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Research Article

Bottlebrush and Myrtle twig canker caused by *Neopestalotiopsis* species: an emerging canker-causing group of fungi in Italy

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Abstract

Pestalotioid fungi were isolated in pure culture from symptomatic plants of *Callistemon laevis*, *C. viminalis*, *Luma apiculata* (marketed as "*Myrtus luma*"), *Myrtus communis* subsp. *tarentina*, and *M. communis* var. *microphylla* (*M. communis* 'Microphylla'), showing twig canker, dieback and defoliation. The isolates were identified to species by ITS, *tef1* and *tub2* sequences, which revealed the presence of six species of *Neopestalotiopsis* (*N. camelliae-oleiferae*, *N. hispanica*, *N. iberica*, *N. rosae*, *N. rosicola*, and *N. zakeelii*) and one species of *Pestalotiopsis* (*P. biciliata*). While most species were isolated only once or twice, the majority of isolates belonged to *N. rosae* (13) and *N. hispanica* (8). Pathogenicity was investigated by pathogenicity tests on all hosts, which confirmed the pathogenicity of all *Neopestalotiopsis* species on at least some of the hosts tested, while *P. biciliata* did not cause any disease symptoms. *Neopestalotiopsis hispanica* and *N. rosae* caused symptoms in all hosts of the present study, while the other *Neopestalotiopsis* species tested showed no symptoms on *Luma apiculata*.

Key words: Bottlebrush, canker, myrtle, *Neopestalotiopsis*, phylogeny

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Introduction

The Bottlebrush (*Callistemon citrinus* (Curtis.) Skeels.) and myrtle (*Myrtus communis* L.) are evergreen shrubs belonging to Myrtaceae. Bottlebrushes refer to *Callistemon* species, a group of plants native to Australia. In recent years, Australian plants, especially *Callistemon* species, have aroused interest in Mediterranean countries as ornamental plants mainly due to their aesthetic value and great adaptability to Mediterranean environmental conditions (Lippi et al. 2003).

Little is known about fungal diseases of bottlebrush. Some species of *Calonectria* (*Ca. cylindrospora*, *Ca. mexicana*, *Ca. pauciramosa*, and *Ca. pseudomexicana*) were reported causing leaf spot, blight, stem lesion and damping-off on different accessions of bottlebrushes in Europe (Pérez-Sierra et al. 2007; Polizzi et al. 2007; Vitale et al. 2013; Aiello et al. 2022) and the African continent (Lombard et al. 2011). Other causal agents of leaf spot have been reported on *C. citrinus*, including species belonging to the genera *Alternaria*, *Botryosphaeria*, *Cercospora*, *Colletotrichum*, *Phyllosticta*, and *Selenophoma* (Alfieri et al. 1984; Pennycook et al. 1989; Gadgil 2005). Recently, in Iran the new species

Xenodidymella iranica (Didymellaceae) was isolated and described from leaf spot and blight of *C. citrinus* (Ahmadpour et al. 2022). In addition, stem canker was reportedly caused by species of *Leptosphaeria* and *Phomopsis*, stem gall by *Cophinforma tumefaciens* (syn. *Sphaeropsis tumefaciens*), and root rot diseases by *Rhizoctonia solani* and species of *Phytophthora* and *Pythium* (Alfieri et al. 1984; Shivas 1989; Erwin and Ribeiro 1996; Cacciola et al. 2009).

Myrtle is widely distributed in the Mediterranean area forming spontaneous bushes, and in Italy is particularly occurring in coastal areas and islands. Two subspecies are currently accepted within this species, *Myrtus communis* subsp. *communis* and subsp. *tarentina* (Medda and Mulas 2021; POWO 2024). Myrtle is cultivated for many different purposes such as pharmaceutical, cosmetic, ornamental (especially due to high vegetative vigor, the bright green color of the leaves, and the abundant multi-colored flowering), and food industries (Mulas et al. 1998; Medda and Mulas 2021). Although myrtle is investigated worldwide under many different aspects due to its manifold uses, few data are available regarding diseases affecting this crop. Amongst fungal diseases, leaf spot and crown and root disease caused by *Ca. mexicana*, *Ca. pauciramosa*, and *Ca. pseudomexicana* have been reported in Europe (Polizzi and Crous 1999; Henricot and Beales 2003) as well as in the United States (Koike and Crous 2001) and in Tunisia (Lombard et al. 2011).

In addition, the myrtle rust disease caused by *Austropuccinia psidii* (syn. *Puccinia psidii*) (Uredinales, Pucciniaceae) is considered an important quarantine threat (Glen et al. 2007; Fensham et al. 2020), which became notorious when it started to infect various species of the Myrtaceae (Coutinho et al. 1998; Glen et al. 2007; Carnegie et al. 2010; Morin et al. 2012; Roux et al. 2013). Recently, Italian provenances of *M. communis* were shown to be highly susceptible to *Austropuccinia psidii*, highlighting a significant threat to myrtle cultivation if the pathogen were accidentally introduced to the Mediterranean (Paap et al. 2023).

In Sicily (southern Italy), recent surveys of a nursery revealed the presence of many myrtle and bottlebrush plants affected by twig cankers and dieback. Investigations conducted in recent years in Sicily, in greenhouses and also in the field, showed an increase of these symptoms in many different crops, especially ornamental plants, caused mainly by Botryosphaeriaceae spp. (Aiello et al. 2020; Gusella et al. 2021; Costanzo et al. 2022; Fiorenza et al. 2022a). Canker-causing pathogens are under investigation worldwide for many different aspects concerning the complex etiology of the diseases, their epidemiology, wide host range and difficulties in management (Guarnaccia et al. 2022). Among the canker-causing pathogens, pestalotioid fungi have been increasingly investigated in recent years (Silva et al. 2020; Diogo et al. 2021; Fiorenza et al. 2022b; Li et al. 2022; Santos et al. 2022; Zheng et al. 2023). Maharachchikumbura et al. (2014) revised *Pestalotiopsis sensu lato* dividing it into three distinct genera, *viz. Pestalotiopsis*, *Pseudopestalotiopsis*, and *Neopestalotiopsis*. These fungi are widely distributed in tropical and temperate areas and are frequently encountered as endophytes and plant pathogens causing stem-end rot, stem and leaf blight, trunk disease, and cankers (Maharachchikumbura et al. 2014). In Sicily, recent studies of young avocado trees showing canker and wood discoloration led to the identification of two *Neopestalotiopsis* species responsible for those symptoms, namely *N. rosae* and *N. siciliana* sp. nov. (Fiorenza et al. 2022b). Moreover, Fiorenza et al. (2022b) noticed frequent necroses and

cankers at the grafting point, reinforcing the assumption that the propagation processes are crucial for pestalotioid infections (Espinoza et al. 2008). Therefore, myrtle and bottlebrush plants showing symptoms of twig canker and dieback were investigated in the present study to provide a deeper insight into etiology. The aims of this study were to: ii) identify and characterize the etiological agents; ii) test their pathogenicity.

Materials and methods

Survey and fungal isolations

The survey was conducted from September 2021 in a nursery in Catania (eastern Sicily). Symptomatic plants of *Callistemon laevis*, *C. viminalis*, *Luma apiculata* (marketed as "*Myrtus luma*"), *Myrtus communis* subsp. *tarentina*, and *M. communis* var. *microphylla* (*M. communis* 'Microphylla'), showing twig canker, dieback and defoliation were brought to the laboratory of the Dipartimento di Agricoltura, Alimentazione e Ambiente for further analyses. Specifically, small pieces of diseased wood tissue from cankered twigs were surface sterilized for 1 min in 1.5% sodium hypochlorite (NaOCl), rinsed in sterile water, dried on sterile absorbent paper in a laminar flow hood and then placed on potato dextrose agar (PDA, Lickson, Italy) amended with 100 mg/L of streptomycin sulfate (Sigma-Aldrich, MO, USA) to prevent bacterial growth. The Petri plates were then incubated at 25 °C for 7 days until the fungal colonies were large enough to be examined. For *M. communis* subsp. *tarentina* and *M. communis* 'Microphylla', isolations were also conducted from the leaves as described above. Colonies of interest were transferred onto fresh PDA plates and then single hypha isolates were obtained from pure cultures. Fungal isolates were stored as mycelial plugs in sterile water in the fungal collection of the laboratory with the strain identifiers ML (isolates from *Luma apiculata*), MT (isolates from *M. communis* subsp. *tarentina*), MP (isolates from *M. communis* 'Microphylla') or CV (isolates from *Callistemon*).

DNA extraction, PCR and sequencing

A total of 26 representative isolates from *Callistemon* spp., as well as from *Luma* and *Myrtus*, were chosen for molecular and phylogenetic analysis. DNA was extracted from single hypha isolates grown on PDA. The mycelium was scraped off and processed following manufacturer's instructions of the Wizard Genomic DNA Purification Kit® (Promega Corporation, WI, USA).

