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RESEARCH PAPER

Characterization of *Campylocarpon pseudofasciculare* associated with black foot of grapevine in southern Brazil

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Summary. The incidence and severity of black foot has been recently increasing in nurseries and vineyards of southern Brazil. The goal of the present study was to characterize *Campylocarpon* isolates associated with black foot of grapevines (*Vitis* spp.) using multi-gene DNA analysis (internal transcribed spacers [ITS rDNA], β-tubulin and histone H3) and morphological characteristics, and to test the pathogenicity of the isolates in grapevine (*Vitis labrusca* cv. Bordô). The three DNA regions analyzed indicated that all the isolates belonged to *Campylocarpon pseudofasciculare*. Their morphology was similar to descriptions published for the species. Isolates exhibited umber- to chestnut-coloured colonies and macroconidia (38.0 × 7.0 µm) predominantly with three septa. All the isolates inoculated in *V. labrusca* cv. Bordô caused typical symptoms of black foot. This is the first report of *Campylocarpon pseudofasciculare* in southern Brazil.

Key words: multi-locus analysis, morphology, pathogenicity, *Vitis labrusca*.

Introduction

Grapevines are an important crop in Brazil, occupying 84,338 ha, with 18,284 kg yield in 2011 (FAOSTAT, 2013). Black foot is one of the most important grapevine soilborne diseases, affecting vineyards in several locations worldwide. This disease was first reported in France in 1961 (Grasso and Magnano Di San Lio, 1975). Black foot was described by Halleen *et al.* (2006) affecting mainly young grapevines, between 2 and 10 years of age. The disease is characterized by root and crown necrosis, reduced root masses, darkened vessels, decreased vigor, dry shoots and plant death (Garrido *et al.*, 2004; Santos *et al.*, 2014).

Ilyonectria P. Chaverri & C. Salgado and *Campylocarpon* Halleen, Schroers & Crous are important pathogens causing typical symptoms of black foot and thus grapevine decline (Halleen *et al.*, 2004, 2006). Gramaje and Armengol (2011) reported that decline symptoms in young grapevines and failure of planting material reduced grape production for raisins, table use and wine since 1990. Among the genera responsible for the disease, *Campylocarpon* is especially relevant. This genus possesses curved macroconidia with (1–) 3–5 (–6) septa, and it does not form microconidia (Halleen *et al.*, 2004). A teleomorphic phase is unknown (Halleen *et al.*, 2004; Chaverri *et al.*, 2011). The genus includes two spe-

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cies: *Campylocarpon fasciculare* Schroers, Halleen & Crous, already reported in South Africa (Halleen *et al.*, 2004), Spain (Alaniz *et al.*, 2011) and Northeast Brazil (Correia *et al.*, 2012), and *Campylocarpon pseudofasciculare*, reported in South Africa (Halleen *et al.*, 2004), Uruguay (Abreo *et al.*, 2010), Peru (Álvarez *et al.*, 2012) and Northeast Brazil (Correia *et al.*, 2012). *Campylocarpon fasciculare* caused 22.5% vine mortality and *C. fasciculare* and *C. pseudofasciculare* mortality of 17.5% (Halleen *et al.*, 2004).

Pathogenicity tests using isolates associated with black foot in rootstocks Ritcher 110 R (*Vitis berlandieri* Planch × *Vitis rupestris* Sch.) (Alaniz *et al*., 2007; Mohammad *et al*., 2009); Ramsey (*Vitis champini* Planch.) (Halleen *et al*., 2004); e 1103 P (*V. berlandieri* × *V. rupestris*) (Alaniz *et al*., 2010; Cabral *et al*., 2012b) have been carried out in other countries. However, studies involving *Vitis labrusca* L. have only been performed by Garrido *et al*. (2004) evaluating only one isolate of *Ilyonectria* sp. (as *Cylindrocarpon destructans* (Zinnsm.) Scholten). *Vitis labrusca* is very important in southern Brazil with many vineyards are established from rooted cuttings. However, *C. pseudofasciculare* causing black foot has not been studied associated with *V. labrusca*. No curative control measures are available to eradicate black foot pathogens in nurseries or vineyards (Oliveira *et al*., 2004). Further research is required on the etiology, the epidemiology and control of the pathogen.

The goal of the present study was to characterize *C. pseudofasciculare* isolates which cause black foot in grapevines of southern Brazil, using multigene DNA analysis [internal transcribed spacers (ITS rDNA) and β-tubulin and histone H3 gene introns and exons] and morphological characteristics, and to determine the pathogenicity of selected representative in rooted cuttings of *Vitis labrusca* cv. Bordô.

