Canadian Journal of Microbiology
Revue canadienne de de microbiologie

Fungal endophytes of wild and hybrid Vitis leaves and their potential for vineyard biocontrol

Journal:	*Canadian Journal of Microbiology*
Manuscript ID	cjm-2016-0740.R1
Manuscript Type:	Article
Date Submitted by the Author:	29-Mar-2017
Complete List of Authors:	Kernaghan , Gavin ; Mount Saint Vincent University, Biology Mayerhofer, Michael; Mount Saint Vincent University, Biology Griffin, Amanda; Mount Saint Vincent University, Biology
Keyword:	fungal diversity, grapevine, next generation sequencing, Nova Scotia

Fungal endophytes of wild and hybrid *Vitis* leaves and their potential for vineyard biocontrol

Gavin Kernaghan*, Michael Mayerhofer and Amanda Griffin, Biology Department, Mount St. Vincent University, 166 Bedford Hwy., Halifax, NS, B3M 2J6, Canada.

*Corresponding author. Tel.: +1 902 457 6328; fax: +1 902 457 6455
E-mail: gavin.kernaghan@msvu.ca

Abstract

Plants are colonized by diverse assemblages of fungal endophytes that have potential as biocontrol agents for a variety of crops, including grapevine. Although the diversity of symbionts can be very high in wild plants, the fungal endophytes of wild *Vitis* plants have not yet been investigated. We surveyed the fungal endophytes of six wild populations of *Vitis riparia,* as well as a cold tolerant, hybrid grapevine in five vineyards (one certified organic), using 454 pyrosequencing. We detected between 43 and 235 OTUs per sample, with the highest richness and diversity in the wild, the lowest in conventional vineyards, and intermediate levels in the organic vineyard. Wild plants supported a range of taxa not seen in the conventional vineyards, and vineyards were dominated by relatively few taxa. We also isolated fungi from the wild plants and tested them for their ability to inhibit pathogens of grapevine. Several wild isolates (e.g. *Ramularia* spp.) were strongly inhibitory to grapevine pathogens. We show that wild *Vitis* supports a distinct and highly diverse community of fungal endophytes, and may represent a rich repository of potential vineyard biocontrol agents.

Key words: fungal diversity, grapevine, next generation sequencing, Nova Scotia

Introduction

Plants tissues support a wide array of endophytic fungi that colonize internally and asymptomatically (Rodriguez et al. 2009; Bacon and White 2000). This diverse group of fungi has recently attracted attention for a variety of reasons, including their potential to protect plants from pathogens, either through competition for space within the plant tissue (Mejía et al. 2008), or by production of antimicrobial secondary metabolites (Aly et al. 2010; Kusari et al 2012; Zheng et al. 2016). Inhibition of pathogens by endophytic fungi has been demonstrated in a variety plant species, and appears to be a valuable strategy for biological control (Backman and Sikora 2008; Wicklow et al. 2005; Hanada et al. 2009; Hue et al. 2009). Advantages over chemical fungicides include persistent, systemic protection, fewer impacts on non-target organisms, and minimal potential for development of resistance mechanisms (Hahn 2014).

Novel methods of disease control are particularly pertinent for vineyards, as grapevine is susceptible to a variety of pathogens, including powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*) and grey mold (*Botrytis cinerea*), which can negatively affect the quality of the final product even at diffuse background levels (Gadoury et al. 2001; Steel et al. 2013). The use of biological control methods in place of fungicides would also aid in the production of certified organic wines, for which there is an increasing demand (Sharples 2000; Point et al. 2012).

In cool regions of North America, hybrid grape varieties obtained by crossing *Vitis vinifera* with native *Vitis* species have become popular due to their tolerance to low temperatures and their relatively high pest resistance. As fungal endophytes can exhibit host preference (Kernaghan and Patriquin 2011; Christian et al. 2016), it is possible that endophytes from native

North American *Vitis* species may be more compatible with hybrids than with the parent *V. vinifera* grown in warmer regions.

Some fungal endophytes are already commercially available for vineyard biocontrol, such as the black yeast *Aureobasidium pullulans,* used against *Botrytis* and postharvest decay (Schena et al. 1999; Chi et al. 2009; Schmid et al. 2011). The mycoparasite *Trichoderma harzianum* can colonize plants endophytically and has been used against a variety of pathogens including powdery mildew (Harman et al. 2004). The secondary metabolites produced by endophytic *Alternaria alternata* are also effective against downy mildew (Musetti et al. 2006).

Other *Vitis* endophytes have also been proposed as potential biocontrol agents (Falk et al. 1996; Brum et al. 2012; Núñez-Trujillo et al. 2012; Cosoveanu et al. 2014), but so far, all have originated from within vineyards. In fact, the diversity of *Vitis* fungal endophytes has only been explored in cultivated plants (Casieri et al. 2009; González and Tello 2011; Pancher et al. 2012) and to the best of our knowledge, there have been no investigations of the fungal endophytes of wild *Vitis*, even though these plants should harbour a greater diversity of microbial symbionts than their domesticated counterparts (Elbeltagy et al. 2000; Zinniel et al. 2002; Campisano et al. 2015).

We therefore set out to investigate the diversity and species composition of fungal endophytes colonizing the leaves of a cultivated hybrid grape variety, and the leaves of one of its native progenitors in eastern Canada using 454 pyrosequencing. We also sought to isolate fungal endophytes from wild *Vitis* leaves, as this habitat may well represent a rich source of vineyard biocontrol agents.

METHODS

Sampling of plant material

Wild grape sampling (samples W1-W6) focused on *Vitis riparia*, the most common wild grape species in eastern Canada. In August 2014, 12 mature *V. riparia* leaves were collected at each of six sampling sites along the shore of Lake Huron, from Bayview Escarpment Nature Reserve (40^{o} 37' N, 80^{o} 43' W) to Awenda Provincial Park (44^{o} 50' N, 80^{o} 01') Ontario. All wild samples were collected from relatively undisturbed, mixed deciduous forests except for W5, which was from a sandy beach habitat.

Vineyard sampling focused on leaves of L'Acadie blanc, Nova Scotia's signature hybrid grape variety. L'Acadie is derived from multiple progenitors, including varieties of *Vitis vinifera* and North American *V. labrusca* and *V. riparia*. In September 2014, 12 mature leaves were collected systematically from each of four traditional vineyards, located in Digby, Kings and Cumberland Counties, Nova Scotia, as well as Gray County, Ontario. One certified organic vineyard in Lunenburg County, Nova Scotia was also sampled.

The Ontario sampling sites experience average daily temperatures from 20.5^{o} C in July to -6.9^{o} C in January, and monthly precipitation from 120 mm in January to 68 mm in April. Average daily temperatures at the Nova Scotia sampling sites are similar; 19.5^{o} C in July and -5.9 in January, but the wettest month is November (134 mm) and the driest is August (78 mm).

Although L'Acadie blanc is relatively disease resistant, conventional vineyards generally control pathogenic fungi with sulfur and the broad spectrum phthalimide captan. Other synthetic compounds, such as triazols and dithiocarbamates may also be applied as required. The organic vineyard (V5) has applied only non-synthetic compounds, such as lime sulfur, potassium bicarbonate, and Bordeaux mixture (copper sulfate and lime).

All samples were kept cool on ice and transported to Mount Saint Vincent University for processing within one week.

Sequencing of fungal endophyte DNA

For DNA extraction, the 12 leaves in each collection were randomly assigned to three sets of four leaves each. Using a sterile cork borer, three, 7mm disks of tissue were excised from each leaf and pooled across each set, resulting in three pooled tissue samples per collection, giving a total of 33 tissue samples (3 pooled samples per collection x 11 collections). Tissue samples were taken such that they included a portion of leaf vein to maximize the diversity of endophytes (Carroll and Petrini 1983). Genomic DNA was extracted from each set of samples using the DNeasy Plant Mini Kit (Qiagen). Tissue was frozen in extraction buffer, ground in a ceramic mortar and then incubated at 65°C for 30 min prior to DNA extraction following the manufacturer's instructions. Fungal ITS rDNA was amplified using a nested approach. rDNA was diluted 1:10 in water and then amplified with Q5® Hot Start High Fidelity Master Mix (New England Biolabs) and 0.2 µM of the fungal specific primers ITS-1F (Gardes and Bruns 1993) and NL6C2 (CAAGYGYTTCCCTTTCAACA - modified from Egger 1995 and Kernaghan et al. 2003) in 25 µl reactions. Cycling parameters were 98°C for 3 min., followed by 25 cycles of 98°C for 30 sec., 58°C for 30 sec. and 72°C for 30 sec. Final extension was at 72°C for 7 min. The second round of amplification used 15µl of 1:10 to1:50 dilutions of the initial PCR reaction as a template (Davey et al. 2012) in 50µl reactions with ITS4 (White et al. 1990) as a reverse primer and 33 forward fusion primers (Integrated DNA Technologies, Inc.) based on ITS7g (Ihrmark et al. 2012). Each fusion primer contained a unique multiplex identifier (MID) sequence (Roche Technical Bulletin 005-2009) for downstream de-multiplexing of the 454 pyrosequencing output. The cycling parameters for the secondary amplification were similar to

those for the initial amplification, but the annealing temperature was increased to 59°C, the extension time decreased to 15 sec., and the number of cycles ranged from 10 to 15 depending on the sample amplified. The 33 PCR products from the secondary amplifications were then purified with the Agencourt AMPure XP® PCR Purification system and sequenced (454 pyrosequencing) with a Roche Genome Sequencer FLX System using Titanium chemistry at the McGill University and Génome Québec Innovation Centre. Sequences have been deposited in the Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under accession SRP077482.

