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Fungal Pathogen Activity and Stress-Dependent Responses of Grapevine Wood to Esca and Drought

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ABSTRACT

Biotic and abiotic stresses alter the physiology of perennial plants, with consequences for fungal endophytes and disease expression. In grapevine, drought inhibits esca disease expression, but the underlying molecular interactions between the plant and fungi are unknown. We combined wood metatranscriptomics, metabolomics, and metabarcoding to investigate these interactions in 30-year-old grapevines and eight wood-pathogenic fungi under conditions of drought or esca leaf symptom expression. Both esca and drought decreased grapevine transpiration, but with different underlying mechanisms that induced specific transcriptomic and metabolic signatures. Similar pathways were also activated, including the phenylpropanoid and stilbenoid synthesis pathways. These stress responses could potentially confer cross-tolerance and elicit different fungal molecular responses. Across all fungi, the total level of putative virulence factors increased significantly under both stresses. Under drought, only the relative abundance of *Phaeomoniella chlamydospora* and gene expression involved in anti-oxidative mechanisms, growth, and reproduction increased. Under esca expression conditions, only the relative abundance of *Fomitiporia mediterranea* and gene expression involved in wood degradation, competition, detoxification, and growth increased. Under drought, induced grapevine defenses and reduced transpiration, together with the low abundance and putatively weak virulence of *F. mediterranea* may account for the inhibition of esca leaf symptom.

1 | Introduction

It has been predicted that global warming will increase abiotic and biotic stresses experienced by crops, including drought (IPCC 2021; Vicente-Serrano et al. 2022) and fungal pathogen pressure (Chaloner et al. 2021). These predictions are of particular concern for perennial plants, such as forest trees (Anderegg et al. 2015; McDowell et al. 2022; Petek-Petrik et al. 2023) and grapevines (Van Leeuwen et al. 2024), which accumulate stress over several decades. Plants host diverse microbiomes that colonize external surfaces and internal tissues (Trivedi et al. 2020). Under certain abiotic and biotic conditions, the microorganisms within these microbial populations may shift from commensals or latent pathogens to a pathogenic state (Kabbage et al. 2015; Mishra et al. 2021). Such plant-environment-microorganism interactions are

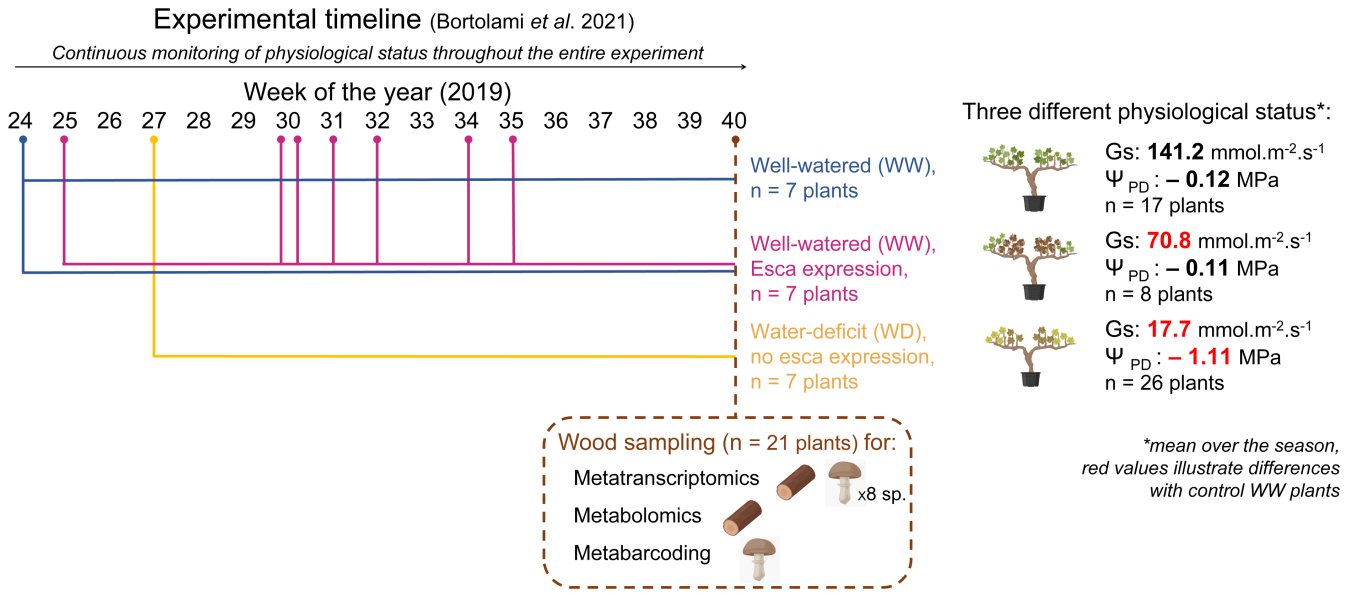

FIGURE 1 | Schematic diagram of the experimental design. *Vitis vinifera* cv. Sauvignon blanc plants ($n = 51$) were uprooted from the vineyard and placed in pots in a greenhouse for the study of interactions between WD and esca as described by Bortolami et al. (2021). At the end of this experiment, wood samples were collected from 21 plants, seven from each of the three treatment groups: Control, WD and plants expressing esca leaf symptoms (the week of the year of leaf symptom onset is indicated for each plant). These treatments resulted in three different plant physiological states according to predawn water potential (Ψ_{PD}) and whole-plant stomatal conductance (G_S) (significant differences from control plants are indicated in red and the full datasets are provided by Bortolami et al. 2021).

known as pathobiomes and lead to disease development (Mina et al. 2020; Vayssier-Taussat et al. 2014; Lv et al. 2023).

Biotic stresses can lead to shifts in the composition of plant fungal communities, with changes in root fungal communities during pine wilt disease (Chu et al. 2016), and in wood fungal communities associated with eutypa dieback and esca in grapevine (Morales-Cruz et al. 2018; Nerva et al. 2022). Abiotic stresses, such as drought, can also alter microbiomes, as shown in drought-affected soils (Preece et al. 2019), and *in planta*, in grapevine root (Carbone et al. 2021) and wood (Gastou et al. 2025), and in grassland root fungal communities (Fu et al. 2022). These shifts may be driven directly by stress effects on endophytic microbiota and indirectly by stress-induced changes in plant physiological status (Desprez-Loustau et al. 2006). Indeed, biotic stresses trigger plant defense responses, such as PAMP-triggered immunity (Jones and Dangl 2006), in which hormonal signaling and reactive oxygen species (ROS) production (Yu et al. 2024) restrict pathogen invasion. However, the endophyte community may perceive these defense responses as stresses (Hamilton et al. 2012). Drought decreases both plant water potential and photosynthesis, resulting in a lower sugar content (Bortolami et al. 2021; Martínez-Vilalta and Garcia-Forner 2017; Zargar et al. 2017). Such physiological changes affect the composition and behavior of wood fungal pathogen communities, as shown in grapevine (Gastou et al. 2025; Goodell 2020; Leal et al. 2024). Gaining deeper insight into how abiotic and biotic stresses modify plant physiological status and how these physiological changes influence the pathobiome at the molecular level in both plant and pathogen, would contribute to a better understanding of the complex interplay between plant physiology, microbial communities, and environmental stresses.

Such issues have been little studied in perennial systems, for which grapevine and complex multifactorial diseases like esca constitute a particularly pertinent model for exploring these interactions. Esca is characterized by foliar scorching, internal wood necrosis caused by various wood pathogens, and a brown stripe along the outer xylem of the trunk (Lecomte et al. 2024; Fontaine et al. 2025). It is associated with yield decline and vine mortality (Dewasme et al. 2022; Li et al. 2017). The incidence of leaf symptoms has been demonstrated to be correlated with the abundance of white rot in the trunk (Maher et al. 2012; Gastou et al. 2025). However, the precise role of each pathogenic species, or even of the entire microbial community, in the onset of esca leaf symptoms remains unclear (Monod, Hofstetter, et al. 2025; Gastou et al. 2025). Multiple factors influence symptom expression, including plant genotype (i.e., grapevine cultivar; Etienne et al. 2024; Gastou et al. 2024), environmental conditions (Dell'Acqua, Gambetta, Comont, et al. 2025; Etienne et al. 2025; Monod, Wilhelm, et al. 2025), and the extent of trunk necrosis (Gastou et al. 2025, 2026; Maher et al. 2012; Ouadi et al. 2019). Interestingly, an experiment on mature grapevines that combined drought with monitoring of esca leaf symptom development showed that both water deficit (WD) and esca leaf symptom expression reduced plant transpiration and carbon assimilation, but through different mechanisms (Bortolami et al. 2021). In drought-stressed plants, low predawn water potential (Ψ_{PD}, around −1 MPa) led to a lower leaf stomatal conductance and lower levels of whole-plant transpiration. By contrast, predawn water potential was unaffected in plants with esca symptoms, which instead presented lower levels of leaf gas exchange and whole-plant transpiration, attributed to a smaller functional canopy area and the lower total chlorophyll content of leaves expressing esca symptoms. This study also revealed an antagonism between drought and esca leaf symptom expression (Bortolami et al. 2021); 30% of well-watered plants developed esca leaf symptoms (as commonly observed in the field), whereas none of the drought-stressed plants expressed symptoms over two consecutive years. This unique experimental framework (summarized in Figure 1) provided new opportunities

for exploring the effects of plant physiological status on the activity of fungal pathogens of wood and for determining whether metatranscriptomic changes can explain the antagonistic interaction between drought and disease expression.

To do so, we analyzed wood samples collected at the end of the Bortolami et al. (2021) study, consisting of 30-year-old grapevines naturally infected with esca and exposed for two consecutive seasons to three conditions: control (asymptomatic, well-watered plants), WD, and esca leaf symptom expression. Internal wood necrosis and the associated *Ascomycota* community were previously studied in this experiment (Gastou et al. 2026). Healthy wood was more abundant in control conditions, and necrotic wood showed lower fungal diversity but higher pathogen abundance than healthy wood. In particular, the well-known grapevine trunk pathogens *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum, Neofusicoccum parvum*, *Diplodia seriata*, *Diaporthe ampelina*, *Eutypa lata*, and *Botryosphaeria dothidea* were found in both healthy wood and necrotic tissues (Gastou et al. 2026). However, basidiomycetes (Hymenochaetaceae), including *Fomitiporia mediterranea* in particular, have also been described as implicated in esca pathogenesis (Moretti et al. 2021). This aspect was not studied by Gastou et al. (2026) but addressed in the present study.

As drought-stressed plants did not develop esca symptoms (Bortolami et al. 2021) but had a high abundance of *Ascomycota* wood pathogens (Gastou et al. 2026), this study aimed to decipher the molecular interaction underlying this antagonism. We used a multi-omics approach combining metatranscriptomics, metabolomics, and metabarcoding. Grapevine transcriptomes and metabolomes were analyzed across control, WD, and esca conditions. We then assessed the effects of these different physiological states on fungal community composition and the expression of virulence-associated genes in the eight grapevine trunk pathogens mentioned above. We hypothesized that: (1) WD may prevent symptom expression by altering the composition of the fungal community (including *Ascomycota* and *Basidiomycota*); (2) wood pathogen activity might be directly inhibited by WD; and/or (3) indirectly affected by stress-induced changes in host physiology, such as a decrease in transpiration, and/or transcriptional and metabolic changes. We also hypothesized that the basidiomycete *Fomitiporia mediterranea* might play a key role in esca pathogenesis and esca-drought antagonism.

2 | Materials and Methods

2.1 | Plant Material and Ecophysiological Status

We sampled 21 out of the 51 *Vitis vinifera* cv. Sauvignon blanc plants from the experimental design described by Bortolami et al. (2021) combining WD and esca leaf symptom expression (Figure 1). Briefly, this experiment consisted of 30-year-old plants naturally infected with wood pathogens associated with esca, uprooted from the vineyard and planted in 20 L pots in a greenhouse. Over two experimental seasons (2018 and 2019), we stopped watering the plants and maintained plants under WD conditions for a period of 3 months (from July to October 2018 and 2019), with a target weekly mean Ψ_{PD} between 0.6 and 1.7 MPa. The plants were not rewatered before sample collection. We checked each plant for the presence of esca leaf symptoms twice weekly from June to October during each season. None of the plants subjected to WD developed esca leaf symptoms. We therefore split these plants ($n = 51$) into three treatment groups at the end of the experiment: asymptomatic WD plants, well-watered plants with esca symptoms, and well-watered asymptomatic plants (hereafter referred to as control plants). Plants in different treatment groups displayed contrasting physiological states (Figure 1). Only WD plants presented low water potentials (mean Ψ_{PD} of −1.11 MPa over the season), whereas both the control plants and the plants with esca symptoms remained unstressed (mean Ψ_{PD} of −0.12 MPa and −0.11, respectively, over the season). However, both WD and esca-symptomatic plants displayed a decrease in whole-plant stomatal conductance (Gs) after the start of the stress (141.18 mmol $m^{-2} s^{-1}$ of mean Gs in control plants, 17.7 mmol $m^{-2} s^{-1}$ under WD conditions and 70.8 mmol $m^{-2} s^{-1}$ on average at the onset of esca leaf symptoms). The physiological differences between these treatments are presented in detail in Bortolami et al. (2021).

2.2 | Wood Sample Collection

At the end of the 2019 season, in early October, a 2 cm transverse section was cut 15 cm from the top of the trunk of 21 plants ($n = 7$ control plants, $n = 7$ WD plants, $n = 7$ plants with esca symptoms, Table S1). Sample collection was done on average 10 weeks after symptom expression (Table S1). The WD plants were still under stress at the time of wood collection (Bortolami et al. 2021). We used a sterilized chisel and a sterile field (radius 30 cm) provided by an electric heater (Microbio, K-Factory, France) to sample healthy wood samples (i.e., wood that is not visibly necrotic). Metatranscriptomic, untargeted metabolomic and fungal metabarcoding analyses were performed on the same healthy wood samples.

