarding the mastic tree and its miraculous resin was collected by Herodotus, Hippocrates (5th century B.C.), Theophrastus (372–278 B.C.), Dioscorides (1st century AD), and Galenus (129–216) [1–4]. Traditional medicine used mastic gum to treat gastrointestinal disorders and skin infections and recognized this crystallized resin as an important ingredient for the preparation of drugs and pharmaceutical potions [2]. Mastic resin and mastic oil are known for their anti-inflammatory, antioxidant, and antiseptic properties [5]. Recently, research turned towards investigating these beneficial traits, which have been partly attributed to the chemical composition of mastic oil and gum [4,6]. Some of the constituents, such as triterpenes, polyphenols, phenolic acids, flavonoids, phytosterols, and natural polymers, are known for their therapeutic properties [4,7,8]. More specifically, the dominant compounds of mastic oil and gum as determined by GC-MS chromatography are α-pinene, β-myrcene, β-pinene, limonene, and β-caryophyllene [6,9,10]. Chios mastic has verified healing effects, when used as an adjunct medicine, against inflammatory bowel diseases, periodontitis, dermatitis, peptic ulcers, non-alcoholic fatty liver disease (NAFLD), and human LDL oxidation [4] and references therein. Its antibacterial action is remarkable, and the inhibition of *Helicobacter pylori*, the

causal agent of peptic ulcers, has been thoroughly studied [11–16]. Reports of the antifungal activity of mastic gum and essential oils are also important since inhibition of important human and plant pathogens, such as *Trichomonas vaginalis, Candida albicans*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Mucor circinelloides*, and *Rhizopus oryzae*, is achieved [4,17]. The essential oil and gum of *P. lentiscus* var. Chia have also found extensive uses in the cosmetic, perfumery, and food industry due to the characteristic aroma they emit [10,18]. For all these reasons, the crystallized resin is considered a product of high economic value, with the price per kilo reaching EUR 91 in 2021 [19]. Moreover, Chios mastic is identified as a Protected Designation of Origin (PDO) product [20].

The mastic tree is a dioecious plant, and gum mastic is obtained only from male trees [1,2]. Traditionally, producers maintain and propagate genotypes (male clones) that are chosen for their stability in the quality and quantity of mastic resin they produce [1,2,21]. Therefore, clonal propagation of mastic trees with hardwood cuttings is the predominant multiplication process mastic producers use [1,2,22]. Nowadays most of the mastic tree propagates are produced in the nurseries of the CMGA.

During the last decade, there have been several reports of trees showing symptoms of defoliation, leaf, and twig blight, brown to black discoloration of woody tissues, wilting, and/or rapid apoplexy in the field and the nursery of the CMGA. The severe quantitative losses on the produced gum in the infected fields, demonstrated the necessity to investigate the causal agents.

Pestalotiopsis-like species have been correlated to the abovementioned symptoms on mastic trees. El Gali (2017) [23], correlated symptoms of dieback and apoplexy of mastic shrubs located in northeastern Libya with *Alternaria alternata*, *Pestalotiopsis* fici, *P. guepinii*, and *P. palmarum*, based on morphological characteristics. Shoot and twig dieback, discoloration of the wood, necrotic lesions, and cankers in the bark of *P. lentiscus* var. Chia were correlated to *P. guepinii,* in diseased mastic tree seedlings grown in Izmir, Turkey [24]. Moreover, recently *A. alternata* was identified as the causal agent of decline and necrosis on olive tree cuttings (*Olea europaea*) in a nursery in northern Greece [25].

This study aimed to determine the dominant fungal pathogens associated with the defoliation, twig blight, wood tissue necrosis, and vessel discoloration observed in fields and nurseries of mastic trees and to compare their impact on two-year-old trees and hardwood cuttings of *P. lentiscus* var. Chia.