Characterization of endophyte communities

All bioinformatic analyses were conducted with the MacQIIME 1.8.0 pipeline (Caporaso et al. 2010). Sequences were de-multiplexed, assessed for quality and labeled using split_libraries.py. Chimeric sequences were then identified and removed using USEARCH 6.1 (drive5.com/usearch) along with a recently updated set of reference sequences (Nilsson 2015). Sequences were then sorted into operational taxonomic units (OTUs ~ species) based on 97% similarity using USEARCH 6.1. A representative set of OTUs was then constructed and taxonomy assigned with the RDP classifier (Wang et al. 2007) in conjunction with the full International Nucleotide Sequence Database (INSD) and the UNITE database adapted for the QIIME pipeline. All singleton sequences were filtered from the data set and samples were rarified (equalized) to the number of sequences in the sample with the lowest sampling depth (1197 sequences). Examples of each of the 50 most abundant OTUs were also manually queried against the INSD database with BLASTn, and a consensus between the BLAST and RDP classifier results used for the final identification.

Statistical analyses of the sequence data were conducted with R 3.2.0 (R Core Team 2013). Diversity analyses utilized the Vegan 2.2-1 package unless otherwise specified. First,

differences among vineyard and wild grape collections were visualized using non-metric multidimensional scaling (nMDS). Statistical significance of the resulting groupings was assessed with permutational multivariate analysis of variance (PERMANOVA) using the adonis function and Sørensen distances.

Species richness and diversity indices (Fisher's alpha) were calculated and significant differences between collecting locations detected by ANOVA, followed by Tukey's HSD. Beta-diversity (the variation in diversity among collections) was assessed with the betadisper function of the Vegan package. Fungal species unique to each treatment were determined through taxonomic Venn analysis with the limma package (vennCounts function), and visualized as Venn diagrams using the Venneuler package.

Isolation of fungal endophytes cultures from wild grape leaves

To obtain cultures of fungal endophytes from the wild grape collections, a sterile cork borer was used to excise three, 7mm disks of tissue from each of the same 12 leaves collected at each of the six wild grape sampling locations (36 samples per site; 216 samples in total). Tissue was excised in a manner similar to that used for the DNA extraction described above, with each sample including a portion of leaf vein. The three tissue samples from each leaf were surface sterilized in 15% hydrogen peroxide for either one, two, or three minutes, followed by a one minute rinse in sterile water. Each tissue sample was then transferred to an individual plate of malt-yeast extract medium (1.5% agar), containing oxytetracycline, streptomycin sulfate and penicillin G to reduce bacterial contamination. Plates were incubated at 23°C and monitored for fungal growth. All emergent filamentous fungi and yeasts were subsequently transferred to fresh malt-yeast extract media without antibiotics.

Screening of endophyte cultures against vineyard pathogens

To screen for biocontrol potential, all isolates were paired with cultures of the pathogen *Botrytis cinerea* (ARSL 141114.14) in confrontation tests. A subset of 15 isolates was also paired with the root pathogen *Cylindrocarpon destructans* (UAMH 10026). Each endophyte culture was transferred to the perimeter of a fresh malt-yeast plate and permitted to grow for one week (or until the mycelium reached the center of the plate in the case of fast growing species), after which the plate was co-inoculated with one of the pathogenic isolates. Fungal growth of each species was measured after one further week of growth. An inhibition index (I) was calculated as follows:

$$I = (HP_{control} - HP_{paired}) + (VP_{control} - VP_{paired}) + D - (HE/2)$$

Where HP is the horizontal growth of the pathogen on the control plate or when paired with an endophyte, VP is the vertical growth of the pathogen, D is the horizontal distance between the pathogen and the endophyte, and HE is the horizontal growth of the endophyte culture.

The thirty isolates with the greatest inhibitory activity against *B. cinerea* ($I > 33$) were sub-cultured by single hyphal tip transfers to fresh media. DNA was extracted from the new cultures with the DNeasy Plant Mini Kit (Qiagen) and amplified using the ITS1F/NL6C2 primer pair described above. Sanger sequencing of the resulting PCR products was conducted using ITS1 and IT4 (White et al. 1990) as sequencing primers. Contig sequences were constructed from each resulting sequence pair using Sequencher 5 software, and the isolates identified by querying against GenBank accessions using BLASTn. Sequences from pure cultures have been deposited in GenBank as KX869937-KX869965.

A further subset of fifteen isolates were re-tested for inhibition of *B. cinerea* after sub-culturing, and all thirty identified cultures were transferred to agar slants in screw cap vials for

storage at 4°C in the Atlantic Root Symbiosis Laboratory (ARSL) at Mount Saint Vincent University, Halifax.

RESULTS

Species richness and diversity of fungal endophytes

Pyrosequncing produced a total of 134,712 individual fungal sequences. After bioinformatic quality control procedures and rarefaction (equalization), 1,197 sequences from each of the 33 samples were utilized for further analyses. A total of 665 operational taxonomic units (OTUs ~ species) were identified, with individual sampling locations supporting between 43 and 235 OTUs, with an overall average of 137.8 OTUs.

An average of 54.8 OTUs were detected in conventional vineyards, while the average number of OTUs in wild samples was 200.5. The organic vineyard was intermediate in fungal endophyte species richness with 94 OTUs. Overall, fungal endophytes were significantly richer in wild samples than in vineyard samples ($p < 0.001$), regardless of whether the organic vineyard was included or not. Endophyte diversity followed a similar trend, with an average Fisher's alpha of 46.1 for wild collections, 9.2 for the vineyard samples, and 17.7 for the organic vineyard. This difference was also significant ($p < 0.001$), with or without the organic vineyard.
Beta diversity was similar between wild and vineyard sampling locations.

The initial exploration of differences in fungal endophytes across sample types using taxonomic Venn analysis indicated that 55.3% of the vineyard fungi also occur in the wild, while only 19% of those found in the wild also occur in vineyards. Overall, only 16.8% of endophytes detected were common to vineyard and wild plants, while 87.9% of the endophytes found in the wild were unique to wild grape (Fig. 1a). When comparing the organic vineyard to a single

representative of a conventional vineyard and a single representative wild collection, 29.8% of the endophytes were shared with the wild and 22.3% were shared with the conventional vineyard. However, 62.8% of endophytes found in the organic vineyard were unique to that location; more than twice the number of unique endophytes found in the conventional vineyard (Fig. 1b).

Wild and vineyard endophyte communities also differed with respect to the fungal taxa present (Fig. 2). Conventional vineyards were dominated by a relatively small number of genera, including *Alternaria,* which was the most abundant genus in conventional vineyards, but was far less common in the organic vineyard and the wild plants. Wild samples also had larger proportions of rare OTU's (<1% of total sequences) than conventional vineyard samples, as well as larger proportions of unassigned taxa - sequences for which there were no, or only ambiguous, genus level matches within the sequence databases. The organic vineyard had fewer rare taxa than wild samples, but exhibited a similar level of unassigned taxa.

Certain genera, including *Ramularia* (4 OTUs), *Subplenodomus* (1 OTU) and *Zymoseptoria* (7 OTUs) were specific to wild samples and not detected in any of the vineyard samples. *Dioszegia* was common in the wild samples and the organic vineyard, but rare in the conventional vineyards. *Aureobasidium* was the most common genus overall, occurring in all sample types, although it was also more abundant in conventional vineyards than in the organic vineyard or in the wild samples. The variation among wild endophyte communities was relatively high, with *Ramularia* and *Subplenodomus* being particularly abundant in individual samples, whereas endophytes from conventional vineyards were more evenly distributed across samples.

