Stress-dependent responses of grapevine wood and fungal pathogen activity under esca and drought

Marie Chambard [1], Dario Cantù [2,3], Giovanni Bortolami [1,4], Ninon Dell'Acqua [1], Nathalie Ferrer [1], Gregory A. Gambetta [5], Jadran F. Garcia [2], Pierre Gastou [1,6], Mélanie Massonnet [2], Samuele Moretti [1], Adam Rochepeau [7,8], Pierre Pétriacq [7,8], Marie Foulongne-Oriol [9], and Chloé E. L. Delmas [1].

[1] INRAE, ISVV, Bordeaux Sciences Agro, Santé et Agroécologie du Vignoble, 33140 Villenave d'Ornon, France

[2] Department of Viticulture and Enology, University of California, Davis, CA, 95616, United States

[3] Genome Center, University of California, Davis, CA, 95616, United States

[4] Plant Ecology Research Laboratory PERL, School of Architecture, Civil and Environmental Engineering ENAC, EPFL, CH-1015 Lausanne, Switzerland.

[5] EGFV, Bordeaux-Sciences Agro, INRAE, Université de Bordeaux, ISVV, 210 chemin de Leysotte, 33882 Villenave d'Ornon, France

[6] Département Sciences de l'Environnement, Univ. Bordeaux, F-33405 Talence, France

[7] University of Bordeaux, INRAE, UMR 1332 BFP, Villenave d'Ornon 33140, France

[8] Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, Villenave d'Ornon 33140, France

[9] INRAE, MYCSA, 33882 Villenave d'Ornon, France

Author for correspondence: Chloé E. L. Delmas (chloe.delmas@inrae.fr)

Key words: esca, grapevine wood, metabolomics, metatranscriptomics, pathogenic fungi, *Vitis vinifera*, water deficit.

Summary

- Biotic and abiotic stresses impact perennial plants, altering plant physiology with consequences on fungal endophytes and disease expression. In grapevine, one of the world's most valuable crops, drought inhibits esca disease expression, but the underlying plant and fungi molecular interactions are unknown.
- We integrated wood metatranscriptomics, metabolomics, and metabarcoding to investigate such interactions in 30 year-old grapevine and eight key wood pathogenic fungi under drought or esca leaf symptom expression.
- While both esca and drought led to reduced grapevine transpiration, they involved distinct transcriptomic and metabolic signatures, including similar pathways activation such as phenylpropanoid and stilbenoid synthesis. Putative fungal virulence factors increased significantly under both stresses. Under drought, only *Phaeomoniella chlamydospora* relative abundance and gene expression, including anti-oxidative mechanisms, growth, and reproduction increased. Under esca expression, only *Fomitiporia mediterranea* relative abundance and gene expression increased, including wood degradation, competition, detoxification, and growth genes.
- Drought and esca had distinct impacts on the transcriptome and metabolome of grapevine wood and overlapping metabolic pathways, which may confer cross-tolerance, eliciting different fungal molecular responses. Grapevine defense mechanisms induced by drought coupled to low transpiration rate, and low abundance and virulence of *F. mediterranea*, may be responsible for esca leaf symptom inhibition under water deficit.

Introduction

Global warming is predicted to increase abiotic and biotic stresses on crops, such as drought (Vicente-Serrano *et al.*, 2022) and fungal pathogen pressure (Chaloner *et al.*, 2021). These predictions are of particular concern for perennial plants, which accumulate stresses over several decades, such as forest trees (Anderegg *et al.*, 2015; McDowell *et al.*, 2022; Petek-Petrik *et al.*, 2023) and grapevines (van Leeuwen et al. 2024). Plants host diverse microbiomes that colonize external surfaces and internal tissues (Trivedi *et al.*, 2020). Under certain abiotic and biotic conditions, micro-organisms can shift toward pathogenic states within microbial populations (Kabbage *et al.*, 2015; Mishra *et al.*, 2021). Such interactions between plant-environment-microorganisms are thus referred to as pathobiomes and lead to disease development (Vayssier-Taussat *et al.*, 2014; Mina *et al.*, 2020; Lv *et al.*, 2023). Plant fungal community composition has been shown to shift in response to biotic stresses such as changes in root fungal communities during pine wilt disease (Chu *et al.*, 2016), or in wood fungal communities associated with eutypa dieback, esca and wood canker in grapevine (Morales-Cruz *et al.*, 2018; Nerva *et al.*, 2022). Abiotic stresses like drought can also alter microbiomes, as shown in drought-affected soils (Preece *et al.*, 2019), as well as *in planta*, including grapevine root (Carbone *et al.*, 2021) and wood (Gastou *et al*. 2025), and grassland root fungal communities (Fu *et al.*, 2022). These shifts can be driven both directly, through the effects of stress on endophytic microbiota, and indirectly through stress-induced changes in the plant physiological status (Desprez-Loustau *et al.*, 2006). Indeed, biotic stresses trigger plant defense responses, such as PAMP-triggered immunity (Jones and Dangl, 2006), involving hormonal signaling and reactive oxygen species (ROS) production (Yu *et al.*, 2024) to restrict pathogen invasion. However, these defense responses can also be perceived as stress by the endophytic community (Hamilton *et al.*, 2012). Abiotic stress such as drought reduces both plant water potential and photosynthesis, leading to decreased sugar content (Martínez-Vilalta and Garcia-Forner, 2017; Zargar *et al.*, 2017; Bortolami *et al.*, 2021). Such physiological changes affect the composition and behavior of the wood fungal pathogens, as shown in grapevine (Goodell, 2020; Leal *et al.*, 2024; Gastou *et al.*, 2025). Gaining a better understanding of how altered plant physiological status, driven by abiotic or biotic stress, shapes the pathobiome at both the plant and pathogen molecular levels could enhance our knowledge of the complex interplay between plant physiology, microbial communities, and biotic and abiotic stresses.

Such questions are particularly under-documented in perennial systems, making grapevine and complex multifactorial diseases like esca an especially pertinent model to explore these

interactions. Esca is characterized by foliar scorching, internal wood necrosis, and a brown stripe along the outer xylem of the trunk (Lecomte *et al.*, 2024), and is associated with yield decline and vine mortality (Li *et al.*, 2017; Dewasme *et al.*, 2022). Symptom expression is multifactorial, influenced by plant genotype (i.e. grapevine cultivar; (Etienne *et al.*, 2024; Gastou *et al.*, 2024), environmental conditions (Dell'Acqua *et al.*, 2025; Etienne *et al.*, 2025; Monod *et al.*, 2025), and the extent of trunk necrosis (Maher *et al.*, 2012; Ouadi *et al.*, 2019; Gastou *et al.*, 2025). Interestingly, an experiment on mature grapevines that combined drought with the monitoring of esca leaf symptom development, showed that both 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 ($\Psi_{PD,}$ around -1MPa) led to decreased leaf stomatal conductance and whole plant transpiration. In contrast, esca symptomatic plants did not show changes in predawn water potential; instead reduced leaf gas exchanges and whole plant transpiration were attributed to lower functional canopy area and decreased total chlorophyll content in symptomatic leaves. Notably, this experiment 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), while none of the drought-stressed plants expressed symptoms over two consecutive years. This unique experimental framework opens new avenues for exploring how plant physiological status influences the activity of wood fungal pathogens, and whether metatranscriptomic changes might explain the antagonistic interaction between drought and disease expression.

To investigate this, we analyzed wood samples collected at the end of the Bortolami *et al*. (2021) experiment, which consisted 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 necroses and associated Ascomycota community has been studied in this experiment (Gastou *et al.*, 2025). Apparently healthy wood was more abundant in the control condition and necrosis were characterized by a lower fungal diversity but higher pathogen abundance comparing to healthy wood, including various Ascomycota (*Botryosphaeriaceae*, *Phaeomoniellaceae*, *Diaporthaceae*, *Togniniaceae*, *Diatrypaceae*). In particular, the well-known grapevine trunk pathogens *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, *Neofusicoccum parvum*, *Diplodia seriata*, *Diaporthe ampelina*, *Eutypa lata*, and *Botryosphaeria dothidea*, were present in healthy wood and necrotic tissues (Gastou *et al.*, 2025). However, Basidiomycetes (*Hymenochaetaceae*), and

in particular *Fomitiporia mediterranea* is usually involved in esca pathogenesis (Moretti et al. 2021) but was not studied in Gastou et al. (2025).

Given that drought-stressed plants did not develop esca symptoms (Bortolami *et al*., 2021) while they were characterized by high abundance of ascomycota wood pathogens (Gastou et al. 2025), we hypothesized that wood pathogen activity might be directly inhibited by WD and/or indirectly affected by stress-induced changes in host physiology. We also hypothesized that the Basidiomycota *Fomitiporia mediterranea* could play a key role in esca pathogenesis and esca-drought antagonism. To unravel the mechanisms underlying this esca-drought antagonism, we applied a multi-omic approach integrating metatranscriptomics, metabolomics, and metabarcoding. Grapevine transcriptomes and metabolomes were analyzed across control, WD, and esca conditions. We then assessed how these distinct physiological states influenced the fungal community composition and the expression of virulence-associated genes from the eight grapevine trunk pathogens mentioned above. Our study provides new insights into the interaction between plant physiological status induced by biotic and abiotic stresses, and pathobiome. We show that WD and esca symptom expression induce both distinct and overlapping metabolic pathways, some of which may confer cross-tolerance (Perincherry *et al.*, 2021), potentially explaining the observed antagonism between drought and esca, rather than fungal activity itself. Importantly, fungal gene expression responses in healthy wood tissue appeared limited and species-specific: *F. mediterranea* responded primarily to esca symptoms, while *P. chlamydospora* responded to drought.

Material and methods

Plant material and ecophysiological status

We sampled 21 *Vitis vinifera* cv. Sauvignon blanc plants from the experimental design described in Bortolami *et al.* (2021) combining WD and esca leaf symptom expression. Briefly, 30-year-old plants, naturally infected and monitored for esca leaf symptoms for seven years, were uprooted from the vineyard in 2018 and planted in 20 L pots in a greenhouse. Plants were irrigated with a nutritive solution ($NH_4H_2PO_4$ 0.1 mM, NH_4NO_3 0.187 mM, KNO_3 0.255 mM, $MgSO_4$ 0.025 mM, Fe 0.002 mM, oligoelements [B, Zn, Mn, Cu, and Mo]). During two experimental seasons (2018 and 2019), the presence of esca leaf symptoms was checked twice per week (from June 2018 to October 2019) on each plant. On the first of July 2018 and 2019, we stopped watering the plants and maintained plants under WD for a period of 3 months (from July to October 2018 and 2019) at a target weekly average Ψ_{PD} between 0.6 and 1.7 MPa. As

none of the plants under WD developed esca leaf symptoms, plants (n=51) were distributed in three treatments at the end of the experiment: WD asymptomatic plants, well-watered esca symptomatic plants, and well-watered asymptomatic plants (hereafter called control plants). Plants presented contrasted physiological status depending on the treatments (Fig. 1). Only WD plants presented low water potentials (average Ψ_{PD} of -1.11 MPa over the season) while both the control and esca-symptomatic plants remained unstressed (averaged Ψ_{PD} of -0.12 MPa and -0.11, respectively over the season). However, both WD plants and esca-symptomatic plants had a reduced whole-plant stomatal conductance (Gs) after the stress began (from an average Gs in control plants of 141.18 $mmol.m^{-2}.s^{-1}$ to 17.7 $mmol.m^{-2}.s^{-1}$ under WD 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).

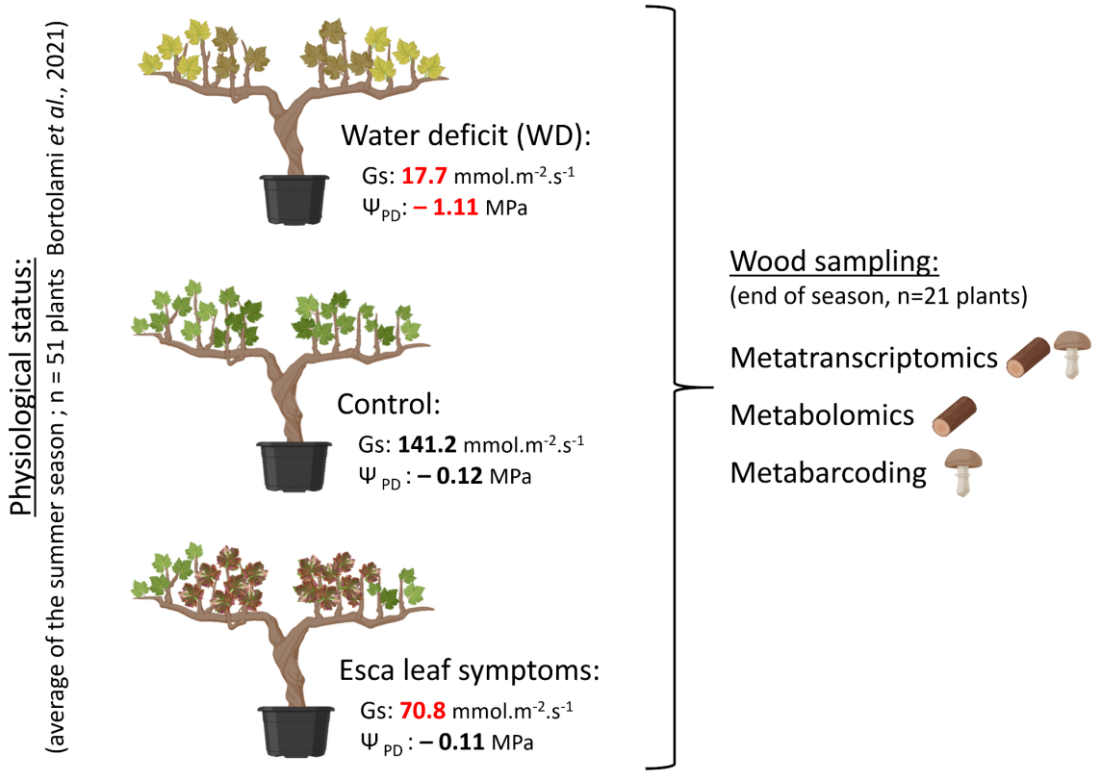

Fig. 1 Scheme of the experimental design. *Vitis vinifera* cv. Sauvignon blanc plants (n=51) were uprooted from the vineyard and placed in pots in a greenhouse for a WD interaction with esca experimentation described in Bortolami *et al*. (2021). Wood samples were collected at the end of this experiment from 21 plants including 7 plants from each of the three treatments: control, WD and esca leaf symptom expression. These treatments resulted in three different plant physiological status based on the predawn water potential (Ψ_{PD}) and whole-plant stomatal

conductance (G_S) (significant differences from control plants are indicated in red; and full datasets are described in Bortolami *et al*. 2021).