The following loci were amplified and sequenced: the terminal 3' end of the small subunit nuclear ribosomal DNA (nSSU rDNA) and the complete internal transcribed spacer region (ITS1-5.8S-ITS2) of the rDNA region with primers V9G (de Hoog and Gerrits van den Ende 1998) and ITS4 (White et al. 1990); a ca 0.5 kb fragment of the translation elongation factor 1-alpha (*tef1*) gene with primers EF1-728F (Carbone and Kohn 1999) and TEFD_iR (Voglmayr et al. 2018); and a ca. 0.95 kb fragment of the beta tubulin (*tub2*) gene with primers T1D (Voglmayr et al. 2019) and BtHV2r (Voglmayr et al. 2016, 2017). PCR products were purified using enzymatic PCR cleanup (Werle et al. 1994) as described in Voglmayr and Jaklitsch (2008). DNA was cycle-sequenced using

the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, U.K.) with the same primers as in PCR. Sequencing was performed on an automated DNA sequencer (3730xl Genetic Analyzer, Applied Biosystems). The resulting DNA sequences were assembled with Lasergene SeqMan Pro (DNASTAR, Madison, WI, USA). The sequences generated during the present study were deposited in Genbank (Table 1).

Analysis of sequence data

For the phylogenetic analysis, a combined matrix of ITS rDNA, *tef1* and *tub2* sequences was produced. The newly generated sequences were aligned to a representative matrix of GenBank sequences of *Neopestalotiopsis* and *Pestalotiopsis*. For *Neopestalotiopsis*, all 88 described species for which sequences were available were included in the matrix, preferentially with ex-type sequences. Sequences of the four taxa of *Pestalotiopsis* which were the closest match to the single *Pestalotiopsis* isolate of the current study were added as outgroup. The GenBank accession numbers of the sequences used in this analysis are given in Table 1.

All alignments were produced with the server version of MAFFT (www.ebi.ac.uk/Tools/mafft), checked and refined using BioEdit version 7.2.6 (Hall 1999). For some sequences, highly deviating leading or trailing sequence regions, which were likely affected by sequencing errors, were removed from the alignment, as well as primer residue sequences that were present in some GenBank sequences. The ITS rDNA, *tef1* and *tub2* matrices were combined for subsequent phylogenetic analysis.

Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 2.0 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA+I substitution model (selected as the best model by Modeltest) with 1000 bootstrap replicates. The matrix was partitioned for different gene regions. For evaluation and discussion of bootstrap support, values below 70% were considered low, between 70 and 90% medium/moderate, above 90% high and 100% maximum.

Maximum parsimony (MP) bootstrap analyses were performed with PAUP v. 4.0a169 (Swofford 2002), with 1 000 bootstrap replicates using five rounds of heuristic search replicates with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect, COLLAPSE command set to MINBRLEN, each replicate limited to 1 million rearrangements) during each bootstrap replicate. All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to minbrlen.

Table 1. Symptomatology observed on different plant species investigated in this study.

Plant species	Symptoms	Disease incidence (%)
Callistemon laevis	small twig canker; dieback and defoliation	30
C. viminalis	small twig canker; dieback and defoliation	30
Luma apiculata	small twig canker, dieback, and low defoliation	20
Myrtus communis 'Microphylla'	small twig canker, dieback, and very low defoliation	20
M. communis subsp. *tarentina*	small twig canker, dieback, and high defoliation	70

Morphological investigation

Morphology of conidia was checked from sporulated cultures on PDA, CMD (CMA: Sigma, St Louis, Missouri; supplemented with 2% (w/v) D(+)-glucose-monohydrate) or 2% MEA (2% w/v malt extract, 2% w/v agar-agar; Merck, Darmstadt, Germany) plates grown at room temperature at ambient light. Conidia, prepared in tap water on microscope slides, were investigated in a Zeiss Axio Imager. A1 compound microscope.

Pathogenicity test

To determine the ability of the fungal species to infect and induce symptoms, seven representative isolates were selected for pathogenicity tests: *Neopestalotiopsis camelliae-oleiferae* ML3, *N. hispanica* ML8, *N. iberica* MP13, *N. rosae* MT32, *N. rosicola* MP29, *N. zakeelii* MP30, and *P. biciliata* MP11 were inoculated on potted plants of *Callistemon laevis*, *C. viminalis*, *M. communis* subsp. *tarentina*, *M. communis* 'Mycrophylla', and *L. apiculata*. Each fungal species was inoculated on three plants (replicate) of each plant species. For each plant, six twigs were inoculated as sub-replicates. The inoculum consisted of a small piece (~ 0.3–0.5 mm^2) of mycelial plug from 15-day-old cultures on PDA. The bark was first gently scraped using a sterile blade and then the mycelial plug was inserted upside down onto the wound. The wounds were sealed with Parafilm to prevent desiccation. Controls consisted of sterile PDA. All inoculated plants were moved to a growth chamber with a 12 h photoperiod and maintained at 25 ± 1 °C. The plants were regularly watered and monitored weekly for development of symptoms. Final symptoms evaluation was conducted 30 days after inoculations. Re-isolations were performed as described above to fulfill Koch's postulates.

Results

Survey and fungal isolations

Symptoms observed in the nursery included twig canker, dieback, internal wood necrosis and defoliation (Fig. 1). Symptomatology details and disease incidence for each plant species are described in Table 1. Fungal isolations constantly yielded *Neopestalotiopsis*-like colonies. A total of 56 *Neopestalotiopsis*-like isolates were stored in the fungal collection.

Phylogenetic analysis and species identification

The combined multilocus matrix used for phylogenetic analyses comprised 2458 characters (539 from the ITS, 978 from *tef1*, and 941 from *tub2*), of which 414 were parsimony informative (66 from ITS, 170 from *tef1*, and 178 from *tub2*). The best ML tree (-lnL = 10329.8215) obtained by RAxML is shown in Fig. 2. While the genus *Neopestalotiopsis* and a few deeper nodes were highly supported, most of the tree backbone received little or no bootstrap support. However, the isolates of the present study could be assigned to species level, based on the results of the phylogenetic analyses (Fig. 2), viz. one species of

Figure 1. Disease symptoms caused by *Neopestalotiopsis* spp. in nursery: blight and defoliation on *Myrtus communis* subsp. *tarentina* **A–B** *Luma apiculata* **C–D** *M. communis* 'Microphylla' **E** *Callistemon viminalis* **F** and *C. laevis* **G**.

Pestalotiopsis and 6 species of *Neopestalotiopsis*: isolate MP11 was revealed as *Pestalotiopsis biciliata*, isolate MP29 as *Neopestalotiopsis rosicola*, ML3 as *N. camelliae-oleiferae*, MP13 as *N. iberica*, and the two isolates CV52 and MP30 as *N. zakeelii*. However, the majority of isolates belonged to two species, viz. eight isolates to *N. hispanica* (syn. *N. vaccinii*) and 13 to *N. rosae* (see Table 2 and Fig. 2).

These results were also confirmed by the nucleotide comparisons (Table 3), which revealed (almost) identical sequences to the ex-type strains of the respective species. While within *N. hispanica* all markers of all isolates were identical with the ex-type sequences, within *N. rosae* slight sequence differences were observed in three isolates, viz. ML1 (1 nucleotide substitution and 1 gap in ITS), CV51 and MP25 (1 gap in ITS and 1 nucleotide substitution in *tef1*). Within *N. camelliae-oleiferae*, the ITS differed from the ex-type sequence by two gaps; however, these are likely sequencing errors as they were located in polyA regions at the immediate beginning of the deposited sequence which do not sufficiently resolved by the Sanger sequencing method. In *N. zakelii*, the ITS and *tub2* sequences of our isolates differed by 3 nucleotide substitutions from the ex-type sequences; however, the difference to the second verified strain (BRIP 72271a) was much less (1 substitution in ITS, identical *tef1* and *tub2* sequences). Also, in *P. biciliata* the ITS differed by 3 and 1 nucleotide substitutions in ITS and *tef1*, respectively, but only by 1 nucleotide substitution in *tef1* from the second verified strain (CBS 790.68). The species identification of these isolates with some differences to the ex-type sequences was also confirmed by BLAST searches of *tef1* and *tub2*, in which the respective species were revealed as closest matches with 100%.

