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# Effects of three elicitors on primary metabolism six days after treatment in healthy *Vitis vinifera* leaves and eight days after treatment in healthy and downy mildew-inoculated leaves

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## ABSTRACT

Elicitors can be used to reduce the application of conventional pesticides against grapevine diseases; however, they may disrupt plant primary metabolism. The long-term effects (more than 6 days after treatment) of applying plant defence stimulators (PDS) to protect grapevine from *Plasmopara viticola* were investigated. We studied the effect of three PDS (acibenzolar-S-methyl/ASM, potassium phosphonate/PHOS, methyl jasmonate/MeJA) and one surfactant (Triton) on gene expression and metabolite production in leaves of *Vitis vinifera* cv. Cabernet-Sauvignon before or after their inoculation with *P. viticola*. The molecular and biochemical results show the impact of these PDS on grapevine metabolism more than 6 days after treatment, and after inoculation.

High-throughput q-RT-PCR revealed that sixty of the primary metabolism genes (ion homeostasis and hormones pathways) were modulated by all treatments, some modulations being specific to a given PDS. Meanwhile, 1H NMR studies revealed variations in metabolite abundances (amines, amino acids, organic acids, polyphenols and sugars), with some being common to all treatments (organic acid increase) and others specific to a single one. By combining two methodological approaches, it was possible to determine the specific effects of each PDS on *Vitis*. All PDS-induced resistance involved modulation of the primary metabolism. This innovative approach can be extended to vineyard studies in order to help better understand the variability of PDS effects *in natura*.

**KEYWORDS:** BTH, Downy mildew, Grapevine, Jasmonate, Phosphite, Metabolomic, Transcriptomic

## INTRODUCTION

Cultivated grapevines (*Vitis vinifera* sp.) are highly susceptible to pathogens, such as *Erysiphe necator* and *Plasmopara viticola*, the causal agents of powdery mildew and downy mildew, respectively. Therefore, grapevines need to be protected in order to produce quality grapes. In vineyard protection, pesticides are generally used, but they can negatively impact the environment (Pesce *et al.*, 2023). For several decades, the use of molecules that stimulate defences has been explored as an alternative and/or complementary strategy for controlling grapevine diseases (Delaunois *et al.*, 2014; Taibi *et al.*, 2023; Reglinski *et al.,* 2023). The aim of inducing plant resistance is to enable a susceptible plant to defend itself against pathogens (Walters *et al.*, 2014) and to limit the intensity of the diseases (Taibi *et al.,* 2023; Kushalappa *et al*., 2016). In grapevines, this typically results in three types of defences: parietal reinforcement (cuticle and cell wall), and the production of phytoalexins (e.g., stilbenes) and PR proteins (e.g., chitinases and β-1,3-glucanase) (Adrian *et al.,* 2024). These defence responses are mediated by phytohormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (Et), and abscissic acid (ABA) (Shigenaga *et al.,* 2017; Walters *et al.,* 2014). Inducers of plant resistance (elicitors or plant defence stimulators (PDS)) can be natural or synthetic compounds, such as analogs of phytohormones (e.g., methyl jasmonate (MeJA), acibenzolar-*S*-methyl (ASM) and ethephon), natural molecules (e.g., laminarin, yeast extract and chitosan), microorganisms (e.g., Bacillus, Trichoderma), and phosphonates (Delaunois *et al*., 2014; Reglinski *et al.,* 2023). ASM, or benzothiadiazole (BTH), is an analog of salicylic acid (Friedrich *et al.*, 1996), which has been reported to display a negative impact on growth and wine quality (Miliordos *et al*, 2023). Phosphonates (or phosphites, PHOS) have the peculiarity of combining direct (fungicide) and indirect (PDS) modes of action (Guest and Grant, 1991; Dufour *et al*., 2013a; Massoud *et al*., 2012). They also have the ability to promote plant growth and yield by acting as biostimulants to counteract abiotic stresses (e.g., thermotolerance and drought) (Wu *et al.*, 2019; Mohammadi *et al*., 2021). They affect sugar metabolism and cause hormonal changes (Wu *et al.*, 2019). MeJA, a derivative of oxilipin acting as jasmonate (JA), enables plants to adapt to environmental stresses and regulates growth and defence responses (Wasternack and Hause, 2013); however, its action can lead to a decrease in sugar and organic acid content, limiting growth and photosynthetic activity (Gould *et al.*, 2021).