The conventional vineyards, the organic vineyard and the wild grape plants also support distinctive endophyte communities at the level of individual OTUs (~species). In the non-metric multidimensional scaling (nMDS) of the most abundant OTUs, samples from the conventional vineyards (including the Ontario vineyard) cluster together toward the right of the diagram, while all the wild samples cluster together on the left. The three sub-samples from the organic vineyard fall between the other two groups, and are also positioned higher on the Y axis (Fig. 3a).

Species characteristic of the conventional vineyards include *Alternaria* sp. (OTU 1), *Sporidiobolus* sp. (OTU 3) and *Rhodotorula mucilaginosa* (OTU 24). The endophyte community within one subsample from the Ontario vineyard (V1) differed slightly from the other conventional vineyards, in that it contained a relatively large amount of a unique species of *Alternaria* (OTU 8)(Fig. 3a). *Dioszegia* sp. (OTU 12), *Dissoconium* sp. (OTU 9) and *Cadophora* sp. (OTU 21) characterized the organic vineyard (Fig. 3a), while the distinctive endophyte community of the wild plants included *Ramularia cynarae* (OTU 15), *Subplenodomus* sp. (OTU 6), Tremellales sp. (OTU 44), *Sydowia* sp. (OTU 23) and *Ramularia* sp. (OTU 15)(Fig. 3b).

Overall, the fungal communities colonizing vineyards and wild plants were significantly different (permanova $p < 0.001$), regardless of whether or not the organic vineyard was included.

Fungal isolates from wild grape

A total of 115 endophytes were isolated from the wild grape leaves. When paired with the pathogen *Botrytis cinerea* in confrontation tests, 85% inhibited the growth of *B. cinerea* to some extent (although only very slightly in some cases)(Fig. 4). ITS sequencing revealed that the 30 isolates most inhibitory to *Botrytis* represented 15 genera and up to 21 species (Table 1). Most were ascomycetes, except for the basidiomycetes *Coprinellus* and *Irpex* (three isolates), and most were filamentous fungi, except for the yeast *Aureobasidium pullulans*. Overall, the inhibition of

Botrytis by isolates purified by single hyphal tip transfers were not significantly different from that of the original isolates, indicating that the inhibitory effects seen in the initial 115 cultures were not the result of mixed cultures.

Cultures with the highest indices of inhibition against the *Botrytis* (I >100) were *Ramularia pratensis* (Isolate 48), followed by *Phoma aliena* (Isolate 44) and *Fusarium acuminatum* (Isolate 4). Several others were also relatively inhibitory, including isolates of *Hypoxylon* and the closely related genus *Biscogniauxia*, as well as *Peyronellaea* and *Lecythophora* (Fig. 4, Table 1). Pairings between isolated endophytes and the root pathogen *Cylindrocarpon destructans* generally resulted in lower inhibition indices than in parings with *Botrytis* (max. I = 50). The endophytes with the greatest inhibition of *Cylindrocarpon* were *Ramularia* sp. (Isolate 76), followed by *Phoma* sp. (Isolate 47), and *Biscogniauxia mediterranea* (Isolate 10).

Pyrosequencing data indicates that of the groups of inhibitory fungi isolated, *Ramularia* spp. were not uncommon (2% of all sequences), but were detected only in the wild samples. *Phoma* accounted for 17% of all sequences and was common in both wild and vineyard samples. Both the Xylariaceae and *Fusarium* appear rare, with fewer than 10 sequences of each detected across all sample types. Neither *Peyronellaea* nor *Lecythophora* sequences were detected by pyrosequencing.

DISCUSSION

Endophyte diversity in vineyards and in wild plants

The use of next generation sequencing allowed us to thoroughly characterize the endophytic fungi of both wild and vineyard grown plants. This high throughput approach is well suited for characterizing highly diverse leaf endophyte communities (Jumpponen and Jones

2009; Bullington and Larkin 2015). To the best of our knowledge, this is the first account of fungal endophytes from wild *Vitis* plants. We found that endophyte species richness and diversity were far higher in wild plants than in conventional vineyards, and that wild, conventional and organically grown *Vitis* support distinctive communities of fungal endophytes with low species overlap. Wild *Vitis* also supports greater numbers of rare and unidentified endophytes, and may represent a rich repository of potential vineyard biocontrol agents.

The high endophyte diversity found in wild *Vitis* compared to cultivated plants may be due to a number of factors: 1) cultivated plants are genetically distinct, in that they have been bred for particular traits (Yuan et al. 2010), 2) wild plants grow in close proximity to other plant species and may share endophytes (Cannon and Simmons 2002), and 3) cultivated plants are subjected to fungicides. Although the effects of fungicides vary depending on the class of fungicide and the composition of the fungal community, application generally decreases endophyte colonization, or at least results in changes in fungal species composition (Mohandoss and Suryanarayanan 2009; Christian et al. 2016; Nettles et al. 2016).

The fact that endophyte species richness and diversity were at an intermediate level in the organic vineyard is evidence of the influence of synthetic fungicides on the fungal communities in the conventional vineyards. However, *Ramularia* and *Zymoseptora* (Mycosphaerellaceae), as well as *Subplenodomus* (Leptosphaeriaceae), were found only in wild plants, and not detected in any vineyards (including the organic vineyard), suggesting that factors other than the application of synthetic fungicides may explain their distribution. Conversely, the basidiomycete yeast *Dioszegia* was common in wild and organic vineyard samples, but not seen in conventional vineyards, suggesting that it may be inhibited by synthetic fungicides, but not the non-synthetic compounds utilized in the organic vineyard.

Pathogenic *Alternaria* spp. and the yeasts *Rhodotorula* and *Sporidiobolus* were detected at much higher levels in the conventional vineyards than in the wild samples or in the organic vineyard. This is part of a general pattern of reduced fungal diversity and the dominance of a few taxa in the conventional vineyards. Although there are several reasons for reduced fungal diversity in conventional vineyards, the lack of such a pattern in the organic vineyard again points to systemic fungicides. *Alternaria* is a common endophyte of cultivated *Vitis* (Casieri et al. 2009; González and Tello 2011; Núñez-Trujillo et al. 2012; Pancher et al. 2012; Cosoveanu et al. 2014) and populations of *Alternaria* infecting a number of other crop plants, including citrus (Vega and Dewdney 2014), crucifers (Iacomi-Vasilescu et al. 2004), pistachio (Avenot et al. 2014) and potato (Fairchild et al. 2013) have exhibited resistance to synthetic fungicides. Our results imply that the strains of endophytic fungi dominating the conventional vineyards (e.g. *Alternaria, Rhodotorula* and *Sporidiobolus*) may have also developed resistance to synthetic fungicides.

Unlike *Alternaria*, the black yeast *Aureobasidium pullulans* was relatively common in most samples. Strains of *A. pullulans* are marketed commercially under the trade name "Botector", as a biocontrol agent against pathogens such as *Botrytis* on grape and other berry crops. It effectively inhibits fungal growth through competition for space and nutrients, and through the production of cell wall degrading enzymes (Chi et al. 2009).

Schmid et al. (2011) found that vineyards which did not apply systemic fungicides (organic vineyards) supported larger populations of *A. pullulans* than conventional vineyards. This was attributed to the ability of *A. pullulans* to oxidize inorganic sulphur (Killham et al. 1981) and absorb and detoxify copper (Gadd and DeRome 1988), enabling it to tolerate the non-synthetic fungicides utilized in the organic vineyards. Conversely, we found *A. pullulans* to be

more abundant in conventional vineyards than in either the organic vineyard or in the wild samples. Although we sampled only one organic vineyard, Pancher et al. (2012) also detected *A. pullulans* more frequently in vineyards using synthetic fungicides than in those using non-synthetic fungicides. One possible explanation for this discrepancy may lie in the nature of the fungicides applied in the organic vineyards, as the organic vineyard in our study applies lime sulfur, which is not recommended for use in conjunction with the *A. pullulans* based biocontrol agent.

Antifungal activity of isolated cultures

Screening of cultures isolated from wild *Vitis* leaves showed relatively high levels of *Botrytis* inhibition by isolates of *Fusarium, Phoma, Lecythophora, Hypoxylon* and *Ramularia.* Although some species in each of these genera are implicated in plant disease to some extent, the effects of fungal endophytes can vary greatly among host species, sometimes eliciting disease symptoms on certain plant species but not others (Redman et al. 2001). For example, *Fusarium circinatum* causes pitch canker on conifer trees, but also colonizes corn and other grasses without inducing negative symptoms (Swett and Gordon 2015).

