Phylogenomics of Plant-Associated Botryosphaeriaceae Species

Jadran F. Garcia¹, Daniel P. Lawrence², Abraham Morales-Cruz¹,²,³, Renaud Travadon², Andrea Minio¹, Rufina Hernandez-Martinez⁴, Philippe E. Rolshausen⁵, Kendra Baumgartner⁶ and Dario Cantu*¹

¹ Department of Viticulture and Enology, University of California, Davis, Davis, CA, United States, ² Department of Plant Pathology, University of California, Davis, Davis, CA, United States, ³ Department of Ecology and Evolutionary Biology, University of California, Irvine, Irvine, CA, United States, ⁴ Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico, ⁵ Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, United States, ⁶ Crops Pathology and Genetics Research Unit, United States Department of Agriculture – Agricultural Research Service, Davis, CA, United States

The Botryosphaeriaceae is a fungal family that includes many destructive vascular pathogens of woody plants (e.g., Botryosphaeria dieback of grape, Panicle blight of pistachio). Species in the genera Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia, Neofusicoccum, and Neoscytalidium attack a range of horticultural crops, but they vary in virulence and their abilities to infect their hosts via different infection courts (flowers, green shoots, woody twigs). Isolates of seventeen species, originating from symptomatic apricot, grape, pistachio, and walnut were tested for pathogenicity on grapevine wood after 4 months of incubation in potted plants in the greenhouse. Results revealed significant variation in virulence in terms of the length of the internal wood lesions caused by these seventeen species. Phylogenomic comparisons of the seventeen species of wood-colonizing fungi revealed clade-specific expansion of gene families representing putative virulence factors involved in toxin production and mobilization, wood degradation, and nutrient uptake. Statistical analyses of the evolution of the size of gene families revealed expansions of secondary metabolism and transporter gene families in Lasiodiplodia and of secreted cell wall degrading enzymes (CAZymes) in Botryosphaeria and Neofusicoccum genomes. In contrast, Diplodia, Dothiorella, and Neoscytalidium generally showed a contraction in the number of members of these gene families. Overall, species with expansions of gene families, such as secreted CAZymes, secondary metabolism, and transporters, were the most virulent (i.e., were associated with the largest lesions), based on our pathogenicity tests and published reports. This study represents the first comparative phylogenomic investigation into the evolution of possible virulence factors from diverse, cosmopolitan members of the Botryosphaeriaceae.

Keywords: grapevine trunk diseases, Botryosphaeria dieback, comparative genomics, gene family evolution, virulence factors, secondary metabolism, cell wall degrading enzymes
INTRODUCTION

The fungal family **Botryosphaeriaceae** (**Botryosphaeriales**, **Dothideomycetes**) was introduced in 1918 by Theissen and Sydow (1918) with **Botryosphaeria** as the type genus. Members of this group have been taxonomically characterized based on the production of large, ovoid to oblong, typically hyaline, aseptate ascospores, which may become brown and septate with age, within bitunicate asci within unilocular or multilocular botryose ascocoma known as pseudothecia (Sivanesan, 1984; Phillips et al., 2005). The asexual states of **Botryosphaeriaceae** exhibit a wide range of conidial morphologies that are taxonomically informative (Phillips et al., 2005). Crous et al. (2006) contributed to stabilize the taxonomy of the genera within the **Botryosphaeriaceae** by employing a natural unit classification scheme, which is also referred to as the “genus-for-genus concept” (Seifert et al., 2000). The distinct asexual morphs were linked to unique sexual morphs on a unit-by-unit basis, which was corroborated with phylogenetic analysis of 28S rDNA sequence data revealing 10 generic clades. The **Botryosphaeriaceae** is currently composed of 24 well-defined genera and more than 200 species (Burgess et al., 2019) that are cosmopolitan in distribution and exist primarily as saprobes, endophytes, or pathogens on a wide array of important perennial plant hosts (Slippers and Wingfield, 2007), in both human-altered (agricultural and urban) and natural ecosystems (forests and riparian areas) (Slippers et al., 2009; Lawrence et al., 2017).

The ecology of Botryosphaeraceous taxa is complex and not fully understood. For example, in spite of being a shoot blight and canker pathogen of pine, **Diplodia sapinea** has been isolated from the bark surface and internal woody tissues of woody twigs from asymptomatic **Pinus** (Petrini and Fisher, 1988), representing what some may consider an “endophytic phase,” in which neither the internal plant tissues from which it is isolated, nor other plant tissues/organs showed apparent symptoms, nor were there negative impacts to host growth at the time of isolation. A similar pattern in the ecology of other **Botryosphaeriaceae** species considered pathogenic, but later being isolated during an endophytic phase, has been documented (Slippers and Wingfield, 2007; Luo et al., 2019; Hrycan et al., 2020). In some cases, abiotic stress (water stress, stress heat, temperature) has been shown to induce severe symptoms in different host plants infected with seemingly innocuous **Botryosphaeriaceae** (Pusey, 1989; Mullen et al., 1991; Smith et al., 1994). This relationship between abiotic stress and more severe symptoms or more rapid colonization has also been reported for pathogenic species, e.g., **Neofusicoccum parvum** causing Botryosphaeria dieback of grape (Luque et al., 2010; Galarneau et al., 2019) and **Botryosphaeria dothidea** causing Pistachio panicle and shoot blight (Ma et al., 2001). Under climate-change scenarios of more frequent temperature extremes and prolonged drought, the interactions between host plants and Botryosphaeriaceae species may transit more readily from endophytic to pathogenic (Desprez-Loustau et al., 2006; Slippers et al., 2007). An increase in **Botryosphaeriaceae** symptom severity in conjunction with other biotic stresses has also been documented in the literature (Old et al., 1990; Lawrence et al., 2018).

Members of the **Botryosphaeriaceae** are probably most well-known as being destructive blight and canker pathogens of planted hosts (Luo et al., 2019). In agricultural settings, for example, they infect a large number of fruit and nut crops, such as almond (Inderbitzin et al., 2010; Gramaje et al., 2012; Nouri et al., 2018; Holland et al., 2020), apple (Phillips et al., 2012), avocado (McDonald et al., 2009), citrus (Linaldeddu et al., 2015), grapevine (Urbez-Torres, 2011), olive (Urbez-Torres et al., 2013), pistachio (Michailides, 1991; Nouri et al., 2019), and walnut (Chen S. et al., 2014). In forest plantations in Australia and South Africa, for example, they infect **Eucalyptus spp**. and **Pinus spp**. (Slippers et al., 2007). Infection is through either wounds to green and woody tissues or through natural openings in flowers, fruit, leaves, and shoots. The pathogens produce enzymes and/or toxins that kill cells and tissues of the various plant organs they attack. Infections of woody tissues of perennial hosts, either deep in the wood or just below the bark, can lead to stunted shoot growth, with eventual shoot death or “dieback.”

Ecological genomic comparisons of phytopathogenic and saprobic fungi suggest that the former possess expanded gene families that generally fall into two main functional categories: (1) lytic capabilities (Massonnet et al., 2018) and (2) putative transporters (Powell et al., 2008). Fungal lignin peroxidases, peroxidases, laccases, and polyphenol oxidases allow fungi to gain access to nutrients and to protect themselves from host defenses while growing in wood (Mayer, 2006; Martinková et al., 2016; Valette et al., 2017). Pathogenic species with the ability to enzymatically decompose a broader diversity of cell wall carbohydrates might be expected to more rapidly colonize, kill, and/or decompose host tissue. Membrane transporters of fungal plant pathogens also play important roles in exporting virulence factors involved in pathogenesis, influx of nutrients, and efflux of host-derived defense antimicrobial compounds (Denny and VanEtten, 1983; Denny et al., 1987). Previous genomic comparisons of phylogenetically diverse wood-infecting pathogens of grape revealed expansions in the repertoire of cell-wall degrading enzymes called carbohydrate-active enzyme (CAZyme) gene families, whose protein products are involved in the synthesis, degradation, and/or modification of glycosidic bonds of plant cell wall constituents, including the main components of wood, cellulose, hemicelluloses, lignin (Morales-Cruz et al., 2015), and significantly so in **Neof.** Further, a recent genomic annotation and in planta transcriptomic study of putative virulence factors of **Neof. parvum** during wood colonization revealed 567 protein-coding genes belonging to 52 different CAZyme families with glycoside hydrolases (GHs), which made up approximately 50% of the pathogen’s cell-wall degrading repertoire (Massonnet et al., 2018). Likewise, Yan et al. (2018) identified 820 CAZymes with at least 10 families that have experienced expansion in the genome of **Lasiodiplodia theobromae** with GHs representing the largest super family involved in the modification of plant cell wall carbohydrates. Genome comparisons of **B. dothidea**, **L. theobromae**, and **Neof. parvum** revealed that the genome of **L. theobromae**, the most virulent of the three species, is expanded in gene families associated with membrane transport, mainly ATP-binding cassette (ABC family), and major facilitator super (MFS) families.
(Yan et al., 2018). That same study reported 17 membrane transport genes that were significantly up-regulated upon host recognition including amino acid transporters and sugar porters. The largest transporter families reported in the genome of Neof. parvum include MFS, Peroxisomal Protein Importer (PPI) family, and the ABC superfamily (Massonnet et al., 2018). In this study we analyze the genome sequences of seventeen Botryosphaeriaceae species representing six genera (Botryosphaeria, Diplodia, Dothiorella, Lasiodiplodia, Neofusicoccum, and Neoscytalidium), which are wood-canker pathogens that attack horticultural crops, namely grape, pistachio, Prunus species (almond and stone fruits apricot, peach, and plum), and walnut. Our objective is to examine through phylogenomic comparisons this comprehensive set of species on one host, grape, to better understand the evolutionary trends within this important fungal family, especially as it pertains to the gene space involving pathogenesis of woody tissues and fungal virulence.