MeJA, ASM and PHOS have been reported to reduce grapevine downy mildew growth by enhancing defence responses (Burdziej *et al.*, 2021; Perazzolli *et al.*, 2008; Pinto *et al.,* 2012; Dufour *et al.*, 2013a; Dufour *et al.*, 2013b; Dufour *et al.*, 2016; Harm *et al*., 2011; Jeandet *et al*., 2023). Studies on the long-term effects of these PDS and their impact on grapevines (healthy or diseased) are scarce (Burdziej *et al.*, 2021; Dufour *et al.*, 2016; Bodin *et al*., 2020; Pagliarani *et al.*, 2020). After PDS treatments, defences remain enhanced over a duration, often being specific to the elicitor and experimental conditions (Jeandet *et al.*, 2023; Adrian *et al.,* 2024). Regarding stilbenes, PHOS and BTH tend to act similarly with an overproduction of pterostilbene, whereas MeJA enhances the production of piceids and ε-viniferin (Burdziej *et al.*, 2021). Several authors have underlined that primary metabolism is essential in order to cover the energy costs relative to plant defence activation, commonly known as the growth-defence tradeoff (Bolton, 2009; Eichmann and Schadfer, 2015). This tradeoff concept is linked to the subtle regulatory processes carried out by the plant to optimise its growth and defenses in a complex environment (Kliebenstein, 2016). In grapevines, elicitors modulate the expression of numerous genes and metabolite production associated with primary metabolism (carbohydrates, amino acids and lipids) (Rojas *et al.*, 2014; Guttierrez-Gamboa *et al.*, 2017; Héloir *et al.*, 2018; Krzyzaniak *et al.*, 2018; Bodin *et al.*, 2020; Lemaître-Guillier *et al.*, 2017; Burdziej *et al.,* 2019). For instance, sulphated laminarin activates primary metabolism, and ASM, PHOS and MeJA increase or decrease amino acid and sugar levels over time. These responses depend strongly on the time of action, the PDS, the dose, the plant (cells, leaves, berries) and the presence or not of a pathogen. In addition, environmental conditions, such as the availability of nitrogen, can influence the cost of treatment with ASM (Dietrich *et al.,* 2005). Plant hormones, especially SA, JA, ET and ABA, highly orchestrate the interplay between growth and defence (Eichmann and Schadfer, 2015; Shigenaga *et al.*, 2017).

The aim of this study was to better understand the effect of three PDS on grapevine metabolism, particularly after long-term treatment. We assessed (i) the expression of genes involved in ion homeostasis and hormone metabolism using a high-throughput microarray, and (ii) the main metabolite (sugars, amino acids, organic acids, polyphenols and amines) abundances using NMR. Finally, we evaluated the benefits of a combined molecular and biochemical approach to underline the action of these PDS on the grapevine, before and after *P. viticola* inoculation.

## MATERIALS AND METHODS

### 1. Plant and pathogen material

#### 1.1. Grapevine plants

*V. vinifera* cv. Cabernet-Sauvignon were grown in a greenhouse (16 h photoperiod-350 µmol/m$^2$/s) from wood cuttings (dormant canes collected in winter before the experiment). Two-month-old plants with 10-12 leaves were used for the experiments.

#### 1.2. Downy mildew

*Plasmopara viticola* monosporangia isolate ORG from the laboratory collection was used. The maintenance and inoculation were performed according to the procedure used by Corio-Costet *et al.* (2010). Briefly, the pathogen was propagated on *V. vinifera* Cabernet-Sauvignon leaves with abaxial surface exposed on moist Whatman paper in Petri dishes. Droplets (15 µL) of freshly produced sporangia suspension that was seven days old were used for inoculation.

