Grapevine Trunk Diseases in British Columbia: Incidence and Characterization of the Fungal Pathogens Associated with Black Foot Disease of Grapevine

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Abstract

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Black foot disease of grapevines, caused by several fungal species in the genera *Campylocarpon*, *Cylindrocarpon*, *Cylindrocladiella*, and *Ilyonectria*, causes significant economic losses to the grapevine industry worldwide. This study represents the first attempt to identify and characterize the fungal pathogens associated with black foot disease of grapevines in British Columbia (BC). Field surveys conducted throughout all grape-growing regions in BC that included assessment of foliar symptomatology and isolations from symptomatic vines showed *Cylindrocarpon/Ilyonectria* spp. occurred in 32 of 90 (35.5%) young vineyards surveyed (≤ 8 year old) and in 41 of 215 (19%) samples collected. In 20 of the 41 (48.8%) samples, *Cylindrocarpon/Ilyonectria* spp. were the sole fungi isolated from symptomatic tissue.

In the rest of the samples, black foot fungi were found to primarily coexist with fungal taxa associated with Petri disease of grapevines. Colony and conidia phenotypical characterization, along with DNA analyses of the internal transcribed spacer region (ITS1-5.8S-ITS2) of the rDNA, and part of the β -tubulin and translation elongation factor 1- α genes, revealed five different black foot fungi occurring in declining young vines in BC, namely *Cylindrocarpon pauciseptatum*, *Ilyonectria liriodendri*, *Ilyonectria macrodidyma*, *Ilyonectria robusta*, and *Ilyonectria torresensis*. Pathogenicity studies showed all five species to be highly virulent in the grapevine rootstock cultivar 3309C. Overall, *I. liriodendri* and *I. macrodidyma* were the most virulent species when inoculated in *Vitis vinifera* ‘Chardonnay’ and rootstock 3309C.

Black foot is a grapevine trunk disease that with Petri disease is part of the young vine decline complex (YVD) (16). Black foot disease symptoms were first described in grapevines in France under the name of “gangrene” in the early 1960s (25), and although the disease is long known to occur in grapes, it has become a worsening problem in both nurseries and young commercial vineyards since the early 1990s (16). Black foot primarily affects young grapevines (up to 8 years old), and symptoms of the disease are characterized by sunken necrotic root lesions with a reduction in root biomass and root hairs. Internally, necrotic tissue and plugged xylem vessels with black inclusion and/or tyloses can be observed in cross-section at the basal end of the trunk. Aboveground, vines can either show lack of spring growth or stunted growth characterized by short shoot internodes and small chlorotic leaves, which lead to eventual death of the vines either during the growing season or during the following winter (5,21,32). Since large investments in vineyard establishment and maintenance are needed before full crop and subsequently cash returns are obtained, a long lifespan for vines is essential for economic success in vineyards. Hence, the decline and death of young vines can cause significant economic losses to the grape industry. Black foot disease is now known to occur in all major grape-growing regions including, Europe (5,35), the Near East (28), Oceania (22,49), South Africa (22), and North and South America (1,6,31,32).

Cylindrocarpon destructans (Zinns.) Scholten was originally thought to be the main and most widespread causal organism of black foot disease worldwide (17,32,35). However, *C. destructans* isolates from symptomatic grapevines from different continents were revealed to be *Cylindrocarpon liriodendri* J.D. MacDonald &

E.E. Butler (teleomorph: *Ilyonectria liriodendri* (Halleen, Rego & Crous) P. Chaverri & C. Salgado) based on morphological characters and multi-gene phylogenies (11,23,34). In addition, although *Cylindrocarpon obtusisporium* (Cooke & Harkness) Wollenweber was identified as the cause of black foot disease in Italy (18) and California (37), the role that this species plays in the syndrome is still uncertain. Morphological similarities between *C. obtusisporium* and *Cylindrocarpon macrodidymum* Schroers, Halleen & Crous (teleomorph: *Ilyonectria macrodidyma* (Halleen, Schroers & Crous) P. Chaverri & C. Salgado) macroconidia along with recent field surveys supported by DNA-based identification methods suggested that *C. obtusisporium* isolates from grapevines were misidentified and were probably *C. macrodidymum* (22,32). Based on recent morphological, molecular, and pathogenicity studies, 18 different fungal species are currently known to be associated with black foot disease of grapevines, including *Campylocarpon fasciculare* Schroers, Halleen & Crous, *Campylocarpon pseudofasciculare* Halleen, Schroers & Crous, *Cylindrocarpon pauciseptatum* Schroers & Crous, *Cylindrocladiella parva* (P.J. Anderson) Boesewinkel, *Cylindrocladiella peruviana* (Batista, J.L. Bezerra & M.P. Herrera) Boesewinkel, *Ilyonectria alcacerensis* A. Cabral, Oliveira & Crous, *Ilyonectria europaea* A. Cabral, Rego & Crous, *Ilyonectria estremocensis* A. Cabral, Nascimento & Crous, *I. liriodendri*, *Ilyonectria lusitanica* A. Cabral, Rego & Crous, *I. macrodidyma*, *Ilyonectria novazelandica* A. Cabral & Crous, *Ilyonectria pseudodestructans* A. Cabral, Rego & Crous, *Ilyonectria robusta* (A.A. Hildebr.) A. Cabral & Crous, *Ilyonectria torresensis* A. Cabral, Rego & Crous, *Ilyonectria vitis* A. Cabral, Rego & Crous, and two as yet uncharacterized *Ilyonectria* species known as *Ilyonectria* sp. 1 and *Ilyonectria* sp. 2 (2,8,10,22–24,32,39).

Grapevines (*Vitis vinifera* L. and *Vitis* spp.) are the third largest fruit crop in Canada, with 11,784 ha under cultivation and a farm gate value of over \$112.5 million CAD in 2011 (42). To date, 16 species of *Cylindrocarpon* Wollenweber have been reported to occur in over 55 hosts in Canada including native and introduced forest tree species as well as economically important annual and perennial crops (7,12,14,40,41). However, the status and impacts of this genus on grapevines has long been overlooked. Seifert and Axelrood (40) first reported one isolate of *Cylindrocarpon*

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destructans var. *destructans* from roots and stems of *V. vinifera* in Vineland, Ontario. However, a later phylogenetic study showed this isolate to be misidentified and was renamed *Cylindrocarpon* sp. (41). A recent field survey conducted by Petit et al. (31) throughout grape-growing regions of northeastern United States and south-eastern Canada revealed that *Cylindrocarpon didymum* (Harting) Wollenweber, *I. liriodendri*, and *I. macrodidyma* occur in black foot symptomatic vines in the province of Québec in Canada. Nowadays, no information is available about the status of black foot disease of grapevines in British Columbia (BC), which after Ontario is the second largest grape-growing province in Canada. Consequently, this study was undertaken to determine the occurrence of black foot disease in young vineyards throughout BC and to characterize the different species associated with this disease in the province by means of morphological characters, multi-gene sequencing, and pathogenicity studies.

Materials and Methods

Field surveys and fungal isolation. To assess the incidence of black foot in BC, 90 young vineyards (≤ 8 years old) were surveyed throughout all grape growing regions of the province, including the Okanagan, the Similkameen, and the Fraser valleys, Vancouver Island, and the Gulf Islands between 2008 and 2012. In total, 215 samples (entire or portions of a plant) were collected and brought to the laboratory to identify the different black foot fungi occurring in symptomatic vascular tissue from grapevines showing characteristic decline in the field. Grapevine samples were first surface-sterilized by submerging them in 4.5% sodium hypochlorite for 5 min and washed twice with sterile distilled water. After air-drying, sampled vines were inspected for presence of necrotic roots, and if detected, fungi from roots were isolated as described by Petit and Gubler (32). Additionally, samples were sectioned to reveal the vascular symptoms in the trunk, and fungi associated with symptomatic tissue were isolated as described by Úrbez-Torres et al. (45). Small pieces of symptomatic tissues from trunk or roots were incubated on potato dextrose agar (PDA) (Difco, Detroit, MI) plates amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich, St Louis, MO) (PDA-tet) at 20°C in the dark until fungal colonies were observed. Colonies were individually transferred to

fresh PDA. Afterwards, pure cultures were obtained by hyphal-tipping from colony margins and by placing them again on fresh PDA plates at 20°C in the dark. Pure culture colonies were used to conduct a preliminary morphological identification of the different fungi isolated from symptomatic grapevine tissue.

Morphological characterization. Among all the different fungi isolated from symptomatic tissue, *Cylindrocarpon*-like species were first tentatively classified in different groups based on previously described macroscopic and microscopic characters of the colony (color, texture, and type of growing margin) and conidia (shape and number of septa), respectively (5,22,32). Pure cultures from representative isolates of each group were selected to measure the length and width of each type of conidium observed (microconidia and one- to five-septa macroconidia) including chlamydospores if present (Table 1). Small colonized agar plugs (approximately 9 mm²) were removed from 15-day-old pure culture colony margins and examined microscopically. Pictures of the different conidia present in each selected sample were taken at $\times 40$ magnification using a Zeiss Axiophot compound microscope with DIC optics and captured using a Nikon D700 digital camera. The length and width of at least 10 conidia per type per isolate were measured using the Image-Pro Plus software (v4.5.1.29, Media Cybernetics, Inc.). For each type of conidium: minimum, lower limit of a 95% interval; mean, upper limit of a 95% interval; and maximum were calculated for both length and width measurements using summary statistics in SAS (v9.3, SAS Institute, Cary, NC). Additionally, macroscopic and microscopic morphological characteristics were compared with reference isolates including ex-type specimens obtained from the Centraalbureau voor Schimmelcultures (CBS) collection in Utrecht, the Netherlands (Table 2).

