Grapevine Trunk Diseases in British Columbia: Incidence and Characterization of the Fungal Pathogens Associated with Esca and Petri Diseases of Grapevine

J. R. Úrbez-Torres, P. Haag, P. Bowen, and D. T. O’Gorman, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada

Abstract

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Esca and Petri disease are two economically important grapevine diseases worldwide. This study reports for the first time the occurrence of both diseases on grapevines in British Columbia (BC) and subsequently in Canada. Visual assessment of 55,699 vines in 118 vineyards revealed a low incidence of esca with only 104 (0.2%) vines showing foliar symptoms. Young vine decline (YVD) was observed in 1,910 (7.8%) of 24,487 monitored young vines and in 52 (8%) of 654 young vines used as re-plants in mature vineyards. In 8 of 51 monitored young vineyards, YVD-affected vines ranged between 15 and 55%. Morphological studies along with DNA analyses of the ITS1-5.8S-ITS2, and part of the β -tubulin, actin, and translation elongation factor 1- α gene regions, allowed us to identify *Cadophora luteo-*

olivacea, *Phaeomoniella chlamydospora*, *Phaeoacremonium iran-
anum*, *Togninia fraxinopennsylvanica*, *Togninia minima*, and the novel species *Phaeoacremonium canadense* and *Phaeoacremonium roseum* from esca and Petri disease infected vines in BC. This study includes for the first time the EF1- α DNA marker in *Phaeoacremonium* spp. delineation. Pathogenicity studies showed all seven fungi to cause vascular symptoms similar to those observed in esca and Petri disease infected vines. Additionally, the “tiger-stripes” foliar symptom of esca was successfully reproduced when healthy potted vines were inoculated with BC isolates of *Pa. chlamydospora*, *Pm. canadense*, *Pm. iran-
ianum*, *T. fraxinopennsylvanica*, and *T. minima*.

Esca and Petri disease are two grapevine trunk diseases (GTD) known to occur wherever grapes (*Vitis vinifera* L. and *Vitis* spp.) are grown (27). The ascomycetous fungi *Phaeomoniella* (*Pa.*) *chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams and *Togninia minima* (Tul. & C. Tul.) Berl. (anamorph: *Phaeoacremonium* (*Pm.*) *aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai) are known to be the main pathogens associated with both diseases (7,8,27). However, up to 24 other species of *Phaeoacremonium* W. Gams, Crous & M.J. Wingf., and several *Cadophora* Lagerberg & Melin species have been reported to occur in either esca or Petri disease symptomatic vines (9,11,18–20,25,26). Additionally, the basidiomycetous taxa, *Fomitiporia mediterranea* M. Fischer, *Fomitiporia punctata* (P. Karst.) Murril, *Phellinus igniarius* (L.) Quel., and to a lesser extent *Stereum hirsutum* (Willd.:Fr.) Pers., are regarded as the causal organisms of the white rot symptom observed in the wood of trunks and/or cordons of esca-infected vines (13,27).

Esca disease, primarily observed on mature vines, was first reported in France in 1898 (32), and it is thought to be as old as vine cultivation (27). However, a dramatic and rapid increase in disease incidence was reported in California, Europe, and South Africa in the 1990s, which was hypothesized to be correlated with several factors including changes in viticulture practices and vineyard management and prohibition of effective fungicides against the disease (6,27). Consequently, much has been written about this complex disease, whose symptoms have been shown to result not only from a biological agent but also from physiological factors affecting the framework of the vine (24,27). Overall, esca infected vines can be recognized in the field by a leaf symptom known as “tiger-stripes”, which is characterized by light-green

and/or chlorotic areas developing between the veins and at the margin of the leaves that eventually turn rust- or reddish-colored throughout the growing season (27). Two other characteristic symptoms of esca-affected vines are gray to dark-brown speckling of the berries known as “black measles”, and sudden wilting of the vine, including shriveling of the fruit that normally occurs in summer and is known as “vine apoplexy” (27,38). Esca vascular symptoms include primarily a white rot characterized by a yellowish spongy mass of wood, usually in the center of the trunk and/or cordons, which can be observed alone or along with dark-brown to black spots in the xylem vessels (27).

Petri disease, on the other hand, affects young vines, and along with black-foot disease of grapevines, caused by species in the genus *Ilyonectria* P. Chaverri & C. Salgado (anamorph: *Cylindrocarpon* Wollenweb.), is part of the young vine decline complex (YVD) (17). YVD symptoms caused by either Petri disease or black-foot fungi are practically indistinguishable in the field and are first recognized by an overall reduction of vine vigor characterized by either lack of or delayed sprouting, stunted growth, chlorotic and/or wilting leaves, and occasional sudden vine collapse (17,39). Vascular symptoms of young vines affected by Petri disease have been described as having a black discoloration of the xylem vessels inside the rootstock and/or self-rooted cultivar, which is a result of tylose formation by the host in response to the fungal colonization of the wood (25,27,38,39). Incidence of esca and Petri disease have been worsening in all grape-producing regions since the late 1990s, including Europe (17,24,27), the Near East (1), North and South America (2,38,39), Oceania (16,29), and South Africa (25), and causing significant economic losses due to yield and quality reductions and vineyard replanting (39).

In Canada, grapes are the third largest fruit crop, with 11,784 ha cultivated and a farm gate value of over \$112.5 million CAD (42). Although the overall cultivated area in Canada is considerably smaller compared with other grape-growing countries, grapes significantly impact the Canadian economy, contributing over \$6.8 billion of total economic impact in 2011 (36). Currently, GTD are one of the main biotic factors limiting both vineyard longevity and productivity, as woody parts of the vine are killed by the fungi. Decline and eventual death of vines have been reported to cause

Corresponding author: J. R. Úrbez-Torres,
E-mail: joseraamon.urbeztorres@agr.gc.ca

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significant economic losses to the raisin and table- and wine-grape industries worldwide (41,49). Currently, no information is available regarding the status of GTD in BC, the second largest grape-growing province in Canada. Consequently, the objectives of this study were (i) to determine the incidence and significance of esca and YVD in BC, and (ii) to characterize by means of morphological, molecular, and pathogenicity studies the different fungi associated with esca and YVD symptoms caused by Petri disease in the province.

Materials and Methods

Field surveys and fungal isolations. Between 2008 and 2012, 191 vineyards representing the most widely grown grape cultivars in BC were surveyed throughout all grape-growing regions of BC, including the Okanagan, Similkameen, and Fraser valleys, and the Vancouver and Gulf islands. Among all surveyed vineyards, 90 were classified as young (≤ 8 years old) and 101 as mature (> 8 years old). To estimate the incidence of esca and YVD in BC, 51 and 67 of these young and mature vineyards, respectively, were selected to monitor for foliar-symptom expression during the 2011 growing season (June to September). In each selected vineyard, a block of approximately 500 vines (10 rows of 50 vines per row) in the middle of the vineyard was inspected twice during the growing season for the presence of characteristic esca, YVD, and other GTD symptoms. In total, 26,112 and 33,237 young and mature grapevines were evaluated, respectively. Vines showing either of the symptoms previously described were flagged and recorded. A representative number of symptomatic vines from each monitored vineyard were collected to correlate foliar symptoms observed in the vineyard with vascular symptoms observed in affected vines and fungi isolated from them. Samples from these monitored blocks were added to the diseased vines collected from the rest of the surveyed vineyards. In total, 463 samples (entire or part of the vine) were collected: 215 from young vineyards and 248 from mature vineyards.

Samples were brought to the laboratory and first inspected for the presence of fungal fruiting bodies (perithecia, pycnidia, and/or pseudothecia) using the dissecting microscope. Samples were then surface-sterilized by submerging them in 4.5% sodium hypochlorite for 5 min and rinsed twice with sterile distilled water. After air-drying, samples were sectioned to reveal the vascular symptoms, and fungal species were isolated by plating small pieces of symptomatic tissue on potato dextrose agar (PDA) (Difco, Detroit, MI) in petri dishes amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich, St Louis, MO) (PDA-tet). YVD symptomatic vines were also inspected for the presence of necrotic roots, and if detected, fungal species from roots were isolated as described by Petit and Gubler (30). PDA-tet petri dishes containing symptomatic tissue from roots, trunk, cordons, and/or spur positions were incubated at 20°C in the dark until fungal colonies were observed. Colonies from the most prevalent fungi observed from symptomatic tissues were transferred individually to fresh PDA, and pure colonies were obtained by hyphal-tipping from colony margins. Hyphal-tips from each colony were plated on fresh PDA and incubated at 20°C in the dark until colony growth was sufficient to conduct a preliminary morphological identification.

