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Potential role of *Fusarium* spp. in grapevine decline

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Summary. Despite studies associating *Fusarium* spp. with grapevine decline since late 1970s, no consensus has been reached regarding the roles these fungi play in grapevine health. Recent studies in British Columbia, Canada, assessed prevalence of grapevine trunk diseases (GTDs) in young and mature vineyards, and the presence of GTD fungi in ready-to-plant nursery material sold in Canada. This study characterized the *Fusarium* spp. isolated from grapevines in BC by sequencing part of the translation elongation factor 1-alpha (*TEF1*) gene, and carried out pathogenicity studies to determine whether *Fusarium* plays a role in grapevine decline. *Fusarium* spp. were isolated from 9.8% of samples collected from young vines (≤ 8-year-old), and 7.3% from mature vines (> 8-year-old), showing decline symptoms in commercial vineyards. *Fusarium* was also isolated from 43.9% of ready-to-plant dormant grapevines analyzed from four nurseries. *Fusarium* incidence varied between plants within the same nursery and between plants from the different nurseries. DNA sequences of *TEF1* allowed identification of *Fusarium oxysporum*, *F. proliferatum*, *F. ramigenum*, and a *Fusarium* sp. Pathogenicity studies were conducted in 1-year-old dormant rooted 'Chardonnay' plants grafted onto '3309C' rootstock, and treatments included: i) whole plant, ii) trimming of roots, iii) cut at rootstock basal ends, and iv) trimming of roots plus cut at rootstock basal ends. Plants were inoculated using standardized methods, and were then planted in a greenhouse. *Fusarium* was compared with *Dactylonectria macrodidyma*, *D. pauciseptata* and *Ilyonectria liriodendri* used as positive controls. The *Fusarium* spp. caused necroses in rootstock roots and basal ends that were similar to those caused by black-foot fungi. *Fusarium* spp. and black-foot fungi reduced root and shoot dry weights when compared with non-inoculated controls, but no statistically significant differences were recorded for most treatments. This study is the first in Canada to identify *Fusarium* spp. from grapevines. Though *Fusarium* was common in these grapevines, pathogenicity tests suggest that the identified *Fusarium* spp. were weakly pathogenic to '3309C' rootstock grapevines.

Keywords. *Fusarium*, grapevine trunk diseases, *Ilyonectria*, Petri disease, translation elongation factor 1-α, *Vitis vinifera*.

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INTRODUCTION

Grapevine trunk diseases (GTDs) are major causes of grapevine decline, resulting in economic losses to grape and wine production industries (Wicks and Davies, 1999; Siebert, 2001; Kaplan *et al*., 2016). These complex diseases are associated with many fungal species, belonging to more than 30 genera, and include black-foot and Petri disease, responsible for grapevine decline and mortality in young vines, and Botryosphaeria dieback, Eutypa dieback, Esca, and Phomopsis dieback, which reduce yields and limit the lifespan of vines in mature vineyards (Gramaje *et al*., 2018). Most GTD fungi infect grapevines through pruning wounds, but those causing black-foot are soilborne (Agustí-Brisach and Armengol, 2013). Several of these fungi are also introduced into vineyards via contaminated grapevine nursery material (Gramaje and Armengol, 2011; Billones-Baaijens *et al*., 2013; Hrycan *et al*., 2023). Some GTD fungi are known to naturally occur in the vascular systems of asymptomatic grapevines, possibly as latent pathogens (González and Tello, 2011; Kraus *et al*., 2019; Hrycan *et al.* 2020).

Fusarium is a species-rich genus, which includes important plant pathogens causing root and crown rots, wilts, blights, and/or cankers in a wide range of annual and perennial crops (Gordon, 2017). Marais (1979) reported *Fusarium* spp. to be commonly isolated from roots and rhizosphere soil of stunted, dying or dead grapevines in South Africa. Grasso (1984) associated death of young grapevines with *Fusarium oxysporum* and *'Cylindrocarpon' destructans* in Sicily, Italy. Highet and Nair (1995) reported *F. oxysporum* to be prevalent in roots of declining 'Semillon' grapevines in the Hunter Valley, Australia. Omer *et al.* (1995), and Granett *et al.* (1998) isolated *F. oxysporum*, *F. roseum* and *F. solani* in high frequencies from roots damaged by grape phylloxera in self-rooted 'Chardonnay' and 'AXR#1' rootstock vines collected from commercial vineyards in California (CA), United States of America. Luque *et al.* (2009) isolated *Fusarium* spp. from wedge-shape cankers, central necroses and wood decay in declining vines from mature vineyards in Spain. Studies from different countries have continued to report *Fusarium* spp. as prevalent in roots, cordons, and/or trunks of grapevines showing decline symptoms (Ziedan *et al*., 2011; Abdullah *et al.* 2015; Chehri, 2017; Bustamante *et al*., 2022; Zhang *et al*., 2023).

Fusarium spp. have been associated with declining grapevines from young and mature vineyards and in grapevine nursery plants. Halleen *et al.* (2003) reported *Fusarium* spp. as the most frequently isolated fungi from asymptomatic rooted cuttings collected 3 months after planting from different commercial nurseries in South Africa. A survey in South Africa by van Coller (2004) identified up to ten *Fusarium* spp. from roots and crowns of declining nursery grapevines. Studies across several nurseries in Spain reported six different *Fusarium* spp. isolated from plants showing decline or vascular wilt symptoms (Pintos *et al*., 2018; Astudillo-Calderon *et al*., 2019). Akgül *et al.* (2023) confirmed *Fusarium* spp. to be commonly found in asymptomatic nursery-produced plants from different geographical regions in Türkiye.