Morphology of the conidia (conidial shape, size of the conidial body, number and coloration of the conidial cells, number and length of appendages) matched the genera and species and was therefore in line with the molecular

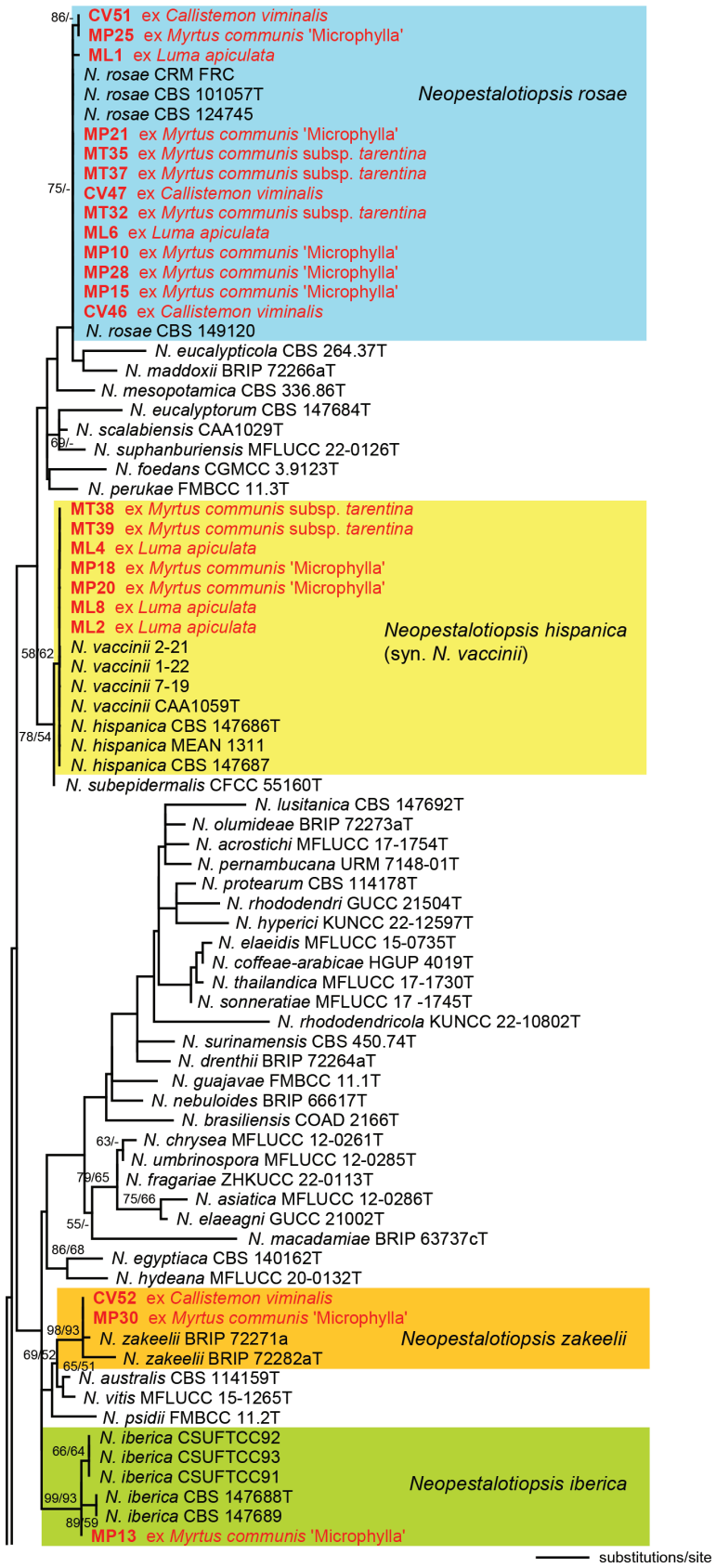

Figure 2. Phylogram of the best ML tree (-lnL = 10329.8215) revealed by RAxML from an analysis of the combined ITS-*tef1-tub2* matrix of *Neopestalotiopsis*, showing the phylogenetic position of the isolates obtained from diseased Myrtaceae. Strains marked by an "T" at the end of the strain designation represent ex-type strains. ML and MP bootstrap support above 50% are given above or below the branches. The broken branches were scaled to one-quarter.

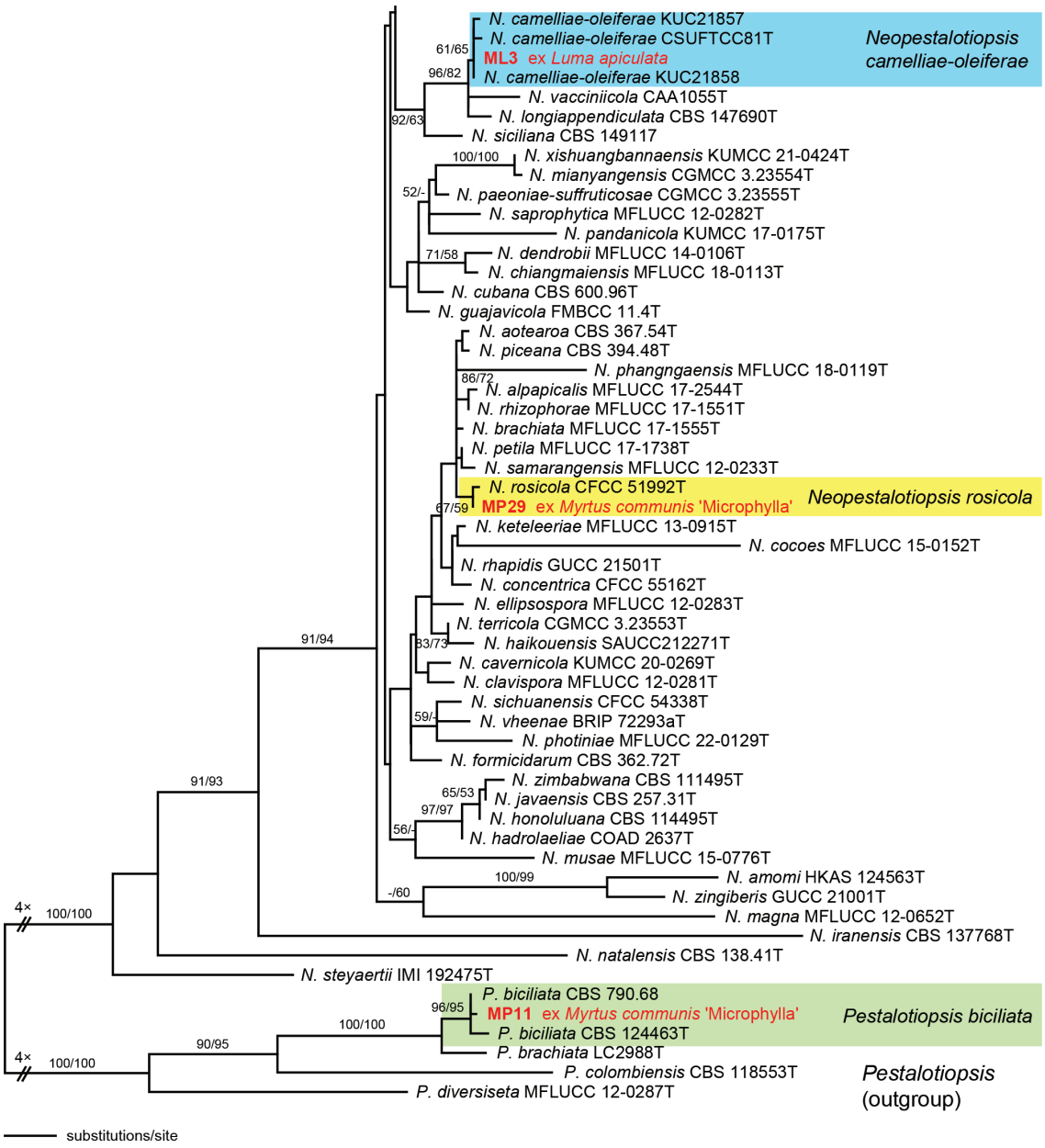

Figure 2. Continued.

identification; however, as within the genus *Neopestalotiopsis* conidial shapes and sizes largely overlap and are highly similar in the bulk of these cryptic species, no species identification is possible based on morphology.

Pathogenicity test

The results of the pathogenicity tests (disease incidence) are shown in Table 4. Although some cankers were visible after one week, the size of the twigs was too thin and small to assess the disease severity (lesion length). Only *N. rosae* and *N. hispanica* caused disease symptoms on all hosts tested, whereas *P. biciliata* did not show pathogenicity. Moreover, the isolates inoculated on wounded twigs caused similar symptoms on different host species and showed twig canker and dieback (Fig. 3).

Table 2. Isolates used in the molecular analyses in this study.