Materials and methods

Fungal isolates

Nine *C. pseudofasciculare* isolates were obtained from symptomatic grapevines: Bordô (*V. labrusca*); VR 043-43 (*V. rotundifolia* Michx. × *V. vinifera* L.) / BRS Violeta (hybrid – *Vitis* spp.); Rupestris du Lot (*V. rupestris*) / Niagara Rosada (*V. labrusca*); Niagara Branca (*V. labrusca*); RR 101-14 (*V. rupestris* × *V. riparia* Michx.) / Isabel (*V. labrusca*); 1103 P (*V. berlandieri* × *V. rupestris*) / Chardonnay (*V. vinifera*); 1103 P (*V. berlandieri* × *V. rupestris*) / Isabel (*V. labrusca*); and Isabel (*V. labrusca*); from different vineyards in the state of Rio Grande do Sul, Brazil, in 2012 (Table 1). Fungal isolates were obtained from the roots and basal regions of plants exhibiting symptoms characteristic of black foot. Symptomatic plants exhibited reduced root mass, necrosis in the roots and crowns, delayed sprouting, decreased vigour, shoot wilting and death. Necrotic wood and root fragments (~3 × 3 × 3 mm) were washed in running water and disinfected with 70% alcohol and 1% sodium hypochlorite, followed by three washes of sterile distilled water of 1 min each. After drying, the fragments were plated onto Petri dishes containing potato dextrose agar (PDA) containing 0.5 g L^{-1} streptomycin sulfate. The Petri dishes were incubated for 7 d at 25ºC in the dark. Following this period, the cultures were checked for morphological characteristics as described by Halleen *et al.* (2004) and Abreo *et al.* (2010). Monosporic isolates were obtained and stored in the mycological collections of the Laboratory of Phytopathology of the Federal University of Santa Maria (Universidade Federal de Santa Maria - UFSM), Santa Maria, Brazil, and of the Embrapa Grape and Wine (Embrapa Uva e Vinho), Bento Gonçalves, Brazil.

Molecular characterization

Fungal mycelia and conidia were collected from the cultures grown in PDA for 2 weeks, at 20°C, in the dark. Pathogen DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method (Dellaporta *et al.*, 1983). The internal transcribed spacer (ITS), β-tubulin (TUB) and histone H3 (HIS) regions were amplified by polymerase chain reaction (PCR), using genomic DNA samples and the primer pairs ITS1 and ITS4 (White *et al.*, 1990), Bt2a and Bt2b, and H3-1a and H3-1b (Glass and Donaldson, 1995), respectively.

The PCR reactions were conducted in a final volume of 50 μL containing 1 μL DNA, 10 μL 5× GoTaq Reaction Buffer (Promega), 1 μL dNTPs mix, 1 μL of each primer, 0.2 μL GoTaq DNA polymerase (Promega) and autoclaved Milli-Q water to reach the final volume. The reactions were performed using a GeneAmp PCR System 2400 thermocycler (Perkin Elmer), under the following temperature conditions: an initial step at 94°C for 2 min, followed by 40 cycles consisting of a denaturation step at 94°C for 30 s, annealing step at 56°C for 30 s and elonga-

Table 1. Details of the *Campylocarpon pseudofasciculare* isolates studied.

Isolate	Origin (Region of Brazil)	Rootstock / Cultivar	GenBank accession numbers		
			ITS	TUB	HIS
Cy1UFSM	Fagundes Varela	Bordô* (*V. labrusca*)	-	KF633144	KF633164
Cy2UFSM	Guaporé	VR 043-43 (*V. rotundifolia* × *V. vinifera*) / BRS Violeta (hybrid – *Vitis* spp.)	KF447564	KF633145	KF633173
Cy3UFSM	Erechim	Rupestris du Lot (*V. rupestris*) / Niagara Rosada (*V. labrusca*)	KF447565	KF633146	KF633166
Cy6UFSM	Garibaldi	Niagara Branca* (*V. labrusca*)	KF447566	KF633147	KF633169
Cy14UFSM	Farroupilha	Bordô* (*V. labrusca*)	KF447567	KF633148	KF633158
Cy17UFSM	Nova Pádua	RR 101-14 (*V. rupestris* × *V. riparia*) / Isabel (*V. labrusca*)	KF656730	KF633149	KF633161
Cy18UFSM	Monte Belo do Sul	1103 P (*V. berlandieri* × *V. rupestris*) / Chardonnay (*V. vinifera*)	KF447568	KF633150	KF633162
Cy19UFSM	Farroupilha	1103 P (*V. berlandieri* × *V. rupestris*) / Isabel (*V. labrusca*)	KF447569	KF633151	KF633163
Cy20UFSM	Nova Roma do Sul	Isabel[a] (*V. labrusca*)	KF447570	KF633152	KF633165

[a] Own-rooted cultivar

tion step at 72°C for 1 min, and a final extension at 72°C for 4 min. At the end of the reaction, the PCR products were kept at 4°C. A negative control containing no DNA was included. The amplified fragments and the control were analyzed by electrophoresis in 0.8% agarose gel stained with ethidium bromide (1 μg L^{-1}), in TAE 1× buffer (0.04 M Tris-acetate + 1 mM EDTA), and visualized under ultraviolet light. The 1 kb Plus DNA Ladder (Invitrogen) was used as a molecular weight marker. The PCR products were purified according to Schmitz and Riesner (2006), using polyethylene glycol 6000 (PEG 6000).