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 esca plants, Table S1). Using a sterilized chisel and in a sterile field (radius 30cm), we sampled the different types of wood separately and obtained samples from apparently healthy wood, black necrotic woody tissue and white rot wood samples. Metatranscriptomic, untargeted metabolomic and fungal metabarcoding analyses were performed on the same wood samples (n=21).

RNA extraction

Frozen wood samples were ground in liquid nitrogen using a Tissue Lyser. RNA extraction was done by the Transcriptome facility (University of Bordeaux, INSERM, PUMA, Neurocentre Magendie, Bordeaux, France) using 0.5 g of tissue and following the modified protocol described in Blanco-Ulate *et al.*, 2013. For each sample, 100 µL mercaptoethanol and 5 mL extraction buffer (CTAB 2%, PVP 2%, Tris 100mM, NaCl 2M, EDTA 25mM, spermidine 0.05g/mL) were added. Samples were vortexed and incubated at 65°C for 10 minutes, then vortexed. An equal amount of CIA (chloroform: IAA 24:1) was added and samples were centrifuged at 4000 rpm for 30 min at 4°C. The supernatant was transferred to new 15 mL Falcon tubes placed on ice and an equal volume of CIA was added. Samples were centrifuged at 4000 rpm for 30 minutes at 4°C. The supernatant was transferred to new tubes and the equivalent of ¼ of the sample volume of 10 mM LiCl was added. Samples were incubated at -20°C for 1 hour. Samples were centrifuged at 4°C for 45 minutes at 4000 rpm. The pellet was dried and resuspended in 100 µL RNase-free water. RNA Cleanup Kit (Quiagen) was then used according to the manufacturer's instructions. RNA quality was assessed with a Fragment Analyzer (Agilent).

Black necrosis and white rot samples appeared to have degraded RNA (RNA concentration < 40ng/µL and RIN < 7.5) and quality samples could not be obtained from these tissues. Thus, a total of 21 high-quality samples, corresponding to apparently healthy wood samples (n=7 from each condition: control, WD, and esca symptomatic plants) were selected for RNA sequencing.

RNA sequencing

RNAseq was performed at the GeT-PlaGe core facility, INRAE Toulouse, France. RNA-seq libraries were prepared according to Illumina's protocols using the Illumina TruSeq Stranded mRNA sample preparation kit to analyze mRNA. (Briefly, mRNA were selected using poly-T beads. Then, RNA were fragmented to generate double stranded cDNA and adaptors were ligated to be sequenced. Eleven cycles of PCR were applied to amplify libraries. Library quality was assessed using an Advanced Analytical Fragment Analyzer (Agilent) and libraries were quantified by QPCR on QuantStudio 6 device (Applied Biosystems, ThermoFisher Scientific) using the Kapa Library Quantification Kit (Roche, KK4824).) Sequencing has been performed on an Illumina NovaSeq 6000 using a paired-end read length of 2x150 pb with the corresponding kits.

Metatranscriptomic bioinformatics and data analyses

The primary scaffolds genome available for *Vitis vinifera* cv. Sauvignon blanc (Urra *et al*., 2023) and grapevine trunk-associated fungi genomes 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 NCBI and Grapegenomics databases (Table S2):

- *P. chlamydospora*, *P. minimum* and *F. mediterranea*, three fungi often associated with trunk necroses 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* associated with the Botryosphaeria dieback (Belair *et al.*, 2022).
- *D. ampelina*, responsible for the Phomopsis dieback (Gonzalez-Dominguez *et al.*, 2022) and Phomopsis cane and leaf spot (Lawrence *et al.*, 2015).
- *E. lata*, responsible for the Eutypa dieback (Rolshausen *et al.*, 2014).

The RNA sequencing resulted in 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 *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 higher than 35 for each base and no adapter sequences.

A first pre-alignment on concatenated genomes of *Vitis vinifera* cv. Sauvignon blanc (Urra *et al.*, 2023) and grapevine trunk-associated fungal genomes as a multi-species reference was performed with Hisat2 v2.2.1 (Kim *et al.*, 2015) and resulted in a mean of 99.15% of overall alignment rate. Samtools v1.20 (Danecek *et al.*, 2021) was then used to keep only high quality alignments (-q 20) with a CIGAR score higher than 50 (-m 50) and remove unaligned sequences (-F 4). This high quality reads alignment resulted in an average of 100M reads per sample. Alignment files were then filtered in order to separate sequences from grapevine and fungi using Samtools view and grep commands. Fungal and grape sequences were then converted to FASTQ sequences and pseudo-aligned on grapevine trunk-associated fungal transcriptomes and genomes or on *Vitis vinifera* cv. Sauvignon blanc primary genome and transcriptome with Salmon v1.10.0 (Patro *et al.*, 2017). Transcripts per million (TPM) and estimated counts were obtained from Salmon quant files.

Differential expression analyses were performed with fungal and grape genes using the DESeq2 R package v4.2.2 (Love *et al.*, 2014). As the number of detected genes and counts were low for some fungi and especially in the control condition, a pseudo-count of 1 was added in the fungal count matrix in order to perform the differential expression analysis between esca and WD conditions without using controls. The grapevine DEG (Differentially Expressed Genes) lists were used for functional category enrichment statistical tests, using the Vitisnet annotation of PN40024 protein-coding genes (Grimplet *et al.*, 2009). Gene count normalization (median of ratios method) was made with DESeq2. Volcano plots, sparse Partial Least Squares Discriminant Analysis (sPLS-DA) and other analyses were carried out using R (R Core Team, 2023) with ggplot2 v4.2.3 and mixomics v6.22.0 packages (Wickham, 2011; Rohart *et al.*, 2017).

Genome annotation

Predicted proteins of the primary contigs of Sauvignon blanc were aligned onto PN40024 proteins (V1 annotation, Jansen *et al.*, 2006) using BLASTP v.2.2.28+. Alignments with an identity greater than 80% and a reciprocal reference:query coverage between 80% and 120% were kept. Alignments with the highest product of identity, query coverage, and reference coverage were selected to determine pairs of homologous genes. The correspondence table between Sauvignon blanc and PN40024 was used in order to assign functional categories from Vitisnet to Sauvignon blanc genes. The functional categories enrichment was calculated in R with Fisher’s tests.

Fungal genomes were annotated for conserved domains of virulence factors and homologies to carbohydrate active enzymes, secondary metabolites, transporters, peroxidases and cytochromes, using 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 with HMMER. A sPLS-DA analysis was performed with the sum of all read counts sharing the same virulence factor annotation per fungus as an input.

Untargeted metabolomics: extraction, mass spectrometry and data analysis

Metabolomic analysis was performed as described in (Dell'Acqua *et al.*, 2025). Semi-polar compounds, including primary and secondary metabolites, were extracted using automated high-throughput ethanol extraction procedures at the MetaboHUB-Bordeaux Metabolome (https://metabolome.u-bordeaux.fr/) from 10 mg of freeze-dried grapevine wood material, following previously established protocols (Luna *et al*., 2020). Untargeted metabolic profiling by UHPLC-LTQ-Orbitrap mass spectrometry (LCMS) was performed as already reported (Dussarrat *et al*., 2022; Martins *et al*., 2022) using an Ultimate 3000 ultra high-pressure liquid chromatography (UHPLC) system coupled to an LTQ-Orbitrap Elite mass spectrometer interfaced with an electrospray ionisation source (ESI, ThermoScientific, Bremen, Germany) operating in negative ion modes as described previously (Luna *et al*., 2020). Wood samples runs for the LCMS analytical sequence were randomised, along with extraction blanks (prepared without plant material and used to rule out potential contaminants detected by untargeted metabolomics), and Quality Control (QC) samples that were prepared by mixing 20 µL from each sample. QC samples were injected every 10 runs and used for i) the correction of signal drift during the analytical batch, and ii) the calculation of coefficients of variation for each metabolomic feature so only the most robust ones are retained for chemometrics (Broadhurst *et al*., 2018). Briefly, MS1 full scan acquisitions at high-resolution (240k) were performed on QC samples and the various standards for exact mass measurements, and all samples were subjected to MS2 Data Dependent Analysis (DDA, 30k resolution) to generate fragmentation information for further annotation (Dell'Acqua et al. 2025).

Raw LCMS data were processed using MS-DIAL v 4.9 (Tsugawa *et al*., 2015). We also implemented retention time correction in MS-DIAL, and the metabolomic signals were normalised according to the LOWESS method based on the QC samples. We performed data curation according to blank check and setting thresholds as signal-to-noise ratio (SN) > 10, coefficient of variation in quality controls < 30%. The 3,184 final features for predictive

metabolomics annotations were performed using in-house chemical library and the FragHUB database (Dablanc *et al*., 2024). Thus, putative annotation of differentially expressed metabolites resulted from MS-DIAL screening of the MS1 detected exact HR *m/z* and MS2 fragmentation patterns (Tsugawa *et al*., 2015). Additionally, the InChiKeys of annotated features were employed within ClassyFire to automatically generate a structural ontology for chemical entities (Djoumbou Feunang *et al.*, 2016). Putative metabolites that were unlikely to be found in plants (*e.g.* Human drugs) were considered as misassigned and therefore reassigned as "Unknown".

Normalization to sample median, square root data transformation and Pareto scaling were applied to metabolomic data using Metaboanalyst 6.0 (Pang *et al.*, 2024). Volcano plots were performed on R using Metaboanalyst fold change and t-test. Thresholds were set at 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 (combining the number of metabolites in the pathway with their importance) were calculated from differentially abundant metabolites lists using the Metaboanalyst pathway analysis tool and *Arabidopsis thaliana* set. In addition to independent analyses, we integrated the transcriptomic and metabolomic datasets to explore alternative potential markers of each stress response. To do so, we used with the MixOmics R package (Rohart *et al.*, 2017) to (i) perform independent sPLS-DA analysis, (ii) to test the correlation between the transcriptomic and metabolomic components, and (iii) to use a heatmap visualization of the top variables of each type of data among the two first components.

Metabarcoding: DNA extraction, amplification and sequencing

For the metabarcoding analysis, the control sample P16 did not result in a high quality library and was discarded, only 20 (n = 6 control plants, n = 7 WD plants, n = 7 esca plants) samples out of 21 left. The same experimental procedure as the one described in Dell'Acqua *et al.*, (2025) was used. Briefly, 60 mg of frozen samples were used and DNA was extracted with the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. All samples (n=20) were diluted to obtain a maximum DNA concentration of 15 ng/µL. A negative extraction control was included in the process. The internal transcribed spacer 1 (ITS1) region of the fungal ITS ribosomal DNA gene (Schoch *et al.*, 2012) was amplified with the ITS1F-ITS2 primer pair. Universal sequences were added to the specific sequences to enable hybridization of adapters and sequencing indexes during 2nd PCR (i.e. mb-ITS1F: 5'

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**CTTGGTCATTTAGAGGAAGTAA**-3' and mb-ITS2: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**GCTGCGTTCTTCATCGATGC**-3', with the specific sequences in bold). The first PCR mixture and cycles are described in Dell'Acqua *et al.*, (2025). The second PCR and the amplicon sequencing (Illumina V3 kit: 2 × 300 bp paired-end reads) were performed by the Genome Transcriptome Facility of Bordeaux according to standard protocols.

Metabarcoding: bioinformatic and data analyses

Bioinformatic analyses were performed according to Dell'Acqua *et al.*, (2025) using the FROGS tools hosted by the Galaxy platform (Escudié *et al.*, 2018). Clustering, chimera removal, OTU (Operational Taxonomic Unit) filter, ITS sequence selection and affiliation tools (blast on the database ITS UNITE Fungi 8.3) of the FROGS software were used to compute the abundance table. The MetabaR package (Zinger *et al.*, 2021), implemented in R v.4.2.1 was used to decontaminate the dataset and remove putatively contaminant OTUs and low-quality samples (highly contaminated or low sequencing depth) based on extraction and PCR blank. OTUs affiliations were controlled through additional blasts against NCBI (nr/nt) nucleotide collection (https://blast.ncbi.nlm.nih.gov/Blast. cgi) and trunkdiseaseID.org, a database specific to grapevine trunk disease pathogens,.

Statistical analyses were performed using R v4.2.1. Alpha and beta diversity metrics were calculated using R phyloseq package v1.34.0 (McMurdie and Holmes, 2013). Shannon, and Simpson alpha diversity indexes of the trunk fungal communities were compared between treatments using ANOVA procedures followed by Tukey's post-hoc tests. A permutational analysis of variance (PERMANOVA) was performed on CLR-transformed data, using R package vegan v2.6-4 (Oksanen *et al.*, 2022), to assess the effect of plant status on the fungal community structure. The relative abundance of eight wood pathogen species, included in the RNA-seq analysis, were compared between treatments using ANOVA followed by Tukey's post-hoc tests.

