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ORIGINAL PAPER

Fusarium oxysporum f. sp. *passiflorae* infecting passionfruit in New Zealand in a changing taxonomic landscape

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Abstract
Fusarium oxysporum f. sp. *passiflorae* (FOP) is reported for the first time in Northland, New Zealand. The identity of this host-specific pathogen was confirmed by pathogenicity testing, morphological characters, and DNA sequencing. Pathogenic strains of *Fusarium oxysporum* secrete unique proteins or effectors, 'secreted in xylem' (*SIX*), which are likely to contribute to host-specific virulence. Sequence analysis of the EF-1a gene, β-tubulin and the effector genes *SIX6* and *SIX9* confirmed that New Zealand isolates belong to FOP. This study confirmed that the three New Zealand EF-1α haplotypes of FOP had identical *SIX6* and *SIX9* sequences, indicating that the same homolog of each gene, *SIX6a* and *SIX9a*, is shared by both haplotypes of FOP. *SIX* genes are rarely detected in non-pathogenic strains of *Fusarium oxysporum* species complex (FOSC) and pathogenicity tests are necessary to confirm its pathogenicity status.

Keyword Diagnostics · Fusarium wilt · *Passiflora edulis* f. *edulis* · *SIX* genes

Introduction

Fusarium oxysporum f. sp. *passiflorae* (FOP), a pathogen belonging to the *Fusarium oxysporum* species complex (FOSC), causes Fusarium wilt disease on *Passiflora edulis* (passionfruit) (Gordon 1965), *P. mollissima* (banana poka) and other *Passiflora* spp. (Gardner 1989). The disease has been reported from Australia (Gordon 1965), Brazil (Fisher and Rezende 2008), Hawaii (Gardner 1989), Malaysia, Panama, South Africa and Venezuela (Fisher and Rezende 2008).

Brazil is the highest passionfruit producer and consumer in the world. In Brazil, FOP is considered a significant disease on passionfruit, responsible for severe economic losses and increased impacts on growers. FOP hyphae enter the root system and on reaching xylem vessels block the transport of water and essential nutrients to parts of the plant. The main symptom associated with FOP is wilting. This can occur at any growth stage of the plant, in any season. Like many species in FOSC, FOP is a soil-borne pathogen that produces chlamydospores. These structures can remain viable for long periods and are very difficult to eliminate once the soil is infected (Silva et al. 2013). In Australia, the initial symptom of this disease on passionfruit vines was wilting, starting from the tip of the stems, and progressing to severe wilting and death within a couple of weeks. In some cases, partial wilt can occur before the complete wilting of passionfruit vines (Liberto and Laranjeira 2005). In North America, infected passionfruit vines exhibit internal dark discolouration of roots and lower stems with severely stunted growth and wilted external appearance (Rooney-Latham et al. 2011).

A number of *Fusarium* species have been associated with passionfruit crown canker disease in New Zealand, the most prevalent being *Fusarium redolens*. There is no known control for crown canker (NZ Passionfruit Growers Association website accessed on 30/10/2020, https://www.passionfruit.org.nz/facts-info/growing-info/diseases). *Fusarium avenaceum, F. equiseti, F. fujikuroi, F. gibbosum, F.*

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lateritium, F. longisporum, F. reticulatum, F. roseum, F. tricinctum, F. tumidum, F. stilboides and *Neocosmospora solani* have been recorded from passionfruit plants in New Zealand (New Zealand Fungi and Bacteria (NZFUNGI 2020), Landcare Research, https://nzfungi.landcareresearch.co.nz site accessed 30/10/2020).

The taxonomy of *Fusarium oxysporum* (Fo) has been considered as one of the most controversial areas within the *Fusarium* genus (Summerell 2019). Phylogenetic inference concluded that there are morphologically different cryptic species within Fo (Laurence et al. 2014). Genetic diversity in FOSC strains is likely due to the horizontal gene transfer and constant outcrossing with other strains in the natural populations (Gordon 2017). Describing and naming of these cryptic taxa is challenging due to the confused multiple sub-species rank in the Fo classification and lack of reference material of previously described species (Lombard et al. 2019). To stabilise the Fo taxonomy, Lombard et al. (2019) epitypified the description for Fo, designating it as a species and recognising twenty-one cryptic phylogenetic species in this species complex, of which fifteen have been formally described as species.

Traditionally Fo has been identified based on the asexual stage only. Recognising species boundaries for Fo due to the absence of the sexual stage and paucity of the asexual morphological features are challenging. The morphological identification of *Fusarium* species is based on several key characters such as colony colour, growth rate, the density of mycelia, types of conidiophores, conidiogenous cells, macroconidia, microconidia, pigment production and formation of chlamydospores (Leslie and Summerell 2006). The colony colour of these species generally varies from white, pink, purple and violet, expressed in response to the unique nutrients a media contains (Teixeira et al. 2017). However, all these key morphological characters are not stable, and variations are observed dependent on media under different environmental conditions (Nelson 1991). Therefore, identification of Fo and special forms based on morphological characters are not reliable for biosecurity decisions and various resistance breeding programmes.

The concept was initiated by Snyder and Hansen (1940) based on host specificity of the pathogenic strains of Fo. The FOSC includes plant pathogenic and non-pathogenic strains. Fo can be distinguished morphologically from other species of *Fusarium* by trained personnel; however, isolates of Fo, whether pathogenic or not, cannot be distinguished from each other. Special forms of Fo are morphologically indistinguishable from non-pathogenic strains and closely related to other Fo special form isolates (Sharma et al. 2018). Plant pathogenic strains are characterised by their ability to infect a specific plant host (Bogale et al. 2007) and are thus known as *formae speciales* (ff. spp., plural; *forma specialis*, f. sp., singular). For example, *Fusarium oxysporum* f. sp. *passiflorae* only infects passionfruit plants. Given that intraspecific groups cannot be identified easily, pathogenicity testing continues to be a fundamental requirement for identification of Fo to *formae speciales* (Recorbet et al. 2003). In addition, many *formae speciales* of Fo are further divided into vegetative compatibility groups (VCGs) based on the capability of isolates to form stable heterokaryons, and/or races which are based on host differential pathogenicity testing (Gordon and Martyn 1997).

Special forms or *formae speciales* of Fo are informal sub-species rank, but they are not under the International Code of Nomenclature (ICN) of algae, fungi, and plants (McNeill et al. 2012). There is no formal requirement for describing sub-species level, and submission of reference material to an internationally recognised repository is required. There are no standard rules for describing new *formae speciales* and the author can choose any name freely. There are confusion and multiple representations of the same strains due to the lack of well-defined nomenclatures. For examples, *forma specialis matthioli* also described as *mathioli* or *matthiolae*. (Hermann and Lecomte 2019). Therefore, Hermann and Lecomte (2019) proposed a minimum requirement for naming a new *forma specialis* or race to avoid the confusion. Up until February 2018, 144 special forms of Fo had been recorded. The availability of living ex-type cultures is limited, which are essentially the reference point for phylogenetic reference (Lombard et al. 2019).

The initial concept of host specificity for *forma specialis* was restricted to a single host plant, but some exception to this rule has been found over time (Hermann and Lecomte 2019). *Fusarium oxysporum* f. sp. *cucumerinum* infected both melon and cucumber (Cafri et al. 2005). Several *formae speciales* have been reported to have a broader host range. Some of the *formae speciales* are pathogenic to several species within the genus, or several genera within the family, or a variety of plants from different families (Hermann and Lecomte 2019). Pathogenic strains of Fo are responsible for causing two different symptoms, such as wilting and rotting. The wilt causing *formae speciales* strains penetrate roots first, travelling towards the vascular systems, resulting in defoliation, and wilting (Olivain and Alabouvette 1999; O'Donnell et al. 1998). In contrast to the wilting strains, the rotting pathogenic strains are not reaching the vascular systems but restricted in the roots and hypocotyl tissues (Jarvis and Shoemaker 1978; Koyyappurath et al. 2016). For example, two different *formae speciales* infect tomato plants: the symptoms responsible for rot causing strains are *formae speciales radicis-lycopersici* and wilt causing strains are *formae speciales lycopersici* (Hermann and Lecomte 2019).