Sequencing was performed using a 3500 XL Genetic Analyzer (Applied Biosystems). The sequenced fragments were analyzed using the BioEdit software (Hall, 1999). The nucleotide sequences obtained from the isolates were compared with sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank). The GenBank sequences with the highest similarity scores were selected and aligned with the sequences obtained from the sequencing, using the ClustalW algorithm. In addition, a phylogenetic analysis was conducted by applying the MEGA software, version 4 (Tamura *et al.*, 2007) with 1,000 bootstrap replicates. The obtained sequences were deposited in GenBank (Table 1).

Morphological characterization

Campylocarpon pseudofasciculare isolates were grown on PDA at 25ºC in the dark for 7–14 d for describing overall macroscopic characteristics (colony transparency, texture, density, colour, growth and growth margin). The Rayner (1970) mycological colour chart was used to determine the colony colour (front and back). For growth measurements, 8 mm diam. mycelial discs were removed from the margin of colonies, placed at the center of Petri dishes containing PDA (five replicates per isolate) and kept at 20ºC in the dark. Colony size was measured after an incubation of 7 d, using a digital caliper in two perpendicular directions, and mean colony diameter was obtained. Sporulation was quantified after 14 d, by adding 20 mL sterile distilled water to the surface of the colony. The surface was scraped using a Drigalski spatula; the obtained suspension was filtered through two layers of gauze, and the conidial concentration was estimated (conidia mL^{-1}) using a Neubauer chamber. Chlamydospores were de-

scribed from 40-d-old PDA cultures (Mohammadi *et al.*, 2009). To measure dimensions of conidia, isolates were grown on synthetic nutrient-poor agar (SNA) (Nirenberg, 1976) to which two 1 cm^2 pieces of filter paper were added and kept at 20°C for 5 weeks in the dark. For each isolate, 30 measurements were performed of each structure (chlamydospores and macroconidia with one, two, three and four septa) using an Olympus BX41 microscope (1000× magnification). Images were captured using a Leica ICC50 digital video camera on a Leica DM500 microscope. Sporodochium formation was described on carnation leaf agar (CLA) (Fisher *et al.*, 1982) after 30 d incubation.

Pathogenicity test

The pathogenicity test was performed for all isolates (Table 1). The inoculum was retrieved from PDA cultures as described above but with 10 mL sterile distilled water added to each colony and the inoculum concentration was adjusted to 10^6 conidia mL^{-1} with sterile distilled water. Four-month-old rooted cuttings of *V. labrusca* cv. Bordô were used. Prior to inoculation, the seedlings were removed from pots, and the roots were washed with running water and slightly pruned using sterile scissors. The roots were dipped in the conidial suspension obtained from each isolate for 60 min (Cabral *et al.*, 2012b), with a total of ten plants inoculated with each isolate. Following inoculation, the plants were transplanted into 1 L plastic bags containing Mec Plant commercial substrate and maintained in a greenhouse at 25 ± 2ºC under a 12 h light/12 h dark photoperiod. A month after the beginning of the experiment, the plants were re-inoculated using a 40 mL conidium suspension (10^6 conidia mL^{-1}) per plant to insure sufficient root infection (Alaniz *et al.*, 2007). The control treatment received only sterile distilled water for both inoculation and re-inoculation. Four months after the beginning of the experiment, the plants were removed from the plastic bags, the roots were washed in running water to eliminate the substrate, and the symptoms were evaluated. For the shoots, a 0 to 5 grading scale was used: 0 = healthy plant (control treatment), 1 = 0 to 20% reduction of leaf mass, 2 = 20 to 40% reduction, 3 = 40 to 60% reduction, 4 = 60 to 80% reduction, and 5 = greater than 80% reduction of leaf mass, drying of the shoot or plant death. Root symptoms for each plant were evaluated according to a scale adapted from Alaniz *et al.* (2007), with grades from 0 to 5: 0 = healthy plant without necrotic lesions, 1 = 0 to 10% reduction of root mass, 2 = 10 to 25% reduction, 3 = 25 to 50% reduction, 4 = more than 50% reduction of root mass, and 5 = plant death. The shoot and root dry mass of each plant was measured, with plant material dried in a forced-air oven at 60°C until a constant weight was reached. For re-isolation of the pathogen, ten wood fragments from the basal region (2 cm above the cutting's lower end) of each plant were superficially disinfected by washing in 70% alcohol and 1% sodium hypochlorite, followed by three washes in sterile distilled water (1 min each). The wood fragments were dried on sterile filter paper and transferred to Petri dishes containing PDA amended with 0.5 g L^{-1} streptomycin sulfate. The plates were incubated at 20ºC for 14 d in the dark. Following that period, the proportion of wood tissue fragments from which *C. pseudofasciculare* colonies were recovered in relation to the total fragments obtained from each plant was calculated. The experimental design was completely randomized. Analysis of variance was performed on the data, followed by a Scott-Knott test ($P \leq 0.05$) whenever a significant effect was observed. The data were $\sqrt{(x + 1)}$ transformed prior to analysis. The SISVAR 5.3 (Sistema de Análise de Variância Para Dados Balanceados [Variance Analysis System for Balanced Data]) software package was used for all ststistical tests (Ferreira, 2010).