2. Materials and Methods

2.1. Sampling and Fungal Isolation

A total of thirty-two (32) fungal isolates were recovered from symptomatic plants in the field on Chios Island (Table 1) and in nurseries of the CMGA. The sampling period was divided into three periods (1: 12–14 May 2019; 2: 29–31 August 2019; 3–7 November 2021). Branches and twigs showing black to dark-brown discoloration of vessels and woody parts were collected from several fields and processed in the Plant Pathology Laboratory (School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece), within 48 h. Other symptoms included leaf spots, defoliation, leaf, and twig blight, bud necrosis, wilting and/or apoplexy of trees, and necrotic bark tissues. Prior to the pathogen isolation process, all samples were disinfected by immersion in 50% Sodium Hypochlorite (NaOCl 5%) for 20 s, following surface disinfection with 70% ethanol for 60 s. The samples were then washed with sterile deionized water for 30 s and left to dry on a sterilized surface in a laminar flow. Small pieces of tissue were then transferred on Petri dishes with Potato Dextrose Agar (PDA, LAbM, Neogen, Lansing, MI, USA), containing 25 mg of chloramphenicol (Sigma-Aldrich, Saint Louis, MO, USA) per liter. Petri dishes were incubated in a growth chamber in the dark at 25 °C for 3–5 days, depending on the colony development. Pure colonies were obtained from single spore cultures using the serial dilution method [26] and then transferred in new PDA dishes where they were routinely kept and re-cultured every two months. Symptomatic leaves showing brown spots and shot hole symptoms, were also sampled in the field, and processed in the laboratory. Sterilization was achieved

by immersion in 70% ethanol for 45 s, and 2 mm pieces of leaf tissue was transferred to the selective PDA dishes at first following growth on PDA dishes for maintenance, as mentioned above.

Table 1. Locations, hosts, isolation data and accession numbers for the isolated fungi that caused symptoms on artificially inoculated mastic trees.

Isolate Information					Accession Numbers				
Isolate Number	**Fungal Species**	**Host, Tissue**	**Location**	**Date**	**ITS**	**β-tubulin**	**Alta-1**	**EndoPG**	**Histone**
M8	*Neopestalotiopsis clavispora*	Cuttings, necrotized buds	Nursery of CMGA	May 2019	OP783346	OP897766	n. a.	n. a.	n. a.
M9	*Alternaria arborescens*	Cuttings, necrotized buds	Nursery of CMGA	May 2019	OP783347	n. a.	OP817016	OP817018	n. a.
M11	*Neopestalotiopsis clavispora*	Mastic tree, wood rots on bark	Field, Chios Island (38°14′07″ N 25°57′38″ E)	August 2019	OP895136	OP897767	n. a.	n. a.	n. a.
M13	*Alternaria alternata*	Mastic tree, necrotic twigs, and branches, necrotic buds	Field, Chios Island (38°13′42″ N 26°00′17″ E)	November 2021	OP895138	n. a.	OP897762	OP897764	n. a.
M15	*Neopestalotiopsis clavispora*	Mastic tree, necrotic twigs, and branches, necrotic buds	Field, Chios Island (38°14′31″ N 26°01′01″ E)	November 2021	OP895137	OP897768	n. a.	n. a.	n. a.
M17	*Alternaria alternata*	Mastic tree, necrotic twigs, and branches with discolorations, leaves with spots	Field, Chios Island (38°12′13″ N 25°59′59″ E)	November 2021	OP783348	n. a.	OP817017	OP817019	n. a.
M18	*Alternaria alternata/ A. tenuissima*	Mastic tree, necrotic twigs, and branches with discolorations, leaves with spots	Field, Chios Island (38°12′13″ N 25°59′59″ E)	November 2021	OP895139	n. a.	OP897763	OP897765	OP897769

n.a. stands for not available.

2.2. Colony Morphology and Microscopy Observation

Colony morphology of all isolates was studied macroscopically and microscopically after 5 and 10 days of incubation in the dark at 25 °C. Conidia were examined after 15 days of incubation and the formation of acervuli by some isolates was also assessed. After macroscopic and microscopic observation of the colonies, 12 isolates were identical, and the 20 remaining isolates were tested for their pathogenicity on mastic trees. Photographs were taken using a digital camera (ZEISS Axiocam ERc 5s Microscope Camera, Appleton Woods Limited, Birmingham, United Kingdom) connected to a microscope (Zeiss. Axio Lab.A1, Crespel & Deiters GmbH, Ibbenbüren, Germany).

2.3. Application of Koch's Postulates

Pathogenicity of 20 fungal isolates, sampled from woody tissues, was confirmed on two-year-old mastic trees and on hardwood cuttings used for clonal propagation of mastic trees. Colonized mycelium plugs (5 mm) were cut from the periphery of 15-day-old pure cultures of the isolates and added on previously wounded trunks, with the mycelium facing the plant tissue. The agar plugs were then covered with parafilm and sprayed with sterile distilled water to maintain humidity. Young trees and cuttings inoculated in the same way using PDA disks were kept as controls. Eight mastic trees and eight hardwood cuttings, respectively, were used per treatment and the experiment was repeated thrice. All plants were grown under controlled greenhouse conditions (20 ± 1/18 ± 1 °C day/night temperature and 60 ± 5/70 ± 5% day/night relative humidity) and inspected weekly for symptoms.

