



**Citation:** Guarnaccia V., Aiello D., Polizzi G., Crous P.W., Sandoval-Denis M. (2019) Soilborne diseases caused by *Fusarium* and *Neocosmospora* spp. on ornamental plants in Italy. *Phytopathologia Mediterranea* 58(1): 127-137. doi: 10.14601/Phytopathol\_Mediterr-23587

**Accepted:** November 19, 2018

**Published:** May 15, 2019

**Copyright:** © 2019 Guarnaccia V., Aiello D., Polizzi G., Crous P.W., Sandoval-Denis M.. This is an open access, peer-reviewed article published by Firenze University Press (<http://www.fupress.com/pm>) and distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Competing Interests:** The Author(s) declare(s) no conflict of interest.

**Editor:** Lizel Mostert, Faculty of AgriSciences, Stellenbosch, South Africa.

## Research Papers

# Soilborne diseases caused by *Fusarium* and *Neocosmospora* spp. on ornamental plants in Italy

VLADIMIRO GUARNACCIA<sup>1,2</sup>, DALIA AIELLO<sup>3,\*</sup>, GIANCARLO POLIZZI<sup>3</sup>, PEDRO W. CROUS<sup>1,4</sup>, MARCELO SANDOVAL-DENIS<sup>1,4</sup>

<sup>1</sup> Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands

<sup>2</sup> DiSAFA, University of Torino, Largo Paolo Braccini, 2, 10095 Grugliasco, TO, Italy

<sup>3</sup> Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy

<sup>4</sup> Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa

\*Corresponding author: [dalia.aiello@unict.it](mailto:dalia.aiello@unict.it)

**Abstract.** During surveys conducted in 2010–2014, several disease symptoms were observed on eight ornamental plant species in different nurseries located in Sicily (Southern Italy). Two *Neocosmospora* and 31 *Fusarium* isolates were recovered from symptomatic plants. Fungus identification was based on morphological characteristics and phylogenetic analyses of fragments of the intergenic spacer (IGS), internal transcribed spacer (ITS) and large subunit (LSU) regions of the rDNA; partial fragments of the beta-tubulin (*TUB*), RNA polymerase largest subunit (*RPB1*), RNA polymerase second largest subunit (*RPB2*) and translation elongation factor 1-alpha (*EF-1 $\alpha$* ) genes. The *Fusarium* species recovered from ornamental plants were *F. agapanthi* (from *Agapanthus africanus*), *F. anthophilum* (*Dasyilirion longissimum*), *F. fujikuroi* (*Trachycarpus princeps*), *F. oxysporum* (*Bougainvillea glabra*, *Cordyline australis* ‘Purpurea’, *Dasyilirion longissimum*, *Eremophila laanii* and *Philotea myoporoides*) and *F. proliferatum* (*T. princeps*), while *N. solani* was isolated from crown and root rot of *Ficus carica*. The pathogenicity of representative isolates collected from each host was tested on seedlings or cuttings grown in a growth chamber. All the *Fusarium* and *Neocosmospora* isolates tested were pathogenic and reproduced symptoms identical to those observed in the field, except for *F. fujikuroi* on *T. princeps* and *F. oxysporum* on *D. longissimum* that were non-pathogenic.

**Keywords.** Morphology, multigene phylogeny, pathogenicity, root rot, wilt.

## INTRODUCTION

During the last decade, Italy has significantly increased production of ornamental plants in nurseries, and several new species and products have been introduced for cultivation in greenhouses and open fields. Movement

of ornamental plants through the peninsula led to the spread of pathogens to new areas, and introduction of new pathogens from abroad (Gullino and Garibaldi, 2006; Polizzi *et al.*, 2012; Aiello *et al.*, 2017, 2018).

In Sicily (Southern Italy), production of ornamentals has increased in the eastern area, where it replaced lemon orchards due to decline in demand for these fruits. Plant growth in nurseries is compromised by several foliar and root diseases, and among these diseases those caused by species of Nectriaceae are exceptionally common (Polizzi *et al.*, 2007; Vitale *et al.*, 2009; Aiello *et al.*, 2014, 2015; Gullino *et al.*, 2015).

*Fusarium* Link *sensu lato* was recently segregated into several *Fusarium*-like genera (i.e., *Bisifusarium* L. Lombard, Crous & W. Gams [*Fusarium dimerum* species complex (SC)], *Neocosmospora* E.F. Sm. [*Fusarium solani* SC] and *Rectifusarium* L. Lombard, Crous & W. Gams [*Fusarium ventricosum* SC]). These taxa are among the most important human, animal or plant pathogens, affecting an extensive variety of hosts (O'Donnell *et al.*, 2008, 2010; Lombard *et al.*, 2015). *Fusarium* and *Fusarium*-like genera are well-known as responsible for diseases on ornamental plants, including flowering crops, herbaceous ornamentals such as begonia, carnation and chrysanthemum, woody ornamentals such as *Bougainvillea*, *Hebe*, *Hibiscus* and *Pyracantha* spp. (Horst and Nelson, 1997; Sinclair and Lyon, 2005; Polizzi *et al.*, 2010a, 2010b, 2011; Bertoldo *et al.*, 2015; Lupien *et al.*, 2017), and palms such as *Arecastrum*, *Phoenix* and *Washingtonia* spp. (Elliott *et al.*, 2004).

Considering the importance of diseases caused by *Fusarium*-like fungi, the high economic losses caused by these pathogens and the relevance of these crops, surveys were conducted over a 5-year period in ornamental nurseries located in the Catania province, eastern Sicily, Italy. During the surveys conducted from 2010 to 2014, large numbers of palms, perennial herbaceous shrubs,

and young cuttings were detected showing symptoms of crown and root rots, damping-off, wilt and dieback. The aims of the present study were to identify the *Fusaria* obtained from these affected ornamentals, using morphological characteristics and DNA sequence analyses, and to evaluate the pathogenicity of representative isolates on the hosts from which they were isolated.