Molecular characterization and multi-gene analysis. Fungal isolates selected for DNA extraction were cultured on PDA and grown to a 3- to 4-cm-diameter colony (Table 1). Total genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA). Oligonucleotide primers ITS1 and ITS4, Bt2a and Bt2b, and EF1 and EF2 were used to amplify the ITS1-5.8S-ITS2 gene (ITS), a portion of the β -tubulin (BT), and part of the translation elongation factor 1- α (EF1- α)

Table 1. *Cylindrocarpon* and *Ilyonectria* species isolated from *Vitis vinifera* in British Columbia used in this study

Species	Isolate ^b	Cultivar ^c	Collector	GenBank accession numbers ^a		
				ITS	BT	EF1- α
<i>C. pauciseptatum</i>	PARC82*	SO4	P. Haag & D.T. O’Gorman	KF511996	KF511968	KF511981
	DAOM 242314					
<i>C. pauciseptatum</i>	PARC407	3309C	J.R. Úrbez-Torres	KF511997	KF511969	KF511982
	DAOM 242315					
<i>I. liriodendri</i>	PARC60	Chardonnay ^d	P. Haag & D.T. O’Gorman	KF511998	KF511970	KF511983
<i>I. liriodendri</i>	PARC72*	Pinot Gris ^d	P. Haag & D.T. O’Gorman	KF511999	KF511971	KF511984
	DAOM 242317					
<i>I. liriodendri</i>	PARC340*	3309C	J.R. Úrbez-Torres	KF512000	KF511972	KF511985
	DAOM 242318					
<i>I. liriodendri</i>	PARC393	SO4	J.R. Úrbez-Torres	KF512001	KF511973	KF511986
<i>I. macrodidyma</i>	PARC345*	3309C	J.R. Úrbez-Torres	KF512002	KF511974	KF511989
	DAOM 242320					
<i>I. macrodidyma</i>	PARC398*	3309C	J.R. Úrbez-Torres	KF512003	KF511975	KF511990
	DAOM 242321					
<i>I. robusta</i>	PARC358*	Pinot Gris ^d	J.R. Úrbez-Torres	KF512004	KF511976	KF511991
	DAOM 242322					
<i>I. robusta</i>	PARC391*	3309C	J.R. Úrbez-Torres	KF512005	KF511977	KF511992
	DAOM 242323					
<i>I. torresensis</i>	PARC14*	3309C	P. Haag & D.T. O’Gorman	KF512006	KF511978	KF511993
	DAOM 242324					
<i>I. torresensis</i>	PARC35*	3309C	P. Haag & D.T. O’Gorman	KF512007	KF511979	KF511994
	DAOM 242325					
<i>I. torresensis</i>	PARC142	3309C	J.R. Úrbez-Torres	KF512008	KF511980	KF511995

^a ITS: internal transcribed spacer. BT: β -tubulin. EF1- α : translation elongation factor 1- α .

^b PARC: Pacific Agri-Food Research Centre. DAOM: Department of Agriculture Ottawa Mycology. Isolates used in the pathogenicity studies are indicated with an asterisk.

^c Grapevine (pure *Vitis vinifera* or rootstock) material from which the fungus was isolated.

^d Self-rooted.

gene, respectively (15,29,48). Each polymerase chain reaction (PCR) contained 2 µl of 10× PCR buffer containing 15 mM MgCl₂, 1.6 µl of 25 mM MgCl₂, 1 µl of 10 mM dNTPs, 0.5 µl of 0.5 mM of each primer, 0.1 µl of Ultra Therm DNA polymerase (BocaScientific, Boca Raton, FL), and 1 µl of DNA template adjusted with micropore filtered water to a final volume of 20 µl. ITS, BT, and EF1-α amplification reactions were carried out on a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA) following previously described ITS (48), BT (15), and EF1-α (29) temperature profiles. Amplification products were purified using a QIAquick PCR purification Kit (QIAGEN Inc., Valencia, CA). Both strands of the ITS, BT, and EF1-α were sequenced using a 48-capillary 3730 DNA Analyzer (Applied Biosystems) in the NAPS Unit at the Michael Smith Laboratories, University of

British Columbia, Vancouver. Sequences were edited and assembled using Lasergene SeqMan Pro version 9.1.1.4 (DNASTAR Inc., Madison, WI). Consensus sequences were then compared with those available in the GenBank database by using the Basic Local Alignment Search Tool (BLAST) to verify the preliminary morphological identification and select closely related sequences for the multi-locus phylogenetic analyses.

Fungal sequences from grapevines from BC were aligned with published GenBank sequences, including ex-type specimens from grapevines and other hosts for comparison using the computer software BioEdit Sequence Alignment Editor Version 7.1.3.0 (19) (Table 2). Alignments were corrected visually and manually edited. Single phylogenetic analyses for each loci data set were conducted using Maximum Parsimony (MP) and the heuristic search option

Table 2. Fungal species used for morphological comparison and phylogenetic analyses

Species	Isolate ^b	Host / Origin	Collector	GenBank accession numbers ^a		
				ITS	BT	EF1-α
<i>Campylocarpon fasciculare</i>	CBS 112613^c	<i>Vitis vinifera</i> / South Africa	F. Halleen	AY677301	AY677221	JF735691
<i>Campylocarpon pseudofasciculare</i>	CBS 112679^c	<i>V. vinifera</i> / South Africa	F. Halleen	AY677306	AY677214	JF735692
<i>Cylindrocarpon cylindroides</i>	CBS 503.67^c	<i>Abies alba</i> / Norway	F. Roll-Hansen	AY677261	JF735436	JF735789
<i>Cylindrocarpon obtusisporium</i>	CBS 183.36^c	<i>Solanum tuberosum</i> / Germany	H.W. Wollenweber	AM419061	AM419085	JF735796
<i>C. obtusisporium</i>	CPC 13544	<i>Prunus armeniaca</i> / Canada	J.A. Traquair	AY295306	JF735443	JF735797
<i>Cylindrocarpon pauciseptatum</i>	CBS 120171^c	<i>Vitis</i> sp. / Slovenia	M. Žerjav	EF607089	EF607066	JF735776
<i>C. pauciseptatum</i>	CBS 120498	<i>Vitis</i> sp. / Slovenia	M. Žerjav	EF607087	EF607072	JF735774
<i>C. pauciseptatum</i>	CBS 100819	<i>Erica melanthera</i> / New Zealand	H.M. Dance	EF607090	EF607067	JF735771
<i>Cylindrocarpon theobromicola</i>	CBS 218.67^c	<i>Theobroma cacao</i> / Papua New Guinea	P.G. Hicks	EF607092	EF607062	KF511967
<i>Ilyonectria alacerensis</i>	CBS 129087	<i>V. vinifera</i> / Portugal	A. Cabral & H. Oliveira	JF735333	AM419111	JF735819
<i>Ilyonectria anthuricola</i>	CBS 564.95	<i>Anthurium</i> sp. / Netherlands	R. Pieters	JF735302	JF735430	JF735768
<i>Ilyonectria coprosmae</i>	CBS 119606	<i>Metrosideros</i> sp. / Canada	G.J. Samuels	JF735260	JF735373	JF735694
<i>Ilyonectria cyclaminicola</i>	CBS 302.93	<i>Cyclamen</i> sp. / Netherlands	M. Hooftman	JF735304	JF735432	JF735770
<i>Ilyonectria estremocensis</i>	CBS 129085	<i>V. vinifera</i> / Portugal	C. Rego & T. Nascimento	JF735320	JF735448	JF735806
<i>Ilyonectria europaea</i>	CBS 129078	<i>V. vinifera</i> / Portugal	C. Rego	JF735294	JF735421	JF735756
<i>Ilyonectria gamsii</i>	CBS 940.97^c	Soil / Netherlands	J.T. Poll	AM419065	AM419089	JF735766
<i>Ilyonectria liliigena</i>	CBS 189.49	<i>Lilium regale</i> / Netherlands	M.A.A. Schipper	JF735297	JF735425	JF735762
<i>Ilyonectria liriodendri</i>	CBS 110.81^c	<i>Liriodendron tulipifera</i> / USA, CA	J.D. MacDonald & E.E. Butler	DQ178163	DQ178170	JF735696
<i>I. liriodendri</i>	CBS 112595 ^c	<i>V. vinifera</i> / South Africa	F. Halleen	AY677263	AY677247	KF511987
<i>I. liriodendri</i>	CBS 112599 ^c	<i>V. vinifera</i> / France	n/a	AY677266	AY677243	KF511989
<i>Ilyonectria lusitanica</i>	CBS 129080	<i>V. vinifera</i> / Portugal	N. Cruz	JF735296	JF735423	JF735759
<i>Ilyonectria macrodidyma</i>	CBS 112615^c	<i>V. vinifera</i> / South Africa	F. Halleen	AY677290	AY677233	JF735836
<i>I. macrodidyma</i>	CBS 112594	<i>V. vinifera</i> / South Africa	F. Halleen	AY677282	AY677231	JF735832
<i>I. macrodidyma</i>	Cy140	<i>V. vinifera</i> / Portugal	C. Rego & T. Nascimento	JF735343	JF735472	JF735840
<i>Ilyonectria mors-panacis</i>	CBS 306.35	<i>Panax quinquefolium</i> / Canada	A.A. Hildebrand	JF735288	JF735414	JF735746
<i>Ilyonectria novozelandica</i>	CBS 113552	<i>Vitis</i> sp. / New Zealand	B. Bonfiglioli	JF735334	AY677237	JF735822
<i>I. novozelandica</i>	Cy116	<i>Vitis vinifera</i> / USA, CA	W.D. Gubler	AJ875322	JF735461	JF735824
<i>Ilyonectria panacis</i>	CBS 129079	<i>P. quinquefolium</i> / Canada	K.F. Chang	AY295316	JF735424	JF735761
<i>Ilyonectria pseudodestructans</i>	CBS 129081	<i>V. vinifera</i> / Portugal	C. Rego	AJ875330	AM419091	JF735752
<i>Ilyonectria radiculicola</i>	CBS 264.65^c	<i>Cyclamen persicum</i> / Sweden	L. Nilsson	AY677273	AY677256	JF735695
<i>Ilyonectria robusta</i>	CBS 308.35	<i>P. quinquefolium</i> / Canada	A.A. Hildebrand	JF735264	JF735377	JF735707
<i>I. robusta</i>	CBS 773.83 ^c	Water in aquarium / Netherlands	J. Hemelraad	AY677276	AY677254	JF735708
<i>Ilyonectria rufa</i>	CBS 153.37	Dune sand / France	F. Moreau	AY677271	AY677251	JF735729
<i>Ilyonectria</i> sp. 1	CBS 162.89	<i>Hordeum vulgare</i> / Netherlands	M. Barth	AM419060	AM419084	JF735799
<i>Ilyonectria</i> sp. 2	CBS 173.37	<i>Pinus laricio</i> / England	T.R. Peace	JF735319	JF735447	JF735803
<i>Ilyonectria torresensis</i>	CBS 129086	<i>V. vinifera</i> / Portugal	A. Cabral	JF735362	JF735492	JF735870
<i>I. torresensis</i>	CBS 112609	<i>Vitis</i> sp. / Australia	M. Sweetingham	AY677289	AY677226	JF735849
<i>I. torresensis</i>	CPC 13533	<i>V. vinifera</i> / Canada	H.F. Dias	AY295332	JF735480	JF735852
<i>Ilyonectria venezuelensis</i>	CBS 102032	Bark / Venezuela	A. Rossman	AM419059	AY677255	JF735760
<i>Ilyonectria vitis</i>	CBS 129082	<i>V. vinifera</i> / Portugal	C. Rego	JF735303	JF735431	JF735769
<i>Neonectria ditissima</i>	CBS 226.31	<i>Fagus sylvatica</i> / Germany	H.W. Wollenweber	JF735309	DQ789869	JF735783
<i>Neonectria faginata</i>	CBS 217.67^c	<i>Fagus grandifolia</i> / Canada	G.L. Stone	HQ840385	JF268730	JF268746
<i>Neonectria macroconidialis</i>	CBS 119596	<i>Astelia</i> sp. / New Zealand	G.J. Samuels	JF735259	JF735372	JF735693
<i>Neonectria major</i>	CBS 240.29	<i>Alnus incana</i> / Norway	H.W. Wollenweber	JF735308	DQ789872	JF735782
<i>Neonectria neomacrospora</i>	CBS 118984	<i>Arceuthobium tsugense</i> / Canada	L. Reitman	JF735311	DQ789882	JF735787
<i>Neonectria ramulariae</i>	CBS 151.29	<i>Malus sylvestris</i> / England	H.W. Wollenweber	JF735314	JF735439	JF735792
<i>Neonectria veuillotiana</i>	NZFS3602	<i>Pinus radiata</i> / New Zealand	n/a	JQ013490	JQ013500	JQ013501