MATERIALS AND METHODS

Isolate Collection and Species Confirmation

All fungal isolates utilized in this study were obtained from internal wood cankers of symptomatic hosts following the protocol of Baumgartner et al. (2013) (Table 1). Total genomic DNA was extracted following Morales-Cruz et al. (2018). The internal transcribed spacer (ITS) and translation elongation factor (TEF) loci were amplified for each isolate via PCR using primers ITS5/ITS4 (White et al., 1990) and EF1-688F/EF1-1251R (Alves et al., 2008). TEF and ITS sequences of each species (including type specimen sequences downloaded from GenBank) were concatenated and aligned using MUSCLE v3.8.31 (Edgar, 2004) with default parameters. The alignment was cleaned with GBLOCKS v. 0.91b (Castresana, 2000) with a minimum block's length of 5 bp and half of the gaps allowed. PhyML (Guindon et al., 2010) was used to calculate the maximum likelihood tree using 100 bootstrap replications, HKY85 substitution model and the subtree-pruning-regrafting method for searching for optimal tree topology. The resulting tree was visualized and edited for presentation using FigTree v1.4.1 (Rambaut, 2012).

Sequencing and Genomes Assemblies

DNA extraction was done following the methods used by Morales-Cruz et al. (2015) using the axenic cultures of the isolated fungi and a CTAB protocol. Sequencing libraries were prepared and sequenced as described in Morales-Cruz et al. (2015). After adapter ligation, libraries were size selected to 550–600 bp using a double-sided size selection with Ampure XP magnetic beads (Beckman Coulter, United States) to remove unused adapter and adapter dimer. Sequencing was carried out on an Illumina HiSeq4000 machine at the DNA Technologies Core at UC Davis. Paired-end reads of 150 bp in length were generated. Raw reads were trimmed for quality (Q > 30) and adapter removal using Trimmomatic v0.36 (Bolger et al., 2014) with options LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:100. Assembly of high-quality reads was made using SPAdes v3.9 (Bankevich et al., 2012) with the careful option and automatic read coverage cutoff. Assembly completeness was assessed using the Core Eukaryotic Genes Mapping Approach (CEGMA v.2.5; Parra et al., 2007) and Benchmarking Universal Single-Copy Orthologs (BUSCO v.1.1; Simão et al., 2015) analysis. RepeatMasker v.4.06 (Smit et al., 1996-2015) with default parameters was used to mask repeats. Gene model prediction was performed with Augustus v.3.2.1 (Stanke et al., 2006) with default parameters and using Neof. parvum gene model as training set. Sequencing data are available at NCBI (BioProject PRJNA673527). Sequencing data of Diplodia seriata (Morales-Cruz et al., 2015) and Neof. parvum (Massonnet et al., 2018) can be retrieved from NCBI under BioProject PRJNA261773 and PRJNA331421, respectively. All genome assemblies and gene models are publicly available at Zenodo (doi: 10.5281/zenodo.4417445).

Functional Annotation

The general annotation of the predicted proteins was assigned based on the similarities with peptides in the GenBank with Blast2GO (Conesa et al., 2005), and to conserved domains in Pfam database (Finn et al., 2016). The functional annotation (Supplementary Table 3) was assigned based on the databases and parameters presented in Supplementary Table 4. CAZymes were annotated with the dbCAN2 (Zhang et al., 2018). The signal peptides were predicted using SignalP 5.0 (Armenteros et al., 2019). The proteins with annotation in both databases were annotated as secreted CAZymes. Secondary metabolites clusters were annotated using antiSMASH 5.0 (Blin et al., 2019). Peroxidases were annotated using a specialized database for fungi called fPoxDB (Choi et al., 2014). CYPED 6.0 was used to annotate the Cytochrome P450 proteins (Fischer et al., 2007). At last, the proteins involved in transportation functions were annotated using the TCDB (Saier et al., 2006, 2016).

Construction of a Clock-Calibrated Phylogenetic Tree

Seventy-three single copy peptides used in Floudas et al. (2012) for fungal phylogeny reconstruction were extracted from the reference strain Saccharomyces cerevisiae 2588C Genome Release 64-2-1 (downloaded from http://www.yeastgenome.org). All these peptides were compared using BLASTP (v.2.6.0+) against the seventeen Botryosphaeriaceae species and two wood-decay basidiomycetes that colonize grape: pathogenic, wood-rotting fungus (with characteristics of both white-rot and brown-rot fungi) Fomitiporia mediterranea and saprobic, white-rot fungus Stereum hirsutum. Fomitiporia mediterranea is one of a complex of pathogens that causes the grapevine trunk disease Esca in Europe, whereas the pathogenicity of S. hirsutum to grape is not known (Fischer, 2006). Twenty-one proteins had exactly one top hit in all the species. The rest of the seventy-three initial proteins were excluded because they were either not present in all the species or had paralogs. Each set of orthologous proteins was aligned using MUSCLE v3.8.31 (Edgar, 2004). Alignments were concatenated and cleaned using GBLOCKS v.
TABLE 1 | Genome assembly summary statistics of the Botryosphaeriaceae species analyzed.

| Species (isolate ID)                      | Isolated from | Assembly size (Mbp) | N. scaffold | N50 (kbp) | L50 (Scaffold #) | CEGMA*** | BUSCO*** |
|------------------------------------------|---------------|---------------------|-------------|-----------|------------------|-----------|----------|
| Botryosphaeria dothidea (0053)           | Grape         | 46                  | 2,425       | 506       | 28               | 99%       | 98%      |
| Diplodia mutila (SBen820)                | Grape         | 46                  | 4,003       | 175       | 67               | 98%       | 98%      |
| Diplodia seriata (DS831)*               | Grape         | 37                  | 811         | 301       | 39               | 98%       | 98%      |
| Dothiorella iberca (Wolf933)             | Apricot       | 37                  | 636         | 412       | 28               | 97%       | 98%      |
| Dothiorella sarmentorum (SBen806)        | Apricot       | 42                  | 1,664       | 242       | 57               | 97%       | 98%      |
| Dothiorella viticola (Wint804)           | Grape         | 37                  | 2,477       | 482       | 25               | 98%       | 98%      |
| Lasiodiplodia citicola (08I-35)          | Walnut        | 44                  | 216         | 1,067     | 13               | 97%       | 98%      |
| Lasiodiplodia exigua (UCR-LTs)           | Grape         | 44                  | 340         | 720       | 15               | 98%       | 98%      |
| Lasiodiplodia missouriana (09-C092)      | Grape         | 44                  | 324         | 883       | 17               | 98%       | 98%      |
| Lasiodiplodia theobromae (MXSCL28)       | Grape         | 44                  | 193         | 883       | 16               | 99%       | 98%      |
| Neofusicoccum australe (UCR-NA2)         | Grape         | 42                  | 2,218       | 385       | 35               | 99%       | 98%      |
| Neofusicoccum hellicenium (02-K91)       | Pistachio     | 43                  | 221         | 768       | 19               | 98%       | 98%      |
| Neofusicoccum mediterraneum (Wint817)    | Grape         | 42                  | 196         | 551       | 24               | 99%       | 98%      |
| Neofusicoccum nonquasitum (05-A04)       | Walnut        | 43                  | 316         | 667       | 19               | 99%       | 98%      |
| Neofusicoccum parvum (UCD646So)**        | Grape         | 43                  | 2,452       | 168       | 74               | 99%       | 98%      |
| Neofusicoccum vitisiforme (05-H02)       | Walnut        | 42                  | 288         | 506       | 24               | 98%       | 98%      |
| Neoscytalidium dimidiatum (UCR-Neo1)     | Grape         | 42                  | 478         | 419       | 31               | 100%      | 98%      |

*Published in Morales-Cruz et al., 2015.
**Published in Massonnet et al., 2018.
***Percentage of complete CEGMA and BUSCO peptides found in the genome assembly.

0.91b (Castresana, 2000; maximum number of contiguous non-conserved positions = 4, minimum length of a block = 10), reducing the initial 21,008 positions to 12,066 informative positions. Clean alignments were imported into BEAUti v1.10.4 to prepare them for BEAST v1.10.4 analysis (Bouckaert et al., 2014). Monophyletic partitions were set for *Ascomycota*, *Basidiomycota*, and *Dothideomycetes* species. Calibrations points were set to 588 and 350 mya on *Ascomycota* and *Dothideomycetes* partition, respectively, according to Beimforde et al. (2014). Six MCMC chains of 1,000,000 steps were launched on BEAST (WAG substitution model, 4 Gamma Categories + Invariant sites, Lognormal relaxed clock, Calibrated Yule Model). The resulting trees were concatenated with LogCombiner v1.10.4 (Bouckaert et al., 2014) and a consensus tree was obtained from TreeAnnotator v1.10.4 (Bouckaert et al., 2014; Supplementary Figure 1). FigTree v1.4.1 (Rambaut, 2012) and Inkscape v1.0.1 (Inkscape Project, 2020) were used to edit the tree for figure presentation.

**Phylogenetic Principal Component Analysis**

The phyl.pca function of the phytools R package (Revell, 2012) was used to create the phylogenetic PCAs. The inputs for this function were the clock calibrated tree and protein matrices of CAZymes and secondary metabolite clusters.