Results

Molecular characterization

Fragments generated in the sequencing reactions with primers ITS1 and ITS4 were 508 to 639 bp long. The histone H3 sequence was of length 515 bp and the β-tubulin sequence was 287 bp.

Due to their genetic similarity, sequences of *C. pseudofasciculare* and *C. fasciculare* were used for constructing phylogenetic dendrograms. *Ilyonectria macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado and *Ilyonectria robusta* (A.A. Hildebr.) A. Cabral & Crous sequences were used as outgroups. Analyses based on the three analyzed regions identified the studied isolates as *C. pseudofasciculare* (Figure 1). The clade comprising sequences on the studied isolates and sequences of reference strains of *C. pseudofasciculare* received satisfactory support in bootstrap analyses. The dendrogram derived from

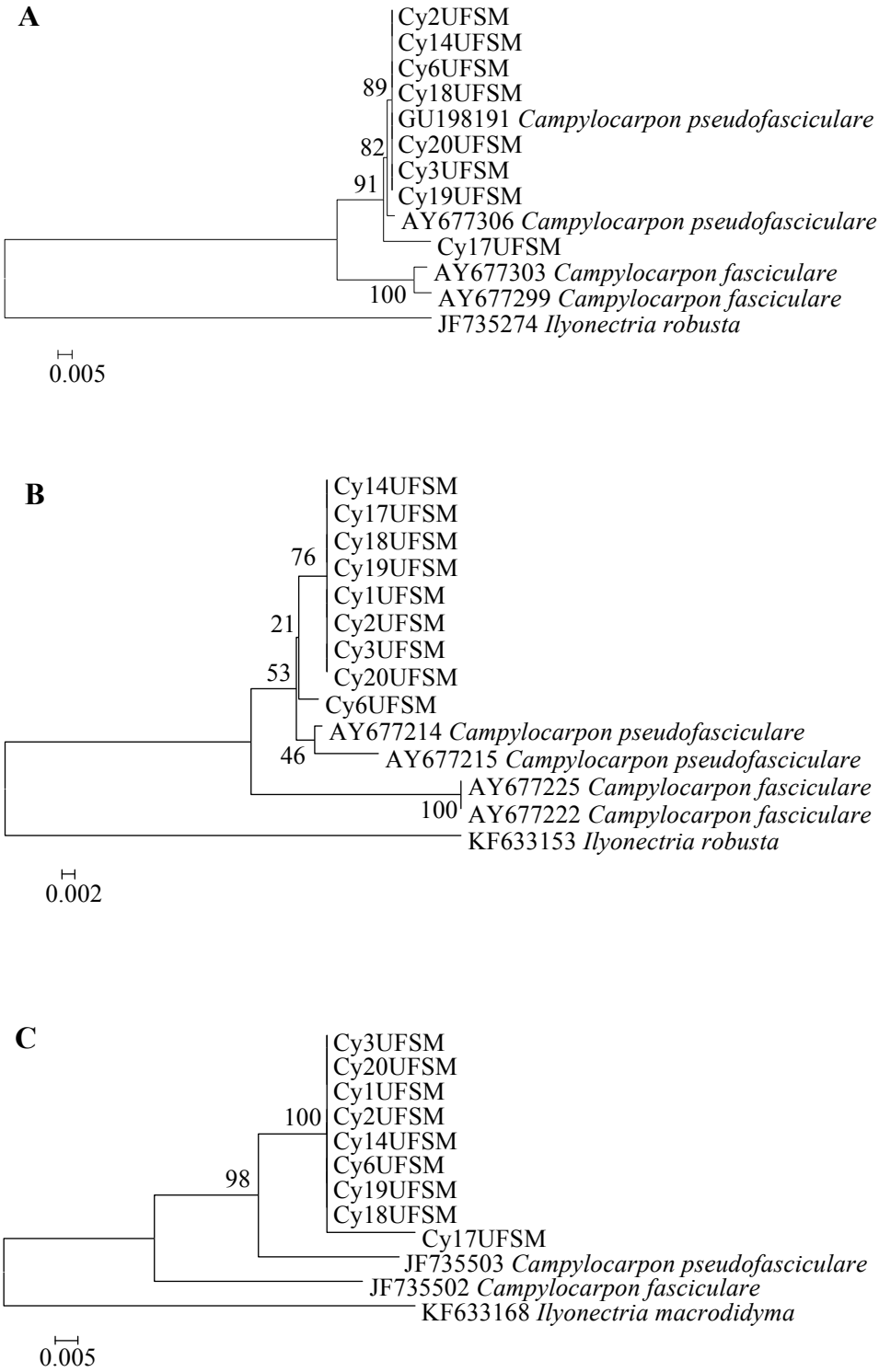

Figure 1. Phylogenetic dendrograms designed for the *Campylocarpon pseudofasciculare* isolates according to "neighbour-joining", derived from sequences of the ITS rDNA (A), β-tubulin (B) and histone H3 (C) regions and based on 1,000 bootstrap replicates. The evolutionary distances were calculated using the Kimura 2-parameter model. The figures at the branches are bootstrap values.