^a GenBank accession numbers in italics were generated in this study. ITS: internal transcribed spacer. BT: β-tubulin. EF1-α: translation elongation factor 1-α.

^b Isolate numbers in bold represent ex-type specimens.

^c Fungal isolates obtained from CBS (Centraalbureau Schimmelcultures, Utrecht, Netherlands) and used for morphological comparison.

with 1,000 random additional sequence replicates with Close-Neighbor-Interchange (CNI) algorithm in MEGA5 (43), and tree topologies were compared (individual trees not shown). A multi-locus phylogenetic analysis was performed on the combined ITS, BT, and EF1- α datasets using MP as previously described. Tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated. The combined ITS, BT, and EF1- α datasets were further analyzed by Neighbor-Joining (NJ) using the Maximum Composite Likelihood method. Bootstrap support was estimated using 1,000 replicates to assess the robustness of each clade in both MP and NJ phylogenetic methods. Sequences from fungal isolates collected in this study were deposited into GenBank, and representative isolates are maintained in the grapevine trunk disease fungal collection at the Pacific Agri-Food Research Centre in Summerland, BC, and in the Canadian Collection of Fungal Cultures in Ottawa, Ontario (Table 1).

Pathogenicity studies. Isolates representing the five different species identified from symptomatic wood tissue were selected to determine their pathogenicity in 'Chardonnay' and 3309C rootstock cultivars (Table 1). Nine isolates for Chardonnay and eight for 3309C rootstock were used. In the first experiment, dormant canes of Chardonnay were collected from an experimental vineyard located at the Pacific Agri-Food Research Centre in Summerland in mid-March of 2012. Six canes per fungal isolate were inoculated using 10-day-old mycelium plugs as described by Úrbez-Torres et al. (45). Twelve additional canes were inoculated with noncolonized PDA plugs for negative controls. Inoculated canes were immediately placed on a metal rack in 6 liter transparent plastic containers with a tight-fitting lid containing 400 ml of distilled water to maintain a humid environment. Treatments were arranged in a completely randomized design with 12 canes per container. Inoculated canes were collected after 31 days of incubation at room temperature and inspected for lesion development. Each cane was sectioned longitudinally through the point of inoculation, and the extent of vascular discoloration was measured upward and downward from the point of inoculation using a digital caliper (Mitutoyo Corporation, Tokyo, Japan). Ten small pieces (approximately 9 mm²) of necrotic tissue from the edge of each lesion were cut and placed on PDA-tet in an attempt to recover the inoculated fungus and complete Koch's postulates. Fungal identity was verified based on colony and conidial morphology.

The second pathogenicity experiment was conducted in 1-year-old dormant rooted vines of 'Baco Noir' grafted on 3309C rootstock. Inoculum from each isolate was prepared as described by Alaniz et al. (5), and conidial concentration was adjusted with a hemacytometer to 10⁶ conidia per ml. Before inoculation, roots were trimmed and disinfested by immersion for 5 min in a 4.5% sodium hypochlorite solution. After air-drying, six vines per fungal isolate were inoculated by immersing the roots and the basal end of the trunk in the spore solution overnight. Twelve control plants were immersed in sterile water. After inoculation, rooted vines were planted in 4-liter pots containing a standard potting soil mixture. Plants were maintained in a greenhouse (27 ± 5°C, 30 to 40% relative humidity) in a completely randomized design and watered as needed. Twenty-one weeks after inoculation, growth characteristics of the grapevines, including total shoot length, average internode length, total pruning weight, length of the longest root, and dry root weight, were compared to the control plants (9). Additionally, roots and the basal end of the rootstock of each plant were inspected for necrosis and vascular discoloration, respectively. Both roots and the basal end of the rootstock were first disinfested as previously described. After air-drying, 10 small pieces of necrotic tissue from two different roots (5 pieces each) were placed on PDA-tet in an attempt to recover the inoculated fungus and complete Koch's postulates. Subsequently, the rootstock was sectioned transversely at the basal end. Transversal sections were scanned and the total necrotic area was calculated using the Fiji image processing computer program (38). Rootstocks were then sectioned longitudinally, and the extent of

vascular discoloration was measured from the basal end using a digital caliper. Ten small pieces of necrotic tissue from the edge of each lesion were cut and placed on PDA-tet in an attempt to recover the inoculated fungus and complete Koch's postulates.

Data from both pathogenicity studies and grapevine growth parameters were independently analyzed by one-way analysis of variance (ANOVA) conducted using the SAS GLM Procedure. Homogeneity of variance was tested using Levene's test. Residuals were visually inspected for each treatment, and when necessary a log₁₀ transformation was used to improve homogeneity of variance. Treatment means were compared using Tukey's test at the 5% significant level.

Results

Incidence of black foot disease of grapevines in BC. Characteristic symptoms of declining young vines were observed in 68 of the 90 young vineyards surveyed (75.5%) and affected all grape-growing regions in BC. During the early part of the growing season (April to June), symptomatic young vines were stunted and characterized by short shoot internodes and chlorotic leaves in either the entire vine or part of it (Fig. 1A and B). Within each vineyard, symptomatic vines appeared to be distributed either as several single vines randomly located throughout the vineyard (Fig. 1A) or as a group of vines located in a specific area of the vineyard (Fig. 1C). Throughout the growing season (July to September), shoots of some of the symptomatic vines progressively dried out beginning with the leaves which consequently dropped and then the clusters which shriveled (Fig. 1D). This vine collapse was observed to affect the entire vine, a single cordon, or just one or more spur positions in a cordon. Vascular symptoms were primarily characterized by dark streaking of the wood in the trunk, especially developing from the basal end (Fig. 1E). Cross-sections of the basal end of symptomatic trunks revealed a necrotic area around the pith, which in some cases was characterized by the presence of tyloses plugging the xylem vessels (Fig. 1F). Depending on the level of infection, the necrotic area extended just 1 to 2 mm from the pith or had affected the entire section of the trunk (Fig. 1G). Additionally, wedge-shape necrotic lesions were occasionally observed in thick mature roots and extending into the basal end of the trunk (Fig. 1H). Many symptomatic vines had sunken necrotic roots, which in some cases were associated with the syndrome known as "J-root" (Fig. 1I). Fungal species associated with black foot disease occurred in 32 of 90 (35.5%) young vineyards surveyed and were isolated from 41 of 215 (19%) samples collected (Table 3). In 20 of the 41 (48.8%) samples, *Cylindrocarpon/ Ilyonectria* spp. were the sole fungi isolated from symptomatic tissue (Table 3). In the rest of the samples, black foot fungi were found to primarily coexist with fungal taxa associated with Petri disease of grapevines and to a lesser extent with other grapevine trunk disease pathogens in the Botryosphaeriaceae family and the *Diaporthe/Phomopsis* genera (Table 3). Although black foot disease pathogens were isolated from necrotic roots observed in vines characterized by either poor or no spring growth, the majority of *Ilyonectria/Cylindrocarpon* spp. in this study were isolated from the necrotic tissue observed at the basal end of the trunk (rootstock or self-rooted cultivar) (Table 3).