Over the last three decades, molecular characterisation of Fo has enabled a greater understanding of the genetic variability within *formae speciales* of Fo and provided sequence variability information which can be used for molecular

diagnostics. The translation elongation factor—1α (EF-1α) is a favourable sequencing target for Fo as it is rich in polymorphic characters and able to resolve intraspecific phylogenetic relationships in the FOSC (O'Donnell et al. 2009). Two intraspecific haplotypes of FOP have been identified previously, although one haplotype was represented by only one isolate (O'Donnell et al. 2009). It is desirable to employ EF-1α gene as a routine sequence target for identification of *Fusarium* and FOSC species followed by one of RPB1 and RPB2 (RNA polymerase II subunit I and RNA polymerase II subunit II) to confirm that identification. Both data sets may provide a reliable diagnostic outcome in many cases (Summerell 2019); however, multi-locus sequencing is not always definitive. For example, the sequencing data must be compared with reference sequences (if available) in the accessible data bank (Summerell 2019). When sequences do not match sequences in the data bank, further analysis is required, such as construction of a comprehensive phylogenetic tree to identify where the isolate resides in relation to other *formae speciales* of Fo. Some *formae speciales* of Fo have polyphyletic origins that is, strains belonging to one group of *formae speciales* may be more genetically related to strains within other *formae speciales*, than with strains within the same *formae speciales* (Lievens et al. 2009a, b; Pinaria et al. 2015). Therefore, conservative gene sequences such as EF-1α, RPB1/RPB2 should be used cautiously for routine identifications and diagnostics.

More recently, fungal effector genes continue to attract significant attention as their role in plant pathogenicity is unravelled. Secreted in xylem (*SIX*) genes are a family of effectors identified initially from isolates of *F. oxysporum* f. sp. *lycopersici*, and subsequently from many more *formae speciales* of Fo (Ma et al. 2010; Czislowski et al. 2018). Currently, 14 *SIX* genes have been reported, and these are generally clustered on lineage- specific (LS) chromosomes, or pathogenicity 'hot spots', outside the Fo core genome and the comparative genomic analysis confirmed that horizontal gene transfer could move the pathogenicity related chromosomes between non-pathogenic and pathogenic strains (Rep et al. 2004; Rep and Kistler 2010).

Pathogenicity genes reside on lineage-specific chromosomes (LS) in both Fo and other pathogenic fungi (Croll and McDonald 2012; Raffaele and Kamoun 2012) and this has been demonstrated in *Fusarium oxysporum* f. sp. *lycopersici* (FOL) that causes tomato wilt disease (Ma et al. 2010).

The distribution and nucleotide sequence of *SIX* genes is variable. For example, in the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense*, not all the known *SIX* genes were found in all the strains; and some *SIX* genes had multiple homologues with variable sequences (Czislowski et al. 2018). The authors identified the *SIX6a* homologue in *F. oxysporum* f. sp. *cubense, lycopersici, medicaginis, melonis, niveum,* and *passiflorae*; and the *SIX9a* homologue in f. sp. *cubense, lycopersici, niveum* and *passiflorae*, but not *medicaginis* and *melonis*. Thus, plant pathogenic strains of *F. oxysporum* may carry zero, one or multiple homologues of each known *SIX* genes.

SIX genes are rarely detected in non-pathogenic Fo from natural ecosystems (Rocha et al. 2016); therefore, their diagnostic utility is two-fold. Variability in the sequence and number of homologues provides favourable molecular diagnostic targets, and pathogenic strains of Fo may be readily distinguishable from non-pathogenic strains, which co-exist in the same niche. For example, *SIX8a* and *SIX8b* homologues were targeted for reliable detection of the 'tropical' race 4 strain of *Fusarium oxysporum* f. sp. *cubense* (Fraser-Smith et al. 2014; O'Neill et al. 2016). Pathogenicity test methods are highly recommended for discriminating host ranges and races, but since this method is time-consuming and laborious, it is not ideal for screening more than 100 different *formae speciales*. Effector genes may be employed for screening several other closely related different *formae speciales* (Lievens et al. 2009a, b). However, pathogenicity testing must be demonstrated for confirmation of Fo to *formae speciales* (Recorbet et al. 2003).

Over the last few years, a decline in mature and young passionfruit vines has been observed in New Zealand's North Island orchards. Early symptoms began with leaf yellowing followed by defoliation, wilting and decline of plants. These symptoms were associated with red-brown discolouration of xylem tissue, spreading into the cortex parenchyma of the stem (Fig. 1). To determine the cause of the disease, a survey of passionfruit orchards was carried out by the AsureQuality Plant Health Laboratory (AQPHL) in association with the New Zealand Passionfruit Growers Association.

Here we report the presence of FOP in passionfruit in New Zealand for the first time. The identification of FOP was validated by pathogenicity testing, morphological examination, and DNA sequence analysis. We also discuss the application of EF-1α, β-tubulin (tub) and *SIX* gene sequencing in molecular diagnostics of FOP and biosecurity implications. Furthermore, the topologies of the *SIX* gene trees were incongruous with the topology of *formae speciales* phylogeny inferred from EF-1a/β-tubulin and concluded that *SIX* genes are essential for *formae speciales* identification.

Materials and methods

Sampling, isolation, and morphological characterisation

In September 2015, samples were collected from symptomatic plants in an orchard in Houhora, Northland and sent

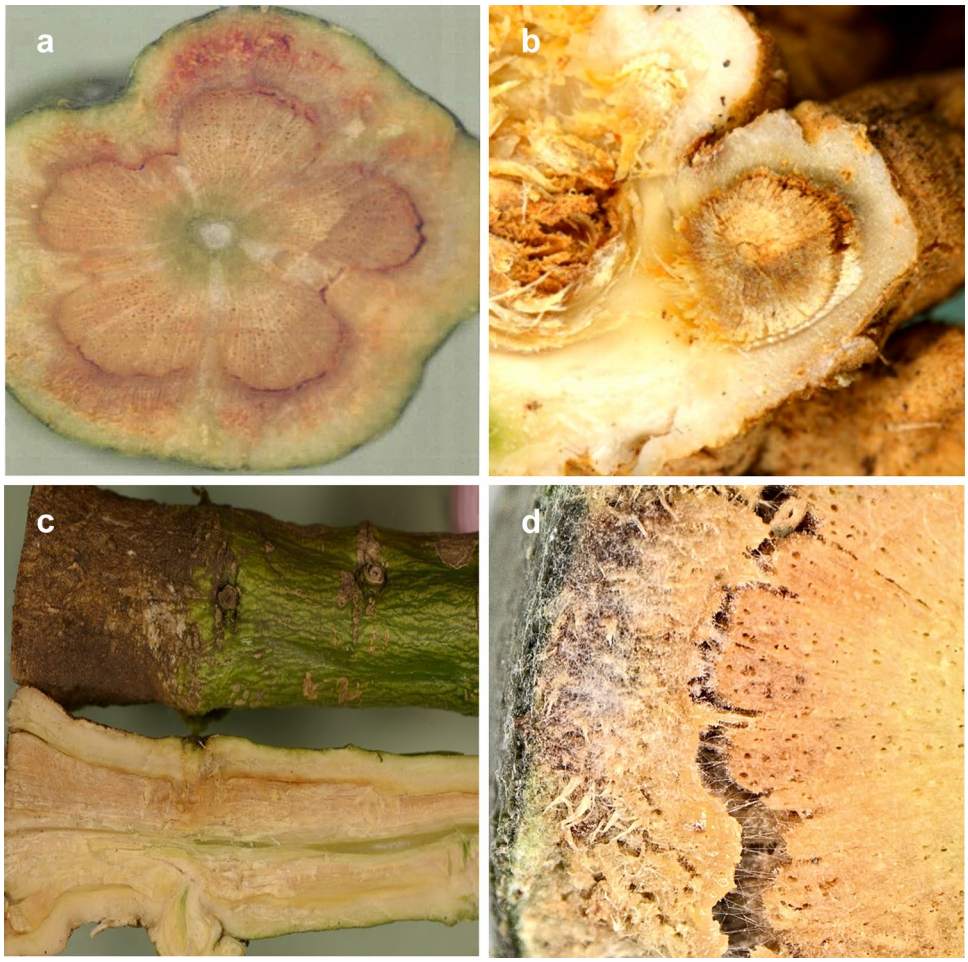

Fig. 1 Symptoms on infected passionfruit plant: **a**, **b** Cross sections of an infected lower stem with red-brown discolouration; **c** longitudinal section of infected lower stem with brown coloured necrotic lesions; **d** mycelial growth on infected lower stem

to AQPHL for diagnostics. Lower stems and roots where red-brown discolouration of vascular and cortex tissue was found, were used for further diagnostics. Affected portions were cut into small pieces, surface sterilized with 1% sodium hypochlorite solution and plated on potato dextrose agar (PDA) media. Media plates were incubated at approximately 25 °C for three days.