2.3 | RNA Extraction

Frozen wood samples ($n = 21$) were ground in liquid nitrogen with a Tissue Lyser. RNA was extracted by the Transcriptome facility (University of Bordeaux, INSERM, PUMA, Neurocentre Magendie, Bordeaux, France) from 0.5 g tissue, according to the modified protocol described by Blanco-Ulate et al. (2013). Each sample was mixed with 100 µL mercaptoethanol and 5 mL extraction buffer (2% CTAB, 2% PVP, 100 mM Tris, 2 M NaCl, 25 mM EDTA, 0.05 g mL^{-1} spermidine). Samples were vortexed and incubated at 65°C for 10 min, then vortexed again. An equal volume of CIA (chloroform:IAA 24:1) was added and the samples were centrifuged at 2575 g for 30 min at 4°C. The supernatant was transferred to a fresh 15 mL Falcon tube, placed on ice and an equal volume of CIA was added. Samples were centrifuged at 2575 g for 30 min at 4°C. The supernatant was transferred to new tubes, and 10 mM LiCl, equivalent to ¼ of the sample volume, was added. Samples were incubated at −20°C for 1 h. They were then centrifuged at 4°C and 2575 g for 45 min. The pellet was dried and resuspended in 100 µL RNase-free water. The RNA Cleanup Kit (Qiagen) was then used according to the manufacturer's instructions. RNA quality was assessed with a fragment analyzer (Agilent).

2.4 | RNA Sequencing

RNA sequencing (RNA-seq) was performed at the GeT-PlaGe core facility, INRAE Toulouse, France. RNA-seq libraries were prepared according to the Illumina TruSeq Stranded mRNA sample preparation kit protocol. In brief, mRNA was selected with poly-T beads and fragmented to generate double-stranded cDNAs, to which adaptors were ligated for sequencing. Libraries were amplified by 11 PCR cycles. Library quality was assessed with an Advanced Analytical Fragment Analyzer (Agilent), and libraries were quantified by quantitative PCR on a QuantStudio 6 device (Applied Biosystems, Thermo Fisher Scientific) with the KAPA Library Quantification Kit (Roche, KK4824). Sequencing was performed on an Illumina NovaSeq 6000 to generate paired-end reads of 2×150 bp with the corresponding kits.

2.5 | Metatranscriptomic, Bioinformatic, and Data Analyses

The primary-scaffold genomes available for *Vitis vinifera* cv. Sauvignon blanc (Urra et al. 2023) and grapevine trunk-associated fungi were concatenated and used as a multi-species reference. Fungal genomes were selected according to their pathogenicity in grapevine wood. Eight fungi were selected from the NCBI and Grapegenomics databases (Table S2):

- *Phaeom chlamydospora, Phaeoa minimum*, and *F. mediterranea*, three fungi often associated with trunk necrosis that occur simultaneously with the expression of esca leaf symptoms (Claverie et al. 2020; Moretti et al. 2021).
- *B. dothidea, D. seriata, N. parvum*, three Botryosphaeriaceae species associated with *Botryosphaeria* dieback (Belair et al. 2022).
- *D. ampelina*, responsible for *Phomopsis* dieback (Gonzalez-Dominguez et al. 2022) and *Phomopsis* cane and leaf spot (Lawrence et al. 2015).
- *E. lata*, responsible for *Eutypa* dieback (Rolshausen et al. 2014).

RNA sequencing generated a mean of 93.34 ± 32.25 M reads for control samples, 82.61 ± 13.27 M reads for WD samples and 104.27 ± 31.35 M for esca samples. FASTQ files were trimmed with Trimmomatic v0.39 (Bolger et al. 2014) with the *slidingwindow* and *illuminaclip* options and fastp v0.23.2 (Chen et al. 2018), and their quality was assessed with FASTQC v0.12.1 (Andrews 2010). Trimmed files contained a mean of 92.53 ± 32.09 M reads for control samples, 81.75 ± 13.13 M reads for WD samples and 103.28 ± 31.10 M for esca samples, with a mean Phred score above 35 for each base and no adapter sequences.

A first pre-alignment against concatenated genomes of *Vitis vinifera* cv. Sauvignon blanc (Urra et al. 2023) and grapevine trunk-associated fungi as a multi-species reference was performed with Hisat2 v2.2.1 (Kim et al. 2015), resulting in a mean overall alignment rate of 99.15%. Samtools v1.20 (Danecek et al. 2021) was then used to retain only high-quality alignments (−q 20) with a CIGAR score above 50 (−m 50) and to remove unaligned sequences (−F 4). This high-quality read alignment contained a mean of 100 M reads per sample. Alignment files were then filtered with the Samtools view and grep commands, to separate the grapevine and fungal sequences. These sequences were then converted to FASTQ sequences and pseudoaligned against grapevine trunk-associated fungal transcriptomes and genomes or the *Vitis vinifera* cv. Sauvignon blanc primary genome and transcriptome with Salmon v1.10.0 (Patro et al. 2017). The number of transcripts per million (TPM) and estimated counts were obtained from Salmon quant files.

Differential expression analyses were performed on fungal and grapevine genes with the DESeq2 R package v4.2.2 (Love et al. 2014). As the numbers of detected genes and counts were low for some fungi, particularly in control conditions, a pseudocount of 1 was added to the fungal count matrix for the analysis of differential expression between the esca and WD samples without the use of controls. A post hoc power analysis was performed using dispersion and effect-size estimates from the DESeq2 differential expression analysis. Genes with $\log2FC \geq 1$ and sufficient mean expression (baseMean > 5) were considered truly differentially expressed, and empirical power was computed within strata of mean expression as the proportion of such genes detected at FDR < 0.05. Lists of grapevine DEG (differentially expressed genes) were used for statistical tests to assess functional category enrichment with the Vitisnet annotation of PN40024 protein-coding genes (Grimplet et al. 2009). Gene-count normalization (median of ratios method) was used with DESeq2. Volcano plots, sparse partial least squares discriminant analysis (sPLS-DA) and other analyses were performed with R (R Core Team 2023), using the ggplot2 v4.2.3 and mixomics v6.22.0 packages (Rohart et al. 2017; Wickham 2011). The mixomics perf() function was used to assess the sPLS-DA model performance and error rate (ER). The ER value, which ranges from 0 to 0.5, indicates the quality of the separation between groups. A value close to 0 indicates good separation, while a value close to 0.5 indicates separation that is almost random.

2.6 | Genome Annotation

We used BLASTP v.2.2.28+ to align the predicted proteins of the primary contigs of Sauvignon blanc with PN40024 proteins (V1 annotation, Jansen et al. 2006). Alignments with an identity greater than 80% and a reciprocal reference:query coverage between 80 and 120% were retained. The alignments with the highest product of identity, query coverage, and reference coverage were selected to identify pairs of homologous genes. The table of correspondence between Sauvignon blanc and PN40024 was used to assign functional categories from Vitisnet to Sauvignon blanc genes. We then assessed functional category enrichment with Fisher's tests performed in R.

Fungal genomes were annotated for conserved virulence factor domains and homology with carbohydrate-active enzymes, secondary metabolites, transporters, peroxidases, and cytochromes, with the dbCAN3 (Zheng et al. 2023),

AntiSMASH (Blin et al. 2023), TCDB (Saier et al. 2020), fPoxDB (Choi et al. 2014), and CYPED (Fischer et al. 2007) databases, containing potential virulence factors with HMMER. An sPLS-DA analysis was performed with the sum of all read counts with the same virulence factor annotation per fungus as an input.

2.7 | Untargeted Metabolomics: Extraction, Mass Spectrometry and Data Analysis

Metabolomic analysis was performed by UHPLC-LTQ-Orbitrap mass spectrometry (LCMS) following the same procedure as previously described (Dell'Acqua, Gambetta, Comont, et al. 2025; Dussarrat et al. 2022; Martins et al. 2022). Semipolar compounds, including primary and secondary metabolites, were extracted with automated high-throughput ethanol extraction procedures at the MetaboHUB-Bordeaux Metabolome (https://metabolome.u-bordeaux.fr/) facility, using 10 mg of freeze-dried grapevine wood material, according to published protocols (Luna et al. 2020). LCMS analyses of wood samples were run in a random sequence, together with extraction blanks (prepared without plant material and used to rule out potential contaminants detected by untargeted metabolomics), and quality control (QC) samples prepared by mixing 20 μL of each sample as done by Dell'Acqua, Gambetta, Comont, et al. (2025). Raw LCMS data were processed with MS-DIAL v 4.9 (Tsugawa et al. 2015) as previously described (Dell'Acqua, Gambetta, Comont, et al. 2025). Data curation was performed on the 13,356 detected features according to blank check and setting thresholds as signal-to-noise ratio (SN) > 10 and coefficient of variation in quality controls < 30%. The final 3184 features retained for predictive metabolomics annotations were annotated with an in-house chemical library and the FragHUB database (Dablanc et al. 2024) as described by Dell'Acqua, Gambetta, Bartlett, et al. (2025). Annotated features were compliant with the Metabolomics Standards Initiative (MSI) confidence levels ranging from MSI2 to MSI4 (Sumner et al. 2007).

Normalization against the sample median, square root data transformation and Pareto scaling were applied to metabolomic data with Metaboanalyst 6.0 (Pang et al. 2024). Volcano plots were generated in R, using Metaboanalyst fold change and *t*-tests. Thresholds were set at a FDR (false-discovery rate) < 0.05 (Welch's *t*-test and Benjamini–Hochberg correction) and a fold change (FC) threshold > 1.5. Pathway enrichment *p*-values and pathway impact were calculated from differentially abundant metabolite lists with the Metaboanalyst pathway analysis tool and the *Arabidopsis thaliana* (At) set, which allowed us to annotate 48.5% of the differentially expressed metabolites. The pathway impact reflects the relative importance of the matched metabolites within a given metabolic pathway and is calculated by summing the importance scores of the matched metabolites within a pathway and normalizing this value by the sum of the importance scores of all metabolites associated with that pathway following Xia and Wishart (2010). In addition to performing independent analyses, we used the transcriptomic and metabolomic datasets to explore alternative potential markers of each stress response. We did this by using the MixOmics R package (Rohart et al. 2017) (1) to perform independent sPLS-DA analyses, (2) to assess the correlation between the transcriptomic and metabolomic components, and (3) to generate a heatmap visualization of the top variables of each type of data among the first two components.

2.8 | Metabarcoding: DNA Extraction, Amplification and Sequencing

For the metabarcoding analysis, the P16 control sample did not generate a library of sufficient quality and was discarded (this sample was not detected as an outlier in the other analyses), resulting in the retention of only 20 ($n = 6$ control plants, $n = 7$ WD plants, $n = 7$ esca plants) of the 21 initial samples for analysis. We used the experimental procedure and the same ITS1F-ITS2 primer pair to amplify the region of the fungal internal transcribed spacer (ITS) ribosomal DNA gene as described by Dell'Acqua, Gambetta, Bartlett, et al. (2025). The first PCR mixture and cycles were also done as described by Dell'Acqua, Gambetta, Bartlett, et al. (2025). The second PCR and amplicon sequencing (Illumina V3 kit: 2 × 300 bp paired-end reads) were performed by the Genome Transcriptome Facility of Bordeaux according to standard protocols.

2.9 | Metabarcoding: Bioinformatic and Data Analyses

Bioinformatic analyses were performed as fully described by Dell'Acqua, Gambetta, Bartlett, et al. (2025), with the FROGS tools hosted by the Galaxy platform (Escudié et al. 2018). OTU affiliations were checked by additional blast analyses against the NCBI (nr/nt) nucleotide collection (https://blast.ncbi.nlm.nih.gov/Blast. cgi) and trunkdiseaseID.org, a database specific for grapevine trunk disease pathogens. Although the ITS region is insufficient for species-level identification of most fungi (as shown by the OTU table presented in Table S3), this cross-reference approach ensures species-level annotation of grapevine trunk pathogens.

Statistical analyses were performed with R v4.2.1. Alpha and beta diversity metrics were calculated with R phyloseq package v1.34.0 (McMurdie and Holmes 2013). The Shannon and Simpson alpha diversity indices of the trunk fungal communities were compared between treatments by ANOVA followed by Tukey's post hoc tests. A permutational analysis of variance (PERMANOVA) was performed on CLR-transformed data, with R package vegan v2.6-4 (Oksanen et al. 2025), to assess the effect of plant status on fungal community structure. The relative abundances of eight wood pathogen species included in the RNA-seq analysis were compared between treatments by ANOVA followed by Tukey's post hoc tests.

3 | Results

3.1 | Effect of Grapevine Ecophysiological Status on Wood Gene Expression Profiles

We assessed grapevine wood responses to esca and WD by transcriptomic profiling using RNA-seq. In total, 2817 genes were identified as differentially expressed (FC ≥ 1.5, $p \leq 0.01$)

