

## RESEARCH ARTICLE

# Grapevine trunk diseases of cold-hardy varieties grown in Northern Midwest vineyards coincide with canker fungi and winter injury

David H. DeKrey<sup>1\*</sup>, Annie E. Klodd<sup>2</sup>, Matthew D. Clark<sup>3</sup>, Robert A. Blanchette<sup>1</sup>

**1** Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, United States of America, **2** University of Minnesota Extension, Farmington, Minnesota, United States of America, **3** Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota, United States of America

\* [dekre004@umn.edu](mailto:dekre004@umn.edu)



## OPEN ACCESS

**Citation:** DeKrey DH, Klodd AE, Clark MD, Blanchette RA (2022) Grapevine trunk diseases of cold-hardy varieties grown in Northern Midwest vineyards coincide with canker fungi and winter injury. PLoS ONE 17(6): e0269555. <https://doi.org/10.1371/journal.pone.0269555>

**Editor:** Hernâni Gerós, Universidade do Minho, PORTUGAL

**Received:** January 24, 2022

**Accepted:** May 23, 2022

**Published:** June 3, 2022

**Copyright:** © 2022 DeKrey et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its [Supporting Information](#) files.

**Funding:** MDC, RAB, AEK, DHD. This research was funded by the Minnesota Agricultural Rapid Response Fund and USDA Hatch Project MIN-22-081 and MIN-22-089. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

## Abstract

Grapevine trunk diseases make up a disease complex associated with several vascular fungal pathogenic species. Surveys to characterize the composition of grapevine trunk diseases have been conducted for most major grape growing regions of the world. This study presents a similar survey characterizing the fungi associated with grapevine trunk diseases of cold-hardy interspecific hybrid grape varieties grown nearly exclusively in the atypical harsh winter climate of Northern Midwestern United States vineyards. From the 172 samples collected in 2019, 640 isolates obtained by culturing were identified by ITS sequencing and represent 420 sample-unique taxa. From the 420 representative taxa, opportunistic fungi of the order Diaporthales including species of *Cytospora* and *Diaporthe* were most frequently identified. Species of *Phaeoacremonium*, *Paraconiothyrium*, and *Cadophora* were also prevalent. In other milder Mediterranean growing climates, species of Xylariales and Botryosphaerales are often frequently isolated but in this study they were isolated in small numbers. No Phaeomoniellales taxa were isolated. We discuss the possible compounding effects of winter injury, the pathogens isolated, and management strategies. Additionally, difficulties in researching and understanding the grapevine trunk disease complex are discussed.

## Introduction

Grapevine trunk diseases (GTDs) make up a disease complex most often associated with several wood-inhabiting fungal species [1] and more recently possibly some bacterial species [2]. Sub-groups of these diseases are frequently categorized by symptomology and or taxonomic designation of causal fungal agents. Common names given to GTDs include Esca [3], folletage or berry shrivel [4], Petri disease, young esca, young vine decline [5], hoja de malvón [6], Botryosphaeria dieback, bot canker, black goo [7], slow stroke [8], eutypiosis, Eutypa dieback [9], black dead arm, dying arm, dead arm [10], swelling arm [11], grapevine leaf stripe disease

[12], Phomopsis dieback, black spot [13], black measles [14], and black foot disease [15]. These diseases can be difficult to diagnose due to their sporadic symptom display and similarity of external and internal symptoms. Such symptoms may include interveinal foliar chlorosis and necrosis or tiger striping, generalized dieback, apoplexy or sudden death, gummosis, vascular streaking, wedge- or V-shaped vascular discoloration, cankers, and wood decay (Fig 1) [16].

GTDs cause serious grapevine health and economic problems and can be found in all grape growing regions of the world [17–19]. In the Northern Midwest United States (NMW), growers often struggle with unproductive cordon sections commonly referred to as “skips in the cordon” or “blind wood” (Fig 1A and 1B). Cordon skips come at the cost of vineyard managers with lower yields and require retraining new cordons. In the past, chemicals such as sodium arsenate was used to control GTDs but health [20] and environmental concerns [21] have eliminated its widespread use. Often the dramatic increase in the incidence of GTDs in the last two decades is associated with the 2003 ban on sodium arsenate [22]. However, the increasing incidence of GTDs in countries which have never used sodium arsenate points to other factors being involved [22]. To date, very few chemical options are available to growers but recent research in the use of biological control agents has shown some promise for controlling specific fungal GTD pathogens [23]. In most situations, development of best practices for GTD management remains the best option for growers. Management strategies can include practices such as variety selection, rootstock selection, training system, pruning timing, double-pruning, wound-protection, multi-trunking, trunk renewal, trunk surgery, debris removal, tool sterilization, and other practices [24]. Management options of GTD pathogens tend to be region specific with considerations to climate, weather, cultural practices, and varieties grown. In the NMW, wine grape growing is a relatively new industry that is increasing at a considerable pace. According to the 2016 University of Minnesota Extension vineyards and grapes status report, planted cold-hardy grapevine varieties increased from 5900 acres to 7580 acres from 2011 to 2015 [25]. However, Tuck et al. also reported an average decrease in yield of 3.5 to 3.2 tons per acre from 2011 to 2015 which indicates a need for better-informed, variety and region-specific GTD management practices. To accomplish this, it is important to identify the GTDs responsible for the problems.

Traditional European *Vitis vinifera* cultivars are not often grown in the NMW due to difficulties brought on by harsh winters and a short growing season. Instead, own-rooted cold-hardy interspecific hybrid grape (CIHG) varieties are widely and often exclusively grown in the region. The genetic contribution of the native riverbank grape (*V. riparia*) provide CIHG varieties developed in Minnesota their cold-hardiness (rated down to -30°C) [26, 27] and some resistance to endemic diseases and insect pests like phylloxera [28, 29]. Over the past four decades, the University of Minnesota has become a leader in the development of several CIHG wine and table grape varieties. The varieties most produced in the region include Marquette, Frontenac blanc, Frontenac, La Crescent, Petite Pearl, Brianna, and Frontenac Gris [30].

As many NMW vineyards are now reaching a decade in age since their first vines were planted, the characteristic cordon skip (Fig 1A and 1B) and dieback (Fig 1D) symptoms of GTDs have begun to appear. In addition, the compounding effect that GTDs and winter injury have on vines is becoming a major concern (Fig 1L). In many other parts of the world where grapes are grown, surveys have been conducted to characterize the region-specific composition of GTD pathogens. In Europe and nearby Mediterranean countries where GTDs were first reported, major causal agents include fungal species of the genera *Eutypa*, *Diplodia*, *Botryosphaeria*, and *Phaeoemoniella* [19]. Similar fungal species have also been identified as major causal agents of GTDs in Australia, New Zealand [31], South Africa [32], China [33–35], and Chile [36, 37] as well as southern US and west coast US [38, 39]. Species of



**Fig 1. Symptoms of grapevine trunk diseases in Northern Midwest vineyards.** Cankers were often associated with skips in the cordons but had rarely wedge-shaped discoloration (A). Cankers more often had irregular shaped xylem reactions (B). Pycnidia were sometimes observed fruiting from cankers (B) and bleached canes (C). Dieback symptoms are common and the result of successive skips starting from tips of cordons (D). Pruning wounds were associated with minor (E), moderate (F), and severe (G) vascular streaking symptoms. Near completely healthy vascular tissue observed in wild *Vitis riparia* vines (H). Infrequent shallow cracks (I), several shallow cracks (J), and deep cracks (K and L) were associated with minor to moderate (I), moderate to severe (J), and severe (K) vascular symptoms. Winter injury often results in deep cracks on the trunk (L). Observations included cankers (ck), skips in the cordons (sk), bleached canes (bc), dieback (db), pruning wounds (pw), shallow cracks (sc), deep cracks (dc), black spotting (bs), black lines (bl), brown-red wood streaking (br), brown to black necrotic streaking (bn), discolored xylem (dx), sometimes healthy tissue (h), and white rot (wr). Bars = 1 cm.

<https://doi.org/10.1371/journal.pone.0269555.g001>

*Fomitiporia* are often the main white-rot pathogen found in older vines in most of these regions as well [19]. Species of *Phaeoacremonium* have been identified in grapevines and other woody hosts in several countries around the world [40]. Species of *Cadophora* are on occasion identified as well notably found in Canada [41]. Species of *Diaporthe* and *Cytospora* have also

been identified in most of these regions though often to a lesser extent and usually in more humid growing regions [32].

However, no surveys have been conducted in the NMW or exclusively on CIHG cultivars. The objective of this study was to identify the major GTD species throughout the grape-growing regions of Minnesota and Wisconsin. Three hypotheses were explored in this study. First, NMW GTDs will have a regionally distinct composition compared to other grape-growing regions of the world given the harsh growing climate and CIHG varieties grown. Second, dieback symptoms and internal vascular streaking can be associated with pruning wounds and winter injury. Third, isolation frequency of fungal genera will significantly differ compared to sample variety, variety berry color, sample section type, and sample county origin.

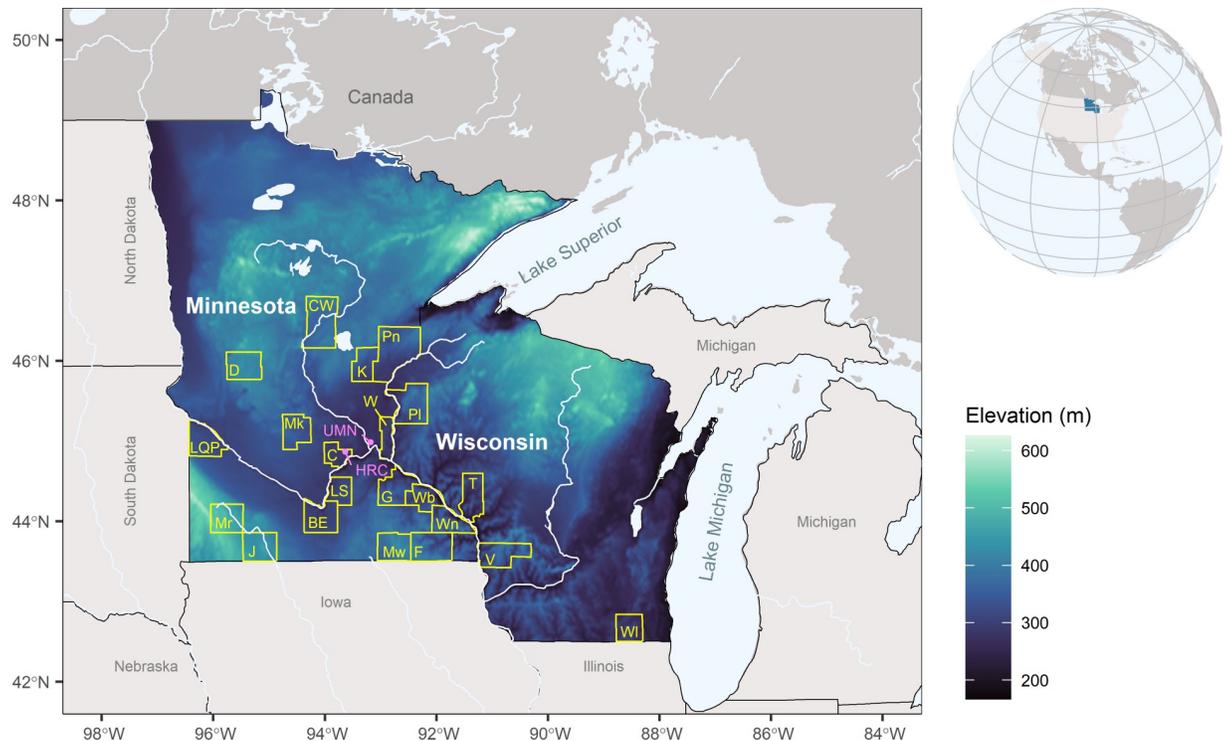
## Methods

### Sample collection

Our sample collection was targeted towards symptomatic grapevines showing skips in the cordons, generalized dieback, reduced productivity, vascular discoloration, vascular decay, or apoplexy (Fig 1). A few externally asymptomatic vines were also collected for comparison. In 2019, a total of 172 samples were collected and brought to the laboratory. Samples were collected throughout both the dormant and growing season of 2019. Some samples were shipped by priority mail. Most samples collected were woody sections of grapevines, especially of cordons and trunks. It is important to note that regular re-trunking is frequently practiced in NMW vineyards and therefore main woody trunks of vines rarely, if ever, exceed ten years in age. Samples were stored at  $-20^{\circ}\text{C}$  until processed. Samples were acquired from 34 vineyards in Minnesota and Wisconsin from a total of 21 counties (Fig 2). However, data reported in this study is down to the county level to conserve anonymity of contributing vineyards. Primarily named CIHG varieties were collected as well as a few wild vines and genetically unique breeding lines.

