Pseudofusicoccum sp. causing shoot canker in peach in Uruguay

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Abstract

We report the presence of a *Pseudofusicoccum* species associated with cankers showing gummosis in peach shoots for the first time. Identification was performed by sequence analysis of the concatenated ITS and EF1- α genes. The *Pseudofusicoccum* sp. was morphologically described and its pathogenicity was evaluated under laboratory conditions.

Keywords Peach · Canker · *Pseudofusicoccum stromaticum*

Pseudofusicoccum is a genus of botryosphaeraceous fungi resembling *Botryosphaeria*, but having a persistent mucilaginous sheath surrounding their conidia (Pavlic et al. 2008). Currently, nine species are known. *Pseudofusicoccum stromaticum*, the type species of the genus, was described from asymptomatic non-native *Acacia* spp. and *Eucalyptus* sp. in Venezuela (Mohali et al. 2006, 2007). This species was later reported as the causal agent of mango and Malay apple dieback (Marques et al. 2013; Silveira et al. 2017), as well as dieback in tropical fruit plants such as cashew, guava and mango in Brazil (Coutinho et al. 2018). Reports include species isolated from dead wood, but mostly isolated from healthy plant tissue, such as *P. olivaceum*, *P. violaceum* and *P. africanum* in South Africa (Mehl et al. 2011; Jami et al. 2018). Other species in the genus are *P. adansoniae*, *P. ardesiacum* and *P. kimberleyense* in Australia, (Pavlic et al. 2008), and *P. adansoniae*, *P. ardesiacum*, *P. artocarpus* and *P. calophylli* in South-Asia (Sharma et al. 2013; Trakunyingcharoen et al. 2015; Jayasiri et al. 2019).

Recently, during a survey of fruit trees and shrubs showing wood decay symptoms in Uruguay (Sessa et al. 2016) a fungus (isolate Fi2364) resembling the macromorphological characteristics of *Pseudofusicoccum* was recovered. The objectives of this work were to identify and characterise this isolate based on macro and

micromorphological characteristics, molecular sequence data from the internal transcribed spacer region (ITS) and part of the translation elongation factor 1- α gene (EF1- α) and pathogenicity assays. The isolation method was as reported by Sessa et al. (2016). Isolate Fi2364 was obtained from Early Grande peach shoots showing cankers with gummosis and it was recovered together with isolates later identified as *Neofusicoccum parvum*. Colony growth at 25 °C, 28 °C, 30 °C and 33 °C was evaluated in order to determine the optimal growth temperature. An eight-mm diameter mycelial plug was placed in the center of a 90-mm diameter PDA plate, and three replicates were incubated at each temperature in the dark. Optimal growth temperature of the Fi2364 colony was 30 °C at which it developed a fluffy white mycelium, which rapidly turned into a dense mycelial mat, pale olivaceous grey coloured, covering the 90 mm diameter Petri dish after 4 days (Fig. 1a). Conidiomata of 1–2 mm were superficial, solitary, circular and covered with hyphae/mycelium. Conidiogenous cells were hyaline and holoblastic, conidia were hyaline, unicellular, aseptate and bacilliform shaped with both apex and base rounded, with an average size of 22.9 (20.3–26.4) $\mu\text{m} \times 5.3$ (4.6–6.3) μm (Fig. 1b). The average length/width ratio of conidia was 4.3. Isolate Fi2364 used in the present study is stored in the culture collection of the Mycology Laboratory of the Universidad de la República (UdelaR). Genomic DNA was extracted according to the protocol of Lee and Taylor (1990) and used in PCR amplification of the ITS gene using primers ITS5 and ITS4 (White et al. 1990) and the EF1- α gene using primers EF1-728F and EF1-986R (Carbone and Khon 1999). Conditions for each PCR were as described by the references cited above.

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Fig. 1 *Pseudofusicoccum* sp. isolated from peach bark, grown on PDA. Upper view of the culture after 4 days **a** and spores in grey scale after lactophenol cotton blue staining **b**. Scale bar: 10 μ m

PCR products were purified and sequenced by Macrogen (Seoul, Korea) and the sequences obtained were assembled and manually corrected using MEGA version 6 software (Tamura et al. 2013). Phylogenetic analysis was performed by Maximum Likelihood and Tamura 3-parameter model using the concatenated ITS and EF1- α sequences of isolate Fi2364 together with 16 *Pseudofusicoccum* spp. sequences retrieved from GenBank sequence database (Table 1). Support of the nodes was determined by analysis of 1,000 bootstrap replicates (Fig. 2). Isolate Fi2364 did not cluster with any sequence from GenBank although it was placed on a single branch next to the *P. stromaticum* clade. Based on alignments of the separate ITS and EF1- α loci between isolate Fi2364 and its closest phylogenetic neighbor *P. stromaticum* (CMW13434), polymorphic positions

were found. The ITS sequences of isolate Fi2364 and *P. stromaticum* (CMW13434) differed in eight nucleotides: C instead of T in position 34 and 109, T instead of C in position 30, 389 and 497, A instead of G in position 111 and 381 and A instead of T in position 177. Also, the partial EF1- α gene sequence differed in three positions: G instead of A in position 34, T instead of a C in position 68 and C instead of T in position 118.