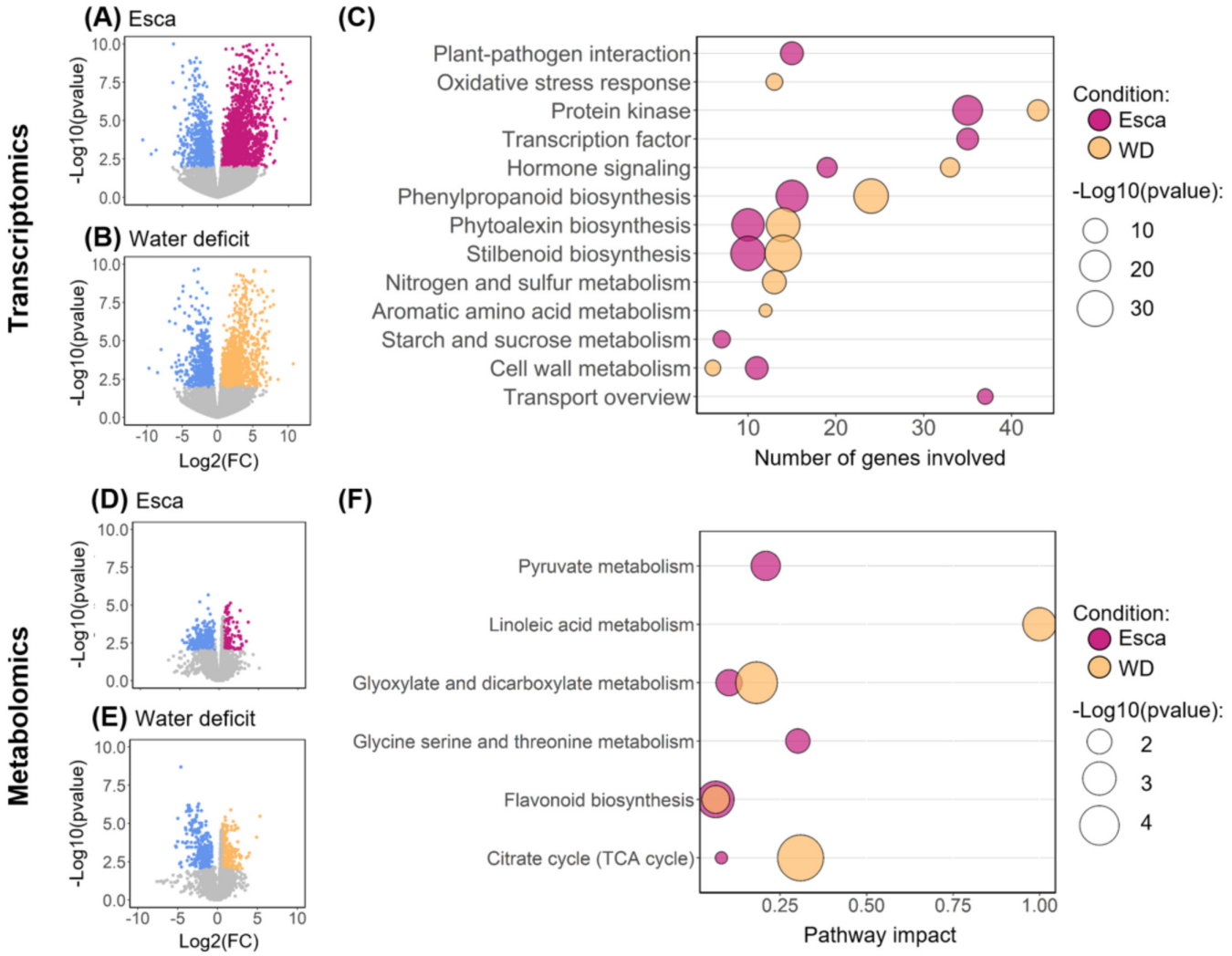

FIGURE 2 | Volcano plots showing the (A, B) differentially expressed genes (FC ≥1.5, $p \leq 0.01$) and (D, E) metabolic features of *V. vinifera* cv. Sauvignon blanc differing (A, D) between the esca and control groups and (B, E) between the WD and control groups. The downregulated genes and metabolic features are represented as blue dots, and the upregulated genes and metabolic features are represented as pink (esca) and yellow (WD) dots. (C) Functional categories displaying enrichment among the genes upregulated in grapevine in response to esca leaf symptoms and WD conditions. (F) Pathways with a $p \leq 0.05$ (enrichment test) and a pathway impact > 0 from the lists of metabolic features displaying enrichment in response to esca and WD. FC, fold change.

in response to the expression of esca leaf symptoms during the summer preceding sampling in the fall (Figure 2A; Table S4). These DEGs consisted of 1816 genes upregulated in the presence of esca symptoms and 1001 genes downregulated in the presence of esca symptoms. WD induced the differential expression of a smaller number of genes, 2347 in total: 1370 upregulated and 977 downregulated (Figure 2B; Table S5). We identified 808 genes upregulated and 363 downregulated in both the WD and esca samples, suggesting the regulation of similar pathways in response to these two stresses.

We investigated the biological mechanisms and pathways affected by both treatments and those affected by only one of the treatments, by performing an analysis of functional category enrichment among DEGs. The functional categories displaying enrichment among the genes upregulated in both the drought and esca groups included "protein kinase," "hormone signaling," "phenylpropanoid biosynthesis," "phytoalexin biosynthesis," "stilbenoid biosynthesis" and "cell wall metabolism" (Figure 2C). By contrast, for some functional categories, enrichment was detected only among the genes most strongly expressed in response to a single treatment. For the WD response, categories such as "oxidative stress response", "nitrogen and sulfur metabolism" and "aromatic amino acid metabolism" were found to be enriched (Figure 2C). Genes encoding proteins involved in ascorbic acid metabolism, glutathione S-transferases, and thioredoxins from these functional categories were identified. In response to esca symptoms, functional categories such as "plant-pathogen interactions," "transcription factors," "starch and sucrose metabolism," and "transport overview" were found to be enriched (Figure 2C). The overexpressed genes from these categories (Table S6) included genes annotated as PR (pathogenesis-related) proteins and transcription factors (TF), such as AP2 (Apetala2), WRKY, zinc finger proteins, and NAC (NAM, ATAF1/2, CUC2) in particular, all of which have been linked to the grapevine response to pathogens and the induction of disease resistance. A large number of genes encoding stilbene

synthases (STS)—key enzymes in stilbene synthesis—and certain phenylalanine ammonia-lyase (PAL) genes were also found to be overexpressed in response to esca leaf symptom expression (17 STS and 3 PAL genes; Table S6). Tethering factors, coat proteins, and ABC multidrug transporters were the most frequently identified protein transport-related genes.

3.2 | Effect of Grapevine Ecophysiological Status on Wood Metabolites

We also investigated the response of grapevine to WD and esca leaf symptom expression at the metabolomic level in healthy trunk samples. A volcano plot analysis identified metabolomic features with differential abundances (DAM; Table S7) in response to esca (Figure 2D) and WD (Figure 2E). WD condition induced a larger number of DAM (332 overabundant and 340 underabundant) than esca (184 overabundant and 272 underabundant). Based on the list of overabundant metabolomic features, we were able to detect an enrichment in certain metabolic pathways (Figure 2F). "Glyoxylate and dicarboxylate metabolism", "flavonoid biosynthesis" and "citrate cycle" were significantly affected in both conditions (esca and WD), with impact scores ranging from 0.06 (flavonoid biosynthesis, WD and esca) to 0.31 (citrate cycle, WD). "Pyruvate metabolism" and "glycine, serine and threonine metabolism" were found to be significantly affected only by esca symptom expression, with impact scores of 0.21 and 0.30, respectively. Only one of the overabundant metabolites was recognized as belonging to the stilbene class (WPPYDDRXZLFEFL-VOTSOKGWSA-N). This metabolite was frequently detected in the esca and WD groups, with fold changes of 1.95 (WD, FDR = 0.04) and 2.38 (esca, FDR = 0.02). A large number of phenylpropanoid compounds were also detected in plants subjected to stress (41 WD, 22 esca), and 15 of these compounds were common to both stresses. The highest pathway impact score was that for "linoleic acid metabolism" in WD conditions. For this pathway, two of four metabolic features were found to be overabundant: linoleic acid and 13(S)-HPODE (hydroperoxyoctadeca-dienoic acid). This pathway had an impact of 1 in the presence of esca symptoms, corresponding to a non-significant *p*-value, and only one metabolic feature was detected (linoleic acid). Finally, "pyruvate metabolism" and "glycine, serine and threonine metabolism" were specifically enriched in response to esca leaf symptom expression.

3.3 | Integration of Grapevine Transcriptome and Metabolome Data

According to the sPLS-DA, transcriptomic data (Figure 3A) were unable to discriminate between samples from the esca and WD groups, whereas samples from control and stressed (esca leaf symptoms or WD) conditions were distinguished moderately well by the second component (Error Rate, ER = 0.38; Figure 3A). The first component seemed to be related to differences between individuals rather than conditions (ER = 0.61; Figure 3A). Metabolomic data clearly distinguished the three physiological states of the plants, with the first component tending to differentiate between the control and stressed conditions (ER = 0.48), whereas the second component differentiated between WD, control and esca conditions (ER = 0.09; Figure 3B). The latent components of each block (the grapevine transcriptome and metabolome datasets) were highly correlated (Pearson's correlation $r = 0.96$).

For each type of data and for the first two components, selection of the 20 variables with the highest loading was the best option for classifying the samples using a heatmap (Figure 3C, Tables S8 and S9). These variables clearly separated the three sets of experimental conditions, demonstrating the existence of different responses to WD and esca. Four main groups were visible on the heatmap: (1) a group of genes displaying enrichment in WD conditions and depletion in the presence of esca symptoms, principally composed of genes encoding a MYND family transcription factor, a protein involved in pyrimidine metabolism, an RNA transporter and a protein involved in plastid organization and biogenesis, together with metabolites such as trehalose and an isoflavonoid (probably ononin); (2) a group of genes displaying a high degree of enrichment in control conditions, including a fatty acid transporter gene, and the genes encoding 2-heptylhexanedioic acid and 9,12,13-trihydroxyoctade c-10-enoic acid; (3) a group of metabolic features overabundant in both stress conditions (WD and esca) including two phenylpropanoids, a benzenoid, a derivative of quinic acid (probably 4,5-dicaffeoylquinic acid), 7-O-methylbavachin, and a terpene lactone; (iv) a group of genes and metabolic features displaying enrichment in the esca group relative to the control and WD groups: three flavonoids recognized as catechins, two benzophenones, a transcription factor from the jasmonate-mediated signaling pathway, and a gene involved in phenylalanine biosynthesis and the oxidative stress response.

3.4 | Effect of Ecophysiological Status on the Trunk Fungal Community, as Assessed by Metabarcoding

In total, 934,497 reads were generated and assigned to 342 fungal operational taxonomic units (OTUs) in apparently healthy trunk samples. The decontaminated dataset (excluding untargeted and putatively contaminant OTUs) contained 789,458 reads across 20 environmental samples, affiliated with 306 OTUs. The decontaminated OTU table is provided in Table S3, and the numbers of reads and OTUs per sample are provided in Table S10.

The fungal communities differed between biological replicates (Figure 4A) and were composed of 80% *Ascomycota*, with three dominant species: *P. chlamydospora* (Eurotiomycetes, 29.6% of the total community), *P. minimum* (Sordariomycetes, 7.4%) and *Pleosporales* sp. (Dothideomycetes, 5.3%). *Basidiomycota* accounted for 16.4% of fungal OTUs, the three most abundant species being *F. mediterranea* (Agaricomycetes, 5.0%), *Peniophora* sp. (Agaricomycetes, 3.7%), and *Phellinus rhamni* (Agaricomycetes, 3.2%). Only 2.1% of OTUs were unaffiliated at the class level.

Neither the observed richness nor the alpha diversity metrics (i.e., Shannon and Simpson indices) of fungal communities from trunk healthy wood samples differed significantly between plant physiological states ($p = 0.21$, $p = 0.51$ and $p = 0.98$, respectively; Figure 4B). However, there was a trend towards lower

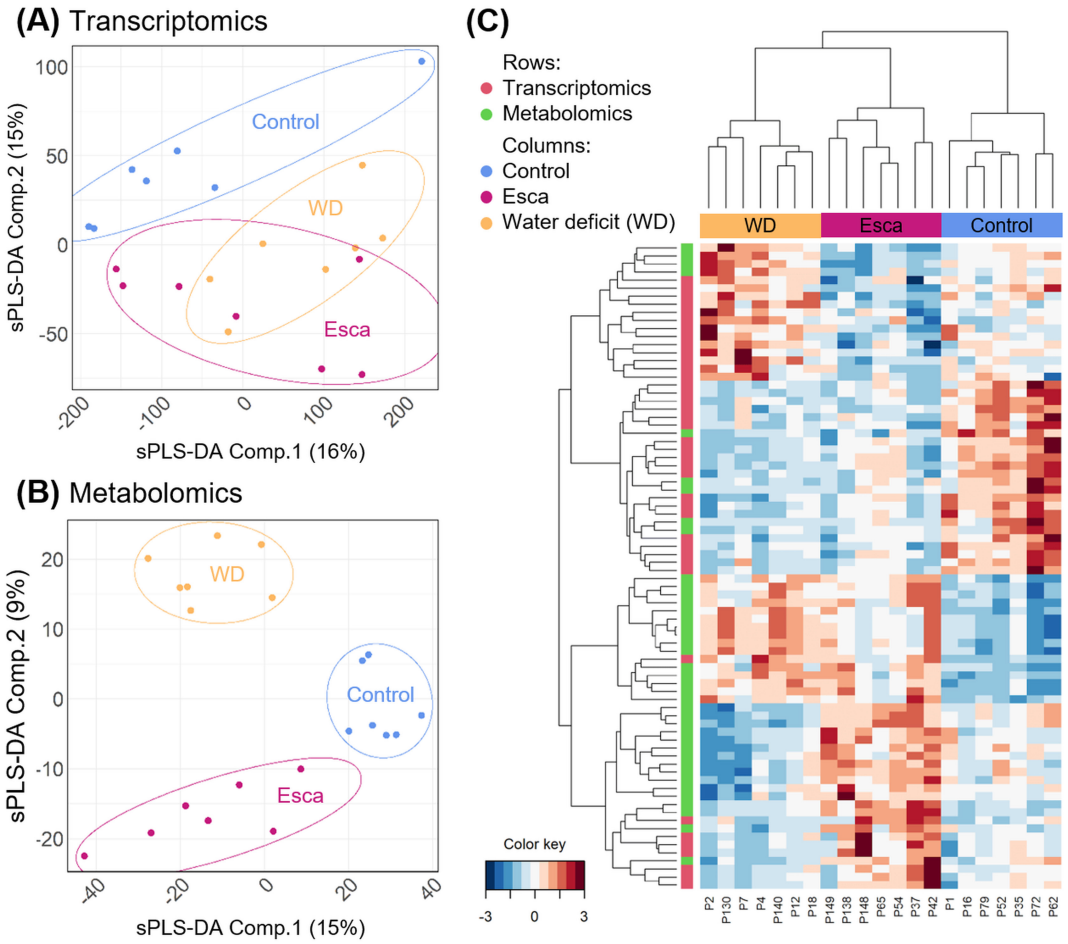

FIGURE 3 | Multi-omic dataset overview of the responses of *V. vinifera* cv. Sauvignon blanc to esca leaf symptom expression and WD. sPLS-DA (sparse partial least squares discriminant analysis) of (A) grapevine transcriptomic (Comp. 1 Error Rate ER = 0.61; Comp. 2 ER = 0.38) and (B) metabolomic (Comp. 1 ER = 0.48; Comp. 2 ER = 0.09) data. (C) Heatmap of 21 individual plants and 40 variables selected with the MixOmics R package for each dataset (components 1 and 2) as best explaining the differences between conditions. Values were scaled and trimmed to the range [−3, 3] for visualization, with lower values shown in blue and higher values in red.

fungal diversity in plants expressing esca leaf symptoms, and even lower in WD plants, than in controls. Regarding beta diversity, PCA and PERMANOVA analyses revealed that plant physiological status accounted for 16.1% of the variance of healthy wood fungal communities ($p = 0.001$; Figure 4C). The structure and composition of these fungal communities differed significantly ($p = 0.006$) between esca and control plants, between WD and control plants ($p = 0.02$), and between WD and esca plants ($p = 0.01$).