### Sample processing

Large diameter vine samples were cut and  $3\text{--}5\text{ mm}^3$  chips excised from the margins of discolored or decayed internal vascular wood tissue or from the edge of cankers. For smaller diameter vine samples, the bark was peeled off and  $3\text{--}5\text{ mm}$  thick discs were cut. Some disks were kept whole while others cut in half or in fourths depending on diameter of the sample. Excised chips were surface sterilized for 30 sec in an aqueous 10% sodium hypochlorite solution, followed by two washes in sterile distilled  $\text{H}_2\text{O}$ , and one wash in 70% EtOH then left to dry in a clean air cabinet prior to plating. Between 3–5 chips per plate were then semi-embedded into 3 different culturing medias including malt extract agar (MEA; 15 g of Difco Bacto-agar, 15 g of Difco Bacto malt extract, and 1 L of deionized water with 0.1 g streptomycin sulphate dissolved in a small amount of 95% EtOH added post-autoclaving once cooled to  $50^{\circ}\text{C}$ ), basidiomycete semi-selective agar (BSA; same as MEA recipe plus 2 g of Difco yeast extract and 0.06 g Aldrich benomyl dissolved in a small amount of 95% EtOH added pre-autoclaving with 2 mL 85% lactic acid added post-autoclaving once cooled to  $50^{\circ}\text{C}$ , adapted from Worrall, 1991) [42], and sabouraud dextrose agar (SDA; same as MEA recipe with 0.1 g Aldrich cycloheximide dissolved in a small amount of deionized water added post-autoclaving once cooled to  $50^{\circ}\text{C}$ , adapted from Harrington, 1981) [43]. Plates were left to incubate at  $20\text{--}23^{\circ}\text{C}$  in darkness and checked daily. Emerging fungi were transferred onto fresh MEA. All cultures were maintained and stored in plastic bins at  $20\text{--}23^{\circ}\text{C}$ .



**Fig 2. Map of counties sampled.** Grapevine wood samples collected from Minnesota and Wisconsin counties traced in yellow included Blue Earth (BE), Carver (C), Crow Wing (CW), Douglas (D), Fillmore (F), Goodhue (G), Jackson (J), Kanabec (K), Lac Qui Parle (LQP), Le Sueur (LS), Meeker (Mk), Mower (Mw), Murray (Mr), Pine (Pn), Polk (Pl), Trempealeau (T), Vernon (V), Wabasha (Wb), Walworth (Wl), Washington (W), and Winona (Wn). Pink points denote locations of the University of Minnesota (UMN) St. Paul campus and the UMN Horticultural Research Center (HRC) where the grape breeding program is located and where several samples were collected. Map constructed in R with the public domain map collections Natural Earth (<https://www.naturalearthdata.com/>) and Terrain Tiles (<https://registry.opendata.aws/terrain-tiles/>).

<https://doi.org/10.1371/journal.pone.0269555.g002>

### Isolate selection

Fungal isolates for each sample were selected by culture macro-morphology on MEA and genetic identification by sequencing the internal transcribed spacer (ITS) genomic region. The primary macro-morphological characteristics considered included isolate color, growth rate, hyphal branching, hyphal depth, hyphal extension, hyphal margin, fruiting, sporulation, and metabolite staining of media. At the start of this study all unidentified cultures with unique morphologies isolated from a single sample were selected for sequencing. Isolates were later selected by macro-morphology in a more targeted manner as the study progressed by choosing unique cultures or cultures similar to known pathogens we previously identified in the isolate collection. Any isolates with questionable, non-descript, or similar culture macro-morphology were sequenced to be sure of their identity.

### DNA extraction and amplification

The DNA of select isolates was extracted using the NaOH protocol according to Osmundson et al. (2013) [44]. Hyphae were scraped using a sterile scalpel from cultures of select isolates on MEA that had grown out larger than 2.5 cm in diameter. Hyphal tissue was transferred to a 1.7 mL microcentrifuge tube with 300  $\mu$ L of 5 mM NaOH and 3 to 5 3.5 mm glass beads. The samples were then vortexed for 1 to 5 min and centrifuged for 30 sec at 10,000 rpm. Then 5  $\mu$ L of supernatant was transferred to new tubes containing 495  $\mu$ L Tris-HCL 5 mM, pH 8.0.

The ITS region of the isolated DNA was targeted for PCR amplification using the ITS1F/4 primer pair [45] according to Blanchette et al. (2016) [46]. Each PCR had a final volume of 25.5  $\mu$ L consisting of 12.5  $\mu$ L GoTaq® Green Master Mix, 9.5  $\mu$ L molecular grade water, 1  $\mu$ L of each primer at 10  $\mu$ M, and 0.5  $\mu$ L bovine serum albumin. The ITS locus was amplified using a Bio-Rad T100™ Thermal Cycler following a program of 94°C for 5 min, 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by a final extension step of 72°C for 5 min. Locus amplification was confirmed by gel electrophoresis of SYBR stained PCR products prior to sequencing. Crude PCR products were Sanger sequenced by ABI 3730xl DNA sequences, Applied Biosystems, Foster City, CA.

### Molecular identification

Sequences were processed using Geneious v9.0. The processed sequences were then identified with the basic local alignment search tool algorithm program for nucleotide sequences (BLASTn) initially against the [TrunkDiseaseID.org](https://www.trunkdiseaseid.org/) [47] database and also against the standard complete NCBI GenBank. Best sequence identity match was selected for by consideration of highest score for published data as denoted in GenBank at the time of BLASTn searches. Identity of isolates were matched to published sequences from taxonomic studies and identified to the species level whenever possible. Isolates with greater than 97% sequences identity match were considered homologous. Pathogenicity of identified fungal species on grapevines were denoted according initially to [TrunkDiseaseID.org](https://www.trunkdiseaseid.org/) [47] and then confirmed and expanded by an assortment of grapevine pathogenicity trials found in published literature. However, most pathogenicity trials for these fungi were conducted on traditional *V. vinifera* grapevine cultivars. Samples were scored as GTD+ upon sequence confirmation of at least one known pathogenic species. Additional isolation and sequencing was discontinued once a sample was designated GTD+.

### Data analysis

Data were analyzed using the R statistical programming language in the RStudio integrated development environment using an assortment of packages but most notably the collection of Tidyverse Packages (v1.3.0) [48], the iNEXT package (v2.0.20) [49] to analyze sample coverage, and the vcd package (v1.4–9) for multivariate analysis. The vcd package was used to explore potential differences in isolation frequencies of genera-level taxa compared to a few variables of interest that are descriptive of the 168 collected woody samples. In brief, Hill numbers are used in the iNEXT package to estimate and then visualize sample completeness [50]. Additionally, diversity Pearson residuals statistics were used to analyze the measure of discrepancy between observed and expected values within the vcd package. For each statistical comparison, a p-value is returned from a corresponding Chi-square test and a residual shaded mosaic plot was produced. Mosaic plots are graphs used for visualizing the comparison of multi-categorical data where both the x- and y-axis are sized proportionally to the input data, i.e. the sum area of the blocks represent 100% of the data and individual blocks are size proportionally to the frequency with which the categories are observed.

### Results

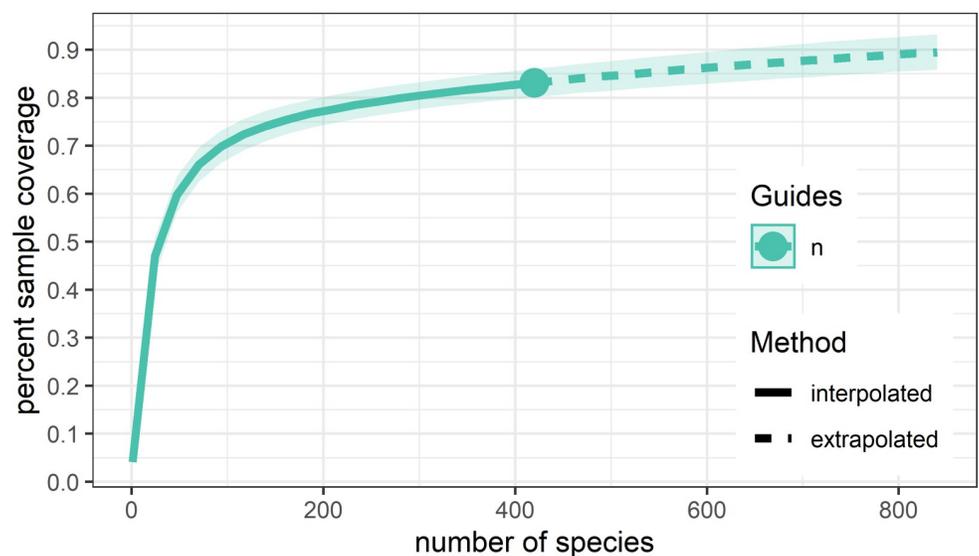
Internal symptoms of GTDs following the terminology of Mugnai et al. 1999 [16] included brown-red wood streaking in a clearly defined wedge-shape from the cambium to pith which is indicative of canker fungi were observed in few of our samples. Cankers more often occurred in irregular forms and were associated with skips in the cordons (Fig 1A and 1B). Centrally diffuse brown-red wood as well as brown to black necrotic streaking originating

from the pith was often associated with pruning wounds (Fig 1E–1G) and cracks (Fig 1I–1L). All samples collected had discolored xylem to some extent and nearly completely healthy vascular tissues were only observed in some cross-sections of wild riverbank grapevines in forest and urban environments not included in this survey (Fig 1H). Cross-sections near pruning spurs often showed discolored wood symptoms without being preceded by diffuse brown-red wood or brown to black streaking (Fig 1E). Concentric black spotting, the result of longitudinal streaking, was also frequently observed, and in some cases, black spotting would begin to coalesce into shorter black lines (Fig 1F). Most samples had severe mottled expression of vascular symptoms especially for brown-red wood streaking, brown to black necrotic streaking, and discolored xylem (Fig 1G). Severe symptoms were sometimes associated with several points of origin from shallow cracking from winter injury or hail damage (Fig 1J). Rarely, if ever, have GTD foliar symptoms been observed in the NMW which possibly may be the result of our overall young vineyards or different climate. Foliar symptoms are more often observed in older vines under particular seasonal conditions [51, 52]. Moreover, lack of foliar symptoms may also be the result of regular re-trunking, a common cultural practice in the NMW. It is uncommon in the NMW for grapevine trunk wood to exceed ten years in age.

All wood samples collected had some degree of internal vascular symptoms including externally healthy samples (Fig 1). From 172 samples with various symptoms that included cankers and vascular discoloration, dieback as well as pruning wounds and cracks from cold injury or other environmental stresses yielded 640 isolates. These isolates represented 420 species-level taxa unique to individual samples. Rarefaction using the 420 representative taxa estimate a sample coverage of 83% that reached to 90% by doubling the number of representative taxa (Fig 3).

We found 32 of the 34 sampled vineyard locations, 20 of 21 counties, in this survey to have at least one GTD+ sample. Of the 172 samples we collected, 142 (83%) had taxa reported as pathogens associated with GTDs. Most samples were cordon sections and of the Marquette variety (Tables 1 and 2). Most taxa are of the phylum Ascomycota (398 isolates, 94.76%) which encompass 19 different orders, 38 genera, and potentially 89 species (Table 3).

The most frequently isolated genera obtained in this study that were known to be associated with GTDs from previous reports included *Cytospora*, *Phaeoacremonium*, *Diaporthe*,



**Fig 3. Rarefaction sample coverage curve.** Observed sample coverage reaches 83% for the 420 sample representative taxa. Extrapolated sample coverage reaches 90% by doubling the number of sample representative taxa.

<https://doi.org/10.1371/journal.pone.0269555.g003>

**Table 1. Type of samples collected from the Northern Midwest in 2019.** The majority of the 172 samples included woody sections of cordon, trunk, root, sucker, shoots, and unknown.

Type	Sample no.	Percent
cordon	113	66%
trunk	32	19%
root	11	6%
sucker	5	3%
shoot	4	2%
unknown	3	2%
slime flux	2	1%
bark	1	1%
basidiocarp	1	1%

<https://doi.org/10.1371/journal.pone.0269555.t001>

*Cadophora*, *Pestalotiopsis*, *Diatrypella*, *Diplodia*, and *Botryosphaeria*, respectively (Fig 4). Of these genera the most frequent species level sequence matches associated with GTDs included *Cy. viticola*, *Ph. fraxinopennsylvanicum*, *Ph. minimum*, *Dpr. ampelina*, *Cd. luteo-olivacea*, *Ps. neglecta*, *Dt. verruciformis*, *Dpl. seriata*, and *Bt. dothidea* (Table 3).

**Table 2. Varieties of the 172 samples collected in the Northern Midwest in 2019.**

Variety	Sample no.	Percent
Marquette	42	24%
La Crescent	28	16%
Frontenac	20	12%
St. Pepin	9	5%
Frontenac Blanc	8	5%
Brianna	7	4%
Frontenac Gris	7	4%
Edelweiss	6	3%
Itasca	6	3%
Marechal Foch	6	3%
unknown	6	3%
Petite Pearl	3	2%
Prairie Star	3	2%
Valiant	3	2%
MN1069	2	1%
MN1016	2	1%
Sabrevois	2	1%
slime flux	2	1%
St. Croix	2	1%
MN43765	1	1%
basidiocarp	1	1%
Millot	1	1%
MN1005	1	1%
Osceola Muscat	1	1%
Riverbank Grape	1	1%
Sauvignon	1	1%
Virginia Creeper	1	1%

<https://doi.org/10.1371/journal.pone.0269555.t002>

Table 3. Taxonomy, isolation frequency, and pathogenicity of fungi identified by ITS with greater than 97% homology match.