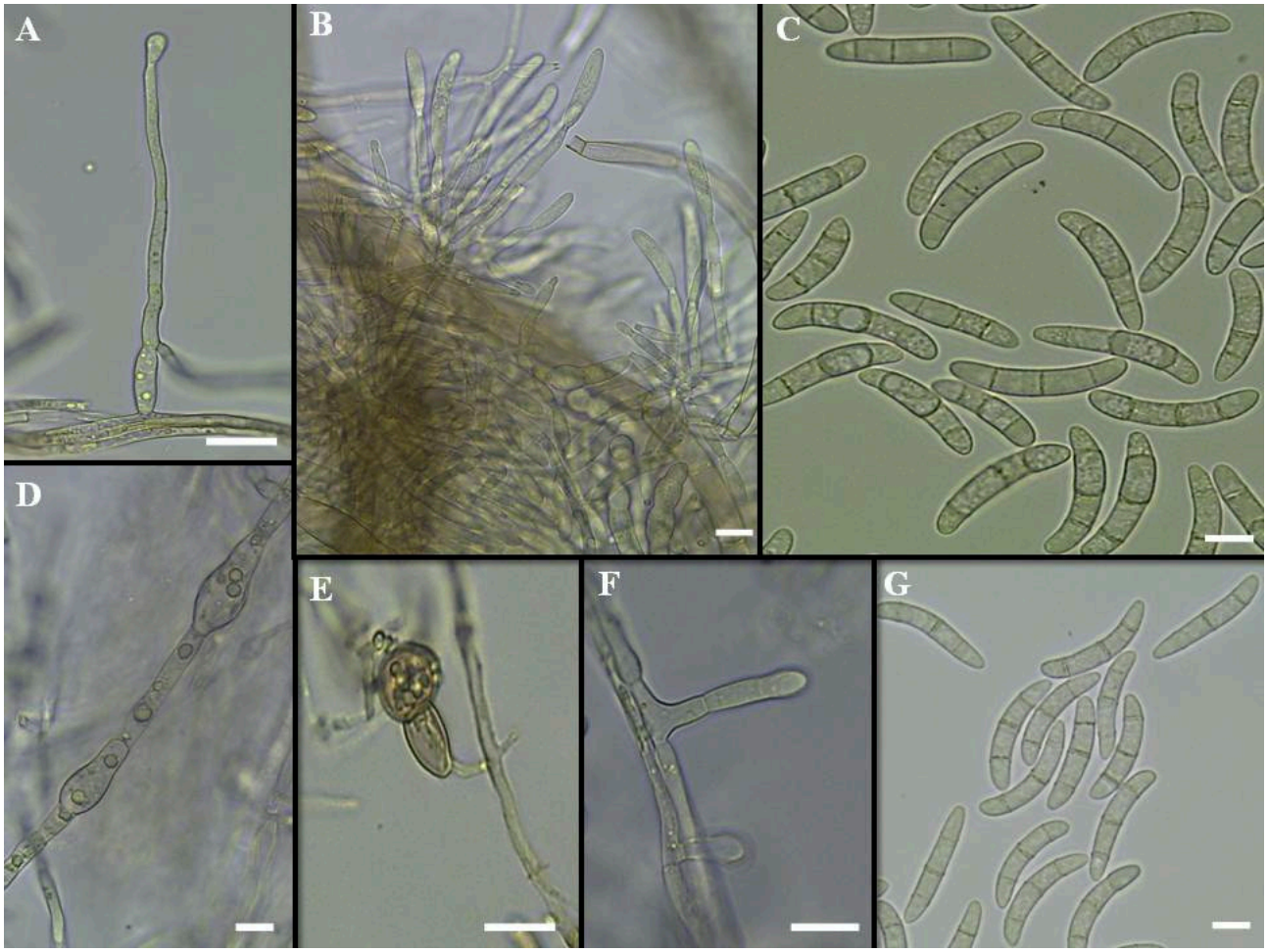

Figure 2. *Campylocarpon pseudofasciculare* (isolate Cy6UFSM): (A, F) simple conidiophores in aerial mycelium; (B) fascicles of branched conidiophores; (C, G) macroconidia; (D, E) chlamydospores in mycelium. Bars = 10 μm.

histone H3 region grouped all new isolates in the same clade, with high bootstrap support (100%), and with 98% similarity to the *C. pseudofasciculare* isolate used as a reference (JF735503).

Morphological characterization

After 10 d of growth on PDA all studied isolates had homogeneous culture transparency, felty to cottony surface texture of colonies, medium colony density, absence of zonation, smooth colony margins, umber to chestnut upper surface colour and sienna to chestnut reverse. All the isolates formed white to light brown sporodochia on the surfaces of the leaves and agar.

The isolates had slightly curved macroconidia with 1–5, mostly 3 septa and had a mean length of 38.0 μm and mean width of 7.0 μm (mean of all isolates). Macroconidia varied in size depending on the number of cells as follows: one-septate (15–36 × 4.5–7 μm); two-septate (16–38 × 5–8 μm); three-septate (29–42 × 5–8 μm); and four-septate (34–45 × 5–8 μm). Macroconidia with five septa were rare. Microconidia were not observed. Chlamydospores were spherical but seen rarely (Figure 2). The observed morphological characters of the nine isolates agreed with those described for *C. pseudofasciculare*, confirming the molecular inferences. Sporulation varied from 5.9×10^5 (isolate Cy3UFSM) to 33.5×10^5 conidia mL^{-1} (Cy14UFSM), showing different capacities for