Morphological characterization. *Cylindrocarpon*-like colonies developing from symptomatic tissue were first identified by the characteristic saffron and/or buff colony color on PDA (10,23,32) (Fig. 2A). Both micro- and macroconidia were produced by all isolates on PDA, and measurements from each type of conidium from each species including the ex-type specimens are given in Table 4. Colony and conidia morphology allowed us to separate all isolates in five different morphological groups by comparison to reference isolates from CBS and previously published data (10,23,32).

The first group included five isolates and was first characterized by a light cream, raised mycelium with slightly lobulated colony margins. With age, the colonies became yellowish to golden-yellow in the center (Fig. 2B). Colony growth on PDA averaged

Fig. 1. Young vine decline symptoms observed in British Columbia. **A and B,** Stunted growth observed in late spring and characterized by chlorotic leaves and short shoot internodes. **C,** Declining of young vines affecting a significant part of the vineyard. **D,** Vine collapse observed during the growing season and characterized by leaf drop and shriveling of clusters. **E,** Dark-brown to black streaking of the wood could be observed in young vines developing from the basal end of the trunk. **F,** Cross sections at the basal end of the trunk revealed vascular necrosis around the pith. **G,** Fungi were observed to colonize the entire section of the trunk. **H,** Cross section of roots from symptomatic plants revealed total rot necrosis (white arrow) and/or wedge-shaped necrosis. **I,** "J-root" syndrome was observed to be associated with many declining young vines.

72 mm diameter after 17 days at 20°C. Morphological characteristics of micro- and macroconidia (Fig. 2G), the latter having predominantly three septa, were consistent with the description of *C. pauciseptatum* (39). Macroconidia of *C. pauciseptatum* isolates from BC were slightly longer than those recorded from the ex-type specimen CBS 120171 (Table 4). The second group included 20 isolates and was characterized by a light to dark brown, flat mycelium, with slightly lobulated light orange colony margin (Fig. 2C). Its colony growth on PDA averaged 78 mm diameter after 17 days at 20°C. Dimensions and morphology of micro- and macroconidia (Fig. 2H), the latter having predominantly one-septum, were in concordance with the *I. liriodendri* published description (23) and the ex-type specimen CBS 110.81 (Table 4). Isolates within this group developed globose to subglobose chlamydospores (8.9–18.8×9.7–15.7 µm) (*n* = 45) formed singly or in chains (Fig. 2I). The third group included 11 isolates and was characterized by a yellowish, felty mycelium, with an even amber-yellow colony margin (Fig. 2D). Colony growth on PDA averaged 47 mm diameter after 17 days at 20°C. Reverse colony was characterized by a strong orange-dark brown color. Colony morphology was in concordance with previous descriptions of *I. macrodidyma* (5,32). Macroconidia were predominantly three septate, but four and five septa were also observed (Fig. 2J). Length of micro- and macroconidia were longer than those recorded from the *I. macrodidyma* ex-type specimen CBS 112615, which only had one- and two-septate macroconidia (Table 4). The fourth group included two isolates and was characterized by a light- to dark-brown, flat mycelium, with an even dark orange colony margin (Fig. 2E). Its colony growth was the fastest among all groups, covering the 85 mm diameter petri plate after 17 days. Macroconidia had predominantly one-septum but two and three septa were also observed (Fig. 2K). Colony morphology and macroconidial shape resemble those described for *I. robusta* (8). However, length of macroconidia from BC isolate PARC358 was longer than those recorded from the *I. robusta* reference isolate CBS 773.83, which only had one- and two-septate macroconidia (Table 3). The last morphological group included three isolates and was characterized by a yellowish, raised mycelium with a pronounced lobulated uneven sepia color colony margin. Isolates from this group had the slowest growth on PDA, reaching only 32 mm diameter after 17 days at 20°C (Fig. 2F). Dimensions and morphology of micro- and macroconidia (Fig. 2L), the latter having predominantly one septum, resembled those described for *I. macrodidyma*. Isolates within this group were first classified as *Cylindrocarpon* sp. and were identified to species based on DNA sequence analyses.

Molecular characterization and multi-gene analysis. PCR amplifications of selected regions of ITS, BT, and EF1- α gave products of approximately 500, 400, and 800 bp, respectively. To evaluate the phylogenetic relationships among the different *Cylindrocarpon/Ilyonectria* isolates obtained from grapevines in BC, their ITS, BT, and EF1- α sequences were analyzed by BLAST to confirm the preliminary morphological identification; to identify isolates from the unidentified morphological group five to species

and to select closely related sequences for the multi-locus phylogenetic analyses (Table 2). Sixty taxa were included in the phylogenetic analysis of the combined ITS, BT, and EF1- α dataset using MP and NJ. The alignment contained 1,923 characters including gaps. The MP evolutionary history generated 33 equally parsimonious trees, of which one is shown in Figure 3. The NJ analysis resulted in a tree with similar topology as the MP tree. The black foot isolates from BC grapevines separated into five different groups based on morphological characteristics, grouped in five different corresponding clades based on the phylogenetic analysis of the combined ITS, BT, and EF1- α dataset. The first clade, with bootstrap values of 90% (MP) and 87% (NJ) consisted of *I. robusta* isolates from BC, which had nearly identical sequences to those of the *I. robusta* ex-type specimen CBS 308.35 from ginseng (*Panax quinquefolium* L.) from Canada (Fig. 3). The second clade, with bootstrap values of 99% (MP) and 100% (NJ) grouped *I. liriodendri* isolates from BC with *I. liriodendri* ex-type specimen CBS 110.81 from tulip tree (*Liriodendron tulipifera* L.) from California and with reference isolates CBS 112595 and CBS 112599 from grapevines from South Africa (Fig. 3). The third clade, with bootstrap values of 99% (MP) and 100% (NJ) included *C. pauciseptatum* isolates from BC, which grouped together with *C. pauciseptatum* isolates from *Vitis* sp. from Slovenia including the ex-type specimen CBS 120171 and with one isolate from ruby shepherd (*Erica melanthera* L.) from New Zealand. The last clade, with bootstrap values of 70% (MP) and 80% (NJ) was formed by two different subclades with bootstrap values lower than 50%, which included BC isolates from morphological groups three and five, preliminarily identified as *I. macrodidyma* and *Cylindrocarpon* sp., respectively (Fig. 3). Within these two subclades, *I. macrodidyma* isolates from BC grouped together with *I. macrodidyma* isolates from grapevines from South Africa and Portugal including the ex-type specimen CBS 112615 (Fig. 3); and *I. torresensis* isolates from BC, which could not be identified to species based on morphological characters, grouped together with *I. torresensis* isolates from grapevines from Australia, Canada, and Portugal, including the ex-type specimen CBS 129086 (Fig. 3).

Pathogenicity studies. The extent of vascular discoloration caused by inoculating the fungal isolates on detached Chardonnay dormant canes averaged between 11 and 5.6 mm 31 days after inoculation and were significantly larger (*P* < 0.0001) than those of controls treated with noncolonized agar plugs (2.1 mm) (Fig. 4A and J). In this experiment, *I. macrodidyma* isolates PARC345 and PARC398 caused the longest lesions among all inoculated fungal species (Fig. 4A). Percent recovery was higher than 90% for all isolates, and fungi obtained from the reisolations were the same as those previously inoculated based on colony and conidia morphology (Fig. 4C). No *Cylindrocarpon/Ilyonectria* sp. was isolated from the controls.

Twenty-one weeks after inoculation, all Baco Noir vines grafted on 3309C rootstock and inoculated with either *Ilyonectria* or *Cylindrocarpon* spp. showed chlorotic leaves with a characteristic scorching at the edge (Fig. 4K). In several of the inoculated plants, a substantial reduction in vigor, characterized by poor shoot

Table 3. Total number of samples yielding black foot fungal species from grapevine vascular symptoms in young vineyards surveyed in British Columbia

Species isolated	No. of samples	Isolated from		
		Trunk ^a	Roots	Trunk ^c + roots
<i>Cylindrocarpon</i> sp.	20	14	2	4
<i>Cylindrocarpon</i> sp. + Petri disease pathogen ^b	13	10	0	3
<i>Cylindrocarpon</i> sp. + Petri disease pathogen ^b + other ^c	4	3	0	1
<i>Cylindrocarpon</i> sp. + other ^c	4	2	0	2
Total number of samples yielding black foot fungi	41			
Total number of samples collected	215			

^a *Cylindrocarpon* spp. were primarily isolated from necrotic tissue observed at the basal end of the trunk.

^b *Cylindrocarpon* spp. were isolated along with Petri disease fungal pathogens (*Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., and *Cadophora* spp.) from the same sample.