Phylum (n)	Family (n)	Genus (n)	Isolate	Host variety	Origin	GenBank
Order (n)		Species (n) [pathogenicity studies]				
<b>Ascomycota (398)</b>						
Diaporthales (98)	Valsaceae (61)	<i>Cytospora</i> (60)				
		<i>Cy. viticola</i> (57) [53]	GI-174	Frontenac Gris	Blue Earth, MN	OM307727
		<i>Cy. piceae</i> (2)	GI-847	Virginia Creeper	Carver, MN	OM307728
		<i>Cy. mali</i> (1)	GI-89	Marquette	Meeker, MN	OM307729
		<i>Valsa</i> (3)				
		<i>Vl. sordida</i> (2)	GI-384	Frontenac	Crow Wing, MN	OM307730
		<i>Vl. salicina</i> (1)	GI-413	Frontenac Gris	Douglas, MN	OM307731
	Diaporthaceae (37)	<i>Diaporthe</i> (38)				
		<i>Dpr. ampelina</i> (31) [54–56]	GI-856	Frontenac	Carver, MN	OM307732
		<i>Dpr. eres</i> (7) [54]	GI-236	Marquette	Goodhue, MN	OM307733
Togniniales (40)	Togniniaceae (40)	<i>Phaeoacremonium</i> (41)				
		<i>Ph. fraxinopennsylvanicum</i> (22) [41]	GI-347	St. Pepin	Goodhue, MN	OM307734
		<i>Ph. minimum</i> (15) [3, 38]	GI-422	Marquette	Douglas, MN	OM307735
		<i>Ph. amstelodamense</i> (1)	GI-212	Edelweiss	Goodhue, MN	OM307736
		<i>Ph. angustius</i> (1) [57]	GI-741	La Crescent	Carver, MN	OM307737
		<i>Ph. canadense</i> (1) [41]	GI-31	St. Pepin	Pine, MN	OM307738
		<i>Ph. hungaricum</i> (1)	GI-75	Osceola Muscat	Wabasha, MN	OM307739
Pleosporales (99)	Didymosphaeriaceae (31)	<i>Paraconiothyrium brasiliense</i> (30) [58, 59]	GI-516	Frontenac	Douglas, MN	OM307740
		<i>Paraphaeosphaeria sporulosa</i> (1)	GI-157	Edelweiss	Wabasha, MN	OM307741
	Didymellaceae (28)	<i>Didymella</i> (16)				
		<i>Dd. pinodella</i> (11)	GI-257	Marquette	Wabasha, MN	OM307742
		<i>Dd. glomerata</i> (2)	GI-116	MN43765	Carver, MN	OM307743
		<i>Dd. pomorum</i> (2)	GI-787	MN1016	Carver, MN	OM307744
		<i>Dd. bellidis</i> (1)	GI-389	La Crescent	Walworth, WI	OM307745
		<i>Epicoccum</i> (10)				
		<i>Ep. nigrum</i> (9)	GI-837	La Crescent	Carver, MN	OM307746
		<i>Ep. sorghinum</i> (1)	GI-223	Marquette	Blue Earth, MN	OM307747
		<i>Nothophoma spiraeae</i> (2)	GI-878	Riverbank Grape	Carver, MN	OM307748
	Pleosporaceae (26)	<i>Alternaria</i> (26)				
		<i>Al. alternata</i> (19)	GI-879	La Crescent	Carver, MN	OM307749
		<i>Al. tenuissima</i> (5)	GI-885	La Crescent	Blue Earth, MN	OM307750
		<i>Al. arborescens</i> (1)	GI-190	Frontenac	Wabasha, MN	OM307751
		<i>Al. infectoria</i> (1)	GI-829	Marquette	Blue Earth, MN	OM307752
	Astrosphaeriellaceae (3)	<i>Pithomyces chartarum</i> (3)	GI-162	Frontenac Gris	Blue Earth, MN	OM307753
	Coniothyriaceae (3)	<i>Coniothyrium palmicola</i> (3)	GI-882	La Crescent	Blue Earth, MN	OM307754
	Phaeosphaeriaceae (3)	<i>Sclerostagonospora</i> (2)				
		<i>Sc. cycadis</i> (1)	GI-141	Marquette	Meeker, MN	OM307755
		<i>Sc. lathyri</i> (1)	GI-140	Marquette	Blue Earth, MN	OM307756
		<i>Neosetophoma cerealis</i> (1)	GI-480	Frontenac Blanc	Crow Wing, MN	OM307757
	undefined family (3)	<i>Microsphaeropsis olivacea</i> (3) [60]	GI-505	Marquette	Lac Qui Parle, MN	OM307758
	Cucurbitariaceae (1)	<i>Neocucurbitaria quercina</i> (1)	GI-37	Marquette	Meeker, MN	OM307759

(Continued)

Table 3. (Continued)

Phylum (n)	Family (n)	Genus (n)	Isolate	Host variety	Origin	GenBank	
Order (n)		Species (n) [pathogenicity studies]					
Hypocreales (49)	Nectriaceae (29)	<i>Fusarium</i> (26)					
		<i>Fs. acuminatum</i> (5)	GI-820	Frontenac	Fillmore, MN	OM307760	
		<i>Fs. equiseti</i> (3)	GI-95	MN1005	Carver, MN	OM307761	
		<i>Fs. solani</i> (3)	GI-376	Frontenac	Crow Wing, MN	OM307762	
		<i>Fs. culmorum</i> (1)	GI-151	La Crescent	Carver, MN	OM307763	
		<i>Ilyonectria liriodendri</i> (1) [61–63]	GI-322	Marechal Foch	Goodhue, MN	OM307764	
		<i>Scolecofusarium ciliatum</i> (1)	GI-149	La Crescent	Carver, MN	OM307765	
		<i>Thyronectria austroamericana</i> (1)	GI-796	slime flux	Carver, MN	OM307766	
	Bionectriaceae (17)	<i>Clonostachys</i> (18)					
		<i>Cln. rosea</i> (16) [60]	GI-870	La Crescent	Polk, MN	OM307767	
		<i>Cln. byssicola</i> (1)	GI-874	Marquette	Trempealeau, WI	OM307768	
	Hypocreaceae (2)	<i>Trichoderma</i> (2)					
		<i>Trc. atroviride</i> (1)	GI-795	slime flux	Carver, MN	OM307769	
		<i>Trc. deliquescens</i> (1)	GI-351	Frontenac Blanc	Fillmore, MN	OM307770	
Xylariales (25)	Sporocadaceae (11)	<i>Pestalotiopsis</i> (10)					
		<i>Ps. neglecta</i> (6)	GI-491	Edelweiss	Trempealeau, WI	OM307771	
		<i>Ps. uvicola</i> (2) [38, 56]	GI-738	Edelweiss	Trempealeau, WI	OM307772	
		<i>Ps. brassicae</i> (1)	GI-403	Marquette	Lac Qui Parle, MN	OM307773	
		<i>Ps. chamaeropsis</i> (1)	GI-231	St. Pepin	Goodhue, MN	OM307774	
		<i>Neopestalotiopsis mesopotamica</i> (1)	GI-220	Frontenac Gris	Blue Earth, MN	OM307775	
		<i>Seimatosporium lichenicola</i> (1)	GI-99	Marquette	Carver, MN	OM307776	
		<i>Seiridium rosarum</i> (1)	GI-352	La Crescent	Murray, MN	OM307777	
	Diatrypaceae (9)	<i>Diatrypella</i>					
		<i>Dt. verruciformis</i> (8) [64, 65]	GI-464	Marquette	Carver, MN	OM307778	
		<i>Dt. pulvinata</i> (1)	GI-416	Valiant	Douglas, MN	OM307779	
		<i>Diatrype stigma</i> (1) [65]	GI-895	Valiant	Crow Wing, MN	OM307780	
	Hypoxylaceae (2)	<i>Hypomontagnella submonticulosa</i> (1)	GI-817	Frontenac Blanc	Blue Earth, MN	OM307781	
		<i>Hypoxylon invadens</i> (1)	GI-350	La Crescent	Blue Earth, MN	OM307782	
	Apiosporaceae (1)	<i>Arthrimum arundinis</i> (1)	GI-70	Frontenac	Carver, MN	OM307783	
	Xylariaceae (2)	<i>Rosellinia corticium</i> (1)	GI-62	St. Croix	Wabasha, MN	OM307784	
	Helotiales (21)	undefined family (18)	<i>Cadophora</i> (16)				
<i>Cd. luteo-olivacea</i> (13) [41, 66–68]			GI-370	La Crescent	Mower, MN	OM307785	
<i>Cd. melinii</i> (2) [68]			GI-316	Marechal Foch	Goodhue, MN	OM307786	
<i>Cd. ferruginea</i> (1)			GI-328	La Crescent	Goodhue, MN	OM307787	
Dermateaceae (1)		<i>Discohainesia oenotherae</i> (1)	GI-442	Frontenac Gris	Winona, MN	OM307788	
Porodiplodiaceae (1)		<i>Porodiplodia vitis</i> (1)	GI-269	Frontenac	Blue Earth, MN	OM307789	
Sclerotiniaceae (1)		<i>Botrytis cinerea</i> (1)	GI-386	Frontenac	Walworth, WI	OM307790	
Dothideales (14)	Sacrotheciaceae (14)	<i>Aureobasidium pullulans</i> (14)	GI-886	La Crescent	Blue Earth, MN	OM307791	
Botryosphaeriales (10)	Botryosphaeriaceae (10)	<i>Diplodia</i> (7)					
		<i>Dpl. seriata</i> (6) [38, 56, 58, 64, 69]	GI-408	La Crescent	Blue Earth, MN	OM307792	
		<i>Dpl. corticola</i> (1) [38, 56]	GI-373	Petite Pearl	Crow Wing, MN	OM307793	
		<i>Botryosphaeria dothidea</i> (2) [38, 56, 70]	GI-225	Marquette	Blue Earth, MN	OM307794	
		<i>Phaeobotryon negundinis</i> (1)	GI-131	MN1005	Carver, MN	OM307795	

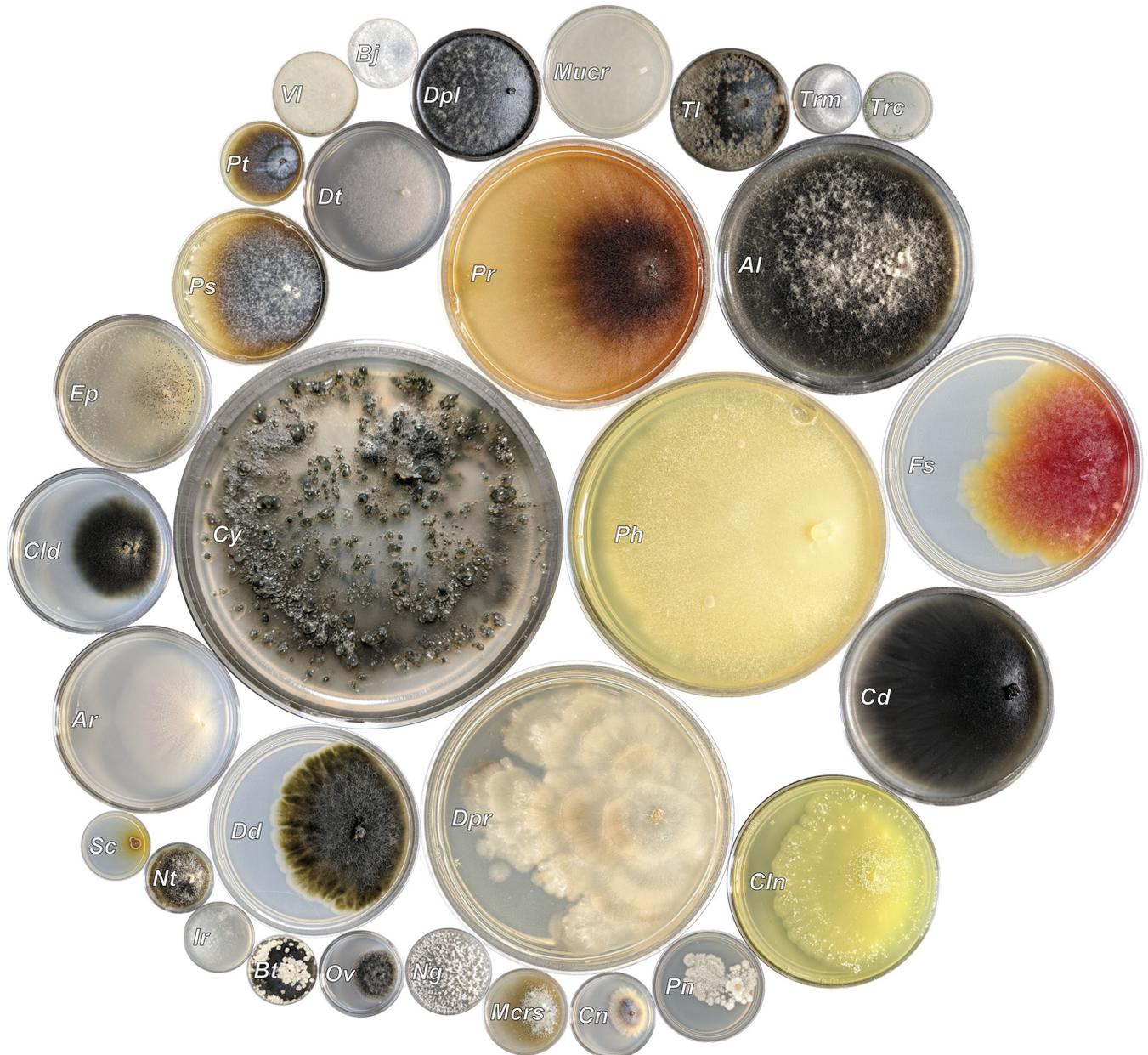
(Continued)