Fusarium oxysporum was consistently isolated from the specimens with disease symptoms and was sub-cultured to obtain pure cultures. These were sent to the Ministry for Primary Industries' Plant Health and Environment Laboratory (PHEL) for further identification as suspect FOP cultures. Colony characteristics of the fungus were examined under stereo and compound microscope on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Prune Extract Agar (PEA) and Corn Meal Agar (CMA) (BBL, Becton, Dickinson and Company, Sparks, MD 21,152 USA) seven days post-inoculation (Fig. 2). A representative culture was submitted to the International Collections of Microorganisms from Plants (ICMP) under accession number ICMP 21871.

Preparation of Prune Extract Media (PEA)

Prune extract was made as per follows: 25 g of finely sliced, pitted prunes were infused in 450 mL tap water for 20 min while simmering. After cooling, the liquid was filtered through two layers of Miracloth into flasks and sterilised for 35 min at 121 °C. Prune extract was stored at 4 °C. PEA was made as per the following: Prune extract 200 ml, sucrose 12 g, yeast extract 2.5 g, agar 32 g and water 1800 ml. All ingredients were mixed and autoclaved for 10 min at 121 °C.

Pathogenicity testing

To confirm the pathogenicity of the isolated Fo, a millet culture of FOP inoculum was prepared from ICMP 21871 culture. Five hundred grams of millet seed (*Pennistem glaucum*) was rinsed in tap water followed by soaking overnight in distilled water in Erlenmeyer flasks. The grain was washed the following day, with distilled water to remove leachates, and autoclaved at 121 °C for 30 min on two

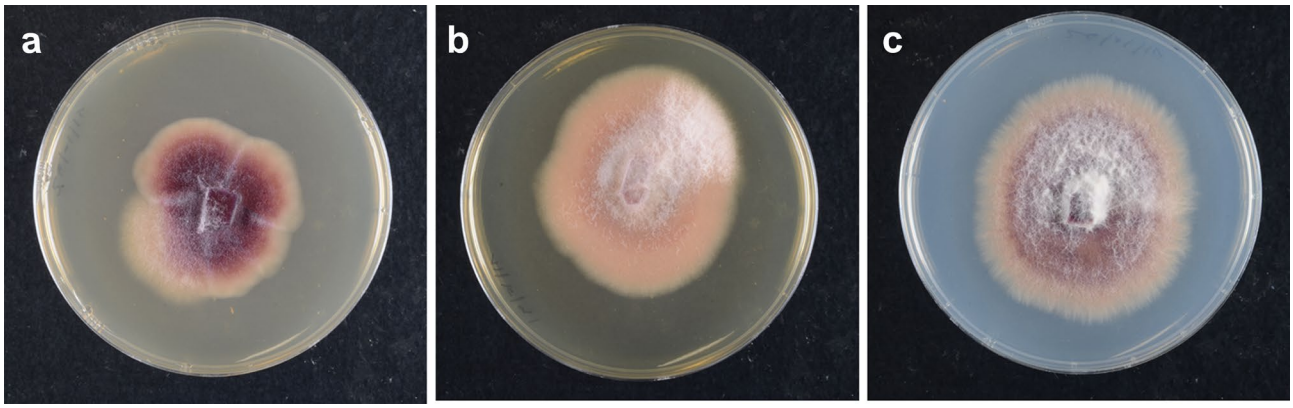

Fig. 2 Seven-day-old colonies of FOP isolate ICMP21871 on **a** Potato Dextrose Agar (PDA); **b** Malt Extract Agar (MEA); and **c** Corn Meal Agar (CMA)

consecutive days. The isolate ICMP 21871 was cultured on PDA for seven days at room temperature under a 12-h light/dark cycle. Five cubes of culture were transferred to one flask of sterile millet grain. A second flask was reserved as a non-inoculated control. Both flasks were shaken once daily. After two weeks, approximately 4 g of inoculated and control millet were plated separately onto PDA and Fo grew consistently from inoculated millet while nothing grew from the non-inoculated millet.

Forty healthy six-week-old passionfruit seedlings (*Passiflora edulis* Sims *f.edulis*) were used for pathogenicity testing. Twenty-five plants were used for FOP pathogenicity test and 15 as controls. One gram of either FOP-colonised millet or sterile millet was gently mixed into the surface layer of potting mix of each passionfruit seedling, for the infection and control treatment respectively. Care was taken to not disturb or damage the roots so that the infection would be natural. The seedlings were placed on trays in a plant growth chamber (Conviron A1000, Thermo Fisher Scientific) and incubated at 25 °C on a 12-h light/dark cycle for 30 days. After completion of the assay, leaves, lower stems and washed roots from both inoculated and control plants were surface sterilised for 1 min in 1% sodium hypochlorite solution, washed twice in sterile deionised water for 1 min, air dried, and plated onto PDA to re-isolate the pathogen.

Molecular characterization

DNA was extracted directly from fungal cultures by bead homogenisation in a CTAB buffer followed by lysis for 25 min at 65 °C. InviMag® Plant DNA Kit (Stratec Molecular, Berlin, Germany) was used to extract DNA using a Kingfisher mL automated DNA extractor (ThermoFisher Scientific, NZ), as per the manufacturer's instructions. The DNA was eluted in 100µL of elution buffer supplied with the kit and stored at -20 °C until required.

In order to confirm the identification of ICMP 21871 as FOP, and those isolates recovered from pathogenicity testing as identical to the inoculating strain ICMP 21871, EF-1α, β-tubulin, *SIX6* and *SIX9* gene regions were amplified by PCR and sequenced. All PCRs were set up using GoTaq® Green Master Mix (Promega, Wisconsin, USA) as per the manufacturer's instruction. PCR primer sequences and annealing temperatures are recorded in Table 1. The reactions were visualised on 1.5% agarose stained with GelRed (Biotium) and forward and reverse strands were sequenced at EcoGene (Auckland, New Zealand).

Table 1 The primers and annealing temperatures used for the amplification of EF-1α, Beta -tubulin, *SIX6* and *SIX9* regions

Primer name	Primer sequences 5′-3′ (F/R)	Annealing °C	Reference
EF-728F/ EF-986R	CAT CGA GAA GTT CGA GAA GG / TAC TTG AAG GAA CCC TTA CC	58 °C	Carbone and Kohn 1999
Bt2a Bt2b	GGT AAC CAA ATC GGT GCT GCT TTC/ ACC CTC AGT GTA GTG ACC CTT GGC	58 °C	Glass and Donaldson 1995
SIX6-F1/ *SIX6*-R1	CTC TCC TGA ACC ATC AAC TT / CAA GAC CAG GTG TAG GCA TT	59 °C	Lievens et al. 2009a, b
FOL *SIX9*	GGG TGG ACC ATA TCA CGA TGT TCG/ GAA TAC CTG AGT GGA GTT GTG TCT TG	64 °C	Taylor et al. 2016

Table 2 List of isolates which were used for sequence analysis to compare ICMP 21871 with other *Fusarium oxysporum* f. sp. *passiflorae* (FOP) strains and closely related fungi. Sequences generated in this study are given in bold type