Results

Grapevine ecophysiological status effect on wood gene expression profiles

To assess the grape wood responses to esca and WD, we performed a transcriptomic profiling using RNA-seq. A total of 2,817 differentially expressed genes (DEG) were observed in

response to the expression of esca leaf symptoms during the summer season preceding the autumn sampling (Fig. 2a; Table S3). These DEGs consisted of 1,816 up-regulated genes and 1,001 down-regulated genes. The WD induced the differential expression of fewer genes, with a total of 2,347, including 1,370 up-regulated and 977 down-regulated genes (Fig. 2b; Table S4). Among these genes, 808 were up-regulated and 363 were down-regulated in both WD and esca samples.

To evaluate the biological mechanisms and pathways affected by both treatments and the treatment-specific ones, we performed an enrichment analysis of the functional categories among the DEGs. The functional categories enriched among the up-regulated genes in response to both treatments (drought and esca leaf symptom expression) included “protein kinase”, “hormone signaling”, “phenylpropanoid biosynthesis”, “phytoalexin biosynthesis”, “stilbenoid biosynthesis” and “cell wall metabolism” (Fig. 2c). In contrast, some functional categories were only enriched among the genes more highly expressed in response to a single treatment. For the WD response, we found categories such as “oxidative stress response”, “nitrogen and sulfur metabolism” and “aromatic amino acid metabolism” (Fig. 2c). Genes coding for ascorbic acid metabolism, glutathione S-transferases and thioredoxins were involved in these functional categories. In response to esca symptoms, functional categories such as “plant-pathogen interactions”, “transcription factors”, “starch and sucrose metabolism”, and “transport overview” were observed (Fig. 2c). Among over-expressed genes involved in these categories, we found genes annotated as PR proteins (pathogenesis-related), transcription factors (TF), in particular AP2 (Apetala2), WRKY, Zinc fingers and NAC (NAM, ATAF1/2, CUC2), all previously linked to grapevine response to pathogens and inducing disease resistance. A large number of genes coding for stilbene synthases (STS), a key enzyme of stilbene synthesis along with some phenylalanine ammonia-lyases genes (PAL), were also found over-expressed in response to esca leaf symptom expression (17 STS and 3 PAL genes). Tethering factors, coat proteins and ABC multidrug transporters were the most identified genes involved in protein transport.

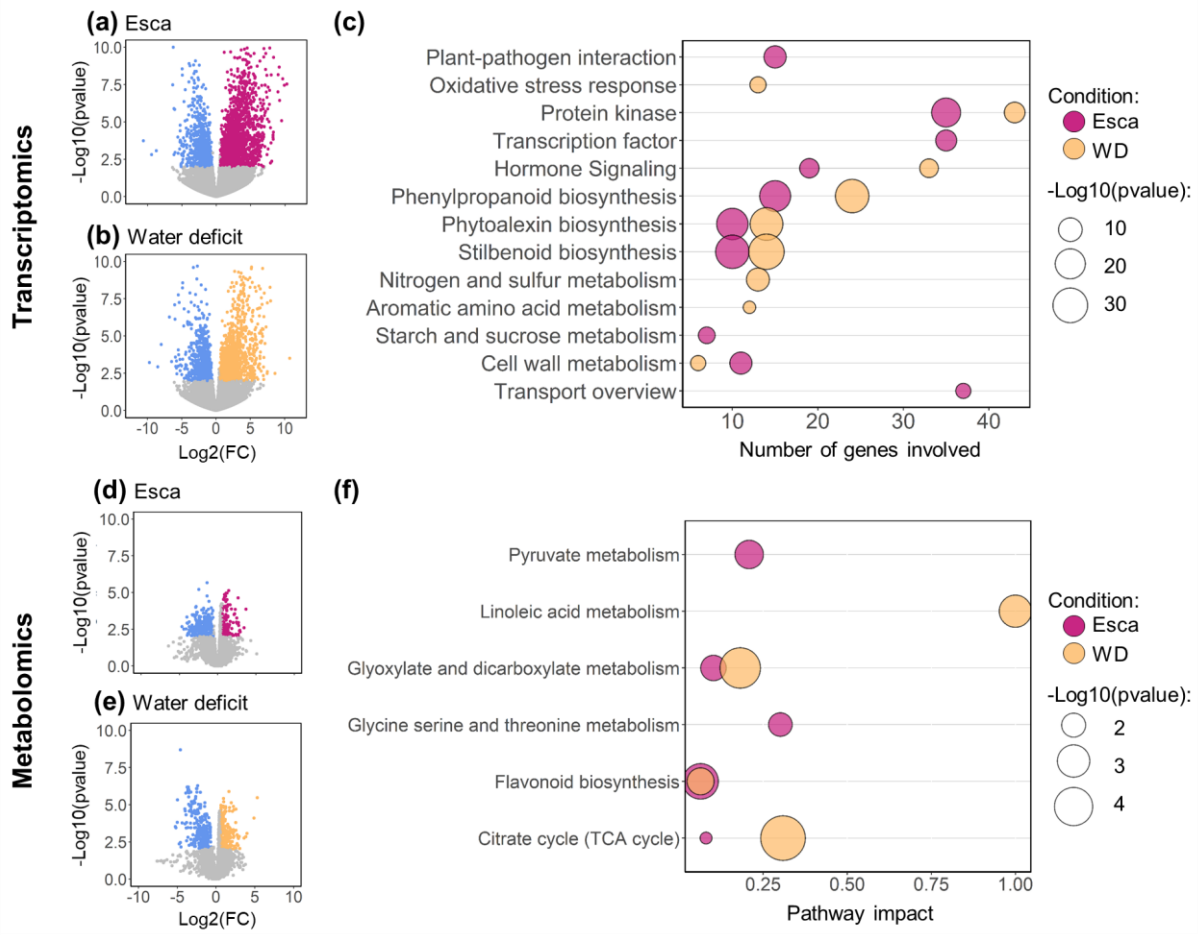

Fig. 2 Volcano plots showing the differentially expressed (FC ≥ 1.5, pvalue ≤ 0.01) genes (a, b) and metabolic features (d, e) of *V. vinifera* cv. Sauvignon blanc (a, d) between esca leaf symptoms and control and (b, e) between WD and control conditions. Down-regulated genes and metabolic features are represented as blue dots, and up-regulated genes and metabolic features are represented as pink (esca) and yellow (WD) dots. (c) Enriched functional categories among grapevine up-regulated genes in response to esca leaf symptoms and WD conditions. (f) Pathways with p value ≤ 0.05 (enrichment test) and a pathway impact > 0 from the lists of enriched metabolic features in response to esca and WD. FC = Fold change.

Grapevine ecophysiological status effect on wood metabolites

Grapevine response to WD and esca leaf symptom expression was also investigated at the metabolome level in healthy trunk samples. A volcano plot analysis showed differentially abundant metabolomic features (DAM; Table S5) in response to esca (Fig. 2d) and WD (Fig. 2e). The WD condition induced more DAM (332 over-abundant and 340 less abundant) than

the esca condition (184 over-abundant and 272 less abundant). The list of over-abundant metabolomic features showed enriched metabolic pathways (Fig. 2f). “Glyoxylate and dicarboxylate metabolism”, “flavonoid biosynthesis” and “citrate cycle” were significantly impacted in both conditions (esca and WD), with impact scores varying between 0.06 (flavonoid biosynthesis, WD and esca) and 0.31 (citrate cycle, WD). “Pyruvate metabolism” and “glycine, serine and threonine metabolism” were found to be significantly impacted only in the esca condition, with an impact score of 0.21 and 0.30 respectively. Among over-abundant metabolites, only one metabolite was recognized as belonging to the stilbene class (WPPYDDRXZLFEFL-VOTSOKGWSA-N). This metabolite was commonly found in response to esca and WD stresses with fold changes of 1,95 (WD, FDR = 0.04) and 2.38 (esca, FDR = 0.02). Along with this metabolite, a large number of phenylpropanoid compounds were also detected in each stress (41 WD, 22 esca), and 15 of them were found in both stresses. We found the higher pathway impact score, corresponding to “linoleic acid metabolism” in the WD condition. For this pathway, two metabolic features over four were found to be over-abundant: linoleic acid and 13(S)-HPODE (hydroperoxyoctadeca-dienoic acid). This pathway had an impact of 1 in the esca condition, a non-significant p-value, and only one metabolic feature detected (linoleic acid). Finally, “pyruvate metabolism”, and “glycine, serine and threonine metabolism”, were specifically enriched in response to esca leaf symptom expression.

Integration of grapevine transcriptome and metabolome

According to the sPLS-DA, transcriptomic data (Fig. 3a) did not allow discrimination between samples from the esca and WD conditions while the samples from the control and stressed (esca leaf symptoms or WD) conditions were moderately discriminated by the second component (R = 0.27, pvalue = 0.01; Fig. 3a). The first component seemed to be related to differences between individuals rather than conditions (Fig. 3a). Metabolomic data clearly distinguished the three plant physiological status conditions (R = 0.62, pvalue = 0.001; Fig. 3b), with the first component tend to differentiate between the control and stressed conditions, while the second component differentiated between WD and esca conditions. The latent components of each bloc (the grapevine transcriptome and metabolome datasets) were highly correlated (Pearson’s correlation r = 0.96).

For each type of data and for the two first components, the selection of 20 variables with the highest loading was the best option to classify the samples using a heatmap (Fig. 3c, table S6

and S7). These variables clearly separated the three experimental conditions, indicating distinct responses to WD and esca. Four main groups were visible on the heatmap: (i) a group of variables enriched in the WD condition and depleted in the esca condition, mainly including genes coding a MYND family transcription factor, an actor of the pyrimidine metabolism, a RNA transporter and a gene involved in plastid organisation and biogenesis as well as metabolites such as trehalose and an isoflavonoid (likely ononin); (ii) a group of genes highly enriched in the control condition, such as a fatty acid transporter gene, 2-Heptylhexanedioic acid and 9,12,13-Trihydroxyoctadec-10-enoic acid; (iii) a group of highly enriched metabolic features in both stress conditions (WD and Esca) such as two phenylpropanoids, a benzenoid, a derivative of quinic acid (likely 4,5-dicaffeoylquinic acid), 7-O-Methylbavachin, and a terpene lactone; (iv) a group of genes and metabolic features enriched in the esca condition compared to the control and WD conditions: three flavonoids recognized as catechins, two benzophenones, a transcription factor from the jasmonate-mediated signalization pathway, and a gene involved in phenylalanine biosynthesis and oxidative stress response.

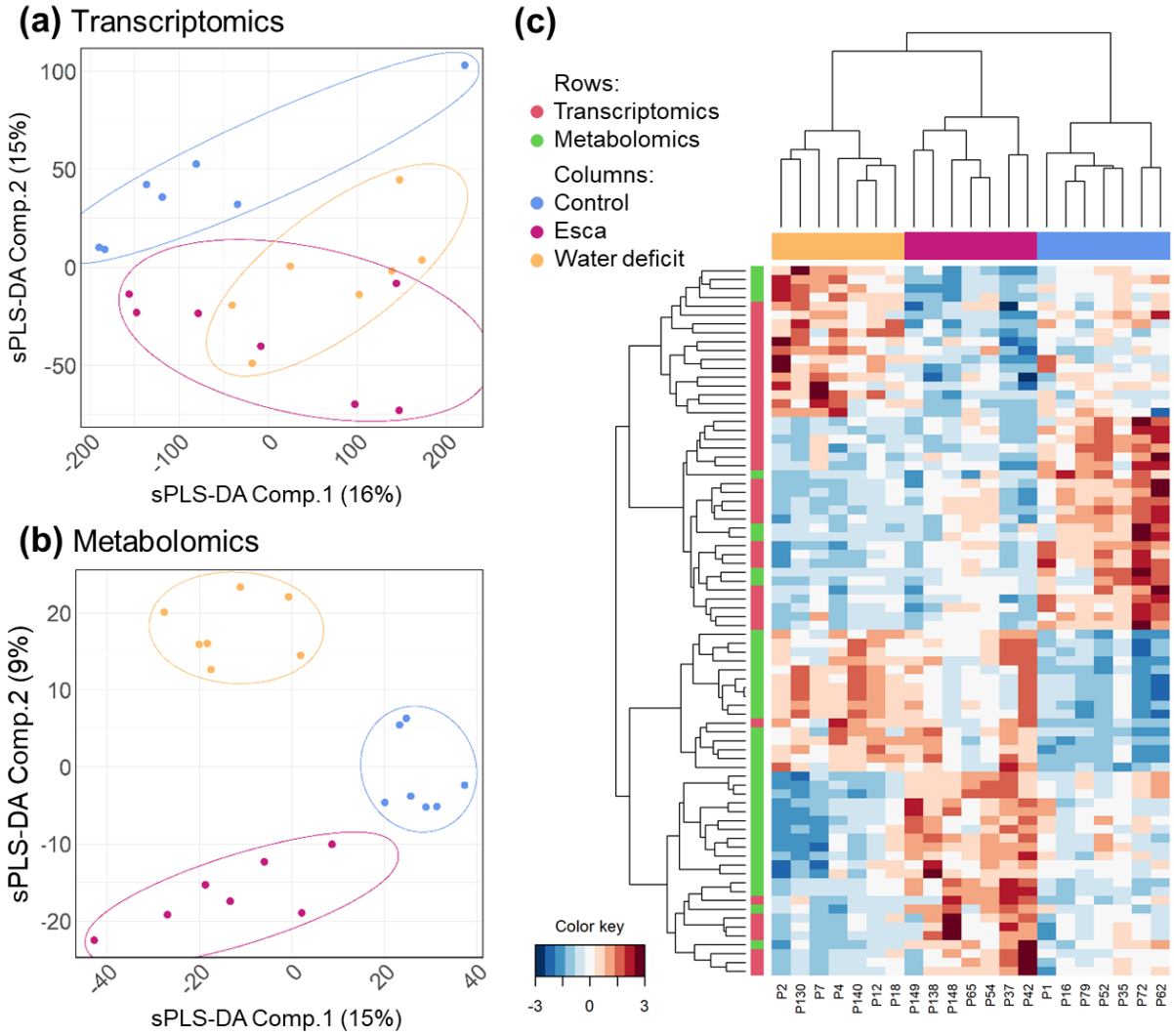

Fig. 3 Multi-omic datasets overview of *V. vinifera* cv. Sauvignon blanc responses to esca leaf symptom expression and WD. sPLS-DA (sparse Partial Least Squares Discriminant Analysis) of (a) grapevine transcriptomic (R = 0.27, pvalue = 0.01) and (b) metabolomic data (R = 0.62, pvalue = 0.001). (c) Heatmap of 21 individual plants and 40 variables selected using the MixOmics R package for each dataset (component 1 and component 2) that best explain the differences between conditions. Values were scaled and trimmed to the range [-3, 3] for visualisation, with lower values shown in blue and higher values in red.