Despite all these studies, the role of *Fusarium* as a true pathogen of grapevines remains unclear. Pathogenicity studies have shown *F. annulatum*, *F. commune*, *F. decemcellulare*, *F. equiseti*, *F. oxysporum*, and *F. solani* are virulent on grapevines, and cause similar symptoms to those in declining young and/or mature vines in nurseries or commercial vineyards (Highet and Nair, 1995; Omer et al., 1999; Ziedan *et al*., 2011; Chehri, 2017; Astudillo-Calderon *et al*., 2019; Bustamante *et al*., 2022; Zhang *et al*., 2023). In contrast, other studies have concluded that *Fusarium* spp. to not play important roles in grapevine decline, and refer to these fungi as common endophytes in *Vitis vinifera* (Marais, 1980; Casieri *et al*., 2009; Pancher *et al*., 2012). Therefore, *Fusarium* spp. found in many studies have not been included in completion of Koch's postulates (Rumbos and Rumbou, 2001; Halleen *et al*., 2003; Luque *et al*., 2009; Moreno-Sanz *et al*., 2013).

Grapevine trunk diseases and consequent vine decline and mortality have been identified as one of the main threats to long-term economic sustainability of the grape and wine industries in British Columbia (BC), Canada. To date, over 40 different fungal species have been identified from young and mature vines showing GTD symptoms (Úrbez-Torres *et al*., 2014a; 2014b; 2015a). These studies have routinely isolated *Fusarium* spp. from declining grapevines in the field or from ready-to-plant nursery vines. Accordingly, the objectives of this study were: i) to characterize the different *Fusarium* spp. isolated from grapevines from commercial vineyards and nursery plants in BC using molecular methods; and ii) to complete pathogenicity studies to determine the role of *Fusarium s*pp. in grapevine health.

MATERIALS AND METHODS

Fusarium *isolates used in this study*

Fusarium isolates were obtained from grapevine samples collected from young and mature commercial vineyards in BC during field surveys conducted between 2010 and 2013 (Úrbez-Torres *et al*., 2014a; 2014b). In

total, 215 and 248 vine samples (entire or part of the vine) showing decline symptoms were collected from 90 young vineyards (≤ 8-years-old) and 101 mature vineyards (> 8-years-old). Fungal isolations were conducted as described by Úrbez-Torres *et al.* (2014a). In addition, 362 ready-to-plant dormant rooted vines, including different scion-rootstock combinations and self-rooted cultivars, were collected between 2014 and 2021 from four nurseries selling grapevines in Canada. Isolations to identify different fungi were conducted from plant roots, basal ends of the rootstocks, graft unions, and scions, with the exception of self-rooted plants, from which samples were taken from roots, scion basal ends, and scions, as described by Hrycan *et al.* (2023). Wood pieces from the different host sections were plated on potato dextrose agar (PDA) (Difco) in Petri plates, amended with tetracycline hydrochloride (0.01%) (Sigma-Aldrich) (PDA-tet), and then incubated at 22°C in the dark until fungal colonies were observed. *Fusarium*-like colonies obtained from sample wood were sub-cultured on PDA. Hyphal tip subculturing onto PDA was then conducted from actively growing colonies, and these pure subcultures were held at 22°C in the dark. Based on colony morphology, a number of isolates representing different geographical locations, host cultivars and nurseries were chosen for molecular and pathogenicity studies. Fifteen to 20 mycelium plugs (0.5 cm diam.) were obtained from each selected pure culture isolate and placed in clear screw-cap glass vials containing autoclaved micropore filtered water. The vials were then maintained at 4°C in the Plant Pathology fungal collection at the Summerland Research and Development Centre (SuRDC), Summerland, BC, Canada until used.

Molecular identification of Fusarium *isolates*

Fusarium isolates selected for DNA extraction were retrieved from the SuRDC fungal collection and revived on PDA at 22°C in the dark. DNA was extracted from actively growing colonies using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc.). Oligonucleotide primers EF1 and EF2 were used to amplify part of the translation elongation factor 1-α (*TEF1*) (O'Donnell *et al.*, 1998). Each polymerase chain reaction (PCR) contained 2 µL of 10× PCR buffer, containing 15 mM $MgCl_2$, 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), and 1 µL of DNA template, adjusted with micropore filtered water to a final volume of 20 µL. Amplification reactions were carried out on a GeneAmp 2700 thermal cycler (Applied Biosystems), with an annealing temperature (T) of 53°C (Geiser *et al.*, 2004). Amplified products were purified using a QIAquick PCR purification Kit (QIAGEN Inc.), and both forward and reverse strands of the *TEF1* were sequenced using a 8-capillary 3500 Genetic Analyzer (Applied Biosystems) at the SuRDC. Sequences were edited and assembled using DNASTAR SeqMan™ Ultra version 17.4.1 (DNASTAR Inc.). Consensus sequences were then subjected to BLASTn queries in the GenBank database to determine species identifications. *Fusarium* sequences collected in this study were deposited into GenBank, and representative isolates are maintained in the SuRDC fungal collection.

Pathogenicity study

Four isolates representing the *Fusarium* spp. identified in this study were selected to inoculate dormant rooted 'Chardonnay' vines grafted onto '3309C' rootstocks. *Dactylonectria macrodydima* (SuRDC-1207), *Dactylonectria pauciseptata* (SuRDC-1248) and *Ilyonectria liriodendri* (SuRDC-1203) isolates, previously identified from black-foot symptomatic vines in BC (Úrbez-Torres *et al.*, 2014a), were included in the study as positive controls. Conidium suspensions from the different *Fusarium* spp. and black-foot fungi were prepared as described by Úrbez-Torres *et al.* (2014a). Plants were inoculated by immersion of the roots and basal end of each plant in a suspension (10^5 conidia mL^{-1}) of each fungus in a separate bucket, and left overnight. Four different treatments were included in this study. These were: 'whole plant' (WP, no cut roots and basal end not cut), 'roots cut' (RC), 'basal end cut' (BC), and 'roots and basal end cut' (RBC). Six plants per fungus/treatment combination were used. Six plants for each treatment were immersed in separate buckets containing non-inoculated autoclaved distilled water as negative controls. After incubation (for approx. 18 h), the plants were retrieved from the buckets and planted into pots (22 L capacity) containing doubled-autoclaved standard potting mix soil, in a greenhouse. All plants were watered and fertilized equally and as needed. Six months after planting, the plants were uprooted and a small portion of roots and rootstock basal end of each plant were collected for fungus re-isolations. These were carried out from the roots (R), basal end (BE) and 1 cm above the basal end (1-BE) of each plant. Fungal isolations and shoot and root dry weights were recorded from each plant using the methods of Úrbez-Torres *et al.* (2014a).