Thirty-five days post-inoculation, discoloration of the trunk, necrotic areas around the buds, and wilting of new leaves were visible on all inoculated trees and cuttings (Figure 1), while controls remained symptomless (Figure 2). Isolations from the treatments resulted in three macroscopically and microscopically distinguished fungi that were repeatedly and constantly present on the PDA dishes. Infection development was evaluated according to the following disease index scale: DI1: Healthy plants—no symptoms; 2: No symptoms on

the aboveground and the root—slight discoloration of vessels, expanding to 1–3 buds that are necrotic; 3: No symptoms on the aboveground and the root–intense discoloration of vessels, expanding to 3–10 buds that are necrotic; 4: Partial wilt of the plant that is evident on the newly sprouted leaves—intense discoloration of vessels throughout the plant; 5: Total wilt of the plant—Necrosis, dieback.

Figure 1. Discoloration of the trunk, necrotic areas around the buds and wilting of new leaves visible on all inoculated trees and cuttings 35 days post inoculation (arrows) (**A**). Visible dark brown discoloration of the vessels of two-year-old mastic trees artificially inoculated with *Neopestalotiopsis clavispora* (arrow) (**B**).

Figure 2. Absence of disease symptoms on hardwood cuttings used for clonal propagation of mastic trees used as controls 35 days post inoculation with uncolonized PDA agar plugs.

The reisolated fungi were macroscopically and microscopically observed, and then molecularly identified, thus proving Koch's postulates fulfillment.

2.4. DNA Extraction, PCR Amplification, and Sequencing

The DNA of all isolates was extracted from mycelium using the DNeasy Blood & Tissue Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's protocol. Two reference genes (ITS and β-tubulin) were targeted for sequence analysis of isolates M8, M11, and M15, and regarding the other isolates, identification was achieved by sequence analysis

of three (3) reference genes (*ITS*, *Alta1*, and *endoPG*), with the further sequence analysis of histone when the result was inconclusive. The ITS1-5.8S-ITS2 region of all single spore isolates was amplified with primers ITS1/ ITS4 [27], and the amplification of β-tubulin for isolates M8, M11, and M15 was performed using the primers Bt2a/Bt2b [28]. Regarding isolates M9, M13, M17, and M18, the endopolygalacturonase (*endoPG*) and the Alternaria major allergen (*Alta1*) genes were amplified using specific primers PG3/PG2b [29] and Alt-for/Alt-rev [30], respectively. When extra specificity was required regarding isolate M18, primers CYLH3F/CYLH3R [31] were used to amplify histone 3 (H3).

PCR conditions and reaction mixtures were those described by Testempasis et al. (2022) [32], with slight modifications. Amplification conditions were similar for all primer pairs, as follows: 94 °C for 30 s; followed by 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 68 °C; and final extension 5 min at 68 °C. PCR products were separated by electrophoresis in 1.5% agarose gel in 1 × TAE buffer (TAE; Tris acetate EDTA) and visualized with MIDORIGreen advance (Nippon Genetics, Düren, Germany) under UV light. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's protocol. Sanger sequencing in both directions was performed using all primer pairs, DNA sequence chromatograms were edited using Geneious Prime® 2022.1.1 (Biomatters Ltd., Auckland, New Zealand) software, and all obtained sequences were compared with sequences in the National Center for Biotechnology Information database using Blastn software. Phylogenetic analysis was carried out with maximum likelihood analysis (Tamura-Nei model), which was performed at the Geneious Prime® 2022.1.1 (Biomatters Ltd., Auckland, New Zealand) software. The hierarchical clustering and tree construction were performed using the UPGMA (unweighted pair group method with arithmetic mean) model in which 1000 rapid bootstrap replicates were run. Regarding the phylogenetic tree of *Neopestalotiopsis* species (Figure S1), *Seiridium camelliae* strain SD096 was used as the outgroup and the accession numbers of all strains used are the following: *Seiridium camelliae* (JQ683725), *Neopestalotiopsis piceana* (MH855130), *Neopestalotiopsis clavispora* (OP783346), *Neopestalotiopsis clavispora* (OP895136), *Neopestalotiopsis clavispora* (OP895137), *Neopestalotiopsis clavispora* (MG729690), *Neopestalotiopsis formicarum* (MH860500), and *Neopestalotiopsis ellipsospora* (KM199343). Regarding the phylogenetic tree of *Alternaria* species (Figure S2), *Alternaria solani* was used as the outgroup and the accession numbers of all strains used are the following: *Alternaria solani* (KJ397978), *Alternaria arborescens* (BMP0582), *Alternaria arborescens* (KY561852), *Alternaria alternata* (BMP0561), *Alternaria alternata* (BMP0591), *Alternaria alternata* (BMP0653), *Alternaria alternata* (EGS_34_016), *Alternaria alternata* (EGS_34_039), *Alternaria alternata* (BMP0660), *Alternaria tenuissima* (EGS_34_015).