Morphological characterization. Among all different fungi isolated from symptomatic tissue, fungal taxa known to be associated with esca and Petri disease were first tentatively identified by comparing colony and conidial characteristics with reference isolates, including ex-type specimens obtained from the Centraalbureau voor Schimmelcultures (CBS) collection, Utrecht, the Netherlands, and from the International Collection for Microorganisms from Plants (ICMP), Lincoln, New Zealand (Table 1). Additionally, morphological characteristics of fungal isolates from BC were compared with published taxonomic descriptions (7,19,25). Colony colors were determined with the color charts of Rayner (33) after 8 and 21 days incubation on PDA at 20°C in the dark. Optimal temperatures for colony growth were determined as described by Úrbez-Torres et al. (46). A 5-mm-diameter plug from

the growing margin of a 3-day-old colony was placed in the center of an 85-mm-diameter PDA-tet petri dish, and three replicates of each isolate were incubated separately between 5 and 40°C with 5°C intervals in the dark. Colony diameter was measured after 21 days of incubation. The experiment was conducted twice. Morphological characteristics of microscopic structures such as mycelium, conidia, conidiophores, and phialides were noted after 21 days incubation on PDA at 20°C in the dark at $\times 100$ magnification using immersion oil in a Zeiss Axiophot compound microscope with DIC optics. Pictures of the different structures were captured using a Nikon D700 digital camera. Length and width of conidia, phialides, and conidiophores were measured using the Image-Pro Plus software (v4.5.1.29, Media Cybernetics, Inc.). Conidia measurements were calculated using summary statistics in SAS (v9.3, SAS Institute, Cary, NC) and included minimum, lower limit of a 95% interval, mean, upper limit of a 95% interval, and maximum for length and width. Conidia length/width (L/W) ratio was also calculated.

Molecular characterization and multi-locus phylogenetic analysis. Fungal isolates selected for molecular characterization (Table 2) were grown on PDA for up to 3 weeks at 20°C in the dark, and total genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA). Oligonucleotide primer sets ITS1/ITS4, Bt1a/Bt1b, Bt2a/Bt2b, EF1-728F/EF1-986R, and ACT-512F/ACT-783R were used to amplify the internal transcribed spacer (ITS) region ITS1-5.8S-ITS2, and portions of the β -tubulin (BT), translation elongation factor 1- α (EF1- α), and actin (ACT) genes, respectively (4,14,28,48). Primer sets ITS1/ITS4, Bt1a/Bt1b, and EF1-728F/EF1-986R were used to amplify *Cadophora* isolates; ITS1/ITS4, Bt2a/Bt2b, EF1-728F/EF1-986R, and ACT-512F/ACT-783R were used to amplify *Togninia/Phaeoacremonium* isolates; and ITS1/ITS4, Bt2a/Bt2b, and EF1-728F/EF1-986R were used to amplify *Pa. chlamydospora* isolates (Table 2). Each polymerase chain reaction (PCR) contained 2 μ l of 10 $\times$ PCR buffer containing 15 mM MgCl₂, 1.6 μ l of 25 mM MgCl₂, 1 μ l of 10 mM dNTPs, 0.5 μ l of 0.5 mM of each primer, 0.1 μ l of *Ultra Therm* DNA polymerase (BocaScientific, Boca Raton, FL), and 1 μ l of DNA template and adjusted with micropore filtered water to a final volume of 20 μ l. PCR amplification reactions were performed in a GeneAmp 2700 thermal cycler (Applied Biosystems, Foster City, CA) following previously described ITS, BT, EF1- α , and ACT temperature profiles (4,14,28,48). Amplification products were purified using a QIAquick PCR purification Kit (QIAGEN Inc., Valencia, CA) and both strands of the ITS, BT, EF1- α , and ACT 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) in order to identify homologous sequences.

Phaeoacremonium sequences obtained from grapevines from BC (Table 2) 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 (21) (Table 1). Alignments were corrected visually and manually edited. A multi-locus phylogenetic analysis was performed on the combined ITS, BT, EF1- α , and ACT datasets using Maximum Parsimony (MP) and the heuristic search option with 1,000 random addition sequence replicates and the Close-Neighbor-Interchange (CNI) algorithm in MEGA5 (44). Tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated. The combined ITS, BT, EF1- α , and ACT datasets was 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. Fungal sequences from BC grapevines were

deposited into GenBank, and representative isolates are maintained in the GTD fungal collection at the Pacific Agri-Food Research Centre in Summerland, BC (PARC Summerland), and in the Canadian Collection of Fungal Cultures in Ottawa, Ontario (Table 2).

Pathogenicity studies. Fourteen isolates representing seven different fungal taxa were selected to assess their pathogenicity in two different studies. In the first study, dormant canes of 'Chardonnay' were collected from an experimental vineyard at PARC Summerland in mid-March of 2012. Detached canes were cut in uniform lengths (approximately 30 cm) and surface-disinfested as previously described. After air-drying, six canes per fungal isolate were inoculated using mycelium plugs collected from the edge of a 2-week-old colony. Mycelium plugs were inserted in a hole made with a drill. Noncolonized PDA agar plugs were used to treat the same number of canes for the negative control treatment. The canes were arranged in a completely randomized design in transparent plastic containers (12 shoots per container) as described by Úrbez-

Torres et al. (45). Thirty-one days after inoculation, the canes were inspected for lesion development. Each cane was sectioned longitudinally, 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 6 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 by morphological comparison against the original and reference isolates obtained from the CBS collection.

The second pathogenicity study was conducted using 1-year-old dormant rooted vines of 'Baco Noir' grafted on 3309C rootstock. Six plants per fungal isolate were inoculated by placing a mycelial plug in the middle part of the rootstock as described by Úrbez-Torres et al. (45). Mycelial plugs were inserted in a hole made with a drill before planting. The same number of control plants was inoculated using noncolonized PDA agar plugs. After inoculation, rooted vines were planted in 4-liter pots containing a Sunshine Mix

Table 1. *Cadophora*, *Phaeoacremonium*/Togninia, and *Phaeomoniella* species used for morphological comparison and phylogenetic analyses