Table 3. (Continued)

Phylum (n)	Family (n)	Genus (n)	Isolate	Host variety	Origin	GenBank
Order (n)		Species (n) [pathogenicity studies]				
Cladosporiales (11)	Cladosporiaceae (11)	<i>Cladosporium</i> (11)				
		<i>Clad. cladosporioides</i> (9)	GI-866	Marquette	Blue Earth, MN	OM307796
		<i>Clad. anthropophilum</i> (1)	GI-209	Marquette	Blue Earth, MN	OM307797
		<i>Clad. westerdijkiae</i> (1)	GI-194	Marquette	Blue Earth, MN	OM307798
	Trichocomaceae (4)	<i>Talaromyces amestolkiae</i> (4)	GI-801	<i>Vitis</i> spp.	Carver, MN	OM307799
Eurotiales (8)	Aspergillaceae (4)	<i>Penicillium</i> (5)				
		<i>Pn. pulvillorum</i> (2)	GI-337	Marquette	Blue Earth, MN	OM307800
		<i>Pn. raistrickii</i> (1)	GI-309	St. Pepin	Goodhue, MN	OM307801
		<i>Pn. simplicissimum</i> (1)	GI-210	Marquette	Blue Earth, MN	OM307802
		<i>Pn. sumatraense</i> (1)	GI-36	Marquette	Meeker, MN	OM307803
Sordariales (6)	Chaetomiaceae (4)	<i>Ovatospora</i> (3)				
		<i>Ov. brasiliensis</i> (2)	GI-828	Marquette	Blue Earth, MN	OM307804
		<i>Ov. mollicella</i> (1)	GI-881	La Crescent	Blue Earth, MN	OM307805
		<i>Chaetomium concavisporum</i> (1)	GI-865	Marquette	Blue Earth, MN	OM307806
	Sordariaceae (1)	<i>Sordaria fimicola</i> (1)	GI-848	Virginia Creeper	Carver, MN	OM307807
Trichosphaeriales (3)	Trichosphaeriaceae (3)	<i>Nigrospora oryzae</i> (3)	GI-846	Edelweiss	Winona, MN	OM307808
Chaetomellales (1)	Chaetomellaceae (1)	<i>Chaetomella raphigera</i> (1)	GI-217	Marquette	Goodhue, MN	OM307809
Chaetothyriales (1)	Herpotrichiellaceae (1)	<i>Rhinochloidiella quercus</i> (1)	GI-486	Marquette	Blue Earth, MN	OM307810
Coniochaetales (1)	Coniochaetaceae (1)	<i>Coniochaeta velutina</i> (1)	GI-472	Brianna	Winona, MN	OM307811
Glomerellales (1)	Glomerellaceae (1)	<i>Colletotrichum acutatum</i> (1)	GI-287	La Crescent	Goodhue, MN	OM307812
Saccharomycetales (1)	Dipodascaceae (1)	<i>Geotrichum candidum</i> (1)	GI-164	Edelweiss	Goodhue, MN	OM307813
Thelebolales (1)	Thelebolaceae (1)	<i>Thelebolus microsporus</i> (1)	GI-88	Frontenac Blanc	Pine, MN	OM307814
Valsariales (1)	Valsariaceae (1)	<i>Valsaria spartii</i> (1)	GI-43	Marquette	Meeker, MN	OM307815
<b>Basidiomycota (15)</b>						
Polyporales (9)	Phanerochaetaceae (3)	<i>Bjerkandera adusta</i> (2)	GI-417	Marquette	Trempealeau, WI	OM307816
		<i>Hyphodermella rosae</i> (1)	GI-823	Frontenac	Fillmore, MN	OM307817
	Irpicaceae (2)	<i>Irpex lacteus</i> (2)	GI-806	<i>Vitis</i> spp.	Carver, MN	OM307818
	Polyporaceae (2)	<i>Trametes versicolor</i> (2)	GI-68	La Crescent	Wabasha, MN	OM307819
	Cerrenaceae (1)	<i>Cerrena unicolor</i> (1)	GI-198	Prairie Star	Carver, MN	OM307820
	Meruliaceae (1)	<i>Phlebia radiata</i> (1)	GI-798	<i>Vitis</i> spp.	Carver, MN	OM307821
Russulales (2)	Peniophoraceae (1)	<i>Peniophora cinerea</i> (1)	GI-342	La Crescent	Blue Earth, MN	OM307822
	Stereaceae (1)	<i>Stereum complicatum</i> (1)	GI-200	Marquette	Goodhue, MN	OM307823
Agaricales (2)	Physalacriaceae (1)	<i>Cylindrobasidium laeve</i> (1)	GI-263	Itasca	Wabasha, MN	OM307824
	Schizophyllaceae (1)	<i>Chondrostereum purpureum</i> (1)	GI-444	Itasca	Le Sueur, MN	OM307825
Hymenochaetales (1)	Hymenochaetaceae (1)	<i>Phellinus conchatus</i> (1)	GI-805	<i>Vitis</i> spp.	Carver, MN	OM307826
Cystofilobasidiales (1)	Mrakiaceae (1)	<i>Tausonia pullulans</i> (1)	GI-61	slime flux	Carver, MN	OM307827
<b>Mucoromycota (7)</b>						
Mucorales (7)	Mucoraceae (7)	<i>Mucor</i> (7)				
		<i>Mucr. circinelloides</i> (6)	GI-71	La Crescent	Vernon, WI	OM307828
		<i>Mucr. moelleri</i> (1)	GI-365	Marquette	Mower, MN	OM307829

Taxonomic rankings from order to species are denoted followed by isolation frequency in parenthesis. The isolation frequency is the count of samples each taxa was isolated from a possible 172 samples. Representative isolates deposited to GenBank are listed for each species along with the sample variety and county origin of that isolate. Pathogenicity studies conducted for each species are listed in brackets following species. See references for complete citations. Highlighted species have associated pathogenicity studies. Highlighted isolates pictured in Fig 4.

<https://doi.org/10.1371/journal.pone.0269555.t003>



**Fig 4. Fungal genera isolated from more than one grapevine sample.** Individual culture image areas are relatively proportional to the number of samples each fungal genus was isolated from. All pictured fungal isolates grown on MEA. *Cytospora* (Cy = 60, GI-174, 80 dpi); *Phaeoacremonium* (Ph = 41, GI-422, 94 dpi); *Diaporthe* (Dpr = 38, GI-449, 47 dpi); *Paraconiothyrium* (Pr = 30, GI-516, 47 dpi); *Alternaria* (Al = 27, GI-879, 36 dpi); *Fusarium* (Fs = 27, GI-151, 17 dpi); *Clonostachys* (Cln = 18, GI-870, 27 dpi); *Cadophora* (Cd = 16, GI-370, 94 dpi); *Didymella* (Dd = 16, GI-257, 21 dpi); *Aureobasidium* (Ar = 14, GI-886, 21 dpi); *Cladosporium* (Cld = 11, GI-866, 21 dpi); *Epicoccum* (Ep = 10, GI-837, 19 dpi); *Pestalotiopsis* (Ps = 10, GI-491, 40 dpi); *Diatrypella* (Dt = 9, GI-464, 15 dpi); *Diplodia* (Dpl = 7, GI-408, 20 dpi); *Mucor* (Mucr = 7, GI-71, 20 dpi); *Talaromyces* (TI = 6, GI-801, 55 dpi); *Penicillium* (Pn = 5, GI-337, 14 dpi); *Coniothyrium* (Cn = 3, GI-882, 21 dpi); *Microsphaeropsis* (Mrcs = 3, GI-505, 20 dpi); *Nigrospora* (Ng = 3, GI-846, 14 dpi); *Ovatospora* (Ov = 3, GI-828, 19 dpi); *Pithomyces* (Pt = 3, GI-162, 28 dpi); *Valsa* (VI = 3, GI-384, 35 dpi); *Bjerkandera* (Bj = 2, GI-417, 28 dpi); *Botryosphaeria* (Bt = 2, GI-225, 35 dpi); *Irpex* (Ir = 2, GI-806, 55 dpi); *Nothophoma* (Nt = 2, GI-878, 31 dpi); *Sclerostagonospora* (Sc = 2, GI-140, 17 dpi); *Trametes* (Trm = 2, GI-68, 9 dpi); and *Trichoderma* (Trc = 2, GI-795, 14 dpi).

<https://doi.org/10.1371/journal.pone.0269555.g004>

There were 15 taxa of Basidiomycota (3.57%) which encompass 5 orders, 11 genera, and 12 species. *Bjerkandera adusta*, *Irpex lacteus*, and *Trametes versicolor* were the most frequently identified Basidiomycota isolated. These fungi were present in 5 samples but were found in

counties not adjacent to one another. Very few taxa of Mucoromycota (7 isolates, 1.68%) were identified. *Mucor circinelloides*, not considered associated with GTD, was isolated from 6 samples which originated from different vineyards in nonadjacent counties.

There were no significant differences in isolation frequencies of genera based on sample berry color (S1 Fig). There is some indication of significant isolation frequency differences of genera by sample variety (S2 Fig) or sample county origin (S3 Fig). For most genera not enough sample representative taxa were obtained to be sure these isolation frequency differences between counties are truly significant. However, *Cadophora* obtained in this study notably had significant differences in isolation frequency between sample section types with a *p*-value of 0.013. *Cadophora* spp. were less often isolated from cordons with a Pearson residual of -2.9 and more frequently isolated from root, trunk, and sucker sections with Pearson residuals between 2.2 to 3.9 (S4 Fig).

## Discussion

The vast majority of the fungal taxa isolated in this study are of the phylum Ascomycota, several of which are considered pathogenic to grapevines (references cited in Table 3). *Cytospora* spp. and *Diaporthe* spp. of the order Diaporthales as well as *Phaeoacremonium* spp. make up the largest majority of isolates identified and are known to be pathogenic on grapevines. Frequent species identification of *Phaeoacremonium* in this study aligns similarly with most other GTD surveys. However, the other major results confirm our first hypothesis that the composition of GTDs for the NMW is different in comparison to most other studied grape growing regions. These fungi in the Diaporthales typically are considered a minor or secondary group of causal agents in other regions where GTD surveys were completed [55, 71–74] but in the study presented here they are the most commonly found GTD fungi in the NMW. The GTD species genera of *Diaporthe* was previously named *Phomopsis* by some other investigators [55, 75]. Species of *Cytospora* have been reported previously as prevalent in cold climate regions [74] and areas of high humidity [32].

The etiology of these Diaporthales pathogens on grapevines has been previously well characterized [76]. In addition to symptoms of internal wood discoloration, pathogenic species of *Cytospora* and *Diaporthe* can induce symptoms of cane bleaching (Fig 1C). They also produce asexual fruiting bodies known as pycnidia which can be found on all affected tissues (Figs 1B, 1C and 4). On succulent green tissue, pycnidia may be surrounded by a halo or half halo of chlorotic tissues or may reside hidden just under the bark of infected vines. Conidio-spores that ooze out from pycnidia serve as a major source of inocula that can re-infect the same host or infect other nearby hosts. Conidia are most often disseminated by rain or irrigation splash but also by contaminated tools and more rarely by wind alone. These species overwinter in colonized wood of canes, spurs, pruning debris, and dormant buds [77]. However, symptoms appear to differ from locations of sample collection and isolation particularly for *Diaporthe* spp. [11, 55, 78, 79]. Symptom differences may be explained by genetic differences of local fungal populations due to the result of horizontal gene transfer of transposable elements for the acquisition or loss of pathogenicity [80, 81]. However, horizontal gene transfer has never been studied in fungal GTD pathogens. Many of the Diaporthales are assumed opportunistic pathogens, causing disease only in stressed or weakened hosts or may live endophytically without causing disease [82]. Since these fungi can colonize wounds, the prevalence of these fungi found may be a result of wounds caused by cold injury.