^c *Cylindrocarpon* spp. were isolated along with other grapevine trunk disease pathogens, including species in the Botryosphaeriaceae family and in the *Diaporthe/Phomopsis* genera.

growth, was observed when compared with the controls. Internal necrosis developing acropetally at the basal end of the 3309C root-stock was observed in all inoculated plants (Fig. 4M and N). The extent of vascular discoloration measured from the basal end averaged between 57.5 and 41.7 mm and was larger ($P < 0.0001$)

than that of the water-treated controls (4.3 mm) (Fig. 4B). In this experiment, *I. liriodendri* isolate PARC70 caused the longest lesion among all inoculated species (Fig. 4B). Percent recovery from the edge of the necrotic lesion varied between 53.3 and 65% (Fig. 4C). Necrotic roots were also observed in all inoculated vines

Fig. 2. Colony and conidia morphology of *Cylindrocarpon/Ilyonectria* species isolated from declining young vines in British Columbia. **A**, *Cylindrocarpon/Ilyonectria* developing from infected roots on PDA-tet. Colony (**B**) and conidia (**G**) morphology of *Cylindrocarpon pauciseptatum* PARC82. Colony (**C**), conidia (**H**) and chlamydospores (**I**) morphology of *Ilyonectria liriodendri* PARC60. Colony (**D**) and conidia (**J**) morphology of *I. macrodidyma* PARC345. Colony (**E**) and conidia (**K**) morphology of *I. robusta* PARC358. Colony (**F**) and conidia (**L**) morphology of *I. torresensis* PARC35. Bars G to L = 10 μm.

(Fig. 4O). Percent recovery from inoculated roots varied between 46.7% for *I. torresensis* isolate PARC35 and *I. robusta* isolate PARC391 and 78.3% for *I. robusta* isolate PARC358 (Fig. 4C). Fungi obtained from reisolation of both rootstock and roots were

the same as the previously inoculated based on colony and conidia morphology. No *Ilyonectria* or *Cylindrocarpon* spp. were reisolated from the controls. The necrotic cross-sectional area measured at the base of the rootstock ranged from 46.8% for *I. robusta*

Table 4. Conidial dimensions of the different *Cylindrocarpon* and *Ilyonectria* species from British Columbia used in this study and comparison with ex-type specimens

Species/isolate ^b	Septa ^c	Macroconidia ^a			Microconidia ^a		
		<i>n</i>	L/W	Length × width (µm)	<i>n</i>	L/W	Length × width (µm)
<i>C. pauciseptatum</i>	PARC82	0	...	...	100	2.1	(5.1-)6.2-6.3-6.4(-12.2)×(2.8-)4.0-4.1-4.2(-5.9)
	1	20	6.4	(25.5-)40.1-44-47.9(-56.2)×(5.2-)6.5-6.8-7.1(8.3)	...	...	...
	2	10	7.2	(32.6-)39.9-43.6-47.3(-50.6)×(5.2-)5.7-6.1-6.5(-7.1)	...	...	...
	3	100	7	(35.4-)46.2-47.3-48.5(-67.5)×(5.2-)6.7-6.8-6.9(-9.3)	...	...	...
	CBS 120171	0	...	...	100	1.6	(4.0-)5.5-5.7-5.9(-10.4)×(2.2-)3.5-3.6-3.7(-5.2)
	1	15	4.6	(31.5-)35.7-37.8-39.8(-44.0)×(6.6-)7.7-8.3-8.9(-10.3)	...	...	...
	2	15	4.8	(32.2-)36.2-37.6-39.1(-41.7)×(6.4-)7.4-7.8-8.2(-8.9)	...	...	...
	3	100	4.9	(31.7-)39.2-39.8-40.4(-49.3)×(6.2-)8.0-8.2-8.4(-10.1)	...	...	...
	PARC60	0	...	...	100	2.3	(3.5-)7.0-7.3-7.6(-10.7)×(2.4-)3.1-3.2-3.3(-4.3)
	1	100	4.7	(14.6-)21.1-21.7-22.4(-29.3)×(3.6-)4.5-4.6-4.8(-5.8)	100	3.1	(8.1-)10.9-11.3-11.6(-15.3)×(2.6-)3.5-3.6-7.1(-4.7)
<i>I. liriodendri</i>	2	35	5.3	(19.0-)24.4-25.5-26.6(-31.9)×(3.3-)4.6-4.9-5.1(-6.4)	...	...	...
	3	20	5	(20.4-)25.2-25.4-25.6(-30.3)×(4.3-)4.9-5.1(-5.7)	...	...	...
	PARC340	0	...	...	100	2.5	(4.3-)7.1-7.5-7.8(-11.9)×(2.0-)2.9-3.1(-4.3)
	1	100	5	(13.2-)21.1-21.8-22.5(-28.0)×(3.1-)4.7-4.8-4.9(-6.9)	60	3.4	(9.5-)11.6-12.0-12.4(-15.3)×(2.7-)3.5-3.6-3.7(-4.6)
	2	15	5.2	(23.6-)26.2-26.6-27.0(-28.5)×(4.3-)5.0-5.1-5.2(-5.8)	...	...	...
	3	10	6	(26.5-)27.6-28.7-29.9(-31.1)×(4.4-)4.5-4.8-5.1(-5.7)	...	...	...
	PARC393	0	...	...	100	2.5	(5.3-)7.7-7.9-8.2(-11.6)×(2.0-)3.1-3.2-3.3(-4.6)
	1	100	4.3	(14.5-)19.0-19.5-20.0(-27.6)×(3.6-)4.4-4.5-4.6(-6.2)	100	3.1	(8.2-)10.7-11.0-11.2(-13.7)×(2.6-)3.5-3.6-7.1(-5.8)
	2	70	4.7	(19.0-)22.7-23.3-23.8(-29.7)×(4.3-)4.8-4.9-5.0(-5.7)	...	...	...
	3	50	4.8	(22.3-)24.7-25.2-25.7(-28.6)×(4.4-)5.1-5.2-5.3(-6.3)	...	...	...
	CBS 110.81	0	...	...	100	2.5	(4.0-)6.9-7.2-7.5(-12.4)×(2.0-)2.8-2.9-3(-3.8)
	1	90	5.1	(14.8-)22.3-23.1-24(-32.1)×(2.9-)4.4-4.5-4.6(-5.9)	100	3.4	(8.5-)11.1-11.4-11.7(-15.7)×(1.7-)3.3-3.4-3.5(-4.9)
	2	40	5.6	(17.4-)25.2-26.6-28.1(-39.4)×(2.9-)4.6-4.8-5.0(-5.9)	...	...	...
	3	100	6.1	(20.9-)31.0-32.0-32.9(-42.3)×(3.8-)5.2-5.3-5.4(-6.8)	...	...	...
	CBS 112595	0	...	...	80	2.7	(3.3-)7.4-7.8-8.1(-11.8)×(1.5-)2.8-2.9-3(-4.6)
<i>I. macrodityna</i>	1	100	5.2	(16.0-)22.6-23.3-24.0(-30.9)×(3.1-)4.4-4.5-4.6(-6.6)	30	3.7	(7.1-)10.8-11.4-12(-14.1)×(2.1-)2.9-3.1-3.3(-4.4)
	2	100	5.6	(20.7-)26.4-26.8-27.2(-31.9)×(3.1-)4.8-4.9-5.0(-6.4)	...	...	...
	3	85	5.7	(23.8-)28.5-28.9-29.3(-33.4)×(3.9-)5.0-5.1-5.2(-6.3)	...	...	...
	PARC345	0	...	...	45	3.8	(12.6-)19.7-19.8-19.9(-22.9)×(3.3-)5.3-5.6-5.9(-7.5)
	1	65	5.9	(30.4-)39.6-41.6-43.5(-62.4)×(5.3-)6.8-7.0-7.2(-8.8)	...	...	...
	2	65	6.5	(35.0-)47.9-49.5-51.0(-64.2)×(5.9-)7.4-7.6-7.8(-9.1)	...	...	...
	3	100	7.2	(41.8-)54.5-55.4-56.4(-69.9)×(5.7-)7.6-7.8-7.9(-9.9)	...	...	...
	4	20	7.9	(52.2-)59.1-61.8-64.4(-71.2)×(6.6-)7.5-7.8-8.1(-9.2)	...	...	...
	5	10	10.1	(53.4-)68.6-77.5-86.5(-94.3)×(7.2-)7.3-7.7-8.1(-9.0)	...	...	...
	PARC398	0	...	...	100	3.5	(10.5-)17.4-17.5-17.6(-23.0)×(3.9-)6.0-6.1-6.2(-8.3)
	1	40	6.4	(37.4-)48.5-50.2-51.9(-61.4)×(6.2-)7.7-7.9-8.2(-10.0)	...	...	...
	2	20	6.2	(46.2-)49.3-50.9-52.6(-59.4)×(7.0-)8.0-8.3-8.6(-9.5)	...	...	...
	3	60	6.6	(44.5-)51.7-52.7-53.7(-62.6)×(6.6-)7.9-8.0-8.2(-9.6)	...	...	...
	4	10	8	(57.0)58.0-61.1-64.2(-70.8)×(7.2-)7.4-7.7-8.0(-8.7)	...	...	...
	CBS 112615	0	...	...	100	2.2	(3.6-)7.4-7.8-8.1(-12.3)×(1.8-)3.5-3.6-3.7(-5.2)
<i>I. robusta</i>	1	60	4	(12.9-)17.5-18.6-19.7(-31.6)×(2.2-)4.5-4.6-4.8(-6.6)	90	2.9	(8.8-)11.6-12.0-12.3(-15.7)×(2.9-)4.0-4.1-4.3(-5.8)
	2	20	5.7	(26.5-)27.5-28.7-29.9(-31.2)×(4.3-)4.7-5.1-5.5(-6.2)	...	...	...
	PARC358	0	...	...	100	2.1	(3.7-)7.1-7.3-7.6(-11.0)×(2.4-)3.4-3.5-3.6(-5.4)
	1	100	4	(14.9-)22.4-23.2-24.0(-31.0)×(4.0-)5.6-5.8-5.9(-9.4)	50	3.1	(7.9-)12.8-13.4-14.1(-18.3)×(3.4-)4.2-4.4-4.5(-5.7)
	2	20	4.4	(14.9-)24.2-26.8-29.4(-34.7)×(5.2-)5.9-6.2-6.4(-6.8)	...	...	...
	3	10	5.5	(26.1-)26.4-29.5-32.6(-33.6)×(4.4-)4.5-5.4-6.3(-7.1)	...	...	...
	CBS 773.83	0	...	...	100	2.2	(3.3-)6.0-6.2-6.4(-9.3)×(1.3-)2.9-3.0-3.1(-4.3)
	1	65	2.6	(9.3-)12.8-13.3-13.7(-19.3)×(3.2-)4.9-5.1-5.3(-7.2)	100	2.5	(6.3-)9.4-9.7-9.9(-12.0)×(2.2-)3.8-3.9-4.0(-5.6)
	2	15	3.8	(18.0-)19.9-21.1-22.2(-24.2)×(4.5-)5.3-5.6-6(-6.8)	...	...	...
	PARC14	0	...	...	50	2.8	(7.6-)8.8-11.0-13.3(-15.4)×(3.1-)3.6-4.0-4.4(-4.6)
<i>I. torresensis</i>	1	100	4.7	(21.7-)28.2-28.8-29.4(-35.2)×(4.4-)6.0-6.1-6.3(-7.7)	...	...	...
	2	10	5.5	(28.1-)30.8-33.0-35.1(-35.6)×(5.2-)5.6-6.0-6.3(-6.6)	...	...	...
	PARC35	0	...	...	35	3.4	(9.4-)12.5-14.2-15.9(-17.9)×(3.6-)3.8-4.2-4.7(-5.3)
	1	100	4.8	(22.4-)28.3-28.8-29.2(-34.3)×(4.0-)5.9-6.1-6.2(-8.3)	...	...	...
	2	10	5.3	(26.5-)29.4-31.7-34(-35.3)×(4.6-)5.6-6.1-6.6(-6.8)	...	...	...
	CBS 129086 ^d	0	...	...	30	2.9	(9.0-)11.8-12.3-12.7(-16.0)×(3.5-)4.2-4.3-4.4(-5.0)
	1	30	4.9	(20.0-)26.5-27.7-28.9(-43.0)×(4.5-)5.5-5.6-5.8(-7.0)	30	3.6	(11.0-)15.0-15.5-16.0(20.0)×(3.5-)4.3-4.4-4.5(-5.5)
	2	30	5.2	(24.0-)31.4-32.5-33.6(-44.0)×(5.0-)6.0-6.2-6.4(-8.0)	...	...	...
	3	30	5.8	(30.0-)38.3-39.4-40.6(-56.0)×(5.0-)6.7-6.8-7.0(-9.0)	...	...	...
	PARC35	0	...	...	35	3.4	(9.4-)12.5-14.2-15.9(-17.9)×(3.6-)3.8-4.2-4.7(-5.3)