Data analyses were carried to determine effects of fungal isolate and experimental treatments on shoot and root dry weights. These data were square root transformed to normalize residuals. Normality was confirmed

using inspection of residuals and Shapiro-Wilk tests. Shoot and root weights were analyzed using analysis of variance and Tukey's Honest Significant Difference test in R Software version 4.3.0 (R Core Team, 2023).

RESULTS

Incidence of Fusarium *in young and mature vineyards in BC, and in nursery grapevines.*

Fusarium was detected in 21 of 215 samples (9.8%) collected from young grapevines (≤ 8 years old) showing decline symptoms in commercial vineyards, and *Fusarium* isolates were mostly obtained from necrotic roots and vascular necroses observed around the stem pith at the bases of rootstocks or self-rooted cultivars (Figure 1). *Fusarium* was detected from 18 of 248 of samples (7.3%) collected from mature vines (> 8 years old) showing dieback in commercial vineyards, and was isolated from different host symptoms, including necrotic roots, wedge-shape cankers, vascular necroses and wood decay in cordons and trunks (Figure 1). Incidence of *Fusarium* in ready-to-plant nursery grapevines is shown in Table 1. *Fusarium* was isolated from 159 of 362 (43.9%) of the ready-to-plant dormant grapevines analyzed from all the assayed nurseries. Overall, the greatest infection was recorded from the graft-union sections of plants, followed by the base of the rootstocks or self-rooted cultivar roots and scions. Nursery C-2021 had the greatest level of infections, with 86.7% of plants yielding *Fusarium*. Nurseries A-2019 and D-2021 followed with, respectively, 70% and 78% of their plants infected with *Fusarium*. Nurseries A-2014 (31.8%) and B-2014 (20%) had the least *Fusarium* infection proportions (Table 1). *Fusarium* incidence also varied depending on the assessment year. Nursery A was sampled in 2014 and 2019. Though the number of plants analyzed in 2019 was less than in 2014, *Fusarium* incidence in 2019 was more than double than in 2014 (Table 1). Incidence of *Fusarium* in the different parts of the plants varied between plants within each nursery, and between nurseries. For example, roots and rootstock bases yielded, respectively, the greatest (73.1%) and least (15.4%) *Fusarium* incidence in nursery C-2021. In contrast, roots and rootstock bases yielded, respectively, the least (4.3%) and greatest (44.9%) *Fusarium* incidence nursery A-2014 (Table 1).

Molecular identification of Fusarium *isolates*

In total, 311 *Fusarium* isolates were obtained in this study, 39 from commercial vineyards and 272 from ready-to-plant dormant rooted nursery plants. Eight isolates from commercial vineyards and 49 isolates from nursery plants were selected for molecular identifications. PCR amplification of the *TEF1* gave products between 650 and 750 bp. BLASTn analyses of the consensus sequences identified four species, including *F. oxysporum*, *F. proliferatum*, *F. ramigenum*, and a *Fusarium* sp. (Table 2). All four species were identified from samples collected from declining vines in commercial vineyards, and *F. oxysporum* and *F. proliferatum* were identified from nursery plants. *Fusarium proliferatum* was the most commonly isolated species from nursery plants (39 of 49 isolates). All *F. oxysporum* and *F. proliferatum* isolates from BC had 100% identity (100% query cover) with previously identified and published sequences in GenBank. Similarly, *F. ramigenum* isolates PARC425 had 99.84% similarity (100% query cover) to GenBank sequences. Isolate PARC428 had 95.40% similarity to *Fusarium napiforme* Genbank, so these isolates were classified as *Fusarium* sp. (Table 2).

Pathogenicity studies

Fusarium spp. caused root necroses in '3309C' in all treatments, including when roots were not cut, and the symptoms were similar to those caused by the black-foot fungi used as positive controls. Statistical analyses showed that effects on mean root dry weights, of isolate (F = 5.813, P = 7.24E^{-06}), treatment (F = 2.784, P = 0.04354), and the isolate by treatment interaction (F = 2.069, P = 0.00728) were statistically significant. The effect of isolate on mean shoot dry weight (F = 4.868, P = 6.81E^{-06}) was significant, but this was not the case for treatment (F = 0.582, P = 0.628) or the isolate by treatment interaction (F = 1.35, P = 0.156). Though not significantly different from the control plants, most inoculated isolates reduced plant root dry weights compared with the non-inoculated controls (Table 3). *Ilyonectria liriodendri* was the only fungus that increased mean shoot dry weight for inoculated plants with the basal ends cut (Table 3). Among all the isolates and treatments, only *D. macrodidyma* and *F. proliferatum* significantly reduced root dry weight when inoculated onto plants with cut roots or basal ends. Similarly, *Fusarium* sp. isolate PARC428 significantly reduced root dry weight when the inoculated plants had cut ends (Table 3). A similar trend was observed for shoot dry weights. With few exceptions, where the inoculated plants had greater shoot dry weights than the non-inoculated controls, most isolates reduced mean shoot dry weights after all of the treatments, but none of these were statistically different from the controls (Table 3).