Effect of ecophysiological status on the trunk fungal community using metabarcoding

A total of 934,497 reads were generated and assigned to 342 fungal Operational Taxonomic Units (OTUs) in apparently healthy trunk samples. The decontaminated dataset (without untargeted and putatively contaminant OTUs) contained 789,458 reads across 20 environmental

samples that were affiliated to 306 OTUs. The decontaminated OTU table is provided in Supplementary Table 8, the number of reads and OTUs per sample are provided in Supplementary Table 9.

Fungal communities varied among biological replicates (Fig. 4a) and were composed of 80% of Ascomycota, with three dominant species: *P. chlamydospora* (Eurotiomycete, 29.6% of the total community), *P. minimum* (Sordariomycete, 7.4%) and *Pleosporales* sp. (Dothideomycete, 5.3%). Basidiomycota represented 16.4% of fungal OTUs, with three most abundant species: *F. mediterranea* (Agaricomycete, 5.0%), *Peniophora* sp. (Agaricomycete, 3.7%) and *Phellinus rhamni* (Agaricomycete, 3.2%). Only 2.1% of OTUs were non-affiliated at the class level.

Neither observed richness nor diversity metrics (i.e. Shannon and Simpson indexes) of fungal communities from trunk healthy wood samples were significantly different between plant physiological status conditions ($P = 0.21$, $P = 0.51$ and $P = 0.98$, respectively; Fig. 4b). However, there was a trend towards a lower fungal diversity in plants expressing esca leaf symptoms, and even lower in WD plants, compared with controls. PCA and PERMANOVA analysis revealed that the plant physiological status significantly explained 16.1% of the variance of healthy wood fungal communities ($P = 0.001$; Fig. 4c). The structure and composition of these fungal communities significantly differed between esca and control plants, and between WD plants and the two other conditions.

We specifically addressed the effect of plant status on the relative abundance of the eight pathogens included in the RNA-seq analysis. Overall, they represented approximately 20% of the fungal community in the control condition and 55% in the esca and WD conditions. Plant status significantly modified the relative abundance of two species over eight: *F. mediterranea* ($P = 10^{-5}$) and *P. chlamydospora* ($P = 0.005$). *F. mediterranea* was more abundant in the healthy wood of esca plants compared to control and WD plants (Fig. 4d). *P. chlamydospora* was more abundant in the wood of the two stressed conditions than in that of control plants (Fig. 4d). No clear differences were observed in the relative abundance of (i) either the 20 most abundant OTUs (Fig. 4a) (ii) or the six remaining species included in the RNA-seq trial (Fig. 4d).

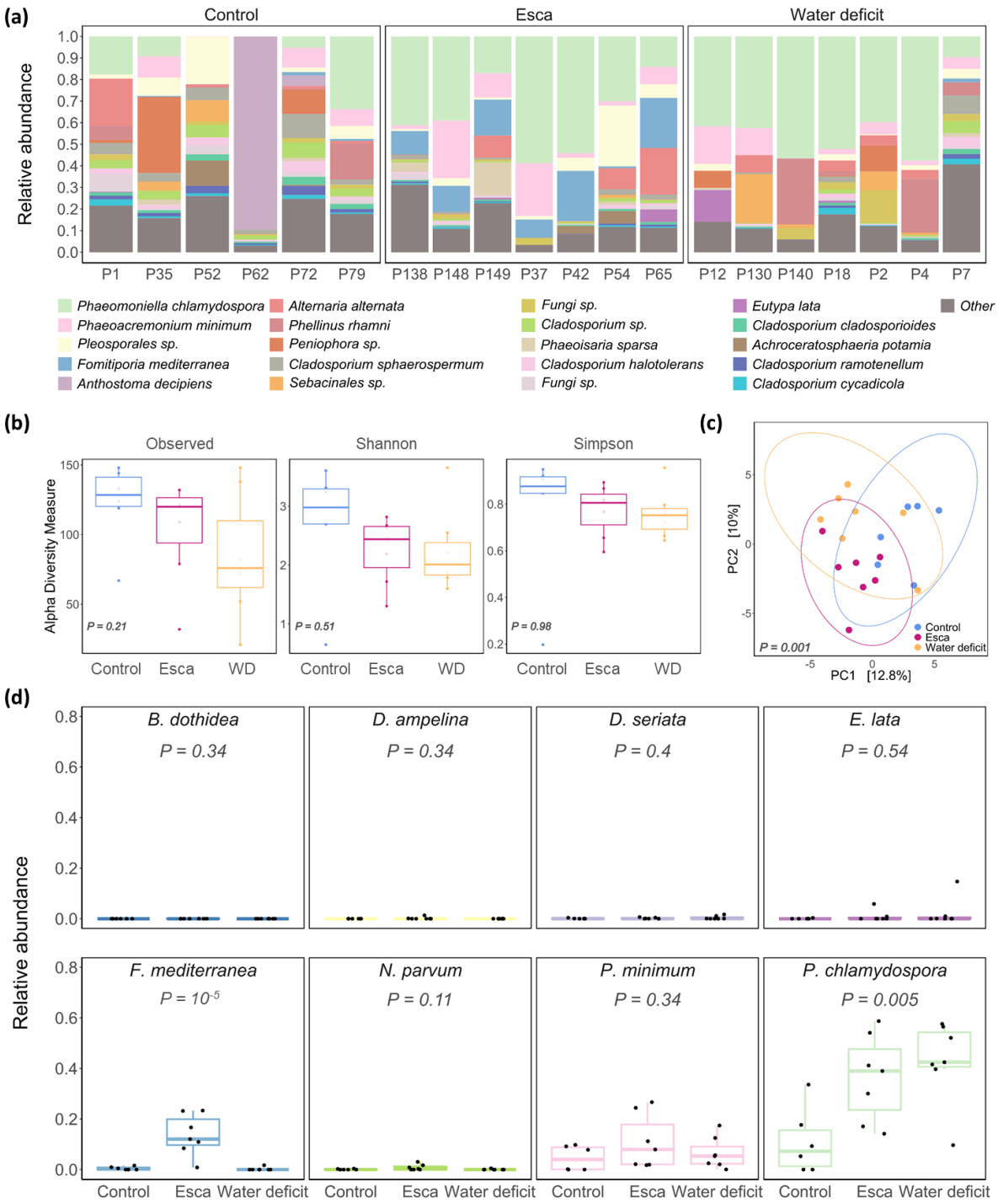

Fig. 4 Fungal metabarcoding of healthy wood samples of *V. vinifera* cv. Sauvignon blanc in the three treatments: control, WD and esca leaf symptom expression. (a) Relative abundance of the top 20 OTUs detected in the control, esca leaf symptom expression and WD conditions per plant sample (x axis labels). (b) Alpha fungal diversity indexes in control, esca and WD conditions. (c) Principal component analysis (beta diversity). (d) Relative abundance of the eight targeted fungal wood pathogens in the three conditions.

Effect of ecophysiological status on grape trunk pathogens gene expression

Metatranscriptomic alignments revealed substantial variation in the number of reads mapped to each fungal species across biological replicates within the same condition, indicating high biological variability (Fig. 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 (2 x 10^2 reads; Fig. 5a) compared to both stress conditions (10^4 reads; Fig. 5b,c). This suggests that fungal activity increases in response to WD and esca stress. In the control condition, *P. chlamydospora* and *P. minimum* accounted for the majority of fungal reads. In esca-symptomatic plants, *F. mediterranea* and *P. chlamydospora* showed the highest transcript levels. Under WD, *P. chlamydospora* was consistently the most transcriptionally active species across all samples.

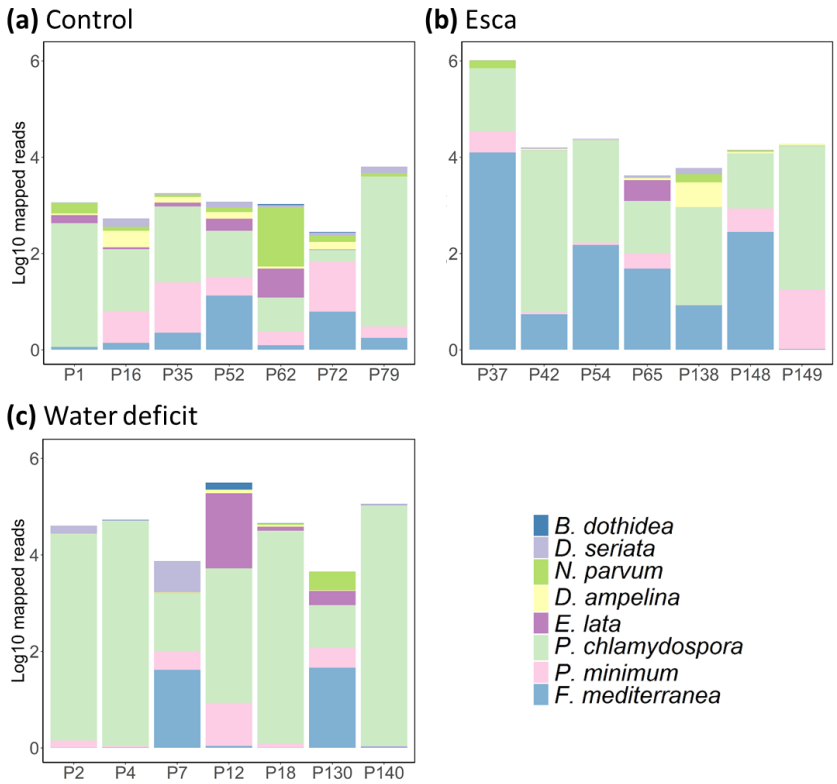

Fig. 5 Number of reads that aligned on the 8 fungal genomes per healthy wood sample (expressed in log10), in control (a), esca leaf symptomatic (b) and WD (c) *Vitis vinifera* cv. Sauvignon blanc plants. Bars were colored according to the percentage of reads per fungus among the total fungal reads.

We annotated putative virulence factors in fungal genomes in the three conditions (Fig. 6a). For *B. dothidea* and *D. seriata*, which had the lowest read counts, only a few virulence factor genes (total mean normalized counts under 100) were found to be expressed in any condition. In the control condition, transporters and cytochrome P450 putative virulence factors were commonly detected in the other six fungi: *N. parvum*, *D. ampelina*, *E. lata*, *P. chlamydospora*, *P. minimum*, and *F. mediterranea* at a lower level than others (Fig. 6a). A few putative peroxidase virulence factors were also detected for these six fungi, but with low total counts. CAZyme putative virulence factors were detected in *N. parvum*, *E. lata*, *P. chlamydospora*, *P. minimum*, and *F. mediterranea*. Secondary metabolite virulence factors were mainly detected in *N. parvum* and *D. ampelina*, as well as in *P. chlamydospora* and *F. mediterranea*. In response to the WD condition, the expression of these putative virulence factors increased in *E. lata*, *P. chlamydospora*, and *P. minimum*. In response to esca leaf symptom expression, putative virulence factors expression increased in *F. mediterranea*, *P. minimum*, *P. chlamydospora*, and *N. parvum* and greatly decreased in *D. ampelina* (Fig. 6a). However, a high and significant increase (x 6.6 times, pvalue = 2.10^{-7}) of putative peroxidase gene expression was observed in *F. mediterranea* in esca compared to the other conditions.

The sPLS-DA analysis of virulence factors expression discriminated between three groups among samples corresponding to the three conditions (control, WD and esca; Fig. 6b). The first component clearly separated the control condition from the two stressed conditions, and the second component tended to separate the WD from the esca condition. Further analysis showed that the variables responsible for the separation of the three conditions (20 highest loadings for each component, table S10) were all *F. mediterranea* and *P. chlamydospora* virulence factors. Transporters (9 from *F. mediterranea* and 3 from *P. chlamydospora*) were mainly responsible for the separation between control and stressed samples. According to the second component of the sPLS-DA, *F. mediterranea* virulence factor genes were associated with esca samples while *P. chlamydospora* virulence factor genes were associated with WD samples. *F. mediterranea* putative virulence factor genes responsible for the separation of esca samples were mainly transporter genes (9 genes over 13). Among them, we found genes coding for a protein involved in the eisosome complex, a mRNA transporter and a protein belonging to the nuclear pore complex. Two genes recognized as CAZymes and more specifically as glucoside hydrolases (GH18 and GH128), a peroxidase (linoleate diol synthase) and a secondary metabolism gene (terpene pathway) were also found. *P. chlamydospora* putative virulence factor genes responsible for the separation of WD samples were also mainly associated with

transporters (5 over 8 genes), such as a lipid transporter (PbgA) and three ATPases. A CAZyme gene from the Auxilary Activity 4 (AA4) family, a cytochrome P450 gene (sfid 149) and a gene involved in the secondary metabolism (type 1 polyketide synthase) were also found.