^a *n*: Total number of conidia measured. L/W: Length width ratio. Length × width: (minimum-)lower limit 95% confidence interval-average-upper limit 95% confidence interval-(maximum).

^b Isolates in bold represent ex-type specimens.

^c Number of septa observed in the different type of both macroconidia and microconidia.

^d Macroconidia and microconidia measurements taken from Cabral et al. (9).

isolate PARC358 to 28.2% for *I. torresensis* isolate PARC14 (Fig. 4D). With the exception of these two isolates, the necrotic area did not differ among the isolates tested.

Grapevine vigor was reduced by some of the fungal treatments. Only vines inoculated with *I. macrodidyma* isolates PARC345 and

PARC340 showed significantly lower shoot weights (6.6 and 7.1 g, respectively) than those from control plants (Fig. 4E). Root dry weights were lower and significantly different for plants inoculated with *I. macrodidyma* isolate PARC345 (3.9 g), *I. robusta* isolate PARC358 (6 g), *I. liriodendri* isolate PARC340 (6.3 g), and *C.*

Fig. 3. One of 33 equally parsimonious trees resulting from a total of 958 positions in the final ITS, BT, and EF1- α combined dataset. Consistency index (CI) = 0.541, retention index (RI) = 0.881, retention composite index (RC) = 0.519. Isolates in bold represent *Cylindrocarpon*/*Ilyonectria* isolated from grapevines from British Columbia. Isolates in italics and underline represent ex-type specimens. Bootstrap values with 1,000 replications are shown above branches for Maximum Parsimony (MP) (only bootstrap values higher than 50% are shown) and below the branches for Neighbor-Joining (NJ). Branches represented with an * were not present in the NJ tree.

Fig. 4. Pathogenicity study of *Cylindrocarpon/Illyonectria* species isolated from declining young vines in British Columbia. **A**, Mean lesion length caused by nine isolates in detached 'Chardonnay' dormant canes. **B**, Mean lesion length caused by eight isolates in grapevine rootstock cv. 3309C measured from the basal end. **C**, Percentage of recovery of *Cylindrocarpon/Illyonectria* species from detached 'Chardonnay' green shoots (■), vascular discoloration in the rootstock developing from the basal end (□) and necrotic roots (□). **D** to **I**, Effect of eight *Cylindrocarpon/Illyonectria* species from BC in vine growth of grapevine 'Baco Noir' grafted on rootstock 3309C recorded as total necrotic area (%) measured 5 mm above the basal end of the rootstock (**D**), total shoot weight (**E**), total dry root weight (**F**), length of the longest root (cm) (**G**), total shoot length (cm) (**H**), and average internode length (cm) (**I**). **J** to **O**, Reproduction of symptoms observed from the pathogenicity tests. **J**, Vascular necrosis caused on detached 'Chardonnay' green shoots by control (1), *I. macrodidyma* PARC345 (2) and PARC398 (3), *I. liriodendri* PARC72 (4), PARC340 (5), *C. pauciseptatum* PARC82 (6), *I. torresensis* PARC35 (7), PARC14 (8), *I. robusta* PARC391 (9) and PARC358 (10). **K**, Leaf symptoms resembling water stress observed in inoculated potted plants. **L**, No symptoms were observed in leaves of control plants. **M**, Vascular necrosis observed in inoculated plants 5 mm above the basal end (below), 3 cm above the basal end (middle), and 7 cm above the basal end (top). **N**, Vascular discoloration marked by black arrows observed to develop acropetally from the basal end of the trunk in inoculated potted plants. **O**, Root necrosis observed in inoculated potted vines.

pauciseptatum isolate PARC82 (6.8 g), than for control plants (11.6 g) (Fig. 4F). Length of the longest root ranged widely from 31.4 cm on average for *I. robusta* isolate PARC391 to 45.3 cm for *I. liriodendri* isolate PARC72, but there were no significant differences among fungal isolates. Only root lengths of isolates PARC391 and PARC345 were significantly shorter than water-treated control plants (Fig. 4G). Inoculated plants had between one and 1.8 shoots on average, whereas control plants averaged 2.4 shoots. Total shoot length ranged widely, and only for *I. liriodendri* isolate PARC340 was total shoot length significantly lower than for control plants (Fig. 4H). Average internode length was not influenced by the fungal inoculants (Fig. 4I).

Discussion

This study represents the first attempt to elucidate the incidence of black foot disease of grapevines and subsequently to identify the fungal species associated with this syndrome in BC. Morphological studies along with multi-gene sequence analyses revealed the identity of five species associated with black foot disease, including *C. pauciseptatum*, *I. liriodendri*, *I. macrodidyma*, *I. robusta*, and *I. torresensis*, which are reported for the first time to occur on grapevines in BC. Among these species, *I. liriodendri* and *I. macrodidyma* were the most prevalent black foot pathogens isolated from both necrotic roots and rootstocks in BC. This is not surprising because both species are known to be the main fungi associated with black foot disease of grapevines elsewhere, and the abundance of either or both species has been similarly found in vineyards in Australia (49), California (32,34), Chile (6), Iran (28), New Zealand (22), Portugal (8,10), eastern Canada (31), Spain (5), South Africa (22,23), and Uruguay (1). This study describes for the first time the occurrence in North America of *C. pauciseptatum*, a recently described *Cylindrocarpon* species that to date has been reported to occur in New Zealand (39), Slovenia (39), South Africa (44), Spain (26), and Uruguay (1). *I. robusta* and *I. torresensis* were the least prevalent black foot fungal species isolated from declining young vines in BC, and whereas the former had until our finding not been found in a country other than Portugal (8), the latter has a wide geographical distribution that includes Australia, California, New Zealand, the Ontario Province in Canada, Portugal, South Africa, and Spain (10).