Species	Strain[1]	Host/Substrate	Origin	GenBank Accession Numbers[2]		
				ITS	*tef1*	*tub2*
Neopestalotiopsis acrostichi	MFLUCC 17-1754^T	*Acrostichum aureum*	Thailand	MK764272	MK764316	MK764338
N. alpapicalis	MFLUCC 17-2544^T	*Rhizophora mucronata*	Thailand	MK357772	MK463547	MK463545
N. amomi	HKAS 124563^T	*Amomum villosum*	China	OP498012	OP653489	OP752133
N. aotearoa	CBS 367.54^T	Canvas	New Zealand	KM199369	KM199526	KM199454
N. asiatica	MFLUCC 12-0286^T	Unidentified tree	China	JX398983	JX399049	JX399018
N. australis	CBS 114159^T	*Telopea* sp.	Australia	KM199348	KM199537	KM199432
N. brachiata	MFLUCC 17-1555^T	*Rhizophora apiculata*	Thailand	MK764274	MK764318	MK764340
N. brasiliensis	COAD 2166^T	*Psidium guajava*	Brazil	MG686469	MG692402	MG692400
N. camelliae-oleiferae	CSUFTCC81^T	*Camellia oleifera*	China	OK493585	OK507955	OK562360
N. camelliae-oleiferae	KUC21857	*Phyllostachys bambusoides*	South Korea	OR654966	OR693485	OR693494
N. camelliae-oleiferae	KUC21858	*Phyllostachys bambusoides*	South Korea	OR654967	OR693486	OR693495
N. camelliae-oleiferae	**ML3**	*Luma apiculata*	**Italy**	**PP146586**	**PP174965**	**PP197178**
N. cavernicola	KUMCC 20-0269^T	Cave rock surface	China	MW545802	MW550735	MW557596
N. chiangmaiensis	MFLUCC 18-0113^T	Dead leaves	Thailand	N/A	MH388404	MH412725
N. chrysea	MFLUCC 12-0261^T	*Pandanus* sp.	China	JX398985	JX399051	JX399020
N. clavispora	MFLUCC 12-0281^T	*Magnolia* sp.	China	JX398979	JX399045	JX399014
N. cocoes	MFLUCC 15-0152^T	*Cocos nucifera*	Thailand	NR_156312	KX789689	N/A
N. concentrica	CFCC 55162^T	*Rosa rugosa*	China	OK560707	OM622433	OM117698
N. coffeae-arabicae	HGUP 4019^T	*Coffea arabica*	China	KF412649	KF412646	KF412643
N. cubana	CBS 600.96^T	Leaf litter	Cuba	KM199347	KM199521	KM199438
N. dendrobii	MFLUCC 14-0106^T	*Dendrobium cariniferum*	Thailand	MK993571	MK975829	MK975835
N. drenthii	BRIP 72264a^T	*Macadamia integrifolia*	Australia	MZ303787	MZ344172	MZ312680
N. egyptiaca	CBS 140162^T	*Mangifera indica*	Egypt	KP943747	KP943748	KP943746
N. elaeagni	GUCC 21002^T	*Elaeagnus pungens*	China	MW930716	MZ203452	MZ683391
N. elaeidis	MFLUCC 15-0735^T	*Elaeis guineensis*	Thailand	ON650690	ON734012	N/A
N. ellipsospora	MFLUCC 12-0283^T	Dead plant materials	China	JX398980	JX399047	JX399016
N. eucalypticola	CBS 264.37^T	*Eucalyptus globulus*	N/A	KM199376	KM199551	KM199431
N. eucalyptorum	CBS 147684^T	*Eucalyptus globulus*	Portugal	MW794108	MW805397	MW802841
N. foedans	CGMCC 3.9123^T	Mangrove plant	China	JX398987	JX399053	JX399022
N. formicidarum	CBS 362.72^T	Dead *Formicidae* (ant)	Ghana	KM199358	KM199517	KM199455
N. fragariae	ZHKUCC 22-0113^T	*Fragaria* × *ananassa*	China	ON553410	ON569076	ON569075
N. guajavae	FMBCC 11.1^T	*Psidium guajava*	Pakistan	MF783085	MH460868	MH460871
N. guajavicola	FMBCC 11.4^T	*Psidium guajava*	Pakistan	MH209245	MH460870	MH460873
N. hadrolaeliae	COAD 2637^T	*Hadrolaelia jongheana*	Brazil	MK454709	MK465122	MK465120
N. haikouensis	SAUCC212271^T	*Ilex chinensis*	China	OK087294	OK104877	OK104870
N. hispanica	CBS 147686^T	*Eucalyptus globulus*	Portugal	MW794107	MW805399	MW802840
N. hispanica	CBS 147687	*Eucalyptus globulus*	Spain	MW794113	MW805401	MW802846
N. hispanica	MEAN 1311	*Eucalyptus globulus*	Portugal	MW794106	MW805400	MW802839
N. hispanica	**ML2**	*Luma apiculata*	**Italy**	**PP146593**	**PP174983**	**PP197171**
N. hispanica	**ML4**	*Luma apiculata*	**Italy**	**PP146594**	**PP174984**	**PP197172**
N. hispanica	**ML8**	*Luma apiculata*	**Italy**	**PP146596**	**PP174985**	**PP197173**
N. hispanica	**MP18**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146599**	**PP174986**	**PP197174**
N. hispanica	**MP20**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146600**	**PP174987**	**PP197175**
N. hispanica	**MT38**	*Myrtus communis* subsp. *tarentina*	**Italy**	**PP146606**	**PP174989**	**PP197176**
N. hispanica	**MT39**	*Myrtus communis* subsp. *tarentina*	**Italy**	**PP146607**	**PP174990**	**PP197177**

Species	Strain[1]	Host/Substrate	Origin	GenBank Accession Numbers[2]		
				ITS	*tef1*	*tub2*
N. honoluluana	CBS 114495^T	*Telopea* sp.	USA	KM199364	KM199548	KM199457
N. hydeana	MFLUCC 20-0132^T	*Artocarpus heterophyllus*	Thailand	MW266069	MW251129	MW251119
N. hyperici	KUNCC 22-12597^T	*Hypericum monogynum*	China	OP498010	OP713768	OP765908
N. iberica	CBS 147688^T	*Eucalyptus globulus*	Portugal	MW794111	MW805402	MW802844
N. iberica	CBS 147689	*Eucalyptus globulus*	Spain	MW794114	MW805403	MW802847
N. iberica	CSUFTCC91	*Camellia oleifera*	China	OK493587	OK507957	OK562362
N. iberica	CSUFTCC92	*Camellia oleifera*	China	OK493588	OK507958	OK562363
N. iberica	CSUFTCC93	*Camellia oleifera*	China	OK493589	OK507959	OK562364
N. iberica	**MP13**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146587**	**PP174967**	**PP197167**
N. iranensis	CBS 137768^T	*Fragaria* × *ananassa*	Iran	KM074048	KM074051	KM074057
N. javaensis	CBS 257.31^T	*Cocos nucifera*	Indonesia	KM199357	KM199548	KM199457
N. keteleeriae	MFLUCC 13-0915^T	*Keteleeria pubescens*	China	KJ023087	KJ023089	KJ023088
N. longiappendiculata	CBS 147690^T	*Eucalyptus globulus*	Portugal	MW794112	MW805404	MW802845
N. lusitanica	CBS 147692^T	*Eucalyptus globulus*	Portugal	MW794110	MW805406	MW802843
N. macadamiae	BRIP 63737c^T	*Macadamia integrifolia*	Australia	KX186604	KX186627	KX186654
N. maddoxii	BRIP 72266a^T	*Macadamia integrifolia*	Australia	MZ303782	MZ344167	MZ312675
N. magna	MFLUCC 12-0652^T	*Pteridium* sp.	France	KF582795	KF582791	KF582793
N. mesopotamica	CBS 336.86^T	*Pinus brutia*	Turkey	KM199362	KM199555	KM199441
N. mianyangensis	CGMCC 3.23554^T	*Paeonia suffruticosa*	China	OP546681	OP723490	OP672161
N. musae	MFLUCC 15-0776^T	*Musa* sp.	Thailand	NR_156311	KX789685	KX789686
N. natalensis	CBS 138.41^T	*Acacia mollissima*	South Africa	NR_156288	KM199552	KM199466
N. nebuloides	BRIP 66617^T	*Sporobolus jacquemontii*	Australia	MK966338	MK977633	MK977632
N. olumideae	BRIP 72273a^T	*Macadamia integrifolia*	Australia	MZ303790	MZ344175	MZ312683
N. paeoniae-suffruticosae	CGMCC 3.23555^T	*Paeonia suffruticosa*	China	OP082292	OP204794	OP235980
N. pandanicola	KUMCC 17-0175^T	*Pandanus* sp.	China	N/A	MH388389	MH412720
N. pernambucana	URM 7148-01^T	*Vismia guianensis*	Brazil	KJ792466	KU306739	N/A
N. perukae	FMBCC 11.3^T	*Psidium guajava*	Pakistan	MH209077	MH523647	MH460876
N. petila	MFLUCC 17-1738^T	*Rhizophora apiculata*	Thailand	MK764276	MK764320	MK764342
N. phangngaensis	MFLUCC 18-0119^T	*Pandanus* sp.	Thailand	MH388354	MH388390	MH412721
N. photiniae	MFLUCC 22-0129^T	*Photinia serratifolia*	China	OP498008	OP753368	OP752131
N. piceana	CBS 394.48^T	*Picea* sp.	UK	KM199368	KM199527	KM199453
N. protearum	CBS 114178^T	*Leucospermum cuneiforme*	Zimbabwe	JN712498	KM199542	KM199463
N. psidii	FMBCC 11.2^T	*Psidium guajava*	Pakistan	MF783082	MH460874	MH477870
N. rhapidis	GUCC 21501^T	*Rhododendron simsii*	China	MW931620	MW980442	MW980441
N. rhizophorae	MFLUCC 17-1551^T	*Rhizophora mucronata*	Thailand	MK764277	MK764321	MK764343
N. rhododendri	GUCC 21504^T	*Rhododendron simsii*	China	MW979577	MW980444	MW980443
N. rhododendricola	KUNCC 22-10802^T	*Rhododendron* sp.	China	OK283069	OK274148	OK274147
N. rosae	CBS 101057^T	*Rosa* sp.	New Zealand	KM199359	KM199523	KM199429
N. rosae	CBS 124745	*Paeonia suffruticosa*	USA	KM199360	KM199524	KM199430
N. rosae	CRM-FRC	*Fragaria* × *ananassa*	Mexico	MN385718	MN268532	MN268529
N. rosae	AC50	*Persea americana*	Italy	ON117810	ON107276	ON209165
N. rosae	**CV46**	*Callistemon viminalis*	**Italy**	**PP146591**	**PP174971**	**PP197179**
N. rosae	**CV47**	*Callistemon viminalis*	**Italy**	**PP146592**	**PP174972**	**PP197180**
N. rosae	**CV51**	*Callistemon viminalis*	**Italy**	**PP146584**	**PP174982**	**PP197191**
N. rosae	**ML1**	*Luma apiculata*	**Italy**	**PP146583**	**PP174973**	**PP197181**
N. rosae	**ML6**	*Luma apiculata*	**Italy**	**PP146595**	**PP174974**	**PP197182**
N. rosae	**MP10**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146597**	**PP174975**	**PP197183**
N. rosae	**MP15**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146598**	**PP174976**	**PP197184**