We specifically addressed the effect of plant physiological status on the relative abundance of the eight pathogens included in the RNA-seq analysis. Overall, they accounted for approximately 20% of the fungal community in control conditions and 55% in the esca and WD groups. Plant physiological status significantly modified the relative abundance of two of the eight species considered: *F. mediterranea* ($p = 0.00009$) and *P. chlamydospora* ($p = 0.005$). *F. mediterranea* was more abundant in the healthy wood of esca plants than in that of control and WD plants (Figure 4D). *P. chlamydospora* was more abundant in the wood of plants from the two stressed groups than in that of control plants (Figure 4d). No clear differences were observed in the relative abundances of (1) the 20 most abundant OTUs (Figure 4A) (2) or the other six species included in the RNA-seq analysis (Figure 4D).

3.5 | Effect of Ecophysiological Status on Grapevine Trunk Pathogen Gene Expression

Metatranscriptomic alignments revealed substantial variation in the number of reads mapping to each fungal species across biological replicates within treatment groups, indicating high biological variability (Figure 5). Across all conditions, the dominant fungal transcripts corresponded to *P. chlamydospora*, *F. mediterranea*, and *P. minimum*. Fungal transcript abundance was markedly lower in control samples (mean = 220.7 ± 710.0 reads; Figure 5A) than in the two sets of stress conditions (mean esca = 19,940.3 ± 98,247.0 reads, mean WD = 10,340.5 ± 29,733.5 reads; Dunn Kruskal-wallis multiple comparison *p*-values adjusted with Holm method = 0.02 for control and esca, and 0.002 for control and WD; Figure 5B,C), suggesting that fungal activity increases in response to WD and esca stress. In control conditions, *P. chlamydospora* and *P. minimum* accounted for the majority of fungal reads. In plants with esca symptoms, transcript levels were highest for *F. mediterranea*

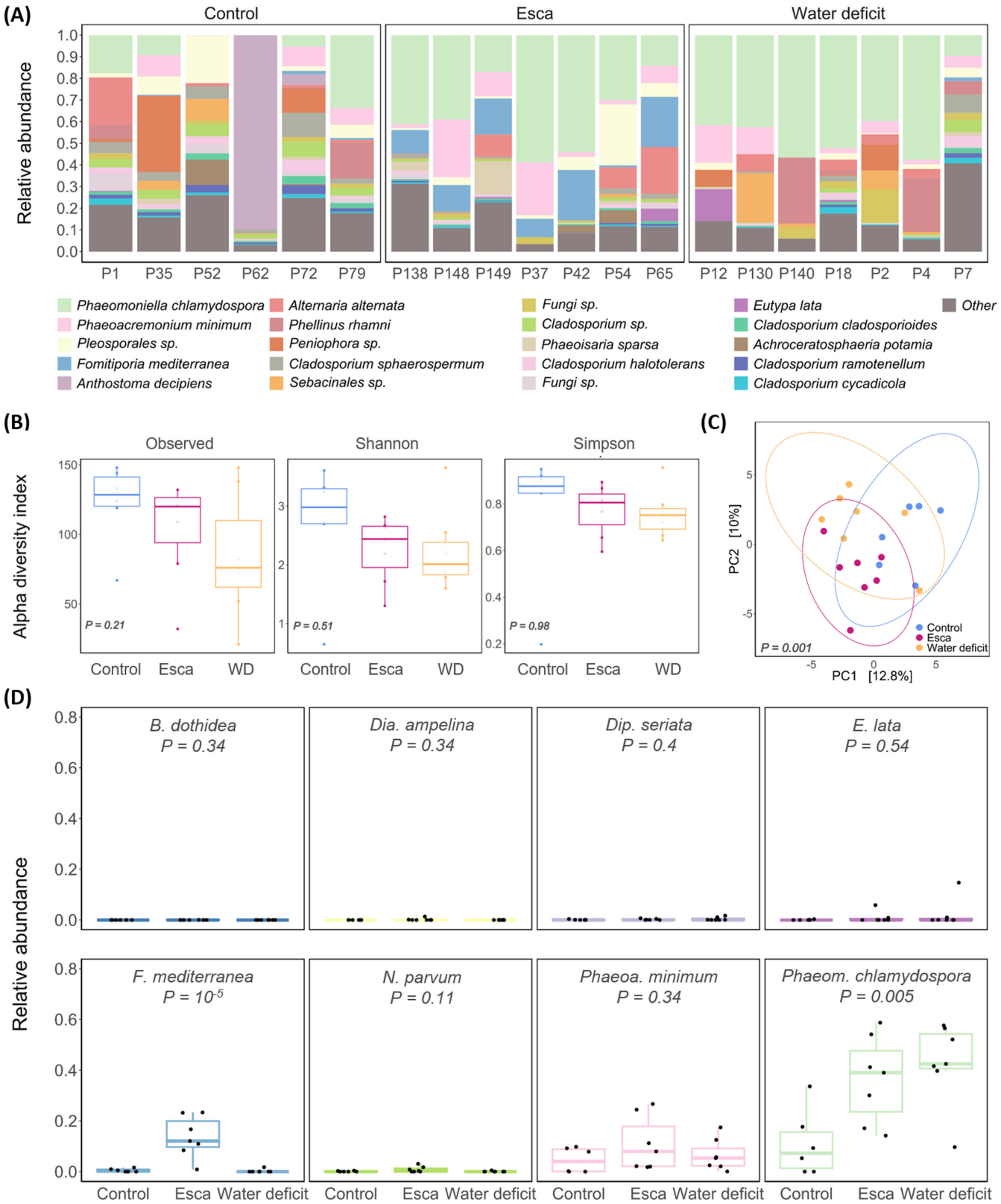

FIGURE 4 | Fungal metabarcoding of healthy wood samples from *V. vinifera* cv. Sauvignon blanc for three treatments: Control, WD, and esca leaf symptom expression. (A) Relative abundance of the top 20 operational taxonomic units (OTUs) per plant sample detected in the control, esca leaf symptom expression, and WD groups (*x*-axis labels). (B) Fungal alpha diversity indices for the control, esca, and WD groups. (C) Principal component analysis (beta diversity). (D) Relative abundances of the eight targeted fungal wood pathogens in the three sets of conditions.

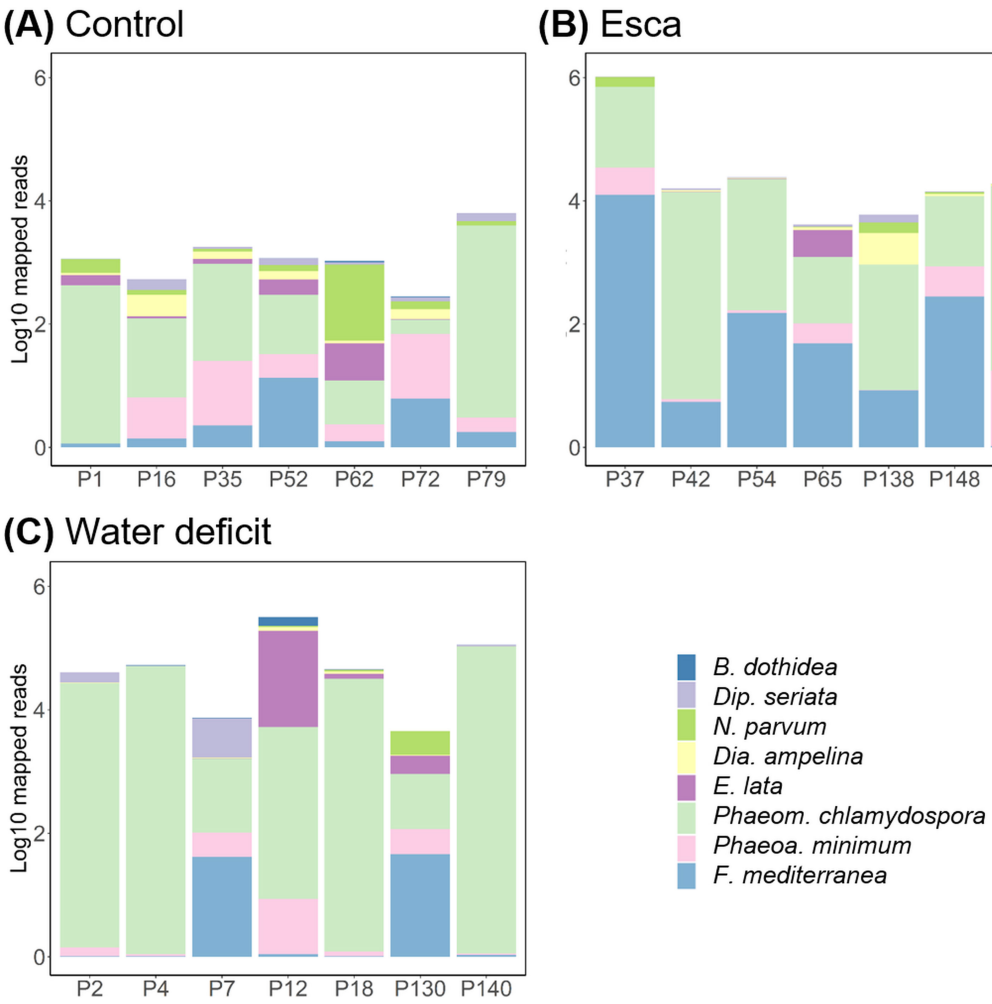

FIGURE 5 | Number of reads aligned with the eight fungal genomes per healthy wood sample (expressed in $\log_{10}$), in control (A), esca-symptomatic (B) and WD (C) *Vitis vinifera* cv. Sauvignon blanc plants. Bars are color-coded according to the percentage of total reads corresponding to each species.

and *P. chlamydospora*. Under WD conditions, transcript levels were consistently highest for *P. chlamydospora* across all samples.

We annotated putative virulence factors in the fungal genomes of plants grown in the three sets of conditions (Figure 6A). For *B. dothidea* and *D. seriata*, which had the lowest read counts, only a few putative virulence factor genes (fewer than 100 total mean normalized counts) were found to be expressed in any of the conditions considered. In control conditions, putative transporter and cytochrome P450 virulence factors were commonly detected in the other six fungi: *N. parvum*, *D. ampelina*, *E. lata*, *P. chlamydospora*, *P. minimum*, and *F. mediterranea*, albeit at lower levels (Figure 6A). A few putative peroxidase virulence factors were also detected for these six fungi, but with low total counts. Putative CAZyme virulence factors were detected in *N. parvum*, *E. lata*, *P. chlamydospora*, *P. minimum*, and *F. mediterranea*. Secondary metabolite virulence factors were detected principally in *N. parvum* and *D. ampelina*, and in *P. chlamydospora* and *F. mediterranea*. In WD conditions, these putative virulence factors were expressed more strongly in *E. lata*, *P. chlamydospora*, and *P. minimum*. In response to esca leaf symptom expression, putative virulence factor expression increased in *F. mediterranea*, *P. minimum*, *P. chlamydospora*, and *N. parvum* and decreased strongly in *D. ampelina* (Figure 6A). However, a large significant increase (x 6.6, $p = 2.10^{-7}$) in putative peroxidase gene expression was observed in *F. mediterranea* in the esca group relative to the other conditions.

The sPLS-DA analysis of putative virulence factor expression distinguished three groups of samples corresponding to the three sets of conditions (control, WD and esca; Figure 6B). The first component clearly separated the control group from the two stressed groups, whereas the second component tended to separate the WD and esca groups. Further analysis showed that the variables separating the three sets of conditions (20 highest loadings for each component, Table S11) were all virulence factors from *F. mediterranea* and *P. chlamydospora*. Transporters (9 from *F. mediterranea* and 3 from *P. chlamydospora*) were the principal elements separating control and stressed samples. According to the second component of the sPLS-DA, *F. mediterranea* virulence factor genes were associated with esca samples whereas *P. chlamydospora* virulence factor genes were associated with WD samples. The putative *F. mediterranea* virulence factor genes identified in esca samples were mostly transporter genes (9 of 13). They included genes encoding a protein involved in the eisosome complex, an mRNA transporter, and a protein