Table 2. Characteristics of the colonies, macroconidia and chlamydospores of *Campylocarpon pseudofasciculare* isolates grown in PDA and SNA media, and pathogenic to *Vitis labrusca* cv. Bordô.

Isolate		Macroconidia dimensions (septa) (µm) [a, b]				Chlamydospore dimensions (µm) [a, c]	Sporulation ($\times 10^5$ conidia mL^{-1}) [c]	Colony diameter (mm) [c]
		1	2	3	4			
Cy1UFSM	Length	–	–	(36–) 38.5 (–41)	(40–) 41.2 (–43)	(6–) 9.5 (–15.5)	15.61	41.34
	Width	–	–	(5–) 6.7 (–8)	(5–) 6.3 (–7)			
Cy2UFSM	Length	(15–) 17.7 (–21)	(16–) 19.5 (–24)	(34.5–) 36.3 (–40) (6–) 7 (–8)	(34–) 38.3 (–42)	(6–) 7.4 (–8.5)	15.78	43.82
	Width	(5–) 5.4 (–6)	(5–) 5.4 (–7)		(6–) 6.8 (–8)			
Cy3UFSM	Length	–	–	(36–) 38.2 (–41)	(39–) 40.4 (–43)	(6–) 8.3 (–10)	5.91	39.39
	Width	–	–	(6.5–) 6.9 (–7)	(6.5–) 6.9 (–8)			
Cy6UFSM	Length	(20–) 27.2 (–34)	(27–) 32.8 (–38)	(32–) 37.8 (–41)	–	(7–) 7.6 (–8.5)	7.63	43.01
	Width	(5.5–) 5.9 (–7)	(6–) 6.5 (–7)	(7–) 7.5 (–8)	–			
Cy14UFSM	Length	–	–	(29–) 37.9 (–41)	(38–) 39.5 (–40.5)	(6.5–) 7.5 (–8.5)	33.51	41.79
	Width	–	–	(7–) 7.1 (–7.5)	(7–) 7.3 (–7.5)			
Cy17UFSM	Length	–	–	(36–) 37.6 (–41)	(37.5–) 40.4 (–43)	(3.5–) 4.4 (–6)	29.53	43.15
	Width			(5.5–) 6.6 (–7)	(6–) 6.9 (–7.5)			
Cy18UFSM	Length	(19–) 27.2 (–36)	(28–) 29.6 (–32)	(35–) 38.1 (–41)	–	(7–) 10 (–12.5)	24.28	44.11
	Width	(4.5–) 5.5 (–7)	(5–) 5.6 (–6)	(6–) 7 (–8)	–			
Cy19UFSM	Length	–	–	(35–) 38.8 (–42)	(37–) 39.5 (–42)	(7–) 8.5 (–10.5)	25.59	43.55
	Width	–	–	(6.5–) 6.8 (–7)	(6.5–) 6.9 (–7)			
Cy20UFSM	Length	(19–) 23.7 (–31)	(22–) 27.2 (–30)	(36–) 38.6 (–41)	(37–) 40.4 (–45)	(7.5–) 11.4 (–12)	6.68	41.03
	Width	(5–) 6.3 (–7)	(6–) 7 (–8)	(6.5–) 7.3 (–8)	(7–) 7.1 (–7.5)			