## MATERIALS AND METHODS

### Field sampling and pathogen isolations

During 2010–2014, surveys were performed in ornamental plant-producing regions located in eastern Sicily (Table 1). The disease incidence (DI) was recorded for each host, based on the number of symptomatic plants in the total of those present in five investigated nurseries. Additionally, approx. 20 plants per species per nursery showing wilt, crown or root rot or damping-off symptoms, were randomly collected for analysis. Fragments (each 5 × 5 mm) of symptomatic tissues were cut from the margins of lesions, surface-sterilised in a sodium hypochlorite solution (10%) for 20 s, followed by 70% ethanol for 30 s, and rinsed three times in sterilised water. Tissue fragments were dried in sterilised filter paper, placed on 2% potato dextrose agar (PDA) amended with 100 µg mL<sup>-1</sup> penicillin and 100 µg mL<sup>-1</sup> streptomycin (PDA-PS), and were incubated at 25°C until characteristic *Fusarium*-like colonies were observed. Pure cultures were obtained by transferring single conidia to fresh PDA, with the aid of a Nikon SMZ1000 dissecting microscope.

### Fungal isolates and morphological characterization

The cultural and micromorphological features of all the isolates included in this study were evaluated fol-

**Table 1.** Hosts, locations, symptoms and incidence (%) of diseases caused by *Fusarium* and *Neocosmospora* in Sicily (Southern Italy).

Hosts	Locations	Geographical coordinates	Collection year	Symptoms	Incidence % <sup>a</sup>
<i>Agapanthus africanus</i>	Carruba, Nursery 1	37.698004, 15.193944	2014	Damping-off	50
<i>Bougainvillea glabra</i>	Carruba, Nursery 1	37.698004, 15.193944	2010	Wilt	30
<i>Cordyline australis</i> 'Purpurea'	Carruba, Nursery 2	37.699090, 15.197300	2014	Wilt	20
<i>Dasyllirion longissimum</i>	Riposto, Nursery 3	37.733699, 15.194320	2014	Wilt	10
<i>Eremophila laanii</i>	Carruba, Nursery 1	37.698004, 15.193944	2010	Wilt	50
<i>Ficus carica</i>	Carruba, Nursery 1	37.698004, 15.193944	2013	Crown and Root rot	50
<i>Phylotoca myoporoides</i>	Milazzo, Nursery 4	38.201964, 15.239530	2013	Wilt	30
<i>Trachycarpus princeps</i>	Grotte, Nursery 5	37.681233, 15.180316	2013	Root rot	40

<sup>a</sup> Number of symptomatic plants on the total of those cultivated.

lowing the procedures of Aoki *et al.* (2003), with some modification as described previously (Sandoval-Denis *et al.*, 2018).

#### DNA extraction, PCR amplification and sequencing

Fungus isolates were grown on PDA for 4-7 d at room temperature, under a natural day/night photoperiod. Total genomic DNA was extracted from fresh mycelium scraped from each colony surface, using the Wizard<sup>®</sup> Genomic DNA purification Kit (Promega Corporation). Fragments of seven nuclear loci, including the translation elongation factor 1-alpha (*EF-1α*), the intergenic spacer region of the rDNA (*IGS*), the internal transcribed spacer region of the rDNA (*ITS*), the large subunit of the rDNA (*LSU*), the RNA polymerase largest subunit (*RPB1*), RNA polymerase second largest subunit (*RPB2*) and beta-tubulin (*TUB*), were PCR amplified as described previously (O'Donnell *et al.*, 2009; 2010; Sandoval-Denis *et al.*, 2018). The PCR products were sequenced using the following primer pairs: EF-1/EF-2 for *EF-1α* (O'Donnell *et al.*, 2008), iNL11/iCNS1 plus the internal sequencing primer pair NLa/CNSa for *IGS* (O'Donnell *et al.*, 2009), ITS4/ITS5 for *ITS* (White *et al.*, 1990), LR0R/LR5 for *LSU* (Vilgalys and Hester, 1990; Vilgalys and Sun, 1994), Fa/G2R for *RPB1* (O'Donnell *et al.*, 2010), 5f2/7cr and 7cf/11ar for *RPB2* (Liu *et al.*, 1999; Sung *et al.*, 2007), and 2Fd/4Rd for *TUB* (Woudenberg *et al.*, 2009). Sequences generated in this study were uploaded to the GenBank and the European Nucleotide Archive (ENA) databases.

#### Phylogenetic analyses and molecular identification

Sequence alignments were performed individually for each locus using MAFFT on the European Bioinformatics Institute (EMBL-EBI) portal (<http://www.ebi.ac.uk/Tools/msa/mafft/>). BLASTn searches on GenBank, and pairwise sequence alignments on the *Fusarium* MLST database of the Westerdijk Fungal Biodiversity Institute (<http://www.westerdijkinstitut.nl/fusarium/>), were performed using *EF-1α* and *RPB2* sequences. This was to assess the distribution of the *Fusaria* isolates among the different *Fusarium* species complexes or *Neocosmospora*. Following this preliminary identification, different loci combinations were selected for each of the *Fusarium* species complexes and *Neocosmospora* isolates, according to the phylogenetic informativeness for each locus as compiled in published literature. These combinations were as follows: *EF-1α*, *ITS*, *RPB1*, *RPB2* and *TUB* for the *F. fujikuroi* species complex (FFSC)

(Edwards *et al.* 2016); *EF-1α* and *IGS* for the *F. oxysporum* species complex (FOSC), and collapsed to haplotypes according to O'Donnell *et al.* (2009); *EF-1α*, *ITS*, *LSU* and *RPB2* for the genus *Neocosmospora* (O'Donnell *et al.*, 2008).

The different gene datasets were analysed independently and combined, using Maximum likelihood (ML) and Bayesian methods (BI) as described previously (Sandoval-Denis *et al.*, 2018).