Pathogenicity tests were conducted on 1-year-old detached shoots of peach and apple trees under laboratory conditions. A mycelial plug from the margin of an actively growing colony (PDA) was inoculated in a V-shaped wound made on each shoot. Wounds were wrapped with Parafilm to avoid desiccation. Control shoots were inoculated with sterile PDA plugs. Inoculated and control shoots (ten each) were placed on an incubation chamber at 25 °C and high humidity levels. After 30 days of incubation, necrotic tissue on the site of inoculation was observed in both hosts, whereas no necrosis was observed on control shoots (Fig. 3). Lesions were significantly higher in peach shoots (12.7 ± 7.5 mm) than apple (8.8 ± 1.1 mm). The pathogen was re-isolated from the margins of necrotic tissues of peach and apple shoots whereas it could not be re-isolated from symptomless control shoots, fulfilling Koch's postulates.

Pseudofusicoccum species are rarely mentioned as primary pathogens in incidence studies of botryosphaeriaceous fungi. On the other hand, species of this genus are frequently reported as endophytes

Table 1 *Pseudofusicoccum* isolates included in the phylogenetic analysis, genes sequenced and accession numbers

Species Name	Isolate	Origin	GenBank acc. no	
			ITS	EF-1 α
<i>P. adansoniae</i>	CBS 122,054*	Australia	EF585532	EF585570
	WAC 13,299	Australia	GU172404	GU172436
<i>P. ardesiacum</i>	CBS 122,062*	Australia	EU144060	EU144075
	WAC 13,294	Australia	GU172405	GU172437
<i>P. artocarp</i>	CPC 22,796*	Thailand	KM006452	KM006483
<i>P. kimberleyense</i>	CBS 122,061*	Australia	EU144059	EU144074
	WAC 13,293	Australia	GU172406	GU172438
<i>P. olivaceum</i>	CBS 124,939*	South Africa	FJ888459	FJ888437
	CMW 22,639	South Africa	FJ888463	FJ888439
	CBS 124,940	South Africa	FJ888462	FJ888438
<i>P. stromaticum</i>	CMW 13,434*	Venezuela	AY693974	AY693975
	CMW 13,435	Venezuela	DQ436935	DQ436936
	CMM 3961	Brazil	JX464103	JX464110
	CMM 4541	Brazil	KT728918	KT728922
<i>P. violaceum</i>	CBS 124,936*	South Africa	FJ888474	FJ888442
	CBS 124,937	South Africa	FJ888458	FJ888440
<i>Pseudofusicoccum</i> sp.	Fi2364	Uruguay	KT191029	<i>MW110607</i>

Sequence numbers in italics were obtained in the present study

*indicates ex-type

Fig. 2 Phylogenetic tree resulting from the Maximum Likelihood analysis of combined ITS and partial EF1- α sequences. Bootstrap support values based on 1000 replicates are shown in the nodes. The tree is outrooted with *Botryosphaeria dothidea* isolate CMW8000

(Sharma et al. 2013; Mehl et al. 2011) or as low virulence pathogens co-occurring with other Botryosphaeriaceae species causing disease symptoms. Co-occurrence with high virulence pathogens include species such as *Neofusicoccum mangroviorum* (Jami et al. 2018), *Lasioidiplodia theobromae* (Silveira et al. 2017), *Botryosphaeria dothidea* and *N. parvum* (Marques et al. 2013; Rolim et al. 2020; Sessa et al. 2016). This could mean that in most cases *Pseudofusicoccum* cannot be signaled as solely responsible for the symptoms observed in the damaged tissues. In this work we studied a *Pseudofusicoccum* isolate associated with symptomatic *Prunus persica* twigs. Based on conidial size and shape

and optimal growth temperature (Phillips et al. 2013) as well as phylogenetic analyses, isolate Fi2364 can be assigned to an unknown species related to *P. stromaticum*. Additional genes like the D1/D2 domain of the Large Subunit (LSU), for which sequences are not yet available at GenBank, should be analysed in order to further clarify the phylogenetic assignment of this isolate (Vu et al. 2019; Crous et al. 2006). Isolate Fi2364 showed moderate virulence on both apple and peach shoots. Although it might be considered as non-hazardous in temperate regions such as Uruguay, its co-occurrence with highly virulent fungi like *N. parvum* suggests a role in the observed symptoms in peach twigs.

Fig. 3 Lesions developed in the pathogenicity evaluation 30 days post inoculation with isolate Fi2364 on peach **a** and apple **c** branches. Uninoculated peach **b** and apple **d** branches

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