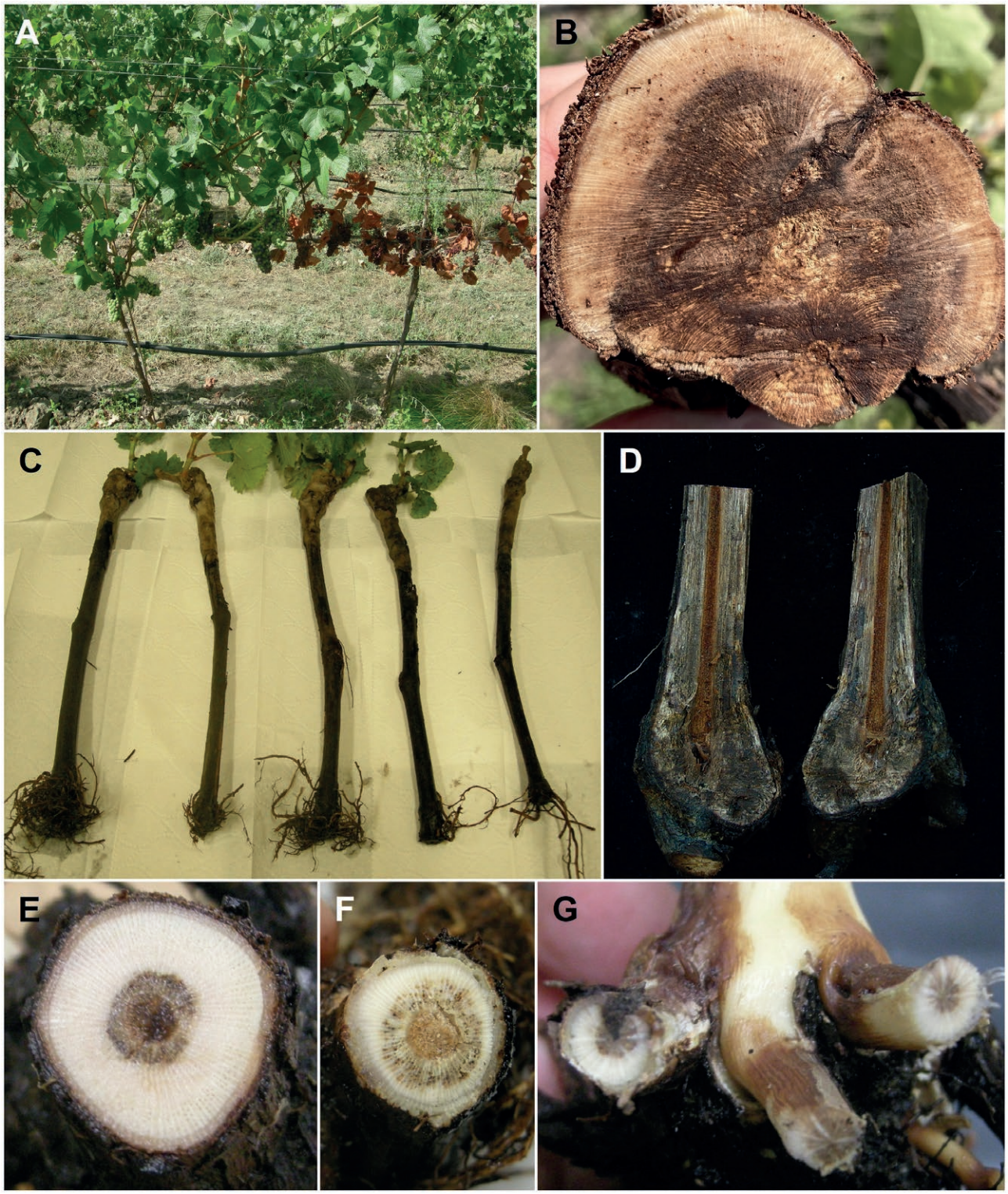

Figure 1. Grapevine decline symptoms from which *Fusarium* spp. were isolated in British Columbia, Canada. A, Decline symptoms and collapse observed in a young 'Merlot' vine in a commercial vineyard. B, Perennial canker, necrosis and soft wood symptoms observed at the base of the trunk of a mature 'Siegerrebe' vine in a commercial vineyard. C, Young vines showing poor shoot growth and root development. D, Vascular necrosis at a rootstock basal end. E and F, Vascular necrosis around the pith of a ready-to-plant nursery vine. **G**, Root necrosis and discolouration in a young vine from a commercial vineyard.

Table 1. Total numbers (and percentages) of plants and plant parts infected with *Fusarium*, that were analyzed from four grapevine nurseries.

Nursery[a]	Plants		Roots	Base	Graft-Union	Scion
	Total	Inf. (%)[b]	Inf. (%)[c]	Inf. (%)[c]	Inf. (%)[c]	Inf. (%)[c]
A-2014	217	69 (31.8)	3 (4.3)	31 (44.9)	27 (39.1)	16 (23.2)
A-2019	45	35 (77.8)	11 (31.4)	20 (57.1)	23 (65.7)	11 (31.4)
B-2014	40	8 (20.0)	4 (50)	3 (37.5)	-	3 (37.5)
C-2021	30	26 (86.7)	19 (73.1)	4 (15.4)	12 (46.2)	5 (19.2)
D-2021	30	21 (70.0)	12 (57.1)	4 (19.0)	14 (66.7)	3 (14.3)
TOTAL	362	159 (43.9)	58 (35.6)	62 (39.0)	76 (47.8)	38 (23.9)

[a] Different letters correspond to different nurseries, and different numbers indicate the years when samples were analyzed from each nursery.
[b] Number (and percentage) of the total analyzed plants that were infected with *Fusarium*.
[c] Number (and percentage) of different plant parts infected with *Fusarium*.