#### Pathogenicity tests

Pathogenicity tests were performed on potted healthy seedlings or cuttings of all symptomatic species recovered with a subset of 16 representative isolates (Table 2). Each experiment was conducted twice, obtaining similar results in both tests. For each experiment three replicates per isolate were used with 20 to 50 plants per replicate. All plants were inoculated by placing two colonised 1 cm<sup>2</sup> plugs (PDA from 9-d-old mycelium cultures, grown at 25 ± 1°C in the dark) at the base of each plant stem. Uninoculated plants for all the host species served as controls. After inoculation, plants were covered with a plastic bag for 48 h and maintained at 25 ± 1°C and 95% relative humidity (RH) under a 12 h fluorescent light/dark regime until the symptoms were observed. All plants were irrigated two to three times per week, and were examined each week for disease symptoms. Disease incidence (DI) was determined for each host species. Fungi were re-isolated from symptomatic tissues and identified, to fulfil Koch's postulates.

## RESULTS

#### Field sampling and pathogen isolations

Symptoms referable to *Fusarium* spp. were detected on eight ornamental species in five nurseries investigated in Eastern Sicily, Italy (Figure 1). The diseases were observed on seedlings and unrooted and rooted cuttings (1 to 12-month-old) during propagation stages in the greenhouses. Disease incidence varied from 10 to 50%, according to the host species (Table 1). The symptoms observed on ornamental plants consisted of damping-off, crown and root rot, and wilt (Table 1).

Damping-off consisted of root rot and stem decay at soil level, and occurred on young seedlings. Rotted roots were dark brown or black, and were partially or completely destroyed. Crown rot sometimes occurred in association with root rot. As consequence of crown and root rot, basal leaves turned necrotic while infected

**Table 2.** Collection details and sequence accession numbers of isolates included in this study, as well as disease incidence from pathogenicity tests conducted with the isolates.

Species	Culture number <sup>a</sup>	Host	Incidence % <sup>b</sup>	GenBank/ENA accession number <sup>c</sup>						
				ITS	EF-1 $\alpha$	IGS	LSU	RPB1	RPB2	TUB
<i>Fusarium agapanthi</i>	CPC 27740 <sup>#</sup>	<i>Agapanthus africanus</i>	100	LS422776	LS420058		LS422776	LS420106	LS420122	LS420041
	CPC 27741 <sup>#</sup>	<i>Agapanthus africanus</i>	100	LS422777	LS420059		LS422777	LS420107	LS420123	LS420042
<i>Fusarium anthophilum</i>	CPC 27742 <sup>#</sup>	<i>Dasylirion longissimum</i>	75	LS422778	LS420060		LS422778	LS420108	LS420124	LS420043
	CPC 27743	<i>Dasylirion longissimum</i>		LS422779	LS420061		LS422779	LS420109	LS420125	LS420044
<i>Fusarium fujikuroi</i>	CPC 27744	<i>Dasylirion longissimum</i>		LS422780	LS420062		LS422780	LS420110	LS420126	LS420045
<i>Fusarium oxysporum</i>	CPC 27719 <sup>#</sup>	<i>Trachycarpus princeps</i>	0	LS422781	LS420063		LS422781	LS420111	LS420127	LS420046
	CPC 27729 <sup>#</sup>	<i>Philotea myoporoides</i>	100		LS420064	LS420138				
	CPC 27730 <sup>#</sup>	<i>Philotea myoporoides</i>	100		LS420065	LS420139				
	CPC 27731	<i>Philotea myoporoides</i>			LS420066	LS420140				
	CPC 27732	<i>Philotea myoporoides</i>			LS420067	LS420141				
	CPC 27733	<i>Philotea myoporoides</i>			LS420068	LS420142				
	CPC 27734 <sup>#</sup>	<i>Eremophila laanii</i>	100		LS420069	LS420143				
	CPC 27735 <sup>#</sup>	<i>Eremophila laanii</i>	100		LS420070	LS420144				
	CPC 27738 <sup>#</sup>	<i>Bougainvillea glabra</i>	100		LS420071	LS420145				
	CPC 27739 <sup>#</sup>	<i>Bougainvillea glabra</i>	100		LS420072	LS420146				
	CPC 27745 <sup>#</sup>	<i>Dasylirion longissimum</i>	0		LS420073	LS420147				
	CPC 27746 <sup>#</sup>	<i>Cordylone australis</i> 'Purpurea'	100		LS420074	LS420148				
CPC 27747 <sup>#</sup>	<i>Cordylone australis</i> 'Purpurea'	100		LS420075	LS420149					
CPC 27748	<i>Cordylone australis</i> 'Purpurea'			LS420076	LS420150					
CPC 27749	<i>Cordylone australis</i> 'Purpurea'			LS420077	LS420151					
CPC 27750	<i>Cordylone australis</i> 'Purpurea'			LS420078	LS420152					
CPC 27751	<i>Cordylone australis</i> 'Purpurea'			LS420079	LS420153					
<i>Fusarium proliferatum</i>	CPC 27711 <sup>#</sup>	<i>Trachycarpus princeps</i>	100	LS422782	LS420080		LS422782	LS420112	LS420128	LS420047
	CPC 27712	<i>Trachycarpus princeps</i>		LS422783	LS420081		LS422783	LS420113	LS420129	LS420048
	CPC 27713	<i>Trachycarpus princeps</i>		LS422784	LS420082		LS422784	LS420114	LS420130	LS420049
	CPC 27714	<i>Trachycarpus princeps</i>		LS422785	LS420083		LS422785	LS420115	LS420131	LS420050
	CPC 27715	<i>Trachycarpus princeps</i>		LS422786	LS420084		LS422786	LS420116	LS420132	LS420051
	CPC 27716	<i>Trachycarpus princeps</i>		LS422787	LS420085		LS422787	LS420117	LS420133	LS420052
	CPC 27717	<i>Trachycarpus princeps</i>		LS422788	LS420086		LS422788	LS420118	LS420134	LS420053
	CPC 27718	<i>Trachycarpus princeps</i>		LS422789	LS420087		LS422789	LS420119	LS420135	LS420054
	CPC 27720	<i>Trachycarpus princeps</i>		LS422791	LS420089		LS422791	LS420121	LS420137	LS420056
	CPC 27736 <sup>#</sup>	<i>Ficus carica</i>	100	LT991945	LT991907		LT991952		LT991914	
CPC 27737 <sup>#</sup>	<i>Ficus carica</i>	100	LT991946	LT991908		LT991953		LT991915		

<sup>a</sup> CPC: Culture collection of P.W. Crous, housed at Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; Strains used in the pathogenicity tests are indicated with #.