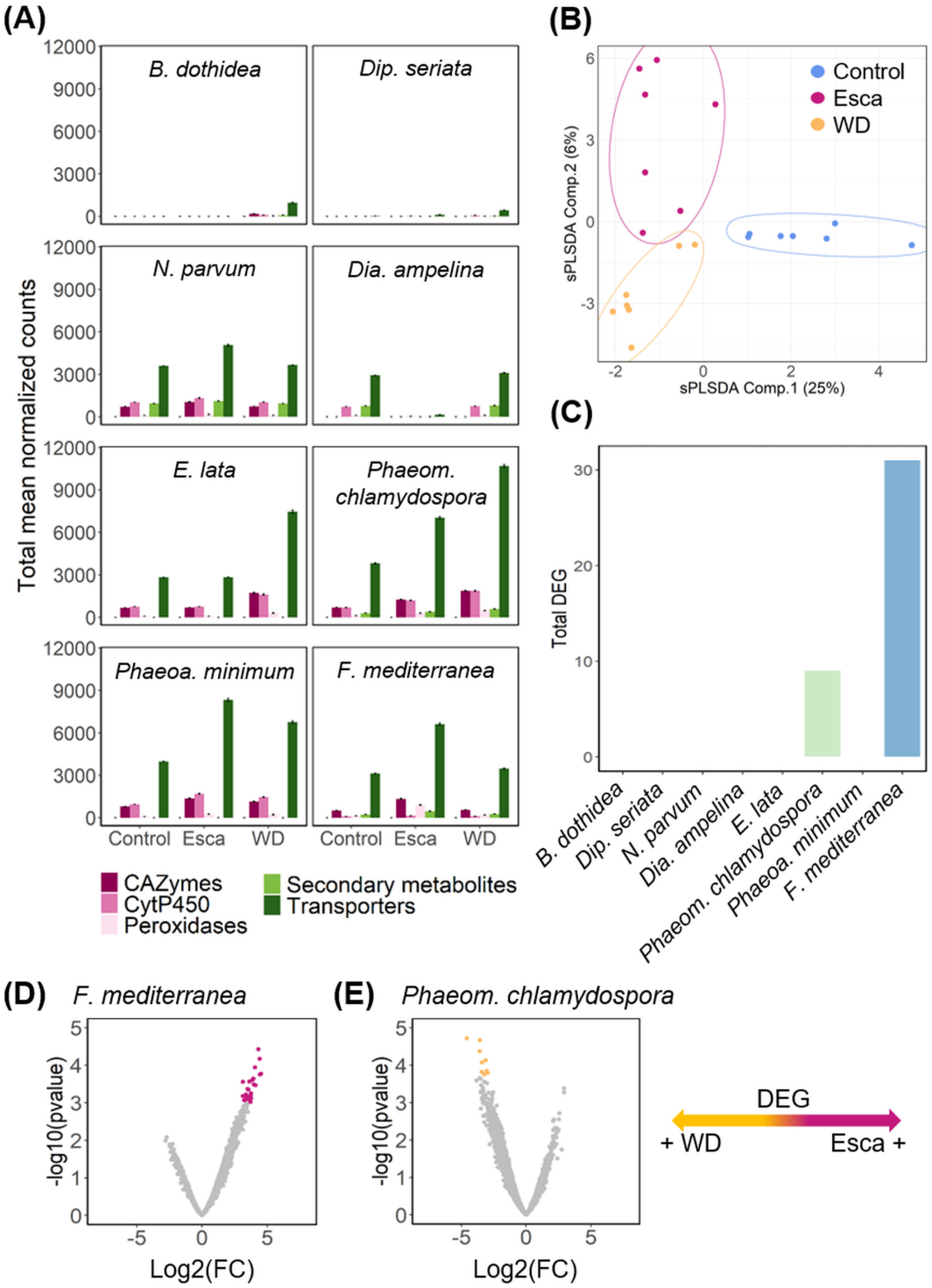

FIGURE 6 | Read counts for putative virulence factors in each of the fungal genomes in control, WD and esca-symptomatic *Vitis vinifera* cv. Sauvignon blanc plants. (A) Normalized expression levels of putative fungal virulence factor genes determined separately for each fungus with the DESeq2 R package. (B) Sparse partial least squares discriminant analysis (sPLS-DA) of the putative virulence genes of all fungi. Analysis of differential expression between esca and WD conditions. (C) Total DEG (FC ≥ 1.5, FDR ≤ 0.05) per fungus identified in the differential expression analysis comparing drought-stressed and esca-symptomatic plants. Volcano plot representation of (D) *F. mediterranea* and (E) *Phaeom chlamydospora* differentially expressed genes (FC ≥ 2, FDR ≤ 0.05) between WD (yellow) and esca expression (pink) conditions, with WD as the reference.

from the nuclear pore complex. Two genes recognized as encoding CAZymes and, more specifically, glucoside hydrolases (GH18 and GH128), a peroxidase (linoleate diol synthase), and a secondary metabolism gene (terpene pathway) were also found. Most of the putative *P. chlamydospora* virulence factor genes associated with WD samples were also transporter genes (5 of 8), including a lipid transporter (PbgA) and three ATPases. A CAZyme gene from the auxiliary activity 4 (AA4) family, a cytochrome P450 gene (sfid 149), and a gene involved in secondary metabolism (type 1 polyketide synthase) were also identified.

The responses of grapevine trunk pathogens to WD and the expression of esca leaf symptoms were compared in a differential analysis. Too few fungal genes were detected in control samples, which were therefore excluded from the analysis. The differential expression analysis therefore compared the effects of the two stresses (WD vs. esca) on gene expression in each fungus (Figure 6). The total number of genes per fungus differentially expressed (FC $\geq$ 1.5, FDR $\leq$ 0.05) between WD and esca conditions (Figure 6C) was low and a post hoc power analysis indicated a low power score (16% for *F. mediterranea*, 1% for *P. chlamydospora* and 0 for other fungi, Table S12). Only two of the eight fungal grapevine trunk pathogens studied presented detectable DEG: *F. mediterranea* (Figure 6D; 31 DEG) and *P. chlamydospora* (Figure 6E; 9 DEG). No DEG could be detected in any of the other fungi. Further investigation of these DEG showed that all the DEG identified in *F. mediterranea* were overexpressed only in esca samples, whereas those of *P. chlamydospora* were overexpressed only in WD samples (Table S13). The *F. mediterranea* DEG overexpressed in esca samples relative to WD samples included genes encoding an ABC transporter, a glucan phosphorylase, a glycosyl hydrolase, an aspartyl protease and a peroxidase. The *P. chlamydospora* genes overexpressed in WD conditions encoded an arylsulfotransferase, an OPT oligopeptide transporter, an SSH4 protein and a calcium-transporting ATPase.

4 | Discussion

Plant physiological status can be altered by various stresses, with consequences for plant-pathogen interactions. The objective of this study was to unravel the molecular responses of the pathobiome to induced abiotic and biotic stresses in grapevine, using *Vitis vinifera*, esca disease, and drought as a model system. In particular, as drought inhibits the expression of esca leaf symptoms (Bortolami et al. 2021), this study aimed to decipher the molecular interaction underlying this antagonism. We first explored the molecular responses of grapevine and wood pathogens to esca leaf symptom expression and WD. We then tested various hypotheses concerning the antagonism between drought and esca symptoms: (1) differences in the composition of the wood fungal community, (2) differences in fungal activity, and (3) differences in metabolism induced by WD.

4.1 | Esca Leaf Symptom Expression Induces a Biotic Stress Response in Grapevine Wood

We previously showed that, in the plants studied here, esca leaf symptom expression induced decreases in transpiring green leaf area, leaf chlorophyll content, photosynthesis, water use efficiency, and total stem nonstructural carbohydrate content (Bortolami et al. 2021). Several weeks after symptom onset (sampling in early fall), we demonstrated that these physiological changes led to changes in the wood transcriptome and metabolome. As expected in the case of fungal diseases of grapevine (Adrian et al. 2012, 2024), esca-symptomatic plants overexpressed genes and overproduced metabolites associated with biotic stress responses and grapevine defense responses. We identified overexpressed genes involved in R proteins, PR proteins, and transcription factors (Dangl and McDowell 2006; Yacoub et al. 2020; Yu et al. 2013, 2016). We also found overexpressed genes involved in the biosynthesis of phenylpropanoids, phytoalexins, stilbenoids and flavonoids, which have been shown to be key players in the response to trunk fungal pathogens and esca response (Billet et al. 2020; Lambert et al. 2013; Ramaroson et al. 2022; Rusjan et al. 2017) as well as in the response to abiotic stresses (Baccouri and Rajhi 2021; Biais et al. 2017; Shen et al. 2022). Furthermore, the synthesis of flavonoids has already been demonstrated in grapevine leaves submitted to an abiotic stress (nitrogen deficiency), in which flavonoid levels were negatively correlated with esca leaf symptom expression (Dell'Acqua, Gambetta, Bartlett, et al. 2025). The accumulation of such defense-associated metabolites in response to esca may protect the plant against stresses occurring after the expression of esca leaf symptoms (Dell'Acqua, Gambetta, Comont, et al. 2025).

Interestingly, a significant enrichment in the "starch and sucrose metabolism" functional category, and particularly genes involved in starch catabolism, was observed in the wood in response to esca leaf symptom expression. Accordingly, esca was found to alter this metabolism, inducing a decrease in stem sucrose and starch content in our plants and altering leaf photosynthesis (Bortolami et al. 2021). Other studies have also reported an effect on starch metabolism in leaves displaying esca symptoms (Ouadi et al. 2021).

4.2 | *F. mediterranea* Is the Fungal Pathogen Responding Most Strongly to Esca Leaf Symptom Expression

Despite the observed increase in the total number of fungal reads in plants with esca symptoms, driven principally by *F. mediterranea* and *P. chlamydospora*, only *F. mediterranea* genes differentiated significantly between esca and the other conditions. Metabarcoding also supported this pattern, with a significant increase in the relative abundances of *P. chlamydospora* and *F. mediterranea* and a marginal increase in *P. minimum* abundance in plants with esca symptoms. Increases in the levels of putative virulence factors of *P. chlamydospora*, *P. minimum*, and *D. ampelina* (Morales-Cruz et al. 2018) and *F. mediterranea* (Nerva et al. 2022) have been documented in plants expressing esca symptoms. However, *Fomitiporia mediterranea*, which specifically causes white rot in wood (Moretti et al. 2021) and is therefore mainly found in this particular wood, is increasingly seen as a key player in esca pathogenesis (Claverie et al. 2020; Fischer and Kassemeyer 2003). Here, we used only healthy non-necrotic wood tissue. Our results indicate that this basidiomycete can actively affect healthy wood, this effect coinciding with the activation of plant defense mechanisms in plants with esca symptoms.

We found that the *F. mediterranea*-specific responses to esca expression and subsequent changes in grapevine physiological status involved four mechanisms that were not observed in asymptomatic control or WD plants. We demonstrated an overexpression of genes associated with growth and developmental processes, such as chitin and cell wall biosynthesis, transporters involved in hyphal growth and reproduction (Xie et al. 2019), a chitinase involved in nutrient acquisition and the

defense of territorial boundaries (Karlsson et al. 2016; Lindahl and Finlay 2006), and the terpene synthesis involved in fungal competition and antifungal activity (El Ariebi et al. 2016). Moreover, we identified genes involved in microenvironment detoxification (Cai et al. 2023; Li et al. 2021; Zhu et al. 2020), including cytochrome b5, suggesting a role in sustaining growth within woody substrates. Genes encoding CAZymes and peroxidases involved in wood degradation and, particularly lignin and cellulose degradation (Janusz et al. 2013; Liu et al. 2017; Qin et al. 2020; Sigoillot et al. 2012), were also shown to be overexpressed. This is not surprising as this white rot fungus has a complete decay arsenal (Moretti et al. 2021). The degradation of healthy wood may underlie the activation of grapevine defense responses described above. An overexpression of *F. mediterranea* genes involved in the mediation of plant stress responses was also observed, including a glycoside hydrolase 128, known to enhance plant susceptibility by inhibiting ROS bursts and callose deposition (Gu et al. 2024), an ABC transporter involved in the efflux of plant defense metabolites (Coleman and Mylonakis 2009), and a peptidase capable of degrading plant proteins (Krishnan et al. 2018).

The activation of these four different mechanisms (growth, detoxification, wood degradation, and plant stress response mediation) in healthy wood from plants expressing esca leaf symptoms suggests that *F. mediterranea* has a high potential to take the upper hand in the competition between fungi upon esca leaf symptom expression (i.e., a specifically altered plant physiological state). The physiological status induced by expression of esca leaf symptoms stimulated *F. mediterranea* growth and territorial competition in healthy wood, probably leading to the onset of wood degradation and white rot production. This observation led to the hypothesis of an esca expression regulatory loop, in which esca expression favors *F. mediterranea* wood colonization, and *F. mediterranea* wood degradation favors esca symptom expression. This loop hypothesis constitutes a change in perspective in our understanding of esca pathogenesis. In accordance with this hypothesis, recent findings have indicated that white rot was significantly more prevalent in symptomatic plants, but only in those that had exhibited symptoms for more than 2 years (Gastou et al. 2025). However, there is growing evidence that trunk surgery or curettage to remove white rot decay due to *F. mediterranea* can decrease both the re-expression of esca symptoms and the abundance of *F. mediterranea* (Bruez et al. 2021; Lecomte et al. 2022; Pacetti et al. 2021), suggesting that a high abundance of *F. mediterranea* in necrotic wood (i.e., a high abundance of white rot tissue) may contribute to esca leaf symptom expression, in addition to being favored by the expression of esca symptoms on leaves. As our study was conducted at a single time point due to the destructive sampling method, future experiments should collect samples at different time points (before, during and after leaf symptom onset) to enhance our understanding of esca pathogenesis.

4.3 | WD Induces a Typical Drought-Stress Response in Grapevine Wood

We previously showed in the plants used here that drought stress induced significant decreases in water potential, whole-plant and leaf gas exchange, and stem and leaf total nonstructural carbohydrate content (Bortolami et al. 2021). The molecular response specific to drought observed in grapevine at the end of the season was similar to that reported for other drought stresses, including the oxidative stress response and hormonal regulation (Braidotti et al. 2024; Carvalho et al. 2015; Tombesi et al. 2015; MacAllister et al. 2019). Several genes or metabolites responsive to abiotic stress have been described as drought response markers, including genes involved in steroid hormone metabolism (Zeng et al. 2024) and in transcription regulation (Mittler et al. 2006). One of these metabolites, trehalose, was identified here. It is involved in stomatal movements and the drought stress response (Gamm et al. 2015; Morabito et al. 2021), consistent with the observed decrease in stomatal conductance. An enrichment in functional categories associated with the drought stress response, such as the "oxidative stress response" and "stilbenoid biosynthesis," was observed in WD conditions. We identified genes encoding proteins involved in the scavenging of ROS, as previously described in responses to oxidative and drought stresses in grapevine (Carvalho et al. 2015; da Fonseca-Pereira et al. 2019; Haider et al. 2017). An enrichment in genes and metabolites involved in the phenylpropanoid, phytoalexin, and stilbenoid biosynthesis functional categories was observed in both the WD and esca groups. In total, 15 *STS* and 2 *PAL* genes were found to be overexpressed in response to WD, suggesting that stilbene compounds, which have already been shown to be associated with the plant response to drought (Valletta et al. 2021), were synthesized.