In January of 2019, an atypical polar vortex occurred in the NMW. In Minnesota on January 30, 2019, the temperature dropped to -33°C (-48°C with wind chill) for the Minneapolis-St. Paul area while the lowest temperature recorded in the state was -39°C (-53°C with wind

chill) [83]. The polar vortex temperatures were well below the lowest ratings for most of the CHIG varieties in many counties. Winter injury was most apparent on Marquette variety grapevines and in vineyards with little wind protection. Exposure of grapevines to these extreme weather conditions resulted in frost cracks of woody tissues and damage to dormant buds. However, winter injury of grapevines more typically occurs by means of sun exposure. Injury occurs when both direct and snow reflected sunlight warms trunks to above freezing during the day followed by a rapid decrease in temperature to below freezing at night. The sudden drop in temperature ruptures just the outer most layer of phloem cells for mild cases while more serious cases kill cambial cells and damage xylem tissues. When this occurs on trees in the NMW it is often referred to as sunscald. On grapevines this could be considered “winter sunscald”, not to be confused with sunscald of grape berries in the summer. Like extreme cold weather exposure, winter sunscald can also result in both shallow and deep frost cracks depending on severity (Fig 1I–1L). Additionally, winter sunscald of grapevines results in a blackened appearance of the bark on the south to southwest facing side of the vine (Fig 1L). In either case of winter injury often the roots and the lower trunk of vines are protected by the insulating snow covering. Thus, trunk replacement by sucker is a viable and common management strategy in the NMW [84]. Regardless, associated observations of winter injury, vascular discoloration, and identification of fungi suggests our second hypothesis is true and that the polar vortex likely predisposed grapevines to these opportunistic canker pathogens.

Wounds, perhaps from winter injury or mechanical pruning, serve as portals for infection by GTD pathogens under conducive weather conditions such as cool spring or fall rains [32, 85–87]. Fungal spores then colonize and spread through the vascular tissue either by hyphal growth or additional sporulation. Many canker pathogens secrete cell wall degrading enzymes or other compounds to spread laterally through xylem tissues eventually circumnavigating and killing the entire cambium. However, the grapevine host produces tyloses, gels, phenolics, and suberin to compartmentalize the damaged tissues and invading microorganisms [88]. However, restricted balanced production of these defensive structures and compounds is essential. Overproduction of occlusions in response to pathogenic infections can lead to extensive hydraulic failure resulting in external foliar symptoms and often vine death [89, 90]. In cross sections the defense response of the grapevine host is seen as a continuum of brown-red wood to brown-black necrotic tissue (Fig 1). Lighter vascular discoloration indicates more recent responding tissues and likely the front of pathogen spread. Darker vascular discoloration indicates long responding tissues and the probable point of pathogen entry [91]. Alternatively, primarily pectinolytic active pathogens degrade gels in xylem vessels and spread longitudinally by spores through the small spaces between tyloses partially occluding xylem conduits [92]. Longitudinal spread of these pathogens is seen in cross section by the host defense response as black spotting and black lines (Fig 1F) [16]. Genomes of *Cytospora* spp. and *Diaporthe* spp. reveal these fungi employ an abundance of cell wall degrading enzymes [93, 94].

Xylem vessel anatomy likely influences host resistance, pathogen spread, and environmental resilience. In the Dutch elm disease pathosystem, smaller diameter xylem vessels appeared to confer some level of resistance to the causal fungal agents *Ophiostoma ulmi* and *O. novo-ulmi* [95]. Reduced vessel diameter permits a more energetically conserved faster occlusion of tissues adjacent to damaged or infected xylem tissues. Pouzoulet et al. (2014; 2017; 2020) conducted histological and pathogenicity studies comparing a few *V. vinifera* grapevine cultivars that had varying susceptibility to GTDs and showed cultivars with smaller diameter xylem vessels may likely confer some resistance [88, 96, 97]. Unfortunately, histological pathogenicity studies of grapevines against vascular pathogens are few and completely lacking for hybrid varieties. However, hybrid varieties as well as traditional cultivars have been studied and show links between vessel anatomy and environmental resilience against freezing and drought

conditions, though more research is needed [98, 99]. Interestingly, developmental histological studies of grapevine xylem tissues have shown plasticity of vessel diameter even within a variety or individual based on early season precipitation [100]. Therefore, abundant early irrigation influence vines to develop larger xylem vessels that can allow for vigorously growing higher yielding grapevines but may also render vines more susceptible to biotic vascular pathogens and abiotic environmental stresses. Moreover, environmental stresses, including extreme weather events, will be more frequent due to climate change which may provide more opportunities for some vascular pathogens [101]. However, the effects of climate change are likely dependent on the pathogens, cultivars, and environments in question. For example, intensive drought conditions have shown both positive [102] or negative [103] effects for grapevines suffering from GTDs.

Species of *Phaeoacremonium* are found in many grape growing countries and often associated with GTDs of young vines. *Phaeoacremonium* spp. are often found to spread through wounds, nursery propagation and grafting [104–112], see review by Gramaje et al. (2015) [40]. *Ph. fraxinopennsylvanicum* is widespread throughout the world on other hosts and has been found in many other investigations in the Midwestern United States [113] but *Ph. minimum* appears to be the most widespread *Phaeoacremonium* spp. throughout grape growing countries.

*Cd. luteo-olivacea* has been isolated from many substrates including soils [114], decaying wood [115–117], and grapevines [68] as well as from various grafting tools [118] and pruning shears [119]. *Cd. luteo-olivacea* is often referred to as a weak pathogen and this fungus was not recognized as pathogenic on grapevine until extended grapevine inoculation studies were conducted (see Table 3). Interestingly, *Cadophora* was the only genus in this study that showed some differences in isolation frequency in comparison with four tested criteria of hypothesis three. No significant differences were observed for any fungal genera isolated compared to variety berry color (S1 Fig). Some researchers have indicated suspicions that red cultivars are more susceptible to GTD pathogens, although much more research is needed [120]. Some significant differences were observed for a few genera compared to sample variety (S2 Fig) or sample county origin (S3 Fig). However, the residuals were only slight for the variety and county origin comparisons and more research is needed to be sure of these correlations. Yet, *Cadophora* spp. were found to be significantly less isolated from cordon sample sections and significantly more isolated from woody sections of trunk, roots, and suckers (S4 Fig). Increased isolation of *Cadophora* spp. from the more central main trunk of the vine may be an indication that infection occurs from the soil or possibly the infection was acquired prior to planting. Additional research of vineyard soils and nursery stock materials would better elucidate the origin of *Cadophora* spp. in NMW grapevines. Additionally, *Ilyonectria liriodendri*, isolated just once in this study, is another weak pathogen often associated with GTDs of roots and often found in nurseries [121, 122].

Fungi considered nonpathogenic to grapevines according to [TrunkDiseaseID.org](https://www.TrunkDiseaseID.org) [47] included *Penicillium*, *Alternaria*, *Didymella*, *Epicoccum*, and *Paraconiothyrium* which were some of the more frequently isolated genera in this study. However, few GTD pathogenicity studies have tested Pleosporales fungi. *Paraconiothyrium* spp. have been demonstrated to be pathogenic on fruit trees and other woody species [105] and potentially pathogenic on grapevines [59]. In the NMW, *Paraconiothyrium* spp. could be a potential pathogen. This fungus was recently found associated with the emerald ash borer and found to cause small cankers on healthy ash trees [113, 123]. Further investigation of the pathogenesis of Pleosporales may prove interesting considering the persistence in isolation and sequencing of these fungi.

Basidiomycota have been also found to play a role in GTDs [124–126]. Often the presence of these wood decay fungi are found mainly in older vines following the colonization of faster

growing host detoxifying pioneering ascomycota [16]. In many parts of the grape growing regions of the world, the primary Basidiomycota associated with GTDs are *Fomitiporia mediterranea* [127] and *Stereum hirsutum* [128]. However, in our study no species of *Fomitiporia* were isolated but a different species of *Stereum* was one of the more frequently isolated Basidiomycota. Additionally, *Trametes versicolor* and *Cerrena unicolor* were also isolated. These fungi are commonly found on forest and shade trees locally (personal observations). Fruiting bodies of these fungi have been found on trunks of grapevines that had advanced stages of GTD symptoms. These Basidiomycota have not been tested for pathogenicity on grapevines but *Trametes versicolor* causes cankers and decay on fruit trees [129] and *Cerrena unicolor* is well characterized on hardwoods where it is an aggressive canker rot pathogen [130].

GTD fungi in the Xylariales, Botryosphaerales, and Phaeomoniellales are of concern in many grape growing countries and this includes fungi in the families Diatrypaceae, Botryosphaeriaceae, and Phaeomoniellaceae [73]. In our study, only 10 Diatrypaceae, and 10 Botryosphaeriaceae isolates were identified. No Phaeomoniellaceae isolates were identified. No species of *Eutypella* or *Eutypa* (both Diatrypaceae), common in some other grape growing regions, were isolated. However, a few isolates not included in analysis closely matched with *Eutypa* but with less than 97% and may prove to be new species following additional detailed taxonomic studies (See S1 Table). *Pestalotiopsis* spp., *Neopestalotiopsis* spp., *Diatrypella verruciformis*, and *Diatrype stigma* of the Xylariales as well as *Diplodia seriata*, *Botryosphaeria dothidea*, *Diplodia corticola*, and *Phaeobotryon negundinis* of the Botryosphaerales represented a minority of the GTD pathogens isolated in our study (Table 3).

At the start of our research project, many grape growers expressed concerns about GTDs in their young vineyards. There was also considerable concern about Botryosphaeriaceae GTD often called “Bot-rot” or otherwise known as Bot canker. Grape growers in the NMW regularly associate any wedge-shaped discoloration of cross-sections of grapevines as Bot-rot. Based on this survey, Botryosphaeriaceae GTD is rare in the NMW. Confusion and concerns of growers, viticultural professionals, and even fellow researchers is understandable given the complexity of GTDs in addition to the many various names used in an attempt to sub-categorize GTDs. Many of these sub-categorized GTDs have been associated with irregular generalized symptoms of grapevines influenced by the cultivar or variety, climate, and environmental conditions [32, 118, 131]. Moreover, many GTD designations rapidly become obsolete with each taxonomic recategorization of fungal species. Fungal taxonomy will likely continue to change as more genetic information is gathered into databases and mycologists strive to dissolve the two-name system for fungi [132].

The isolation frequency differences of these typically important GTD groups in other grape growing regions is especially notable. Such differences could possibly be correlated with the different climate of the NMW as compared to the many other grape growing regions which typically have more seasonally mild, often Mediterranean climates. Several spore trapping studies from various countries have attempted to characterize sporulation events of various GTD pathogens in correlation with varying weather measurements [87, 133–135]. Given the drastically different GTDs composition of the NMW, additional studies using spore trapping would prove insightful to obtain a better understanding of the GTD pathogens in the NMW.

Notably, culturing methods may present bias as some faster growing fungal species such as those of *Cytospora*, *Diaporthe*, *Diplodia*, and *Botryosphaeria* may outgrow slower growing species such as those of *Phaeoacremonium*, *Phaeomoniella*, and many basidiomycota. This bias of culture-based studies would benefit being paired with modern metagenomic techniques to characterize all potential microorganismal species present in a substrate. However, many metagenomic techniques also present bias such as detection of non-viable organisms or unintended preferential over-identification. Thus, metagenomic techniques also benefit being

paired with classical culture-based techniques. Additionally, inclusion of culture-based methods allows for the curation of live microbial collections for use in future pathogenicity or characterization studies. Therefore, future studies which use both classical culturing and metagenomic techniques would better elucidate the NMW GTD complex. This combination of classical and modern techniques has been demonstrated effective in recent local studies of Heterobasidion Root Rot [136]. Regardless, in the study we present here the sample coverage curve (Fig 3) was observed to have reached a plateau providing confidence all major fungal species were identified in this study. Moreover, the use of three types of media allowed for frequent isolation of slow growing fungal species such as those of *Phaeoacremonium* as well as infrequent isolation of fast growing fungal species such as those of the Botryosphaerales. Therefore, confidence is assured that the isolation frequency of these fungi identified is representative for grapevines of the NMW.

In this study we revealed a large diversity of fungal species associated with cold-hardy hybrid grapevines in the NMW. A handful of these isolates (those with less than 97% sequence match provided in S1 Table) could potentially be revealed as new species following additional detailed taxonomic studies. However, the majority of fungal species we identified show Diaporthales predominate GTDs in the NMW. Diaporthales GTD species, *Cytospora* and *Diaporthe*, are generally opportunistic fungi and largely spread to new hosts within short distances by asexual conidia via rain splash or contaminated tools. Basic understanding of these opportunistic pathogens lifecycles emphasizes the benefit growers would gain from more intentional phytosanitary practices such as prompt removal and destruction of pruning debris as well as the regular sanitization of tools. Pruning debris and diseased canes left unpruned have recently been shown to be a major source of *Diaporthe* GTD Inoculum [135]. Our current recommendation for grape growers in the NMW is to prune in the dormant winter season during a period of cold and dry weather. Recommendations on pruning timing could be fine-tuned by epidemiological spore trapping studies in NMW vineyards and may possibly allow for some degree of GTDs forecasting. Vineyard spore trapping could also provide the opportunity for broader biosurveillance of invasive pathogenic microbial species of forest, shade, and orchard trees.