<sup>b</sup> Percentage calculated from the number of symptomatic plants relative to the total number inoculated.

<sup>c</sup> ENA: European Nucleotide Archive; EF-1 $\alpha$ : Translation elongation factor 1-alpha; IGS: Intergenic spacer region of the rDNA; ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; LSU: Partial large subunit of the rDNA; RPB1: RNA polymerase second largest subunit; RPB2: RNA polymerase second largest subunit; TUB: Beta-tubulin.



**Figure 1.** Natural and artificial symptoms referable to *Fusarium* and *Neocosmospora* spp. **a, b.** Wilt of *Bougainvillea glabra* cuttings (a) and *Cordyline australis* seedlings (b). **c.** Root rot with subsequent leaf chlorosis of *Trachycarpus princeps*. **d.** Damping-off of *Agapanthus africanus*. **e.** Wilt of *Phylotea myoporoides*. **f to i.** Vascular discoloration and wilt after *Fusarium oxysporum* inoculation of *Bougainvillea glabra* (f and g), *Eremophila laanii* (h) and *Phylotea myoporoides* (i). **j.** Crown and root rot caused by *Neocosmospora solani* (= *Fusarium solani*) on *Ficus carica* (left) compared with control plants (right).



**Table 3.** Characteristics of the data partitions used for phylogenetic analyses in this study.

Species <sup>a</sup>	Data partition <sup>b</sup>	ML evolutionary model <sup>c</sup>	Number of characters <sup>d</sup>				
			Total	Conserved	Variable	Informative	BI unique sites
<i>F. fujikuroi</i> SC	ITS	SYM+I	459	420	39	32	50
	<i>RPB1</i>	SYM+I+G	1279	1038	241	148	195
	<i>RPB2</i>	GTR+I+G	1570	1251	319	216	332
	<i>EF-1<math>\alpha</math></i>	SYM+G	455	317	133	76	148
	<i>TUB</i>	SYM+I+G	507	389	117	65	140
<i>F. oxysporum</i> SC	<i>EF-1<math>\alpha</math></i>	GTR+I+G	591	448	143	101	67
	IGS	GTR+I+G	2190	1420	744	554	218
<i>Neocosmospora</i>	ITS	GTR+I+G	496	404	90	65	117
	LSU	GTR+I+G	481	450	31	14	25
	<i>RPB2</i>	GTR+I+G	1603	1235	365	243	275
	<i>EF-1<math>\alpha</math></i>	GTR+G	324	222	97	48	107

<sup>a</sup> SC: species complex.

<sup>b</sup>*EF-1 $\alpha$* : Translation elongation factor 1-alpha. IGS: Intergenic spacer region of the rDNA. ITS: Internal transcribed spacer regions of the rDNA and 5.8S region. *RPB1*: RNA polymerase largest subunit. *RPB2*: RNA polymerase second largest subunit. *TUB*: Beta-tubulin.

<sup>c</sup> G: Gamma distributed rate variation among sites. GTR: Generalised time-reversible. I: Proportion of invariable sites. ML: Maximum-likelihood; SYM: Symmetrical model.

<sup>d</sup> BI: Bayesian inference.

plants sometimes wilted and died. Wilted plants had conspicuous vascular brown discolourations from the crown to the canopy.

A total of 33 monosporic *Fusarium*-like isolates were collected (Table 2). Among these, two isolates were obtained from damping-off, 12 from root rot, and 19 were from wilted plants.

#### Phylogenetic analyses and species identification

Pairwise sequence alignments on the *Fusarium* MLST database and GenBank BLASTn searches demonstrated that 16 isolates belonged to the FOSC and 15 isolates to the FFSC, while two isolates were assigned to the genus *Neocosmospora* (*F. solani* species complex) (Table 2).

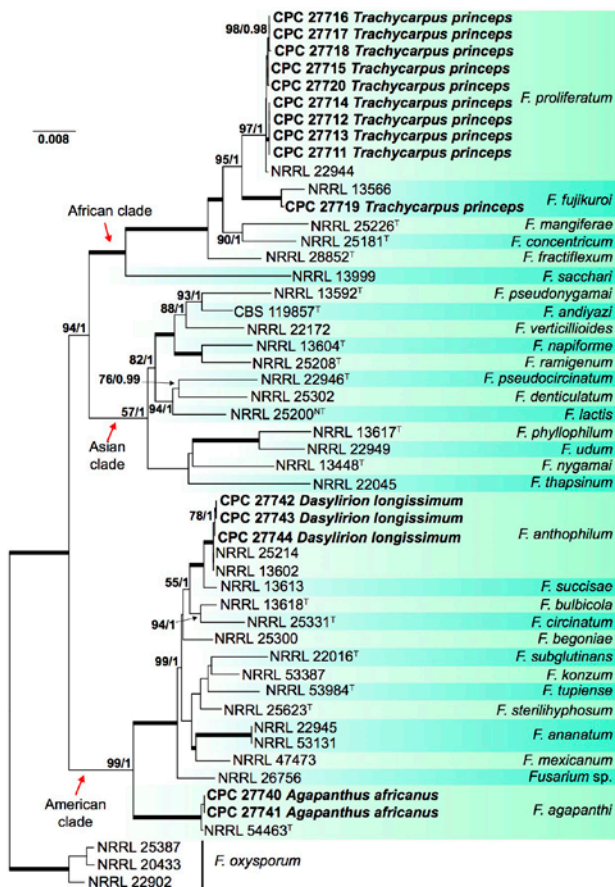
Subsequent more inclusive multilocus phylogenetic analyses identified a total of five *Fusarium* spp. and one *Neocosmospora* sp. The alignment characteristics and statistics are summarized in Table 3. The phylogenetic analyses of the 15 FFSC isolates from ornamentals revealed a total of four species (*F. agapanthi* O'Donnell, T. Aoki, J. Edwards & Summerell, *F. anthophilum* (A. Braun) Wollenw., *F. fujikuroi* Nirenberg and *F. proliferatum*) from different hosts (Figure 2). Isolates belonging to FOSC were studied based on a two-gene analysis using *EF-1 $\alpha$*  and IGS sequences and incorporated in the original alignments previously published by O'Donnell *et al.* (2009) including representatives of 257 known FOSC haplotypes. The FOSC isolates from ornamentals