Fusarium acuminatum successfully inhibited *Botrytis* in the present study, and is not known to cause disease on grapevine. However, this species is implicated in mycotoxin production (Visconti et al. 1989), and may therefore be a poor choice for vineyard biocontrol. Other, more promising endophytic *Fusarium* species exist, but were not detected in the present study, such as *F. proliferatum,* which is active against powdery mildew of grape (*Plasmopara viticola*)(Falk et al. 1996).

Isolates of *Phoma* and the related *Peyronellaea* also inhibited *Botrytis* in culture. Although many species in both genera are involved in disease processes, some endophytic

Phoma species produce potent antimicrobial secondary metabolites (Strobel et al. 2011; Arora et al. 2016), which may have potential as new biopesticides against other fungal pathogens.

Another somewhat inhibitory endophyte isolated from wild *Vitis* leaves was *Lecythophora hoffmannii.* Although mainly isolated from soil, it also grows endophytically, and has been isolated previously from *Vitis* (young *V. vinifera* buds)(Casieri et al. 2009). Rosa et al. (2012) showed that extracts of endophytic *Lecythophora* spp. from *Smallanthus* (Asteraceae) inhibit plant pathogenic fungi, and Sugijanto et al. (2011) demonstrated that an endophytic species of *Lecythophora* produces the antifungal compound lecythomycin.

We also isolated inhibitory species of *Hypoxylon* and *Biscogniauxia* (Xylariaceae) from wild plants. Although some species of Xylariaceae (e.g. *H. mammatum* and *Rosellinia* spp.) can cause root rots, cankers and needle blights in woody plants (Whalley 1996), an endophytic species of *Xylaria* has been isolated from leaves of cultivated *Vitis labrusca* that inhibits the vineyard pathogen *Fusarium oxysporum* (Brum et al. 2012). Also, an endophytic species of *Hypoxylon* produces a wide variety of volatile organic compounds with antimicrobial activity against the plant pathogens *Botrytis*, *Phytophthora, Cercospora*, and *Sclerotinia* (Tomsheck et al. 2010; Ul-Hassan et al. 2012). Xylariaceous fungi from wild *Vitis* may therefore warrant further investigation with respect to their potential as vineyard biocontrol agents.

Isolates of *Ramularia* were also inhibitory to fungal pathogens. Of the 115 isolates, *Ramularia pratensis* (Isolate 48) had the highest inhibition index against *Botrytis*, and of the 15 isolates tested against *Cylindrocarpon, Ramularia* sp. (Isolate 76) showed the highest level. *Ramularia* species range from endophytes to pathogens, the latter often being associated with host specific leaf spot disease (Videira et al. 2015, 2016). The related *Pseudocercospora brachypus* causes angular leaf spot only on muscadine grape in the southern Unites States (Chen

and Lamikanra 1997), and *P. vitis* is considered a "minor foliage disease" of grapevine in warm climates (Pearson and Goheen 1988). However, our isolates of *Ramularia* grow slowly in culture, and appear to be good examples of fungi that focus their energy on the production of antimicrobial secondary metabolites rather than rapid growth (Mejía et al. 2008). Therefore, even if these isolates were to prove inappropriate for use as direct biocontrol agents due to pathogenicity to hybrid grape cultivars, their metabolites should be further explored as potential biopesticides (Reino et al. 2008; Wang et al. 2013).

We have demonstrated that wild *Vitis* plants support highly diverse communities of fungal endophytes. As several of these inhibit pathogenic fungi *in vitro,* and some do not appear to be present in the vineyards sampled, they likely represent a rich source of potential vineyard biocontrol agents. Future work should involve the inoculation of cultivated *Vitis* seedlings with endophytes cultured from wild plants in order to assess the reaction of the host to colonization (Yang et al. 2016). If no symptoms are elicited, plants could then be inoculated with fungal pathogens to assay for improved disease resistance. It would also be prudent to test for fungicide tolerance in any proposed biocontrol fungi (Murphy et al. 2016), as it is clear that fungicide application impacts fungal endophyte communities. Isolates that improve pathogen resistance and exhibit fungicide tolerance could then be utilized as part of a larger integrated pest management program.

ACKNOWLEDGEMENTS

This project was funded through an Engage grant (EGP #469598-14) from the Natural Science and Engineering Research Council of Canada (NSERC). We are also indebted to the members of Grape Growers Association of Nova Scotia for their helpful information and cooperation.

References

Aly, A.H., Debbab, A., Kjer, J. and Proksch, P. 2010. Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers. 41: 1-16.

Arora, P., Wani, Z.A., Nalli, Y., Ali, A., and Riyaz-Ul-Hassan, S. 2016. Antimicrobial potential of thiodiketopiperazine derivatives produced by *Phoma* sp., an endophyte of *Glycyrrhiza glabra* Linn. Microb. Ecol. 72: 802-812.

Avenot, H.F., Van Den Biggelaar, H., Morgan, D.P., Moral, J., Joosten, M., and Michailides, T.J. 2014. Sensitivities of baseline isolates and boscalid-resistant mutants of *Alternaria alternata* from pistachio to fluopyram, penthiopyrad, and fluxapyroxad. Plant Dis. 98: 197-205.

Backman, P.A., and Sikora, R.A. 2008. Endophytes: an emerging tool for biological control. Biol. Control, 46: 1-3.

Bacon, C.W., and White, J.F. 2000. Microbial endophytes. Marcel Deker, Inc., NY.

Brum, M.C.P., Araujo, W.L., Maki, C.S., and Azevedo, J.L. 2012. Endophytic fungi from *Vitis labrusca* L. ('Niagara Rosada') and its potential for the biological control of *Fusarium oxysporum.* Genet. Mol. Res. 11: 4187-4197.

Bullington, L.S., and Larkin, B.G. 2015. Using direct amplification and next-generation sequencing technology to explore foliar endophyte communities in experimentally inoculated western white pines. Fungal Ecol 17: 170-178.

Campisano, A., Pancher, M., Puopolo, G., Puddu, A., Lòpez-Fernàndez, S., Biagini, B., et al. 2015. Diversity in endophytic populations reveals functional and taxonomic diversity between wild and domesticated grapevines. Am. J. Enol. Vitic. 66: 12-21.

Cannon, P.F., and Simmons, C.M. 2002. Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. Mycologia, 94: 210-220.

Caporaso, J., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods, 7: 335-336.

Carroll, G., and Petrini, O. 1983. Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycologia, 75: 53-63.

Casieri L., Hofstetter, V.A., Viret, O.L., and Gindro, K.A. 2009. Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol. Mediterr. 48: 73-83.

Chen, J., and Lamikanra, O. 1997. Resistance of muscadine grapes to angular leaf spot (*Mycosphaerella angulata* Jenkins) in North Florida. HortScience, 32: 94-95.

Chi, Z., Wang, F., Chi, Z., Yue, L., Liu, G., and Zhang, T. 2009. Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. Appl. Microbiol. Biotechnol. 82: 793-804.

Christian, N., Sullivan, C., Visser, N.D., and Clay, K. 2016. Plant host and geographic location drive endophyte community composition in the face of perturbation. Microb. Ecol. 72: 621-632.

Cosoveanu, A., Gimenez-Mariño, C., Cabrera, Y., Hernandez, G., and Cabrera, R. 2014. Endophytic fungi from grapevine cultivars in Canary Islands and their activity against phytopatogenic fungi. Intl. J. Agri. Crop Sci. 7: 1497-1503.

Davey, M.L., Heegaard, E., Halvorsen, R., Ohlson, M., and Kauserud, H. 2012. Seasonal trends in the biomass and structure of bryophyte-associated fungal communities explored by 454 pyrosequencing. New Phytol. 195: 844-856.

Egger, K. 1995. Molecular analysis of ectomycorrhizal communities. Can. J. Bot. 73 (Suppl. 1): S1415–S1422.

Elbeltagy, A., Nishioka, K., Suzuki, H., Sato, T., Sato, Y.I., Morisaki, H., et. al. 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. Soil Science and Plant Nutrition, 46: 617-629.

Fairchild, K.L., Miles, T.D., and Wharton, P.S. 2013. Assessing fungicide resistance in populations of *Alternaria* in Idaho potato fields. Crop Prot. 49: 31-39.

Falk, S.P., Pearson R.C., Gadoury, D.M., Seem, R.C., Sztejnberg. A. 1996. *Fusarium proliferatum* as a biocontrol agent against grape downy mildew. Phytopathology 86: 1010–1017.

Gadd, G.M., and de Rome, L. (1988). Biosorption of copper by fungal melanin. Appl. Microbiol. Biotechnol. 29: 610-617.