Knowing the prevalence of GTDs in the NMW provides insight for the development of improved management practices. Similar studies of GTD pathogens spread from nurseries of cold-hardy grapevine hybrid varieties would also provide insight to improved propagation practices as well as yield less stressed, higher quality, and more vigorous growing nursery stock plants for growers. Assessment of these hybrid varieties against a panel of GTD pathogens may reveal novel evidence of resistance or susceptibility that would be useful for grape breeders. The development of cost-effective rapid molecular assays for the most prevalent GTDs in the NMW would be a useful tool to measure the effectiveness of practices or the variability of variety susceptibility to GTDs.

## Supporting information

**S1 Table. Isolates with less than 97% homology match.** Taxonomic rankings from order to species are denoted followed by isolation frequency in parenthesis. The isolation frequency is the count of samples each taxa was isolated from a possible 172 samples. Isolates deposited to GenBank are listed for each species along with the sample variety and county origin of that isolate. Pathogenicity studies conducted for each species are listed in brackets following species. See references for complete citations. Highlighted species have associated pathogenicity studies.

(DOCX)

**S1 Fig. Mosaic plot of genus level taxa isolated from sample varieties.**  
(TIF)

**S2 Fig. Mosaic plot of genus level taxa isolated from sample variety berry color.**  
(TIF)

**S3 Fig. Mosaic plot of genus level taxa isolated from sample section types.**  
(TIF)

**S4 Fig. Mosaic plot of genus level taxa isolated from counties.**  
(TIF)

## Acknowledgments

We thank Owen Geier and Brian Prior for technical support and the staff and management of all the contributing Northern Midwestern vineyards that provided samples for this study.

## Author Contributions

**Conceptualization:** David H. DeKrey.

**Data curation:** David H. DeKrey.

**Formal analysis:** David H. DeKrey.

**Funding acquisition:** Matthew D. Clark.

**Investigation:** David H. DeKrey.

**Methodology:** David H. DeKrey.

**Project administration:** David H. DeKrey.

**Resources:** David H. DeKrey, Robert A. Blanchette.

**Software:** David H. DeKrey.

**Supervision:** Matthew D. Clark, Robert A. Blanchette.

**Validation:** David H. DeKrey.

**Visualization:** David H. DeKrey.

**Writing – original draft:** David H. DeKrey.

**Writing – review & editing:** David H. DeKrey, Annie E. Klodd, Matthew D. Clark, Robert A. Blanchette.

## References

1. Bertsch C, Ramírez-Suero M, Magnin-Robert M, Larignon P, Chong J, Abou-Mansour E, et al. Grapevine trunk diseases: complex and still poorly understood. *Plant Pathology*. 2013; 62: 243–265. <https://doi.org/10.1111/j.1365-3059.2012.02674.x>
2. Haidar R, Yacoub A, Vallance J, Compant S, Antonielli L, Saad A, et al. Bacteria associated with wood tissues of Esca-diseased grapevines: functional diversity and synergy with *Fomitiporia mediterranea* to degrade wood components. *Environmental Microbiology*. 2021; 23: 6104–6121. <https://doi.org/10.1111/1462-2920.15676> PMID: 34288352
3. Larignon P, Dubos B. Fungi associated with esca disease in grapevine. *European Journal of Plant Pathology*. 1997; 103: 147–157. <https://doi.org/10.1023/A:1008638409410>
4. Galloway BT, Fairchild DG. Diseases of the Grape in Western New York. *The Journal of Mycology*. 1891; 6: 95–99. <https://doi.org/10.2307/3752563>

5. Scheck H, Vasquez S, Fogle D, Gubler WD. Grape growers report losses to black-foot and grapevine decline. *Cal Ag*. 1998; 52: 19–23. <https://doi.org/10.3733/ca.v052n04p19>
6. The Gatica M. “Hoja de Malvón” grape disease in Argentina. *Phytopathologia Mediterranea*. 2000; 1–5. <https://doi.org/10.1400/57809>
7. Pascoe IG. Grapevine trunk diseases in Australia: diagnostics and taxonomy. Symptoms and occurrence of grape declines. Fort Valley, VA: International Ampelography Society and International Council on Grapevine Trunk Diseases; 1998. pp. 56–77.
8. Fontaine F, Gramaje D, Armengol J, Smart R, Nagy ZA, Borgo M, et al. Grapevine trunk diseases. A review. OIV Publications; 2016.
9. Moller WJ, Kasimatis AN. Dieback of grapevines caused by *Eutypa armeniacae*. *Plant Disease Reporter*. 1978; 62: 254–258.
10. Lehoczky J. Black dead-arm disease of grapevine caused by *Botryosphaeria stevensii* infection. *Acta Phytopathologica Academiae Scientiarum Hungaricae*. 1974; 9: 319–327.
11. Kajitani Y, Kanematsu S. *Diaporthe kyushuensis* sp. nov., the teleomorph of the causal fungus of grapevine swelling arm in Japan, and its anamorph *Phomopsis vitimegaspora*. *Mycoscience*. 2000; 41: 111–114. <https://doi.org/10.1007/BF02464318>
12. Fontaine F, Pinto C, Vallet J, Clément C, Gomes AC, Spagnolo A. The effects of grapevine trunk diseases (GTDs) on vine physiology. *Eur J Plant Pathol*. 2016; 144: 707–721. <https://doi.org/10.1007/s10658-015-0770-0>
13. Hewitt WB. *Phomopsis* cane and leaf spot. *Compendium of grape diseases*. APS Press; 1988. pp. 17–18.
14. Chiarappa L. Wood decay of the grapevine and its relationship with black measles disease. *Phytopathology*. 1959;49.
15. Halleen F, Schroers HJ, Groenewald JZ, Crous PW. Novel species of *Cylindrocarpon* (*Neonectria*) and *Campylocarpon* gen. nov. associated with black foot disease of grapevines (*Vitis* spp.). *Studies in Mycology*. 2004; 50: 431–455.
16. Mugnai L, Graniti A, Surico G. Esca (black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Disease*. 1999; 83: 404–418. <https://doi.org/10.1094/PDIS.1999.83.5.404> PMID: 30845530
17. Kaplan J, Travadon R, Cooper M, Hillis V, Lubell M, Baumgartner K. Identifying economic hurdles to early adoption of preventative practices: The case of trunk diseases in California winegrape vineyards. *Wine Economics and Policy*. 2016; 5: 127–141. <https://doi.org/10.1016/j.wep.2016.11.001>
18. Baumgartner K, Hillis V, Lubell M, Norton M, Kaplan J. Managing grapevine trunk diseases in California’s southern San Joaquin Valley. *Am J Enol Vitic*. 2019; 70: 267–276. <https://doi.org/10.5344/ajev.2019.18075>
19. Guerin-Dubrana L, Fontaine F, Mugnai L. Grapevine trunk disease in European and Mediterranean vineyards: occurrence, distribution and associated disease-affecting cultural factors. *Phytopathologia Mediterranea*. 2019; 58: 49–71. [https://doi.org/10.13128/Phytopathol\\_Mediterr-25153](https://doi.org/10.13128/Phytopathol_Mediterr-25153)
20. Lee T-C, Ho I-C. Modulation of cellular antioxidant defense activities by sodium arsenite in human fibroblasts. *Arch Toxicol*. 1995; 69: 498–504. <https://doi.org/10.1007/s002040050204> PMID: 8526746
21. Tanner CC, Clayton JS. Persistence of arsenic 24 years after sodium arsenite herbicide application to Lake Rotorua, Hamilton, New Zealand. *New Zealand Journal of Marine and Freshwater Research*. 1990; 24: 173–179. <https://doi.org/10.1080/00288330.1990.9516412>
22. Rezgui A, Vallance J, Ben Ghnaya-Chakroun A, Buez E, Dridi M, Demasse RD, et al. Study of *Lasi-diodiplodia pseudotheobromae*, *Neofusicoccum parvum* and *Schizophyllum commune*, three pathogenic fungi associated with the grapevine trunk diseases in the North of Tunisia. *Eur J Plant Pathol*. 2018; 152: 127–142. <https://doi.org/10.1007/s10658-018-1458-z>
23. Van Jaarsveld WJ, Halleen F, Mostert L. *In vitro* screening of *Trichoderma* isolates for biocontrol of black foot disease pathogens. *Phytopathologia Mediterranea*. 2020; 59: 465–471.
24. Mondello V, Songy A, Battiston E, Pinto C, Coppin C, Trotel-Aziz P, et al. Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Disease*. 2018; 102: 1189–1217. <https://doi.org/10.1094/PDIS-08-17-1181-FE> PMID: 30673583
25. Tuck B, Gartner W, Appiah G. *Vineyards and grapes of the North*. University of Minnesota; 2016 Dec. Available: <http://conservancy.umn.edu/handle/11299/197814>.
26. Hemstad PR, Luby JJ. Utilization of *Vitis riparia* for the development of new wine varieties with resistance to disease and extreme cold. VII International Symposium on Grapevine Genetics and Breeding 528. 2000. pp. 487–496. <https://doi.org/10.17660/ActaHortic.2000.528.70>

27. Hemstad PR, Luby JL. La Crescent, a new cold hardy, high quality, white wine variety. VIII International Conference on Grape Genetics and Breeding 603. 2003. pp. 719–722. <https://doi.org/10.17660/ActaHortic.2003.603.100>
28. Clark MD. Development of Cold Climate Grapes in the Upper Midwestern U.S. Plant Breeding Reviews. John Wiley & Sons, Ltd; 2019. pp. 31–60. <https://doi.org/10.1002/9781119616801.ch2>
29. Yin L, Karn A, Cadle-Davidson L, Zou C, Underhill A, Atkins P, et al. Fine mapping of leaf trichome density revealed a 747-kb region on chromosome 1 in cold-hardy hybrid wine grape populations. *Frontiers in Plant Science*. 2021;12. <https://doi.org/10.3389/fpls.2021.587640> PMID: 33746993
30. Clark M, Tuck B. Minnesota grape production statistics: 2020—estimates for the yield, production, and pricing data of the Minnesota grape industry. 2021. Available: <http://conservancy.umn.edu/handle/11299/224882>.
31. Billones-Baaijens R, Savocchia S. A review of Botryosphaeriaceae species associated with grapevine trunk diseases in Australia and New Zealand. *Australasian Plant Pathology*. 2019; 48: 3–18. <https://doi.org/10.1007/s13313-018-0585-5>
32. Van Niekerk JM, Bester W, Halleen F, Crous PW, Fourie PH. The distribution and symptomatology of grapevine trunk disease pathogens are influenced by climate. *Phytopathologia Mediterranea*. 2011; 50: S98–S111.
33. Ye Q, Jia J, Manawasinghe IS, Li X, Zhang W, Mugnai L, et al. *Fomitiporia punicata* and *Phaeoacremonium minimum* associated with Esca complex of grapevine in China. *Phytopathology Research*. 2021; 3: 11. <https://doi.org/10.1186/s42483-021-00087-w>
34. Yan J-Y, Xie Y, Zhang W, Wang Y, Liu J-K, Hyde KD, et al. Species of Botryosphaeriaceae involved in grapevine dieback in China. *Fungal Diversity*. 2013; 61: 221–236. <https://doi.org/10.1007/s13225-013-0251-8>
35. Li H, Li RY, Wang H. New Disease for Wine-making Grape—Eutypa Dieback. *Liquor Making Science and Technology*. 2007; 5: 48.
36. Díaz GA, Latorre BA. Infection Caused by *Phaeoconiella chlamydospora* Associated with Esca-like Symptoms in Grapevine in Chile. *Plant Disease*. 2014; 98: 351–360. <https://doi.org/10.1094/PDIS-12-12-1180-RE> PMID: 30708428
37. Díaz GA, Auger J, Besoain X, Bordeu E, Latorre BA. Prevalence and pathogenicity of fungi associated with grapevine trunk diseases in Chilean vineyards. *Ciencia e investigación agraria*. 2013; 40: 327–339. <https://doi.org/10.4067/S0718-16202013000200008>
38. Urbez-Torres JR, Peduto F, Striegler RK, Urrea-Romero KE, Rupe JC, Cartwright RD, et al. Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Diversity*. 2012; 52: 169–189. <https://doi.org/10.1007/s13225-011-0110-4>
39. Gubler W, Rolshausen P, Trouillas F, Urbez-Torres JR, Voegel T, Leavitt GM, et al. Grapevine trunk diseases in California: Research update. *Practical Winery & Vineyards*. 2005; 1–9.
40. Gramaje D, Mostert L, Groenewald JZ, Crous PW. *Phaeoacremonium*: From esca disease to phaeo-hyphomycosis. *Fungal Biology*. 2015; 119: 759–783. <https://doi.org/10.1016/j.funbio.2015.06.004> PMID: 26321726
41. Urbez-Torres JR, Haag P, Bowen P, O’Gorman DT. Grapevine trunk diseases in British Columbia: incidence and characterization of the fungal pathogens associated with esca and petri diseases of grapevine. *Plant Disease*. 2014; 98: 469–482. <https://doi.org/10.1094/PDIS-05-13-0523-RE> PMID: 30708729
42. Worrall JJ. Media for selective isolation of hymenomyces. *Mycologia*. 1991; 83: 296–302. <https://doi.org/10.1080/00275514.1991.12026013>
43. Harrington TC. Cycloheximide sensitivity as a taxonomic character in ceratocystis. *Mycologia*. 1981; 73: 1123–1129. <https://doi.org/10.1080/00275514.1981.12021447>
44. Osmundson TW, Eyre CA, Hayden KM, Dhillon J, Garbelotto MM. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. *Molecular Ecology Resources*. 2013; 13: 66–74. <https://doi.org/10.1111/1755-0998.12031> PMID: 23121735
45. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology*. 1993; 2: 113–118. <https://doi.org/10.1111/j.1365-294x.1993.tb00005.x> PMID: 8180733
46. Blanchette RA, Held BW, Hellmann L, Millman L, Buntgen U. Arctic driftwood reveals unexpectedly rich fungal diversity. *Fungal Ecology*. 2016; 23: 58–65. <https://doi.org/10.1016/j.funeco.2016.06.001>
47. Lawrence DP, Travadon R, Nita M, Baumgartner K. TrunkDiseaseID.org: a molecular database for fast and accurate identification of fungi commonly isolated from grapevine wood. *Crop Protection*. 2017; 102: 110–117. <https://doi.org/10.1016/j.cropro.2017.08.017>

48. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, et al. Welcome to the Tidyverse. *Journal of Open Source Software*. 2019; 4: 1686. <https://doi.org/10.21105/joss.01686>
49. Hsieh TC, Ma KH, Chao A. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*. 2016; 7: 1451–1456. <https://doi.org/10.1111/2041-210X.12613>
50. Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, et al. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. *Ecological Monographs*. 2014; 84: 45–67. <https://doi.org/10.1890/13-0133.1>
51. Sosnowski MR, Shtienberg D, Creaser ML, Wicks TJ, Lardner R, Scott ES. The Influence of Climate on Foliar Symptoms of Eutypa Dieback in Grapevines. *Phytopathology*. 2007; 97: 1284–1289. <https://doi.org/10.1094/PHTO-97-10-1284> PMID: 18943686
52. Marchi G, Peduto F, Mugnai L, Di Marco S, Calzarano F, Surico G. Some observations on the relationship of manifest and hidden esca to rainfall. *Phytopathologia Mediterranea*. 2006; 45: S117–S126.
53. Lawrence DP, Travadon R, Pouzoulet J, Rolshausen PE, Wilcox WF, Baumgartner K. Characterization of *Cytospora* isolates from wood cankers of declining grapevine in North America, with the descriptions of two new *Cytospora* species. *Plant Pathology*. 2017; 66: 713–725. <https://doi.org/10.1111/ppa.12621>
54. Lawrence D, Travadon R, Baumgartner K. Diversity of *Diaporthe* species associated with wood cankers of fruit and nut crops in northern California. *Mycologia*. 2015; 107: 926–940. <https://doi.org/10.3852/14-353> PMID: 26240309
55. Úrbez-Torres JR, Peduto F, Smith RJ, Gubler WD. Phomopsis dieback: a grapevine trunk disease caused by *Phomopsis viticola* in California. *Plant Disease*. 2013; 97: 1571–1579. <https://doi.org/10.1094/PDIS-11-12-1072-RE> PMID: 30716818
56. Úrbez-Torres JR, Adams P, Kamas J, Gubler WD. Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *Am J Enol Vitic*. 2009; 60: 497–507.
57. Aroca A, Raposo R. Pathogenicity of *Phaeoacremonium* Species on Grapevines. *Journal of Phytopathology*. 2009; 157: 413–419. <https://doi.org/10.1111/j.1439-0434.2008.01513.x>
58. Cloete M, Fourie PH, Damm U, Crous PW, Mostert L. Fungi associated with die-back symptoms of apple and pear trees, a possible inoculum source of grapevine trunk disease pathogens. *Phytopathologia Mediterranea*. 2011; 50: S176–S190.
59. Elena G, Bruez E, Rey P, Luque J. Microbiota of grapevine woody tissues with or without esca-foliar symptoms in northeast Spain. *Phytopathologia Mediterranea*. 2018. [https://doi.org/10.14601/Phytopathol\\_Mediterr-23337](https://doi.org/10.14601/Phytopathol_Mediterr-23337)
60. Bahmani Z, Abdollahzadeh J, Amini J, Evidente A. *Biscogniauxia rosacearum* the charcoal canker agent as a pathogen associated with grapevine trunk diseases in Zagros region of Iran. *Sci Rep*. 2021; 11: 14098. <https://doi.org/10.1038/s41598-021-93630-w> PMID: 34239007
61. Berlanas C, Ojeda S, López-Manzanares B, Andrés-Sodupe M, Bujanda R, del Pilar Martínez-Diz M, et al. Occurrence and diversity of black-foot disease fungi in symptomless grapevine nursery stock in Spain. *Plant Disease*. 2020; 104: 94–104. <https://doi.org/10.1094/PDIS-03-19-0484-RE> PMID: 31738690
62. Cabral A, Cecilia R, CROUS P, Oliveira H. Virulence and cross-infection potential of *Ilyonectria* spp. to grapevine. *Phytopathologia Mediterranea*. 2012; 51: 340–354.
63. Probst CM, Ridgway HJ, Jaspers MV, Eirian Jones E. Pathogenicity of *Ilyonectria liriodendri* and *Dactylonectria macrodidyma* propagules in grapevines. *Eur J Plant Pathol*. 2019; 154: 405–421. <https://doi.org/10.1007/s10658-018-01664-0>
64. Lawrence DP, Travadon R, Baumgartner K. Novel *Seimatosporium* species from grapevine in northern California and their interactions with fungal pathogens involved in the trunk-disease complex. *Plant Disease*. 2018; 102: 1081–1092. <https://doi.org/10.1094/PDIS-08-17-1247-RE> PMID: 30673434
65. Trouillas FP, Gubler WD. Pathogenicity of diatrypaceae species in grapevines in California. *Plant Disease*. 2010; 94: 867–872. <https://doi.org/10.1094/PDIS-94-7-0867> PMID: 30743554
66. Halleen F, Mostert L, Crous PW. Pathogenicity testing of lesser-known vascular fungi of grapevines. *Australasian Plant Pathology*. 2007; 36: 277–285. <https://doi.org/10.1071/AP07019>
67. Raimondo ML, Carlucci A, Ciccarone C, SADDALLAH A, Francesco L. Identification and pathogenicity of lignicolous fungi associated with grapevine trunk diseases in southern Italy. *Phytopathologia Mediterranea*. 2019; 58: 639–662.
68. Travadon R, Lawrence DP, Rooney-Latham S, Gubler WD, Wilcox WF, Rolshausen PE, et al. *Cado-phora* species associated with wood-decay of grapevine in North America. *Fungal biology*. 2015; 119: 53–66. <https://doi.org/10.1016/j.funbio.2014.11.002> PMID: 25601149

69. Úrbez-Torres JR, Leavitt GM, Guerrero JC, Guevara J, Gubler WD. Identification and pathogenicity of *Lasiodiplodia theobromae* and *Diplodia seriata*, the causal agents of bot canker disease of grapevines in Mexico. *Plant Disease*. 2008; 92: 519–529. <https://doi.org/10.1094/PDIS-92-4-0519> PMID: 30769642
70. Marsberg A, Kemler M, Jami F, Nagel JH, Postma-Smidt A, Naidoo S, et al. *Botryosphaeria dothidea*: a latent pathogen of global importance to woody plant health. *Molecular Plant Pathology*. 2017; 18: 477–488. <https://doi.org/10.1111/mpp.12495> PMID: 27682468
71. Baranek M, Armengol J, Holleínová V, Pečenka J, Calzarano F, Peňázová E, et al. Incidence of symptoms and fungal pathogens associated with grapevine trunk diseases in Czech vineyards: first example from a north-eastern European grape-growing region. *Phytopathologia Mediterranea*. 2018; 57: 449–458. [https://doi.org/10.14601/Phytopathol\\_Mediterr-22460](https://doi.org/10.14601/Phytopathol_Mediterr-22460)
72. Mundy DC, Brown A, Jacobo F, Tennakoon K, Woolley RH, Vanga B, et al. Pathogenic fungi isolated in association with grapevine trunk diseases in New Zealand. *New Zealand Journal of Crop and Horticultural Science*. 2020; 48: 84–96. <https://doi.org/10.1080/01140671.2020.1716813>
73. Kaliterna J, Miličević T, Cvjetković B. Grapevine trunk diseases associated with fungi from the diaportheaceae family in Croatian vineyards. *Arhiv za higijenu rada i toksikologiju*. 2012; 63: 471–478. <https://doi.org/10.2478/10004-1254-63-2012-2226> PMID: 23334042
74. Rolshausen PE, Wilcox W, Baumgartner K. Distribution and occurrence of fungi associated with grapevine trunk diseases in Northeastern American vineyards. *Phytopathologia Mediterranea*. 2010; 49: 105.
75. van Niekerk JM, Groenewald JZ, Farr DF, Fourie PH, Halleen F, Crous PW. Reassessment of *Phomopsis* species on grapevines. *Austral Plant Pathol*. 2005; 34: 27. <https://doi.org/10.1071/AP04072>
76. Mostert L, Crous PW, Petrini O. Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. *Sydowia*. 2000; 52: 46–58.
77. Anderson HW, Colby AS. Dead arm disease of grape in Illinois. *The Plant Disease Reporter*. 1943; 27: 245–246.
78. Baumgartner K, Fujiyoshi PT, Travadon R, Castlebury LA, Wilcox WF, Rolshausen PE. Characterization of species of *Diaporthe* from wood cankers of grape in eastern North American vineyards. *Plant Disease*. 2013; 97: 912–920. <https://doi.org/10.1094/PDIS-04-12-0357-RE> PMID: 30722541
79. Dissanayake AJ, Liu M, Zhang W, Chen Z, Udayanga D, Chukeatirote E, et al. Morphological and molecular characterisation of *Diaporthe* species associated with grapevine trunk disease in China. *Fungal Biology*. 2015; 119: 283–294. <https://doi.org/10.1016/j.funbio.2014.11.003> PMID: 25937058
80. Fitzpatrick DA. Horizontal gene transfer in fungi. *FEMS Microbiology Letters*. 2012; 329: 1–8. <https://doi.org/10.1111/j.1574-6968.2011.02465.x> PMID: 22112233
81. Casacuberta E, González J. The impact of transposable elements in environmental adaptation. *Molecular Ecology*. 2013; 22: 1503–1517. <https://doi.org/10.1111/mec.12170> PMID: 23293987
82. Christensen CM. Studies on the biology agric. *Valsa sordida* and *Cytospora chrysosperma*. *Phytopathology*. 1940;30. Available: <https://www.cabdirect.org/cabdirect/abstract/19401101375>.
83. Cold Outbreak: January 27–31, 2019. In: Minnesota Department of Natural Resources [Internet]. [cited 19 Dec 2021]. Available: <https://www.dnr.state.mn.us/climate/journal/cold-outbreak-january-27-31-2019.html>.
84. Svyantek A, Köse B, Stenger J, Auwarter C, Hatterman-Valenti H. Cold-hardy grape cultivar winter injury and trunk re-establishment following severe weather events in North Dakota. *Horticulturae*. 2020; 6: 75. <https://doi.org/10.3390/horticulturae6040075>
85. van Niekerk JM, Calitz FJ, Halleen F, Fourie PH. Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. *Eur J Plant Pathol*. 2010; 127: 375–390. <https://doi.org/10.1007/s10658-010-9604-2>
86. Ayres M, Billones -Baaijens Regina, Savocchia S, Scott E, Sosnowski M. Trunk diseases: susceptibility of pruning wounds to grapevine trunk disease pathogens. *Wine & Viticulture Journal*. 31: 48–50. <https://doi.org/10.3316/informit.523883064121148>
87. Kraus C, Voegelé RT, Fischer M. Temporal development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD-associated fungi. *Microb Ecol*. 2019; 77: 866–876. <https://doi.org/10.1007/s00248-018-1280-3> PMID: 30397796
88. Pouzoulet J, Scudiero E, Schiavon M, Rolshausen PE. Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeoaniella chlamydospora* in grapevine. *Frontiers in Plant Science*. 2017; 8: 1442. <https://doi.org/10.3389/fpls.2017.01442> PMID: 28871268
89. Bortolami G, Gambetta GA, Delzon S, Lamarque LJ, Pouzoulet J, Badel E, et al. Exploring the Hydraulic Failure Hypothesis of Esca Leaf Symptom Formation. *Plant Physiology*. 2019; 181: 1163–1174. <https://doi.org/10.1104/pp.19.00591> PMID: 31455632