3. Results

Pathogenicity tests demonstrated that 11 out of 20 isolates were able to cause pathogenic symptoms in mastic tree (Table 1). Observation of colony and conidia morphology lead to the conclusion that isolates M8, M11, and M15 were identical and identified as *N. clavispora*. Moreover, M13 and M17 were also identical and identified as *A. alternata*. The typical symptoms caused by *N. clavispora* isolates included wilting, extended bud necrosis, discoloration of vessels, and bark cankers both on young mastic trees and on hardwood propagation cuttings (Figure 3). *A. arborescens* caused wilting, limited bud necrosis, vessel, and bark discoloration, on both artificially inoculated two-year-old trees and hardwood cuttings of mastic trees (Figure 4). The disease index assessment revealed that *N. clavispora* caused the most severe symptoms on the two-year-old trees, whereas in the case of hardwood cutting, *A. arborescens* provoked a higher disease index (Figure 5). Both *N. clavispora* and *A. arborescens* were constantly reisolated from all artificially inoculated trees and cuttings and in the case of *N. clavispora* the formation of acervuli and slimy conidial masses were evident on the PDA cultures 14 days post isolation (Figure 6). *A. alternata* caused wilting, bud necrosis, wood decay, bark, and vessel discoloration, on young mastic trees under controlled conditions and in the field (Figure 7). Moreover, necrotic leaf spots were caused

on two-year-old trees, underlying the complex symptomatology of this plant pathogenic fungus (Figure 7).

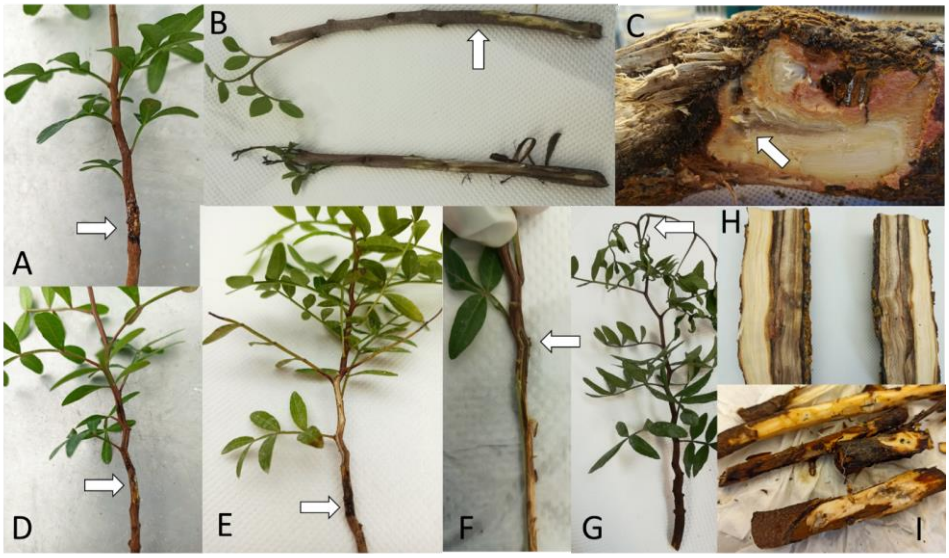

Figure 3. Disease symptoms on *Pistacia lentiscus* var. Chia artificially inoculated with *Neopestalotiopsis clavispora*: Two-year-old tree showing bark discoloration and cankers, as well as acervuli of the fungus (arrow) (**A**). Vessel discoloration and bud necrosis on hardwood cuttings (arrow) (**B**). Wood decay and vessel discoloration (arrow) on mastic tree branches in the field (Chios Island, 38°14′07″ N 25°57′38″ E) (**C**). Discoloration of bark and vessels (arrows) of two-year-old trees without bud necrosis (**D**–**F**). Intense wilting of young leaves, vessel discoloration and bark cankers (**G**). Wood decay and vessel discoloration on mastic tree branches and twigs, with defoliation symptoms in the field (Chios Island, 38°14′07″ N 25°57′38″ E) (**H**,**I**).