Species ^b	Isolate ^c	Host / Origin / Collector	GenBank accession numbers ^a			
			ITS	BT	EF1- α	ACT
<i>Cadophora fastigiata</i>	CBS 226.30	<i>Pinus strobus</i> / Canada / C.W. Fritz	AY249073	...	...	...
<i>C. finlandica</i>	CBS 444.86	<i>Pinus sylvestris</i> / Finland / C.J.K. Wang	AF486119	...	...	...
<i>C. gregata</i>	CBS 132.51	<i>Glycine max</i> / MO, USA / D.W. Chamberlain	U66731	...	...	...
<i>C. hiberna</i>	CBS 110521	<i>Robinia pseudoacacia</i> / PA, USA / G.F. Bills	AF530462	...	...	...
<i>C. luteo-olivacea</i> *	CBS 357.51	<i>Malus sylvestris</i> / Italy / G. Goidánich	AY249068	<i>KF764682^d</i>	<i>KF764611</i>	...
<i>C. malorum</i>	CBS 266.31	n.a. / n.a. / F.D. Heald	AY249057	...	...	...
<i>C. melinii</i> *	CBS 268.33	Lumber and wood pulp / Sweden / E. Melin	AY249072	...	...	...
<i>Phaeoacremonium alvesii</i> *	CBS 110034	Human / Brazil / S.H. Alves	<i>KF764558</i>	AY579301	<i>KF764612</i>	AY579234
<i>Pm. amstelodamense</i>	CBS 110627	Human / The Netherlands / J. Bruins	<i>KF764559</i>	AY579295	<i>KF764613</i>	AY579228
<i>Pm. angustius</i> *	CBS 249.95	<i>Vitis vinifera</i> / CA, USA / P. Larignon	AF197974	AF246814	<i>KF764614</i>	DQ173127
<i>Pm. armeniacum</i> *	ICMP 17421	<i>Vitis</i> sp. / New Zealand / B.S. Weir	EU770224	EU596526	<i>KF764615</i>	EU595463
<i>Pm. australiense</i> *	CBS 113589	<i>V. vinifera</i> / Australia / T. Knaggs	<i>KF764560</i>	AY579296	<i>KF764616</i>	AY579229
<i>Pm. cinereum</i> *	CBS 123909	<i>V. vinifera</i> / Iran / H. Mohammadi	<i>KF764561</i>	FJ517161	<i>KF764617</i>	FJ517153
<i>Pm. croatiense</i> *	CBS 123037	<i>V. vinifera</i> / Croatia / B. Cvjetković	EU863526	EU863482	<i>KF764618</i>	EU863514
<i>Pm. fuscum</i>	CBS 120856	<i>Prunus salicina</i> / South Africa / U. Damm	EU128050	EU128098	<i>KF764619</i>	EU128141
<i>Pm. globosum</i> *	ICMP 16988	<i>Vitis</i> sp. / New Zealand / B.S. Weir	EU770229	EU596525	<i>KF764620</i>	EU595466
<i>Pm. griseorubrum</i> *	CBS 111657	Human / MD, USA / D. Sutton	<i>KF764562</i>	AY579294	<i>KF764621</i>	AY579227
<i>Pm. hispanicum</i> *	CBS 123910	<i>V. vinifera</i> / Spain / D. Gramaje	<i>KF764563</i>	FJ517164	<i>KF764622</i>	FJ517156
<i>Pm. hungaricum</i> *	CBS 123036	<i>V. vinifera</i> / Hungary / B.T. Dula	EU863525	EU863483	<i>KF764623</i>	EU863515
<i>Pm. inflatipes</i> *	CBS 391.71	<i>Quercus virginiana</i> / TX, USA / R.S. Halliwell	<i>KF764564</i>	AF246805	<i>KF764624</i>	AY579259
<i>Pm. iraniamum</i> *	CBS 101357	<i>Actinidia chinensis</i> / Italy / F. Calzarano	AY179942	DQ173097	<i>KF764625</i>	DQ173120
<i>Pm. occidentale</i> *	ICMP 17037	<i>Vitis</i> sp. / New Zealand / B.S. Weir	EU770226	EU596524	<i>KF764626</i>	EU595460
<i>Pm. pallidum</i>	CBS 120862	<i>Prunus armeniaca</i> / South Africa / U. Damm	EU128053	EU128103	<i>KF764627</i>	EU128144
<i>Pm. prunicola</i>	CBS 120858	<i>P. salicina</i> / South Africa / U. Damm	EU128047	EU128095	<i>KF764628</i>	EU128137
<i>Pm. scolymus</i> *	CBS 113597	<i>V. vinifera</i> / South Africa / M. Groenewald	<i>KF764575</i>	AF246800	<i>KF764629</i>	AY579224
<i>Pm. sicilianum</i> *	CBS 123034	<i>V. vinifera</i> / Italy / L. Mugnai	EU863523	EU863488	<i>KF764630</i>	EU863520
<i>Pm. subulatum</i> *	CBS 113584	<i>V. vinifera</i> / South Africa / L. Mostert	<i>KF764565</i>	AY579298	<i>KF764631</i>	AY579231
<i>Pm. tardicrescens</i>	CBS 110573	Human / TX, USA / Levi	<i>KF764566</i>	AY579300	<i>KF764632</i>	AY579233
<i>Pm. theobromatis</i>	CBS 111586	<i>Theobroma gileri</i> / Ecuador / H.C. Evans	<i>KF764567</i>	DQ173106	<i>KF764633</i>	DQ173132
<i>Pm. tuscanum</i> *	CBS 123033	<i>V. vinifera</i> / Italy / L. Mugnai	EU863522	EU863458	<i>KF764634</i>	EU863490
<i>Pm. venezuelense</i> *	CBS 651.85	Human / Venezuela / M.B. de Albornoz	<i>KF764568</i>	AY579320	<i>KF764635</i>	AY579256
<i>Phaeomoniella chlamydospora</i> *	CBS 117179	<i>V. vinifera</i> / South Africa / L. Mostert	<i>KF764544</i>	<i>KF764683</i>	<i>KF764636</i>	...
<i>Togninia africana</i>	CBS 120863	<i>P. armeniaca</i> / South Africa / U. Damm	EU128052	EU128100	<i>KF764637</i>	EU128142
<i>T. argentinensis</i>	CBS 777.83	Soil / Argentina / A. Martínez	<i>KF764569</i>	DQ173108	<i>KF764638</i>	DQ173135
<i>T. austroafricana</i> *	CBS 112949	<i>V. vinifera</i> / South Africa / L. Mostert	<i>KF764570</i>	DQ173099	<i>KF764639</i>	DQ173122
<i>T. fraxinopennsylvanica</i> *	CBS 101585	<i>V. vinifera</i> / CA, USA / L.M. Morton & v. der Water	AF295328	<i>KF764684</i>	<i>KF764640</i>	DQ173137
<i>T. griseo-olivacea</i>	CBS 120857	<i>P. armeniaca</i> / South Africa / U. Damm	EU128049	EU128097	<i>KF764641</i>	EU128139
<i>T. kraidenii</i> *	CBS 110118	<i>V. vinifera</i> / South Africa / G. van Coller	<i>KF764571</i>	AY579324	<i>KF764642</i>	AY579261
<i>T. minima</i> *	CBS 631.94	<i>V. vinifera</i> / Italy / L. Mugnai	AF266647	<i>KF764685</i>	<i>KF764643</i>	<i>KF764521</i>
<i>T. novae-zelandiae</i>	CBS 110156	<i>Cupressus macrocarpa</i> / New Zealand / J. Reid & S. Reid	<i>KF764572</i>	DQ173109	<i>KF764644</i>	DQ173138
<i>T. vibratilis</i>	CBS 117115	<i>Fagus sylvatica</i> / France / J. Fournier & G. Corriol	<i>KF764573</i>	DQ649063	<i>KF117115</i>	DQ649064
<i>T. viticola</i> *	CBS 101738	<i>V. vinifera</i> / France / P. Larignon	<i>KF764574</i>	AF192391	<i>KF764646</i>	DQ173131

^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 . ACT: actin.

^b Fungal species reported to occur in grapevines (*Vitis vinifera* and/or *Vitis* sp.) are indicated with an asterisk.

^c Isolates used for morphological comparison and phylogenetic analyses. Isolate numbers in bold represent ex-type specimens. CBS (Centraalbureau voor Schimmelcultures, Utrecht, Netherlands), ICMP (International Collection for Microorganisms from Plants, Lincoln, New Zealand).

^d Part of the β -tubulin gene amplified using Bt1a/Bt1b primers.

1 potting soil mixture (75 to 85% Canadian Sphagnum peat moss, horticultural grade perlite, dolomitic limestone; Sun Gro Horticulture Canada Ltd.), placed in a greenhouse (27 ± 5°C, 30 to 40% relative humidity), and watered as needed. After budbreak, inoculated plants were inspected visually weekly for foliar symptom development. Twenty-one weeks after inoculation, grapevine growth characteristics, including total shoot length, average internode length, and total pruning weight, were recorded and compared to the control plants. Rootstocks were then sectioned longitudinally and the extent of vascular discoloration was measured as previously described. Reisolation and identification of the inoculated fungi were conducted as previously described.

Data from both pathogenicity studies were analyzed independently by one-way analysis of variance (ANOVA) using SAS GLM Procedure (SAS Institute). 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% significance level.