**Pathogenicity Tests**

The pathogenicity of 17 species of *Botryosphaeriaceae* was evaluated on potted grapevines (*Vitis vinifera* 'Pinot noir' clone 777) in replicate experiments in the greenhouse (18 treatments × 10 replicate plants per treatment × 2 experiments = 360 total plants). Hardwood cuttings obtained from a commercial nursery were propagated in May/June 2016 following the protocol of Travadon et al. (2013). Inoculations took place after callusing and before planting in pots in the greenhouses. For inoculations, the commonly used agar plug method was employed (e.g., Chacón et al., 2020). This method has been shown to produce lower variability in lesion length than the method with mycelial suspensions for the pathogens *Neof. parvum* and *Di. seriata* (Guan et al., 2016). For inoculations, a power drill was used to wound (5 mm wide × 3 mm deep) the cutting, approximately 2 cm below the apical node. A 5-mm agar plug from a 7-day culture on PDA was aseptically inserted into the wound and sealed with Vaseline and parafilm to prevent inoculum desiccation. Cuttings were coated in melted paraffin wax (Gulf Wax; Royal Oak Enterprises, LLC, Roswell, GA, United States) and potted in a sterile potting mix amended with slow-release fertilizer (Osmocote® Pro 24-4-9, Scotts, Marysville, OH, United States). The plants were watered twice per week for 16 weeks. Ten plants were used for each isolate and ten plants were mock-inoculated with sterile PDA. Plants were

---

**Computational Analysis of Gene Family Evolution (CAFE)**

BLASTP (e-value < 10⁻⁵) was used to group proteins in families based on sequence similarity followed by Markov clustering with MCL (Van Dongen, 2000; Enright et al., 2002). The 10,158 families with at least one protein in no less than four species were used with the clock calibrated tree as input for the CAFE v4.2.1 (De Bie et al., 2006) analysis. CAFE was run in the default mode with the option -s to optimize the lambda parameter to 0.00155948837239, and a P-value threshold of 0.01 (option -p). To evaluate significant expansions or contractions of a specific branch, Viterbi P-values were calculated for each significant family.
arranged in a completely randomized design in two separate greenhouses at the University of California Experiment Station in Davis from June 2016 to October 2016 [natural sunlight photoperiod, 25 ± 1 C (day), 18 ± 3 C (night)]. The second experiment was initiated 1 week after the first experiment. The length of internal wood discoloration extending out from the inoculation site up and down the stem (lesion length) was measured approximately 16 weeks after inoculation in October 2016. First, plants were inspected for foliar symptoms. Then the newly developed green shoots, roots, and bark of each plant were removed and discarded, and the woody stems were surface sterilized in 1% sodium hypochlorite for 2 min and rinsed with deionized water. The length of each stem was recorded and cut longitudinally to expose wood discoloration, the length of which was measured with a digital caliper. From each plant, ten small wood pieces were cut from the margin of the lesion with flame-sterilized scalpels and plated onto PDA in an attempt to recover the inoculated fungi. Inoculated pathogens, identified based on colony morphology, were re-isolated from all samples.

Lesion lengths were used as a measure of pathogenicity. Normality and homogeneity of variances were evaluated using Shapiro–Wilk’s and Levene’s tests, respectively. ANOVA was used to determine whether there were differences in lesion length among treatments. ANOVA was performed in R using the lesion size as a function of the inoculation treatment and the experiment. Means were compared for significant effects (P < 0.05) by Tukey’s HSD post hoc test.

RESULTS

Genome Assembly, Gene Prediction, and Virulence Factor-Focused Functional Annotation

To expand the genomic information for Botryosphaeriaceae, we de novo assembled the genomes of fifteen species isolated from multiple hosts. We included the previously published genomes of Neof. parvum and Di. seriata in the comparative genomics analysis (Table 1 and Supplementary Table 1). For all seventeen species, pathogenicity was evaluated using inoculations of potted grapevines (Figure 1). All seventeen species produced dark necrotic lesions in the woody stems extending upward and downward from the point of inoculation at 15 weeks post inoculation. Overall, Lasiidiplodia and Neofusicoccum spp. were the most aggressive, while Diplodia and Dothiorella spp. caused the smallest lesions.

All genomes were sequenced using illumina technology at coverage 171 ± 10× (Supplementary Table 1). On average, sequencing illumina reads were assembled into 1,066 ± 308 scaffolds (N50 length: 577.64 ± 64.53 kbp; L50 scaffold count: 27.9 ± 4 scaffolds). The total genome size assembly varied from 37 Mbp for Dothiorella viticola to 46 Mbp for B. dothidea with an average of 42.37 ± 0.69 Mbp. The expected and assembled genome size had a discrepancy of less than ten percent on average (4.4 ± 2.2 Mbp), which suggests near completeness of the assemblies (Supplementary Table 1). This result was confirmed by CEGMA (Parra et al., 2007) and BUSCOs (Simão et al., 2015) analyses, which reported an average 98.3 ± 0.2% and 98.1 ± 0.1% completeness, respectively (Table 1). Interspersed repeats only accounted for 1.87 ± 0.003% of the genome assemblies. Among the classified elements, long-terminal-repeats (LTR) were the most abundant, ranging from a total of 315 kbp in B. dothidea to 26 kbp in Neoscytalidiumdimidiatum (Supplementary Table 2).

The predicted protein-coding genes in the seventeen genomes varied from 10,827 in Dothiorella iberica to 13,492 in Dothiorella sarmentorum. On average 12,193 ± 193 CDS were found per species (Table 2).

The predicted genes of the seventeen genomes were annotated using general databases for protein domains (Pfam), gene ontology (GO), as well as more specialized databases related to putative virulence factors. The last group included carbohydrate-active enzymes (CAZymes), cytochrome P450s, peroxidases, usually associated with host colonization and wood degradation, and secondary metabolism gene clusters, including toxins production, and cellular transporters (Supplementary Table 4 and Table 3). A total of 229,251 predicted protein-coding genes were annotated (Table 3).

Carbohydrate-Active Enzymes Are Especially Abundant in the Genomes of Neofusicoccum Species

A wide variety of monosaccharides can be linked to many different types of molecules (proteins, lipids, nucleic acids and, sugar themselves) and converting these glycoconjugates into one of the most structurally diverse substrates (Cantarel et al., 2009). CAZymes are the group of enzymes responsible for the assembly and breakdown of these diverse substrates (Lombard et al., 2014). Not all CAZymes contribute to the pathogenicity of the microorganisms, however, predicting them
TABLE 2 | Gene model predictions statistics of the Botryosphaeriaceae species analyzed.

| Species              | Repeat content (bp) | N. CDS | Mean protein size (AA) | BUSCO* | Mean gene density (genes/10 kbp) | SD |
|----------------------|---------------------|--------|------------------------|--------|-------------------------------|----|
| Botryosphaeria dothidea | 2,608,021 (5.67%)  | 12,424 | 478  | 98% | 2.14 | 1.9 |
| Diplodia mutila      | 2,877,891 (6.28%)  | 11,947 | 491  | 98% | 1.74 | 1.9 |
| Di. seriata          | 1,144,284 (2.05%)  | 11,085 | 493  | 98% | 2.93 | 1.8 |
| Dothiorella iberica  | 1,350,049 (3.69%)  | 10,827 | 491  | 98% | 3.02 | 1.8 |
| Do. sarmentorum      | 1,687,353 (4.02%)  | 13,942 | 474  | 98% | 2.90 | 2.5 |
| Do. viticola         | 950,252 (2.60%)    | 11,235 | 473  | 98% | 2.19 | 1.9 |
| Lasiodiplodia citricola | 825,529 (1.89%)  | 12,376 | 492  | 98% | 3.19 | 1.4 |
| L. exigua            | 771,071 (1.77%)    | 12,399 | 492  | 98% | 3.12 | 1.5 |
| L. missouriana       | 833,148 (1.89%)    | 12,448 | 494  | 98% | 3.12 | 1.5 |
| L. theobromae        | 820,166 (1.88%)    | 12,434 | 493  | 98% | 3.20 | 1.4 |
| Neofusicoccum australe | 2,055,816 (4.92%) | 12,104 | 488  | 98% | 2.29 | 1.9 |
| Neof. hellenicum     | 1,078,449 (2.53%)  | 12,433 | 486  | 98% | 3.27 | 1.4 |
| Neof. mediterraneum  | 960,302 (2.26%)    | 12,541 | 484  | 98% | 3.36 | 1.4 |
| Neof. nonquaesitum   | 1,010,201 (2.35%)  | 12,602 | 487  | 98% | 3.24 | 1.5 |
| Neof. parvum         | 2,170,098 (5.00%)  | 13,942 | 474  | 98% | 2.90 | 2.5 |
| Neof. vitifusiforme  | 1,405,151 (3.37%)  | 12,112 | 485  | 98% | 3.22 | 1.5 |
| Neoscytalidium dimidiatum | 1,099,801 (2.61%) | 11,067 | 478  | 98% | 3.00 | 1.5 |

*Percentage of complete BUSCO peptides found in the predicted proteome.

TABLE 3 | Number of protein coding genes annotated per functional category.

| Species              | Total genes | Annotated genes | Secondary metabolites involved genes | P450s CAZymes | Secreted CAZymes | Peroxidases | Signal Peptides | Transporters | % Secreted CAZymes of total secreted |
|----------------------|-------------|-----------------|-------------------------------------|---------------|-----------------|-------------|-----------------|-------------|-------------------------------------|
| Botryosphaeria dothidea | 12,424      | 11,877          | 809                                 | 857           | 485             | 280         | 56              | 1,341       | 2,505                                |
| Diplodia mutila      | 11,947      | 11,240          | 407                                 | 675           | 433             | 242         | 55              | 1,198       | 2,362                                |
| Di. seriata          | 11,085      | 10,535          | 300                                 | 620           | 432             | 251         | 53              | 1,114       | 2,238                                |
| Dothiorella iberica  | 10,827      | 10,237          | 374                                 | 609           | 399             | 229         | 41              | 1,029       | 2,185                                |
| Do. sarmentorum      | 13,942      | 13,226          | 457                                 | 652           | 487             | 259         | 50              | 1,596       | 3,143                                |
| Do. viticola         | 11,235      | 10,663          | 283                                 | 594           | 395             | 218         | 46              | 1,053       | 2,339                                |
| Fomitiporia mediterranea | 11,338     | 11,338          | 205                                 | 639           | 315             | 178         | 52              | 780         | 1,965                                |
| Lasiodiplodia citricola | 12,376      | 11,831          | 564                                 | 785           | 465             | 266         | 54              | 1,298       | 2,492                                |
| L. exigua            | 12,399      | 11,838          | 578                                 | 785           | 474             | 271         | 55              | 1,287       | 2,499                                |
| L. missouriana       | 12,448      | 11,902          | 572                                 | 787           | 470             | 267         | 54              | 1,305       | 2,507                                |
| L. theobromae        | 12,434      | 11,822          | 610                                 | 784           | 465             | 270         | 54              | 1,279       | 2,490                                |
| Neofusicoccum australis | 12,104      | 11,709          | 654                                 | 800           | 488             | 291         | 61              | 1,334       | 2,530                                |
| Neof. hellenicum     | 12,433      | 12,015          | 662                                 | 868           | 501             | 281         | 62              | 1,369       | 2,546                                |
| Neof. mediterraneum  | 12,122      | 12,052          | 658                                 | 843           | 504             | 288         | 61              | 1,372       | 2,549                                |
| Neof. nonquaesitum   | 12,602      | 12,229          | 661                                 | 918           | 514             | 296         | 60              | 1,394       | 2,604                                |
| Neof. parvum         | 12,679      | 12,328          | 639                                 | 908           | 504             | 298         | 59              | 1,393       | 2,588                                |
| Neof. vitifusiforme  | 12,112      | 11,715          | 575                                 | 817           | 501             | 289         | 62              | 1,322       | 2,492                                |
| Neoscytalidium dimidiatum | 11,067      | 10,701          | 489                                 | 746           | 452             | 247         | 48              | 1,139       | 2,357                                |
| Saccharomyces cerevisiae | 5,917       | 5,917           | 48                                  | 215           | 136             | 44          | 21              | 306         | 1,478                                |
| Stereum hirsutum     | 14,066      | 14,066          | 426                                 | 652           | 394             | 230         | 52              | 1,070       | 2,150                                |