Re-isolation proportions of the different inoculated fungi depended on experimental treatment (Table 4). None of the fungal pathogens were re-isolated from the non-inoculated controls. *Fusarium solani*, *Ilyonectria robusta* and *I. torresensis* were isolated from roots of some control plants, from the 'whole plant' and 'roots trimmed' treatments. Black-foot pathogens and *Fusarium* spp. were re-isolated from between 20 and 100% from the roots of 'whole plants'. Re-isolation percentages from roots were greater for all fungi in the 'roots trimmed' treatment (60 to 100%), and were even greater (80 to 100%) from the 'roots and basal end cut' treatment (Table 4). Only *I. liriodendri*, *D. macrodidyma* and *Fusarium* sp. PARC428 were re-isolated from the basal ends of 'whole plants', though at low proportions (20 to 40%). Re-isolation percentages were greater from the basal ends from the 'roots trimmed' (20 to 60%), 'basal end cut' (40 to 100%) and 'roots and basal end cut' (80 to 100%) treatments (Table 4). No fungal pathogens were re-isolated from 1 cm above the basal ends from the 'whole plant' treatment, and very re-isolation was recorded for *D. pauciseptata*, *F. oxysporum* and *F. proliferatum* from the 'roots trimmed' treatment (Table 4). Re-isolation of all fungi from 1 cm above the basal ends increased from the 'basal end cut' (20 to 60% isolation) or 'roots and basal end cut' (20 to 80%) treatments (Table 4).

DISCUSSION

Seventeen different *Fusarium* species have been isolated and identified from asymptomatic and symptomatic grapevines (Highet and Nair, 1995; van Coller, 2004; Chehri, 2017; Kraus *et al.*, 2019; Bustamante *et al.*, 2022; Li *et al.*, 2023). The present study identified *F. oxysporum*, *F. proliferatum*, *F. ramigenum*, and a potentially novel *Fusarium* sp., from young and mature grapevines with GTDs symptoms, from vineyards in BC, Canada. Previous studies have identified more than 40 fungal species belonging to 14 genera, from declining vines in BC (Úrbez-Torres *et al.*, 2014a; 2014b; 2015a). The present study adds *Fusarium* spp. to the group of fungi associated with GTD symptoms in Canada.

Molecular identification of *Fusarium* spp. in this study was achieved using amplification of part of the *TEF1* using primers EF1 and EF2 (O'Donnell *et al.*, 1998), since this approach has been reported to be the most informative for identification and discrimination among *Fusarium* spp. (Geiser *et al.*, 2004). In addition, the use of short sequences obtained from other *TEF1* primers has been shown wrongly identify *Fusarium* spp. (Torres-Cruz *et al.*, 2022). Other loci, such as the RNA polymerase largest (*RPB1*) and the second largest subunit (*RPB2*) have been shown to effectively discriminate among *Fusarium* spp. in single or multi-loci phylogenetic analyses (O'Donnell *et al.*, 2013). Identification of isolates in the present study as belonging to the *F. oxysporum* species complex and as *F. proliferatum* and *F. ramigenum* using the *TEF1* is likely to be accurate, but further research including the *RPB1* and/or *RPB2* would allow further characterization of isolate PARC428, and determining if it is a novel *Fusarium* sp. or belong to an already known species.

Fusarium was isolated from 8.4% of samples collected from young and mature grapevines showing different vascular symptoms in roots, trunks, cordons, and spurs. Previous studies investigating fungi associated with GTDs symptoms or diversity of fungal communities in grapevine wood have identified *Fusarium* spp. at different levels of abundance. Marais *et al.* (1979), Granett *et al.* (1998), Abdullah *et al.* (2015), Moreno-Sanz *et al.* (2013), and Li *et al.* (2023) found *Fusarium* spp. to be among the most prevalent fungi isolated from mature grapevines showing decline symptoms. In contrast, other studies have reported incidence of *Fusarium* to be low in grapevines with characteristic GTD symptoms (Luque *et al.*, 2009). The incidence of *Fusarium* isolated from vines with GTD symptoms in vineyards in BC was low, and these results were similar to those reported by Luque *et al.* (2009). Since *Fusarium* spp. are primarily soilborne, the low incidence of *Fusarium* in the present study could be because most of the samples analyzed were symptomatic aerial plant parts, such as cordons and spurs. Incidence of *Fusarium* in the present study was greater

Table 2. *Fusarium* isolates from grapevines from British Columbia identified in this study.