belonged to 15 different haplotypes: isolates CPC 27748 and 22749, from *Cordyline australis* 'Purpurea' showed identical DNA sequences, and corresponded to haplotype 122 of FOSC; isolate CPC 27733 from *Philotoca myoporoides* belonged to haplotype 188, while each of the remaining isolates corresponded to a previously undescribed haplotype (Figure 3). The phylogeny of the genus *Neocosmospora* was based on *EF-1 $\alpha$* , ITS, LSU and *RPB2* sequences, and showed that two isolates from *Ficus carica* (CPC 27736 and 27737) belonged to *N. solani* (Martius) L. Lombard & Crous (= *F. solani*) (Figure 4).

#### Pathogenicity tests

Fourteen *Fusarium* and two *Neocosmospora* isolates tested were pathogenic to the different inoculated original hosts, and produced symptoms similar to those observed on diseased plants in nurseries (Figure 1). Two isolates were non-pathogenic. Damping-off occurred on *Agapanthus africanus*, crown and root rot on *F. carica* and root rot with subsequent leaf chlorosis appeared on *Trachycarpus princeps*. The remaining host plants showed vascular discolouration and wilted. The DI (%) caused by *Fusarium* and *Neocosmospora* species on different hosts ranged from 75 to 100%, after 15 d to 3 months (Table 2).

All *F. agapanthi*, *F. proliferatum*, and *N. solani* isolates were pathogenic, and caused 100% DI on *A. africanus*, *T. princeps* and *F. carica*, whereas *F. anthophilum*

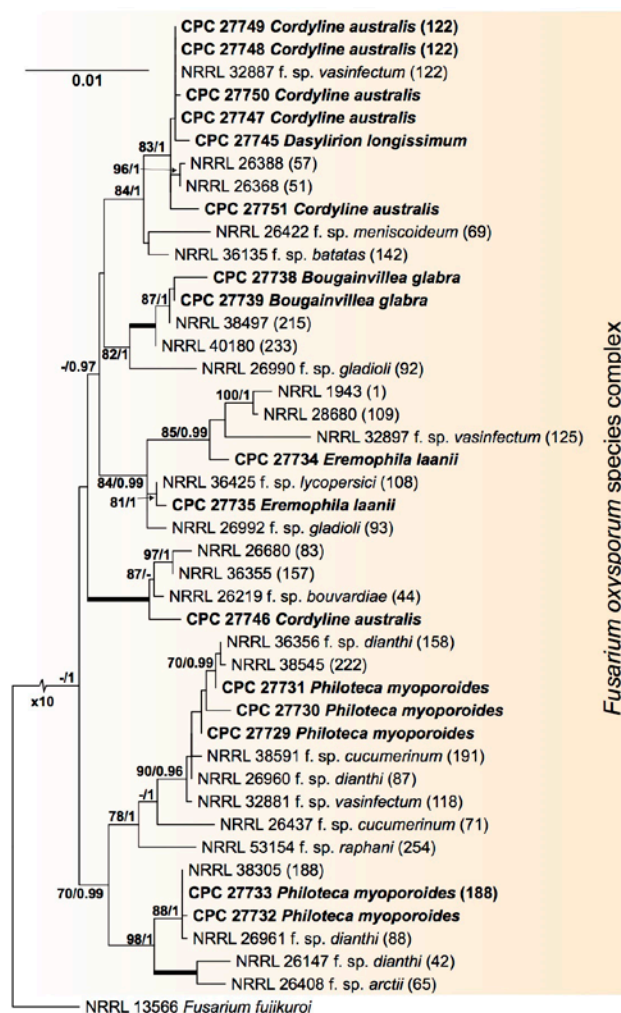


**Figure 2.** Maximum-likelihood (ML) phylogram of the *Fusarium fujikuroi* species complex obtained from combined ITS, *RPB1*, *RPB2*, *EF-1α* and *TUB* sequences. Branch lengths are proportional to distance. Numbers on the nodes are ML bootstrap values greater than 55%; and Bayesian posterior probability values greater than 0.95. Full supported branches and isolates obtained from ornamentals plants are indicated in bold. Ex-type strains are indicated with <sup>T</sup> and ex-neotype strains are indicated with <sup>NT</sup>.

caused disease with lower DI on *Dasylium longissimum* (75%). *Fusarium oxysporum* isolates gave high DI (100%) on *Bougainvillea glabra*, *C. australis* ‘Purpurea’, *Eremophila laanii* and *P. myoporoides*, but was non-pathogenic on *D. longissimum*. Similarly, *F. fujikuroi* caused no symptoms on the original host *T. princeps*. The pathogens were re-isolated from the artificially inoculated plants, and were identified as previously described, fulfilling Koch’s postulates. No symptoms were observed on control (uninoculated) plants.