Isolate number	Species name	Location	Host	Gene bank accession numbers			
				EF	*SIX6*	*SIX9*	tub2
NRRL 22346	*Fusarium ambrosium*	India	*Camellia sinensis*	FJ240350	N/A	N/A	N/A
K13 15	*Fusarium avenaceum*	Poland	*Brassica oleracea*	N/A	N/A	N/A	KU852663
UCR4511	*Fusarium euwallaceae*	USA	*Platanus racemosa*	N/A	N/A	N/A	MK108959
T50	*Fusarium cerealis*	Turkey	*Triticum* sp.	GU370496	N/A	N/A	N/A
WN99	*Fusarium culmorum*	Syria	*Triticum* sp.	GU370494	N/A	N/A	N/A
ATCC 204258	*Fusarium culmorum*	Holland	*Triticum* sp.	GU370495	N/A	N/A	N/A
NRRL 25475	*Fusarium culmorum*	Denmark	*Hordeum vulgare*	N/A	N/A	N/A	AF212780
CS3005	*Fusarium graminearum*	Australia	*Triticum* sp.	GU370497	N/A	N/A	N/A
ATCC 60309	*Fusarium graminearum*	Canada	*Triticum* sp.	GU370498	N/A	N/A	N/A
F10102005	*Fusarium graminearum*	USA	Unknown	GU370499	N/A	N/A	N/A
MAFF 240270	*Fusarium graminearum*	Japan	Unknown	N/A	N/A	N/A	AB587040
CBS 130300	*Fusarium nirenbergiae*	USA	Human toe	N/A	N/A	N/A	MH485107
NRRL 22549	*Fusarium nirenbergiae*	Brazil	*Passiflora edulis*	MH484973	N/A	N/A	N/A
CBS 129.81	*Fusarium nirenbergiae*	USA	*Chrysanthemum* sp.	N/A	N/A	N/A	MH485067
CBS 102030	*Fusarium odoratissimum*	Malaysia	*Musa sapientum*	N/A	N/A	N/A	MH485080
CBS 130310	*Fusarium odoratissimum*	Australia	*Musa* sp.	N/A	N/A	N/A	MH485104
F203	*Fusarium oxysporum*	China	*Vigna radiata*	N/A	MF314838	N/A	N/A
F268	*Fusarium oxysporum*	China	*Vigna radiata*	N/A	MF314839	N/A	N/A
NRRL 52694	*Fusarium oxysporum*	Colombia	*Zulia pubescens*	JF740777	N/A	N/A	N/A
06603B	*Fusarium oxysporum*	New Zealand	*Passiflora edulis*	**MW162623**	N/A	N/A	**MW328518**
06603C	*Fusarium oxysporum*	New Zealand	*Passiflora edulis*	**MW162624**	N/A	N/A	**MW328519**
06603D	*Fusarium oxysporum*	New Zealand	*Passiflora edulis*	**MW162625**	N/A	N/A	**MW328520**
CBS 144134	*Fusarium oxysporum*	Germany	*Solanum tuberosum*	N/A	N/A	N/A	MH485135
CBS 144135	*Fusarium oxysporum*	Germany	*Solanum tuberosum*	N/A	N/A	N/A	MH485136
FOPS025	*Fusarium oxysporum*	Brazil	*Phaseolus vulgaris*	N/A	KP681651	N/A	N/A
FOP16	*Fusarium oxysporum*	USA	*Glycine max*	N/A	KP681652	N/A	N/A
FOP52	*Fusarium oxysporum*	USA	*Glycine max*	N/A	KP681650	N/A	N/A
FOP31	*Fusarium oxysporum*	Netherlands	*Glycine max*	N/A	KP681649	N/A	N/A
FOP58	*Fusarium oxysporum*	USA	*Glycine max*	N/A	KP681654	N/A	N/A
NRRL34936	*Fusarium oxysporum*	USA	*Solanum lycopersicum*	N/A	KP681655	N/A	N/A
N/A	*Fusarium oxysporum*	N/A	N/A	N/A	N/A	HQ260603	N/A
RBG5841	*Fusarium oxysporum*	Australia	Soil	N/A	N/A	KR855737	N/A
RBG5827	*Fusarium oxysporum*	Australia	Soil	N/A	N/A	KR855735	N/A
RBG5829	*Fusarium oxysporum*	Australia	Soil	N/A	N/A	KR855736	N/A
RBG5850	*Fusarium oxysporum*	Australia	Soil	N/A	N/A	KR855738	N/A
RBG5885	*Fusarium oxysporum*	Australia	Soil	N/A	N/A	KR855733	N/A
FUS2	*Fusarium oxysporum* f. sp. *cepae*	UK	*Allium cepa*	N/A	N/A	KP964976	N/A
BRIP40340	*Fusarium oxysporum* f. sp. *cubense*	Australia	*Musa* sp.	N/A	KX435007	N/A	N/A
BRIP62895	*Fusarium oxysporum* f. sp. *cubense*	Australia	*Musa* sp.	N/A	N/A	KX435015	N/A
N/A	*Fusarium oxysporum* f. sp. *lycopersici*	N/A	N/A	N/A	FJ755835	N/A	N/A
FRL4273	*Fusarium oxysporum* f. sp. *lycopersici*	Australia	*Solanum lycopersicum*	N/A	N/A	KR855730	N/A
RBG6924	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	KR855739	N/A	N/A

Table 2 (continued)

Isolate number	Species name	Location	Host	Gene bank accession numbers			
				EF	*SIX6*	*SIX9*	tub2
FRL4120	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	N/A	KR855729	N/A
NTDPI36955	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrulus* sp.	N/A	N/A	KX435041	N/A
FRL4121	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	N/A	KR855726	N/A
RBG5771	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	N/A	KR855725	N/A
FRL4119	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	N/A	KR855727	N/A
FRL1583	*Fusarium oxysporum* f. sp. *niveum*	Australia	*Citrullus lanatus*	N/A	N/A	KR855728	N/A
12105b	*Fusarium oxysporum* f. sp. *niveum*	USA	*Citrullus lanatus*	N/A	N/A	N/A	KX369332
F2-53	*Fusarium oxysporum* f. sp. *niveum*	USA	*Citrullus lanatus*	N/A	N/A	N/A	KX369333
ATCC 90245	*Fusarium oxysporum* f. sp. *phaseoli*	USA	*Phaseolus vulgaris*	N/A	KP964967	N/A	N/A
RBG6471	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855776	N/A	N/A
RBG6451	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855773	N/A	N/A
RBG6438	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855778	N/A	N/A
RBG6447	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855772	N/A	N/A
RBG6399	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855779	N/A	N/A
RBG6498	*Fusarium oxysporum* f. sp. *pisi*	Australia	*Pisum sativum*	N/A	KR855777	N/A	N/A
Afu-3	*Fusarium oxysporum* f. sp. *radicis-cucumerinum*	Crete	*Cucumis sativus*	N/A	GQ268958	N/A	N/A
RBG5063	*Fusarium oxysporum* f. sp. *cucumerinum*	Australia	*Cucumis sativus*	N/A	KR855757	N/A	N/A
R207	*Fusarium oxysporum* f. sp. *dianthi*	UK	*Dianthus caryophyllus*	N/A	N/A	KP964980	N/A
Fod 86	*Fusarium oxysporum* f. sp. *dianthi*	Spain	Soil	N/A	N/A	N/A	KJ433894
Fod 87	*Fusarium oxysporum* f. sp. *dianthi*	Spain	Soil	N/A	N/A	N/A	KJ433895
FOXN7	*Fusarium oxysporum* f. sp. *narcissi*	UK	*Narcissus* sp.	N/A	N/A	KP964979	N/A
FOXN139	*Fusarium oxysporum* f. sp. *narcissi*	UK	*Narcissus* sp.	N/A	N/A	KP964978	N/A
06603E	*Fusarium oxysporum* f. sp. *passiflorae*	New Zealand	*Passiflora edulis*	**MW162626**	**MW328513**	**MW328515**	**MW328521**
06603F	*Fusarium oxysporum* f. sp. *passiflorae*	New Zealand	*Passiflora edulis*	**MW162627**	**MW328514**	**MW328516**	**MW328522**
ICMP 21871	*Fusarium oxysporum* f. sp. *passiflorae*	New Zealand	*Passiflora edulis*	**MH230158**	**MH230160**	**MH230159**	**MW328517**
BRIP28044	*Fusarium oxysporum* f. sp. *passiflorae*	Australia	*Passiflora edulis*	KX434919	KX435045	KX435047	N/A