Species	Strain[1]	Host/Substrate	Origin	GenBank Accession Numbers[2]		
				ITS	*tef1*	*tub2*
N. rosae	**MP21**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146601**	**PP174977**	**PP197185**
N. rosae	**MP25**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146588**	**PP174988**	**PP197186**
N. rosae	**MP28**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146602**	**PP174978**	**PP197187**
N. rosae	**MT32**	*Myrtus communis* subsp. *tarentina*	**Italy**	**PP146603**	**PP174979**	**PP197188**
N. rosae	**MT35**	*Myrtus communis* subsp. *tarentina*	**Italy**	**PP146604**	**PP174980**	**PP197189**
N. rosae	**MT37**	*Myrtus communis* subsp. *tarentina*	**Italy**	**PP146605**	**PP174981**	**PP197190**
N. rosicola	CFCC 51992^T	*Rosa chinensis*	China	KY885239	KY885243	KY885245
N. rosicola	**MP29**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146590**	**PP174968**	**PP197168**
N. samarangensis	MFLUCC 12-0233^T	*Syzygium samarangense*	Thailand	JQ968609	JQ968611	JQ968610
N. saprophytica	MFLUCC 12-0282^T	*Magnolia* sp.	China	JX398982	JX399048	JX399017
N. scalabiensis	CAA1029^T	*Vaccinium corymbosum*	Portugal	MW969748	MW959100	MW934611
N. sichuanensis	CFCC 54338^T	*Castanea mollissima*	China	MW166231	MW199750	MW218524
N. siciliana	CBS 149117	*Persea americana*	Italy	ON117813	ON107273	ON209162
N. sonneratiae	MFLUCC 17-1745^T	*Sonneronata alba*	Thailand	MK764280	MK764324	MK764346
N. steyaertii	IMI 192475^T	*Eucalytpus viminalis*	Australia	KF582796	KF582792	KF582794
N. subepidermalis	CFCC 55160^T	*Rosa rugosa*	China	OK560699	OM622425	OM117690
N. suphanburiensis	MFLUCC 22-0126^T	dead stem of unid. plant	Thailand	OP497994	OP753372	OP752135
N. surinamensis	CBS 450.74^T	Soil under *Elaeis guineensis*	Suriname	KM199351	KM199518	KM199465
N. terricola	CGMCC 3.23553^T	*Paeonia suffruticosa*	China	OP082294	OP204796	OP235982
N. thailandica	MFLUCC 17-1730^T	*Rhizophora mucronata*	Thailand	MK764281	MK764325	MK764347
N. umbrinospora	MFLUCC 12-0285^T	unidentified plant	China	JX398984	JX399050	JX399019
N. vaccinii	CAA1059^T	*Vaccinium corymbosum*	Portugal	MW969747	MW959099	MW934610
N. vaccinii	22-Jan	*Vaccinium corymbosum*	Serbia	OQ316613	OQ342778	OQ473026
N. vaccinii	21-Feb	*Vaccinium corymbosum*	Serbia	OQ316612	OQ342777	OQ473025
N. vaccinii	19-Jul	*Vaccinium corymbosum*	Serbia	OQ316611	OQ342776	OQ473024
N. vacciniicola	CAA1055^T	*Vaccinium corymbosum*	Portugal	MW969751	MW959103	MW934614
N. vheenae	BRIP 72293a^T	*Macadamia integrifolia*	Australia	MZ303792	MZ344177	MZ312685
N. vitis	MFLUCC 15-1265^T	*Vitis vinifera*	China	KU140694	KU140676	KU140685
N. xishuangbannaensis	KUMCC 21-0424^T	*Kerivoula hardwickii*	China	ON426865	OR025973	OR025934
N. zakeelii	BRIP 72282a^T	*Macadamia integrifolia*	Australia	MZ303789	MZ344174	MZ312682
N. zakeelii	BRIP 72271°	*Macadamia integrifolia*	Australia	MZ303788	MZ344173	MZ312681
N. zakeelii	**CV52**	*Callistemon viminalis*	**Italy**	**PP146585**	**PP174969**	**PP197170**
N. zakeelii	**MP30**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146589**	**PP174970**	**PP197169**
N. zimbabwana	CBS 111495^T	*Leucospermum cuneiforme*	Zimbabwe	JX556231	KM199545	KM199456
N. zingiberis	GUCC 21001^T	*Zingiber officinale*	China	MW930715	MZ683389	MZ683390
Pestalotiopsis biciliata	CBS 124463^T	*Platanus* × *hispanica*	Slovakia	KM199308	KM199505	KM199399
P. biciliata	CBS 790.68	*Taxus baccata*	Netherlands	KM199305	KM199507	KM199400
P. biciliata	**MP11**	*Myrtus communis* 'Microphylla'	**Italy**	**PP146582**	**PP174966**	**PP197166**
P. brachiata	LC2988^T	*Camellia* sp.	China	KX894933	KX895150	KX895265
P. colombiensis	CBS 118553^T	*Eucalyptus grandis* × *urophylla*	Colombia	KM199307	KM199488	KM199421
P. diversiseta	MFLUCC 12-0287^T	Dead plant material	China	NR_120187	JX399073	JX399040

Table 3. Sequence comparison of differences in number of nucleotide substitutions and gaps between the sequences of isolates of the present study and the ex-type sequences of the respective species.

Species	No. of isolates	Sequence difference to ex-type strain (nucleotide substitutions/gaps)		
		ITS	*tef1*	*tub2*
Neopestalotiopsis camelliae-oleiferae	1	(2)*/0	0/0	0/0
N. hispanica (syn. *N. vaccinii*)	8	0/0	0/0	0/0
N. iberica	1	0/0	1/0	0/0
N. rosae	13	0-1/0-1	0-1/0	0/0
N. rosicola	1	0/0	1/0	0/0
N. zakeelii	2	3/0	0/0	2/0
Pestalotiopsis biciliata	1	3/0	1/1	0/0

*Gaps located at the immediate beginning of the ITS in polyA regions, likely representing sequencing errors.

Table 4. Results of pathogenicity tests conducted in this study.