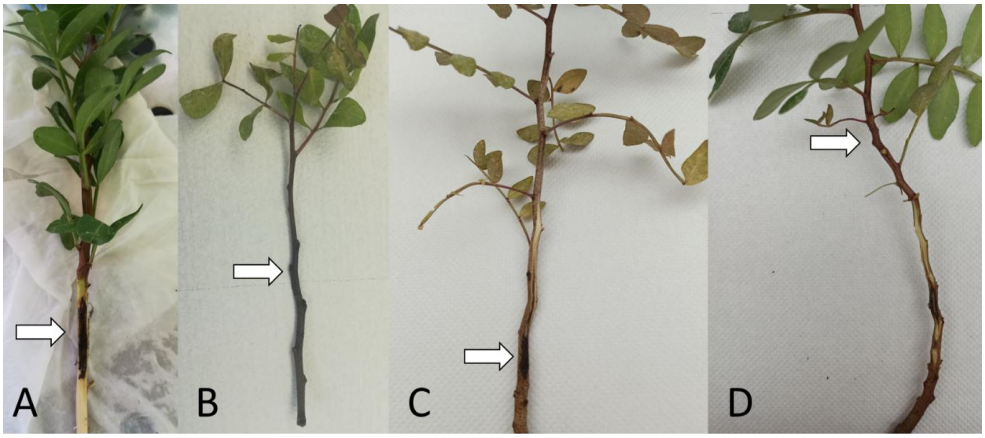

Figure 4. Disease symptoms on *Pistacia lentiscus* var. Chia artificially inoculated with *Alternaria arborescens*: Two-year-old tree showing vessel discoloration and bud necrosis (arrow) (**A**). Hardwood cuttings of mastic tree showing bark discoloration (arrow) (**B**). Wilting, vessel discoloration and bud necrosis on young mastic trees (arrow) (**C**). Extended bud necrosis and vessel discoloration on two-year-old mastic trees (**D**).

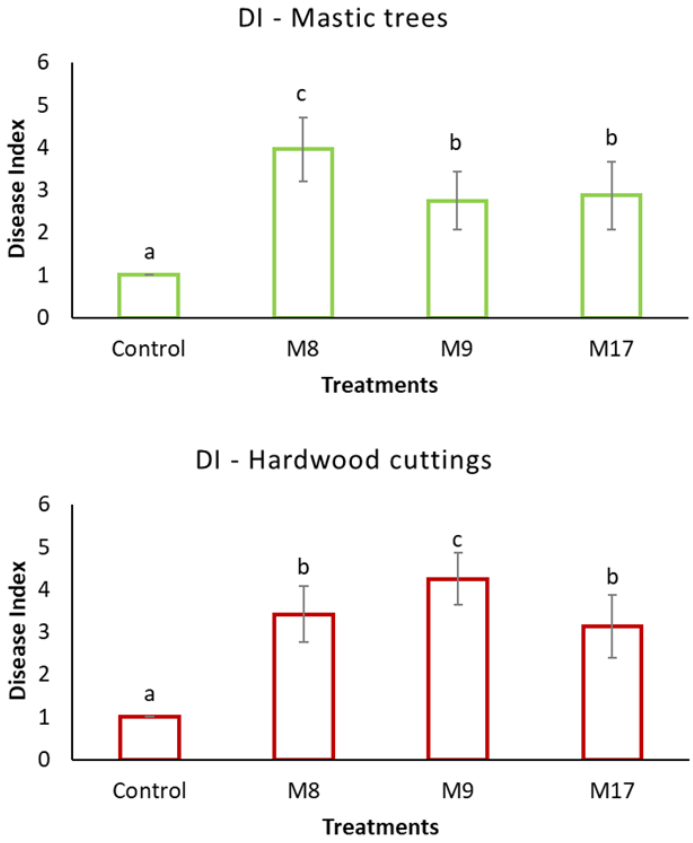

Figure 5. Severity of disease caused by *Neopestalotiopsis clavispora* M8, *Alternaria arborescens* M9, and *A. alternata* M17, which were artificially inoculated on two-year-old mastic trees and hardwood cuttings grown in pots under greenhouse conditions. Control plants were inoculated only with PDA plugs, without the pathogen. Disease development was assessed 35 days after inoculation. The experiment was repeated three times. Different letters indicate significant differences according to Tukey's test at $p \leq 0.05$. Error bars represent standard deviation.