Gadoury, D.M., Seem, R.C., Pearson, R.C., Wilcox, W.F., and Dunst, R.M. 2001. Effects of powdery mildew on vine growth, yield, and quality of concord grapes. Plant Dis. 85: 137-140.

Gardes, M., and Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.

González, V., and Tello, M.L. 2011. The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers. 47: 29-42.

Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. J. Chem. Biol. 7: 133-141.

Hanada, R.E., Pomella, A.W., Soberanis, W., Loguercio, L.L., and Pereira, J.O. 2009. Biocontrol potential of *Trichoderma martiale* against the black-pod disease (*Phytophthora palmivora*) of cacao. Biol Control 50: 143-149.

Harman, G.E., Howell, C.R., Viterbo, A., Chet I., and Lorito M. 2004. *Trichoderma* spp.: Opportunistic avirulent plant symbionts. Nat. Rev. Microbiol. 2: 43–56.

Hue, A.G., Voldeng, H.D., Savard, M.E., Fedak, G., Tian, X., and Hsiang, T. 2009. Biological control of fusarium head blight of wheat with *Clonostachys rosea* strain ACM941. Can. J. Plant Pathol. 31: 169-179.

Iacomi -Vasilescu, B., Avenot, H., Bataillé-Simoneau, N., Laurent, E., Guénard, M., and Simoneau, P. 2004. In vitro fungicide sensitivity of *Alternaria* species pathogenic to crucifers and identification of *Alternaria brassicicola* field isolates highly resistant to both dicarboximides and phenylpyrroles. Crop Prot. 23: 481-488.

Ihrmark, K., Bödeker, I.T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., et al. 2012. New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. FEMS Microbiol. Ecol. 82: 666-677.

Jumpponen, A., and Jones, K.L. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytol 184: 438-448.

Kernaghan, G., and Patriquin, G. 2011. Host associations between fungal root endophytes and boreal trees. Microb. Ecol. 62: 460-473.

Kernaghan, G., Sigler, L., and Khasa, D. 2003. Mycorrhizal and root endophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA sequence analysis. Microb. Ecol. 45: 128-136.

Killham, K., Lindley, N.D., and Wainwright, M. 1981. Inorganic sulfur oxidation by *Aureobasidium pullulans*. Appl. Environ. Microbiol. 42: 629-631.

Kusari, S., Hertweck, C., Spiteller, M. 2012. Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem. Biol. 19: 792–798.

Mejía, L.C., Rojas, E.I., Maynard, Z., Bael, S.V., Arnold, A.E., Hebbar, P., et al. 2008. Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. Biol. Control, 46: 4-14.

Mohandoss, J., and Suryanarayanan, T. S. 2009. Effect of fungicide treatment on foliar fungal endophyte diversity in mango. Sydowia, 61: 11-24.

Murphy, B.R., Doohan, F.M., and Hodkinson, T.R. 2016. A seed dressing combining fungal endophyte spores and fungicides improves seedling survival and early growth in barley and oat. Symbiosis. doi:10.1007/s13199-016-0418-7.

Musetti, R., Vecchione, A., Stringher, L., Borselli, S., Zulini, L., Marzani, C., et al. 2006. Inhibition of sporulation and ultrastructural alterations of grapevine downy mildew by the endophytic fungus *Alternaria alternata*. Phytopathology 96: 689-698.

Nettles, R., Watkins, J., Ricks, K., Boyer, M., Licht, M., Atwood, L.W., et al. 2016. Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. Appl. Soil Ecol. 102: 61-69.

Nilsson, R.H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterseher, et al. 2015. A comprehensive, automatically updated fungal ITS sequence dataset for

reference-based chimera control in environmental sequencing efforts. Microbes Environ. 30: 145–150.

Núñez-Trujillo, G., Cabrera, R., Burgos-Reyes, R.L., Silva, E.D., Giménez, C., Cosoveanu, A., et al. 2012. Endophytic fungi from *Vitis vinifera* L. isolated in Canary Islands and Azores as potential biocontrol agents of *Botrytis cinerea* Pers.: Fr. J. Hortic. Sci. Biotech. 16: 1-6.

Pancher, M., Ceol, M., Corneo, P.E., Longa, C.M.O., Yousaf, S., Pertot, I., et al. 2012. Fungal endophytic communities in grapevines (*Vitis vinifera* L.) respond to crop management. Appl. Environ. Microbiol. 78: 4308-4317.

Pearson, R.C., and Goheen, A.C. 1988. Compendium of Grape Diseases, 4th ed. American Phytopathological Society, St. Paul, Mn.

Point, E., Tyedmers, P., and Naugler, C. 2012. Life cycle environmental impacts of wine production and consumption in Nova Scotia, Canada. J. Clean. Prod. 27: 11-20.

R Core Team 2013. R: A language and environment for statistical computing, 3.2.0 ed, Vienna, Austria.

Redman, R.S., Dunigan, D.D., and Rodriguez, R.J. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? New Phytol. 151: 705-716.

Reino, J.L., Guerrero, R.F., Hernandez-Galan, R., and Collado, I.G. 2008. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev. 7: 89-123.

Rodriguez, R.J., White, J.F., Arnold, A.E., and Redman, R.S. 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182: 314-330.

Rosa, L.H., Tabanca, N., Techen, N., Pan, Z., Wedge, D.E., and Moraes, R.M. 2012. Antifungal activity of extracts from endophytic fungi associated with *Smallanthus* maintained *in vitro*

as autotrophic cultures and as pot plants in the greenhouse. Can. J. Microbiol. 58: 1202-1211.

Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F., et al. Droby, S. 1999. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. Postharvest Biol. Technol. 17: 189-199.

Schmid, F., Moser, G., Müller, H., Berg, G. 2011. Functional and structural microbial diversity in organic and conventional viticulture: organic farming benefits natural biocontrol agents. Appl. Environ. Microbiol. 77: 2188–2191.

Sharples, L. 2000. Organic Wines-The UK Market: A shift from 'niche market' to 'mainstream' position? International Journal of Wine Marketing, 12: 30-41.

Steel, C.C., Blackman, J.W., and Schmidtke, L.M. 2013. Grapevine bunch rots: impacts on wine composition, quality, and potential procedures for the removal of wine faults. J. Agric. Food Chem. 61: 5189-5206.

Strobel, G., Singh, S.K., Riyaz-Ul-Hassan, S., Mitchell, A.M., Geary, B., and Sears, J. 2011. An endophytic/pathogenic *Phoma* sp. from creosote bush producing biologically active volatile compounds having fuel potential. FEMS Microbiol. Lett. 320: 87-94.

Sugijanto, N.E., Diesel, A., Rateb, M., Pretsch, A., Gogalic, S., Zaini, N.C., et al. 2011. Lecythomycin, a new macrolactone glycoside from the endophytic fungus *Lecythophora* sp. Nat. Prod. Commun. 6: 677-678.

Swett, C.L., and Gordon, T.R. 2015. Endophytic association of the pine pathogen *Fusarium circinatum* with corn (*Zea mays*). Fungal Ecol. 13: 120-129.

Tomsheck, A.R., Strobel, G.A., Booth, E., Geary, B., Spakowicz, D., Knighton, B., et al. 2010.

Hypoxylon sp., an endophyte of *Persea indica*, producing 1,8-cineole and other bioactive volatiles with fuel potential. Microb. Ecol. 60: 903–914.

Ul-Hassan, S.R., Strobel, G.A., Booth, E., Knighton, B., Floerchinger, C., and Sears, J. 2012. Modulation of volatile organic compound formation in the Mycodiesel-producing endophyte *Hypoxylon* sp. CI-4. Microbiology 158: 465-473.

Vega, B., and Dewdney, M.M. 2014. Distribution of QoI resistance in populations of tangerine-infecting *Alternaria alternata* in Florida. Plant Dis. 98: 67-76.

Videira, S.I.R., Groenewald, J.Z., Verkley, G.J., Braun, U., and Crous, P.W. 2015. The rise of *Ramularia* from the *Mycosphaerella* labyrinth. Fungal biology, 119: 823-843.

Videira, S.I.R., Groenewald, J.Z., Braun, U., Shin, H.D., and Crous, P.W. 2016. All that glitters is not *Ramularia*. Stud. Mycol. 49-163.

Visconti, A., Mirocha, C.J., Logrieco, A., Bottalico, A., and Solfrizzo, M. 1989. Mycotoxins produced by *Fusarium acuminatum*. Isolation and characterization of acuminatin: a new trichothecene. J. Agric. Food Chem. 37: 1348-1351.

Wang, Q., Garrity, G.M., Tiedje, J M., and Cole, J.R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73: 5261-5267.