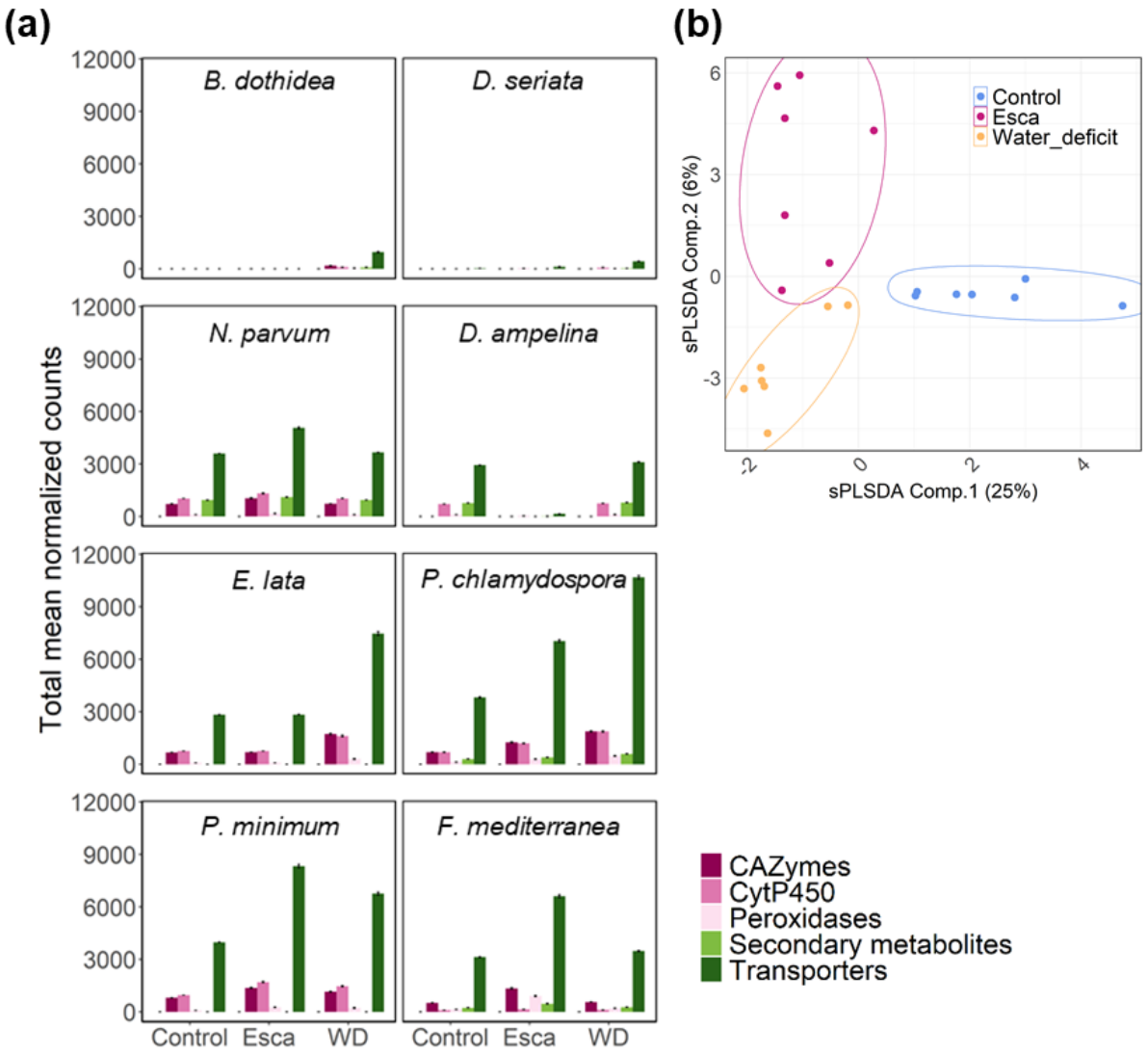

Fig. 6 Putative virulence factors in fungal genomes for each fungus in control, WD and esca leaf symptomatic *Vitis vinifera* cv. Sauvignon blanc plants. (a) Normalized expression levels of putative fungal virulence factors genes performed with the DESeq2 R package for each fungus separately. (b) Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) of putative virulence genes of all fungi.

The responses of grape trunk pathogens to WD and the expression of esca leaf symptoms were compared by a differential analysis. As the control samples had too few detected fungal genes,

they were not used and the differential expression analysis compared the two stresses (WD vs. esca) in each fungus (Fig. 7). The total number of differentially expressed genes (FC ≥ 1.5, FDR ≤ 0.05) between WD and esca conditions per fungus (Fig. 7a) was low. Among the 8 fungal grape trunk pathogens observed, only two showed differentially expressed genes (DEG): *F. mediterranea* (31 DEG) and *P. chlamydospora* (9 DEG). Other fungi did not have any DEG. Further observation of these DEG showed that for *F. mediterranea*, the detected DEG were only over-expressed in esca samples, and for *P. chlamydospora* the detected DEG were only over-expressed in WD samples (Table S11). Among *F. mediterranea* DEGs over-expressed in esca samples compared to WD samples, genes coding an ABC transporter, a glucan phosphorylase, a glycosyl hydrolase, an aspartyl protease, or a peroxidase were detected. *P. chlamydospora* genes over-expressed in WDcondition were coding an arylsulfotransferase, an OPT oligopeptide transporter, a SSH4 protein and a calcium-transporting ATPase.

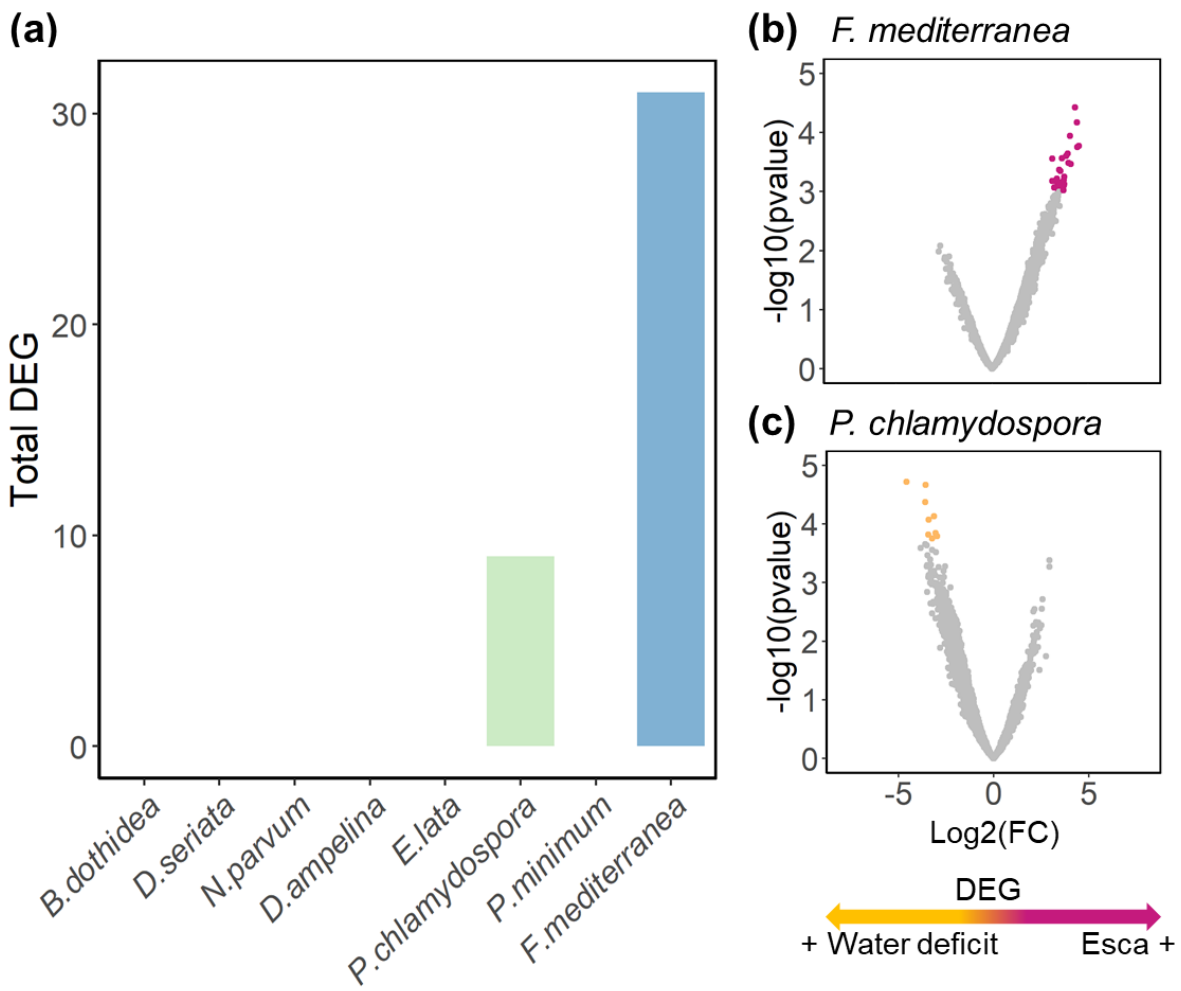

Fig. 7 Differential expression analysis between Esca and WD. **(a)** Total DEG (FC ≥ 1.5, FDR ≤ 0.05) per fungus resulting from the differential expression analysis comparing drought-

stressed and esca symptomatic plants. Volcanoplot representation of **(b)** *F. mediterranea* and **(c)** *P. chlamydospora* differentially expressed genes (FC ≥ 2, FDR ≤ 0.05) between WD (yellow) and esca leaf symptoms expression (pink) conditions, with WD condition as a reference.

Discussion

Plant physiological status can be altered by various stresses, with subsequent consequences for plant interactions with pathogens. The objective of this study was to unravel the molecular responses of the pathobiome to grapevine-induced abiotic and biotic stresses using *Vitis vinifera*, esca disease and drought as a model system. In particular, as drought inhibited the expression of esca leaf symptoms (Bortolami *et al.*, 2021), this study aimed at deciphering the putative molecular interaction underlying this antagonism. We first explored the molecular responses of grapevine and wood pathogens to esca leaf symptom expression and WD. Then, we explored different hypotheses underlying the drought and esca antagonism: (i) different wood fungal community composition, (ii) different fungal activity, and (iii) different grapevine metabolism induced by WD.

Esca leaf symptom expression induced a biotic stress response in grapevine wood

In the plants used in this study, we previously showed that esca leaf symptom expression induced a decrease in transpiring green leaf area, leaf chlorophyll content, photosynthesis, water use efficiency, and total nonstructural carbohydrate stem content (Bortolami *et al*. 2021). Several weeks after symptom onset (early autumnal sampling), we demonstrated here that these physiological changes led to wood transcriptomic and metabolomic changes. As expected in the case of grapevine fungal disease (Adrian *et al.*, 2012, 2024), esca symptomatic plants over-expressed and over-produced genes and metabolites associated with biotic stress responses and grapevine defense responses. We identified over-expressed genes coding for R proteins (resistance) and involved in effector detection and defense mechanism activation (Dangl and McDowell, 2006), as well as important actors in grapevine defense responses such as PR proteins and transcription factors (Yu *et al.*, 2013, 2016; Yacoub *et al.*, 2020). Furthermore, esca expression induced the biosynthesis of phenylpropanoid, phytoalexin, stilbenoid and flavonoid, as these functional categories were enriched in wood of esca symptomatic plants. Phenylpropanoids are specialized metabolites involved in plant defense against biotic and

abiotic stresses (Savoi *et al.*, 2016; Ramaroson *et al.*, 2022). Stilbene compounds have an antioxidative activity (Wei *et al.*, 2016; Biais *et al.*, 2017) and are key metabolites in grapevine response to trunk pathogens (Amalfitano *et al.*, 2000; Rusjan *et al.*, 2017; Stempien *et al.*, 2017; Billet *et al.*, 2020; Jeandet *et al.*, 2023). They accumulate less rapidly and to lower levels in grapevine cultivars with high susceptibility to a wood fungal pathogen inoculation (Lambert *et al.*, 2013). Flavonoids and more specifically catechins have already been associated with grapevine defense mechanisms in response to the expression of esca leaf symptoms (Fontaine *et al.*, 2016; Rusjan *et al.*, 2017). Furthermore, it was also shown that flavonoids were synthesized in grapevine leaves submitted to an abiotic stress (nitrogen deficiency), and the level of flavonoids was negatively correlated with esca leaf symptom expression (Dell'Acqua *et al.*, 2025). Flavonoid compounds also present an antioxidative activity (Shen *et al.*, 2022), as well as terpenes (Baccouri and Rajhi, 2021) which were also accumulated in response to the esca condition. The accumulation of such defense-associated metabolites in response to esca might have a protective effect against stresses occurring after the expression of esca leaf symptoms.

Many genes involved in transport of macromolecules such as proteins and multidrug transporters were also detected in the grapevine specific response to esca, suggesting an hypothetical transport of defense-associated molecules in the wood. These genes are involved in vesicle docking and fusion (Sztul and Lupashin, 2009), in the formation of small coated vesicles (Bonifacino and Lippincott-Schwartz, 2003), in detoxification processes, but also in ion flux regulation, plant growth processes and stilbene transport (Martinoia *et al.*, 2002; Çakır *et al.*, 2023; Martínez Márquez *et al.*, 2024, Preprint). They are often associated with plant immunity (Yun and Kwon, 2017).

Interestingly, the "starch and sucrose metabolism" functional category, in particular genes involved in starch catabolism, were significantly enriched in the wood in response to esca leaf symptom expression. Accordingly, esca was shown to alter this metabolism as it induced a decrease in sucrose and starch stem content in our plants and altered leaf photosynthesis (Bortolami *et al.*, 2021). In addition, other studies also showed that starch metabolism seemed to be impacted in esca symptomatic grapevine leaves (Ouadi *et al.*, 2021).