Dishes were incubated in a growth chamber (22 ± 2 °C) with a daily photoperiod of 16 h light/8 h dark. One day after inoculation, the droplets were removed and the dishes were incubated six days before disease measurement.

## 2. Chemicals

ASM (acibenzolar-*S*-methyl or *S*-methyl benzo[1,2,3,] thiadiazole-7-carthioate, Bion® 50WG, Syngenta, Switzerland), and phosphonates (PHOS) (mono- and dipotassium salts of phosphorous acid, PHOS-01F34®, De Sangosse, France) were used. Methyl jasmonate (MeJA, 95 %) and its wetting agent, Triton X-100 (Triton), were purchased from Sigma® (USA). Methanol-*d*4 (99.80 % D), $D_2O$ (99.90 % D, Euriso-Top) and TMSP (98 % D, Euriso-Top) were purshased from Euriso-Top (St-Aubin and Gif-sur-Yvette, France) and calcium formate at Sigma-Aldrich

## 3. Treatments and inoculation with *P. viticola*

### 3.1. Elicitation

A total of 70 cuttings and 14 plants per condition were used. The treatments were: MeJA at 1.09 g/L in Triton solution at 0.1 % (co-formulant), ASM at 2 g/L, PHOS at 1.5 g/L, Triton at 0.1 % and distilled water. MeJA was dissolved in 1 % EtOH before being added to the Triton solution. All solutions were sprayed on grapevine leaves using a micro-diffuser (Ecospray®).

Leaves were collected at two stages: i) 6 days after treatment (6 dpt) and ii) 8 days after treatment, 2 days after inoculation (8dpt-2dpt and 8dpt) (Figure 1A). Leaf L4 (the fourth below the apex) was harvested from each plant. From collected leaves, foliar discs (25 mm-wide) were cut out for *P. viticola* assays, and the leaves were cut in half and stored at -80 °C for further analysis. After being thoroughly rinsed with water, the leaves were inoculated (8dpt-2dpi) or not (8dpt) with downy mildew. Then 48 h later (8dpt-2dpi), the leaves were cut in half and the samples were stored at -80 °C.

### 3.2. Inoculation

On the sixth day of treatment, 6 leaves (L4) from 6 different plants per treatment were inoculated with *P. viticola* fresh sporangia suspension (7-days old) as described by Corio-Costet *et al.* (2010), with droplets (15 or 3 per leaf or disc, respectively) of 15 µL of spore suspension (8500 sporangia/mL). Afterwards, leaves were incubated at 22 °C (16 h day/8 h night photoperiod -25 $\mu E/m^2/s$). Two days after inoculation, one disc from each inoculated leaf was placed in a Petri dish (6 discs per dish) and incubated for 5 days before disease measurement.

### 3.3. Disease intensity measurement

Seven days after inoculation, growth and sporulation of *P. viticola,* for each drop (3 per disc) and each disc (6 replicates), were measured to estimate disease intensity (Corio-Costet *et al.*, 2011; Dufour *et al.*, 2013a). A nonparametric test (Kruskal-Wallis) using R x64 3.0.3 software was used to calculate the mean values for sporulation inhibition. The significant differences were determined by Tukey's test at the 5 % significance level.

### 4. Samples preparation for NMR analysis

For 6 dpt or 8dpt-2dpi, each leaf was split in two and extemporaneously frozen under liquid nitrogen. Three batches of 2 leaves (3 replicates) underwent this procedure (Figure 1A). Samples were stored at -80°C for gene expression and NMR analyses. The extraction was done on the freeze-dried leaves (50 mg) with 1 mL of a buffer as used by Burdziej *et al*., 2019. The 3-(trimethylsilyl) propanoic-2,2,3,3-d4 acid sodium salt and calcium formate were used as NMR chemical shift reference, and internal standard for the quantification of metabolites, respectively. After extraction, the sample preparation procedure was carried out as previously described (Burdziej *et al.*, 2019).