Results

Incidence of esca and YVD of grapevines in BC. Esca and YVD symptomatic vines were observed in all grape-growing regions surveyed in BC (Fig. 1). Overall, vines with characteristic esca and/or YVD symptoms were observed in 21 and 75 out of 191 vineyards surveyed, respectively (Table 3). Within the 118 blocks selected to monitor disease symptom expression, esca and/or YVD symptoms were recorded in 19 and 48 vineyards, respectively. While the percentage of young and mature vineyards yielding esca symptomatic vines was very similar, the percentage of vineyards

Table 2. *Cadophora luteo-olivacea*, *Phaeoacremonium/Togninia*, and *Phaeomoniella chlamydospora* species isolated from grapevines in British Columbia used in this study

Species	Isolate ^b	Cultivar ^c	Collector	GenBank accession numbers ^a			
				ITS	BT	EF1- α	ACT
<i>Cadophora luteo-olivacea</i>	PARC223 DAOM 242316	3309C	J.R. Úrbez-Torres	KF764522	KF764647*	KF764576	...
<i>C. luteo-olivacea</i>	PARC312 DAOM242319	Chardonnay	P. Haag & D.T. O'Gorman	KF764523	KF764648*	KF764577	...
<i>C. luteo-olivacea</i>	PARC313	Chardonnay	J.R. Úrbez-Torres	KF764524	KF764649*	KF764578	...
<i>C. luteo-olivacea</i>	PARC314	Chardonnay	J.R. Úrbez-Torres	KF764525	KF764650*	KF764579	...
<i>Phaeoacremonium canadense</i>	PARC327 DAOM 242366	3309C	J.R. Úrbez-Torres	KF764526	KF764651	KF764580	KF764499
<i>Pm. canadense</i>	PARC392	3309C	J.R. Úrbez-Torres	KF764527	KF764652	KF764581	KF764500
<i>Pm. canadense</i>	PARC394	Pinot Noir	J.R. Úrbez-Torres	KF764528	KF764653	KF764582	KF764501
<i>Pm. iraniamum</i>	PARC70 DAOM 242361	Shiraz	P. Haag & D.T. O'Gorman	KF764529	KF764654	KF764583	KF764502
<i>Pm. iraniamum</i>	PARC107	Shiraz	J.R. Úrbez-Torres	KF764530	KF764655	KF764584	KF764503
<i>Pm. iraniamum</i>	PARC188	Cabernet Franc	J.R. Úrbez-Torres	KF764531	KF764656	KF764585	KF764504
<i>Pm. iraniamum</i>	PARC194 DAOM 242362	Sauvignon Blanc	J.R. Úrbez-Torres	KF764532	KF764657	KF764586	KF764505
<i>Pm. roseum</i>	PARC273	Gewürztraminer	J.R. Úrbez-Torres	KF764533	KF764658	KF764587	KF764506
<i>Pm. roseum</i>	PARC281 DAOM 242365	Kerner	J.R. Úrbez-Torres	KF764534	KF764659	KF764588	KF764507
<i>Phaeomoniella chlamydospora</i>	PARC13	Gewürztraminer	P. Haag & D.T. O'Gorman	KF764535	KF764660	KF764589	...
<i>Pa. chlamydospora</i>	PARC50 DAOM 242367	Merlot	P. Haag & D.T. O'Gorman	KF764536	KF764661	KF764590	...
<i>Pa. chlamydospora</i>	PARC100	Shiraz	J.R. Úrbez-Torres	KF764537	KF764662	KF764591	...
<i>Pa. chlamydospora</i>	PARC124 DAOM 242368	Riesling	J.R. Úrbez-Torres	KF764538	KF764663	KF764592	...
<i>Pa. chlamydospora</i>	PARC143	3309C	J.R. Úrbez-Torres	KF764539	KF764664	KF764593	...
<i>Pa. chlamydospora</i>	PARC315	Chardonnay	J.R. Úrbez-Torres	KF764540	KF764665	KF764594	...
<i>Pa. chlamydospora</i>	PARC316	Chardonnay	J.R. Úrbez-Torres	KF764541	KF764666	KF764595	...
<i>Pa. chlamydospora</i>	PARC326	Pinot Noir	J.R. Úrbez-Torres	KF764542	KF764667	KF764596	...
<i>Pa. chlamydospora</i>	PARC328	Pinot Noir	J.R. Úrbez-Torres	KF764543	KF764668	KF764597	...
<i>Togninia fraxinopennsylvanica</i>	PARC88 DAOM 242363	Riesling	J.R. Úrbez-Torres	KF764545	KF764669	KF764598	KF764508
<i>T. fraxinopennsylvanica</i>	PARC133	Chardonnay	J.R. Úrbez-Torres	KF764546	KF764670	KF764599	KF764509
<i>T. fraxinopennsylvanica</i>	PARC140 DAOM 242364	Chardonnay	J.R. Úrbez-Torres	KF764547	KF764671	KF764600	KF764510
<i>T. fraxinopennsylvanica</i>	PARC143B	3309C	J.R. Úrbez-Torres	KF764548	KF764672	KF764601	KF764511
<i>T. minima</i>	PARC158	Cabernet Sauvignon	J.R. Úrbez-Torres	KF764549	KF764673	KF764602	KF764512
<i>T. minima</i>	PARC172	Sauvignon Blanc	J.R. Úrbez-Torres	KF764550	KF764674	KF764603	KF764513
<i>T. minima</i>	PARC187	Cabernet Franc	J.R. Úrbez-Torres	KF764551	KF764675	KF764604	KF764514
<i>T. minima</i>	PARC220	Cabernet Sauvignon	J.R. Úrbez-Torres	KF764552	KF764676	KF764605	KF764515
<i>T. minima</i>	PARC249 DAOM 242326	Pinot Noir	J.R. Úrbez-Torres	KF764553	KF764677	KF764606	KF764516
<i>T. minima</i>	PARC341	Gewürztraminer	J.R. Úrbez-Torres	KF764554	KF764678	KF764607	KF764517
<i>T. minima</i>	PARC349 DAOM 242360	Shiraz	J.R. Úrbez-Torres	KF764555	KF764679	KF764608	KF764518
<i>T. minima</i>	PARC369	3309C	J.R. Úrbez-Torres	KF764556	KF764680	KF764609	KF764519
<i>T. minima</i>	PARC395	SO4	J.R. Úrbez-Torres	KF764557	KF764681	KF764610	KF764520

^a ITS: internal transcribed spacer. BT: β -tubulin. EF1- α : translation elongation factor 1- α . ACT: actin. * Part of the β -tubulin gene amplified using Bt1a/Bt1b primers.

^b PARC: Pacific Agri-Food Research Centre. DAOM: Department of Agriculture Ottawa Mycology.

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

Fig. 1. Esca and young vine decline foliar and vascular symptoms observed in British Columbia. **A**, Early esca foliar symptoms on a Merlot leaf. **B**, "Black measles" symptoms on berries of a Merlot vine. **C**, Spur and cordon cross-sections showing characteristic esca light-brown discoloration of the xylem and/or dark vascular necrosis. Xylem vessels plugged with tyloses can be observed. **D**, Longitudinal section of a cordon showing dark streaking of the wood. Black tyloses plugging vascular vessels can be observed. **E**, Characteristic poor vigor and stunted growth observed in young vine decline affected vines. **F**, Sudden vine collapse observed during the growing season is characterized by leaf drop and shrivelled clusters. **G**, Light- to dark-brown discoloration and tyloses observed at the basal end of the rootstock of a vine affected by Petri disease. **H**, Longitudinal section of a Petri disease infected young vine showing the dark streaking of the wood at the basal end of the rootstock.

yielding YVD symptomatic vines was significantly higher in young than in mature vineyards. In this study, only 104 vines of 55,699 monitored vines showed esca foliar (Fig. 1A) and/or fruit symptoms (Fig. 1B), with 69 of them observed in young vineyards. Among all esca symptomatic vines, there were only two mature plants with “vine apoplexy” in the 2011 growing season. A total of 1,910 (7.8%) of 24,487 vines in young monitored blocks showed characteristic YVD symptoms, from which 569 (29.8%) collapsed and died (Fig. 1F) by the end of the growing season. Additionally, 52 YVD symptomatic vines were found in mature vineyards from young vines (2- to 5-year-old) used as replants in previous years, from which 11 (21.1%) collapsed and died by the end of the 2011 growing season. The incidence of esca and YVD symptomatic vines varied among the monitored blocks. Esca incidence was lower than 5% in all of the 19 monitored blocks yielding esca symptomatic vines. In the majority of these blocks (13 of 19), the number of esca symptomatic vines ranged between one and five. The highest number of esca symptomatic vines, 21, was observed in one young block. Similarly, incidence of YVD was lower than 5% in the majority of the monitored blocks (31 out of 44) yielding YVD symptomatic vines. Incidence of YVD was between 5 and 10% and between 15 and 20% in five and four blocks, respectively. The highest incidence of YVD was recorded in four separated blocks with 32, 33.2, 38.8, and 54.3% of the total monitored vines showing characteristic YVD symptoms. In the four mature vineyards positive for YVD, 12.5, 14.9, 15.1, and 17.4% of the vines used as re-plants in previous years showed characteristic YVD symptoms.