F. mediterranea was the fungal pathogen that responded most to esca leaf symptom expression

Despite the observed increase of total fungal reads abundance in esca symptomatic plants, mainly driven by *F. mediterranea* and *P. chlamydospora*, only *F. mediterranea* genes significantly differentiated esca from other conditions. We also observed this pattern using metabarcoding, showing a significant increase in *P. chlamydospora* and *F. mediterranea* relative abundances as well as a marginal increase in *P. minimum* abundance in esca symptomatic plants. Increased putative virulence factors of *P. chlamydospora*, *P. minimum* and *D. ampelina*(Morales-Cruz *et al.*, 2018) and of *F. mediterranea* (Nerva *et al.*, 2022) was previously documented in plants expressing esca symptoms. However, *Fomitiporia mediterranea*, specifically causing white rot in wood (Moretti *et al.*, 2021), is increasingly seen as a key player in esca pathogenesis (Fischer and Kassemeyer, 2003; Claverie *et al.*, 2020). Here we only used healthy non-necrotic wood tissue showing that this Basidiomycete is able to actively impact healthy wood, which coincides with the activation of plant defence mechanisms in esca condition.

We demonstrated that *F. mediterranea* specific responses to esca expression and subsequent grapevine physiological status involved four different mechanisms.. We evidenced an over-expression 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 defense of territorial boundaries (Lindahl and Finlay, 2006; Karlsson *et al.*, 2016), and terpene synthesis involved in fungal competition and antifungal activity (El Ariebi *et al.*, 2016). Moreover, we found genes involved in the detoxification of the microenvironment (Zhu *et al.*, 2020; Li *et al.*, 2021; Cai *et al.*, 2023) such as a cytochrome b5 were highlighted, suggesting its ability to maintain growth and development in wood. CAZymes and peroxidases genes involved in wood degradation and more specifically in lignin and cellulose degradation (Sigoillot *et al.*, 2012; Janusz *et al.*, 2013; Liu *et al.*, 2017; Qin *et al.*, 2020), were also observed. This was not surprising as this white rot fungus has a complete decay arsenal largely associated with esca wood symptoms (Moretti *et al.*, 2021). The degradation of healthy wood might be responsible for the grapevine defense response activation described above. In response to defense activation, some *F. mediterranea* genes involved in the plant stress response mediation were also observed. Among them, we observed a glycoside hydrolase 128 which was found to increase plant susceptibility by inhibiting ROS burst and callose deposition (Gu *et al.*, 2024), an ABC transporter involved in plant defense metabolites

efflux (Coleman and Mylonakis, 2009) and a peptidase which can degrade plant proteins (Krishnan *et al.*, 2018).

The activation of these four different mechanisms (growth, detoxification, wood degradation, and plant stress response mediation), occurring in healthy wood from plants expressing esca leaf symptoms, suggests a high capacity from *F. mediterranea* to take the upper hand in the competition among fungi upon esca leaf symptoms expression (i.e. specific altered plant physiological status). The expression of esca leaf symptoms stimulated *F. mediterranea* growth and territorial competition in trunk's healthy wood and is thus likely followed by the onset of white rot wood degradation. This is a paradigm change in the understanding of esca pathogenesis as usually it is hypothesized that *F. mediterranea* is playing an important role in leaf symptom expression (Moretti *et al.*, 2021) and not that the physiology induced by esca leaf symptoms could favor *F. mediterranea*. However, it is worth mentioning that there is increasing evidence that the use of trunk surgery or curettage, which consists of the removal of the white rot decay produced by *F. mediterranea*, is able to decrease esca symptom re-expression along with *F. mediterranea* abundance (Bruez *et al.*, 2021; Pacetti *et al.*, 2021; Lecomte *et al.*, 2022), suggesting that a high abundance of *F. mediterranea* in necrotic wood (i.e high abundance of white rot tissue) might contribute to esca leaf symptom expression, in addition to being favoured by it. There is probably a threshold in *F. mediterranea*-white rot abundance at specific grapevine phenological stages associated with esca expression (Maher *et al.*, 2012), as observed with latent pathogenic fungi switching to pathogenic mode (Mishra *et al.*, 2021), fungal quorum sensing events (Padder *et al.*, 2018), and interactions with bacterial community (Bruez *et al.*, 2020; Haidar *et al.*, 2024).

Water deficit induced a typical drought stress response in grapevine wood

In the plants used in this study, we previously showed that the drought stress applied induced a significant decrease in water potential, whole-plant and leaf gas exchanges, and total nonstructural carbohydrate leaf and stem content (Bortolami *et al*. 2021). Overall, the molecular grapevine-specific response to drought observed at the end of the season was comparable to other drought stresses observed in the literature, including oxidative stress response and hormonal regulation (Carvalho *et al*., 2015; Tombesi *et al*., 2015; MacAllister *et al*., 2019; Braidotti *et al*., 2024). Several genes or metabolites responsive to abiotic stress were highlighted as drought response markers, including genes involved in the steroid hormone metabolism (Zeng *et al.*, 2024) and in transcription regulation (Mittler *et al.*, 2006). Among highlighted

metabolites, we detected trehalose that is involved in stomatal movements and drought stress response (Gamm *et al.*, 2015; Morabito *et al.*, 2021), in line with the decreased stomatal conductance observed. Functional categories associated with drought stress response were enriched in the WD condition (such as: "oxidative stress response" or "stilbenoid biosynthesis"). We found genes acting in reactive oxygen species scavenging, as previously described in response to oxidative and drought stresses in grapevine (Carvalho *et al*., 2015; Haider *et al*., 2017; da Fonseca-Pereira *et al.*, 2019). Genes and metabolites involved in the phenylpropanoid, phytoalexin, and stilbenoid biosynthesis functional categories were enriched in the WD condition, as they were in response to esca leaf symptom expression. A total of 15 STS and 2 PAL genes were found over-expressed in response to WD, suggesting the synthesis of stilbene compounds, which was shown to be associated with plant response to drought (Valletta *et al*., 2021).

P. chlamydospora was the fungal pathogen that responded most to drought

Trunk fungal community richness and diversity slightly decreased in response to drought stress, which correlated with the increase in *P. chlamydospora* relative abundance in WD samples, as previously observed in the literature (Leal *et al.*, 2024; Gastou *et al.*, 2025). However, the relative abundance of other studied wood fungal pathogens, including *F. mediterranea*, were similar between control and WD, which were both free of esca symptoms. This result suggests that drought stress may have favored *P. chlamydospora* over the other fungal species in healthy wood under WD. In addition, the putative virulence factor expression of *P. chlamydospora*, *E. lata* and *P. minimum* increased in response to WD. However, only *P. chlamydospora* putative virulence factor genes significantly drove the difference between esca and WD samples, in particular (i) transporters, known to be involved in nutrition, formation and germination of spores, pathogenesis and post-translational regulation (Adachi *et al.*, 2003; Mandujano-González *et al.*, 2016), (ii) carbohydrate active enzymes involved in growth and virulence (Pan *et al.*, 2021), and (iii) enzymes known for their role in toxin synthesis (Choquer *et al.*, 2005; Baker *et al.*, 2006), and in anti-oxidative mechanisms (Castaño *et al.*, 2022). The observation of differentially expressed genes between WD and esca conditions highlighted *P. chlamydospora* genes involved in growth and reproduction (Masloff *et al.*, 2002; Menotta *et al.*, 2008; Yang *et al.*, 2008; Cuperlovic-Culf and Culf, 2014). However, as wood decay-associated genes did not seem to be clearly highlighted as important variables in our analysis,

we can hypothesize that wood degradation activity of *P. chlamydospora* is not impacted by WD. Therefore, *P. chlamydospora* might be able to grow and degrade wood at low water content conditions, as discussed with other fungal species such as *Coniophora sp*. (Brischke and Alfredsen, 2020). Furthermore, as *P. chlamydospora* activity and abundance was favored under WD condition in the absence of esca leaf symptom expression, this suggests that this fungus might not be directly linked to esca expression, contrary to previous hypotheses in the literature (Mondello *et al.*, 2018; Claverie *et al.*, 2020; Hrycan *et al.*, 2020).

Exploring the underlying mechanisms of WD x esca antagonism

A first hypothesis to consider in the antagonism between WD and esca leaf symptom expression is the decrease in plant transpiration that likely prevents the transport of fungal toxins or plant elicitors from the wood to the leaves, as previously hypothesized (Bortolami *et al.*, 2021). This hypothesis is difficult to test due to the short-lived nature of the signal and its low concentration in sap or plant tissue. Here, we investigated putative fungal virulence factor genes in eight trunk pathogens which might be used in future research on the transport of fungal virulence factors through the xylem in the context of esca pathogenesis.

A second hypothesis was that WD might alter the composition of the fungal wood community, resulting in the absence of esca leaf symptom expression. We used healthy wood samples to address this hypothesis, which includes functional sapwood, directly impacted by drought in its water (i.e. low water potential) and carbon balances (i.e., lower NSC). Metabarcoding analysis showed significant differences in the beta-diversity metric between symptomatic (esca) and asymptomatic (WD and control) plants, supporting this second hypothesis. In particular, *F. mediterranea* relative abundance was significantly lower under WD compared with esca symptomatic plants, and similar to control conditions. Thus, the two physiological states without leaf symptoms (control and WD) presented low relative abundance of *F. mediterranea* in contrast with esca symptomatic plants.

The third hypothesis was that WD might negatively impact the wood fungal pathogen activity, in particular inhibiting virulence factors and thus esca pathogenesis. As the total fungal reads increased in stressed conditions, WD seemed to increase overall fungal activity in contrast with this hypothesis. Furthermore, the expression of putative virulence factors of the three fungi mainly associated with esca symptom expression in the literature was not inhibited or reduced in response to WD. However, an important difference between WD and esca was that *P. chlamydospora* increased its virulence under WD, whereas *F. mediterranea* increased its

virulence following esca leaf symptom expression. One explanation to the antagonism between WD and esca leaf symptom expression could be that WD might have inhibited *F. mediterranea* activity at the time of application (before the onset of esca expression), resulting in the inhibition of a putative switch of *F. mediterranea* to its pathogenic mode, and later resulting in the observed low relative abundance of this species in healthy wood in WD plants. Indeed, *F. mediterranea* may have to switch to a pathogenic mode (Mishra *et al.*, 2021) before symptom onset (early summer or even before during grapevine active growth), inducing esca leaf symptom expression later on (and the increase of *F. mediterranea* abundance).

Finally, the last hypothesis is that molecular grapevine response to WD might have inhibited the expression of esca leaf symptoms. Grapevines responded to WD and esca conditions with distinct and shared molecular signatures at the transcriptome and metabolome levels, activating some common stress response pathways. Indeed, plants' response to stress involves the activation of pathways often regulating the plant tolerance to both biotic and abiotic stressors, which is described as cross-tolerance processes (Nadeem *et al.*, 2023). In particular, stress-responsive actors were over-expressed in both esca and WD conditions. Auxin signaling, described for its role in drought stress tolerance (Braidotti *et al.*, 2024) and interaction with biotic stress response (Kazan and Manners, 2009), was the only hormone-related pathway responding to both esca and WD. Specialized metabolites involved in both biotic and abiotic stress tolerance (stilbenoids), were also detected in both conditions. The activation of these biotic and abiotic stress-responsive pathways may have played a key role in the protection of grapevine against esca leaf symptom expression upon drought stress.

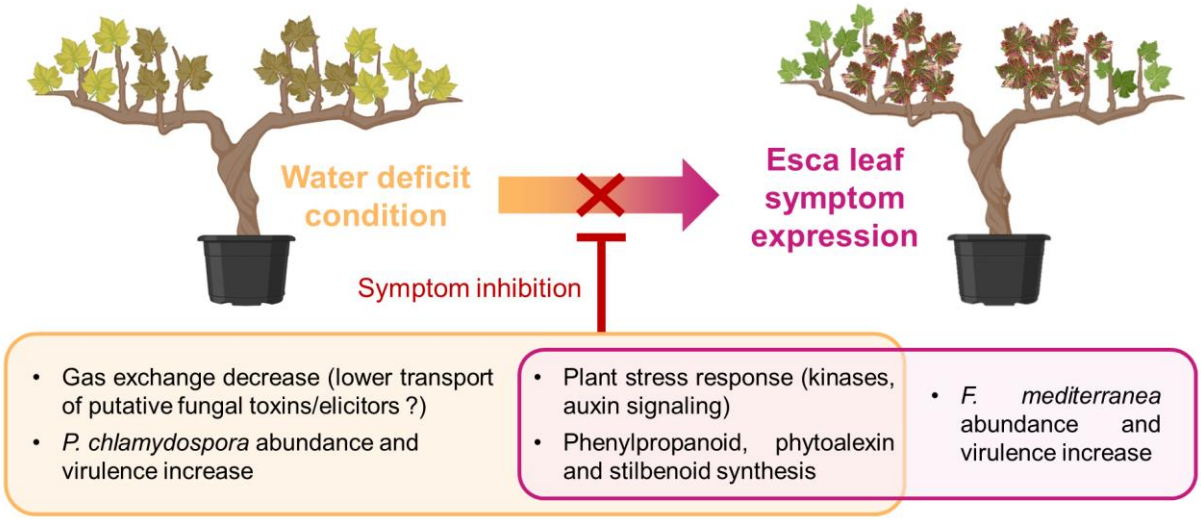

Fig. 8 Overview of the underlying molecular mechanisms that might be responsible for the observed antagonism between water deficit and esca leaf symptoms expression.

In conclusion, our findings revealed the grapevine molecular stress response signatures to WD and esca leaf symptom expression include common and specific pathways. The plant physiology under biotic and abiotic stresses did not affect wood fungal pathogens in the same way; some did not respond to either stress while others did, which suggest a role of fungal ecology and competition for resources. We also highlighted that the inhibition of esca leaf symptom expression under drought might result from a plant defense priming coupled with low transpiration, and the similar *F. mediterranea* abundance and putative virulence as in control plants (Fig. 8). We also observed that *P. chlamydospora* might not be involved in esca leaf symptom expression, as this fungus was highly abundant and active despite the lack of leaf symptoms under WD. Finally, our results also open new perspectives in *F. mediterranea* biology suggesting that white rot might be a response to esca physiology and stimulate *F. mediterranea* growth in healthy wood. Overall, this integrative work highlights that both plant and fungal physiology has to be studied if we are to unravel the underlying mechanisms of perennial plant dieback in the context of global changes.