– Absent.
[a] Averages were derived from measurements of 30 structures (conidia and chlamydospore) for each isolate (range minimum and maximum in parentheses).
[b] SNA medium with the addition of two pieces of 1 cm^2 filter paper.
[c] PDA medium.

conidium production by the different isolates. Colonies reached a diameter at day 7 between 39 and 44 mm. Chlamydospore diameter varied from (3.5–) 4.4 (–6) μm (Cy17UFSM) to (7.5–) 11.4 (–12) μm (Cy20UFSM) (Table 2).

Pathogenicity test

All tested *C. pseudofasciculare* isolates were pathogenic to *V. labrusca* cv. Bordô. After 4 months, the inoculated plants were dead or showed a combination of disease symptoms consisting of decreased root masses, root and crown necrosis, vessel darkening, shoot mass reduction and wilting. All studied isolates had negative effects on plant shoots, significantly differing from the control plants. The isolate Cy18UFSM had a significantly lower disease score (2.0) than the remaining isolates and thus was the least virulent. The severity of score for root symptoms, varied from 2.2 to 4 for the inoculated plants, significantly differing from the control treatment. However, the isolates did not statistically differ from each other (Table 3). The lowest re-isolation percentage from wood fragments was observed for Cy19UFSM (60%), although it did not differ significantly from that for isolates Cy3UFSM, Cy14UFSM, Cy18UFSM and Cy20UFSM, confirming Koch's postulates. The control plants yielded 0% *C. pseudofasciculare* re-isolation.

Discussion

This is the first study reporting *C. pseudofasciculare* from vineyards in Rio Grande do Sul, Brazil. The isolates obtained from black foot in different grapevine genetic material from southern Brazil were identified as *C. pseudofasciculare* on the basis of a multi-locus molecular analysis and morphological characterization. The other species of the genus, *C. fasciculare*, has also been identified associated with grapevines, however, from other locations (Halleen *et al.*, 2004; Alaniz *et al.*, 2011; Correia *et al.*, 2012). The isolates used in the present study were obtained from symptomatic grapevines, as in the study performed in Uruguay by Abreo *et al.* (2010). Halleen *et al.* (2004) obtained isolates from asymptomatic plants in South African vineyards.

Abreo *et al.* (2010), on the basis of ITS sequences only, separated isolates associated with black foot into six species: *C. pseudofasciculare*; *Cylindrocarpon de-*

Table 3. Mean shoot and root severity scores, and re-isolations percentages, for different *Campylocarpon pseudofasciculare* isolates inoculated on to *Vitis labrusca* cv. Bordô, after 4 months incubation in a greenhouse.

Isolate	Shoot disease severity[a, b]	Root disease severity[b, c]	% Re-isolation
Control	0.0 a[d]	0.0 a[d]	0.0 a[d]
Cy1UFSM	5.0 c	2.8 b	90.0 c
Cy2UFSM	3.8 c	3.8 b	90.0 c
Cy3UFSM	5.0 c	2.6 b	72.5 b
Cy6UFSM	5.0 c	4.0 b	90.0 c
Cy14UFSM	3.0 c	3.2 b	72.5 b
Cy17UFSM	4.4 c	3.4 b	90.0 c
Cy18UFSM	2.0 b	2.2 b	70.0 b
Cy19UFSM	4.0 c	3.8 b	60.0 b
Cy20UFSM	3.0 c	3.8 b	72.5 b