### 4.1. NMR spectroscopy

For recording one-dimensional $^1$H-NMR spectra, a 600 MHz AVANCE III spectrometer (Bruker, Wissembourg, France) was used. The same parameters of processing, acquisition and assignment as that mentionned in Burdziej *et al.* (2019) were followed. 2D-NMR experiments based on COSY and J-resolved enabled the identification of quercetin-3-*O*-glucoside and *trans*-feruloyl acid derivative. The semi-quantification of compounds was also carried out as described by Burdziej *et al.* (2019). The relative quantification of the metabolites was determined by the ratio of the intensities of the analyte peak integrals and those of the internal standard.

### 4.2. Gene expression analysis by RT-qPCR

Each sample, at 6 and 8 days (6 dpt and 8dpt-2dpi), was equivalent to 2 half-leaves from 2 different plants. Three samples (3 replicates x 2 half-leaves) were considered for each treatment, as we had 6 different plants (Figure 1A). Total RNA was extracted using the Spectrum™ Plant Total RNA kit (Sigma®) as described by Burdziej *et al.* (2021). After action of DNase I (Bellée *et al.*, 2018), 10 µg of total RNA were reverse transcripted in a mix of 2 µM oligo-$d(T)_{15}$, ribonuclease inhibitor and M-MLV reverse transcriptase according to the manufacturer's instructions (Invitrogen®). High-throughput gene expression was quantified using microfluidic dynamic array (Fluidigm) technology with primer sets of the « Biostim96 » chip designed by Bodin *et al.* (2020). Genes are listed in Table S1. Three reference genes (*VvGAPDH*, *VvTHIORYLS8, VvTIP41*) were used to normalise the expressions (Bodin *et al.,* 2020). The Fluidigm technology is a dynamic microfluidic array of microchannels interconnected by external inputs and outputs (primers, samples, reagents). Liquids are injected and withdrawn from the chip using automated active systems. Each reaction is checked against the Cq curve for each sample and gene tested. Compared to conventional q-PCR (Dufour *et al.*, 2016), this high-throughput quantification method has excellent reproducibility and is very economical. Briefly, using a 96-well plate containing 3 replicates per PDS treatment and the controls, 30 experimental conditions are analysed per plate. In parallel, a plate containing 95 primers for different genes is used. All primers are tested on all samples using robotic microfluidics. In this study, the genes of interest were distributed among different categories (carbon metabolism,