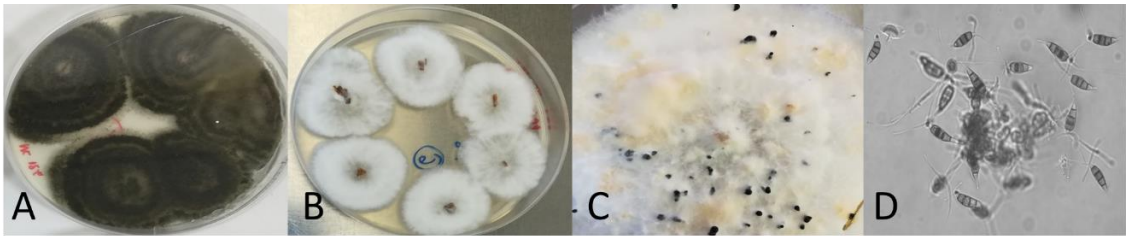

Figure 6. PDA culture of *Alternaria arborescens* constantly reisolated from artificially inoculated mastic trees with symptoms of bud necrosis and discoloration (**A**). Culture of *Neopestalotiopsis clavispora* constantly reisolated from artificially inoculated mastic trees with symptoms of brown discoloration of the vessels, bud necrosis, and wilting (**B**). Acervuli and conidial slimy masses formed by *N. clavispora* in colonies on PDA (**C**). Conidia of *N. clavispora* located in important quantities in the fungal acervuli (**D**).

Disease severity was observed on mastic trees and cuttings inoculated with all other isolates of *N. clavispora* and *A. alternata* as well, proving that the symptoms that were caused by those fungi were of similar intensity, respectively (Figure 8). Once again, the disease caused by isolates M11 and M15 of *N. clavispora* was more severe on rooted mastic trees, whereas *A. alternata* M13 was more virulent on hardwood cuttings (Figure 8).

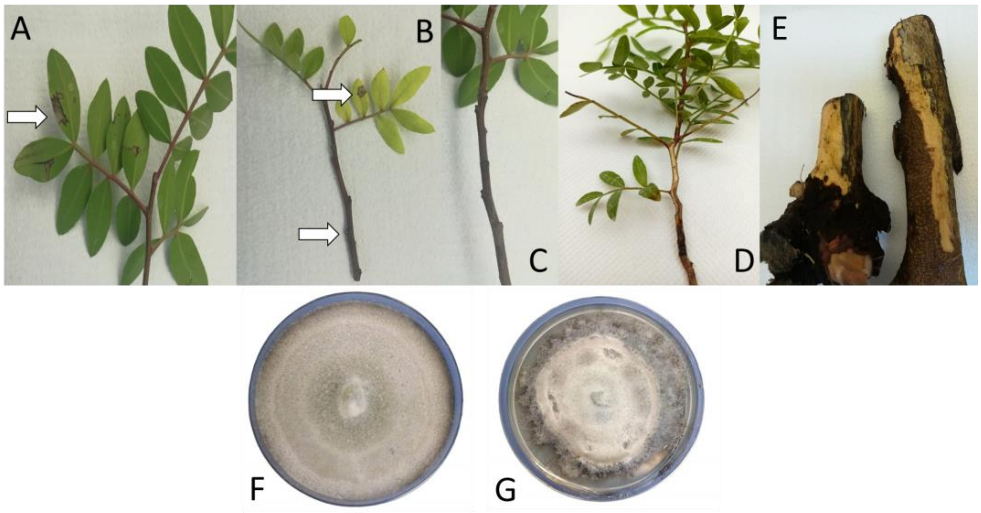

Figure 7. Disease symptoms on *Pistacia lentiscus* var. Chia artificially inoculated with *Alternaria alternata*: Two-year-old tree showing necrotic leaf spots (arrows) (**A**,**B**). Two-year-old tree showing necrotic leaf spots, bark, and vessel discoloration (arrows) (**B**–**D**). Wood decay and vessel discoloration on branches in the field (Chios Island, 38°12′13″ N 25°59′59″ E) (**E**). PDA culture of *Alternaria alternata* M17 (**F**) and PDA culture of *Alternaria alternata* /*A. tenuissima* M18 (**G**).