in conjunction with signal peptides is widely used to obtain information about plant pathogen cell wall degrading enzymes (Floudas et al., 2012; Suzuki et al., 2012; Blanco-Ulate et al., 2014; Jones et al., 2014; Morales-Cruz et al., 2015). An average of 20.9 ± 0.3% of the predicted secreted proteins among all seventeen Botryosphaeriaceae genomes shared similarity with the CAZymes in the dbCAN2 database (Zhang et al., 2018). Glycoside Hydrolases (GH) and Auxiliary Activity CAZymes (AA) were the two groups with the most predicted proteins. GHs were especially abundant in Neofusicoccum spp. with an average of 336 ± 4 proteins compared to 303 ± 7 for the rest of the Botryosphaeriaceae species in this study (Supplementary...
Table 5). A total of 15 putative genes of GH3 were present in Neofusicoccum nonquaeatum and Lasiodiplodia missourianna, as well as 14 in Neof. parvum, Lasiodiplodia citriola, and Lasiodiplodia exigua. GH3 and GH43 families activities include β-glucosidases, β-xyllosidases, glucanases, L-arabinofuranosidase, galactanase and others related to the hydrolysis of plant cell wall components into more simple sugars (Faure, 2002; Polizeli et al., 2005; Cairns and Esen, 2010; Knob et al., 2010; Sampredo et al., 2017).

Auxiliary activities (AAs) were also more abundant in Neofusicoccum (127 ± 3) than the rest of the Botryosphaeriaceae species (100 ± 4). The AA3 family was the most abundant with numerous copies in the genus Neofusicoccum (Figure 2), ranging from 21 to 26 predicted proteins in Neof. parvum (UCD64650). The genome of B. dothidea was predicted to possess 23 AA3 proteins. The AA3 and AA9 families include cellobiose dehydrogenases, alcohol oxidases, pyranose oxidase, acting over more complex substrates of the plant cell wall like cellulose and/or lignin (Daniel et al., 1994; Henriksson et al., 2000; Harreither et al., 2011; Hernández-Ortega et al., 2012).

Neofusicoccum, Botryosphaeria, and Lasiodiplodia Species Encode the Largest Number of Predicted P450s

Cytochrome P450 enzyme evolution is thought to contribute to the adaptation of organisms to new ecological niches. The functions may vary from primary metabolism, detoxification of xenobiotic compounds, to producing a vast variety of secondary metabolites (Črešnar and Petrič, 2011; Moktali et al., 2012; Chen W. et al., 2014). These features sometimes play essential roles in pathogenesis (Črešnar and Petrič, 2011; Moktali et al., 2012). The P450s were classified in superfamilies as described by Fischer et al. (2007). Neofusicoccum, Botryosphaeria, and Lasiodiplodia species encoded a larger number of predicted P450 (859 ± 20, 857, and 785 ± 1, respectively) compared to Diplodia and Dothiorella species (648 ± 28 and 618 ± 17, respectively). Neof. nonquaeatum and Neof. parvum showed the highest number of predicted P450s genes with 918 and 908, respectively (Table 3 and Supplementary Table 6). CYP53, CYP51, and CYP504 were the most abundant across all the species. CYP53 was especially numerous in Neofusicoccum (137 ± 4 genes), Lasiodiplodia (128 ± 1 gene), and Botryosphaeria (119 ± 4 genes) species compared to the other genera (93 ± 8 genes). On the other hand, the CYP51 was very consistent in the Botryosphaeriaceae family (from 93 to 105 genes). Other superfamilies like CYP706, CYP102, and CYP3 show the same pattern of higher representation in Neofusicoccum, Lasiodiplodia, and Botryosphaeria than in Diplodia and Dothiorella species.

Peroxidases Are Most Abundant in the Genomes of Neofusicoccum Species

Fungal peroxidases are oxidoreductases that catalyze the oxidation of various compounds ranging from ligninolysis to the detoxification of host-derived reactive oxygen species and have been shown to contribute to virulence (Molina and Kahmann, 2007; Guo et al., 2010; Choi et al., 2014). The annotation of these peroxidases was based on the manually curated Fungal Peroxidases Database fPoxDB (Choi et al., 2014). Neofusicoccum species encoded the largest number of predicted peroxidases (61 ± 0), followed by Lasiodiplodia, Botryosphaeria, and Diplodia with an average of 54 ± 1 annotated genes (Table 3). Dothiorella, with only 46 ± 3 was the genus with the least number of annotated peroxidases in the Botryosphaeriaceae (Supplementary Table 7). Hybrid Ascorbate-Cytochrome C peroxidases were more abundant in Neofusicoccum, Botryosphaeria, and Lasiodiplodia, ranging from 8 to 11 genes, while haloperoxidases were more abundant in the genus Neofusicoccum (11 ± 0 genes).

The Genomes of Botryosphaeria, Neofusicoccum, and Lasiodiplodia Species Have the Largest Number of Secondary Metabolism Gene Clusters

Secondary metabolites play important roles in fungal development and interactions with other organisms, including plant hosts (Keller, 2019). Phytotoxic metabolites, e.g., melleins, produced by Neof. parvum both in vitro and in the wood of symptomatic grape are thought to be associated with pathogenesis (Abou-Mansour et al., 2015). In fungi, the genes encoding the functions responsible for the biosynthesis of secondary metabolites are physically grouped in clusters of contiguous genes (Brakhage, 2013; Keller, 2019), which typically comprise a central biosynthetic gene as well as genes involved in post-synthesis modification of the metabolites and cellular transport.

Using antiSMASH 5 (Blin et al., 2019), we detected an average of 43 ± 3 biosynthetic gene clusters (BGCs) in the seventeen Botryosphaeriaceae. The Type I Polyketide synthase cluster (TIPKS) and the Non-ribosomal peptide synthetase-like fragment (NRPS-like) together accounted for 47% of all annotated BGCs. BGCs were most abundant in Botryosphaeria, Neofusicoccum, and Lasiodiplodia species with an average of 57 ± 8, 56 ± 1, and 49 ± 1 BGCs, respectively. In these genera, we also found the larger number of genes per BGC (11 ± 1, 12 ± 0, and 12 ± 0, respectively; Supplementary Table 9). In Neofusicoccum spp., 169 ± 7 genes were associated with TIPKS, 154 ± 12 in Lasiodiplodia and 175 ± 19 in Botryosphaeria (Figure 2). For these secondary metabolites as well as classes, we found fewer genes in the genomes of Diplodia and Dothiorella species (Supplementary Table 10).

Toxins and other secondary metabolites are exported by cellular transporters (Del Sorbo et al., 2000). Homologies with the Transporter Classification Database (TCDB; Saier et al., 2006) were used to annotate hypothetical protein transporters. Overall, the Electrochemical Potential-driven Transporters was the most prominent group across all the species representing 31 ± 1% of the annotated transporters followed by the Primary Active Transporters (19%) and the Incompletely Characterized Transport Systems (19%). More specifically, The Major Facilitator Superfamily (MFS) (TCDB code 2.A.1) represented the highest number in all the species but was especially abundant.
FIGURE 2 | Number of protein-coding genes annotated as P450s, secondary metabolism, and secreted CAZymes. The heatmap includes only the annotations with the highest number of genes across all genomes.

in *Neofusicoccum*, *Lasiodiplodia*, and *Botryosphaeria* from 455 to 514 predicted genes (Supplementary Table 8). The genome of *Dothiorella sarmentorum* encodes a higher number of genes in the ATP-binding Cassette (ABC; 134 genes) superfamily compared to the other fungi analyzed (59 ± 1 genes). Both MFS and ABC transporters can be involved in toxin secretion and defense responses (Del Sorbo et al., 2000; Perlin et al., 2014).

**Estimation of Gene Family Expansion and Contraction and Evaluation of Functional Enrichment**

We further evaluated the differences in putative virulence factors to identify gene families that have significantly expanded or contracted in specific lineages by statistical analysis of the evolution of the size of gene families using Computational Analysis of gene Family Evolution (CAFE; De Bie et al., 2006). CAFE estimates the global birth and death rate of gene families and identifies those families that have an accelerated rate of gain or loss (Hahn et al., 2005; De Bie et al., 2006). CAFE uses a clock-calibrated phylogenetic tree and gene family sizes in all the species’ genomes as input. We included *F. mediterranea* and *St. hirsutum*, two well-known wood decay basidiomycetes related to the white-rot symptom in Esca disease of grapevines, and *Sa. cerevisiae*. These additional species were used as calibration points for the estimated dates of monophyletic partition of *Ascomycota* (588 mya) and *Dothideomycetes* (350 mya) as in Floudas et al. (2012) and Beimforde et al. (2014). To construct the phylogenetic tree, we identified twenty-one single-copy protein sequences that were previously used to study phylogenetic relationships across fungi (Floudas et al., 2012). The phylogenetic tree was built using a multiple alignment comprising 12,066 amino acid positions. The topology of the clock-calibrated tree was confirmed independently (Supplementary Figure 2) using ITS (Internal Transcribed Spacer) and TEF (Translation Elongation Factor), and was consistent with published ones (Phillips et al., 2008; Chen S. et al., 2014; Thambugala et al., 2014).