Wang, X., Radwan, M.M., Taráwneh, A.H., Gao, J., Wedge, D.E., Rosa, L.H., et al. 2013. Antifungal activity against plant pathogens of metabolites from the endophytic fungus *Cladosporium cladosporioides*. J. Agric. Food Chem. 61: 4551-4555.

Whalley, A. 1996. The xylariaceous way of life. Mycol. Res. 100: 897-922.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to

Methods and Applications. Edited by M.A. Innes, D.H. Gelfand, J.J. Sninsky and T.J. White. Academic Press, NY. pp. 315-322.

Wicklow, D.T., Roth, S., Deyrup, S.T., and Gloer, J.B. 2005. A protective endophyte of maize: *Acremonium zeae* antibiotics inhibitory to *Aspergillus flavus* and *Fusarium verticillioides*. Mycol. Res. 109: 610-618.

Yang, M., Ma, M., Yuan, M., Huang, Z., Yang, W., Zhang, H., et al. 2016. Fungal endophytes as a metabolic fine-tuning regulator for wine grape. PLoS ONE 11: e0163186. doi:10.1371/journal.pone

Yuan, Z.L., Zhang, C.L., Lin, F.C., and Kubicek, C.P. 2010. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. Appl. Environ. Microbiol. 76: 1642-1652.

Zheng, Y.K., Qiao, X.G., Miao, C.P., Liu, K., Chen, Y.W., Xu, L.H., et al. 2016. Diversity, distribution and biotechnological potential of endophytic fungi. Ann. Microbiol. 66: 529-542.

Zinniel, D.K., Lambrecht, P., Harris, N.B., Feng, Z., Kuczmarski, D., Higley, P., et al. 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl. Environ. Microbiol. 68: 2198-2208.

Table 1. Identity, accession numbers and sampling locations of fungal endophyte cultures selected for identification.

Isolate	Location	ARSL[a]/GenBank accession numbers	GenBank match	% similarity	Identity
1	W1	260914.10/KX869937	HQ414607	100	*Lecythophora hoffmannii*
2	W1	260914.11/KX869938	AJ246218	99	*Hypoxylon fragiforme*
3	W4	190914.16/ KX869939	EU167565	99	*Peyronellaea pinodella*
4	W6	311014.19/KX869940	KJ082098	99	*Fusarium acuminatum*
10	W3	271014.4/KX869941	GQ377490	100	*Biscogniauxia mediterranea*
17	W2	271014.15/KX869942	FJ185160	99	*Coprinellus* sp.
20	W3	141114.8/KX869943	EF026134	99	*Biscogniauxia mediterranea*
24	W3	141114.18/KX869944	GQ377490	99	*Biscogniauxia mediterranea*
40	W3	141114.7/KX869945	HM469419	99	*Penicillium* sp.
44	W4	190914.17/ KX869946	KC311486	99	*Phoma aliena*
46	W2	071114.22/KX869947	EF155523	99	*Hypoxylon fragiforme*
47	W4	271014.16/KX869948	KF367550	98	*Phoma* sp.
48	W6	190914.15/KX869949	EU019284	98	*Ramularia pratensis*
51	W5	141114.14	NA	NA	*Botrytis cinerea*[b]
54	W3	141114.21/KX869950	JQ009309	96	*Hypoxylon* sp.
59	W6	260914.12/KX869951	KC311486	99	*Phoma aliena*
73	W4	071114.11/KX869952	FM991735	99	*Epicoccum nigrum*
74	W1	051214.25/KX869953	JX290573	99	*Irpex lacteus*
75	W4	051214.27/KX869954	JN578625	87	*Phoma* sp.
76	W2	051214.22/KX869955	EU019284	96	*Ramularia* sp.
77	W2	051214.23/KX869956	JN578625	99	*Peyronellaea pinodella*
87	W6	071114.1/KX869957	AF462431	96	*Rhizosphaera* sp.
91	W3	051214.10/KX869958	JF340278.1	99	*Fimetariella rabenhorstii*
95	W5	050914.3/ KX869959	JX188092	100	*Aureobasidium pullulans*
97	W5	051214.9/KX869960	JX188092	99	*Aureobasidium pullulans*
99	W5	071114.4/KX869961	JX496118	98	*Paraconiothyrium variabile*
103	W4	191214.3/KX869962	JX290578	99	*Irpex lacteus*
108	W5	071115.5/KX869963	KF512824	100	*Epicoccum nigrum*
112	W6	191214.7/KX869964	EU019284	96	*Ramularia* sp.
113	W6	051214.4/KX869965	KC164754	99	*Epicoccum nigrum*

[a] Atlantic Root Symbiosis Laboratory.

[b] Identified by microscopy.