In total, the fungal species associated with esca and/or Petri disease causing YVD symptoms were identified in 96 (50.3%) of 191 vineyards surveyed. Esca and/or Petri disease fungal species were isolated from 94 (43.7%) of 215 samples collected in young vineyards and from 83 (33.5%) of 248 samples collected in mature vineyards (Table 4). Esca and Petri disease fungal species were primarily isolated from dark streaking of the wood observed in spurs, cordons, and/or trunks in mature vineyards (Fig. 1C and D). In young vineyards, those fungal species were primarily isolated from necrotic tissue observed in the rootstock, particularly at the basal end (Fig. 1G and H). Esca and Petri disease fungal species were isolated either alone or in different combinations from the same sample (Table 4). Additionally, those fungi were often isolated along with other GTD fungal pathogens, including species in the Botryosphaeriaceae and Diatrypaceae families and in the *Ilyonectria* and *Diaporthe* Nitschke/Phomopsis (Sacc.) Sacc. genera (Table 4).

Morphological characterization. Fungal species associated with esca and Petri disease and isolated from symptomatic tissue were first tentatively identified based on macroscopic (colony) and microscopic (hyphae, conidiophores, conidia) phenotypic characteristics on PDA (7,19,25) and by comparison to ex-type reference isolates obtained from both CBS and ICMP fungal collections (Table 1). Based on these morphological characters, *Cadophora luteo-olivacea* (J.F.H. Byma) T.C. Harr. & McNew, *Pa. chlamydospora*

spora, and different species of *Phaeoacremonium* were identified from symptomatic vines. Among these, *Pa. chlamydospora* and *T. minima* were the most prevalent taxa in both young and mature vineyards. *Cadophora luteo-olivacea* was isolated from 21 and 6 samples, respectively, from young and mature vineyards (Table 4). Colony morphology of *Phaeoacremonium* isolates PARC281 and PARC327 was observed to differ significantly when first compared with reference *Phaeoacremonium* isolates, and thus they were selected for further morphological characterization. Several isolates of each morphologically identified species were selected for molecular characterization to confirm identification to species (Table 2).

Molecular characterization and multi-locus analysis. BLAST analyses showed that the ITS *C. luteo-olivacea* DNA sequences from grapevines from BC shared 99 and 100% sequence homology with *C. luteo-olivacea* isolates from GenBank, including the ex-type isolate CBS 141.41. Additionally, BT and EF1- α *C. luteo-olivacea* sequences from BC shared 99 and 100% sequence homology with reference isolate CBS 357.51 as well as with several *C. luteo-olivacea* isolates from grapevines from Spain (19). BLAST analyses showed ITS and BT *Pa. chlamydospora* DNA sequences from grapevines from BC to share 99 and 100% sequence homology with *Pa. chlamydospora* isolates from GenBank, including the ex-type isolate CBS 229.95. Additionally, EF1- α *Pa. chlamydospora* DNA sequences from BC shared 99 and 100% sequence homology with reference isolate CBS 117179.

Table 4. Total number of samples yielding esca and/or Petri disease fungal species from grapevine vascular symptoms in young and mature vineyards surveyed in British Columbia

	Samples collected	
	Young vineyards	Mature vineyards
Total samples collected	215	248
Total with esca and/or Petri disease pathogens	94	83
Species isolated		
<i>Phaeomoniella chlamydospora</i>	17	16
<i>Phaeoacremonium</i> sp.	22	9
<i>Cadophora</i> sp.	5	4
<i>Phaeomoniella</i> + <i>Phaeoacremonium</i> sp.	8	4
<i>Phaeomoniella</i> + <i>Cadophora</i> sp.	1	...
<i>Phaeomoniella</i> + <i>Phaeoacremonium</i> + <i>Cadophora</i> sp.	1	...
<i>Phaeomoniella</i> + <i>Phaeoacremonium</i> sp. + other ^a	6	6
<i>Phaeomoniella</i> + <i>Cadophora</i> sp. + other ^a	4	...
<i>Phaeomoniella</i> + other ^a	11	21
<i>Phaeoacremonium</i> sp. + <i>Cadophora</i> sp.	5	...
<i>Phaeoacremonium</i> sp. + other ^a	9	21
<i>Cadophora</i> sp. + other ^a	5	2

^a Other: Fungi associated with grapevine trunk diseases other than esca and Petri disease such as species in the Botryosphaeriaceae and Diatrypaceae families and *Ilyonectria/Cylindrocarpum* and *Diaporthe/Phomopsis* genera.

Table 3. Incidence of esca and young vine decline (YVD) in British Columbia

	Vineyards surveyed (%)			Monitored blocks (%)			Monitored vines within blocks (%)				
	No.	Esca ^a	YVD ^b	No.	Esca ^a	YVD ^b	No. ^c	Esca ^d	YVD ^e	Collapsed ^f	Apoplectic
Young ^g	90	8 (8.8)	68 (75.5)	51	8 (15.7)	44 (86.3)	24,487	69 (0.3)	1,910 (7.8)	569 (29.8)	0
Mature ^h	101	13 (12.9)	7 (6.9)	67	11 (16.4)	4 (6)	31,212	35 (0.1)	52 (0.2)	11 (21.1)	2 (5.7)
Total	191	21 (11)	75 (39.3)	118	19 (16.1)	48 (40.7)	55,699	104 (0.2)	1,962 (3.5)	580 (29.5)	2 (1.9)

^a Number of vineyards/blocks with at least one esca symptomatic vine.

^b Number of vineyards/blocks with at least one YVD symptomatic vine.

^c Total number of vines included in the study. Total does not include vines dead prior to 2011 for unknown cause and new vines planted in the spring of 2011.

^d Total number of esca symptomatic vines. Percent calculated from number of vines included in the study (c).

^e Total number of YVD symptomatic vines. Percent calculated from number of vines included in the study (c).

^f Total number of YVD vines that collapsed at the end of the 2011 growing season.

^g Vineyards $\leq$ 8 years old.

^h Vineyards $>$ 8 years old.

Fig. 2. One of 19 equally most parsimonious trees resulting from a total of 773 positions in the final internal transcribed spacer (ITS), β -tubulin (BT), elongation factor (EF1- α), and actin (ACT) *Phaeoacremonium* combined dataset. Length = 1,272 steps. Consistency index (CI) = 0.430, retention index (RI) = 0.768, retention composite index (RC) = 0.363. Isolates in bold represent *Phaeoacremonium* species 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) and below the branches for Neighbor-Joining (NJ) (only bootstrap value higher than 50% is represented). Branches represented with an * were not present in the NJ tree.

To evaluate the phylogenetic relationships among the different *Phaeoacremonium* isolates obtained from grapevines in BC, their ITS, BT, EF1- α , and ACT sequences were analyzed using BLAST to select closely related sequences for the multi-locus phylogenetic analyses (Table 2). A total of 56 taxa were included in the combined ITS, BT, EF1- α , and ACT phylogenetic analysis using MP and NJ. The MP evolutionary history generated 19 equally most parsimonious trees (Length = 1,280 steps, CI = 0.432, RI = 0.767, RC = 0.365), of which one is shown in Figure 2. The NJ analysis resulted in a tree with similar topology as the MP tree. Phylogenetic analysis of the combined ITS, BT, EF1- α , and ACT datasets grouped *Phaeoacremonium* isolates from grapevines from BC in five different well-supported clades. The first clade, with bootstrap values of 96% (MP) and 97% (NJ) grouped the nine *T. minima* isolates from BC with the *T. minima* reference isolate CBS 631.94 from *V. vinifera* from Italy (Fig. 2). Based on the multigene analyses, *T. minima* isolates from BC showed a considerable intraspecific DNA variation from the ex-type specimen CBS 246.91. The second clade, with bootstrap values of 97% (MP) and 95% (NJ) grouped *Phaeoacremonium iranianaum* L. Mostert, Gräfenhan, W. Gams & Crous isolates from BC with *Pm. iranianaum* ex-type

specimen CBS 101357 from *Actinidia chinensis* in Italy (Fig. 2). A third clade, with bootstrap values of 97% (MP) and 100% (NJ) included four *Togninia fraxinopennsylvanica* (T.E. Hinds) Georg Hausner, Eyyólfssd. & J. Reid isolates from BC, which grouped together with the ex-type specimen CBS 101585 from *V. vinifera* from California (Fig. 2). Five isolates from BC, morphologically identified as two potential novel *Phaeoacremonium* species, fell into two distinct clades and did not group with any known *Phaeoacremonium* sp. Isolates PARC273 and PARC281 grouped in a well-supported clade with bootstrap values of 97% (MP) and 100% (NJ) and were phylogenetically closely related to *Pm. angustius* ex-type isolate CBS 249.95 (Fig. 2). Isolates PARC327, PARC392, and PARC394 grouped in a separated well-supported clade with bootstrap values of 99% (MP) and 100% (NJ). Accordingly, and along with the phenotypical characters previously recorded, these *Phaeoacremonium* spp. from grapevines from BC were proven to be distinct from known species; and thus are newly described.