[a] Shoot symptoms were rated using following scale: 0 = healthy (control treatment), 1 = 0 to 20% shoot mass reduction, 2 = 20 to 40% reduction, 3 = 40 to 60% reduction, 4 = 60 to 80% reduction, and 5 = > 80% shoot mass reduction, drying or plant death.
[b] Transformed means (√(x+1)).
[c] Root symptoms were rated using the following scale: 0 = healthy with no lesions, 1 = 0 to 10% root mass reduction, 2 = 10 to 25% reduction, 3 = 25 to 50% reduction, 4 = > 50% root mass reduction, and 5 = death [Adapted from Alaniz *et al.* (2007)].
[d] In each column, values followed by the same letter do not differ according to Scott–Knott ($P \leq 0.05$).

structans var. *crassum* (Wollenw.) C. Booth; *Cyl.* (*Ilyonectria*) *liriodendri* J. D. MacDon. & E. E. Butler; *Cyl.* (*Ilyonectria*) *macrodydimum* Schroers, Halleen & Crous; *Cyl. olidum* var. *crassum* Gerlach; and *Cyl. pauciseptatum* Schroers & Crous. High bootstrap values were also reported for the sequencing of the ITS rDNA region and β-tubulin, grouping the two *Campylocarpon* species in different clades (Halleen *et al.*, 2004). Alaniz *et al.* (2007) reported little variation in partial sequences of β-tubulin obtained from several *Ilyonectria macrodidyma* isolates. Cabral *et al.* (2012a; 2012c) analyzed isolates of the *I. macrodidyma* and *I. radicicola* complex and observed histone H3 as a more efficient region for differentiating the species. The combination of sequencing results from different DNA regions increases the reliability of identification at the species level.

The use of molecular DNA analysis techniques has allowed the development of rapid, sensitive and specific methods for phytopathogen diagnosis. The Rayner (1970) colour chart, used in the present study for describing colonies, has also been used elsewhere in studies of the causal agents of black foot of grapevine (Cabral *et al.*, 2012a, 2012c; Reis *et al.*, 2013).

Halleen *et al.* (2004) reported that *C. fasciculare* and *C. pseudofasciculare* are phylogenetically closely related. When looking for conidiophore fascicles in *C. pseudofasciculare*, they only observed isolated, mostly branched, conidiophores. *Campylocarpon pseudofasciculare* exhibited produced few conidia and no conidiophore fascicles, which could be an artifact of culturing the fungus in sub-optimal conditions. In the present study, conidiophore fascicles were observed in *C. pseudofasciculare* isolates on CLA, SNA and PDA media. The size range of macroconidia obtained in the present study were similar to those observed by Halleen *et al.* (2004) for two *C. pseudofasciculare* isolates, except for the presence of one-septate macroconidia, observed in the present study. *Campylocarpon fasciculare* was reported by Alaniz *et al.* (2011) to display three-septate macroconidia measuring (37.5–) 48 (–52.5) × (6.3–) 7.5 (–8.8) μm.

According to Abreo *et al.* (2010), the presence of chlamydospores is the main characteristic that separates the two known *Campylocarpon* species. Species of *Campylocarpon* form brownish colonies while those of *Thelonectria* (*Neonectria*) *mammoidea* have violet shades. *Campylocarpon* is also characterized by brownish hyphae (Halleen *et al.*, 2004). *Thelonectria mammoidea* was also associated with grapevine black foot in USA and Canada (Petit *et al.*, 2011).

The pathogenicity test described here showed that all isolates studied resulted in symptoms typical of black foot in American grapevines cv. Bordô, affecting roots and shoots. The plants also exhibited symptoms typical of the disease when inoculated with *Ilyonectria* sp. (as *Cyl. destructans*) (Garrido *et al.*, 2004). Grapevines inoculated with *C. fasciculare* displayed symptoms after 20 d, consisting of decreased vigour, chlorosis and interveinal necrosis in leaves, necrotic lesions with decreased root mass and plant death (Alaniz *et al.*, 2011).

Alaniz *et al.* (2007) tested the pathogenicity of *I. macrodidyma* and *I. liriodendri* isolates, which also cause black foot, to 110 R rootstocks and observed less symptom severity in roots (disease scores between 0.93 and 2.15) than those observed in the present study. This is possibly due to genetic differences between the fungal species causing black foot and also to the plant material used in the assay, exhibiting different levels of susceptibility. Inoculation with *C. pseudofasciculare* obtained from roots of asymptomatic nursery grapevines from South Africa led to the death of approximately 17.5% of cv. Ramsey plants (Halleen *et al.*, 2004).

Campylocarpon pseudofasciculare is associated with grapevines exhibiting typical symptoms of black foot in southern Brazil. The isolates displayed a range of morphological characteristics, and were pathogenic for *V. labrusca* cv. Bordô. The present study will serve as a basis for future studies of the epidemiology of this disease and possibilities for effective management of black foot in Brazilian vineyards.

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