Isolate[a]	Species	Host	Source[b]	*TEF1*[c]
SuRCD-1207*	*Dactylonectria macrodydima*	*V. riparia* x *V. rupestris*	3309C (base)[f]	KF511989
SuRDC-1248*	*Dactylonectria pauciseptata*	*V. riparia* x *V. rupestris*	3309C (base)[f]	KF511982
PARC420*	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	3309C (base)[f]	OR398349
PARC422	*Fusarium oxysporum*	*Vitis vinifera*	Riesling (trunk)[f]	OR398350
SuRDC-1271	*Fusarium oxysporum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398356
SuRDC-1282	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	101-14 (base)[n]	OR398353
SuRDC-1283	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	101-14 (base) [n]	OR398354
SuRDC-1296	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	101-14 (base) [n]	OR398358
SuRDC-1298	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	101-14 (graft-union)[n]	OR398359
SuRDC-1300	*Fusarium oxysporum*	*V. riparia* x *V. rupestris*	3309C (base)[n]	OR398355
SuRDC-1306	*Fusarium oxysporum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398351
SuRDC-1307	*Fusarium oxysporum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398352
SuRDC-1308	*Fusarium oxysporum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398357
PARC40	*Fusarium proliferatum*	*Vitis vinifera*	Chardonnay (roots)[f]	OR398360
PARC45	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (cordon)[f]	OR398361
PARC64	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (roots)[f]	OR398362
PARC416*	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[f]	OR398367
SuRDC-1256	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398390
SuRDC-1258	*Fusarium proliferatum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398379
SuRDC-1260	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398386
SuRDC-1261	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398402
SuRDC-1262	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (base)[n]	OR398389
SuRDC-1263	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398392
SuRDC-1264	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398394
SuRDC-1265	*Fusarium proliferatum*	*Vitis vinifera*	Pinoy Noir (scion)[n]	OR398383
SuRDC-1266	*Fusarium proliferatum*	*Vitis vinifera*	Chardonnay (scion)[n]	OR398363
SuRDC-1267	*Fusarium proliferatum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398364
SuRDC-1268	*Fusarium proliferatum*	*Vitis vinifera*	Chardonnay (scion)[n]	OR398368
SuRDC-1269	*Fusarium proliferatum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398369
SuRDC-1270	*Fusarium proliferatum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398381
SuRDC-1272	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (graft-union)[n]	OR398370
SuRDC-1273	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398393
SuRDC-1274	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (graft-union)[n]	OR398371
SuRDC-1275	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398372
SuRDC-1276	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR338399
SuRDC-1277	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	101-14 (graft-union)[n]	OR398373
SuRDC-1278	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	101-14 (graft-union)[n]	OR398365
SuRDC-1280	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398374
SuRDC-1281	*Fusarium proliferatum*	*Vitis riparia*	Riparia Gloire (base)[n]	OR398382
SuRDC-1285	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (graft-union)[n]	OR398380
SuRDC-1286	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398385
SuRDC-1287	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398395
SuRDC-1288	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398396
SuRDC-1289	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398387
SuRDC-1290	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	101-14 (base)[n]	OR398388
SuRDC-1291	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398375
SuRDC-1292	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398376
SuRDC-1293	*Fusarium proliferatum*	*Vitis vinifera*	Chardonnay (scion)[n]	OR398984
SuRDC-1294	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398377

(Continued)

Table 2. (Continued).

Isolate[a]	Species	Host	Source[b]	*TEF1*[c]
SuRDC-1295	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	101-14 (graft-union)[n]	OR398378
SuRDC-1297	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	101-14 (graft-union)[n]	OR398366
SuRDC-1299	*Fusarium proliferatum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[n]	OR398391
SuRDC-1301	*Fusarium proliferatum*	*Vitis vinifera*	Pinot Noir (scion)[n]	OR398400
SuRDC-1302	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	420A (graft-union)[n]	OR398397
SuRDC-1303	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (graft-union)[n]	OR398398
SuRDC-1304	*Fusarium proliferatum*	*V. berlandieri* x *V. riparia*	SO4 (base)[n]	OR398401
PARC425*	*Fusarium ramigenum*	*V. riparia* x *V. rupestris*	3309C (graft-union)[f]	OR398403
PARC428*	*Fusarium* sp.	*V. riparia* x *V. rupestris*	3309C (roots)[f]	OR398404
SuRDC-1205*	*Ilyonectria liriodendri*	*V. riparia* x *V. rupestris*	3309C (roots)[f]	KF511985

[a] PARC: Pacific Agri-Food Research Centre. SuRDC: Summerland Research and Development Centre. CBS: Centraal Bureau voor Schimmelcultures. * Isolates selected for the pathogenicity assessments.
[b] f: isolate obtained from commercial vineyard. n: isolate obtained from nursery .
[c] *TEF1*: Translation elongation factor 1-α.

in young vines (≤ 8 years old) primarily showing characteristic black-foot symptoms, including root rot and vascular necrosis surrounding pith tissues at the rootstock bases and in self-rooted plants. This agrees with most studies that have shown high abundance of *Fusarium* in samples from symptomatic roots and basal areas of trunks (Marais *et al*., 1979; Grannet *et al*., 1998; Highet and Nair, 1995). Therefore, in order to better understand the associating between *Fusarium* spp. and vine decline in BC, further research should include assessments of the belowground status of symptomatic plants.

Fusarium incidence has been reported to be usually greater in ready-to-plant nursery grapevines than in young and/or mature vines from commercial vineyards. The present study showed that 43.9% of all analyzed nursery plants were infected by *Fusarium*, and in one nursery up to 87% incidence of infection was detected. The high prevalence of *Fusarium* spp. isolated from nursery material sold in Canada agrees with previous research in other countries (Halleen et al., 2003; van Coller, 2004; Pintos *et al*., 2018; Astudillo-Calderon *et al*., 2019; Akgül *et al*., 2023).

Only two species, *F. oxysporum* and *F. proliferatum* (i.e. low species diversity) were identified from nursery stocks in the present study. These results are of pertinent because the nursery plants came from different geographical regions, so greater species diversity was expected. Previous studies have identified greater *Fusarium* species diversity from nursery plants, including *F. oxysporum* and *F. proliferatum* (van Coller, 2004; Pintos *et al*., 2018). In contrast and similarly to the present study, other investigations have identified one *Fusarium* sp. from surveyed nursery plants (Astudillo-Calderon *et al*., 2019; Zhang *et al*., 2023). Several factors could explain these differences, but since morphological identification of *Fusarium* spp. is challenging, possible loss of diversity may have occurred in the present study as a result of the initial morphological classifications. Previous studies have shown molecular detection to be more informative than traditional culturing when determining the incidence of GTD fungi from grapevine nursery material (Úrbez-Torres *et al*., 2015b; Hrycan *et al*., 2023). Only traditional plating was conducted in the present study, so *Fusarium* incidence in nursery plants may have been greater if a molecular method was used. *Fusarium* incidence varied between plants within each nursery, between sections within the individual plants, and between plants from the different nurseries. These results were similar to those of Hrycan *et al.* (2023).