Host	*Neopestalotiopsis* spp. isolates						
	N. rosae **MT32**	***N. rosicola*** **MP29**	***N. hispanica*** **ML8**	***N. iberica*** **MP13**	***N. zakeelii*** **MP30**	***N. camelliae-oleiferae*** **ML3**	***P. biciliata*** **MP11**
Callistemon laevis	+	+	+	+	+	+	-
C. viminalis	+	+	+	+	+	+	-
Luma apiculata	+	-	+	-	-	-	-
Myrtus communis 'Microphylla'	+	+	+	+	+	+	-
M. communis subsp. *tarentina*	+	+	+	+	-	+	-

"+" symptoms; "–" symptomless.

Discussion

In the present study, the following fungal species were described causing twig canker, dieback and defoliation on *Callistemon*, *Luma* and *Myrtus* spp.: *Neopestalotiopsis camelliae-oleiferae*, *N. hispanica* (syn. *N. vaccinii*), *N. iberica*, *N. rosae*, *N. rosicola*, *N. zakeelii*, and *Pestalotiopsis biciliata*. In the group of fungi characterized in this study the species *N. hispanica* and *N. rosae*, based on frequency of isolations and ability to induce symptoms in all tested plant species, probably play a key role in the disease development. *Neopestalotiopsis* species, as well as other species belonging to other taxa within the Sporocadaceae are known worldwide causing different symptoms on many hosts, such as leaf spot, flower and fruit blight, twig canker and crown rot (Rebollar-Alviter et al. 2020; Santos et al. 2022; Shahriar et al. 2022; Guterres et al. 2023). Similar symptoms to those observed in this study have been reported for *N. iberica* on *Eucalyptus globulus* in Portugal (Diogo et al. 2021) and on *Synsepalum dulcificum* in China (You et al. 2024), for *N. rosae* on blueberry in Peru, and Portugal (Rodríguez-Gálvez et al. 2020; Santos et al. 2022), and on avocado in Italy (Fiorenza et al. 2022b), for *N. rosicola* on *Rosa chinensis* in China (Jiang et al. 2018), for *N. hispanica* (syn. *N. vaccinii*) on *E. globulus* in Portugal and Spain (Diogo et al. 2021) and on blueberry in Portugal (Santos et al. 2022). However, *N. camelliae-oleiferae* was also reported causing foliar symptoms on *Camellia oleifera* in

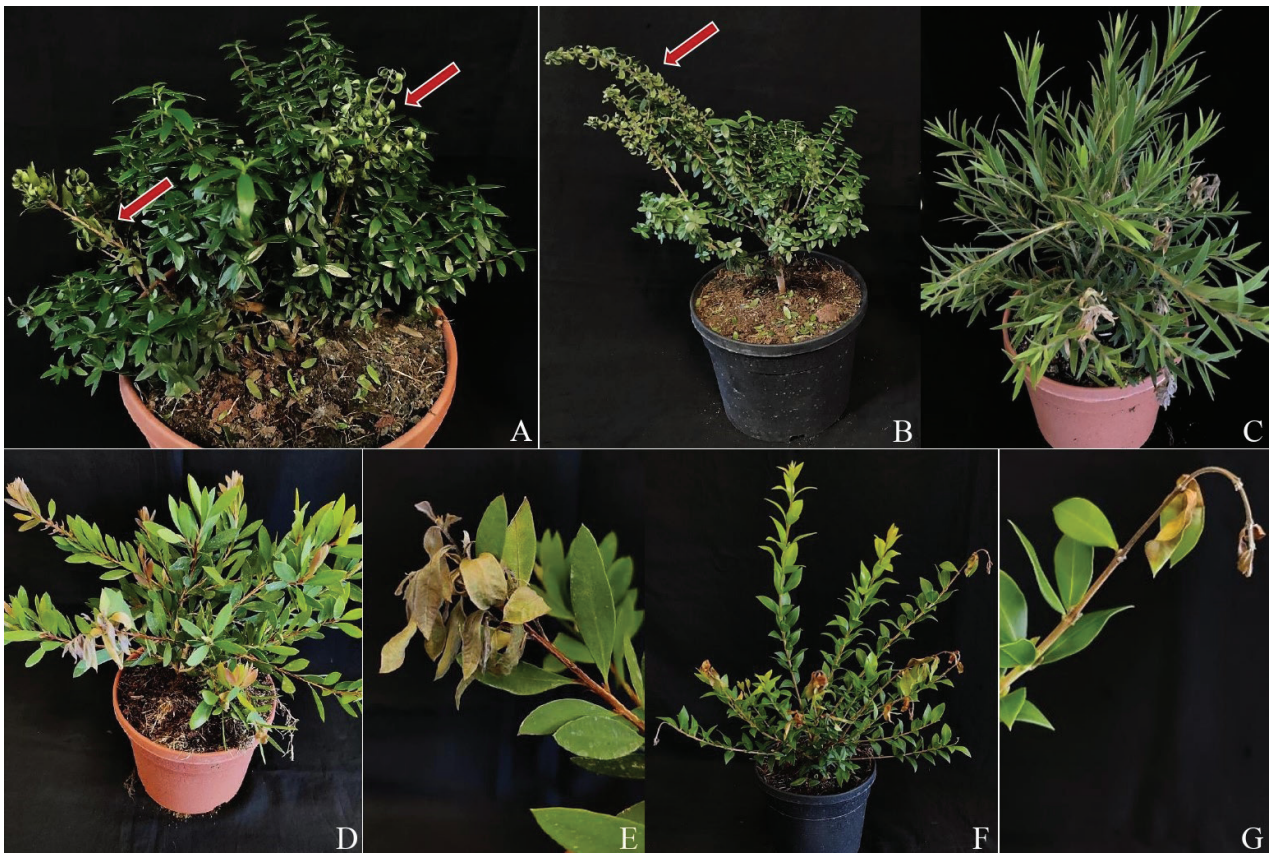

Figure 3. Symptoms caused by artificial inoculation with *Neopestalotiopsis rosae* MT32 on *Myrtus communis* 'Microphylla' **A** *Myrtus communis* subsp. *tarentina* (red arrow) **B** *Callistemon viminalis* (red arrow) **C** *Callistemon laevis* **D–E** *Luma apiculata* **F–G**.

China (Li et al. 2021), and *N. zakeelii* was described in 2021, associated with flower diseases of *Macadamia integrifolia* in Australia (Prasannath et al. 2021). Although *N. zakeelii* was reported from macadamia inflorescences showing dry flowers, Koch's postulates were not conducted and the pathogenicity was not demonstrated (Prasannath et al. 2021). In our study it is the first time that *N. zakeelii* is reported to be associated with symptoms of canker and dieback and our study confirms, for the first time, the pathogenic activity on *C. laevis* and *viminalis* and on *M. communis* 'Microphylla' causing necrotic lesions of woody tissues. On the other hand, different results have been obtained regarding pathogenicity of *P. biciliata*. This species was reported around the world causing also twig canker and dieback on different hosts such as blueberry, *Pinus pinea*, *Quercus coccifera* and *Pistacia lentiscus* (Hlaiem et al. 2022 a, b; Santos et al. 2022), whereas in our study, although isolated from cankered tissues, the tested isolate did not show any pathogenic activity on these hosts. This result is not surprising, since many other studies demonstrated that pestalotioid fungi can occur as saprobionts or generalist endophytes (Wu et al. 1982; Agarwal and Chauhan 1988; Yanna and Hyde 2002; Osono and Takeda 2006; Hu et al. 2007; Rajulu et al. 2014; Prakash et al. 2015; Park et al. 2016; Reddy et al. 2016). The results of our study showed the potential fungal diversity involved in twig canker. In fact, as highlighted in this study, to investigate these kinds of diseases, such as canker and dieback, the whole fungal diversity and the interactions among all the species involved must be taken into consideration.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualisation: DA, GP. Formal analysis: GG, GRL, HV. Funding acquisition: GP. Investigation: DA, GRL, GP. Methodology: DA, GP, GRL, HV. Software: HV. Supervision: DA, GP, HV. Validation: DA, GP, HV. Writing – original draft: DA, GG, GRL, HV. Writing – review and editing: DA, GG, GRL, GP, HV.

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Data availability

All of the data that support the findings of this study are available in the main text

References

Agarwal AK, Chauhan S (1988) A new species of the genus *Pestalotiopsis* from Indian soil. Indian Phytopathology 41: 625–627.

Ahmadpour SA, Mehrabi-Koushki M, Farokhinejad R, Asgari B (2022) *Xenodidymella iranica* sp. nov. and new hosts of *X. glycyrrhizicola* in Iran. Tropical Plant Pathology 47(3): 430–441. https://doi.org/10.1007/s40858-022-00491-3

Aiello D, Gusella G, Fiorenza A, Guarnaccia V, Polizzi G (2020) Identification of *Neofusicoccum parvum* causing canker and twig blight on *Ficus carica* in Italy. Phytopathologia Mediterranea 59(1): 213–218. https://doi.org/10.36253/phyto-10798

Aiello D, Guarnaccia V, Vitale A, LeBlanc N, Shishkoff N, Polizzi G (2022) Impact of Calonectria diseases on ornamental horticulture: Diagnosis and Control strategies. Plant Disease 106(7): 1773–1787. https://doi.org/10.1094/PDIS-11-21-2610-FE

Alfieri Jr SA, Langdon KR, Wehlburg C, Kimbrough JW (1984) Index of Plant Diseases in Florida (Revised). Florida Department of Agriculture and Consumer Service, Division of Plant Industry. Bulletin 11: 1–389.