Taxonomy

***Phaeoacremonium roseum* J.R. Úrbez-Torres, P. Haag & D.T. O'Gorman sp. nov.**

Mycobank: MB 805782

Fig. 3

Fig. 3. *Phaeoacremonium roseum* isolate PARC281 (DAOM 242365) macroscopic and microscopic phenotypic characteristics. **A**, Colony after 21 days on potato dextrose agar in 9-cm petri dish incubated at 20°C. **B and C**, Erect and single conidiophores with a lateral phialide. **D**, Subcylindrical conidiophore. **E**, Phialides and conidiogenous cells with prominent collarettes rising from hyphae. **F**, Type I and type II phialides. **G**, Type II phialides with prominent collarettes. **H to J**, Type I phialides. **K**, adeleophiliade. **L and M**, Conidia. Bars: B, C, E, G, L, and M = 5 µm; D, K, F, H, I, and J = 2 µm.

Etymology. Named after the characteristic rose to pinkish hue of a band developed at the edge of the colony on PDA.

Aerial structures in vitro on PDA. Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to five. Hyphae smooth to slightly granulate, hyaline with no warts observed, 1.8–2.5 μm wide. *Conidiophores* short, mostly unbranched, arising from aerial or submerged hyphae, elongate to subcylindrical, up to five-septate, mostly one- or two-septate, occasionally showing prominent collarettes and/or percurrent rejuvenations, hyaline, smooth to slightly granulate, (11.2–)15.9–17.5–19.1(–33.6) $\times$ (1.4–)2.0–2.1–2.2(–3.4) μm ($n = 45$). *Phialides* terminal or lateral, mostly monophiliadic, hyaline, smooth, collarettes. Type I phialides predominate, mostly elongate-ampulliform, some subcylindrical, widened at the base, lacking a basal septum, smooth, hyaline, (1.0–)3.2–3.4–3.7(–6.0) $\times$ (0.6–)1.1–1.2(–1.9) μm ($n = 127$). Type II phialides elongate-ampulliform to

subcylindrical, collarettes, hyaline, smooth to slightly granulate, (5.4–)7.5–7.9–8.3(–11.5) $\times$ (0.9–)1.3–1.4–1.4(–1.9) μm ($n = 55$). No type III phialides observed. *Conidia* mostly oblong-elliptical, some allantoid, hyaline, smooth to slightly granulate, aseptate, (3.2–)4.1–4.1–4.2(–6.2) $\times$ (1.1–)1.4–1.4–1.5(–2.1) μm , L/W = 2.9 ($n = 100$).

Cultural characteristics. Colonies reaching a diameter of 24.3 mm after 21 days at 25°C. Minimum temperature for growth 15°C, optimum 25°C, maximum 35°C. Colonies on PDA hilly, felt-like, with irregular and undulating margin. After 7 days, white with rose (71b) to rose pink (71f) margin above, amber yellow (21'b) with coral red (5') margin in reverse. After 21 days, white to pinkish vinaceous (5''d) with rose pink (71f) margin becoming livid red (69''i) at the leading edge above, amber yellow (21'b) with coral red (5') margin in reverse. Yellow pigment produced that diffused into the agar ahead of the leading edge of the colony.

Fig. 4. *Phaeoacremonium canadense* isolate PARC327 (DAOM 242366) macroscopic and microscopic phenotypic characteristics. **A**, Colony after 21 days on potato dextrose agar in 9-cm petri dish incubated at 20°C. **B**, Grouped hyphae with solitary conidiogenous cells. **C**, Hyphae aggregated and coiled. **D**, Elongate, unbranched conidiophore attenuated at the base. **E**, Subcylindrical conidiophores. **F** to **H**, Type I phialides. **I**, adelophialide. **J**, Type II phialide with prominent collarette. **K**, Type II (right) and type I (left) phialides. **L**, Navicular type III phialide. **M**, Erect type III phialide. **N** and **O**, Conidia. Bars: B and C = 10 μm ; D, E, and K to O = 5 μm ; F, G, H, I, and J = 2 μm .

Substrate. *Vitis vinifera*

Known distribution. British Columbia, Canada.

HOLOTYPE: Canada, British Columbia, Okanagan Valley, isolated from *V. vinifera* cv. Kerner showing vascular discoloration in the trunk in May 2011, J. R. Úrbez-Torres, ex-type culture PARC281/DAOM 242365.

Additional specimens examined. Canada, British Columbia, Okanagan Valley, isolated from *V. vinifera* cv. Gewürztraminer showing vascular discoloration in the trunk in May 2011, J. R. Úrbez-Torres, PARC273.

Notes. DNA sequence analyses revealed *Pm. roseum* to be closely related to *Pm. angustius*. *Phaeoacremonium angustius* lacks both the distinct rose to pink color at the margin of the colony and the yellow pigment that diffuses into the agar ahead of the leading edge of the colony. *Phaeoacremonium globosum* and *Pm. occidentale* are the other species producing yellow pigment diffusing into the agar.

Phaeoacremonium canadense J.R. Úrbez-Torres, P. Haag & D.T. O'Gorman sp. nov.

Mycobank: MB 805783

Fig. 4

Etymology. Named after Canada, where this species was collected.

Aerial structures in vitro on PDA. Mycelium consisting of branched, septate hyphae that occur singly or in bundles of up to seven. Hyphae smooth to slightly tuberculate, granulate, hyaline to light-brown en masse, no warts observed, 1.2–2.1 µm wide. Bundles occasionally coiled with conidiophores projecting outwards. Coils 8.7 to 46.1 µm, average = 21.1 µm ($n = 10$) in diameter. Conidiophores short, unbranched, arising from aerial or submerged hyphae, elongate-ampulliform to subcylindrical, occasionally navicular, mostly one-septate, some two- or three-septate, collarettes, hyaline, smooth to slightly granulate, (11.9–)17.6–18.8–19.9(–32.1) × (1.6–)2.0–2.1–2.2(–2.8) µm ($n = 61$). Phialides terminal or lateral, monophiliadic, hyaline, smooth to slightly granulate, collarettes. Type I and II predominant. Type I phialides mostly cylindrical, some widened at the base, lacking a basal septum, (1.2–)2.7–3.1–3.5(–4.9) × (0.8–)1.2–1.3–1.4(–1.8) µm ($n = 31$). Type II phialides elongate-ampulliform to subcylindrical, some with a constricted base, (5.1–)6.7–7.2–7.6(–9.5) × (1.1–)1.5–1.6–1.7(–2.5) µm ($n = 34$). Type III phialides mostly subcylindrical, some elongate with the base attenuated, (10.0–)11.1–11.7–12.2(–19.3) × (1.2–)1.4–1.5–1.6(–2.1) µm ($n = 22$). Conidia mostly oblong-elliptical, some obovate allantoid, biguttulate, hyaline, aseptate, (3.1–)4.2–4.4–4.5(–7.0) × (1.3–)1.9–1.9–2.0(–3.1) µm, L/W = 2.3 ($n = 100$).

Cultural characteristics. Colonies reaching a diameter of 26.2 mm after 21 days at 30°C. Minimum temperature for growth 15°C, optimum 30°C, maximum 35°C. Colonies on PDA flat, cottony,

with irregular and filiform margin. After 7 days, pale olivaceous-gray (21''''f) above, smoke gray (21''''d) in reverse. After 21 days, pale olivaceous-gray (21''''f) to olivaceous gray (23''''i) with a grayish yellow-green (27''''d) margin above, smoke gray (21''''d) with an olivaceous buff (21''''d) margin in reverse.