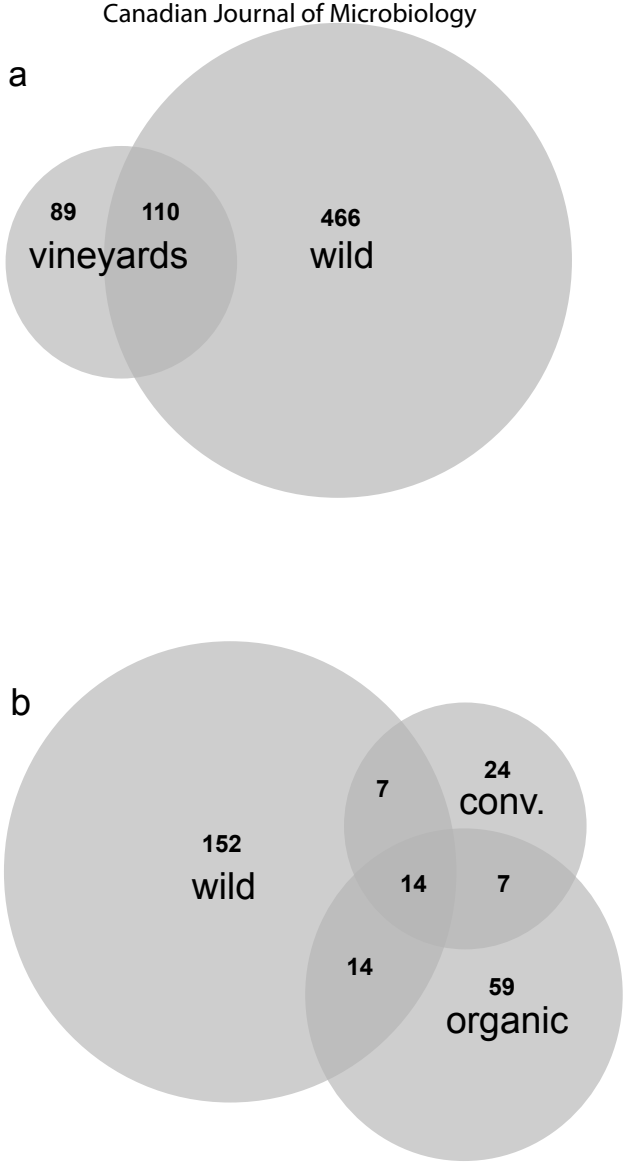
Figure legends

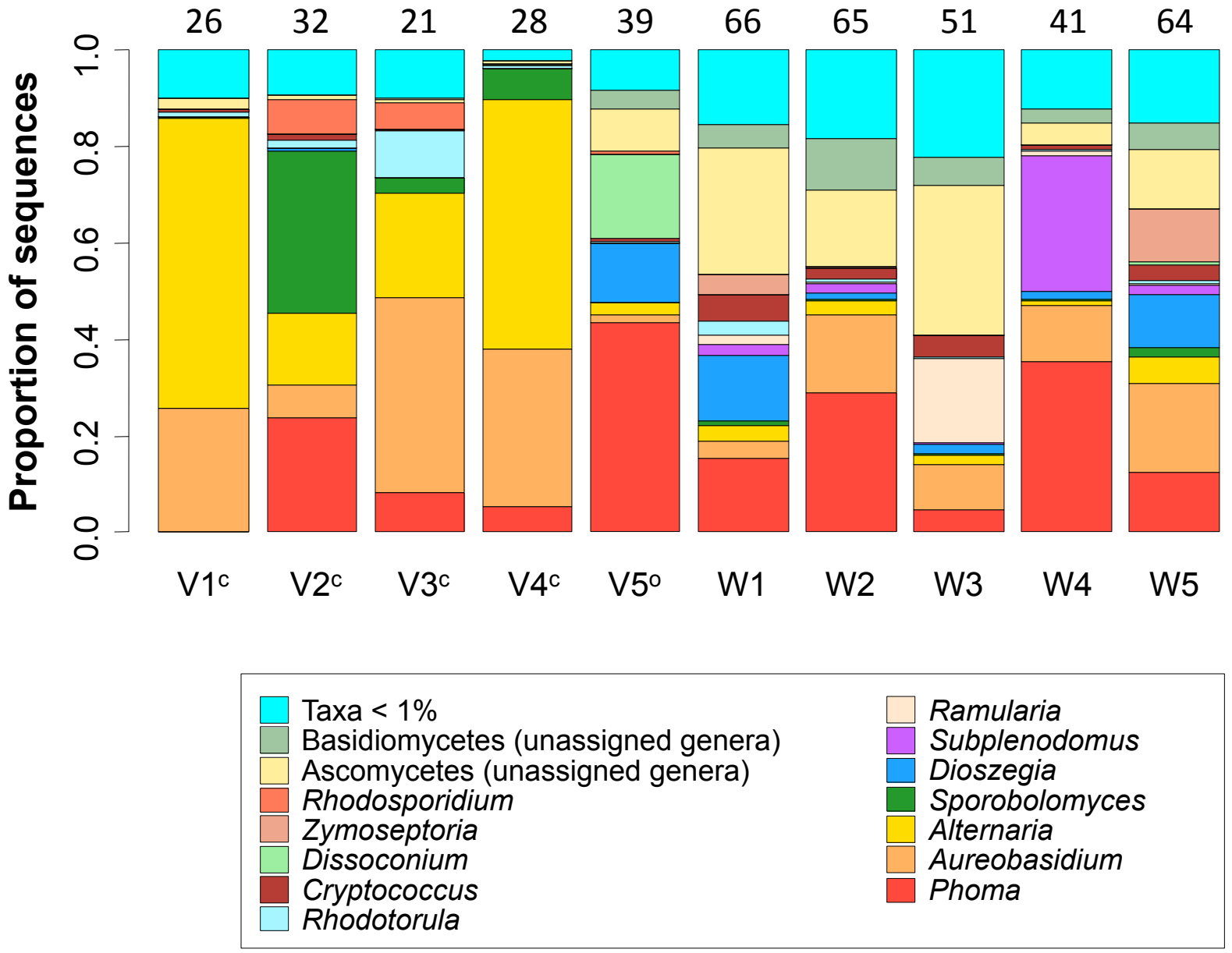
Figure 1a,b. Venn diagrams comparing the number of unique and shared OTUs in (a) all wild and vineyard samples, and (b) one representative wild sample, one representative conventional vineyard sample and the organic vineyard sample.

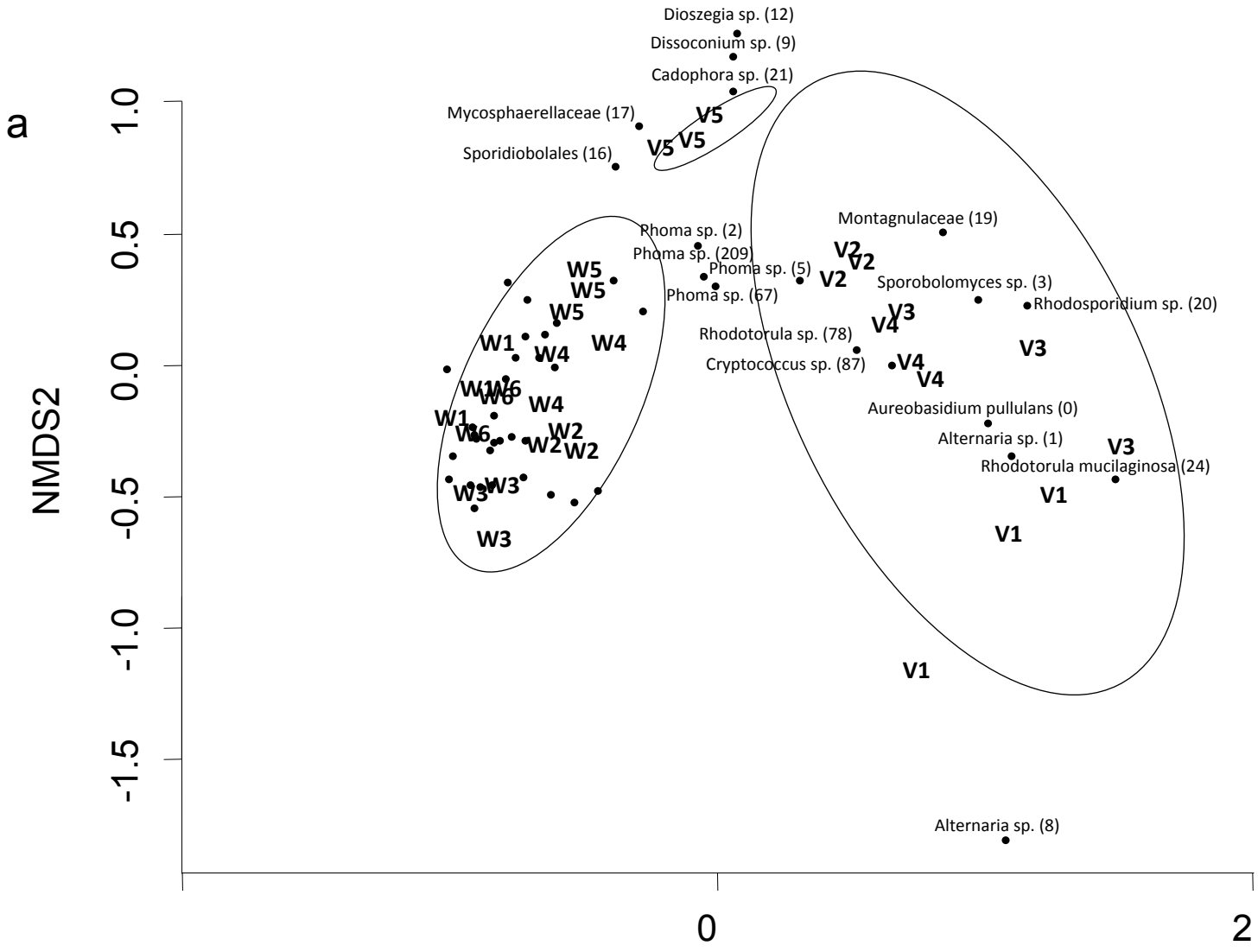
Figure 2. Proportions of sequences assigned to individual fungal genera, as well as rare (<1% of total sequences) and unassigned sequences for each vineyard (V) and wild (W) sample. Proportions within each bar represent and average of three subsamples from each location. Superscripts on vineyard sample labels indicate conventional (c) and organic (o) cultivation. Numbers at the top of each bar indicate the total number of OTUs detected in that sample.