DISCUSSION

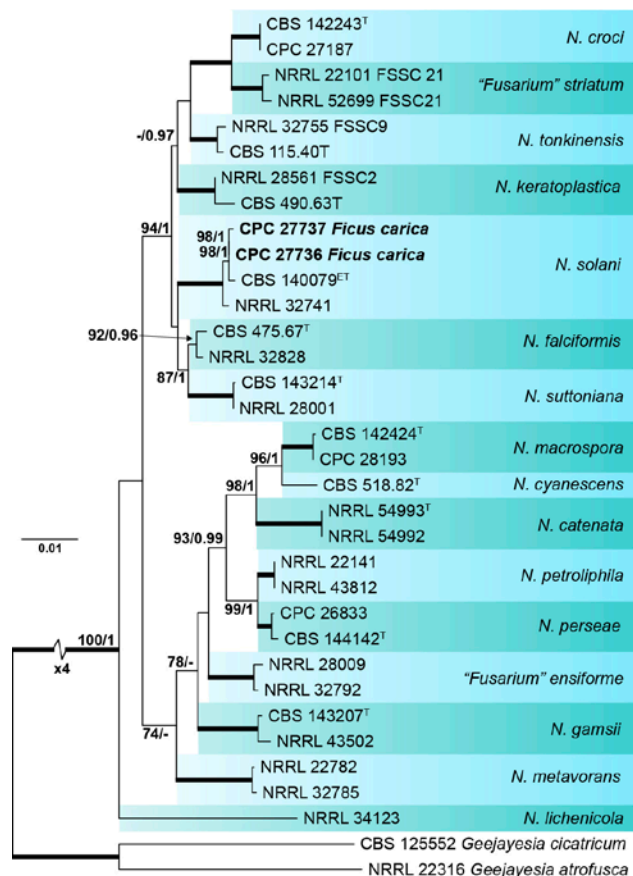
The most important plant pathogenic *Fusarium* species is the soil-borne *F. oxysporum* Schldtl. (Gordon and



**Figure 3.** Maximum-likelihood (ML) phylogram of the *Fusarium oxysporum* species complex obtained from combined *EF-1α* and IGS sequences of isolates obtained in this study and representatives of the most closely related haplotypes. Branch lengths are proportional to distance. Numbers on the nodes are ML bootstrap values greater than 55%; and Bayesian posterior probability values greater than 0.95. Full supported branches and isolates obtained from ornamentals plants are indicated in bold. Numbers between parentheses indicate the corresponding haplotype.

Martyn, 1997; Gullino *et al.*, 2012), currently encompassing nearly 150 *formae speciales* (ff. spp.) and races. The broad host plant range of this fungus includes valuable ornamental plants such as *Chrysanthemum*, *Dianthus*, *Gerbera*, *Gladiolus*, and *Lilium* spp. (Engelhard and Woltz, 1971; Linderman, 1981; Farr and Rossman, 2018), on which it causes symptoms ranging from vascular wilt to crown and root rot (Engelhard and Woltz, 1971; Linderman, 1981).

*Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg is another important species, which has



**Figure 4.** Maximum-likelihood (ML) phylogram of *Neocosmospora* (= *Fusarium solani* species complex) obtained from combined *EF-1 $\alpha$* , ITS, LSU and *RPB2* sequences. Branch lengths are proportional to distance. Numbers on the nodes are ML bootstrap values greater than 55%; and Bayesian posterior probability values greater than 0.95. Full supported branches and isolates obtained from ornamentals plants are indicated in bold. Ex-type and ex-epitype strains are indicated with <sup>T</sup> and <sup>ET</sup> respectively.

been described as the causal agent of blight, dieback and wilt of several palms belonging to the genera *Chamaerops*, *Phoenix*, *Ravenea*, *Trachycarpus* and *Washingtonia* (Polizzi and Vitale, 2003; Armengol *et al.*, 2005).

In the present study, two *Neocosmospora* and 31 *Fusarium* isolates were recovered from eight ornamental species in Sicily over a 5-year period. Disease symptoms were observed in five ornamental nurseries, and included damping-off, crown and/or root rot, and wilt. The isolates obtained from symptomatic tissues were identified based on single and multilocus phylogenetic analyses of seven loci (*EF-1 $\alpha$* , IGS, ITS, LSU, *RPB1*, *RPB2*, and *TUB*), as well as morphological characters. Our study revealed considerable diversity in the composition of *Fusarium*-like fungal populations recovered from nurseries.

As confirmed in the pathogenicity tests, all the *F. oxysporum* isolates caused symptoms except on *D. longissimum*. However, this host showed disease symptoms when inoculated with *F. anthophilum*. The inoculated isolate of *F. fujikuroi* produced no symptoms on *T. princeps*, while the remaining *Fusarium* species investigated, *F. agapanthi*, *F. proliferatum* and *N. solani*, were pathogenic to the respective tested hosts from which they were isolated.

*Fusarium* and *Neocosmospora* species are widespread in nurseries in Italy (Polizzi *et al.*, 2003; 2010a; 2010b; 2011; Bertoldo *et al.*, 2015), where they represent a limiting factor for production of ornamental plants cultivated in Sicily. These pathogenic species have very broad host ranges worldwide (Farr and Rossman, 2018). However, there are no known reports of diseases caused by *F. anthophilum* on *D. longissimum*. Moreover, *F. agapanthi* was originally described as pathogenic on *Agapanthus praecox* in Australia and Italy (Edwards *et al.*, 2016). However, this pathogen was isolated in the present study, causing serious seedling damping-off of *A. africanus*, suggesting that it may also be more prevalent on other species of *Agapanthus*. Previous studies have reported *F. proliferatum* associated with palms belonging to the genera *Chamaerops*, *Phoenix*, *Trachycarpus* and *Washingtonia* (Polizzi and Vitale, 2003; Armengol *et al.*, 2005). Our study presents a new report for *F. proliferatum* as a pathogen of *T. princeps*.

The FOSC includes soil-borne pathogens responsible for vascular wilts, stem cankers, rots, and damping-off of a wide range of agronomical and horticulturally important crops (Baayen *et al.*, 2000; Michielse and Rep, 2009; O'Donnell, 2009). Members of this complex collectively represent the most commonly found and economically important species complex within *Fusarium*. *Fusarium oxysporum* was the predominant species found in all the nurseries sampled, and unlike other species, it was recovered from multiple hosts. Recently, Polizzi *et al.* (2010a; 2010b; 2011) identified *F. oxysporum* associated with wilt diseases of *B. glabra*, *E. laanii* and *P. myoporoides*. However, no reports were previously known of diseases caused by *F. oxysporum* on *C. australis*, as reported here.