Acknowledgments

The authors thank Thierry Leste-Lasserre from the Transcriptome facility (University of Bordeaux, INSERM, PUMA, Neurocentre Magendie, Bordeaux, France) for the advice and technical contribution to the RNA extractions. This work was performed in collaboration with the GeT core facility, Toulouse, France (GeT, https://doi.org/10.15454/1.5572370921303193E12), and was supported by France Génomique

National infrastructure, funded as part of “Investissement d’avenir” program managed by Agence Nationale pour la Recherche (contract ANR-10-INBS-09). We specifically thank Jérôme Lluch and Sophie Valière from the GeT core facility.

This work received financial support from the French government in the framework of the IdEX Bordeaux University "Investments for the Future" program / GPR Bordeaux Plant Sciences, and the MetaboHUB infrastructure funded by the Agence Nationale de la Recherche under the France 2030 program (MetaboHUB ANR-11-INBS-0010 ; MetEx+ ANR-21-ESRE-0035; MetaboHUB (JVCE) ANR-24-INBS-0012). It was also supported by the PHYSIOPATH (22001150) and ESCAPADE (22001436) projects (programme ‘Plan National Dépérissement du Vignoble’, FranceAgriMer/CNIV), and the Champion program framework INRAE—UC Davis. Research in the Cantu lab was supported by the California Department of Food and Agriculture, California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (Grant# 20-1062-000-SA; 21-0427000-SA; 22-1588-000-SA; 23-0706-000-SA) and by the Ray Rossi Endowment.

Competing interests

The authors have no competing interests to declare.

Author contributions

C.E.L.D. and M.F.O designed the present study, obtained fundings and co-supervised the research. C.E.L.D., G.A.G. and G.B. conceived the greenhouse experimentation; G.B. and N.F. sampled the tissues and grounded the samples; M.C. designed and realized the RNAseq bioinformatics pipeline under the supervision of C.E.L.D., M.F.O. and D.C.; J.F.G. and M.M. contributed to the metatransciptomic analysis. N.F. carried out the DNA extraction and PCR reactions for the metabarcoding; P.G. analysed the metabarcoding datasets. S.M. contributed to fungal functional result interpretation. A.R., P.P. and the MetaboHUB-Bordeaux team carried out untargeted metabolomic measurements (extraction and MS-DIAL analysis); N.D.A. prepared the samples for metabolomics; M.C. and N.D.A. analysed the metabolomic dataset. M.C. integrated the different datasets, produced the figures and wrote the first version of the paper under C.E.L.D. supervision; All authors participated in different stages of the project, contributed to scientific discussion and critically revised and approved the final version of the manuscript.

ORCID

Data availability

All sequencing datasets (RNAseq and ITS metabarcoding) are available in the ENA database under the accession number PRJEB76229. Metabolomic data is available in the MetaboLights database under the accession number REQ20250521210653.

Bibliography

Adachi K, Nelson GH, Peoples KA, DeZwaan TM, Skalchunes AR, Heiniger RW, Shuster JR, Hamer L, Tanzer MM. 2003. Sequence analysis and functional characterization of the dialkylglycine decarboxylase gene DGD1 from Mycosphaerella graminicola. Current Genetics **43**, 358–363.

Adrian M, Corio-Costet M-F, Calonnec A, Cluzet S, Poinssot B, Trouvelot S, Wiedemann-Merdinoglu S, Viaud M. 2024. Grapevine defence mechanisms when challenged by pathogenic fungi and oomycetes. Advances in Botanical Research. Academic Press.

Adrian M, Trouvelot S, Gamm M, Poinssot B, Héloir M-C, Daire X. 2012. Activation of Grapevine Defense Mechanisms: Theoretical and Applied Approaches. In: Mérillon JM, Ramawat KG, eds. Plant Defence: Biological Control. Dordrecht: Springer Netherlands, 313–331.

Amalfitano C, Evidente A, Mugnai L, Tegli S, Bertelli E, Surico G. 2000. Phenols and Stilbene Polyphenols in the Wood of Esca-Diseased Grapevines. Phytopathologia mediterranea. APRIL, 2000, 1000–1006.

Anderegg WRL, Flint A, Huang C, Flint L, Berry JA, Davis FW, Sperry JS, Field CB. 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.

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

Baker SE, Kroken S, Inderbitzin P, Asvarak T, Li B-Y, Shi L, Yoder OC, Turgeon BG. 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, Grau AL, Chong J, Tian X, Luo J, Guan X, Pensec F. 2022. Pathogenicity Factors of Botryosphaeriaceae Associated with Grapevine Trunk Diseases: New Developments on Their Action on Grapevine Defense Responses. Pathogens **11**, 951.

Biais B, Krisa S, Cluzet S, Da Costa G, Waffo-Teguo P, Mérillon J-M, Richard T. 2017. Antioxidant and Cytoprotective Activities of Grapevine Stilbenes. Journal of Agricultural and Food Chemistry **65**, 4952–4960.

Billet K, Malinowska MA, Munsch T, *et al.* 2020. Stilbenoid-Enriched Grape Cane Extracts for the Biocontrol of Grapevine Diseases. In: Mérillon J-M, Ramawat KG, eds. Plant Defence: Biological Control. Cham: Springer International Publishing, 215–239.

Blanco-Ulate B, Vincenti E, Powell AL, Cantu D. 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, Shaw S, Augustijn HE, *et al.* 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research **51**, W46–W50.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**, 2114–2120.

Bonifacino JS, Lippincott-Schwartz J. 2003. Coat proteins: shaping membrane transport. Nature Reviews Molecular Cell Biology **4**, 409–414.

Bortolami G, Gambetta GA, Cassan C, Dayer S, Farolfi E, Ferrer N, Gibon Y, Jolivet J, Lecomte P, Delmas CEL. 2021. Grapevines under drought do not express esca leaf symptoms. Proceedings of the National Academy of Sciences **118**.

Braidotti R, Falchi R, Calderan A, Pichierri A, Vankova R, Dobrev PI, Griesser M, Sivilotti P. 2024. Multi-hormonal analysis and aquaporins regulation reveal new insights on drought tolerance in grapevine. Journal of Plant Physiology **296**, 154243.

Brischke C, Alfredsen G. 2020. Wood-water relationships and their role for wood susceptibility to fungal decay. Applied Microbiology and Biotechnology **104**, 3781–3795.

Bruez É, Cholet C, Thibon C, Redon P, Lacampagne S, Martignon T, Giudici M, Darriet P, Gény L. 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.

Bruez E, Vallance J, Gautier A, Laval V, Compant S, Maurer W, Sessitsch A, Lebrun M-H, Rey P. 2020. Major changes in grapevine wood microbiota are associated with the onset of esca, a devastating trunk disease. Environmental Microbiology **22**, 5189–5206.

Cai E, Jia H, Feng R, Zheng W, Li L, Zhang L, Jiang Z, Chang C. 2023. Cytochrome c-peroxidase modulates ROS homeostasis to regulate the sexual mating of Sporisorium scitamineum. Microbiology Spectrum **11**, e02057-23.

Çakır B, Jalili H, Turgay G. 2023. Genome-wide analysis of the ABCB gene family in Vitis vinifera: its expression patterns in berries and its responses to iron and heavy metal stresses. The Journal of Horticultural Science and Biotechnology **98**, 591–607.

Carbone MJ, Alaniz S, Mondino P, Gelabert M, Eichmeier A, Tekielska D, Bujanda R, Gramaje D. 2021. Drought Influences Fungal Community Dynamics in the Grapevine Rhizosphere and Root Microbiome. Journal of Fungi **7**, 686.

Carvalho LC, Vidigal P, Amâncio S. 2015. Oxidative stress homeostasis in grapevine (Vitis vinifera L.). Frontiers in Environmental Science **3**.

Castaño JD, Muñoz-Muñoz N, Kim YM, Liu J, Yang L, Schilling JS. 2022. Metabolomics Highlights Different Life History Strategies of White and Brown Rot Wood-Degrading Fungi. (K McMahon, Ed.). mSphere **7**, e00545-22.

Chaloner TM, Gurr SJ, Bebber DP. 2021. Plant pathogen infection risk tracks global crop yields under climate change. Nature Climate Change **11**, 710–715.

Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor.

Bioinformatics **34**, i884–i890.

Choi J, Détry N, Kim K-T, Asiegbu FO, Valkonen JP, Lee Y-H. 2014. fPoxDB: fungal peroxidase database for comparative genomics. BMC Microbiology **14**, 117.

Choquer M, Dekkers KL, Chen H-Q, Cao L, Ueng PP, Daub ME, Chung K-R. 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, Wang C, Wang H, Chen H, Tang M. 2016. Pine wilt disease alters soil properties and root-associated fungal communities in Pinus tabulaeformis forest. Plant and Soil **404**, 237–249.

Claverie M, Notaro M, Fontaine F, Wery J. 2020. Current knowledge on Grapevine Trunk Diseases with complex etiology: a systemic approach. Phytopathologia Mediterranea **59**, 29–53.

Coleman JJ, Mylonakis E. 2009. Efflux in Fungi: La Pièce de Résistance. PLOS Pathogens **5**, e1000486.

Cuperlovic-Culf M, Culf AS. 2014. Role of Histone Deacetylases in Fungal Phytopathogenesis: A Review. International Journal of Modern Botany *in press*.

Danecek P, Bonfield JK, Liddle J, *et al.* 2021. Twelve years of SAMtools and BCFtools. GigaScience **10**, giab008.

Dangl JL, McDowell JM. 2006. Two modes of pathogen recognition by plants. Proceedings of the National Academy of Sciences **103**, 8575–8576.

Dell'Acqua N, Gambetta GA, Comont G, Ferrer N, Rochepeau A, Pétriacq P, Delmas CEL. 2025. Nitrogen nutrition impacts grapevine esca leaf symptom incidence, physiology, and metabolism. Journal of Experimental Botany, eraf172.

Desprez-Loustau M-L, Marçais B, Nageleisen L-M, Piou D, Vannini A. 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science **63**, 597–612.

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

Djoumbou Feunang Y, Eisner R, Knox C, *et al.* 2016. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. Journal of Cheminformatics **8**, 61.

El Ariebi N, Hiscox J, Scriven SA, Müller CT, Boddy L. 2016. Production and effects of volatile organic compounds during interspecific interactions. Fungal Ecology **20**, 144–154.

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. 2018. FROGS: Find, Rapidly, OTUs with Galaxy Solution. (B Berger, Ed.). Bioinformatics **34**, 1287–1294.

Etienne L, Fabre F, Martinetti D, Frank E, Michel L, Bonnardot V, Guérin-Dubrana L, Delmas CEL. 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, Martinetti D, Frank E, Bonnardot V, Michel L, Guerin Dubrana L, Delmas CEL. 2025. Identification of ecoclimatic indicators of esca disease through 20 years of large-scale vineyard monitoring in France. Plant Disease doi: 10.1094/PDIS-01-25-0228-RE.

Fischer M, Kassemeyer H-H. 2003. Fungi associated with Esca disease of grapevine in Germany. Vitis **42**, 109–116.

Fischer M, Knoll M, Sirim D, Wagner F, Funke S, Pleiss J. 2007. The Cytochrome P450 Engineering Database: a navigation and prediction tool for the cytochrome P450 protein family. Bioinformatics **23**, 2015–2017.

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

Fontaine F, Pinto C, Vallet J, Clément C, Gomes AC, Spagnolo A. 2016. The effects of grapevine trunk diseases (GTDs) on vine physiology. European Journal of Plant Pathology **144**, 707–721.

Fu W, Chen B, Jansa J, *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, Héloir M-C, Adrian M. 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, Bortolami G, Ferrer N, Gambetta GA, Moretti S, Vallance J, Delmas CEL. 2025. Differential impacts of drought and esca expression on Ascomycota fungi in the trunks and young organs of mature grapevines. bioRxiv, 2025.07.03.663063.

Gastou P, Irvine AD, Arcens C, Courchinoux E, This P, Leeuwen C van, Delmas C. 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**.

Gonzalez-Dominguez E, Caffi T, Paolini A, Mugnai L, Latinović N, Latinović J, Languasco L, Rossi V. 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**.

Goodell B. 2020. Fungi Involved in the Biodeterioration and Bioconversion of Lignocellulose Substrates. In: Benz JP, Schipper K, eds. Genetics and Biotechnology. Cham: Springer International Publishing, 369–397.

Grimplet J, Cramer GR, Dickerson JA, Mathiason K, Van Hemert J, Fennell AY. 2009. VitisNet: “Omics” Integration through Grapevine Molecular Networks. (NJ Provart, Ed.). PLoS ONE **4**, e8365.

Gu X, Cao Z, Li Z, Yu H, Liu W. 2024. Plant immunity suppression by an β-1,3-glucanase of the maize anthracnose pathogen Colletotrichum graminicola. BMC Plant Biology **24**, 339.

Haidar R, Compant S, Robert C, *et al.* 2024. Two Paenibacillus spp. strains promote grapevine wood degradation by the fungus Fomitiporia mediterranea: from degradation experiments to genome analyses. Scientific Reports **14**, 15779.

Haider MS, Zhang C, Kurjogi MM, Pervaiz T, Zheng T, Zhang C, Lide C, Shangguan L, Fang J. 2017. Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq analysis. Scientific Reports **7**, 13134.

Hamilton CE, Gundel PE, Helander M, Saikkonen K. 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Diversity **54**, 1–10.