90. Bortolami G, Farolfi E, Badel E, Burlett R, Cochard H, Ferrer N, et al. Seasonal and long-term consequences of esca grapevine disease on stem xylem integrity. *Journal of Experimental Botany*. 2021; 72: 3914–3928. <https://doi.org/10.1093/jxb/erab117> PMID: 33718947
91. Agrios GN. *Plant Pathology*. fifth. Elsevier Inc.; 2005.
92. Valtaud C, Larignon P, Roblin G, Fleurat-Lessard P. Developmental and ultrastructural features of *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum* in relation to xylem degradation in esca disease of the grapevine. *Journal of Plant Pathology*. 2009; 91: 37–51.
93. Yin Z, Liu H, Li Z, Ke X, Dou D, Gao X, et al. Genome sequence of *Valsa* canker pathogens uncovers a potential adaptation of colonization of woody bark. *New Phytologist*. 2015; 208: 1202–1216. <https://doi.org/10.1111/nph.13544> PMID: 26137988
94. Morales-Cruz A, Amrine KCH, Blanco-Ulate B, Lawrence DP, Travadon R, Rolshausen PE, et al. Distinctive expansion of gene families associated with plant cell wall degradation, secondary metabolism, and nutrient uptake in the genomes of grapevine trunk pathogens. *BMC Genomics*. 2015; 16: 469. <https://doi.org/10.1186/s12864-015-1624-z> PMID: 26084502
95. Blanchette RA. Degradation of the lignocellulose complex in wood. *Can J Bot*. 1995; 73: 999–1010. <https://doi.org/10.1139/b95-350>
96. Pouzoulet J, Pivovarov AL, Santiago LS, Rolshausen PE. Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. *Frontiers in Plant Science*. 2014; 5: 253. <https://doi.org/10.3389/fpls.2014.00253> PMID: 24971084
97. Pouzoulet J, Rolshausen PE, Charbois R, Chen J, Guillaumie S, Ollat N, et al. Behind the curtain of the compartmentalization process: Exploring how xylem vessel diameter impacts vascular pathogen resistance. *Plant, Cell & Environment*. 2020; 43: 2782–2796. <https://doi.org/10.1111/pce.13848> PMID: 32681569
98. Knipfer T, Eustis A, Brodersen C, Walker AM, Mcelrone AJ. Grapevine species from varied native habitats exhibit differences in embolism formation/repair associated with leaf gas exchange and root pressure. *Plant, Cell & Environment*. 2015; 38: 1503–1513. <https://doi.org/10.1111/pce.12497> PMID: 25495925
99. Smith MS, Centinari M. Young grapevines exhibit interspecific differences in hydraulic response to freeze stress but not in recovery. *Planta*. 2019; 250: 495–505. <https://doi.org/10.1007/s00425-019-03183-6> PMID: 31089803
100. Lovisolo C, Schubert A. Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *Journal of Experimental Botany*. 1998; 49: 693–700. <https://doi.org/10.1093/jxb/49.321.693>
101. Garrett KA, Nita M, De Wolf ED, Esker PD, Gomez-Montano L, Sparks AH. Chapter 24—Plant pathogens as indicators of climate change. In: Letcher TM, editor. *Climate Change* (Third Edition). Elsevier; 2021. pp. 499–513. <https://doi.org/10.1016/B978-0-12-821575-3.00024-4>
102. Bortolami G, Gambetta GA, Cassan C, Dayer S, Farolfi E, Ferrer N, et al. Grapevines under drought do not express esca leaf symptoms. *Proceedings of the National Academy of Sciences*. 2021; 118. <https://doi.org/10.1073/pnas.2112825118> PMID: 34675082
103. Galarneau ERA, Lawrence DP, Travadon R, Baumgartner K. Drought Exacerbates Botryosphaeria Dieback Symptoms in Grapevines and Confounds Host-based Molecular Markers of Infection by *Neofusicoccum parvum*. *Plant Disease*. 2019; 103: 1738–1745. <https://doi.org/10.1094/PDIS-09-18-1549-RE> PMID: 31082329
104. Whiteman SA, Stewart A, Ridgway HJ, Jaspers MV. Infection of rootstock mother-vines by *Phaeoconiella chlamydospora* results in infected young grapevines. *Australasian Plant Pathology*. 2007; 36: 198–203. <https://doi.org/10.1071/AP07012>
105. Damm U, Verkley GJM, Crous PW, Fourie PH, Haegi A, Riccioni L. Novel *Paraconiothyrium* species on stone fruit trees and other woody hosts. *Persoonia*. 2008; 20: 9–17. <https://doi.org/10.3767/003158508X286842> PMID: 20467483
106. Groenewald M, Kang J-C, Crous PW, Gams W. ITS and  $\beta$ -tubulin phylogeny of *Phaeoacremonium* and *Phaeoconiella* species. *Mycological Research*. 2001; 105: 651–657. <https://doi.org/10.1017/S0953756201004282>
107. Gramaje D, Alaniz S, Pérez-Sierra A, Abad-Campos P, García-Jiménez J, Armengol J. First report of *Phaeoacremonium mortoniae* causing Petri disease of grapevine in Spain. *Plant Disease*. 2007; 91: 1206–1206. <https://doi.org/10.1094/PDIS-91-9-1206A> PMID: 30780685
108. Mohammadi H. First report of *Phaeoacremonium mortoniae* associated with grapevine decline in Iran. *Plant Disease*. 2011; 95: 1034–1034. <https://doi.org/10.1094/PDIS-05-11-0361> PMID: 30732074

109. Crous PW, Gams W, Wingfield MJ, van Wyk PS. *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia*. 1996; 88: 786–796. <https://doi.org/10.1080/00275514.1996.12026716>
110. Gatica M, Dupont J, Magnin S, Césari C. *Phaeoacremonium* species and *Phaeomoniella chlamydospora* in vines showing “hoja de malvón” and young vine decline symptoms in Argentina. *Phytopathologia Mediterranea*. 2001; 1000–1008. <https://doi.org/10.1400/14644>
111. Cottral E, Pascoe I. Developments in grapevine trunk diseases research in Australia. *Phytopathologia Mediterranea*. 2000; 39: 68–75. <https://doi.org/10.1400/57813>
112. Essakhi S, Mugnai L, Crous PW, Groenewald JZ, Surico G. Molecular and phenotypic characterisation of novel *Phaeoacremonium* species isolated from esca diseased grapevines. *Persoonia*. 2008; 21: 119–134. <https://doi.org/10.3767/003158508X374385> PMID: 20396582
113. Held BW, Simeto S, Rajtar NN, Cotton AJ, Showalter DN, Bushley KE, et al. Fungi associated with galleries of the emerald ash borer. *Fungal Biology*. 2021; 125: 551–559. <https://doi.org/10.1016/j.funbio.2021.02.004> PMID: 34140151
114. Arenz BE, Held BW, Jurgens JA, Farrell RL, Blanchette RA. Fungal diversity in soils and historic wood from the Ross Sea Region of Antarctica. *Soil Biology and Biochemistry*. 2006; 38: 3057–3064. <https://doi.org/10.1016/j.soilbio.2006.01.016>
115. Blanchette RA, Held BW, Jurgens JA, McNew DL, Harrington TC, Duncan SM, et al. Wood-destroying soft rot fungi in the historic expedition huts of Antarctica. *Applied and Environmental Microbiology*. 2004 [cited 21 Jan 2022]. <https://doi.org/10.1128/AEM.70.3.1328-1335.2004> PMID: 15006750
116. Blanchette RA, Held BW, Jurgens J, Stear A, Dupont C. Fungi attacking historic wood of Fort Conger and the Peary Huts in the High Arctic. *PLOS ONE*. 2021; 16: e0246049. <https://doi.org/10.1371/journal.pone.0246049> PMID: 33497418
117. Rusman Y, Held BW, Blanchette RA, He Y, Salomon CE. Cadopherone and colomitide polyketides from *Cadophora* wood-rot fungi associated with historic expedition huts in Antarctica. *Phytochemistry*. 2018; 148: 1–10. <https://doi.org/10.1016/j.phytochem.2017.12.019> PMID: 29366851
118. Gramaje D, Mostert L, Armengol J. Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. *Phytopathologia Mediterranea*. 2011; 50: S112–S126.
119. Agustí-Brisach C, León M, García-Jiménez J, Armengol J. Detection of grapevine fungal trunk pathogens on pruning shears and evaluation of their potential for spread of infection. *Plant Disease*. 2015; 99: 976–981. <https://doi.org/10.1094/PDIS-12-14-1283-RE> PMID: 30690978
120. Murolo S, Romanazzi G. Effects of grapevine cultivar, rootstock and clone on esca disease. *Australasian Plant Pathology*. 2014; 43: 215–221. <https://doi.org/10.1007/s13313-014-0276-9>
121. Agustí-Brisach C, Mostert L, Armengol J. Detection and quantification of *Ilyonectria* spp. associated with black-foot disease of grapevine in nursery soils using multiplex nested PCR and quantitative PCR. *Plant Pathology*. 2014; 63: 316–322. <https://doi.org/10.1111/ppa.12093>
122. del Pilar Martínez-Diz M, Andrés-Sodupe M, Berbegal M, Bujanda R, Díaz-Losada E, Gramaje D. Droplet digital PCR technology for detection of *Ilyonectria liriodendri* from grapevine environmental samples. *Plant Disease*. 2020; 104: 1144–1150. <https://doi.org/10.1094/PDIS-03-19-0529-RE> PMID: 32053059
123. Rajtar NN, Held BW, Blanchette RA. Fungi from galleries of the emerald ash borer produce cankers in ash trees. *Forests*. 2021; 12: 1509. <https://doi.org/10.3390/f12111509>
124. Biodiversity Fischer M. and geographic distribution of basidiomycetes causing esca-associates white rot in grapevine: a worldwide perspective. *Phytopathologia Mediterranea*. 2006; 1–13.
125. Fischer M, García G. V. An annotated checklist of European basidiomycetes related to white rot of grapevine. *Phytopathologia Mediterranea*. 2015; 54: 281–298.
126. Brown AA, Lawrence DP, Baumgartner K. Role of basidiomycete fungi in the grapevine trunk disease esca. *Plant Pathology*. 2020; 69: 205–220. <https://doi.org/10.1111/ppa.13116>
127. Fischer M, Edwards J, Cunnington JH, Pascoe IG. Basidiomycetous pathogens on grapevine: a new species from Australia-*Fomitiporia australiensis*. *Mycotaxon*. 2005; 92: 85–96.
128. Tomoiaga LL, Chedea VS. Grapevine trunk diseases, anagement in vineyards from central Transylvania. *BUASVMCN-HORT*. 2020; 77: 117. <https://doi.org/10.15835/buasvmcn-hort:2019.0041>
129. Dilley MA, Covey RP. Survey of wood decay and associated hymenomycetes in central Washington apple orchards. *Plant Disease*. 1980; 64: 561.
130. Enebak SA, Blanchette RA. Canker formation and decay in sugar maple and paper birch infected by *Cerrena unicolor*. *Canadian Journal of Forest Research*. 2011 [cited 21 Jan 2022]. <https://doi.org/10.1139/x89-031>

131. Claverie M, Notaro M, Fontaine F, Wery J. Current knowledge on grapevine trunk diseases with complex etiology: a systemic approach. *Phytopathologia Mediterranea*. 2020; 59: 29–53.
132. Wingfield MJ, De Beer ZW, Slippers B, Wingfield BD, Groenewald JZ, Lombard L, et al. One fungus, one name promotes progressive plant pathology. *Molecular Plant Pathology*. 2012; 13: 604–613. <https://doi.org/10.1111/j.1364-3703.2011.00768.x> PMID: 22146077
133. Silva F, Santos K, Tamiris R, Armengol J, Rossi V, Michereff S, et al. Temporal conidial dispersal pattern of Botryosphaeriaceae species in table-grape vineyards in Northeastern Brazil. *Phytopathologia Mediterranea*. 2018; 57: 547–556. [https://doi.org/10.14601/Phytopathol\\_Mediterr-24240](https://doi.org/10.14601/Phytopathol_Mediterr-24240)
134. Urbez-Torres JR, Gispert C, Trouillas FP. Spore dispersal of *Eutypella* species under desert grape-growing conditions of southern California. *Phytopathologia Mediterranea*. 2020; 59: 485–501. <https://doi.org/10.14601/Phyto-11807>
135. González-Domínguez E, Caffi T, Languasco L, Latinovic N, Latinovic J, Rossi V. Dynamics of *Dia-portha ampelina* conidia released from grape canes that overwintered in the vineyard. *Plant Disease*. 2021; 105: 3092–3100. <https://doi.org/10.1094/PDIS-12-20-2639-RE> PMID: 33755509
136. Otto EC, Held BW, Gould TJ, Blanchette RA. Fungal Diversity in Multiple Post-harvest Aged Red Pine Stumps and Their Potential Influence on Heterobasidion Root Rot in Managed Stands Across Minnesota. *Frontiers in Fungal Biology*. 2021;2. Available: <https://www.frontiersin.org/article/10.3389/ffunb.2021.782181>.