Results from the present study add to those that have reported high incidence of *Fusarium* spp. in ready-to-plant nursery material (Halleen *et al*., 2003; Pintos *et al*., 2018; Astudillo-Calderon *et al*., 2019; Akgül *et al*., 2023). Previous studies have investigated GTD fungi during nursery propagation processes, and the health status of nursery plants. (Gramaje and Armengol, 2011; Billones-Baaijens *et al*., 2013; Pintos *et al*., 2018; Hrycan *et al*., 2023; Akgül *et al*., 2023). Therefore, the high incidence of *Fusarium* confirmed in nursery material in several countries should be further investigated, to determine the main sources of infections during nursery propagation processes. Furthermore, the role of *Fusarium* as a common grapevine endophyte should be investigated.

Despite the numerous reports of *Fusarium* spp. as fungal pathogens of grapevines, there is no consensus regarding the role of *Fusarium* in grapevine health and

Table 3. Mean root and shoot dry weights for 'Chardonnay' grapevine plants grafted onto '3309C' rootstock and inoculated with spore suspensions of different *Fusarium* and black-foot fungi, in four experimental treatments.

Isolate / Species	Mean root dry weight (g)				Mean shoot dry weight (g)			
	WP[a]	RC[b]	BC[c]	RBC[d]	WP[a]	RC[b]	BC[c]	RBC[d]
Control	10.30 ± 6.84 a	9.00 ± 4.25 a	6.28 ± 5.27 ab	7.66 ± 3.35 a	6.98 ± 3.15 a	7.47 ± 1.43 a	4.98 ± 1.77 a	7.62 ± 5.85 a
SuRDC-1205 / *I. liriodendri*	4.02 ± 3.59 a	5.64 ± 5.50 ab	12.36 ± 3.90 a	4.62 ± 2.01 a	3.85 ± 0.81 a	6.54 ± 2.44 a	9.05 ± 4.18 a	5.82 ± 1.39 a
SuRCD-1207 / *D. macrodydima*	6.62 ± 1.69 a	3.35 ± 1.16 b	3.52 ± 0.63 b	3.78 ± 1.60 a	4.45 ± 1.12 a	5.77 ± 3.11 a	4.58 ± 0.42 a	7.74 ± 1.38 a
SuRDC-1248 / *D. pauciseptata*	3.48 ± 2.50 a	4.38 ± 1.16 ab	4.14 ± 2.08 ab	5.80 ± 2.98 a	4.74 ± 0.50 a	5.08 ± 1.80 a	3.05 ± 2.19 a	3.29 ± 2.94 a
PARC420 / *F. oxysporum*	6.80 ± 1.69 a	4.04 ± 1.42 ab	5.38 ± 2.62 ab	4.68 ± 2.72 a	6.65 ± 1.54 a	8.40 ± 4.39 a	6.82 ± 2.55 a	7.47 ± 3.66 a
PARC416 / *F. proliferatum*	5.06 ± 0.63 a	3.76 ± 0.63 b	3.56 ± 1.15 b	2.42 ± 1.24 a	6.67 ± 0.66 a	5.60 ± 1.29 a	5.69 ± 1.50 a	5.17 ± 1.76 a
PARC425 / *F. ramigenum*	9.46 ± 3.93 a	4.16 ± 1.48 ab	7.50 ± 1.43 ab	5.60 ± 3.76 a	7.66 ± 2.31 a	5.42 ± 1.04 a	7.28 ± 2.65 a	6.75 ± 4.15 a
PARC428 / *F usarium* sp.	8.70 ± 3.44 a	7.98 ± 3.62 ab	3.65 ± 0.91 b	6.26 ± 2.26 a	5.98 ± 1.59 a	5.66 ± 2.78 a	4.68 ± 1.37 a	6.15 ± 1.64 a

Means in each column accompanied by the same letter are not significantly different ($P = 0.05$), Tukey's Honest Significant Difference test.
[a] WP: 'whole plant' untouched roots and basal end of the rootstock.
[b] RC: roots cut and basal end of the rootstock untouched.
[c] BC: basal end of the rootstock cut and roots untouched.
[d] RBC: roots cut and basal end of the rootstock cut.

Table 4. Percentage reisolations of *Fusarium* and black-foot fungi from different parts of grapevine plants after inoculations with different pathogen isolates.

Isolate / Species	WP[a]			RC[b]			BC[c]			RBC[d]		
	R[e]	BE[f]	1 cm BE[g]	R[e]	BE[f]	1 cm BE[g]	R[e]	BE[f]	1 cm BE[g]	R[e]	BE[f]	1 cm BE[g]
Control	0	0	0	0	0	0	0	0	0	0	0	0
SuRDC-1205 / *I. liriodendri*	80	20	0	100	40	0	100	80	20	100	100	80
SuRCD-1207 / *D. macrodydima*	60	20	0	80	40	0	60	100	20	100	100	40
SuRDC-1248 / *D. pauciseptata*	40	0	0	60	60	20	60	100	60	100	80	40
PARC420 / *F. oxysporum*	100	0	0	100	20	20	80	80	20	80	80	20
PARC416 / *F. proliferatum*	100	0	0	100	60	20	80	40	20	80	100	40
PARC425 / *F. ramigenum*	20	0	0	60	0	0	80	100	40	80	100	40
PARC428 / *Fusarium* sp.	20	40	0	60	0	0	60	40	20	80	80	40

[a] WP: 'whole plant' untouched roots and basal end of the rootstock.
[b] RC: roots cut and basal end of the rootstock untouched.
[c] BC: basal end of the rootstock cut and roots untouched.
[d] RBC: roots cut and basal end of the rootstock cut.
[e] R: roots.
[f] BE: basal end of the rootstock.
[g] 1 cm BE: one cm above the cut done at the basal end of the rootstock.