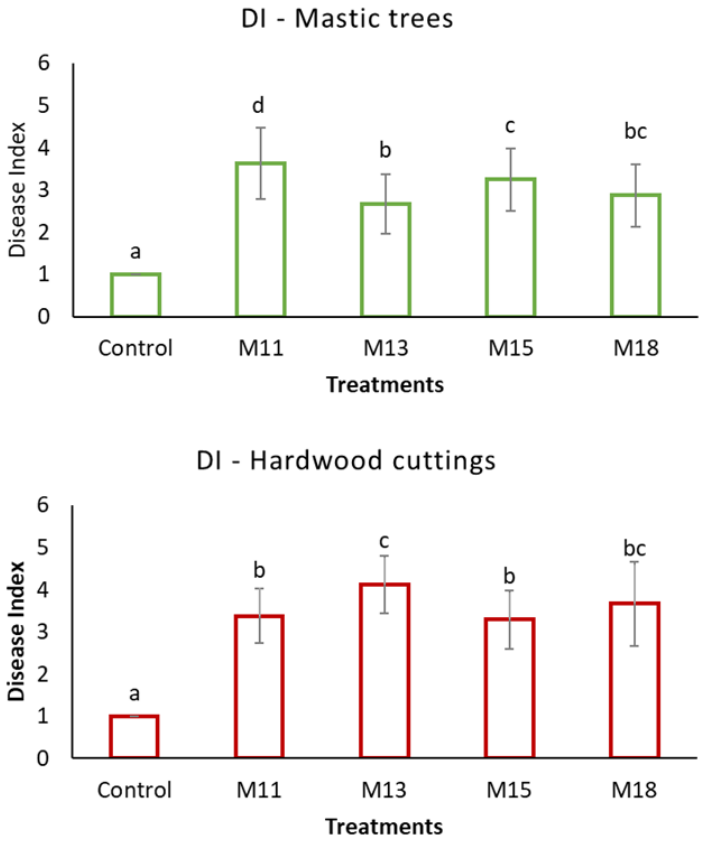

Figure 8. Severity of disease caused by *Neopestalotiopsis clavispora* M11 and M15, and *Alternaria alternata* M13 and M18 that were artificially inoculated on two-year-old mastic trees and hardwood cuttings grown in pots under greenhouse conditions. Control plants were inoculated only with PDA plugs, without the pathogen. Disease development was assessed 35 days after inoculation. The experiment was repeated three times. Different letters indicate significant differences according to Tukey's test at $p \leq 0.05$. Error bars represent standard deviation.

4. Discussion

Following the continuous reports of mastic trees showing wilting, wood decay, leaf spots, bark, and vessel discoloration, this investigation began to define the causal agents of these symptoms. After sampling in the nursery of the CMGA and several problematic fields, some specific pathogens were consistently isolated. The fungi *N. clavispora*, *A. arborescens*, and *A. alternata* were identified based on culture morphology and targeted gene sequence analysis. The species identification process regarding *Neopestalotiopsis* genera is controversial and according to Maharachchikumbura et al. (2012) [33], who evaluated specific regions or genes for their ability to act as reliable species-defining regions, ITS region, β-tubulin, and translation elongation 1 (tef1) genes were the more accurate choice for species definition [33]. The genera of *Pestalotiopsis*, *Pseudopestalotiopsis*, and *Neopestalotiopsis* comprise a wide variety of species (>235) that are important plant pathogens [33–35]. *N. clavispora* is a member of the *Pestalotiopsis* group and, as such, is widely distributed in the tropics and recently its geographical distribution has extended in temperate ecosystems as well. Endophytic stages of *N. clavispora* in mangrove trees (*Bruguiera sexangular*, *Rhizophora harrisonii*, and *Phoenix reclinata*) are mentioned in some studies [36–38], showing a possible ability to switch life modes and endure unfavorable conditions, but the interest is focused on its plant pathological aspect. In detail, *N. clavispora* has been proven to attack and cause important symptoms on strawberry plants (*Fragaria* × *ananassa*) in Spain, Argentina, Italy, and Uruguay [39–42], on pecan (*Carya illinoinensis*) and macadamia (*Macadamia integrifolia*) in Brazil [43,44], on China rose (*Rosa sinensis*) and the evergreen climber *Kadsurra coccinea* in China [45,46], and on blueberry (*Vaccinium corymbosum*) in Spain [47].