Table 2 (continued)

Isolate number	Species name	Location	Host	Gene bank accession numbers			
				EF	*SIX6*	*SIX9*	tub2
FRL1584	*Fusarium oxysporum* f. sp. *passiflorae*	Australia	*Passiflora edulis*	KR855944	KR855780	KR855722	N/A
RBG5775	*Fusarium passiflorae* f. sp. *passiflorae*	Australia	*Passiflora edulis*	KR855931	KR855781	KR855723	N/A
RBG6380	*Fusarium passiflorae* f. sp. *passiflorae*	Australia	*Passiflora edulis*	KR855934	KR855782	KR855724	N/A
N/A	*Fusarium oxysporum* f. sp. *passiflorae*	Australia	*Passiflora edulis*	N/A	HM467130	N/A	N/A
CDFA 591	*Fusarium oxysporum* f. sp. *passiflorae*	USA	*Passiflora edulis*	JF332039	N/A	N/A	N/A
NRRL 38273	*Fusarium oxysporum* f. sp. *passiflorae*	USA	*Passiflora edulis*	FJ985362	N/A	N/A	N/A
N/A	*Fusarium oxysporum* f. sp. *passiflorae*	USA	N/A	N/A	N/A	N/A	AF008540
CMM4864	*Fusarium oxysporum* f. sp. *passiflorae*	Brazil	N/A	MH712505	N/A	N/A	N/A
CMM4865	*Fusarium oxysporum* f. sp. *passiflorae*	Brazil	N/A	MH712506	N/A	N/A	N/A
F021	*Fusarium oxysporum* f. sp. *passiflorae*	Portugal		MK430070	N/A	N/A	N/A
9722–2-17	*Fusarium poae*	Norway	Unknown	N/A	N/A	N/A	AF404220
9722 -2-1B (714)	*Fusarium poae*	Norway	Unknown	N/A	N/A	N/A	AF404215
NRRL 25028	*Fusarium proliferatum*	India	*Melanapsis glomerata*	JF740705	N/A	N/A	N/A
NRRL 25029	*Fusarium proliferatum*	India	*Scirpophaga excerptalis*	JF740706	N/A	N/A	N/A
WN61	*Fusarium pseudograminearum*	Tunisia	*Triticum* sp.	GU324916	N/A	N/A	N/A
NRRL 28065	*Fusarium pseudograminearum*	South Africa	*Medicago* sp.	N/A	N/A	N/A	AF212786
NRRL 28334	*Fusarium pseudograminearum*	South Africa	*Medicago* sp.	N/A	N/A	N/A	AF212787
NRRL 25101	*Fusarium solani*	Mexico	*Boophilus* sp.	JF740728	N/A	N/A	N/A
NRRL 25100	*Fusarium solani*	Mali	Soil	JF740727	N/A	N/A	N/A
Q205	*Fusarium solani*	Saudi Arabia	*Phoenix dactylifera*	N/A	N/A	N/A	KJ544186
Q6	*Fusarium solani*	Saudi Arabia	*Phoenix dactylifera*	N/A	N/A	N/A	KJ544187
NRRL 62579	*Fusarium* sp.	USA	*Euwallacea validus*	KC691538	N/A	N/A	N/A
NRRL 62580	*Fusarium* sp.	USA	*Euwallacea validus*	KC691539	N/A	N/A	N/A
NRRL 54727	*Fusarium* sp.	Israel	*Persea americana*	JQ038012	N/A	N/A	N/A
NRRL 62626	*Fusarium* sp.	USA	*Persea americana*	KC691532	N/A	N/A	N/A
Cy262	*Ilyonectria torresensis*	Portugal	*Vitis vinefera*	JF735879	N/A	N/A	JF735501
CBS 101018	*Neocosmospora solani*	Italy	*Rubus idaeus*	N/A	N/A	N/A	KM232062

Abbreviations of various isolates sequences used in this study and culture collections where these isolates were collected: *ATCC* The American Type Culture Collection, *BRIP* Biosecurity Queensland Plant Pathology Herbarium, *FRL* Fusarium Research Laboratory, *ICMP* International collection of Microbiology from Plants, *NRRL* Agriculture Research Service Culture Collection, *RBG* Royal Botanic Gardens

Sequence analysis was performed using Geneious v10.0.6 (Biomatters Ltd, New Zealand). Forward and reverse sequence reads were assembled into contigs and automatically trimmed. The contigs were analysed by BLAST (Altschul et al. 1990) then aligned and compared with known EF-1α, *SIX6*, *SIX9* and β tubulin sequences respectively, other known FOP isolates, and closely related, pathogenic and non-pathogenic strains of Fo (O'Donnell et al. 2009; Rocha et al. 2016; Czislowski et al. 2018) using Clustal W (Thompson et al. 1994).

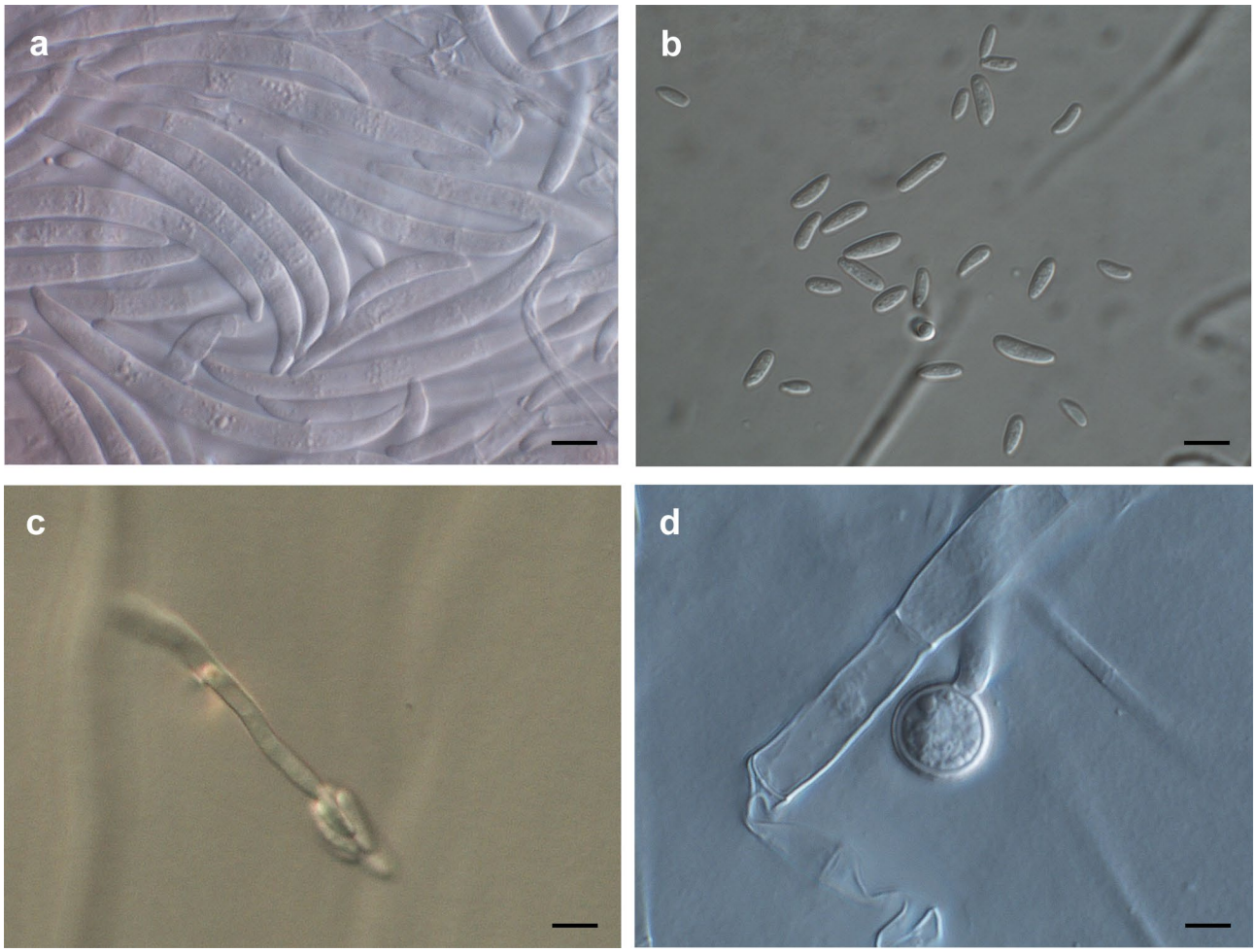

Fig. 3 Morphological characters of FOP isolate ICMP21871. **a** Macroconidia, **b** Microconidia, **c** Monophialides, **d** Chlamydospores. Scale bar = 10 µm. All these structures were observed on PEA media