The present study is also the first report of *N. solani* causing crown and root rot of *F. carica* cuttings. This plant species is often cultivated for fruit production, and thousands of cuttings cultivated for ornamental purposes were investigated because serious losses were observed from crown and root rot, leading to plant death. *Neocosmospora* is a species-rich genus containing at least 60 phylogenetically distinct species (O'Donnell, 2000; Zhang *et al.*, 2006; O'Donnell *et al.*, 2008; Nalim *et al.*,



2011). These fungi generally cause crown and/or root rot of infected host plants, while symptoms on above-ground plant portions may manifest as cankers, wilting, stunting and chlorosis, or as lesions on stems and/or leaves (Coleman, 2016; Guarnaccia *et al.*, 2018).

The high disease incidence observed in the investigated ornamental nurseries probably depends on the prevailing climatic conditions, farming practices and environmental conditions such as temperature, humidity, irrigation systems or the use of non-disinfected plant growth substrates. Potted plant production could promote infections, since plants are frequently stressed due to being containerised during the production processes. Moreover, several wounds can occur during transplanting. Thus, prevention is a major strategy to control *Fusarium* diseases, and an accurate diagnosis of *Fusaria* species occurring in a particular area is significant for the selection of effective disease management strategies.

This study provides the first overview of *Fusarium* and *Neocosmospora* diversity associated with diseased ornamental plants in Southern Italy, and includes information on the pathogenicity of these fungi. It also provides the first reports of several new pathogen/host combinations, such as *N. solani* associated with crown and root rot of *F. carica*, and *F. agapanthi*, *F. anthophilum*, *F. oxysporum* and *F. proliferatum* as pathogens, respectively, of *A. africanus*, *D. longissimum*, *C. australis* and *T. princeps*.

#### ACKNOWLEDGEMENTS

The authors thank Dr Pietro Formica for support with specimen collection and fungal isolations. This research was funded by Research Project 2016-2018 “Emergent Pests and Pathogens and Relative Sustainable Management Strategies”, financed by University of Catania.

#### LITERATURE CITED

- Aiello D., Guarnaccia V., Vitale A., Cirvilleri G., Granta G., ... Crous P.W., 2014. *Ilyonectria palmarum* sp. nov. causing dry basal stem rot of Areaceae. *European Journal of Plant Pathology* 138: 347–359.
- Aiello D., Guarnaccia V., Epifani F., Perrone G., Polizzi G., 2015. ‘*Cylindrocarpon*’ and *Ilyonectria* species causing root and crown rot disease of potted laurustinus plants in Italy. *Journal of Phytopathology* 163: 675–680.
- Aiello D., Polizzi G., Crous P.W., Lombard L., 2017. *Pleio-carpon* gen. nov. and a new species of *Ilyonectria* causing basal rot of *Strelitzia reginae* in Italy. *IMA Fungus* 8: 65–76.
- Aiello D., Hansen Z.R., Smart C.D., Polizzi G., Guarnaccia V., 2018. Characterisation and mefenoxam sensitivity of *Phytophthora* spp. from ornamental plants in Italian nurseries. *Phytopathologia Mediterranea* 57: 245–256.
- Aoki T., O’Donnell K., Homma Y., Lattanzi A.R., 2003. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex - *F. virguliforme* in North America and *F. tucumaniae* in South America. *Mycologia* 95: 660–684.
- Armengol J., Moretti A., Perrone G., Vicent A., Bengoechea J.A., García-Jiménez J., 2005. Identification, incidence and characterization of *Fusarium proliferatum* on ornamental palms in Spain. *European Journal of Plant Pathology* 112: 123–131.
- Baayen R.P., O’Donnell K., Bonants P.J.M., Cigelnik E., Kroon L.P.N.M., ... Waalwijk C., 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic *formae speciales* causing wilt and rot diseases. *Phytopathology* 90: 891–900.
- Bertoldo C., Gilardi G., Spadaro D., Gullino M.L., Garibaldi A., 2015. Genetic diversity and virulence of Italian strains of *Fusarium oxysporum* isolated from *Eustoma grandiflorum*. *European Journal of Plant Pathology* 141: 83–97.
- Coleman J.J., 2016. The *Fusarium solani* species complex: ubiquitous pathogens of agricultural importance. *Molecular Plant Pathology* 17: 146–158.
- Edwards J., Auer D., de Alwis S.K., Summerell B., Aoki T., ... O’Donnell K., 2016. *Fusarium agapanthi* sp. nov., a novel bikaverin and fusarubin-producing leaf and stem spot pathogen of *Agapanthus praecox* (African lily) from Australia and Italy. *Mycologia* 108: 981–992.
- Elliott M.L., Broschat T.K., Uchida J.Y., Simone G.W., 2004. Compendium of ornamental palm diseases and disorders. St. Paul, MN, APS Press.
- Engelhard A.W., Woltz S.S., 1971. *Fusarium* wilt of *chrysanthemum*: symptomatology and cultivar reactions. In: *Proceedings of the Florida State Horticultural Society*, 84: 351–354.
- Farr D.F., Rossman A.Y., 2018. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved February 05, 2018, from <https://nt.ars-grin.gov/fungal-databases>.
- Gordon T.R., Martyn R.D., 1997. The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology* 35: 111–128.