Black foot and Petri disease, the latter primarily caused by *Phaeoconiella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams and *Togninia minima* (Tul. & C. Tul.) Berl. (anamorph: *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai), have been historically considered two separate grapevine trunk diseases responsible for the decline of young vines (16,21). However, it is currently well-accepted that vascular and foliar symptomatology associated with black foot resembles that caused by Petri disease, and thus, disease diagnosis of individual maladies is extremely difficult based solely on field observations (16). Furthermore, *Ilyonectria/Cylindrocarpon* spp. have been broadly shown to be isolated along with Petri disease pathogens from the same infected vine (13,16,35). Therefore, both diseases have been recently considered to constitute the young vine decline (YVD) complex (16). In this study, YVD symptoms were recorded in 68 of 90 vineyards surveyed. However, black foot disease from symptomatic vines in the field could only be confirmed when isolation and identification of the fungal pathogens from the vascular necrotic tissue was conducted. Additionally, results from this study revealed that the number of samples from which *Ilyonectria/Cylindrocarpon* spp. were the sole pathogen isolated were almost identical to those from which black foot fungi were found along with Petri disease or other grapevine trunk pathogens. Accordingly, although black foot can be considered as the sole cause of YVD in BC, results from this study further support black foot as part of the YVD disease complex (16). *Ilyonectria/Cylindrocarpon* spp. are soilborne pathogens, and thus, black foot diagnosis in the field has been historically based on the observation of sunken necrotic roots from symptomatic vines (21,23). However, recent studies have shown Petri disease patho-

gens to be also isolated from grapevine roots and soil (20,36). Interestingly, the majority of *Ilyonectria/Cylindrocarpon* isolates obtained in this study were not isolated from infected roots but from the base of the trunk where Petri disease pathogens also commonly occur. Based on isolation data, incidence of black foot fungal pathogens observed in the current study was higher than that recently reported by Petit et al. (31) in grape-growing regions of northeastern United States and southeastern Canada where black foot fungi were identified in 28.6% of vineyards surveyed (4 out of 14) and in 13.3% of samples collected (12 out of 90).

Colony and conidia morphology have been extensively used to identify *Cylindrocarpon/Ilyonectria* spp. associated with black foot disease of grapevines (5,22,23,32,39). However, while phenotypic characters are valuable for the identification of some species (11,22,39), they are insufficient for distinguishing others. Furthermore, identification of *Cylindrocarpon/Ilyonectria* spp. based only on morphology has become difficult due to the broad number of species identified, including cryptic species from grapevines and other hosts. Although we were able to separate all isolates into five morphological groups, identification of *Cylindrocarpon/Ilyonectria* isolates from BC to species was challenging on the sole basis of phenotypical characteristics even with access to several ex-type specimens for comparison. Therefore, molecular analyses were required to accurately identify to species the *Cylindrocarpon/Ilyonectria* isolates from BC. Phylogenetic analyses of individual ITS, BT, and EF1- α datasets allowed us to precisely identify and separate *C. pauciseptatum*, *I. liriodendri*, and *I. robusta* isolates from BC, which fell into well-supported clades along with their respective ex-type specimens (individual trees not shown). However, individual phylogenies as well as the combined ITS+BT and ITS+EF1- α analyses were not able to differentiate between *I. torresensis* and *I. macrodidyma* isolates from BC, including the ex-types. Differentiation between *I. torresensis* and *I. macrodidyma* was only possible when BT+EF1- α or ITS+BT+EF1- α combined datasets were analyzed. High genetic diversity among *I. liriodendri* and *I. macrodidyma* isolates has been detected since phylogenetic analyses were included in *Cylindrocarpon* studies (1,22,23,41). However, multi-gene sequence analyses by using the BT, histone H3, and EF1- α have recently resolved part of this genetic diversity, revealing 12 and six new taxa within, respectively, the *I. liriodendri* and *I. macrodidyma* species complex from different hosts, including *I. torresensis* within the latter species complex (8,10). Interestingly, based on these recent findings, *I. torresensis* was already isolated from roots and stems of grapevines in Ontario, Canada, in 1998 but was incorrectly identified as *Cylindrocarpon destructans* var. *destructans* based solely on phenotypic characters (40).

This study revealed that the grapevine rootstock 3309 Couderc (=3309C, *Vitis riparia* $\times$ *Vitis rupestris*), which is the most widely planted rootstock cultivar in BC, is highly susceptible to infection by several *Cylindrocarpon/Ilyonectria* spp. The rootstock SO4, the second most widely planted in BC, has been shown in previous studies to be among the most susceptible to infection by *I. liriodendri* and *I. macrodidyma* in California (30) and in Spain (4), respectively. Petit (30) showed AXR1 to be among the least susceptible rootstocks tested, and Riparia, 039-16, and Freedom to show no susceptibility to black foot disease. Consequently, and due to the extensive number of rootstocks planted in BC vineyards, a larger scale susceptibility trial, including the two most prevalent grapevine rootstocks and self-rooted cultivars of *V. vinifera*, is needed to provide BC growers with further information for selecting rootstocks for new plantings. Pathogenicity of all five *Cylindrocarpon/Ilyonectria* spp. found on declining young vines in BC has been characterized in previous studies conducted in different grape-growing regions (9,22,32). A recent pathogenicity study involving 60 different isolates from 14 *Cylindrocarpon/Ilyonectria* spp. concluded that root lesions, vascular discoloration, and necrosis in the basal end of the vine trunks were symptoms difficult to quantify (9). On the other hand, black foot pathogenicity studies conducted in California (32) and Spain (5) showed

root rot severity as a quantifiable parameter. We found that although root lesions were observed in all inoculated plants, lesion severity is a challenging factor to precisely quantify. However, length of vascular discoloration measured from the base of the rootstock, and necrotic area measured cross-sectionally at the stem base, were used successfully to quantify disease symptoms in the current study. Among the five species identified in this study, *I. macrodidyma* was the most virulent on Chardonnay canes. Overall, *I. macrodidyma* isolate PARC345 and *I. liriodendri* PARC340 caused the most severe reductions in total dry root weight (66 and 47% reduction, respectively), total shoot length (38.4 and 49.7%), and shoot weight (50 and 42%) compared with the control, which agrees with results obtained previously (9,32). Short shoot internodes have been described as a characteristic symptom of YVD. Interestingly, no effects of infection on internode length were detected in this study. Short internode symptoms are commonly observed in the field 2 to 3 years after infected vines are planted, and thus a long-term pathogenicity trial may be required to reproduce this type of symptom.

Cylindrocarpon/Ilyonectria spp. are soilborne pathogens that can remain in the soil after an infected plant or the entire crop has been removed (44). Other than grapevines, several other economically important perennial crops, including apple (*Malus domestica* L.) (44), ginseng (*Panax quinquefolius* L.) (41), olive (*Olea europaea* L.) (46), and peach (*Prunus persica* L.) (50) were shown to be susceptible to *Cylindrocarpon/Ilyonectria* infection, which causes significant root rot and consequent decline. Furthermore, a recent study revealed *Cylindrocarpon/Ilyonectria* isolates from nongrape hosts to be as virulent to grapevines as those obtained from grapes (9). In the current study, two of the 32 vineyards in which *Cylindrocarpon/Ilyonectria* spp. were isolated from declining vines were previously ginseng plantations. However, further studies are required to confirm that the young vines were infected by inoculum already established in the soil. *Cylindrocarpon/Ilyonectria* spp. have long been known to be part of the pathogen complex implicated in apple replant disease, which is a devastating problem in apple characterized by the stunted growth in young trees planted in soils previously cultivated to apple (27). In a study of replant disease in BC, Utkhede and Thomas (47) concluded that grapes should not be planted after apples without soil treatment to reduce inoculum. None of the vineyards from which black foot pathogens were isolated in the current study were established in pulled out apple orchards. However, removal of apple trees for vineyard establishment is a common practice in BC. This information may be useful to growers judging the risk of planting grapes following apples or other crops susceptible to infection caused by *Cylindrocarpon/Ilyonectria* spp., and in assessing whether to conduct costly tests for pathogens and treat soils prior to planting.

In the current study, all but two vineyards containing *Cylindrocarpon/Ilyonectria* spp. were established on noncultivated lands or at sites previously planted with grapevines. The black foot inoculum may have pre-existed in the soil previously planted with grapevines, but it is improbable that high inoculum levels of *Cylindrocarpon/Ilyonectria* spp. were present in the noncultivated soils. Studies conducted during the past decade have thoroughly documented that grapevine transplants can become infected by black foot pathogens during propagation at nurseries before establishment in vineyards (13,16,22). However, practices can be implemented at nurseries to control black foot infections, including hot water treatments, fungicide baths, and soil amendments with biological agents such as *Trichoderma* spp. and/or mycorrhizal fungi (3,16,21,33). Although grapevines have been planted in BC for more than 100 years, the industry has undergone a conversion to *V. vinifera* varieties and planted more than 10,000 acres of vineyards in the last 25 years using transplants produced in different countries. Consequently, it is recommended that the transplants be acquired from registered nurseries that produce disease-free plants and practice strategies to avoid and control black foot contamination. Further studies to determine the quality and infection status

of the planting material being currently used in BC should be encouraged.