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

Jansen RK, Kaittanis C, Saski C, Lee S-B, Tomkins J, Alverson AJ, Daniell H. 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, Kucharzyk KH, Pawlik A, Staszczak M, Paszczynski AJ. 2013. Fungal laccase, manganese peroxidase and lignin peroxidase: Gene expression and regulation. Enzyme and Microbial Technology **52**, 1–12.

Jeandet P, Trotel-Aziz P, Jacquard C, Clément C, Mohan C, Morkunas I, Khan H, Aziz A. 2023. Use of Elicitors and Beneficial Bacteria to Induce and Prime the Stilbene Phytoalexin Response: Applications to Grapevine Disease Resistance. Agronomy **13**, 2225.

Jones JDG, Dangl JL. 2006. The plant immune system. Nature **444**, 323–329.

Kabbage M, Yarden O, Dickman MB. 2015. Pathogenic attributes of *Sclerotinia sclerotiorum*: Switching from a biotrophic to necrotrophic lifestyle. Plant Science **233**, 53–60.

Karlsson M, Stenlid J, Lindahl B. 2016. Functional differentiation of chitinases in the white-rot fungus *Phanerochaete chrysosporium*. Fungal Ecology **22**, 52–60.

Kazan K, Manners JM. 2009. Linking development to defense: auxin in plant–pathogen interactions. Trends in Plant Science **14**, 373–382.

Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. Nature Methods **12**, 357–360.

Krishnan P, Ma X, McDonald BA, Brunner PC. 2018. Widespread signatures of selection for secreted peptidases in a fungal plant pathogen. BMC Evolutionary Biology **18**, 7.

Lambert C, Khiook ILK, Lucas S, Télef-Micouleau N, Mérillon J-M, Cluzet S. 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 DP, Travadon R, Baumgartner K. 2015. Diversity of Diaporthe species associated with wood cankers of fruit and nut crops in northern California. Mycologia **107**, 926–940.

Leal C, Bujanda R, Carbone MJ, Kiss T, Eichmeier A, Gramaje D, Maldonado-González MM. 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, Bénétreau C, Diarra B, Meziani Y, Delmas C, Fermaud M. 2024. Logistic modeling of summer expression of esca symptoms in tolerant and susceptible cultivars in Bordeaux vineyards. OENO One **58**.

Lecomte P, Cholet C, Bruez E, *et al.* 2022. Recovery after curettage of grapevines with esca leaf symptoms. Phytopathologia Mediterranea **61**, 473–489.

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

Li W, Li P, Zhou X, Situ J, Lin Y, Qiu J, Yuan Y, Xi P, Jiang Z, Kong G. 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**.

Lindahl BD, Finlay RD. 2006. Activities of chitinolytic enzymes during primary and secondary colonization of wood by basidiomycetous fungi. New Phytologist **169**, 389–397.

Liu Y, Jiang X, Guan D, Zhou W, Ma M, Zhao B, Cao F, Li L, Li J. 2017. Transcriptional analysis of genes involved in competitive nodulation in Bradyrhizobium diazoefficiens at the presence of soybean root exudates. Scientific Reports **7**, 10946.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology **15**, 550–571.

Lv T, Zhan C, Pan Q, Xu H, Fang H, Wang M, Matsumoto H. 2023. Plant pathogenesis: Toward multidimensional understanding of the microbiome. iMeta **2**, e129.

MacAllister S, Mencuccini M, Sommer U, Engel J, Hudson A, Salmon Y, Dexter KG. 2019. Drought-induced mortality in Scots pine: opening the metabolic black box. Tree Physiology **39**, 1358–1370.

Maher N, Piot J, Bastien S, Vallance J, Rey P, Guérin-Dubrana L. 2012. Wood necrosis in esca-affected vines: types, relationships and possible links with foliar symptom expression. OENO One **46**, 15–27.

Mandujano-González V, Villa-Tanaca L, Anducho-Reyes MA, Mercado-Flores Y. 2016. Secreted fungal aspartic proteases: A review. Revista Iberoamericana de Micología **33**, 76–82.

Martínez Márquez A, Martins V, Selles S, Gerós H, Corchete P, Bru-Martinez R. 2024. The grapevine ABC transporter B family member 15 (VvABCB15) is a transresveratrol

transporter out of grapevine cells. Frontiers doi: 10.3389/fpls.2024.1450638. [Preprint].

Martínez-Vilalta J, Garcia-Forner N. 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant, Cell & Environment **40**, 962–976.

Martinoia E, Klein M, Geisler M, Bovet L, Forestier C, Kolukisaoglu Ü, Müller-Röber B, Schulz B. 2002. Multifunctionality of plant ABC transporters – more than just detoxifiers. Planta **214**, 345–355.

Masloff S, Jacobsen S, Pöggeler S, Kück U. 2002. Functional analysis of the C6 zinc finger gene *pro1* involved in fungal sexual development. Fungal Genetics and Biology **36**, 107–116.

McDowell NG, Sapes G, Pivovaroff A, *et al.* 2022. Mechanisms of woody-plant mortality under rising drought, CO2 and vapour pressure deficit. Nature Reviews Earth & Environment **3**, 294–308.

McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. (M Watson, Ed.). PLoS ONE **8**, e61217.

Menotta M, Amicucci A, Basili G, Polidori E, Stocchi V, Rivero F. 2008. Molecular and functional characterization of a Rho GDP dissociation inhibitor in the filamentous fungus Tuber borchii. BMC Microbiology **8**, 57.

Mina D, Pereira JA, Lino-Neto T, Baptista P. 2020. Impact of plant genotype and plant habitat in shaping bacterial pathobiome: a comparative study in olive tree. Scientific Reports **10**, 3475.

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

Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu J-K. 2006. Gain- and Loss-of-Function Mutations in Zat10 Enhance the Tolerance of Plants to Abiotic Stress. FEBS letters **580**, 6537–6542.

Mondello V, Songy A, Battiston E, Pinto C, Coppin C, Trotel-Aziz P, Clément C, Mugnai L, Fontaine F. 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, Wilhelm M, Viret O, Gindro K, Croll D, Zufferey V, Hofstetter V. 2025. Identifying the pedoclimatic conditions most critical in the susceptibility of a grapevine cultivar to esca disease. OENO One **59**.

Morabito C, Secchi F, Schubert A. 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, Allenbeck G, Figueroa-Balderas R, Ashworth VE, Lawrence DP, Travadon R, Smith RJ, Baumgartner K, Rolshausen PE, Cantu D. 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, Pacetti A, Pierron R, *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, Khan A, Gupta R, Hashem M, Alamri S, Siddiqui MA, Ahmad F. 2023. Stress combination: When two negatives may become antagonistic, synergistic or additive for plants? Pedosphere **33**, 287–300.

Nerva L, Garcia JF, Favaretto F, *et al.* 2022. The hidden world within plants: metatranscriptomics unveils the complexity of wood microbiomes. (M Gifford, Ed.). Journal of Experimental Botany **73**, 2682–2697.

Ouadi L, Bruez E, Bastien S, Vallance J, Lecomte P, Domec J-C, Rey P. 2019. Ecophysiological impacts of Esca, a devastating grapevine trunk disease, on Vitis vinifera L. PLOS ONE **14**, e0222586.

Ouadi L, Bruez E, Bastien S, Yacoub A, Coppin C, Guérin-Dubrana L, Fontaine F, Domec J-C, Rey P. 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, Moretti S, Pinto C, Compant S, Farine S, Bertsch C, Mugnai L. 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.

Padder SA, Prasad R, Shah AH. 2018. Quorum sensing: A less known mode of communication among fungi. Microbiological Research **210**, 51–58.

Pan S, Tang L, Pan X, Qi L, Yang J. 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, Lu Y, Zhou G, *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, Duggal G, Love MI, Irizarry RA, Kingsford C. 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nature Methods **14**, 417–419.

Perincherry L, Stępień Ł, Vasudevan SE. 2021. Cross-Tolerance and Autoimmunity as Missing Links in Abiotic and Biotic Stress Responses in Plants: A Perspective toward Secondary Metabolic Engineering. International Journal of Molecular Sciences **22**, 11945.

Petek-Petrik A, Petrík P, Lamarque LJ, Cochard H, Burlett R, Delzon S. 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, Verbruggen E, Liu L, Weedon JT, Peñuelas J. 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, Li B, Zhou S. 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.

Ramaroson M-L, Koutouan C, Helesbeux J-J, Le Clerc V, Hamama L, Geoffriau E, Briard M. 2022. Role of Phenylpropanoids and Flavonoids in Plant Resistance to Pests and Diseases. Molecules **27**, 8371.

Rohart F, Gautier B, Singh A, Cao K-AL. 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLOS Computational Biology **13**, e1005752.

Rolshausen PE, Baumgartner K, Travadon R, Fujiyoshi P, Pouzoulet J, Wilcox WF. 2014. Identification of Eutypa spp. Causing Eutypa Dieback of Grapevine in Eastern North America. Plant Disease **98**, 483–491.

Rusjan D, Persic M, Likar M, Biniari K, Mikulic-Petkovsek M. 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 MH, Reddy VS, Moreno-Hagelsieb G, *et al.* 2020. The Transporter Classification Database (TCDB): 2021 update. Nucleic Acids Research **49**, D461–D467.

Savoi S, Wong DCJ, Arapitsas P, Miculan M, Bucchetti B, Peterlunger E, Fait A, Mattivi F, Castellarin SD. 2016. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). BMC Plant Biology **16**, 67.

Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. 2022. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. Food Chemistry **383**, 132531.

Sigoillot J-C, Berrin J-G, Bey M, Lesage-Meessen L, Levasseur A, Lomascolo A, Record E, Uzan-Boukhris E. 2012. Chapter 8 - Fungal Strategies for Lignin Degradation. In: Jouanin L, Lapierre C, eds. Lignins. Advances in Botanical Research. Academic Press, 263–308.

Stempien E, Goddard M-L, Wilhelm K, Tarnus C, Bertsch C, Chong J. 2017. Grapevine Botryosphaeria dieback fungi have specific aggressiveness factor repertory involved in wood decay and stilbene metabolization. PLOS ONE **12**, e0188766.

Sztul E, Lupashin V. 2009. Role of vesicle tethering factors in the ER–Golgi membrane traffic. FEBS Letters **583**, 3770–3783.

Tombesi S, Nardini A, Frioni T, Soccolini M, Zadra C, Farinelli D, Poni S, Palliotti A. 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Scientific Reports **5**, 12449.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: from community assembly to plant health. Nature Reviews Microbiology **18**, 607–621.

Urra C, Sanhueza D, Pavez C, *et al.* 2023. Identification of grapevine clones via high-throughput amplicon sequencing: a proof-of-concept study. G3 Genes|Genomes|Genetics **13**, jkad145.

Vayssier-Taussat M, Albina E, Citti C, *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**.

Vicente-Serrano SM, Peña-Angulo D, Beguería S, Domínguez-Castro F, Tomás-Burguera M, Noguera I, Gimeno-Sotelo L, El Kenawy A. 2022. Global drought trends and future projections. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **380**, 20210285.

Wei Y-J, Zhao S-R, Li J-M, Xue B. 2016. Stilbene profiles in different tissues of Vitis vinifera L. cv. Cabernet Sauvignon and a comparison of their antioxidant activity. Australian Journal of Grape and Wine Research **22**, 226–231.

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

Xie Q, Chen A, Zhang Y, *et al.* 2019. ESCRT-III accessory proteins regulate fungal development and plant infection in Fusarium graminearum. Current Genetics **65**, 1041–1055.

Yacoub A, Magnin N, Gerbore J, Haidar R, Bruez E, Compant S, Guyoneaud R, Rey P. 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, El-Ganiny AM, Bray GE, Sanders DAR, Kaminskyj SGW. 2008. *Aspergillus nidulans hypB* encodes a Sec7-domain protein important for hyphal morphogenesis. Fungal Genetics and Biology **45**, 749–759.

Yu Y-H, Li X-Z, Wu Z-J, Chen D-X, Li G-R, Li X-Q, Zhang G-H. 2016. VvZFP11, a Cys2His2-type zinc finger transcription factor, is involved in defense responses in Vitis vinifera. Biologia Plantarum **60**, 292–298.

Yu X-Q, Niu H-Q, Liu C, Wang H-L, Yin W, Xia X. 2024. PTI-ETI synergistic signal mechanisms in plant immunity. Plant Biotechnology Journal **22**, 2113–2128.

Yu Y, Xu W, Wang J, Wang L, Yao W, Yang Y, Xu Y, Ma F, Du Y, Wang Y. 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.

Yun HS, Kwon C. 2017. Vesicle trafficking in plant immunity. Current Opinion in Plant Biology **40**, 34–42.

Zargar SM, Gupta N, Nazir M, Mahajan R, Malik FA, Sofi NR, Shikari AB, Salgotra RK. 2017. Impact of drought on photosynthesis: Molecular perspective. Plant Gene **11**, 154–159.

Zeng G, Wan Z, Xie R, Lei B, Li C, Gao F, Zhang Z, Xi Z. 2024. 24-epibrassinolide enhances drought tolerance in grapevine (Vitis vinifera L.) by regulating carbon and nitrogen metabolism. Plant Cell Reports **43**, 219.

Zheng J, Ge Q, Yan Y, Zhang X, Huang L, Yin Y. 2023. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. Nucleic Acids Research **51**, W115–

W121.

Zhu Z, Yang M, Bai Y, Ge F, Wang S. 2020. Antioxidant-related catalase CTA1 regulates development, aflatoxin biosynthesis, and virulence in pathogenic fungus Aspergillus flavus. Environmental Microbiology **22**, 2792–2810.

Zinger L, Lionnet C, Benoiston A-S, Donald J, Mercier C, Boyer F. 2021. metabaR: An r package for the evaluation and improvement of DNA metabarcoding data quality. Methods in Ecology and Evolution **12**, 586–592.