4.4 | *Phaeom chlamydospora* Is the Fungal Pathogen Most Responsive to Drought

Trunk fungal community richness and diversity were slightly decreased by drought stress, a decrease correlated with an increase in the relative abundance of *P. chlamydospora* in WD samples, as previously reported (Gastou et al. 2026; Leal et al. 2024). However, the relative abundances of other fungal pathogens of wood studied, including *F. mediterranea*, were similar in control and WD samples, both of which were free from esca symptoms. This result suggests that drought stress may have favored *P. chlamydospora* over the other fungal species in healthy wood under WD conditions. Furthermore, expression of the putative virulence factors of *P. chlamydospora*, *E. lata* and *P. minimum* increased in response to WD. However, only the putative virulence factor genes of *P. chlamydospora* drove the difference between esca and WD samples. These putative virulence factors included, in particular (1) transporters known to be involved in nutrition, the formation and germination of spores, pathogenesis, and posttranslational regulation (Adachi et al. 2003; Mandujano-González et al. 2016), (2) carbohydrate-active enzymes involved in growth and virulence (Pan et al. 2021), and (3) enzymes known to be involved in toxin synthesis (Baker et al. 2006; Choquer et al. 2005), and antioxidant mechanisms (Castaño et al. 2022). Investigations of the differentially expressed genes between WD and esca conditions highlighted the role of *P. chlamydospora* genes involved in growth and reproduction (Cuperlovic-Culf and Culf 2014; Masloff et al. 2002; Menotta et al. 2008; Yang et al. 2008). However, as wood decay-associated genes did not appear to be important in our analysis, we can hypothesize that the wood-degrading activity of *P. chlamydospora* is not affected by WD.

P. chlamydospora may, therefore, be able to grow and degrade wood with a low water content, as reported for other fungal species, such as *Coniophora* sp. (Brischke and Alfredsen 2020). Furthermore, as WD increases *P. chlamydospora* activity and abundance in the absence of esca leaf symptom expression, this fungus may not be directly linked to esca expression, contrary to the hypotheses put forward in previous studies (Claverie et al. 2020; Hrycan et al. 2020; Mondello et al. 2018).

4.5 | Exploring the Mechanisms Underlying the Antagonism Between WD and Esca

The first hypothesis we considered here was that WD might alter the composition of the wood fungal community, preventing the expression of esca symptoms on leaves. We tested this hypothesis with healthy wood samples, including functional sapwood, in which water and carbon balances are directly affected by drought, resulting in a low water potential and a lower NSC. Metabarcoding analysis revealed significant differences in the beta-diversity metric between symptomatic (esca) and asymptomatic (WD and control) plants, supporting this hypothesis. In particular, the relative abundance of *F. mediterranea* was significantly lower under WD conditions than in plants with esca symptoms, reaching levels similar to those in control conditions. Thus, the two physiological states in which the plants did not express esca symptoms (control and WD) were associated with a lower relative abundance of *F. mediterranea* than in plants with esca symptoms.

The second hypothesis we tested was that WD might have a negative effect on the activity of fungal wood pathogens, inhibiting their virulence factors and, thus, esca pathogenesis. The total number of fungal reads was greater in stressed conditions, suggesting that WD actually increased overall fungal activity, contrary to this hypothesis. Furthermore, expression of the putative virulence factors of the three fungi most frequently reported to be associated with esca symptom expression was not inhibited or decreased by WD. However, one important difference between WD and esca conditions was the increase in response to WD of the gene expression associated with putative virulence factors of *P. chlamydospora*, whereas that of *F. mediterranea* increased in response to esca leaf symptom expression. *F. mediterranea* may need to switch to the pathogenic mode (Mishra et al. 2021) before symptom onset (early summer or even before during active growth of the grapevine), subsequently inducing esca leaf symptom expression and an increase in *F. mediterranea* abundance at a later time point. The WD applied may have prevented this switch as we observed similar *F. mediterranea* activity in WD and control plants. Alternatively, the antagonism between WD and esca leaf symptom expression might be due to the decrease in transpiration in the plant potentially preventing the transport of fungal molecular signals or plant elicitors from the wood to the leaves. This is difficult to test due to the probably short-lived nature of the signal and its low concentration in sap or plant tissue.

The third hypothesis we considered is that the molecular response of the grapevine to WD might have inhibited the expression of esca leaf symptoms. Grapevines responded to WD and esca by the induction of overlapping but different molecular signatures at the transcriptome and metabolome levels, with the activation of some common stress response pathways. Indeed, the plant stress response involves the activation of pathways that regulate plant tolerance to both biotic and abiotic stressors, a phenomenon known as cross-tolerance (Nadeem et al. 2023). Auxin signaling, which has been reported to be involved in drought-stress tolerance (Braidotti et al. 2024) and interactions with biotic stress responses (Kazan and Manners 2009), was the only hormone-related pathway found to respond to both esca and WD. Genes and specialized metabolites involved in both biotic and abiotic stress tolerance (phenylpropanoids and more specifically stilbenoids) were also detected in both WD and esca conditions, with a higher fold change in esca samples (mean log2(FC) = 5.2 for genes and 5.9 for metabolites) than in WD samples (mean log2(FC) = 4.2 for genes and 2.8 for metabolites). The activation of these biotic and abiotic stress-response

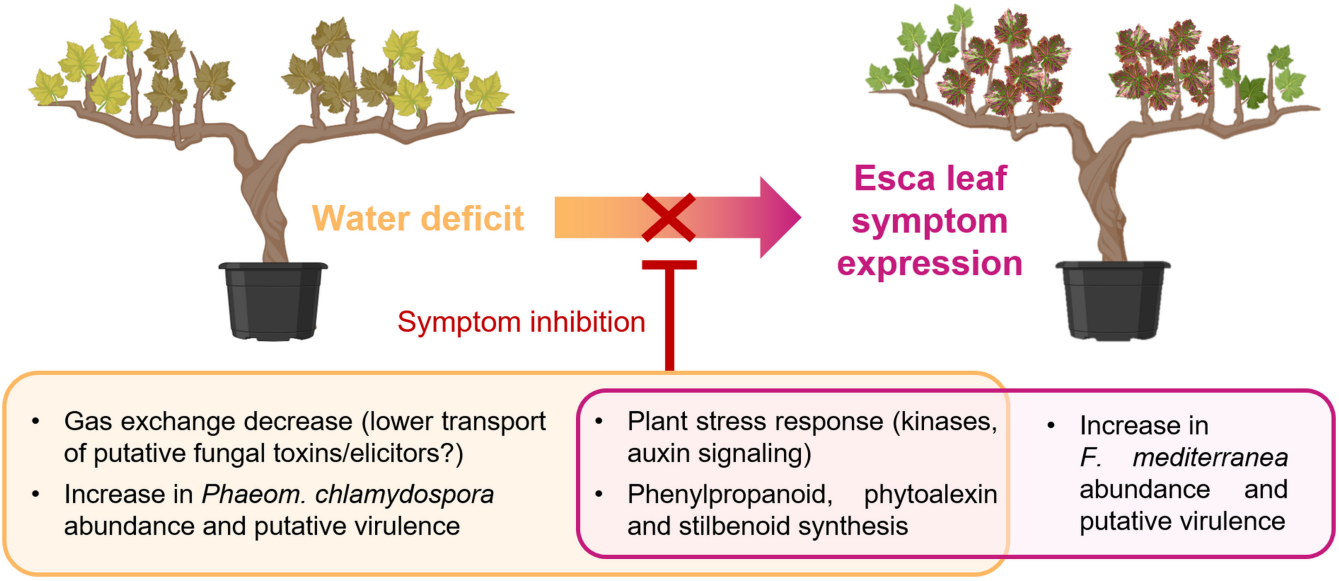

FIGURE 7 | Overview of the underlying molecular mechanisms potentially accounting for the observed antagonism between WD and esca leaf symptom expression.

pathways may have played a key role in the protection of grapevine against esca leaf symptom expression upon drought stress.

In conclusion, our findings reveal that the inhibition of esca leaf symptom expression under drought conditions may result from the priming of plant defenses coupled with low rates of transpiration, and an abundance and putative virulence of the *Basydiomycete* species *F. mediterranea* similar to levels observed in control plants (Figure 7). Interestingly, some pathogens did not appear to respond to either stress, whereas others did, suggesting that the ecology of fungi and their competition for resources could be important factors to explore. Overall, this integrative study highlights the importance of studying both plant and fungal physiology if we are to unravel the mechanisms underlying perennial plant dieback in the context of global changes.

Author Contributions

Chloé E.L. Delmas and Marie Foulongne-Oriol designed this study, obtained the funding and jointly supervised the research. Chloé E.L. Delmas, Gregory A. Gambetta and Giovanni Bortolami conceived the greenhouse experiment. Giovanni Bortolami and Nathalie Ferrer sampled and ground tissues. Marie Chambard designed and implemented the RNAseq bioinformatics pipeline under the supervision of Chloé E.L. Delmas, Marie Foulongne-Oriol, and Dario Cantù. Jadran F. Garcia and Mélanie Massonnet contributed to the metatranscriptomic analysis. Nathalie Ferrer performed the DNA extraction and PCR for metabarcoding. Pierre Gastou analyzed the metabarcoding datasets. Samuele Moretti contributed to the interpretation of functional category analyses on fungi. Adam Rochepeau, Pierre Pétriacq and the MetaboHUB-Bordeaux team performed untargeted metabolomic measurements (extraction and MS-DIAL analysis). Ninon Dell'Acqua prepared the samples for metabolomics; Marie Chambard and Ninon Dell'Acqua analyzed the metabolomic dataset. Marie Chambard integrated the different datasets, produced the figures and wrote the first version of the paper under the supervision of Chloé E.L. Delmas. All authors were involved in the various stages of the project, contributed to scientific discussion and critically revised and approved the final version of the manuscript.

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Data Availability Statement

All sequencing datasets (RNAseq and ITS metabarcoding) are available from the ENA database under accession number PRJEB76229. Metabolomics data are available from the MetaboLights database under accession number REQ20250521210653. The codes for the bioinformatics analyses have been deposited on the INRAE forge at the following link: https://forge.inrae.fr/marie.chambard/gpr_bps_rnaseq1.

References

Adachi, K., G. H. Nelson, K. A. Peoples, et al. 2003. "Sequence Analysis and Functional Characterization of the Dialkylglycine Decarboxylase Gene DGD1 From *Mycosphaerella graminicola*." *Current Genetics* 43: 358–363.

Adrian, M., M.-F. Corio-Costet, A. Calonnec, et al. 2024. "Grapevine Defence Mechanisms When Challenged by Pathogenic Fungi and Oomycetes." *Advances in Botanical Research. Academic Press* 110: 101–195.

Adrian, M., S. Trouvelot, M. Gamm, B. Poinssot, M.-C. Héloir, and X. Daire. 2012. "Activation of Grapevine Defense Mechanisms: Theoretical and Applied Approaches." In *Plant Defence: Biological Control*, edited by J. M. Mérillon and K. G. Ramawat, 313–331. Springer Netherlands.

Anderegg, W. R. L., A. Flint, C. Huang, et al. 2015. "Tree Mortality Predicted From Drought-Induced Vascular Damage." *Nature Geoscience* 8: 367–371.

Andrews, S. 2010. "Babraham Bioinformatics - Fastqc a Quality Control Tool for High Throughput Sequence Data." Accessed November 17, 2023. https://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Baccouri, B., and I. Rajhi. 2021. "Potential Antioxidant Activity of Terpenes." In *Terpenes and Terpenoids-Recent Advances*. IntechOpen.

Baker, S. E., S. Kroken, P. Inderbitzin, et al. 2006. "Two Polyketide Synthase-Encoding Genes Are Required for Biosynthesis of the Polyketide Virulence Factor, T-Toxin, by Cochliobolus Heterostrophus." *Molecular Plant-Microbe Interactions* 19: 139–149.

Belair, M., A. L. Grau, J. Chong, et al. 2022. "Pathogenicity Factors of Botryosphaeriaceae Associated With Grapevine Trunk Diseases: New Developments on Their Action on Grapevine Defense Responses." *Pathogens* 11: 951.

Biais, B., S. Krisa, S. Cluzet, et al. 2017. "Antioxidant and Cytoprotective Activities of Grapevine Stilbenes." *Journal of Agricultural and Food Chemistry* 65: 4952–4960.

Billet, K., M. A. Malinowska, T. Munsch, et al. 2020. "Stilbenoid-Enriched Grape Cane Extracts for the Biocontrol of Grapevine Diseases." In *Plant Defence: Biological Control*, edited by J.-M. Mérillon and K. G. Ramawat, 215–239. Springer International Publishing.

Blanco-Ulate, B., E. Vincenti, A. L. Powell, and D. Cantu. 2013. "Tomato Transcriptome and Mutant Analyses Suggest a Role for Plant Stress Hormones in the Interaction Between Fruit and Botrytis Cinerea." *Frontiers in Plant Science* 4: 142.

Blin, K., S. Shaw, H. E. Augustijn, et al. 2023. "antiSMASH 7.0: New and Improved Predictions for Detection, Regulation, Chemical Structures and Visualisation." *Nucleic Acids Research* 51: W46–W50.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. "Trimmomatic: A Flexible Trimmer for Illumina Sequence Data." *Bioinformatics* 30: 2114–2120.

Bortolami, G., G. A. Gambetta, C. Cassan, et al. 2021. "Grapevines Under Drought Do Not Express Esca Leaf Symptoms." *Proceedings of the National Academy of Sciences* 118: e2112825118.

Braidotti, R., R. Falchi, A. Calderan, et al. 2024. "Multi-Hormonal Analysis and Aquaporins Regulation Reveal New Insights on Drought Tolerance in Grapevine." *Journal of Plant Physiology* 296: 154243.

Brischke, C., and G. Alfredsen. 2020. "Wood-Water Relationships and Their Role for Wood Susceptibility to Fungal Decay." *Applied Microbiology and Biotechnology* 104: 3781–3795.

Bruez, É., C. Cholet, C. Thibon, et al. 2021. "Influence of Curettage on Esca-Diseased *Vitis vinifera* L. cv. Sauvignon Blanc Plants on the Quality of Musts and Wines." *OENO one* 55: 171–182.

Cai, E., H. Jia, R. Feng, et al. 2023. "Cytochrome c-Peroxidase Modulates ROS Homeostasis to Regulate the Sexual Mating of Sporisorium Scitamineum." *Microbiology Spectrum* 11: e02057-23.

Carbone, M. J., S. Alaniz, P. Mondino, et al. 2021. "Drought Influences Fungal Community Dynamics in the Grapevine Rhizosphere and Root Microbiome." *Journal of Fungi* 7: 686.

Carvalho, L. C., P. Vidigal, and S. Amâncio. 2015. "Oxidative Stress Homeostasis in Grapevine (*Vitis vinifera* L.)." *Frontiers in Environmental Science* 3: 20.

Castaño, J. D., N. Muñoz-Muñoz, Y. M. Kim, J. Liu, L. Yang, and J. S. Schilling. 2022. "Metabolomics Highlights Different Life History Strategies of White and Brown Rot Wood-Degrading Fungi." *mSphere* 7: e00545-22.