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Literature Cited

- Abreo, E., Martínez, S., Bettucci, L., and Lupo, S. 2010. Morphological and molecular characterisation of *Campylocarpon* and *Cylindrocarpon* spp. associated with black foot disease of grapevines in Uruguay. *Australas. Plant Pathol.* 39:446-452.
- Agusti-Brisach, C., Alaniz, S., Gramaje, D., Pérez-Sierra, A., Armengol, J., Landeras, E., and Izquierdo, P. M. 2012. First report of *Cylindrocladiella parva* and *C. peruviana* associated with black-foot disease of grapevine in Spain. *Plant Dis.* 96:1381.
- Alániz, S., Abad-Campos, P., García-Jiménez, J., and Armengol, J. 2011. Evaluation of fungicides to control *Cylindrocarpon liriodendri* and *Cylindrocarpon macrodidymum* in vitro, and their effect during the rooting phase in the grapevine propagation process. *Crop Prot.* 30:489-494.
- Alániz, S., García-Jiménez, J., Abad-Campos, P., and Armengol, J. 2010. Susceptibility of grapevine rootstocks to *Cylindrocarpon liriodendri* and *C. macrodidymum*. *Sci. Hortic.* 125:305-308.
- Alániz, S., León, M., Vicent, A., García-Jiménez, J., Abad-Campos, P., and Armengol, J. 2007. Characterization of *Cylindrocarpon* species associated with black foot disease of grapevines in Spain. *Plant Dis.* 91:1187-1193.
- Auger, J., Esterio, M., and Pérez, I. 2007. First report of black foot disease of grapevine caused by *Cylindrocarpon macrodidymum* in Chile. *Plant Dis.* 91:470.
- Booth, C. 1966. The genus *Cylindrocarpon*. *Mycol. Pap.* 104:1-56.
- Cabral, A., Groenewald, Z. J., Rego, C., Oliveira, H., and Crous, P. W. 2012. *Cylindrocarpon* root rot: Multi-gene analysis reveals novel species within the *Ilyonectria radicola* species complex. *Mycol. Prog.* 11:655-688.
- Cabral, A., Rego, C., Crous, P. W., and Oliveira, H. 2012. Virulence and cross-infection potential of *Ilyonectria* spp. to grapevine. *Phytopathol. Mediterr.* 51:340-354.
- Cabral, A., Rego, C., Nascimento, T., Oliveira, H., Groenewald, Z., and Crous, P. W. 2012. Multi-gene analysis and morphology reveal novel *Ilyonectria* species associated with black foot disease of grapevines. *Fungal Biol.* 116:62-80.
- Chaverri, P., Salgado, C., Hirooka, Y., Rossman, A. Y., and Samuels, G. J. 2011. Delimitation of *Neonectria* and *Cylindrocarpon* (*Nectriaceae*, *Hypocreales*, *Ascomycota*) and related genera with *Cylindrocarpon*-like anamorphs. *Stud. Mycol.* 68:57-78.
- Farr, D. F., and Rossman, A. Y. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved May 10, 2013, from <http://nt.ars-grin.gov/fungaldbases/fungushost/fungushost.cfm>
- Gimenez-Jaime, A., Aroca, A., Raposo, R., Garcia-Jimenez, J., and Armengol, J. 2006. Occurrence of fungal pathogens associated with grapevine nurseries in Spain. *J. Phytopathol.* 154:598-602.
- Ginns, J. H. 1986. Compendium of plant diseases and decay fungi in Canada 1960-1980. Research Branch Agriculture Canada Publ. 1813.
- Glass, N. L., and Donaldson, G. 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous Ascomycetes. *Appl. Environ. Microbiol.* 61:1323-1330.
- Gramaje, D., and Armengol, J. 2011. Fungal trunk pathogens in the grapevine propagation process: Potential inoculum sources, detection, identification, and management strategies. *Plant Dis.* 95:1040-1055.
- Grasso, S. 1984. Infezioni di *Fusarium oxysporum* e di *Cylindrocarpon destructans* associate a una moria di giovani piante di vite in Sicilia. *Inform. Fitopatol.* 1:59-63.
- Grasso, S., and Magnano di San Lio, G. 1975. Infezioni di *Cylindrocarpon obtusisporium* su piante di vite in Sicilia. *Vitis* 14:36-39.
- Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid S.* 41:95-98.
- Halleen, F., Crous, P. W., and Petrini, O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. *Australas. Plant Path.* 32:47-52.
- Halleen, F., Fourie, P. H., and Crous, W. C. 2006. A review of black foot disease of grapevines. *Phytopathol. Mediterr.* 45:S55-S67.
- Halleen, F., Schroers, H. J., Groenewald, J. Z., and Crous, P. W. 2004. Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Stud. Mycol.* 50:431-455.
- Halleen, F., Schroers, H. J., Groenewald, J. Z., Rego, C., Oliveira, H., and Crous, P. W. 2006. *Neonectria liriodendri* sp. nov., the main causal agent of

- black foot disease of grapevines. *Stud. Mycol.* 55:227-234.
24. Jones, E. E., Brown, D. S., Bleach, C. M., Pathrose, B., Barclay, C., Jaspers, M. V., and Ridgway, H. J. 2012. First report of *Cylindrocladiella parva* as a grapevine pathogen in New Zealand. *Plant Dis.* 96:144.
25. Maluta, D. R., and Larignon, P. 1991. Pied-noir: Mieux vaut prevenir. *Viti* 11:71-72.
26. Martin, M. T., Martin, L., Cuesta, M. J., and Garcia-Benavides, P. 2011. First report of *Cylindrocarpon pauciseptatum* associated with grapevine decline from Castilla y Leon, Spain. *Plant Dis.* 95:361.
27. Mazzola, M., and Manici, M. 2012. Apple replant disease: Role of microbial ecology in cause and control. *Annu. Rev. Phytopathol.* 50:45-65.
28. Mohammadi, H., Alániz, S., Banihashemi, Z., and Armengol, J. 2009. Characterization of *Cylindrocarpon liriodendri* associated with black foot disease of grapevine in Iran. *J. Phytopathol.* 157:642-645.
29. O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Nat. Acad. Sci.* 95:2044-2049.
30. Petit, E. 2005. Characterization and control of black foot disease of grapevine in California. Ph.D. diss. University of California, Davis.
31. Petit, E., Barriault, E., Baumgartner, K., Wilcox, W. F., and Rolshausen, P. E. 2011. *Cylindrocarpon* species associated with black-foot of grapevines in northeastern United States and southeastern Canada. *Am. J. Enol. Vitic.* 62:177-183.
32. Petit, E., and Gubler, W. D. 2005. Characterization of *Cylindrocarpon* species, the cause of black foot disease of grapevines in California. *Plant Dis.* 89:1051-1059.
33. Petit, E., and Gubler, W. D. 2006. Influence of *Glomus intraradices* on black foot disease caused by *Cylindrocarpon macrodidymum* on *Vitis rupestris* under controlled conditions. *Plant Dis.* 90:1481-1484.
34. Petit, E., and Gubler, W. D. 2007. First report of *Cylindrocarpon liriodendri* causing black foot disease of grapevines in California. *Plant Dis.* 91:1060.
35. Rego, C., Oliveira, H., Carvalho, A., and Phillips, A. J. L. 2000. Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. *Phytopathol. Mediterr.* 39:76-79.
36. Rooney-Latham, S., Eskalen, A., and Gubler, W. D. 2001. Recovery of *Phaeomoniella chlamydospora* and *Phaeoacremonium inflatipes* from soil and grapevine tissue. *Phytopathol. Mediterr.* 40:S351-S356.
37. Scheck, H. J., Vasquez, S. J., Gubler, W. D., and Fogle, D. 1998. First report of black-foot disease, caused by *Cylindrocarpon obtusisporium*, of grapevine in California. *Plant Dis.* 82:448.
38. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., and Cardona, A. 2012. Fiji: An open-source platform for biological-image analysis. *Nat. Methods* 9:676-682.
39. Schroers, H.-J., Žerjav, M., Munda, A., Halleen, F., and Crous, P. W. 2008. *Cylindrocarpon pauciseptatum* sp. nov., with notes on *Cylindrocarpon* species with wide, predominantly 3-septa macroconidia. *Mycol. Res.* 112:82-92.
40. Seifert, K. A., and Axelrood, P. E. 1998. *Cylindrocarpon destructans* var. *destructans*. *Can. J. Plant Pathol.* 20:115-117.
41. Seifert, K. A., McMullen, C. R., Yee, D., Reeleder, R. D., and Dobinson, K. F. 2003. Molecular differentiation and detection of ginseng-adapted isolates of the root rot fungus *Cylindrocarpon destructans*. *Phytopathology* 93:1533-1542.
42. Statistics Canada. Fruit and Vegetable Production 2011. Catalogue no 22-003-XWE, vol. 80, no. 2. Online publication retrieved from <http://www.statcan.gc.ca/pub/22-003-x/22-003-x2011002-eng.pdf>
43. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol. Biol. Evol.* 28:2731-2739.
44. Tewoldemedhin, Y. T., Mazzola, M., Mostert, L., and McLeod, A. 2011. *Cylindrocarpon* species associated with apple tree roots in South Africa and their quantification using real-time PCR. *Eur. J. Plant. Pathol.* 129:637-651.
45. Úrbez-Torres, J. R., Adams, P., Kamas, J., and Gubler, W. D. 2009. Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *Am. J. Enol. Vitic.* 60:497-507.
46. Úrbez-Torres, J. R., Peduto, F., and Gubler, W. D. 2012. First report of *Ilyonectria macrodidyma* causing root rot of olive trees (*Olea europaea*) in California. *Plant Dis.* 96:1378.
47. Utkhede, R. S., and Thomas, S. C. 1988. Determining the occurrence of replant disease in British Columbia orchard and vineyard soils by pasteurization. *Can. Plant Dis. Surv.* 68:149-151.
48. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols, A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.
49. Whitelaw-Weckert, M. A., Nair, N. G., Lamont, R., Alonso, M., Priest, M. J., and Huang, R. 2007. Root infection of *Vitis vinifera* by *Cylindrocarpon liriodendri* in Australia. *Australas. Plant Pathol.* 36:403-406.
50. Yaseen, T., Ahmed, Y., D'Onghia, A. M., and Digiaro, M. 2012. First report of *Cylindrocarpon pauciseptatum* associated with root rot and decline of peach in southern Italy (Apulia region). *Plant Dis.* 96:764.