- Guarnaccia V., Sandoval-Denis M., Aiello D., Polizzi G., Crous P.W., 2018. *Neocosmospora perseae* sp. nov., causing trunk cankers on avocado in Italy. *Fungal Systematics and Evolution* 1: 131–140.
- Gullino M.L., Garibaldi A., 2006. Evolution of fungal diseases of ornamental plants and main implications for their management. In: *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (J.A. Teixeira da Silva, ed.), Global Science Books, London, UK, 464–471.
- Gullino M.L., Katan J., Garibaldi A., 2012. The genus *Fusarium* and the species that affect greenhouse vegetables and ornamentals. In: *Fusarium wilts of greenhouse vegetable and ornamental crops* (M.L. Gullino, J. Katan, A. Garibaldi, ed.), APS Press, St. Paul, MN, USA, 5–9.
- Gullino M.L., Daughtrey M.L., Garibaldi A., Elmer W.H., 2015. Fusarium wilts of ornamental crops and their management. *Crop Protection* 73: 50–59.
- Horst R.K., Nelson P.E., 1997. Compendium of chrysanthemum diseases. St. Paul, MN, APS Press.
- Linderman R.G., 1981. Fusarium disease of flowering bulb crops. In: *Fusarium: Disease, Biology, and Taxonomy* (P.E. Nelson, T.A. Toussoun, R.J. Cook, ed.), The Pennsylvania State University Press, University Park, PA, USA, 129–142.
- Liu Y.J., Whelen S., Hall B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. *Molecular Biology and Evolution* 16: 1799–1808.
- Lombard L., Van der Merwe N.A., Groenewald J.Z., Crous P.W., 2015. Generic concepts in Nectriaceae. *Studies in Mycology* 80: 189–245.
- Lupien S.L., Dugan F.M., Ward K.M., O'Donnell K., 2017. Wilt, crown, and root rot of common rose mallow (*Hibiscus moscheutos*) caused by a novel *Fusarium* sp. *Plant Disease* 101: 354–358.
- Michielse C.B., Rep M., 2009. Pathogen profile update: *Fusarium oxysporum*. *Molecular Plant Pathology* 10: 311–324.
- Nalim F.A., Samuels G.J., Wijesundera R.L., Geiser D.M., 2011. New species from the *Fusarium solani* species complex derived from perithecia and soil in the Old World tropics. *Mycologia* 103: 1302–1330.
- O'Donnell K., 2000. Molecular phylogeny of the *Nectria haematococca*–*Fusarium solani* species complex. *Mycologia* 92: 919–938.
- O'Donnell K., Sutton D.A., Fothergill A., McCarthy D., Rinaldi M.G., ... Geiser D.M., 2008. Molecular phylogenetic diversity, multilocus haplotype nomenclature, and *in vitro* antifungal resistance within the *Fusarium solani* species complex. *Journal of Clinical Microbiology* 46: 2477–2490.
- O'Donnell K., Gueidan C., Sink S., Johnston P.R., Crous P.W., ... Sarver B.A., 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology* 46: 936–948.
- O'Donnell K., Sutton D.A., Rinaldi M.G., Sarver B.A., Balajee S.A., ... Geiser D.M., 2010. Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. *Journal of Clinical Microbiology* 48: 3708–3718.
- Polizzi G., Vitale A., 2003. First report of Fusarium blight on majesty palm caused by *Fusarium proliferatum* in Italy. *Plant Disease* 87: 1149.
- Polizzi G., Vitale A., Aiello D., Dimartino M.A., Parlavecchio G., 2007. First report of damping-off and leaf spot caused by *Cylindrocladium scoparium* on different accessions of bottlebrushes cuttings in Italy. *Plant Disease* 91: 769.
- Polizzi G., Aiello D., Guarnaccia V., Vitale A., Perrone G., Epifani F., 2010a. First report of Fusarium wilt on *Eremophila* spp. caused by *Fusarium oxysporum* in Italy. *Plant Disease* 94: 1509.
- Polizzi G., Aiello D., Guarnaccia V., Vitale A., Perrone G., Stea G., 2010b. First report of Fusarium wilt of paper flower (*Bougainvillea glabra*) caused by *Fusarium oxysporum* in Italy. *Plant Disease* 94: 483.
- Polizzi G., Aiello D., Guarnaccia V., Vitale A., Perrone G., Stea G., 2011. First report of Fusarium wilt on *Philotheca myoporoides* caused by *Fusarium oxysporum* in Italy. *Plant Disease* 95: 877.
- Polizzi G., Vitale A., Aiello D., Guarnaccia V., Crous P.W., Lombard L., 2012. First report of *Calonectria ilicicola* causing a new disease on Laurus (*Laurus nobilis*) in Europe. *Journal of Phytopathology* 160: 41–44.
- Sandoval-Denis M., Guarnaccia V., Polizzi G., Crous P.W., 2018. Symptomatic citrus trees reveal a new pathogenic lineage in *Fusarium* and two new *Neocosmospora* species. *Persoonia* 40: 1–25.
- Sinclair W.A., Lyon H.H., 2005. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, NY, p. 660.
- Sung G.H., Sung J.M., Hywel-Jones N.L., Spatafora J.W., 2007. A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. *Molecular Phylogenetics and Evolution* 44: 1204–1223.
- Vilgalys R., Hester M., 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology* 172: 4238–4246.
- Vilgalys R., Sun B.L., 1994. Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleuro-*

- rotus revealed by phylogenetic analysis of ribosomal DNA sequences. In: *Proceedings of the National Academy of Sciences of the United States of America* 91: 4599–4603.
- Vitale A., Aiello D., Castello I., Dimartino M.A., Parlavecchio G., Polizzi G., 2009. Severe outbreak of crown rot and root rot caused by *Cylindrocladium pauciramosum* on strawberry tree in Italy. *Plant Disease* 93: 842.
- White T.J., Bruns T., Lee S., Taylor J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: a guide to methods and applications* (M.A. Innes, D.H. Gelfand, J.J. Sninsky, T.J. White, ed.), USA: Academic Press, NY, 315–322.
- Woudenberg J.H.C., Aveskamp M.M., de Gruyter J., Spiers A.G., Crous P.W., 2009. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* 22: 56–62.
- Zhang N., O'Donnell K., Sutton D.A., Nalim F.A., Summerbell R.C., ... Geiser D.M., 2006. Members of the *Fusarium solani* species complex that cause infections in humans and plants are common in the environment. *Journal of Clinical Microbiology* 44: 2186–2190.