Cacciola SO, Scibetta S, Pane A, Faedda R, Rizza C (2009) *Callistemon citrinus* and *Cistus salvifolius*, two new hosts of *Phytophthora* taxon *niederhauserii* in Italy. Plant Disease 93(10): 1075–1075. https://doi.org/10.1094/PDIS-93-10-1075A

Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/00275514.1999.12061051

Carnegie AJ, Lidbetter JR, Walker J, Horwood MA, Tesoriero L, Glen M, Priest M (2010) *Uredo rangelii*, a taxon in the guava rust complex, newly recorded on *Myrtaceae* in Australia. Australasian Plant Pathology 39(5): 463–466. https://doi.org/10.1071/AP10102

Costanzo MB, Gusella G, Fiorenza A, Leonardi GR, Aiello D, Polizzi G (2022) *Lasiodiplodia citricola*, a new causal agent of *Acacia* spp. dieback. New Disease Reports 45(2): e12094. https://doi.org/10.1002/ndr2.12094

Coutinho TA, Wingfield MJ, Alfenas AC, Crous PW (1998) Eucalyptus rust – a disease with the potential for serious international implications. Plant Disease 82(7): 819–825. https://doi.org/10.1094/PDIS.1998.82.7.819

De Hoog GS, Gerrits van den Ende AHG (1998) Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses 41: 183–189. https://doi.org/10.1111/j.1439-0507.1998.tb00321.x

Diogo E, Gonçalves CI, Silva AC, Valente C, Bragança H, Phillips AJ (2021) Five new species of *Neopestalotiopsis* associated with diseased *Eucalyptus* spp. in Portugal. Mycological Progress 20(11): 1441–1456. https://doi.org/10.1007/s11557-021-01741-5

Erwin DC, Ribeiro OK (1996) Phytophthora Diseases worldwide. The American Phytopathological Society, Saint Paul, Minnesota, USA, 599 pp.

Espinoza JG, Briceño EX, Keith LM, Latorre BA (2008) Canker and twig dieback of blueberry caused by *Pestalotiopsis* spp. and a *Truncatella* sp. in Chile. Plant Disease 92(10): 1407–1414. https://doi.org/10.1094/PDIS-92-10-1407

Fensham RJ, Carnegie AJ, Laffineur B, Makinson RO, Pegg GS, Wills J (2020) Imminent extinction of Australian Myrtaceae by fungal disease. Trends in Ecology & Evolution 35(7): 554–557. https://doi.org/10.1016/j.tree.2020.03.012

Fiorenza A, Aiello D, Costanzo MB, Gusella G, Polizzi G (2022a) A New Disease for Europe of *Ficus microcarpa* caused by Botryosphaeriaceae species. Plants 11(6): 727. https://doi.org/10.3390/plants11060727

Fiorenza A, Gusella G, Aiello D, Polizzi G, Voglmayr H (2022b) *Neopestalotiopsis siciliana* sp. nov. and *N. rosae* causing stem lesion and dieback on avocado plants in Italy. Journal of Fungi 8(6): 562. https://doi.org/10.3390/jof8060562

Gadgil PD (2005) Fungi on trees and shrubs in New Zealand. Fingi of New Zeland Vol. 4, Fungal Diversity Press, Hong Kong, 437 pp.

Glen M, Alfenas AC, Zauza EAV, Wingfield MJ, Mohammed C (2007) *Puccinia psidii*, a threat to the Australian environment and economy – a review. Australasian Plant Pathology 36(1): 1–16. https://doi.org/10.1071/AP06088

Guarnaccia V, Kraus C, Markakis E, Alves A, Armengol J, Eichmeier A, Compant S, Gramaje D (2022) Fungal trunk diseases of fruit trees in Europe: Pathogens, spread and future directions. Phytopathologia Mediterranea 61(3): 563–599. https://doi.org/10.36253/phyto-14167

Gusella G, Costanzo MB, Aiello D, Polizzi G (2021) Characterization of *Neofusicoccum parvum* causing canker and dieback on *Brachychiton* species. European Journal of Plant Pathology 161(4): 999–1005. https://doi.org/10.1007/s10658-021-02379-5

Guterres DC, Silva MA, Martins MD, Azevedo DMQ, Lisboa DO, Pinho DB, Furtado GQ (2023) Leaf spot caused by *Neopestalotiopsis* species on *Arecaceae* in Brazil. Australasian Plant Pathology 52(1): 47–62. https://doi.org/10.1007/s13313-022-00893-6

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Henricot B, Beales P (2003) First record of *Cylindrocladium pauciramosum* on myrtle (*Myrtus communis*) in Portugal. Plant Pathology 52(3): 420–420. https://doi.org/10.1046/j.1365-3059.2003.00842.x

Hlaiem S, Yangui I, Della Rocca G, Barberini S, Danti R, Ben Jamaa ML (2022a) First report of *Pestalotiopsis biciliata* causing dieback on *Quercus coccifera* and *Pistacia lentiscus* in Tunisia. Canadian Journal of Plant Pathology 44(4): 534–541. https://doi.org/10.1080/07060661.2022.2032831

Hlaiem S, Yangui I, Rocca GD, Barberini S, Danti R, Jamâa MLB (2022b) First report of *Pestalotiopsis biciliata* associated with twig canker and dieback of *Pinus pinea* in Tunisia. Journal of Plant Pathology 104(1): 393–394. https://doi.org/10.1007/s42161-021-00956-5

Hu HL, Jeewon R, Zhou DQ, Zhou TX, Hyde KD (2007) Phylogenetic diversity of endophytic *Pestalotiopsis* species in *Pinus armandii* and *Ribes* spp.: Evidence from rDNA and β-tubulin gene phylogenies. Fungal Diversity 24: 1–22.

Jiang N, Bonthond G, Fan XL, Tian CM (2018) *Neopestalotiopsis rosicola* sp. nov. causing stem canker of *Rosa chinensis* in China. Mycotaxon 133(2): 271–283. https://doi.org/10.5248/133.271

Koike ST, Crous PW (2001) First report of a root and crown rot disease of myrtle in California caused by *Cylindrocladium pauciramosum.* Plant Disease 85(4): 448–448. https://doi.org/10.1094/PDIS.2001.85.4.448C

Li L, Yang Q, Li H (2021) Morphology, Phylogeny, and Pathogenicity of Pestalotioid Species on *Camellia oleifera* in China. Journal of Fungi (Basel, Switzerland) 7(12): 1080. https://doi.org/10.3390/jof7121080

Li Y, Zhang S, Wang T, Zhou S, Wu Y, Huang X, Lin H, Su X (2022) First report of *Taxus media* branch blight caused by *Neopestalotiopsis clavispora* in China. Plant Disease 106(12): 3214. https://doi.org/10.1094/PDIS-12-21-2840-PDN

Lippi G, Serra G, Vernieri P, Tognoni F (2003) Response of potted *Callistemon* species to high salinity. Acta Horticulturae 609: 247–250. https://doi.org/10.17660/ActaHortic.2003.609.36

Lombard L, Polizzi G, Guarnaccia V, Vitale A, Crous PW (2011) *Calonectria* spp. causing leaf spot, crown and root rot of ornamental plants in Tunisia. Persoonia 27(1): 73–79. https://doi.org/10.3767/003158511X615086

Maharachchikumbura SS, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014) *Pestalotiopsis* revisited. Studies in Mycology 79(1): 121–186. https://doi.org/10.1016/j.simyco.2014.09.005

Medda S, Mulas M (2021) Fruit quality characters of myrtle (*Myrtus communis* L.) selections: Review of a domestication process. Sustainability 13(16): 8785. https://doi.org/10.3390/su13168785

Morin L, Aveyard R, Lidbetter JR, Wilson PG (2012) Investigating the host-range of the rust fungus *Puccinia psidii sensu lato* across tribes of the family *Myrtaceae* present in Australia. PLoS ONE 7(4): e35434. https://doi.org/10.1371/journal.pone.0035434

Mulas M, Cani MR, Brigaglia N (1998) Characters useful to cultivation in spontaneous populations of *Myrtus communis* L. Acta Horticulturae 457(457): 271–278. https://doi.org/10.17660/ActaHortic.1998.457.34

Osono T, Takeda H (2006) Fungal decomposition of *Abies* needle and *Betula* leaf litter. Mycologia 98(2): 172–179. https://doi.org/10.1080/15572536.2006.11832689

Paap T, Santini A, Rodas CA, Granados GM, Pecori F, Wingfield MJ (2023) *Myrtus communis* in Europe threatened by the pandemic and South African strains of the myrtle rust pathogen *Austropuccinia psidii* (Sphaerophragmiaceae, Pucciniales). In: Jactel H, Orazio C, Robinet C, Douma JC, Santini A, Battisti A, Branco M, Seehausen L, Kenis M (Eds) Conceptual and technical innovations to better manage invasions of alien pests and pathogens in forests. NeoBiota 84: 41–46. https://doi.org/10.3897/neobiota.84.95823

Park S, Lee SY, Lee JJ, Back CG, Ten L, Lee HB, Jung HY (2016) First report of *Neopestalotiopsis australis* isolated from soil in Korea. The Korean Journal of Mycology 44: 360–364. https://doi.org/10.4489/KJM.2016.44.4.360

Pennycook SR, Young JM, Fletcher MJ, Fry PR (1989) Plant diseases recorded in New Zealand. Vol. 3. Auckland, Plant Disease Division. D.S.I.R.