Chaloner, T. M., S. J. Gurr, and D. P. Bebber. 2021. "Plant Pathogen Infection Risk Tracks Global Crop Yields Under Climate Change." *Nature Climate Change* 11: 710–715.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. "Fastp: An Ultra-Fast All-In-One FASTQ Preprocessor." *Bioinformatics* 34: i884–i890.

Choi, J., N. Détry, K.-T. Kim, F. O. Asiegbu, J. P. Valkonen, and Y.-H. Lee. 2014. "fPoxDB: Fungal Peroxidase Database for Comparative Genomics." *BMC Microbiology* 14: 117.

Choquer, M., K. L. Dekkers, H.-Q. Chen, et al. 2005. "The CTB1 Gene Encoding a Fungal Polyketide Synthase Is Required for Cercosporin Biosynthesis and Fungal Virulence of Cercospora Nicotianae." *Molecular Plant-Microbe Interactions* 18: 468–476.

Chu, H., C. Wang, H. Wang, H. Chen, and M. Tang. 2016. "Pine Wilt Disease Alters Soil Properties and Root-Associated Fungal Communities in Pinus Tabulaeformis Forest." *Plant and Soil* 404: 237–249.

Claverie, M., M. Notaro, F. Fontaine, and J. Wery. 2020. "Current Knowledge on Grapevine Trunk Diseases With Complex Etiology: A Systemic Approach." *Phytopathologia Mediterranea* 59: 29–53.

Coleman, J. J., and E. Mylonakis. 2009. "Efflux in Fungi: La Pièce de Résistance." *PLoS Pathogens* 5: e1000486.

Cuperlovic-Culf, M., and A. S. Culf. 2014. "Role of Histone Deacetylases in Fungal Phytopathogenesis: A Review." *International Journal of Modern Botany* 4: 48–60.

da Fonseca-Pereira, P., D. M. Daloso, J. Gago, et al. 2019. "The Mitochondrial Thioredoxin System Contributes to the Metabolic Responses Under Drought Episodes in Arabidopsis." *Plant and Cell Physiology* 60: 213–229.

Dablanc, A., S. Hennechart, A. Perez, et al. 2024. "FragHub: A Mass Spectral Library Data Integration Workflow." *Analytical Chemistry* 96: 12489–12496.

Danecek, P., J. K. Bonfield, J. Liddle, et al. 2021. "Twelve Years of SAMtools and BCFtools." *GigaScience* 10: giab008.

Dangl, J. L., and J. M. McDowell. 2006. "Two Modes of Pathogen Recognition by Plants." *Proceedings of the National Academy of Sciences* 103: 8575–8576.

Dell'Acqua, N., G. A. Gambetta, M. K. Bartlett, et al. 2025. "The Reduced Canopy Area in Esca-Symptomatic Grapevine Plants Leads to Lower Canopy Transpiration and Mitigates Water Stress." *Plant Physiology* 199, no. 1: kiaf361.

Dell'Acqua, N., G. A. Gambetta, G. Comont, et al. 2025. "Nitrogen Nutrition Impacts Grapevine Esca Leaf Symptom Incidence, Physiology, and Metabolism." *Journal of Experimental Botany* 76: 3225–3242.

Desprez-Loustau, M.-L., B. Marçais, L.-M. Nageleisen, D. Piou, and A. Vannini. 2006. "Interactive Effects of Drought and Pathogens in Forest Trees." *Annals of Forest Science* 63: 597–612.

Dewasme, C., S. Mary, G. Darrieutort, J.-P. Roby, and G. A. Gambetta. 2022. "Long-Term Esca Monitoring Reveals Disease Impacts on Fruit Yield and Wine Quality." *Plant Disease* 106: 3076–3082.

Dussarrat, T., S. Prigent, C. Latorre, et al. 2022. "Predictive Metabolomics of Multiple Atacama Plant Species Unveils a Core Set of Generic Metabolites for Extreme Climate Resilience." *New Phytologist* 234: 1614–1628.

El Ariebi, N., J. Hiscox, S. A. Scriven, C. T. Müller, and L. Boddy. 2016. "Production and Effects of Volatile Organic Compounds During Interspecific Interactions." *Fungal Ecology* 20: 144–154.

Escudié, F., L. Auer, M. Bernard, et al. 2018. "FROGS: Find, Rapidly, OTUs With Galaxy Solution." *Bioinformatics* 34: 1287–1294.

Etienne, L., F. Fabre, D. Martinetti, et al. 2024. "Exploring the Role of Cultivar, Year and Plot Age in the Incidence of Esca and Eutypa Dieback: Insights From 20 Years of Regional Surveys in France." *Plant Pathology* 73: 2344–2358.

Etienne, L., D. Martinetti, E. Frank, et al. 2025. "Identification of Ecoclimatic Indicators of Esca Disease Through 20 Years of Large-Scale Vineyard Monitoring in France." *Plant Disease*. https://doi.org/10.1094/PDIS-01-25-0228-RE.

Fischer, M., and H.-H. Kassemeyer. 2003. "Fungi Associated With Esca Disease of Grapevine in Germany." *Vitis* 42: 109–116.

Fischer, M., M. Knoll, D. Sirim, F. Wagner, S. Funke, and J. Pleiss. 2007. "The Cytochrome P450 Engineering Database: A Navigation and Prediction Tool for the Cytochrome P450 Protein Family." *Bioinformatics* 23: 2015–2017.

Fontaine, F., F. P. Trouillas, J. Armengol, and A. Eskalen. 2025. "Fungal Trunk Diseases: A Global Threat to Grapevines." *Annual Review of Phytopathology* 63: 577–602.

Fu, W., B. Chen, J. Jansa, et al. 2022. "Contrasting Community Responses of Root and Soil Dwelling Fungi to Extreme Drought in a Temperate Grassland." *Soil Biology and Biochemistry* 169: 108670.

Gamm, M., M.-C. Héloir, and M. Adrian. 2015. "Trehalose and Trehalose-6-Phosphate Induce Stomatal Movements and Interfere With ABA-Induced Stomatal Closure in Grapevine." *OENO one* 49: 165–171.

Gastou, P., G. Bortolami, N. Ferrer, et al. 2026. "Differential Impacts of Drought and Esca Expression on Ascomycota Fungi in the Trunks and Young Organs of Mature Grapevines." *Phytobiomes Journal* 10: 57.

Gastou, P., T. Carayol, G. Comont, et al. 2025. "Wood Composition, Rather Than Microbial Communities, Underpins Varietal Differences in Wood Degradation and Esca Foliar Symptom Expression in Grapevine." Preprint, bioRxiv 2025.12.09.693160.

Gastou, P., A. D. Irvine, C. Arcens, et al. 2024. "Large Gradient of Susceptibility to Esca Disease Revealed by Long-Term Monitoring of 46 Grapevine Cultivars in a Common Garden Vineyard." *OENO One* 58, no. 2.

Gonzalez-Dominguez, E., T. Caffi, A. Paolini, et al. 2022. "Development and Validation of a Mechanistic Model That Predicts

Infection by *Diaporthe ampelina*, the Causal Agent of Phomopsis Cane and Leaf Spot of Grapevines." *Frontiers in Plant Science* 13: 872333.

Goodell, B. 2020. "Fungi Involved in the Biodeterioration and Bioconversion of Lignocellulose Substrates." In *Genetics and Biotechnology,* edited by J. P. Benz and K. Schipper, 369–397. Springer International Publishing.

Grimplet, J., G. R. Cramer, J. A. Dickerson, K. Mathiason, J. Van Hemert, and A. Y. Fennell. 2009. "VitisNet: "Omics" Integration Through Grapevine Molecular Networks." *PLoS One* 4: e8365.

Gu, X., Z. Cao, Z. Li, H. Yu, and W. Liu. 2024. "Plant Immunity Suppression by an β-1,3-Glucanase of the Maize Anthracnose Pathogen Colletotrichum Graminicola." *BMC Plant Biology* 24: 339.

Haider, M. S., C. Zhang, M. M. Kurjogi, et al. 2017. "Insights Into Grapevine Defense Response Against Drought as Revealed by Biochemical, Physiological and RNA-Seq Analysis." *Scientific Reports* 7: 13134.

Hamilton, C. E., P. E. Gundel, M. Helander, and K. Saikkonen. 2012. "Endophytic Mediation of Reactive Oxygen Species and Antioxidant Activity in Plants: A Review." *Fungal Diversity* 54: 1–10.

Hrycan, J., M. Hart, P. Bowen, T. Forge, and J. R. Úrbez-Torres. 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.

IPCC. 2021. "Climate Change 2021: The Physical Science Basis." In *Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by V. Masson-Delmotte, P. Zhai, A. Pirani, et al., 2391. Cambridge University Press.

Jansen, R. K., C. Kaittanis, C. Saski, et al. 2006. "Phylogenetic Analyses of Vitis (Vitaceae) Based on Complete Chloroplast Genome Sequences: Effects of Taxon Sampling and Phylogenetic Methods on Resolving Relationships Among Rosids." *BMC Evolutionary Biology* 6: 32.

Janusz, G., K. H. Kucharzyk, A. Pawlik, M. Staszczak, and A. J. Paszczynski. 2013. "Fungal Laccase, Manganese Peroxidase and Lignin Peroxidase: Gene Expression and Regulation." *Enzyme and Microbial Technology* 52: 1–12.

Jones, J. D. G., and J. L. Dangl. 2006. "The Plant Immune System." *Nature* 444: 323–329.

Kabbage, M., O. Yarden, and M. B. Dickman. 2015. "Pathogenic Attributes of Sclerotinia Sclerotiorum: Switching From a Biotrophic to Necrotrophic Lifestyle." *Plant Science* 233: 53–60.

Karlsson, M., J. Stenlid, and B. Lindahl. 2016. "Functional Differentiation of Chitinases in the White-Rot Fungus Phanerochaete Chrysosporium." *Fungal Ecology* 22: 52–60.

Kazan, K., and J. M. Manners. 2009. "Linking Development to Defense: Auxin in Plant–Pathogen Interactions." *Trends in Plant Science* 14: 373–382.

Kim, D., B. Langmead, and S. L. Salzberg. 2015. "HISAT: A Fast Spliced Aligner With Low Memory Requirements." *Nature Methods* 12: 357–360.

Krishnan, P., X. Ma, B. A. McDonald, and P. C. Brunner. 2018. "Widespread Signatures of Selection for Secreted Peptidases in a Fungal Plant Pathogen." *BMC Evolutionary Biology* 18: 7.

Lambert, C., I. L. K. Khiook, S. Lucas, N. Télef-Micouleau, J.-M. Mérillon, and S. Cluzet. 2013. "A Faster and a Stronger Defense Response: One of the Key Elements in Grapevine Explaining Its Lower Level of Susceptibility to Esca?" *Phytopathology* 103: 1028–1034.

Lawrence, D. P., R. Travadon, and K. Baumgartner. 2015. "Diversity of Diaporthe Species Associated With Wood Cankers of Fruit and Nut Crops in Northern California." *Mycologia* 107: 926–940.

Leal, C., R. Bujanda, M. J. Carbone, et al. 2024. "Drought Influences the Structure, Diversity, and Functionality of the Fungal Community Inhabiting the Grapevine Xylem and Enhances the Abundance of *Phaeomoniella chlamydospora*." *Phytobiomes Journal* 8: 529–539.

Lecomte, P., C. Bénétreau, B. Diarra, Y. Meziani, C. Delmas, and M. Fermaud. 2024. "Logistic Modeling of Summer Expression of Esca Symptoms in Tolerant and Susceptible Cultivars in Bordeaux Vineyards." *OENO One* 58, no. 1.

Lecomte, P., C. Cholet, E. Bruez, et al. 2022. "Recovery After Curettage of Grapevines With Esca Leaf Symptoms." *Phytopathologia Mediterranea* 61: 473–489.

Li, S., F. Bonneu, J. Chadoeuf, D. Picart, A. Gégout-Petit, and L. Guérin-Dubrana. 2017. "Spatial and Temporal Pattern Analyses of Esca Grapevine Disease in Vineyards in France." *Phytopathology* 107: 59–69.

Li, W., P. Li, X. Zhou, et al. 2021. "A Cytochrome B5-Like Heme/Steroid Binding Domain Protein, PlCB5L1, Regulates Mycelial Growth, Pathogenicity and Oxidative Stress Tolerance in *Peronophythora litchii*." *Frontiers in Plant Science* 12: 783438.

Lindahl, B. D., and R. D. Finlay. 2006. "Activities of Chitinolytic Enzymes During Primary and Secondary Colonization of Wood by Basidiomycetous Fungi." *New Phytologist* 169: 389–397.

Liu, Y., X. Jiang, D. Guan, et al. 2017. "Transcriptional Analysis of Genes Involved in Competitive Nodulation in Bradyrhizobium Diazoefficiens at the Presence of Soybean Root Exudates." *Scientific Reports* 7: 10946.

Love, M. I., W. Huber, and S. Anders. 2014. "Moderated Estimation of Fold Change and Dispersion for RNA-Seq Data With DESeq2." *Genome Biology* 15: 550–571.

Luna, E., A. Flandin, C. Cassan, et al. 2020. "Metabolomics to Exploit the Primed Immune System of Tomato Fruit." *Metabolites* 10: 96.

Lv, T., C. Zhan, Q. Pan, et al. 2023. "Plant Pathogenesis: Toward Multidimensional Understanding of the Microbiome." *iMeta* 2: e129.

MacAllister, S., M. Mencuccini, U. Sommer, et al. 2019. "Drought-Induced Mortality in Scots Pine: Opening the Metabolic Black Box." *Tree Physiology* 39: 1358–1370.

Maher, N., J. Piot, S. Bastien, J. Vallance, P. Rey, and L. Guérin-Dubrana. 2012. "Wood Necrosis in Esca-Affected Vines: Types, Relationships and Possible Links With Foliar Symptom Expression." *OENO One* 46: 15–27.

Mandujano-González, V., L. Villa-Tanaca, M. A. Anducho-Reyes, and Y. Mercado-Flores. 2016. "Secreted Fungal Aspartic Proteases: A Review." *Revista Iberoamericana de Micología* 33: 76–82.

Martínez-Vilalta, J., and N. Garcia-Forner. 2017. "Water Potential Regulation, Stomatal Behaviour and Hydraulic Transport Under Drought: Deconstructing the Iso/Anisohydric Concept." *Plant, Cell & Environment* 40: 962–976.