Substrate. *Vitis vinifera*

Known distribution. British Columbia, Canada.

HOLOTYPE: Canada, British Columbia, Okanagan Valley, isolated from 3309C rootstock cv. with vascular discoloration in vine showing young vine decline symptoms in June 2011, J. R. Úrbez-Torres, ex-type culture PARC327/DAOM 242366.

Additional specimens examined. Canada, British Columbia, Shuswap Valley, isolated from SO4 rootstock cv. showing dark streaking of the wood in October 2011, J. R. Úrbez-Torres, PARC392. Canada, British Columbia, Okanagan Valley, isolated from *V. vinifera* cv. Pinot Noir showing dark streaking of the wood in October 2011, J. R. Úrbez-Torres, PARC394.

Notes. *Phaeoacremonium canadense* can be distinguished by the distinct grayish yellow-green margin of the colony and the pronounced irregular, filiform margin on PDA. Hyphae of *Pm. canadense* aggregate and form characteristic coils. Other *Phaeoacremonium* spp. with hyphae aggregating in coils include *Pm. armeniacum*. Biguttulate conidia of *Pm. canadense* can be observed after 3–5 days.

Pathogenicity studies. Mean extent of vascular discoloration caused by 14 fungal isolates on detached Chardonnay dormant canes 31 days after inoculation is shown in Figure 5A. All isolates caused wood necrosis that developed upward and downward from the point of inoculation (Fig. 6A). Additionally, some of the isolates caused dark streaking of the wood, a characteristic vascular symptom associated with both esca and Petri diseases of grapevine. In this experiment, *Pm. iraniana* isolate PARC70 and *Pa. chlamydospora* isolate PARC124 caused the largest (8.9 mm) and smallest (3.8 mm) lesions, respectively. Although length of vascular necrosis was not significantly different than the natural necrosis observed in the controls in nine of the 14 treatments, percent recovery was higher than 80% for all treatments and reisolated fungi were the same as the previously inoculated based on colony and conidia morphology. *Cadophora luteo-olivacea*, *Pa. chlamydospora*, and *Togninia/Phaeoacremonium* spp. were not reisolated from the control plants.

Twenty-one weeks after inoculation, all 3309C rootstock inoculated with *C. luteo-olivacea*, *Pa. chlamydospora*, and *Togninia/Phaeoacremonium* spp. showed vascular discoloration, primarily in the form of dark streaking of the wood, which developed upward and downward from the point of inoculation (Fig. 5B). Vascular discoloration caused by all 14 fungal isolates was significantly greater than the natural necrosis observed in the controls (Fig. 6B).

Fig. 5. Pathogenicity studies of *Cadophora luteo-olivacea*, *Phaeoacremonium chlamydospora*, and *Phaeoacremonium* spp. isolated from esca and declining young vines in British Columbia. **A.** Mean lesion length caused by 14 isolates in detached dormant canes of 'Chardonnay' 31 days after inoculation. **B.** Mean lesion length caused by 14 isolates in grapevine rootstock 3309C 21 weeks after inoculation. Mean lesion length is based on six replicates per isolate. Means followed by different letters differ significantly ($P < 0.05$) according to Tukey's. Bars represent standard error of the mean.

Togninia minima isolate PARC249 and *C. luteo-olivacea* isolate PARC223 caused the largest (68.9 mm) and smallest (14.6 mm) lesions, respectively. Percent recovery for *Pa. chlamydospora* and *Togninia/Phaeoacremonium* isolates ranged from 61 to 93%. Percent recovery for both *C. luteo-olivacea* isolates was 51%. Reisolated fungi were the same as the previously inoculated based on colony and conidia morphology. *Cadophora luteo-olivacea*, *Pa. chlamydospora*, and *Togninia/Phaeoacremonium* spp. were not reisolated from the control plants.

Approximately 15 weeks after inoculation, grapevines inoculated with *Pa. chlamydospora* isolate PARC50 and *Pm. canadense* isolate PARC327 started to show distinctive esca “tiger-striped” foliar symptoms characterized first by a light interveinal chlorosis of the leaves that developed into a dark-brown color with scorched tissue between the veins (Fig. 6C to G). Eighteen weeks after inoculation, grapevines inoculated with *Pa. iraniana* isolates PARC70 and PARC194, *T. fraxinopennsylvanica* isolate PARC88, and *T. minima* isolate PARC249 showed similar “tiger-striped” esca symptoms. Sudden collapse and characteristic “apoplexy” symptoms were observed in two of the six plants inoculated with *T. fraxinopennsylvanica* isolate PARC88 1 week after observing the first foliar symptoms. Twenty-one weeks after inoculation, severe “tiger-striped” esca foliar symptoms, including leaf drop, were observed in all plants inoculated with the mentioned isolates. No characteristic esca foliar symptoms were recorded in plants inoculated with the other isolates and control plants.

Grapevine vigor indices, including total shoot weight, total shoot length, and average internode length were measured 21 weeks after inoculation. Among all treatments, only grapevines inoculated with *Pm. roseum* isolate PARC273 showed a significant ($F = 2.04$, $P = 0.0253$) total shoot weight reduction (6.1 g) when compared to the controls (11.25 g). There were no differences in total shoot length ($F = 1.31$, $P = 0.2246$) and average internode length ($F = 1.33$, $P = 0.2109$) between inoculated plants and the controls.

Discussion

In North America, esca and Petri diseases of grapevine have long been studied in California (5,37,38,39), and more recently, field surveys revealed esca disease to also occur throughout mature vineyards in Arkansas and Missouri (47). However, little is known about the occurrence and severity of these diseases in the rest of grape-growing regions of North America, which account for approximately 20% of the total bearing acres on the continent. This study provides important information about the current status of both esca and Petri disease in BC, an emerging grape-growing region whose wine and grape industry contributed over \$2.01 billion to the Canadian national economy in 2011 (36). This research revealed for the first time the occurrence of esca and Petri diseases of grapevine in BC and Canada. Morphological studies along with DNA-based molecular identification revealed the identity of seven fungal species associated with esca and Petri diseases in BC, namely *Cadophora luteo-olivacea*, *Phaeoacremonium chlamydospora*, *Phaeoacremonium canadense*, *Phaeoacremonium iraniana*, *Phaeoacremonium roseum*, *Togninia fraxinopennsylvanica*, and *Togninia minima*. Among these taxa, only *T. minima* was previously known to occur in Canada on pin cherry (*Prunus pennsylvanica* L. f.) (22). *Cadophora luteo-olivacea*, *Pa. chlamydospora*, *T. fraxinopennsylvanica*, and *T. minima* are isolated routinely from esca- and Petri disease-affected vines and have a cosmopolitan distribution (12). Their discovery in BC vineyards further expands the broad known geographical distribution of these species. Moreover, this study revealed for the first time the occurrence of *Pm. iraniana* in America. Other *Phaeoacremonium* spp. previously reported in Canada include *Phaeoacremonium krajdienii* L. Mostert, Summerb. & Crous, *Phaeoacremonium parasiticum* (Ajello, Georg & C.J.K. Wang) W. Gams, Crous & M.J. Wingf., and *Phaeoacremonium venezuelense* L. Mostert, Summerb. & Crous; although all were isolated from human infections (26).

Visual assessment of 55,699 vines in 118 vineyards in 2011 found only 104 plants (0.2%) with characteristic esca foliar and fruit symptoms. Therefore, if only foliar symptomatology is considered, the incidence of esca in BC would be considered very low compared with that found in European grape-growing regions. For instance, the incidence of plants showing esca foliar symptoms was about 21.1% in the Ampulia region of Italy (31), 2.6% in the Marmara region of Turkey (10), and 1.3% in Austria (35). Esca foliar symptoms mainly manifest themselves in mature vines, with the highest incidence and severity found in vineyards older than 20 years (27,31,35). Although grapevines have long been cultivated in BC, over 60% of the current acreage has been gradually planted after 1999 (3). Therefore, the low esca incidence observed in BC could be a result of the relative youth of the vineyards throughout the province. Interestingly, 69 of the 104 affected vines (66.3%) recorded in BC were found in young vineyards. These results show that esca foliar and fruit symptoms are not limited to mature vines, and agree with results obtained by Pollastro et al. (31) showing that symptoms of esca occurred in 12% of vineyards younger than 10 years old in the Ampulia region of Italy. Most studies conducted to assess the incidence of esca throughout European vineyards revealed either a significant discontinuity or a sudden increase in the total number of esca symptomatic vines from year to year (31,34,43). It has been suggested that abiotic and physiological seasonal factors directly contribute to esca symptom expression, and thus disease incidence varies from season to season (24,27). Consequently, a long-term field study is required to confirm whether esca incidence is actually very low in BC or follows similar discontinuity and/or rapid increase patterns as reported from European vineyards.