The gene families were computed using a Markov Cluster algorithm (MCL) that groups putative orthologs and paralogs (Enright et al., 2002). In total, 237,976 proteins of the 20 fungal genomes were clustered into gene families ($e$-value < $1e^{-6}$). These family sizes and the clock calibrated tree produced by
BEAST allowed CAFE to detect 666 families (35,498 genes) across all the species with a significantly higher than expected rate of gene gain/loss ($P \leq 0.01$). The numbers of gene families expanded and contracted for each branch of the phylogeny are shown in Figure 3. The parent branches of the Neofusicoccum, Lasiodiplodia, and Botryosphaeria clades show a positive rate of gene gain/losses (+0.45, +0.14, and +0.44, respectively), which suggest an expansion of some set of proteins. On the other hand, the parent branches of Diplodia, Dothiorella, and Basidiomycetes (Stereum and Fomitiporia) clades present a negative rate of gain losses (−0.40, −0.49, and −0.70, respectively). Saccharomyces cerevisiae, as seen in previous studies (Morales-Cruz et al., 2015), showed the lowest rate with −1.63.

The 35,498 genes in the significantly expanded or contracted families were analyzed with a Fisher’s Exact test to identify functional enrichments within those families. We found that families were analyzed with a Fisher’s Exact test to identify specifically abundant in L. missouriana pattern. The expanded secondary metabolite proteins were shown in Figure 3, as seen in previous studies (Morales-Cruz et al., 2015), and the PCA was carried out using the Phyl.PCA (Revell, 2009). At the same time, we observe a close cluster of Neofusicoccum species which are separated from the other groups mostly by the abundance of AA1, AA3, and GH5. In addition, the genus Lasiodiplodia is tightly clustered together with B. dothidea. This is driven by the abundance of AA9, GH28, and GH3, with the last family being more abundant in Lasiodiplodia species. The close clustering of Neofusicoccum, Botryosphaeria, and Lasiodiplodia is driven mostly by their similar profile of GH16 and AA3. Neoscytalidium dimidiatum is well separated from the rest of the species by the higher presence of GH76 and PL3 proteins.

The PCA on secondary metabolite genes shows a similar separation of the most virulent genera from the others (Figure 5). Lasiodiplodia species are grouped together by similarly high profiles of T1PKS, Beta-lactone and T1PKS/NRPS clusters. Neofusicoccum species are grouped due to high numbers of terpene synthases and NRPS-like clusters. Botryosphaeria dothidea is separated because of its high abundance of NRPS, T1PKS, Terpenes, Beta-lactone, and NRPS-like clusters.

**DISCUSSION**

In this study, we describe the genome sequences of seventeen well-known canker-causing fungal species in the Botryosphaeriaceae. The genomes assembled coupled with *in planta* experiments allowed us to start analyzing the pathogenicity levels and the virulence factor profiles within this important fungal family. The level of completeness of the assembled genomes is consistent across all the drafts based on the expected and assembled genome sizes. This behavior is also confirmed by the high representation of conserved genes (Parra et al., 2007; Simão et al., 2015). The completeness of the genomes, as well as the protein-coding genes and the repetitive DNA content, are similar to those of other wood-colonizing fungi of grape, such as *Diaporthella ampelina* DA912 (Morales-Cruz et al., 2015), *Di. seriata* DS831 (Morales-Cruz et al., 2015), and *L. theobromae* LA-SOL3 (Félix et al., 2019). Apart from the estimated completeness of the genomes, it is necessary to understand some of the limitations of the short reads technology, like copy number errors, chimeric contigs, and under-representation of repetitive regions (Alkan et al., 2011; Treangen and Salzberg, 2012).

The functional annotation of the seventeen *Botryosphaeriaceae* species presents a broad and variable...
profile of virulence factors that are used in different ways by fungi to colonize and survive in their hosts (Schulze-Lefert and Panstruga, 2011; Peyraud et al., 2019). The results show a great variation in the number of genes identified with a functional category, and these differences were usually associated with the genus of each species like those observed by Baroncelli et al. (2016) in Colletotrichum and Morales-Cruz et al. (2015) in other grapevine trunk pathogens. Researchers are inclined to think that the gene content is associated with the lifestyle and the variety of hosts (Zhao et al., 2013; Lo Presti et al., 2015; Baroncelli et al., 2016). The expansion or contraction of a gene family usually occurs on functions that are under positive or negative selection. For instance, the genes related to host colonization and defense are under high pressure, therefore, it is common to encounter duplications or even losses. On the other hand, genes related to growth are more conserved and usually selected against these changes (Wapinski et al., 2007). Gene duplication events are crucial as they are considered to be one of the main processes that generate functional innovation (Zhang, 2003; Ohno, 2013). This process plays one of the most important roles in fungal adaptation and divergence (Gladieux et al., 2014).

Host colonization during infection is mostly driven by gene expression of some groups of well-known proteins, namely, the secreted CAZymes, cytochrome P450 monoxygenases, peroxidases, and secondary metabolite-producing proteins (Massonnet et al., 2018). The Botryosphaeriaceae family has a variable profile of these sets of genes, with the most virulent and aggressive species having, on average, greater

**FIGURE 3** Clock calibrated phylogenetic tree showing the number of gene families significantly expanded (red), contracted (blue), and their average pattern (black). Calibrations points of the Ascomycota partition (A), and Dothideomycetes partition (B).
numbers of annotated genes in these categories (Table 3). In grape and pistachio, species in the genera *Neofusicoccum* and *Lasiodiplodia*, are typically more virulent than species in the genera *Diplodia* and *Dothiorella* (Úrbez-Torres et al., 2008; Úrbez-Torres and Gubler, 2009; Nouri et al., 2019). GH functions of β-glucosidases, β-xylosidases, glucanases, L-arabinofuranosidase, and galactanase were present in all the pathogens in this study and significantly more in *Neofusicoccum* and *Lasiodiplodia*. In the same way as the GH, AA functions like cellobiose dehydrogenases, alcohol oxidases, pyranose
oxidase were more abundant among *Neofusicoccum* species and *B. dothidea*. GH and AA play a critical role in the degradation of the host cell wall compounds (Kubicek et al., 2014), which is involved with the degree of pathogenicity within these genera, albeit on grape, the host we examined. Marsberg et al. (2017); Massonnet et al. (2018), and Félix et al. (2019), found similar numbers of CAZymes in *Neof. parvum*, *L. theobromae*, and *B. dothidea*, respectively. P450s are instrumental to the development of all organisms. These enzymes are involved in many aspects of primary and secondary metabolisms and are responsible for xenobiotic detoxification and degradation (Čresná and Petrič, 2011; Moktali et al., 2012). Virulence may in part reflect the ability of some species to better tolerate and, further, to metabolize phenolic compounds produced by the host. Both *Neof. parvum* and *Di. seriata* can eliminate the stilbene piceid and its derivative resveratrol in vitro (Stempień et al., 2017), but the former is better able to tolerate resveratrol derivatives amelopsin A, hopeaphenol, isohopeaphenol, miyabenol C, and e-viniferin, which are produced at higher levels in planta in response to *Neof. parvum* versus *Di. seriata* infection (Lambert et al., 2012). Therefore, it is not unexpected to see a variable profile amongst genera in the *Botryosphaeriaceae* family and even within a single genus. As presented in Figure 2, some superfamilies are abundant in *Neofusicoccum*, *Lasiodiplodia* and *Botryosphaeria* genera, but other superfamilies are especially more numerous in the Basidiomycetes species included in this study. On the other hand, for most of the superfamilies presented, *Sa. cerevisiae* shows a considerable lack of such annotated genes, but CYP53 and CYP578 the counts are comparable with the rest of the species. This variation is sourced by the constant evolution and adaptation of the microorganism and hosts to their specific environment (Yan et al., 2018).

As plants evolve new defense mechanisms and compounds against pathogens, the fungi diversify their methods to degrade these compounds or generate new metabolites to attack their hosts (Deng et al., 2007; Yan et al., 2018). The *Botryosphaeriaceae* species in this study and the two Basidiomycetes present a set of fungal peroxidases that range from 41 to 62. As for the previous putative virulence factors, *Neofusicoccum*, *Lasiodiplodia*, and *Botryosphaeria* genera have the most annotated peroxidases, however, in this case, *Diplodia* also showed a comparable amount. Manganese peroxidase was only found in the two basidiomycetes. This enzyme has a critical role in the degradation of lignocellulose compounds by basidiomycetes (Elisashvili and Kachlishvili, 2009; Liers et al., 2011), therefore it is very common in white-rot fungi such as *F. mediterranea* and *St. hirsutum* (Morgenstern et al., 2010; Lee et al., 2015). The former enzyme was found in higher numbers in the genus *Neofusicoccum* compared to other genera within the family. The hybrid ascorbate-cytochrome C peroxidase was overrepresented in the genera *Neofusicoccum*, *Lasiodiplodia*, and *Botryosphaeria* and is associated directly with the detoxification of ROS (Zámocký et al., 2014; Wang et al., 2016; Segal and Wilson, 2018). The wide array of transporters annotated in this study suggests a high adaptation to toxic compounds, either produced by other microorganisms, the host, or potentially chemical synthesized fungicides (Stergiopoulos et al., 2002). The number of proteins in the Major Facilitator Superfamily (MFS) and Superfamily in *Neofusicoccum*, *Lasiodiplodia*, and *Botryosphaeria* were more numerous than the other *Botryosphaeriaceae* species. Protein members of the MFS family may have different functions in the influx/efflux of molecules between cells and the exterior environment, and several cases of fungicide resistances have been associated with the overexpression of certain MFS channels (Stergiopoulos et al., 2002; Gulshan and Moye-Rowley, 2007; Dos Santos et al., 2014; Chen et al., 2017). The former genera have been reported to have lower sensitivities to almost full resistance to different synthetic fungicides (Wang et al., 2010; Tennakoon et al., 2019; Li et al., 2020). Similar behavior was observed in *Do. sarmentorum*, were the ATP-binding Cassette (ABC) is
highly represented. The ABC superfamily plays different roles in fungicide resistance, mycelial growth, and overall pathogenicity (Stergiopoulos et al., 2002; Qi et al., 2018). In addition, the array of secondary metabolite gene clusters is more expanded in the Botryosphaeriaceae family than in the Basidiomycetes except for of secondary metabolite gene clusters is more expanded in the fungicide resistance, mycelial growth, and overall pathogenicity highly represented. The ABC superfamily plays different roles in Garcia et al. Phylogenomics of Neofusicoccum annotation of putative virulence factors in a significantly higher than expected rate of gain/loss. The gene families of the proteins analyzed in this study have expansions in three of the four species in this study. Also, Neofusicoccum australe appears to be actively evolving, showing major expansions in three of the four species in this study. Also, B. dothidea and three Neofusicoccum species (Neof. parvum, Neofusicoccum australe, and Neofusicoccum mediterraneum) show an expansion of these families. On the other side, Neos. dimidiatum, B. dothidea, Do. sarmentorum, L. exigua, and L. missouriana are actively expanding their secondary metabolite gene clusters. Finally, the wide variety of transporters present in fungi is the result of the positive selection pressure over them. The need of the fungi to adapt to new environments and hosts had selected for multiple mutations that diversifies the transporters functions (Gladieux et al., 2014). The MFS (2.A.1) displays the largest effect of expansion and contraction among all the species. Botryosphaeria dothidea, L. missouriana, L. exigua, and Di. mutila appear to be actively expanding the MFS transporters. However, Neos. dimidiatum, Di. seriata, Neofusicoccum vitifusiforme, Neof. australe, and Neof. mediterraneum are contracting MFS transporters.