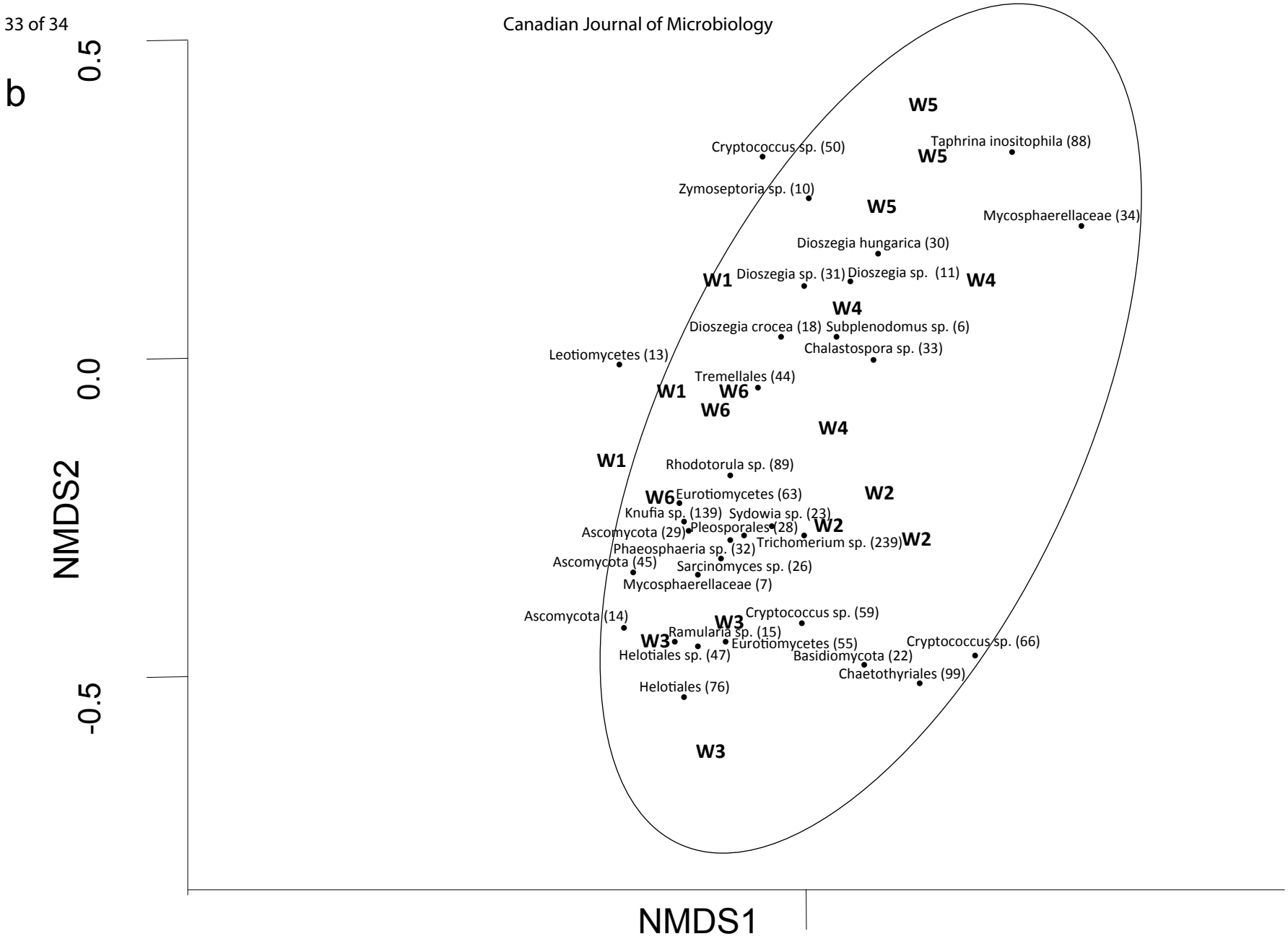
Figure 3a,b. Non-metric multidimensional scaling (nMDS) biplot of the 50 most common OTUs associated with all samples (a) and an enlargement of the region of the diagram showing only the wild collections for clarity (b). Sampling locations are represented by bold letters V1-V5 (vineyard) and W1-W6 (wild). The three sub-samples from each location are plotted separately. V1-V4 are conventional vineyards and V5 uses organic practices. Fungal endophyte OTUs are represented by labeled dots. Ellipses represent 95% confidence limits.

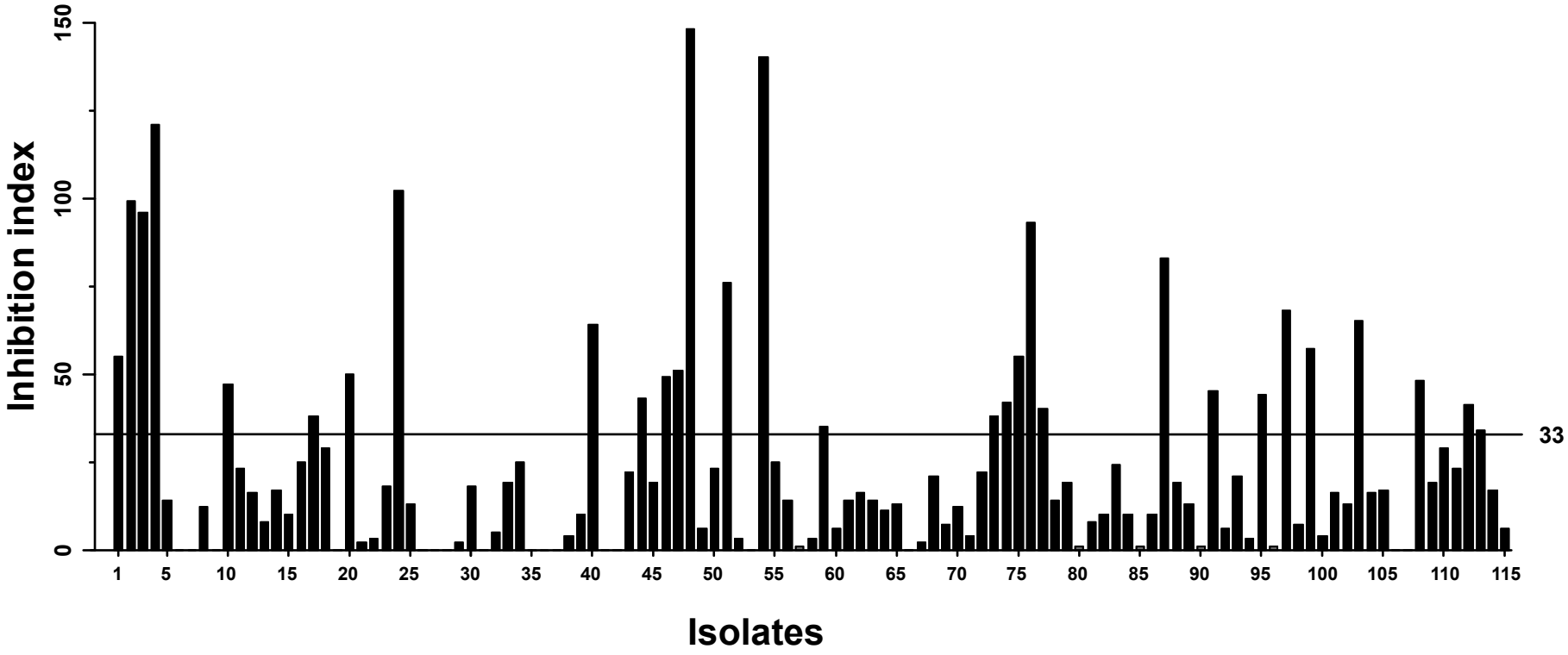
Figure 4. Inhibition of *Botrytis cinerea* by each endophyte isolate. Isolates with inhibition indices > 33 (horizontal line) were further processed and identified by sequencing.


a
89
110
vineyards
466
wild
b
152
wild
7
24
conv.
14
7
14
59
organic


26
32
21
28
39
66
65
51
41
64
73
Proportion of sequences
0.0
0.2
0.4
0.6
0.8
1.0
V1c
V2c
V3c
V4c
V5o
W1
W2
W3
W4
W5
W6
Taxa < 1%
Basidiomycetes (unassigned genera)
Ascomycetes (unassigned genera)
Rhodosporidium
Zymoseptoria
Dissoconium
Cryptococcus
Rhodotorula
Ramularia
Subplenodomus
Dioszegia
Sporobolomyces
Alternaria
Aureobasidium
Phoma


a
Dioszegia sp. (12)
Dissoconium sp. (9)
Cadophora sp. (21)
Mycosphaerellaceae (17)
Sporidiobolales (16)
Phoma sp. (2)
Phoma sp. (209)
Phoma sp. (5)
Phoma sp. (67)
Montagnulaceae (19)
Sporobolomyces sp. (3)
Rhodosporidium sp. (20)
Rhodotorula sp. (78)
Cryptococcus sp. (87)
Aureobasidium pullulans (0)
Alternaria sp. (1)
Rhodotorula mucilaginosa (24)
Alternaria sp. (8)
V1
V2
V3
V4
V5
W1
W2
W3
W4
W5
W6
NMDS1
NMDS2
1.0
0.5
0.0
-0.5
-1.0
-1.5
0
2


b
0.5
0.0
-0.5
NMDS2
NMDS1
-1
W5
Cryptococcus sp. (50)
Taphrina inositophila (88)
W5
Zymoseptoria sp. (10)
W5
Mycosphaerellaceae (34)
Dioszegia hungarica (30)
W1
Dioszegia sp. (31)
Dioszegia sp. (11)
W4
W4
Dioszegia crocea (18)
Subplenodomus sp. (6)
Chalastospora sp. (33)
Leotiomycetes (13)
Tremellales (44)
W1
W6
W6
W4
W1
Rhodotorula sp. (89)
W6
Eurotiomycetes (63)
W2
Knufia sp. (139)
Sydowia sp. (23)
Ascomycota (29)
Pleosporales (28)
W2
Phaeosphaeria sp. (32)
Trichomerium sp. (239)
W2
Ascomycota (45)
Sarcinomyces sp. (26)
Mycosphaerellaceae (7)
Ascomycota (14)
Cryptococcus sp. (59)
W3
Ramularia sp. (15)
W3
Eurotiomycetes (55)
Cryptococcus sp. (66)
Helotiales sp. (47)
Basidiomycota (22)
Chaetothyriales (99)
Helotiales (76)
W3


Inhibition index
150
100
50
0
33
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
Isolates