Phylogenetic analyses

Chromatograms were analysed and assembled using the Staden package v1.6.0 9 (Staden et al. 1998) and the multiple sequence alignment was performed with ClustalX v2.0.11 (Thompson et al. 1994) with default parameters. Complete details of various strains of *Fusarium* sequences employed in the analyses have been provided in Table 2. The phylogenetic analyses were performed with RAxML v7.0.4 (Stamatakis et al. 2008) employing maximum-likelihood (ML) bootstrap analyses. We performed 1000 bootstrap replicates with the thorough bootstrap algorithm on these individual datasets and estimated the base frequencies for each dataset separately. Trees were visualized in Figtree v1.3.1 (Rambaut 2009).

Results

On PDA, the fungus isolated from symptomatic plants produced abundant pale pink sporodochia. Macroconidia were usually three septate, 27–35 × 4–5 µm, slightly curved and thin-walled. Microconidia were abundant on aerial mycelium and formed in false heads on monophialides. Microconidia were non-septate, 6–15 × 2 -3 µm, hyaline, smooth walled and oval shaped. Chlamydospores formed three weeks after inoculation in Prune Extract Agar (PEA) medium and were abundant, single, terminal, and mostly found on surface hyphae (Fig. 3). These morphological characters are consistent with the description of Fo (Leslie and Summerell 2006).

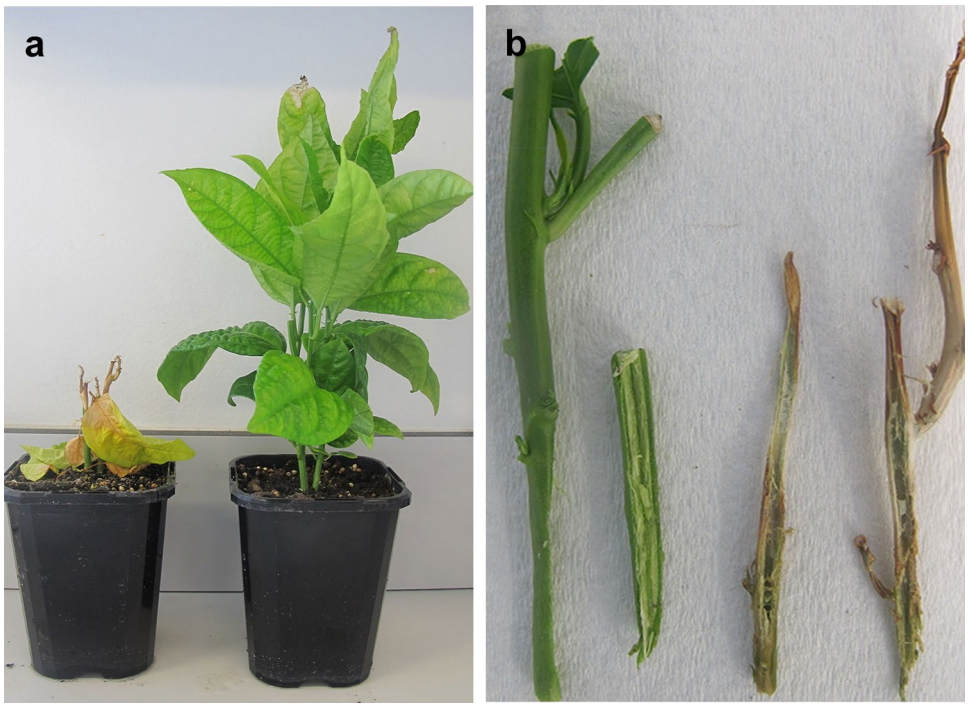

Fig. 4 Pathogenicity test of FOP isolate ICMP21871. **a** Six-week-old passionfruit seedlings showing disease symptoms on the inoculated plant (left) and healthy control plant (right); **b** stems from infected (right) and healthy plants (left)

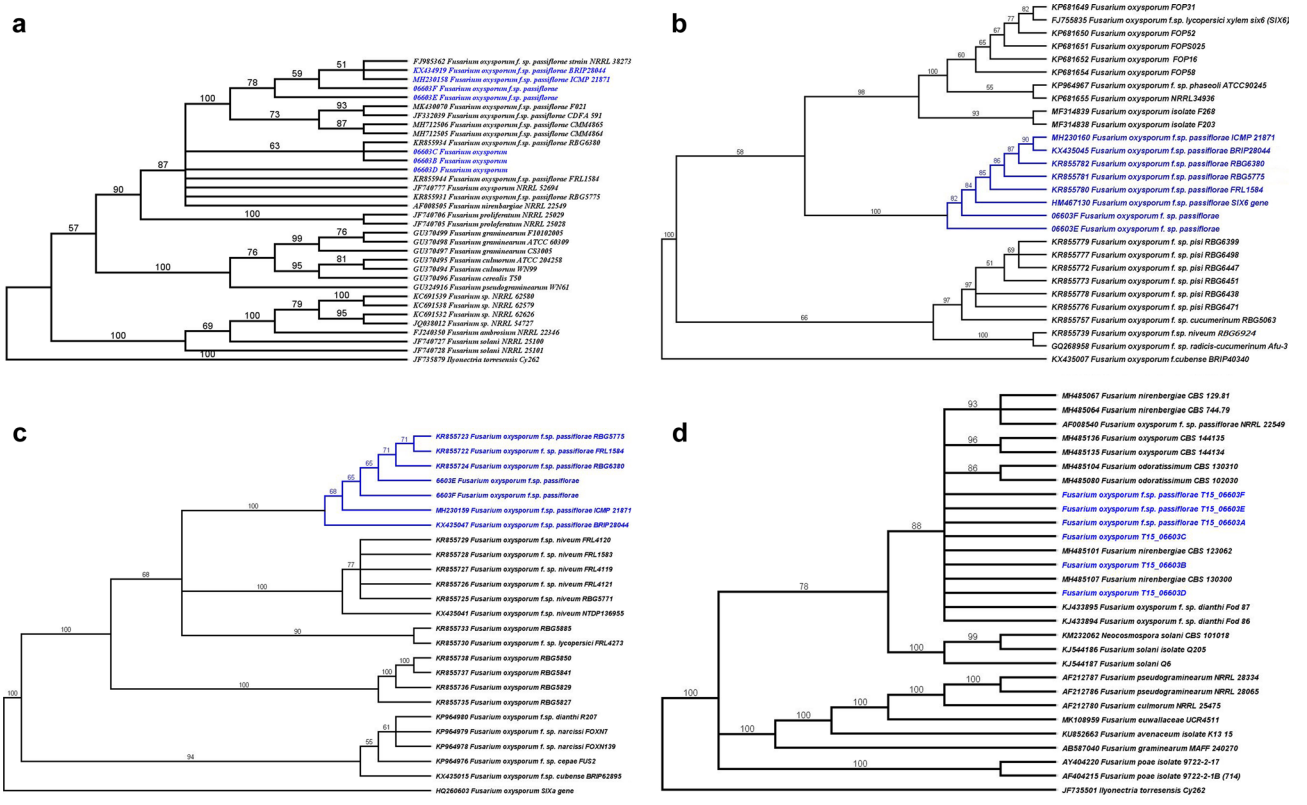

Fig. 5 Maximum Likelihood (ML) tree based on the translation elongation factor 1-alpha (EF-1α) **a**, Secreted in Xylem (*SIX6* **b**, *SIX9* **c** and β- tubulin **d**. sequences are showing phylogenetic relationship between FOP, *Fusarium oxysporum formae speciales* and *Fusarium* spp. All these sequences were retrieved from GenBank. ML bootstrap values are shown at the nodes and all the NZ isolates are highlighted in blue

The passionfruit plants inoculated in the pathogenicity assay showed leaf discolouration and blight symptoms after 20 days, followed by severe wilting and defoliation at 30 days post inoculations. *Fusarium oxysporum* was consistently isolated from the symptomatic leaves, lower stems, and roots of inoculated plants. No wilt symptoms were observed on control plants which remained healthy; no fungi were isolated from these (Fig. 4).