its association with GTD symptoms. This could be due to lack of consistency in results from pathogenicity tests conducted with *Fusarium*. Marais *et al.* (1979) reported that three isolates of *Fusarium* (species unknown) caused root rot when inoculated onto three different grape rootstocks. That study also showed no statistically significant differences on root or shoot masses between *Fusarium* inoculated plants and non-inoculated controls. These results convinced the authors that *Fusarium* was not a pathogen of grapevines, but was an endophyte,

so *Fusarium* was not included in pathogenicity studies investigating effects of other soilborne fungi on grapevine health (Marais *et al.*, 1980). In contrast, Highet and Nair (1995) showed that *F. oxysporum* caused root rot on 99% of inoculated self-rooted 'Semillon' plants, although no other plant health parameters were measured. Similarly, Ziedan *et al.* (2011) fulfilled Koch's postulates when all the *F. oxysporum* isolates used in that study caused root rot and wilting of self-rooted 'Crimson' plants. Pathogenicity studies have also confirmed *F. equiseti* as a cause of root rot and wilting on 'Tempranillo' grafted onto '110R' (Astudillo-Calderon *et al.*, 2019), *F. annulatum* to cause vascular necroses on 'Chardonnay' (Bustamante *et al.*, 2022), and *F. commune* to cause root necroses and yellowing of leaves on 'Marselan' (Zhang *et al.*, 2023), but no other plant health parameters were considered in these studies.

In the present study, all four assessed *Fusarium* spp. caused root necroses in '3309C' plant, from all the experimental treatments including when roots were not cut. These symptoms were similar to those caused by the black-foot isolates used as positive experimental controls. Plants inoculated with *Fusarium* and black-foot isolates had reduced root and shoot dry weights when compared with negative controls, no matter the experimental treatment. However, with very few exceptions, data analyses gave no statistically significant differences between inoculated and non-inoculated plants. The results are similar to those reported by Marais (1979), and similar results were obtained with the well-known pathogens *D. macrodidyma*, *D. pauciseptata*, and *I. liriodendri*. *Fusarium* and black-foot fungi were re-isolated from roots from the 'whole plant' treatment, suggesting that these fungi do not need wounds for entry into host root tissues. However, results from the present study showed that fungal colonization of roots and rootstock basal ends increased when plants were wounded. Fungi were also capable of colonizing up to 1 cm above the basal ends when wounded. These results are similar to those reported by Grannett *et al.* (1998), where *Fusarium* spp. were recovered in greater incidence from roots damaged by the grape-phylloxera in CA, than from undamaged roots.

Fusarium may be a secondary pathogen on grapevines, as reported by Marais (1979), and wounded host tissues would facilitate vascular colonization. This is an important result, because poor quality nursery material with weak roots or poor callusing at the rootstock bases could be susceptible to *Fusarium* colonization. The differences observed among pathogenicity studies could be due to differences in host susceptibility to *Fusarium*. Omer *et al.* (1999) assessed effects of *Fusarium* on eight grapevine rootstocks, and concluded that rootstock type played a significant role in infection. 'Self-rooted' 'Carignan' and AXR#1 rootstocks were the most susceptible to root necrosis, while *Fusarium* infections did not cause necroses on '3309C', '420A', '5C', and 'Freedom'. In the present study, *Fusarium* spp. caused root necrosis on '3309C'. Based on available studies, including the present one, *Fusarium* spp. were more successful at causing root necrosis and plant wilt on 'self-rooted' *V. vinifera* than on rootstocks. This could explain why *Fusarium*, though present in grapevines, may not be a primary cause of grapevine decline, as most vineyards have vines grafted onto phylloxera resistant rootstocks. Self-rooted vines from nursery B-2014 showed the least *Fusarium* infection when compared with the rootstock material from the other nurseries. This could have given low *Fusarium* inoculum levels in nursery B-2014 during the propagation process, or could have resulted from effective sanitation strategies applied by that nursery (Gramaje and Armengol, 2011). However, since graft unions were the plant parts in the other three nurseries from which *Fusarium* was most isolated, lack of graft-union wounding in self-rooted material most likely resulted in the low incidence observed.

It is well-known that several GTD fungi occur in asymptomatic grapevines, so it has been suggested that these fungi may be latent pathogens transitioning from endophytic to pathogenic states under abiotic and/or biotic host stress conditions. Initial inoculum concentrations of these fungi may also affect this transition (Hrycan *et al.*, 2020). Results from the present study showed *Fusarium* to occur in declining field-grown vines, and at high incidence in ready-to-plant nursery material. However, the pathogenicity studies indicated that *Fusarium* was an opportunist or weak pathogen of grapevines. The same conclusion could be made from the pathogenicity tests with the three black-foot fungi used as positive controls. Abiotic and biotic factors have been associated with disease severity and fungal growth in other pathosystems. Light soil types increased severity of soybean Fusarium root rot symptoms compared with symptoms developing in sandy loam and silt loam soils (Yan and Nelson, 2022). *Fusarium pseudograminearum* biomass increased in drought stressed barley seedlings (Liu and Liu, 2016). For biotic factors, Li *et al.* (2023) reported that *Fusarium* spp. were more prevalent in GTD-symptomatic grapevine roots and rhizospheres than in for asymptomatic plants. That study also showed that disease indices were increased when *Fusarium* spp. were co-inoculated with *D. macrodidyma* than with individual inoculations, suggesting that *Fusarium* could enhance disease severity when in the presence of other GTD

fungi. Further research should investigate *Fusarium* inoculum thresholds in ready-to-plant nursery grapevine material and determine correlation of this information with studies assessing effects of abiotic and/or biotic factors on *Fusarium* infected grapevines. Also, there is no information from *Fusarium* pathogenicity studies conducted under natural field conditions. These types of research will increase understanding of the roles of *Fusarium* spp. in grapevine health.

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