El Gali and partners (2017) [23] depicted *A. alternata* as the dominant pathogen, causing extended brown leaf spot on mastic trees, in addition to *Pestalotiopsis fici, P. guepinii*, and *P. palmarum*, which were defined as the causal agents of leaf blight, leaf tip death, and silvery gray leaf spots, respectively, on mastic shrubs. *A. alternata* is an opportunistic fungus with worldwide distribution, which is often identified as the causal agent of leaf spot disease in various hosts including trees such as platan (*Platanus acerifolia*) in China [48], olive tree (*Olea europaea*) in Pakistan [49] and apricot (*Prunus armeniaca*) in Iraq [50], but also in shrubs such as pomegranate (*Punica granatum*) in Israel [51]. Reports regarding the ability of this fungus to cause wilting, wood and bud necrosis, or branch decay are very scarce and hosts include Thuja (*Thuja occidentalis*) in Kazakhstan [52], and olive trees and olive tree cuttings (*O. europaea*) in Greece [25,53]. Similarly, *A. arborescens* is often identified as the causal agent of leaf spots and leaf blotch on various herbaceous hosts, and more rarely on woody plants such as almond (Prunus dulcis), apple (*Malus domestica*), and date palm (*Phoenix dactylifera*) [54–57].

Subsequently, the correlation of the symptoms *A. alternata* and *A. arborescens* caused on mastic trees is of great importance since little is known about the fact that those fungi can also cause bud necrosis, vessel discoloration, and twig blight, in addition to the leaf spots they provoke.

Mastic cultivation follows organic farming strategies and given the economic value of its resin, the necessity to define the causal agents of reduced production is crucial. This study correlated the most significant symptoms observed in fields and nurseries of mastic trees with three dominant fungal pathogens. The comparison of their impact showed that the trees were significantly affected by all fungal isolates. *N. clavispora* caused more intense symptoms on two-year-old trees, and the *Alternaria* species affected hardwood cuttings of *P. lentiscus* var. Chia more intensively as compared to control plants and to *N. clavispora.* The devastating symptoms that were observed in the nursery of CMGA, in combination with the economic importance of this specific host, underlines the importance of the present study, in terms of alerting the mastic tree growers towards finding a solution and inhibiting the propagation of *N. clavispora*, *A. arborescens*, and *A. alternata* on the island of Chios.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres14010010/s1, Figure S1. The phylogenetic tree was

generated by Geneious Prime®2022.1.1 (Biomatters Ltd, New Zealand) software analyzing the data set of ITS gene as downloaded from the National Center for Biotechnology Information (NCBI) database. The Tamura—Nei genetic distance model was used, and the tree was constructed according to UPGMA method. *Seiridium camelliae* strain SD096 was used as the outgroup and the strains isolated within this study are written in blue. The accession numbers of all strains used are the following: *Seiridium camelliae* (JQ683725), *Neopestalotiopsis piceana* (MH855130), *Neopestalotiopsis clavispora* (OP783346), *Neopestalotiopsis clavispora* (OP895136), *Neopestalotiopsis clavispora* (OP895137), *Neopestalotiopsis clavispora* (MG729690), *Neopestalotiopsis formicarum* (MH860500), and *Neopestalotiopsis ellipsospora* (KM199343); Figure S2. The phylogenetic tree was generated by Geneious Prime®2022.1.1 (Biomatters Ltd, New Zealand) software analyzing the data set of ITS gene as downloaded from the National Center for Biotechnology Information (NCBI) database. The Tamura—Nei genetic distance model was used, and the tree was constructed according to UPGMA method. *Alternaria solani* was used as the outgroup and the strains isolated within this study are written in blue. The accession numbers of all strains used are the following: *Alternaria solani* (KJ397978), *Alternaria arborescens* (BMP0582), *Alternaria arborescens* (KY561852), *Alternaria alternata* (BMP0561), *Alternaria alternata* (BMP0591), *Alternaria alternata* (BMP0653), *Alternaria alternata* (EGS_34_016), *Alternaria alternata* (EGS_34_039), *Alternaria alternata* (BMP0660), *Alternaria tenuissima* (EGS_34_015).

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