Sequence analysis of the EF-1α from ICMP 21871 showed that the isolate was identical to the FOP isolate BRIP28044, but not the other FOP isolates FRL 1584, RBG5775 and RBG6380. There are 11 bp differences between the isolate BRIP28044 and the other isolates. The ML phylogenetic analyses revealed that the EF-1α and β-tubulin could not resolve the species identity of FOP accurately from other strains of *F. oxysporum* (Fig. 5a, d). On the other hand, the phylogenetic analyses employing *SIX6* gene produced a well-supported clade for all the FOP from the current study, along with the Australian isolates from passionfruit and other substrates (Fig. 5b). Both phylogenetic trees produced polytomy, which could be due to these genes not being able to discriminate the sub species level identifications in Fo. However, the ML analyses employing the *SIX9* gene has produced a strong clade (with an ML value of 100) comprising all the FOP from our study with that of all other strains reported so far (Fig. 5c). The same tree also revealed that FOP is phylogenetically closer to Fo, provided the other two strains viz. *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *phaseoli* could be wrongly identified, as falling under the same clade (Fig. 5c).

Discussion

In this study, we describe the first record of FOP in New Zealand. Pathogenicity testing confirmed that Fo isolate ICMP 21871, isolated from wilting passionfruit plants, was the causal agent of the disease symptoms observed. The purple passionfruit (*Passiflora edulis* f. *edulis*) is grown on approximately 38 hectares in New Zealand, producing around 125 tonnes of fruit per year for both local and export markets (New Zealand Passionfruit Growers Inc. 2020). The purple passionfruit is the only commercial variety grown in the subtropical regions of New Zealand. Until the mid-1930s, the successful cultivation of passionfruit was relatively easy. Since then, the incidence of the disease has made commercial production more difficult, reducing yields and increasing costs for growers. Commercial passionfruit production in New Zealand is a small industry and passionfruit in the home garden is limited to warm frost-free areas unless grown undercover. FOP is expected to be widespread wherever passionfruit plants are grown in New Zealand. While FOP can cause significant damage to passionfruit orchards overseas, it is unlikely to cause significant economic damage in New Zealand. The industry is conducting management practices for the control of *Fusarium* species in orchards. There are no known effective control measures to cure the disease; therefore, efforts to reduce the risk of infection are considered worthwhile. However, passionfruit growers using systemic fungicides to minimise different *Fusarium* infections in their orchards. For example, application of soil fumigation in *Fusarium oxysporum* infested sites and incorporation of systemic fungicides in potting mix to control root infection in passionfruit seedlings. The site selection is important to reduce frost and weather damage or covering orchard site with frost protection cloth to reduce frost damage and subsequent *Fusarium* infection. Other agricultural practices like careful application of fertilisers, herbicides to minimise injury to the base of the passionfruit plants for avoiding *Fusarium* infection. It is also recommended that the base of the plant remain free from grass and weeds which encourage fungal activity and harbour slugs and snails. Plants suffering from FOP and other *Fusarium* canker diseases should be carefully removed and destroyed by burning (NZ Passionfruit Growers Association website accessed on 30/10/2020, https://www.passionfruit.org.nz/facts-info/growing-info/diseases). Planting resistant varieties shows promise in controlling incidence of FOP, but breeding resistant varieties are proving challenging (Yamashiro and Cardoso 1982).

While the conserved EF-1α locus is able to resolve intraspecific phylogenetic relationships in this species complex (O'Donnell et al. 2009), its utility as a molecular diagnostic region may require some caution. An example for this would be where Rocha et al. (2016) isolated Fo from an undisturbed, natural ecosystem, which shared an identical EF-1α sequence to the international isolate of FOP, FRL1584. While unlikely to be a common occurrence, it is important to use caution when using the polymorphism-rich EF-1α locus for diagnostics, without providing additional supporting data. For example, according to Van Dam (2016), Fo is considered polyphyletic, that is, clonal lineages of the same *forma specialis* of Fo may have incongruent conserved genes, but identical, host-specific, effector genes responsible for pathogenicity profiles.

In this study, ML sequence analysis showed that conserved EF-1α gene from the NZ isolate of FOP ICMP 21871 was identical to the previously published FOP isolate BRIP28044, but not FOP isolates FRL1584, RBG5775 and RBG6380, supporting the conclusion of O'Donnell et al. (2009), that FOP has at least two different EF-1α haplotypes. Both haplotypes, however, share the same homologs of the effector genes, *SIX6* and *SIX9*. Given the challenge of diagnosing strains of *formae speciales* of Fo from conserved genes such as EF-1α, effector genes could be used for the characterisation of host-specificity within *formae speciales*

of Fo, followed by the development of diagnostic markers for host-specific strains, for example the 'tropical' race 4 (TR4) stain of *Fusarium oxysporum* f. sp. *cubense* (O'Neill et al. 2016). These results reinforce the need to understand the complexities of the FOSC in order to carefully interpret results and ensure the use of the most appropriate gene regions for diagnostics. Pathogenicity genes are attractive targets for molecular diagnostics as they may screen out environmental fungi which might co-exist in the same niche (Lievens et al. 2009a, b). When different haplotypes of the same pathogen share the same effector homologs, developing a matrix of conserved and effector diagnostic targets may be desirable to mitigate the risk of introducing similar haplotypes with new or unknown pathogenicity.

Recently, an epitype was designated for *F. oxysporum* (Lombard et al. 2019). Fifteen cryptic taxa were described as *Fusarium* species after resolving multi-locus phylogenetic analysis of conservative gene sequences (EF-1α, RPB2 and β-tubulin 2), and subtle morphological differences (Lombard et al. 2019). The FOP isolate included in this study (CBS 744.79 = BBA 62355 = NRRL 22349) was assigned to the new species, *Fusarium nirenbergiae* based on (Lombard et al. 2019). In addition, another *formae specialis*, *Fusarium oxysporum* f. sp. *cubense*, TR4 was assigned to the new species *Fusarium odoratissimum* (Maryani et al. 2019).

Such a controversial proposal of raising subspecies level to species level, creates confusion for practitioners and legislators. To avoid such a contentious situation, the *forma specialis* status of Fo could nominally be restricted to isolate strains within FOSC. Further, all the current strains of *formae speciales* should be confirmed by conservative gene sequence analysis, pathogenicity assays and, characterisation of effector genes (Summerell 2019). Without additional sequence data relative to the composition of *SIX* genes in CBS 744.79, it is beyond the scope of this study to compare and discuss the effector attributes of this isolate.

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Bogale M, Wingfield BD, Wingfield MJ, Steenkamp ET (2007) Species-specific primers for *Fusarium redolens* and a PCR-RFLP technique to distinguish among three clades of *Fusarium oxysporum*. FEMS Microbiol Lett 271:27–32

Cafri D, Katan J, Katan T (2005) Cross-pathogenicity between *formae speciales* of *Fusarium oxysporum*, the pathogens of cucumber and melon. J Phytopathol 153:615–622

Carbone I, Kohn L (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3):553–556. https://doi.org/10.2307/3761358

Croll D, McDonald BA (2012) The accessory genome as a cradle for adaptive evolution in pathogens. PloS Pathog 8:e1002608

Czislowski E, Fraser-Smith S, Zander M, O'Neill WT, Meldrum RA, Tran-Nguyen LTT, Batley J, Aitken EAB (2018) Investigation of the diversity of effector genes in the banana pathogen, *Fusarium oxysporum* f. sp. *cubense*, reveals evidence of horizontal gene transfer. Mol Plant Pathol 19:1155–1171. https://doi.org/10.1111/mpp.12594

dos Santos Silva A, de Oliveira EJ, Haddad F, Laranjeira FF, de Jesus ON, de Oliveira SAS, de Carvalho Costa MAP, de Freitas JPX (2013) Identification of passionfruit genotypes resistant to *Fusarium oxysporum* f. sp. *passiflorae*. Trop Plant Pathol 38(3):236–242