Phylo PCAs results support the idea that within the Botryosphaeriaceae family, Neofusicoccum, Lasiodiplodia, and Botryosphaeria genera are the most virulent (Úrbez-Torres et al., 2008; Úrbez-Torres and Gubler, 2009). There was a very clear separation of these species from the Diplodia, Dothiorella, and Neoscytalidium. The secreted CAZymes that cause the clustering of the Neofusicoccum species are usually associated with laccases, cellulbiose dehydrogenases, and cellulase activities. These enzymes usually target components of the plant cell wall such as lignin, cellulose, cellulbiose (Cameron and Aust, 2001; Zamocky et al., 2006; Fillat et al., 2016; Di Francesco et al., 2020). Among the functions driving the clustering of Lasiodiplodia and Botryosphaeria, the lytic polysaccharide monooxygenases (LPMOs, AA9) are one of the most important. They have a role in the oxidative degradation of various biopolymers such as cellulose and chitin. LPMOs can increase the activity of cellulases highly, and now, they are used in a mixture for the preparation of biofuels (Frommhagen et al., 2018; Labourel et al., 2020). Therefore, this set of enzymes may facilitate the colonization and infection of their hosts. The separation of Neos. dimidiatum from the rest of species is caused by GH78 which includes mannanases, α-glucosidase enzymes and the PL3 family of pectate lyases. As Neos. dimidiatum is also known for infecting the fruits and soft tissues of their hosts (Marques et al., 2013; Nouri et al., 2018), this set of enzymes seems to be well developed.

Lasiodiplodia species have a wide array of secondary metabolites. Their profile varies according to the species, isolate, and even the host (Salvatore et al., 2020). These metabolites are often synthesized by clusters of TIPKS, TIPKS/NRPS, and some beta-lactones (Félix et al., 2019; Salvatore et al., 2020), which are some of the major drivers for their clustering in the phylo-PCA (Figure 5). Neofusicoccum, besides the previous gene clusters, also have lamanypenes and NRPS, which drives their clustering in the PCA. Similar results have been presented by Morales-Cruz et al. (2015) and Massonnet et al. (2018). Very little literature is available about the effect of the secondary metabolites of B. dothidea on their plant host; however, this fungus is known for its remarkable ability to produce secondary metabolites in vitro (Wang et al., 2018), which recently have been studied for their potential use as commercial antioxidants (Xiao et al., 2014; Druzman et al., 2020; Valente et al., 2020).

Few of the fungi in this study have been characterized in terms of their interactions with wood and individual wood components, their activation of cell-wall degrading enzymes, or their ability to tolerate phenolic compounds. Therefore, it is difficult to connect the pattern of gene family evolution to such aspects of fungal biology, especially in a comparative way among so many species, none of which have all been compared at once on a single host. The pathogenicity test on young, rooted grapevine plants raises some interesting observations. First, L. theobromae, Neof. parvum, and Neof. australe are among the species that induced the most prominent lesions in the plants. These results are consistent with those of Úrbez-Torres and Gubler (2009), who found these species to be highly virulent on grape. This same study found Di. mutila, Di. seriata, Do. iberica, and Do. viticola to be weakly virulent, which is congruent with the results presented by this study. Although most Neofusicoccum species present high numbers of putative virulence factors, the targets for these may be variable within the genus. In this pathogenicity experiment, the isolates of Neof. vitifusiforme, Neof. nonguaesitum, and Neof. hellenicum were isolated from active cankers in walnut and pistachio trees, and even if some of these...
species can develop disease in grapevine, their virulence on *Vitis vinifera* may not be the same. Finally, the reasons why lesions produced by *B. dothidea* were not significantly different from the control are difficult to assess with certainty, but studies had reported *B. dothidea* to be weakly or moderately pathogenic on grapevine (Úrbez-Torres and Gubler, 2009; Pitt et al., 2013). Some researchers remark that in potted plants after 5–6 weeks of inoculation with *B. dothidea*, the plants were not different compared to the control, whereas the other species in that study showed poor bud development and stunted green shoot growth (Úrbez-Torres and Gubler, 2009). Also, in other studies, this species is presented as endophytic and latent pathogen and they suggest that the environmental conditions can have a significant effect on the development of the disease (Piškur andJurc, 2011; Marsberg et al., 2017).

**DATA AVAILABILITY STATEMENT**

Sequencing data are available at NCBI (BioProject PRJNA673527). Sequencing data of Diplodia seriata and *Neof. parvum* can be retrieved from NCBI under BioProject PRJNA261773 and PRJNA321421, respectively. All genome assemblies and gene models are publicly available at Zenodo (doi: 10.5281/zenodo.4417445).

**REFERENCES**

Abou-Mansour, E., Débieux, J. L., Ramirez-Suero, M., Bénard-Gellon, M., Magnin-Robert, M., Spagnolo, A., et al. (2015). Phytotoxic metabolites from Neofusicoccum parvum, a pathogen of Botryosphaeria dieback of grapevine. *Phytochemistry* 115, 207–215. doi: 10.1016/j.phytochem.2015.01.012

Alkan, C., Sajjadian, S., and Eichler, E. E. (2011). Limitations of next-generation genome sequence assembly. *Nat. Methods* 8, 61–65. doi: 10.1038/nmeth0112.1527

Alves, A., Crous, P. W., Correia, A., and Phillips, A. J. L. (2008). Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Divers.* 28, 1–13.

Andolfi, A., Migliaccio, D., Luque, J., Surico, G., Cimmino, A., and Evidente, A. (2011). Phytotoxins produced by fungi associated with grapevine trunk diseases. *Toxins* 3, 1569–1605. doi: 10.3390/toxins3121569

Armeros, J. J. A., Tarigos, K. D., Sonderby, C. K., Petersen, T. N., Winther, O., Brunak, S., et al. (2019). SignaLP 5.0 improves signal peptide predictions using deep neural networks. *Nat. Biotechnol.* 37, 420–423. doi: 10.1038/s41587-019-0036-x

Baumgartner, K., Fujiyoshi, P. T., Travadon, R., Castlebury, L. A., Wilcox, W. F., and Rolshausen, P. E. (2013). Characterization of species of Diaporthe from wood cankers of grape in eastern North American vineyards. *Plant Dis.* 97, 912–920. doi: 10.1094/PDIS-04-12-0357-RE

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., et al. (2012). SPAdes: a new genome assembly algorithm and its applications for single-cell sequencing. *Nat. Biotechnol.* 30, 1473–1477. doi: 10.1038/nbt.2421.

Baroncelli, R., Amby, D. B., Zapparata, A., Sarrocco, S., Vannacci, G., Le Floch, G., et al. (2018). Evaluation of six red grapevine cultivars inoculated with *Neofusicoccum parvum*. *Eur. J. Plant Pathol.* 158, 811–815. doi: 10.1007/s10658-020-0211-9

Christodoulou, J., Chacón, J. L., Gramaje, D., Izquierdo, P. M., Martínez, J., and Mena, A. (2020). *Diplodia seriata* and *Botryosphaeria alternata* are the causal agents of grapevine trunk disease in the north of Spain. *Plant Dis.* 104, 1673–1680. doi: 10.1094/PDIS-06-20-01550-R

Cantarel, B. L., Coutinho, P. M., Andrade, M. A., Normal, B., and Henrissat, B. (2004). A new algorithm for the automated annotation of glycoside hydrolases. *Bioinformatics* 20, 2317–2323. doi: 10.1093/bioinformatics/bth243

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552. doi: 10.1093/molbev/17.3.540

Daubresse, M., Gambetta, S., and Bruni, M. (2011). Expression of clusters of secreted enzymes in different grapevine cultivars. *BMC Genomics* 12, 390. doi: 10.1186/1471-2164-12-390

Debboun, A. B., Amara, A., and Boyer, A. (2000). The ratio of the expression of the enzyme carbamoylphosphate synthetase in two grapevine cultivars. *BMC Plant Biol.* 10, 13. doi: 10.1186/1471-2229-10-13