Martins, J., P. Pétriacq, A. Flandin, et al. 2022. "Genotype Determines Arbutus Unedo L. Physiological and Metabolomic Responses to Drought and Recovery." *Frontiers in Plant Science* 13: 1011542.

Masloff, S., S. Jacobsen, S. Pöggeler, and U. Kück. 2002. "Functional Analysis of the C6 Zinc Finger Gene pro1 Involved in Fungal Sexual Development." *Fungal Genetics and Biology* 36: 107–116.

McDowell, N. G., G. Sapes, A. Pivovaroff, et al. 2022. "Mechanisms of Woody-Plant Mortality Under Rising Drought, CO2 and Vapour Pressure Deficit." *Nature Reviews Earth & Environment* 3: 294–308.

McMurdie, P. J., and S. Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PLoS One* 8: e61217.

Menotta, M., A. Amicucci, G. Basili, E. Polidori, V. Stocchi, and F. Rivero. 2008. "Molecular and Functional Characterization of a Rho GDP Dissociation Inhibitor in the Filamentous Fungus *Tuber borchii*." *BMC Microbiology* 8: 57.

Mina, D., J. A. Pereira, T. Lino-Neto, and P. Baptista. 2020. "Impact of Plant Genotype and Plant Habitat in Shaping Bacterial Pathobiome: A Comparative Study in Olive Tree." *Scientific Reports* 10: 3475.

Mishra, S., A. Bhattacharjee, and S. Sharma. 2021. "An Ecological Insight Into the Multifaceted World of Plant-Endophyte Association." *Critical Reviews in Plant Sciences* 40: 127–146.

Mittler, R., Y. Kim, L. Song, et al. 2006. "Gain- and Loss-of-Function Mutations in Zat10 Enhance the Tolerance of Plants to Abiotic Stress." *FEBS Letters* 580: 6537–6542.

Mondello, V., A. Songy, E. Battiston, et al. 2018. "Grapevine Trunk Diseases: A Review of Fifteen Years of Trials for Their Control With Chemicals and Biocontrol Agents." *Plant Disease* 102: 1189–1217.

Monod, V., V. Hofstetter, O. Viret, V. Zufferey, K. Gindro, and D. Croll. 2025. "Landscape-Scale Endophytic Community Analyses in Replicated Grapevine Stands Reveal That Dieback Disease Is Unlikely To Be Caused by Specific Fungal Communities." *Applied and Environmental Microbiology* 91, no. 7: e00782-25.

Monod, V., M. Wilhelm, O. Viret, et al. 2025. "Identifying the Pedoclimatic Conditions Most Critical in the Susceptibility of a Grapevine Cultivar to Esca Disease." *OENO One* 59, no. 1.

Morabito, C., F. Secchi, and A. Schubert. 2021. "Grapevine TPS (Trehalose-6-Phosphate Synthase) Family Genes Are Differentially Regulated During Development, Upon Sugar Treatment and Drought Stress." *Plant Physiology and Biochemistry* 164: 54–62.

Morales-Cruz, A., G. Allenbeck, R. Figueroa-Balderas, et al. 2018. "Closed-Reference Metatranscriptomics Enables in Planta Profiling of Putative Virulence Activities in the Grapevine Trunk Disease Complex." *Molecular Plant Pathology* 19: 490–503.

Moretti, S., A. Pacetti, R. Pierron, et al. 2021. "Fomitiporia Mediterranea M. Fisch., the Historical Esca Agent: A Comprehensive Review on the Main Grapevine Wood Rot Agent in Europe." *Phytopathologia Mediterranea* 60: 351–379.

Nadeem, H., A. Khan, R. Gupta, et al. 2023. "Stress Combination: When Two Negatives May Become Antagonistic, Synergistic or Additive for Plants?" *Pedosphere* 33: 287–300.

Nerva, L., J. F. Garcia, F. Favaretto, et al. 2022. "The Hidden World Within Plants: Metatranscriptomics Unveils the Complexity of Wood Microbiomes." *Journal of Experimental Botany* 73: 2682–2697.

Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2025. "vegan: Community Ecology Package. 2.7-2."

Ouadi, L., E. Bruez, S. Bastien, et al. 2019. "Ecophysiological Impacts of Esca, a Devastating Grapevine Trunk Disease, on *Vitis vinifera* L." *PLoS One* 14: e0222586.

Ouadi, L., E. Bruez, S. Bastien, et al. 2021. "Sap Flow Disruption in Grapevine Is the Early Signal Predicting the Structural, Functional, and Genetic Responses to Esca Disease." *Frontiers in Plant Science* 12: 695846.

Pacetti, A., S. Moretti, C. Pinto, et al. 2021. "Trunk Surgery as a Tool to Reduce Foliar Symptoms in Diseases of the Esca Complex and Its Influence on Vine Wood Microbiota." *Journal of Fungi* 7: 521.

Pan, S., L. Tang, X. Pan, L. Qi, and J. Yang. 2021. "A Member of the Glycoside Hydrolase Family 76 Is Involved in Growth, Conidiation, and Virulence in Rice Blast Fungus." *Physiological and Molecular Plant Pathology* 113: 101587.

Pang, Z., Y. Lu, G. Zhou, et al. 2024. "MetaboAnalyst 6.0: Towards a Unified Platform for Metabolomics Data Processing, Analysis and Interpretation." *Nucleic Acids Research* 52: W398–W406.

Patro, R., G. Duggal, M. I. Love, R. A. Irizarry, and C. Kingsford. 2017. "Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression." *Nature Methods* 14: 417–419.

Petek-Petrik, A., P. Petrík, L. J. Lamarque, H. Cochard, R. Burlett, and S. Delzon. 2023. "Drought Survival in Conifer Species Is Related to the Time Required to Cross the Stomatal Safety Margin." *Journal of Experimental Botany* 74: 6847–6859.

Preece, C., E. Verbruggen, L. Liu, J. T. Weedon, and J. Peñuelas. 2019. "Effects of Past and Current Drought on the Composition and Diversity of Soil Microbial Communities." *Soil Biology and Biochemistry* 131: 28–39.

Qin, J., B. Li, and S. Zhou. 2020. "A Novel Glycoside Hydrolase 74 Xyloglucanase CvGH74A Is a Virulence Factor in Coniella Vitis." *Journal of Integrative Agriculture* 19: 2725–2735.

R Core Team. 2023. *R: A Language and Environment for Statistical*. R Foundation for Statistical Computing.

Ramaroson, M.-L., C. Koutouan, J.-J. Helesbeux, et al. 2022. "Role of Phenylpropanoids and Flavonoids in Plant Resistance to Pests and Diseases." *Molecules* 27: 8371.

Rohart, F., B. Gautier, A. Singh, and K.-A. L. Cao. 2017. "mixOmics: An R Package for Omics Feature Selection and Multiple Data Integration." *PLoS Computational Biology* 13: e1005752.

Rolshausen, P. E., K. Baumgartner, R. Travadon, P. Fujiyoshi, J. Pouzoulet, and W. F. Wilcox. 2014. "Identification of Eutypa spp. Causing Eutypa Dieback of Grapevine in Eastern North America." *Plant Disease* 98: 483–491.

Rusjan, D., M. Persic, M. Likar, K. Biniari, and M. Mikulic-Petkovsek. 2017. "Phenolic Responses to Esca-Associated Fungi in Differently Decayed Grapevine Woods From Different Trunk Parts of 'Cabernet Sauvignon'." *Journal of Agricultural and Food Chemistry* 65: 6615–6624.

Saier, M. H., V. S. Reddy, G. Moreno-Hagelsieb, et al. 2020. "The Transporter Classification Database (TCDB): 2021 Update." *Nucleic Acids Research* 49: D461–D467.

Shen, N., T. Wang, Q. Gan, S. Liu, L. Wang, and B. Jin. 2022. "Plant Flavonoids: Classification, Distribution, Biosynthesis, and Antioxidant Activity." *Food Chemistry* 383: 132531.

Sigoillot, J.-C., J.-G. Berrin, M. Bey, et al. 2012. "Chapter 8-Fungal Strategies for Lignin Degradation." In *Lignins. Advances in Botanical Research*, edited by L. Jouanin and C. Lapierre, 263–308. Academic Press.

Sumner, L. W., A. Amberg, D. Barrett, et al. 2007. "Proposed Minimum Reporting Standards for Chemical Analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI)." *Metabolomics* 3: 211–221.

Tombesi, S., A. Nardini, T. Frioni, et al. 2015. "Stomatal Closure Is Induced by Hydraulic Signals and Maintained by ABA in Drought-Stressed Grapevine." *Scientific Reports* 5: 12449.

Trivedi, P., J. E. Leach, S. G. Tringe, T. Sa, and B. K. Singh. 2020. "Plant–Microbiome Interactions: From Community Assembly to Plant Health." *Nature Reviews Microbiology* 18: 607–621.

Tsugawa, H., T. Cajka, T. Kind, et al. 2015. "MS-DIAL: Data-Independent MS/MS Deconvolution for Comprehensive Metabolome Analysis." *Nature Methods* 12: 523–526.

Urra, C., D. Sanhueza, C. Pavez, et al. 2023. "Identification of Grapevine Clones via High-Throughput Amplicon Sequencing: A Proof-Of-Concept Study." *G3: Genes, Genomes, Genetics* 13: jkad145.

Valletta, A., L. M. Iozia, and F. Leonelli. 2021. "Impact of Environmental Factors on Stilbene Biosynthesis." *Plants* 10: 90.

Van Leeuwen, C., G. Sgubin, B. Bois, et al. 2024. "Climate Change Impacts and Adaptations of Wine Production." *Nature Reviews Earth & Environment* 5: 258–275.

Vayssier-Taussat, M., E. Albina, C. Citti, et al. 2014. "Shifting the Paradigm From Pathogens to Pathobiome: New Concepts in the Light of Meta-Omics." *Frontiers in Cellular and Infection Microbiology* 4: 77659.

Vicente-Serrano, S. M., D. Peña-Angulo, S. Beguería, et al. 2022. "Global Drought Trends and Future Projections." *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 380: 20210285.

Wickham, H. 2011. "ggplot2." *WIREs Computational Statistics* 3: 180–185.

Xia, J., and D. S. Wishart. 2010. "MetPA: A Web-Based Metabolomics Tool for Pathway Analysis and Visualization." *Bioinformatics* 26: 2342–2344.

Xie, Q., A. Chen, Y. Zhang, et al. 2019. "ESCRT-III Accessory Proteins Regulate Fungal Development and Plant Infection in Fusarium Graminearum." *Current Genetics* 65: 1041–1055.

Yacoub, A., N. Magnin, J. Gerbore, et al. 2020. "The Biocontrol Root-Oomycete, Pythium Oligandrum, Triggers Grapevine Resistance and Shifts in the Transcriptome of the Trunk Pathogenic Fungus, *Phaeomoniella chlamydospora*." *International Journal of Molecular Sciences* 21: 6876.

Yang, Y., A. M. El-Ganiny, G. E. Bray, D. A. R. Sanders, and S. G. W. Kaminskyj. 2008. "Aspergillus Nidulans hypB Encodes a Sec7-Domain Protein Important for Hyphal Morphogenesis." *Fungal Genetics and Biology* 45: 749–759.

Yu, X.-Q., H.-Q. Niu, C. Liu, H.-L. Wang, W. Yin, and X. Xia. 2024. "PTI-ETI Synergistic Signal Mechanisms in Plant Immunity." *Plant Biotechnology Journal* 22: 2113–2128.

Yu, Y., W. Xu, J. Wang, et al. 2013. "The Chinese Wild Grapevine (*Vitis pseudoreticulata*) E3 Ubiquitin Ligase Erysiphe Necator-Induced RING Finger Protein 1 (EIRP1) Activates Plant Defense Responses by Inducing Proteolysis of the VpWRKY11 Transcription Factor." *New Phytologist* 200: 834–846.

Yu, Y.-H., X.-Z. Li, Z.-J. Wu, et al. 2016. "VvZFP11, a Cys2His2-Type Zinc Finger Transcription Factor, Is Involved in Defense Responses in *Vitis vinifera*." *Biologia Plantarum* 60: 292–298.

Zargar, S. M., N. Gupta, M. Nazir, et al. 2017. "Impact of Drought on Photosynthesis: Molecular Perspective." *Plant Gene* 11: 154–159.

Zeng, G., Z. Wan, R. Xie, et al. 2024. "24-Epibrassinolide Enhances Drought Tolerance in Grapevine (*Vitis vinifera* L.) by Regulating Carbon and Nitrogen Metabolism." *Plant Cell Reports* 43: 219.

Zheng, J., Q. Ge, Y. Yan, X. Zhang, L. Huang, and Y. Yin. 2023. "dbCAN3: Automated Carbohydrate-Active Enzyme and Substrate Annotation." *Nucleic Acids Research* 51: W115–W121.

Zhu, Z., M. Yang, Y. Bai, F. Ge, and S. Wang. 2020. "Antioxidant-Related Catalase CTA1 Regulates Development, Aflatoxin Biosynthesis, and Virulence in Pathogenic Fungus *Aspergillus flavus*." *Environmental Microbiology* 22: 2792–2810.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Description of the plant samples used for this study. **Table S2:** References of the wood pathogenic fungal genomes used in the metatranscriptomic approach. **Table S3:** Decontaminated OTU table. **Table S4:** Differentially expressed grapevine genes in response to esca leaf symptom expression. **Table S5:** Differentially expressed grapevine genes in response to water deficit. **Table S6:** Over-expressed grapevine genes in response to esca and water deficit (Over-expression column) annotated with their functional category and subcategory and *Arabidopsis thaliana* (At) corresponding genes. **Table S7:** Differentially abundant metabolic features in response to esca leaf symptom expression (Comparison = Esca) and water deficit (Comparison = WD) compared to control condition. **Table S8:** Grapevine transcriptomic variables with the 20 highest loading for each component. **Table S9:** Grapevine metabolomic variables with the 20 highest loading for each component. **Table S10:** Number of reads and OTUs per sample. **Table S11:** Putative virulence factors with the 20 highest loading for each component. **Table S12:** Post hoc power analysis performed on DESeq2 differential expression analysis. **Table S13:** Differentially expressed fungal genes between WD and esca conditions.