Pérez-Sierra A, Alvarez LA, Leon M, Abad-Campo P, Armengol J, Garcia-Jimenez J (2007) First report of leaf spot, blight, and stem lesions caused by *Cylindrocladium pauciramosum* on Callistemon in Spain. Plant Disease 91(8): 1057–1057. https://doi.org/10.1094/PDIS-91-8-1057C

Polizzi G, Crous PW (1999) Root and collar rot of milkwort caused by *Cylindrocladium pauciramosum*, a new record for Europe. European Journal of Plant Pathology 105(4): 407–411. https://doi.org/10.1023/A:1008759230180

Polizzi G, Vitale A, Aiello D, Dimartino MA, Parlavecchio G (2007) First report of damping-off and leaf spot caused by *Cylindrocladium scoparium* on different accessions of bottlebrush cuttings in Italy. Plant Disease 91(6): 769–769. https://doi.org/10.1094/PDIS-91-6-0769B

POWO (2024) Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet. http://www.plantsoftheworldonline.org/ [Retrieved 22 Feb. 2024]

Prakash CP, Thirumalai E, Rajulu MG, Thirunavukkarasu N, Suryanarayanan TS (2015) Ecology and diversity of leaf litter fungi during early-stage decomposition in a seasonally dry tropical forest. Fungal Ecology 17: 103–113. https://doi.org/10.1016/j.funeco.2015.05.004

Prasannath K, Shivas RG, Galea VJ, Akinsanmi OA (2021) *Neopestalotiopsis* species associated with flower diseases of *Macadamia integrifolia* in Australia. Journal of Fungi 7(9): 771. https://doi.org/10.3390/jof7090771

Rajulu MBG, Lai LB, Murali TS, Gopalan V, Suryanarayanan TS (2014) Several fungi from fire-prone forests of southern India can utilize furaldehydes. Mycological Progress 13(4): 1049–1056. https://doi.org/10.1007/s11557-014-0992-0

Rebollar-Alviter A, Silva-Rojas HV, Fuentes-Aragón D, Acosta-González U, Martínez-Ruiz M, Parra-Robles BE (2020) An emerging strawberry fungal disease associated with root rot, crown rot and leaf spot caused by *Neopestalotiopsis rosae* in Mexico. Plant Disease 104(8): 2054–2059. https://doi.org/10.1094/PDIS-11-19-2493-SC

Reddy MS, Murali TS, Suryanarayanan TS, Rajulu MG, Thirunavukkarasu N (2016) *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. Fungal Ecology 24: 70–75. https://doi.org/10.1016/j.funeco.2016.09.002

Rodríguez-Gálvez E, Hilário S, Lopes A, Alves A (2020) Diversity and pathogenicity of *Lasiodiplodia* and *Neopestalotiopsis* species associated with stem blight and dieback of blueberry plants in Peru. European Journal of Plant Pathology 157(1): 89–102. https://doi.org/10.1007/s10658-020-01983-1

Roux J, Greyling I, Coutinho TA, Verleur M, Wingfeld MJ (2013) The Myrtle rust pathogen, *Puccinia psidii*, discovered in Africa. IMA Fungus 4(1): 155–159. https://doi.org/10.5598/imafungus.2013.04.01.14

Santos J, Hilário S, Pinto G, Alves A (2022) Diversity and pathogenicity of pestalotioid fungi associated with blueberry plants in Portugal, with description of three novel species of *Neopestalotiopsis*. European Journal of Plant Pathology 162(3): 539–555. https://doi.org/10.1007/s10658-021-02419-0

Shahriar SA, Nur-Shakirah AO, Mohd MH (2022) *Neopestalotiopsis clavispora* and *Pseudopestalotiopsis camelliae-sinensis* causing grey blight disease of tea (*Camellia sinensis*) in Malaysia. European Journal of Plant Pathology 162(3): 709–724. https://doi.org/10.1007/s10658-021-02433-2

Shivas RG (1989) Fungal and bacterial diseases of plants in Western Australia. Journal of the Royal Society of Western Australia 72: 1–62.

Silva AC, Diogo E, Henriques J, Ramos AP, Sandoval-Denis M, Crous PW, Bragança H (2020) *Pestalotiopsis pini* sp. nov., an emerging pathogen on stone Pine (*Pinus pinea* L.). Forests 11(8): 805. https://doi.org/10.3390/f11080805

Silvestro D, Michalak I (2012) raxmlGUI: A graphical front-end for RAxML. Organisms Diversity and Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis E (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swofford DL (2002) PAUP* 4.0b10: Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA. https://doi.org/10.1111/j.0014-3820.2002.tb00191.x

Vitale A, Crous PW, Lombar L, Polizzi G (2013) Calonectria diseases on ornamental plants in Europe and the Mediterranean basin: An overview. Journal of Plant Pathology 95: 463–476.

Voglmayr H, Aguirre-Hudson MB, Wagner HG, Tello S, Jaklitsch WM (2019) Lichens or endophytes? The enigmatic genus *Leptosillia* in the Leptosilliaceae fam. nov.(Xylariales), and *Furfurella* gen. nov.(Delonicicolaceae). Persoonia-Molecular Phylogeny and Evolution of Fungi 42(1): 228–60. https://doi.org/10.3767/persoonia.2019.42.09

Voglmayr H, Castlebury LA, Jaklitsch WM (2017) *Juglanconis* gen. nov. on Juglandaceae, and the new family Juglanconidaceae (Diaporthales). Persoonia 38: 136–155. https://doi.org/10.3767/003158517x694768

Voglmayr H, Friebes G, Gardiennet A, Jaklitsch WM (2018) *Barrmaelia* and *Entosordaria* in Barrmaeliaceae (fam. nov., Xylariales) and critical notes on *Anthostomella*-like genera based on multigene phylogenies. Mycological Progress 17: 155–177. https://doi.org/10.1007/s11557-017-1329-6

Voglmayr H, Gardiennet A, Jaklitsch WM (2016) *Asterodiscus* and *Stigmatodiscus*, two new apothecial dothideomycete genera and the new order Stigmatodiscales. Fungal Diversity 80: 271–284. https://doi.org/10.1007/s13225-016-0356-y

Voglmayr H, Jaklitsch WM (2008) Prosthecium species with *Stegonsporium* anamorphs on Acer. Mycological Research 112: 885–905. https://doi.org/10.1016/j.mycres.2008.01.020

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a guide to methods and amplifications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Werle E, Schneider C, Renner M, Völker M, Fiehn W (1994) Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Research 22: 4354–4355. https://doi.org/10.1093/nar/22.20.4354

Wu CG, Tseng HY, Chen ZC (1982) Fungi inhabiting on *Schoenoplectus triqueter* (L.) Palla (I). Taiwania 27: 35–38.

Yanna Ho WH, Hyde KD (2002) Fungal succession on fronds of *Phoenix hanceana* in Hong Kong. Fungal Diversity 10: 185–211.

You LQ, Senanayake IC, Liu JW, Luo M, Huang YH, Dong ZY (2024) *Neopestalotiopsis iberica* induces leaf spots on *Synsepalum dulcificum* in China. Journal of Phytopathology 172(1): e13258. https://doi.org/10.1111/jph.13258

Zheng X, Liu X, Li X, Quan C, Li P, Chang X, Gu J, Khaskheli MI, Gong G (2023) *Pestalotiopsis* species associated with blueberry leaf spots and stem cankers in Sichuan province of China. Plant Disease 107(1): 149–156. https://doi.org/10.1094/PDIS-07-21-1550-RE