Fisher I, Rezende JM (2008) Diseases of Passionflower (*Passiflora* spp.). Global Science Books

Fraser-Smith S, Czislowski E, Meldrum RA, Zander M, O'Neill W, Balali GR, Aitken EAB (2014) Sequence variation in the putative effector gene *SIX8* facilitates molecular differentiation of *Fusarium oxysporum* f. sp. *cubense*. Plant Pathol 63:1044–1052

Gardner DE (1989) Pathogenicity of *Fusarium oxysporum* f. sp. *passiflore* to Banana Poka and other *Passiflora* spp. in Hawaii. Plant Dis 73:476–478

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61(4):1323–1330. https://doi.org/10.1128/AEM.61.4.1323-1330.1995

Gordon DR, Martyn RD (1997) The evolutionary biology of *Fusarium oxysporum*. Annu Rev Phytopathol 35:111–128

Gordon TR (2017) *Fusarium oxysporum* and the *Fusarium* wilt syndrome. Annu Rev Phytopathol 55:23–29

Gordon WL (1965) Pathogenic strains of *Fusarium oxysporum*. Can J Botany 43:1309–1318

Hermann VE, Lecomte C (2019) Current status of *Fusarium oxysporum formae speciales* and races. Phytopathology 109:512–530. https://doi.org/10.1094/PHYTO-08-18-0320-RVW

Jarvis WR, Shoemaker RA (1978) Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679–1680

Koyyappurath S, Atuahiva T, Le Guen R, Batina H, Le Squin H, Gautheron N, Hermann E, Peribe J, Jahiel M, Steinberg C, Liew ECY, Alabouvette C, Besse P, Dron M, Sache I, Laval V, Grisoni M (2016) *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. Plant Patho J 65:612–625

Laurence MH, Summerell BA, Burgess LW, Liew ECY (2014) Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. Fungal Biol 118:374–384

Leslie JF, Summerell BA (2006) The *Fusarium* laboratory manual. Blackwell Publishing Iowa, USA

Liberato JR, Laranjeira FF (2005) *Fusarium* wilt of passionfruit (*Fusarium oxysporum* f. sp. *passiflorae*) Updated on 12/21/2007 9:21:11 AM Available online: PaDIL - http://www.padil.gov.au

Lievens B, Houterman PM, Rep M (2009) Effector gene screening allows unambiguous identification of *Fusarium oxysporum* f. sp. *lycopersici* races and discrimination from other *formae speciales*. FEMS Microbiol Lett 300:201–215

Lievens B, Van Baarlen P, Verreth C, Kerckhove V, Rep M, Bart PHJ, Thomma, (2009) Evolutionary relationships between *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-lypersici* isolates inferred from mating type, elongation factor-1α and exopolygalacturonase sequences. Mycol Res 113:1181–1191

Lombard L, Sandoval-Denis M, Lamprecht SC, Crous PW (2019) Epitypification of *Fusarium oxysporum* – clearing the taxonomic chaos. Persoonia - Molecular Phylogeny and Evolution of Fungi 43. https://doi.org/10.3767/persoonia.2019.43.01

Ma LJ, van der Does H., Borkovich, K et al. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464, 367–373 doi10. 1038/nature08850

Mariyani N, Lombard L, Poerba YS, Subandiyah, Crous PW, Kema GHJ 2019 Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. Stud Mycol. 2019 Mar; 92:155–194. Epub 2018 Jul 5. PMID: 30122796; PMCID: PMC6086327. https://doi.org/10.1016/j.simyco.2018.06.003

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prudhomme Van Reine WF, Smith GF, Wiersema JH, Turland NJ (2012) International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Gantner Verlag KG [Regnum Vegetabile no. 154].

Nelson PE (1991) Recent Advances in *Fusarium* Systematics: History of *Fusarium* systematics. Phytopathology 81:1045–1048

New Zealand Fungi and Bacteria (NZFUNGI) (2020) Landcare Research, https://nzfungi.landcareresearch.co.nz. Accessed 30 October 2020

New Zealand Passionfruit Growers Inc (2020) https://www.passionfruit.org.nz/facts-info/growing-info/diseases Accessed 30 October 2020

O'Donnell K, Gueidan C, Sink S et al (2009) A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genet Biol 46(12):936–948

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. PNAS USA 95:2044–2049

Olivain C, Alabouvette C (1999) Process of tomato root colonization by a pathogenic strain of *Fusarium oxysporum* f. sp. *lycopersici* in comparison with a non-pathogenic strain. New Phytol 141:497–510

O'Neill WT, Henderson J, Pattemore, JA, O'Dwyer C, et al. (2016) Detection of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 strain in northern Queensland. Australas Plant Dis Notes 11:33.

Pinaria AG, Laurence MH, Burgess LW, Liew ECY (2015) Phylogeny and origin of *Fusarium oxysporum* f. sp. *vanillae* in Indonesia. Plant Pathol 64:1358–1365

Raffaele S, Kamoun S (2012) Genome evolution in filamentous plant pathogens: why bigger can be better. Nat Rev Microbiol 10:417–430

Rambaut A (2009) FigTree, Version 1.3.1. Available at: http://tree.bio.ed.ac.uk/software/figtree. Accessed 11 March 2020

Recorbet G, Steinberg C, Olivan C, Trouvelot S, Gaudot ED, Gianinazzi S, Alabouvette C (2003) Wanted: pathogenesis-related marker molecules for *Fusarium oxysporum*. New Phytol 159:73–92

Rep M, Kistler HC (2010) The genomic organization of plant pathogenicity in *Fusarium* species. Curr Opin Plant Biolo 13:420–426

Rep M, van der Does HC, Meijer M et al (2004) A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I-3-mediated resistance in tomato. Mol Microbiol 53:1373–1383

Rocha LO, Laurence MH, Ludowici VA et al (2016) Putative effector genes detected in *Fusarium oxysporum* from natural ecosystems of Australia. Plant Pathol 65:914–929. https://doi.org/10.1111/ppa.12472

Rooney-Latham S, Blomquist CL, Scheck HJ (2011) First report of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *passiflorae* on passion fruit in North America. Plant Dis 95(11):1478. https://doi.org/10.1094/PDIS-03-11-0261

Sharma KD, Hemlata, Rathour R, Kapila RK, Paul YS (2018) Detection of pea wilt pathogen *Fusarium oxysporum* f. sp. *pisi* using DNA-based markers. J Plant Biochem Biotechnol 27, 342–350 https://doi.org/10.1007/s13562-018-0443-0

Snyder WC, Hansen HN (1940) The species concept in *Fusarium*. Am J Bot 27:64–67

Staden R, Beal K, Bonfield JK (1998) The Staden package. In: Misener S, Krawetz S (eds) Computer methods in molecular biology. Humana Press, Totowa, NJ, pp 115–130

Stamatakis A, Hoover P, Rougemont J (2008) A rapid boot-strap algorithm for the RAxML Web servers. Syst Biol 57:758–771

Summerell BA (2019) Resolving *Fusarium*: Current status of the genus. Annu Rev Phytopatholo 57:327–329

Taylor A, Vagany V, Jackson AC, Harrison RJ, Rainoni A, Clarkson, JP (2016) Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*. Mol Plant Pathol 17(7):1032–1047.

Teixeria LM, Coelho L, Donizete TN (2017) Characterization of *Fusarium oxysporum* isolates and resistance of passionfruit genotypes to Fusariosis. Rev Bras Fruti 39(3):e-415. Epub Aug 17, 2017. ISSN 1806–9967. https://doi.org/10.1590/0100-29452017415

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22(22):4673–4680

Van Dam P, Fokkens L, Schmidt SM, Linmans JH, Kistler HC, Ma LJ, Rep M (2016) Effector profiles distinguish *formae speciales* of *Fusarium oxysporum*. Environ Microbiol 18:4087–4102

Yamashiro T, Cardoso RMG (1982) Ocorrência de murcha de Fusarium em maracujá-doce· (*Passiflora alata* Ait) no Estado de São Paulo. Summa Phytopathologica 8:57