Garcia et al. (2021). Phylogenomics of *Botryosphaeriaceae*. *Front. Microbiol.* 12, 652802. doi: 10.3389/fmicb.2021.652802/full#supplementary-material
Druzian, S. P., Pinheiro, L. N., Susin, N. M. B., Dal Prá, V., Mazutti, M. A., Di Francesco, A., Rusin, C., Di Foggia, M., Marceddu, S., Rombolà, A., Botelho, Dos Santos, S. C., Teixeira, M. C., Dias, P. J., and Sá-Correia, I. (2014). MFS Desprez-Loustau, M. L., Marçais, B., Nageleisen, L. M., Piou, D., and Vannini, A. Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. doi: 10.1093/nar/gkh340 Elisashvili, V., and Kachishvili, E. (2009). Physiological regulation of laccase and manganese peroxidase production by white-rot Basidiomycetes. J. Biotechnol. 144, 37–42. doi: 10.1016/j.jbiotec.2009.06.020 Enright, A. J., Van Dongen, S., and Ouzounis, C. A. (2002). An efficient algorithm for large-scale detection of protein families. Nucleic Acids Res. 30, 1575–1584. doi: 10.1093/nar/30.7.1575 Faure, D. (2002). The family-3 glycoside hydrolases: from housekeeping functions to host-microbe interactions. Appl. Environ. Microbiol. 68, 1485–1490. doi: 10.1128/ae.68.4.1485-1490.2002 Félix, C., Meneses, R., Gonçalves, M. F., Tillemann, L., Duarte, A. S., Jorrín-Novo, J. V., et al. (2019). A multi-omics analysis of the grapevine pathogen Lasiobipodia theobromae reveals that temperature affects the expression of virulence-and pathogenicity-related genes. Sci. Rep. 9:13144. Fillat, U., Martín-Sampedro, R., Macaya-Sanz, D., Martín, J. A., Ibarra, D., Martínez, M. J., et al. (2016). Screening of eucalyptus wood endophytes for laccase activity. Process Biochem. 51, 589–598. doi: 10.1016/j.procbio.2016.02.006 Finn, R. D., Coggill, P., Eberhardt, R. Y., Eddy, S. R., Mistry, J., Mitchell, A. L., et al. (2016). The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res. 44, D279–D285. Fischer, M. (2006). Biodiversity and geographic distribution of basidiomycetes causing esca-associated white rot in grapevine: a worldwide perspective. Phytopathol. Mediterranea 45, S30–S42. Fischer, M., Knoll, M., Sirim, D., Wagner, F., Funke, S., and Pleiss, J. (2007). The Cytochrome P450 engineering database: a navigation and prediction tool for the cytochrome P450 protein family. Bioinformatics 23, 2015–2017. doi: 10.1093/bioinformatics/btm268 Floudas, D., Binder, M., Riley, R., Barry, K., Blanchette, R. A., Henrisatt, R., et al. (2012). The Paleozioc origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336, 1715–1719. Frommhen, M., Westphal, A. H., Van Berkel, W. J., and Kabel, M. A. (2018). Distinct substrate specificities and electron-donating systems of fungal lytic polysaccharide monooxygenases. Front. Microbiol. 9:1080. Galanarneu, E. R., Lawrence, D. P., Travodon, R., and Baumgartner, K. (2019). Drought exacerbates botryosphaeria dieback symptoms in grapevines and confounds host-based molecular markers of infection by Neofusicoccum parvum. Plant Dis. 103, 1738–1745. doi: 10.1094/pdis-09-18-1549-re Gladieux, P., Ropars, J., Badouin, H., Branca, A., Aguleta, G., De Vienne, D. M., et al. (2014). Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. Mol. Ecol. 23, 753–773. doi: 10.1111/mec.12631 Goodell, B., Qian, Y., and Jellison, J. (2008). Fungal decay of wood: soft rot-brown rot-white rot. ACS Symp. Ser. 9–31. doi: 10.1021/bk-2008-0982.ch002 Gramaje, D., Agusti-Brisach, C., Pérez-Sierra, A., Moraléjo, E., Olmo, D., Mostert, L. J. Z. E., et al. (2012). Fungal tunn pathogen associated with wood decay of almond trees on Mallorca (Spain). Persoonia 28, 1–13. doi: 10.37671/003158512x626155 Guan, X., Essakhli, S., Laloue, H., Nick, P., Bertsch, C., and Chong, J. (2016). Mining new resources for grape resistance against Botryosphaeriaceae: a focus on Vitis vinifera subsp. sylvestris. Plant Pathol. 65, 273–284. doi: 10.1111/ppa.12405 Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., and Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. doi: 10.1093/sysbio/sys010 Gulshan, K., and Moye-Rowley, W. S. (2007). Multidrug resistance in fungi. Eurakaryot. Cell 6, 1933–1942. doi: 10.1128/ec.00254-07 Guo, M., Guo, W., Chen, Y., Dong, S., Zhang, X., Zhang, H., et al. (2010). The basic leucine zipper transcription factor Moaft mediates oxidative stress responses and is necessary for full virulence of the rice blast fungus Magnaporthe oryzae. Mol. Plant Microbe Interact. 23, 1053–1068. doi: 10.1094/mpi-23-8-1053 Hahn, M. W., De Bie, T., Stajich, J. E., Nguyen, C., and Cristianini, N. (2011). A multi-omics analysis of the grapevine pathogen Botryosphaeriaceae: a focus on Vitis vinifera subsp. sylvestris. Plant Pathol. 65, 273–284. doi: 10.1111/ppa.12405 Hahn, M. W., De Bie, T., Stajich, J. E., Nguyen, C., and Cristianini, N. (2005). Estimating the tempo and mode of gene family evolution from comparative genomic data. Genome Res. 15, 1153–1160. doi: 10.1101/gr.35 67905 Harreither, W., Sigmund, C., Augustin, M., Narciso, M., Rabinovich, M. L., Gorton, L., et al. (2011). Catalytic properties and classification of cellobiose dehydrogenases from ascomycetes. Appl. Environ. Microbiol. 77, 1804–1815. doi: 10.1128/aem.02052-10 Henriksson, G., Johansson, G., and Pettersson, G. (2000). A critical review of cellobiose dehydrogenases. J. Biotechnol. 78, 93–113. doi: 10.1016/s0168-1656(00)00206-6 Hernández-Ortega, A., Ferreira, P., and Martínez, A. T. (2012). Fungal aryl-alcohol oxidase: a Peroxidase-producing flavonozyme involved in lignin degradation. Appl. Microbiol. Biotechnol. 93, 1395–1410. doi: 10.1007/s00253-011-3836-8 Hofrichter, M., Ullrich, R., Pecyna, M. J., Liers, C., and Lundell, T. (2010). New and classic families of secreted fungal heme peroxidases. Appl. Microbiol. Biotechnol. 87, 871–897. doi: 10.1007/s00253-010-2633-0
Holland, L. A., Trouillas, F. P., Nouri, M. T., Lawrence, D. P., Crespo, M., Doll, D. A., et al. (2020). Fungal pathogens associated with canker diseases of almond in california. Plant Dis. 105, 346–360. doi: 10.1094/psd-10-19-2128-re

Hrycan, J., Hart, M., Bowen, P., Forge, T., and Úbeda-Torres, J. R. (2020). Grapevine trunk disease fungi: their roles as latent pathogens and stress factors that favour disease development and symptom expression. Phytopathologia Mediterranea 59, 395–424.

Inderbitzin, P., Bostock, R. M., Trouillas, F. P., and Michailides, T. J. (2010). A six locus phylogeny reveals high species diversity in Botryosphaeriaceae from California almond. Mycologia 102, 1350–1368. doi: 10.3852/10-006

Inkscape Project (2020). Inkscape. Available online at: from https://inkscape.org (accessed July 15, 2020).

Jones, L., Riaz, S., Morales-Cruz, A., Amrine, K. C., McGuire, B., Gubler, W. D., et al. (2014). Adaptive genomic structural variation in the grape powdery mildew pathogen, Erysiphe necator. BMC Genomics 15:1081. doi: 10.1186/1471-2164-15-1081

Keller, N. P. (2019). Fungal secondary metabolism: regulation, function and drug discovery. Nat. Rev. Microbiol. 17, 167–180. doi: 10.1038/s41579-018-0121-1

Knob, A., Terrasan, C. F., and Carmona, E. C. (2010). β-Xylosidases from filamentous fungi: an overview. World J. Microbiol. Biotechnol. 26, 389–407. doi: 10.1007/s11274-009-0190-4

Kubicke, C. P., Starr, T. L., and Glass, N. L. (2014). Plant cell wall–degrading enzymes and their secretion in plant-pathogenic fungi. Annu. Rev. Phytopathol. 52, 427–451. doi: 10.1146/annurev-phyto-102313-045831

Labourè, A., Frandsen, K. E., Zhang, F., Brouilly, N., Grisel, S., Haon, M., et al. (2020). A fungal family of lytic polysaccharide monooxygenase-like copper proteins. Nat. Chem. Biol. 16, 345–350. doi: 10.1038/s41589-019-0438-8

Lambert, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B. (2010). The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42, D490–D495.

Lata, M., Eustar-Lessard, P., Rousseau, L., Roblin, G., and Berjeaud, I. M. (2010). Inhibitory effects of polypeptides secreted by the grapevine pathogens Phaeomoniella chlamydospora and Phaeacromium aleophilum on plant cell activities. Physiol. Mol. Plant Pathol. 74, 403–411. doi: 10.1016/j.pmpp.2010.06.007

Luo, Y., Lichtenberg, P. S. F., Niederholzer, F. J. A., Lightle, D. M., Felts, D. G., and Michailides, T. J. (2019). Understanding the process of latent infection of canker-causing pathogens in stone fruit and nut crops in California. Plant Dis. 103, 2374–2384. doi: 10.1094/psd-11-18-1963-re

Lucie, J., Martos, S., and Garcia-Figueroes, F. (2010). Effects of water stress and inoculation with Eutypa lata and Neofusicoccum parvum on young grapevine plants. Phytopathologia Mediterranea 49:120.