Similar to the observations with esca, the incidence of young vine decline and vine plant failures has been steadily increasing since the early 1990s in grape-growing regions worldwide (6,17,25,39). In 1998, Sidoti et al. (40) observed YVD to affect about 15% of the vines in a vineyard in Sicily (Italy) only 1 year after being planted. Also in the late 1990s, several young vineyards in Australia had lost 50% of their vines, and many of the survivors were infected and declining (29). However, large and thorough field studies on the incidence of YVD in vineyards, similar to those conducted on esca, are limited and based solely on estimations. Therefore, to our knowledge, this study represents the first attempt to assess the incidence of YVD over an entire grape-growing region. Evaluation of 24,487 vines in 51 young BC vineyards revealed that 7.8% of plants manifested characteristic YVD symptoms. Moreover, 8% of the young vines used as re-plants in mature vineyards manifested characteristic YVD symptoms. Although YVD includes both Petri disease and black foot, results from this study revealed that fungi associated with Petri disease were more prevalent than those known to cause black foot in YVD-symptomatic vines in BC. However, lack of both similar field studies from other grape-growing regions and studies on the economic impacts of YVD makes it difficult to evaluate the seriousness of the 7.8% YVD affliction. Although the majority of young vineyards surveyed had YVD in less than 5% of monitored vines, the number of YVD-affected vines ranged from 8 to 55% in 10 vineyards. Because a long lifespan for vines is essential for economic success in vineyards, especially considering the large investments in vineyard establishment and maintenance, it is plausible that losses of this magnitude (8 to 55% of vines) may cause substantial untenable economic loss.

Since 1996, when the genus *Phaeoacremonium* was first identified (8), 25 species of *Phaeoacremonium* had been isolated from grapevines and identified based on their cultural, morphological, and molecular characters (8,9,11,16,18,20,25,26). Interestingly, the majority of these species (19 of 25) have been identified within the last 8 years, which may be a response to a significant upsurge in the number of field surveys conducted since the early 2000s spurred by increases in esca and Petri disease incidences in grape-growing regions worldwide. In the present study, two novel *Phaeoacremonium* spp., namely *Pm. canadense* and *Pm. roseum*,

were isolated from grapevines showing characteristic esca and Petri disease vascular symptoms. Discovery of the novel species increases to 27 and 39, respectively, the total number of *Phaeoacremonium* spp. occurring in grapevine and in this genus. Species delimitation within *Phaeoacremonium* based solely on cultural and morphological characteristics is challenging, and thus molecular analyses of part of the actin and β -tubulin gene regions have, to date, been used routinely for species delineation (11,18,25,26). In the present study, part of the EF1- α and the ITS gene barcodes, respectively, have been added to 34 and 18 different *Phaeoacremonium* spp., including ex-type specimens. Translation elongation

factor 1- α alone or combined with other DNA markers in multi-locus phylogenetic analyses significantly increased phylogenetic resolution, and thus species delineation in complex taxonomic groups of fungi, such as Botryosphaeriaceae Theiss. & P. Syd. (23), *Diaporthe* Nitschke (15), and *Fusarium* Link (28). Consequently, as observed in this study, adding the EF1- α DNA marker will improve molecular characterization and significantly increase phylogenetic resolution within *Phaeoacremonium*.

Phaeomoniella chlamydospora and *T. minima* are the most prevalent and widely distributed species found in esca- and Petri disease-affected vines (25). Hence, both species have been ex-

Fig. 6. Vascular and foliar symptoms observed in grapevines used in the pathogenicity tests and inoculated with *Cadophora luteo-olivacea*, *Phaeomoniella chlamydospora*, and *Phaeoacremonium* spp. isolates from British Columbia. **A**, Vascular necrosis and dark streaking of the wood caused on detached 'Chardonnay' green shoots 31 days after inoculation by control (1), *Pa. chlamydospora* PARC50 (2) and PARC124 (3), *Togninia minima* PARC249 (4) and PARC349 (5), *Phaeoacremonium iraniamum* PARC70 (6) and PARC194 (7), *C. luteo-olivacea* PARC223 (8), *Togninia fraxinopennsylvanica* PARC88 (9) and PARC140 (10), *Phaeoacremonium roseum* PARC273 (11) and PARC281 (12), and *Phaeoacremonium canadense* PARC327 (13) and PARC394 (14). **B**, Vascular necrosis and dark streaking of the wood caused on rootstock 3309C 21 weeks after inoculation by control (1), *Pa. chlamydospora* PARC50 (2), *T. minima* PARC249 (3), *Pm. iraniamum* PARC70 (4), *T. fraxinopennsylvanica* PARC88 (5), *Pm. roseum* PARC273 (6), and *Pm. canadense* PARC327 (7). **C and D**, Development of characteristic "tiger-stripes" esca symptoms on leaves of 'Baco Noir' of vines inoculated with *Pm. canadense* isolate PARC327 16 weeks after inoculation (C) and 19 weeks after inoculation (D). Black arrow shows interveinal chlorotic areas (C) that became scorched as symptoms progress. **E and F**, Vine inoculated with *Pa. chlamydospora* isolate PARC50 showing characteristic "tiger-striped" esca foliar symptoms 19 weeks after inoculation. **G**, Leaves showing esca foliar symptoms from vines inoculated with *Pm. iraniamum* isolate PARC70 21 weeks after inoculation.

tensively studied, and as a result, their association with both vascular and foliar symptoms has been demonstrated (25,27). Results from this study further support previous research since both fungi were not only the most prevalent species isolated from esca- and Petri disease-infected vines in BC but also vascular and characteristic esca “tiger-striped” foliar symptoms were reproduced when healthy potted vines were inoculated with BC isolates of *Pa. chlamydospora* and *T. minima*. Although many new *Phaeoacremonium* spp. have been identified from esca- and Petri disease-infected plants, there is a lack of evidence for pathogenicity in most studies (9,11,18,20,25,26), making it difficult to evaluate whether the majority of *Phaeoacremonium* spp. are involved in disease development. Results from both pathogenicity tests in this study confirm that *C. luteo-olivacea* and all *Phaeoacremonium* spp. other than *T. minima* found in BC cause dark vascular streaking of the wood, a characteristic wood symptom observed in both esca- and Petri disease-infected vines. Additionally, “tiger-striped” esca foliar symptoms were reproduced when healthy potted vines were inoculated with the novel species *Pm. canadense* and the known *Pm. iraniana* and *T. fraxinopennsylvanica*. These results provide irrefutable evidence that fungi other than *Pa. chlamydospora* and *T. minima* can be virulent on grapevines and cause esca foliar symptoms. Thus, future molecular, epidemiological and chemical studies investigating the poorly understood cause of esca foliar symptoms should consider including a larger number of *Phaeoacremonium* spp. Moreover, although no esca foliar symptom was reproduced with isolates of the novel species *Pm. roseum*, the healthy potted vines inoculated with this species showed a significant reduction in total shoot weight, which could indicate a role for *Pm. roseum* in the decline of young vines.

Accurate identification, diagnostics, and monitoring of fungal pathogens is critical to developing effective management strategies. Accordingly, this study has completed the first step by identifying and characterizing the fungi associated with esca and Petri disease of grapevines in BC as well as revealing declining of young vines as an important disease issue in BC that needs further investigation. It has been demonstrated that many of the pathogens responsible for causing YVD can be introduced into a vineyard with the planting material (17). Expansion of the different BC grape-growing regions depends on the importation of vine transplants. Consequently, the information generated in this study could be used to develop species-specific molecular detection tools to facilitate epidemiological studies and to determine the health status of planting material entering in BC.

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