Ma, Z., Boehm, E. W., Luo, Y., and Michailides, T. J. (2001). Population structure of Botryosphaeria dothidea from pistachio and other hosts in California. Phytopathology 91, 665–672. doi: 10.1094/PHYTO.2001.91.7.665

Marques, M. W., Lima, N. B., de Morais, M. A., Michereff, S. J., Phillips, A. J., and Câmara, M. P. (2013). Botryosphaeria, Neofusicoccum, Neoscytalidium and Pseudofuscosium species associated with mango in Brazil. Fungal Divers. 61, 195–208. doi: 10.1007/s11235-013-0258-1

Marsberg, A., Kemler, M., Jani, F., Nagel, J. H., Postma-Smith, A., Naidoo, S., et al. (2017). Botryosphaeria dothidea: a latent pathogen of global importance to woody plant health. Mol. Plant Pathol. 18, 477–488. doi: 10.1111/mpp.12495

Martinková, L., Kotík, M., Marková, E., and Homolková, L. (2016). Biodegradation of phenolic compounds by Basiomyces cultura and its phenol oxidases: a review. Chemosphere 149, 373–382. doi: 10.1016/j.chemosphere.2016.01.022

Massonnet, M., Morales-Cruz, A., Figueroa-Balderas, R., Lawrence, D. P., Baumgartner, K., and Cantu, D. (2018). Condition-dependent co-regulation of genomic clusters of virulence factors in the grapevine trunk pathogen Neofusicoccum parvum. Mol. Plant Pathol. 19, 21–34. doi: 10.1111/mpp.12491

Mayer, A. M. (2006). Polyphenol oxidases in plants and fungi: going places! a review. Phytochemistry 67, 2318–2331. doi: 10.1016/j.phytochem.2006.08.006

Mayer, A. M., Staples, R. C., and Gil-ad, N. L. (2001). Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. Phytochemistry 58, 33–41. doi: 10.1016/s0031-9422(01)00187-x

McDonald, V., Lynch, S., and Eskenal, A. (2009). First report of Neofusicoccum australis, N. luteum, and N. parvum associated with avocado branch canker in California. Plant Dis. 93:967. doi: 10.1094/psd-93-9-0967b

Michailides, T. J. (1991). Pathogenicity, distribution, sources of inoculum, and infection courts of Botryosphaeria dothidea on pistachio. Phytopathology 81, 566–573. doi: 10.1094/PHYTO-81-566

Moktali, V., Park, J., Fedorova-Abrams, N. D., Park, B., Choi, J., Lee, Y. H., et al. (2012). Systematic and searchable classification of cytochrome P450 proteins encoded by fungal and oomycete genomes. BMC Genomics 13:525. doi: 10.1186/1471-2164-13-525

Molina, L., and Kahmann, R. (2007). An Ustilago maydis gene involved in H2O2 detoxification is required for virulence. Plant Cell 19, 2293–2309. doi: 10.1105/tpc.107.052332

Mora-Cruz, A., Allenbeck, G., Figueroa-Balderas, R., Ashworth, V. E., Lawrence, D. P., Travon, D., et al. (2018). Closed-reference metatranscriptomics enables in planta profiling of putative virulence activities in the grapevine trunk disease complex. Mol. Plant Pathol. 19, 490–503. doi: 10.1111/mpp.12544

Mora-Cruz, A., Amrine, K. C., Blanco-Ulate, B., Lawrence, D. P., Travon, D., Rolshausen, P. E., et al. (2015). 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 16:469. doi: 10.1186/ps1-05-13-0523-re

Morgenstern, I., Robertson, D. L., and Hibbett, D. S. (2010). Characterization of three mnp genes of Fomitopsis mediterranea and report of additional class II peroxidases in the order Hymenochaetales. Appl. Environ. Microbiol. 76, 6431–6440. doi: 10.1128/aem.00547-10

Mullen, J. M., Gilliam, C. H., Hagan, A. K., and Morgan-Jones, G. (1991). Canker of dogwood caused by Lasiodiplodia theobromae, a disease influenced by drought stress or cultivar selection. Plant Dis. 75, 886–889. doi: 10.1094/psd-75-0886

Nouri, M. T., Lawrence, D. P., Holland, L. A., Doll, D. A., Kallsen, C. E., Culumber, C. M., et al. (2019). Identification and pathogenicity of fungal species associated with canker diseases of pistachio in California. Plant Dis. 103, 2397–2411. doi: 10.1094/psd-10-18-1717-re

Nouri, M. T., Lawrence, D. P., Yaghmour, M. A., Michailides, T. J., and Trouillas, F. P. (2018). Neoscytalidium dimidiatum causing canker, shoot blight and fruit rot of almond in California. Plant Dis. 102, 1638–1647. doi: 10.1094/psd-12-17-1967-re

Ohno, S. (2013). Evolution by Gene Duplication. Berlin: Springer.
Treangen, T. J., and Salzberg, S. L. (2012). Repetitive DNA and next-generation sequencing: computational challenges and solutions. Nat. Rev. Genet. 13, 36–46. doi: 10.1038/nrg3117

Urbéz-Torres, J. R. (2011). The status of Botryosphaeriaceae species infecting grapevines. Phytopathology 101, 558–545.

Urbéz-Torres, J. R., and Gubler, W. D. (2009). Pathogenicity of Botryosphaeriaceae species isolated from grapevine cankers in California. Plant Dis. 93, 584–592. doi: 10.1094/PDIS-93-6-0584

Urbéz-Torres, J. R., Leavitt, G. M., Guerrero, J. C., Guevara, J., and Gubler, W. D. (2008). Identification and pathogenicity of Lasiodiplodia theobromae and Diplodia seriata, the causal agents of bot canker disease of grapevines. Plant Dis. 92, 519–529. doi: 10.1094/pdis-92-4-0519

Urbéz-Torres, J. R., Peduto, F., Vossen, P. M., Krueger, W. H., and Gubler, W. D. (2013). Olive twig and branch dieback: etiology, incidence, and distribution in California. Plant Dis. 97, 231–244. doi: 10.1094/pdis-04-12-0390-re

Valente, I. D. L., Confortin, T. C., Luft, L., Todero, I., Quadros, G. P., Tomato, D., et al. (2020). Effects of ultrasound on submerged fermentation for producing antioxidant metabolites from Botryosphaeria dothidea. Br. J. Chem. Eng. 37, 475–484. doi: 10.1007/s43153-020-00044-8

Valette, N., Perrot, T., Sormani, R., Gelhaye, E., and Morel-Rouhier, M. (2017). Antifungal activities of wood extracts. Fungal Biol. Rev. 31, 113–123. doi: 10.1016/j.fbr.2017.01.002

Van Dongen, S. M. (2000). Graph Clustering by Flow Simulation. Doctoral dissertation, Netherlands, University of Utrecht.

Wang, B., Liang, X., Gleason, M. L., Zhang, R., and Sun, G. (2018). Comparative genomics of Botryosphaeria dothidea and B. kuwatsukai, causal agents of apple ring rot, reveals both species expansion of pathogenicity-related genes and variations in virulence gene content during speciation. IMA Fungus 9, 243–257. doi: 10.5598/imafungus.2018.09.02.02

Wang, M., Sun, X., Yu, D., Xu, J., Chung, K., and Li, H. (2016). Genomic and transcriptomic analyses of the tangerine pathotype of Alternaria alternata in response to oxidative stress. Sci. Rep. 6:23437.

Wang, Y., Zhang, W., Liu, B., Luan, B., and Wang, P. (2010). Research on resistance and geographical distribution of Botryosphaeria dothidea from apple to Tebuconazole in Shandong province. J. Fruit Sci. 27, 961–964.

Wapinski, I., Pfeffer, A., Friedman, N., and Regev, A. (2007). Natural history and evolutionary principles of gene duplication in fungi. Nature 449, 54–61. doi: 10.1038/nature06107

White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications 18, 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1

Xiao, J., Zhang, Q., Gao, Y. Q., Tang, J. J., Zhang, A. L., and Gao, J. M. (2014). Secondary metabolites from the endophytic Botryosphaeria dothidea of Melia azedarach and their antifungal, antibacterial, antioxidant, and cytotoxic activities. J. Agric. Food Chem. 62, 3584–3590. doi: 10.1021/jf501551s

Yan, J. Y., Zhao, W. S., Chen, Z., Xing, Q. K., Zhang, W., Chethana, K. T., et al. (2018). Comparative genome and transcriptome analyses reveal adaptations to opportunistic infections in woody plant degrading pathogens of Botryosphaeriaceae. DNA Res. 25, 87–102. doi: 10.1093/dnares/dsx040

Zámocký, M., and Obinger, C. (2010). “Molecular phylogeny of heme peroxidases,” in Biocatalysis Based on Heme Peroxidases, eds E. Torres and M. Ayala (Berlin; Heidelberg: Springer), 7–35. doi: 10.1007/978-3-642-12627-7_2

Zámocký, M., Gasselhuber, B., Furtmüller, P. G., and Obinger, C. (2014). Turning points in the evolution of peroxidase–catalase superfamily: molecular phylogeny of hybrid heme peroxidases. Cell. Mol. Life Sci. 71, 4681–4696. doi: 10.1007/s00018-014-1643-y

Zamocký, M., Ludwig, R., Peterbauer, C., Hallberg, B. M., Divine, C., Nicholls, P., et al. (2006). Cellobiose dehydrogenase—a flavocytochrome from wood-degrading, phytopathogenic and saprotrophic fungi. Curr. Protein Pept. Sci 7, 255–280. doi: 10.2174/138920306777452367

Zhang, H., Yohe, T., Huang, L., Entwistle, S., Wu, P., Yang, Z., et al. (2018). dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 46, W95–W101.

Zhang, J. (2003). Evolution by gene duplication: an update. Trends Ecol. Evol. 18, 292–298. doi: 10.1016/s0169-5347(03)00033-8

Zhao, Z., Liu, H., Wang, C., and Xu, J. R. (2013). Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. BMC Genomics 14:274. doi: 10.1186/1471-2164-14-274

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Garcia, Lawrence, Morales-Cruz, Travadon, Minio, Hernandez-Martinez, Rohsenaht, Baumgartner and Cantu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.