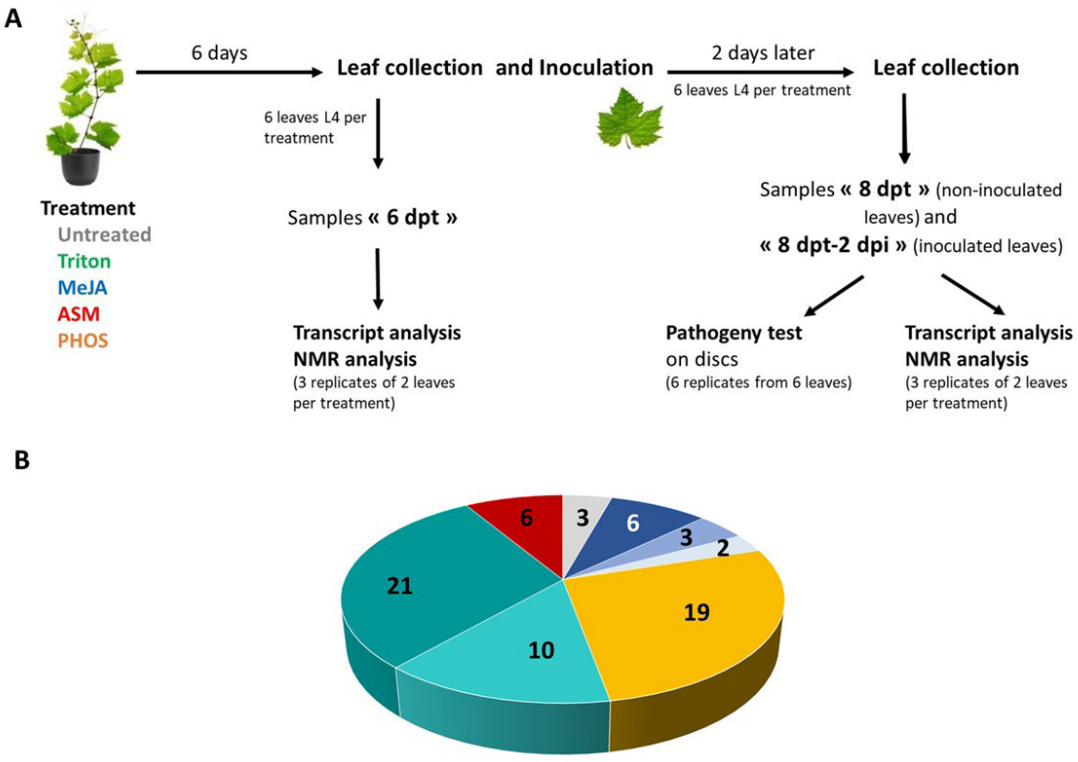


**FIGURE 1.** (A) Experimental diagram (MeJA = methyl jasmonate; ASM = acibenzolar-S-methyl; PHOS = phosphonate). (B) Number of genes in the different categories on the Biostim-96 chip. Grey = housekeeping genes; dark blue = carbon metabolism; medium blue = nitrogen metabolism; light blue = cell division; orange = hormone metabolism; medium green = aquaporins; dark green: ion homeostasis; red = stress responses and defence genes.

nitrogen metabolism, cell division, hormonal metabolisms, homeostasis and aquaporins, oxido-reduction processes, defense and housekeeping genes (Figure 1B). Statistical analysis of gene expression was performed on the mean of the 3 biological replicates relative to the control.

Combining the data with the default distance metric set to Pearson correlation, hierachical clustering analysis was conducted using the Multiple Array Viewer software, version 4.9.0. Gene expression of treated-uninoculated or treated-inoculated leaves was analysed relative to untreated plants at 6 dpt or 8dpt-2dpi.

### 5. Statistical analysis

For gene expression and NMR data, statistical analyses were performed using the R Studio software (3.6.2 version). Data were subjected to one-way ANOVA, and means were separated by Tukey's test ($p < 0.05$) (glht function {multcomp}). Relative gene expression was observed as differentially expressed for a $P$ value < 0.05 in rank-based nonparametric multiple comparisons (Dunnet_test, nparcom function {nparcomp}). In order to determine the individual variability of the treatments, multiple factor analyses were performed (MFA function {FactoMineR}).

## RESULTS

### 1. General effect of the three PDSs

#### 1.1. Gene expression data

At 6 days, the number of regulated genes varied between 18 (MeJA, PHOS) and 32 (ASM). PHOS and MeJA equally modulated the genes. ASM led to 1.66 times more up-regulated than down-regulated genes. It should be noted that Triton led to numerous up-regulations (Figure 2A).

Two days after inoculation, the expression of 9 to 25 genes were modulated in the treated leaves. After inoculation, ASM modulated the fewest genes and, MeJA and PHOS modulated the most up-regulation (Figure 2B).

#### 1.2. NMR data

Six days after treatment, all treatments significantly increased the content of organic acids between 1.8- and 3.3-fold. ASM led to the highest quantities, followed by PHOS, Triton and MeJA treatments (Figure 3A). Similarly, polyphenol abundance was significantly higher in the treated plants than in the control plants. MeJA treatment reduced total sugars.

As at 6 dpt, at 8dpt-2dpi, the most significant increase obtained after inoculation with all treatments was in the relative abundance of the organic acids, except for MeJA.

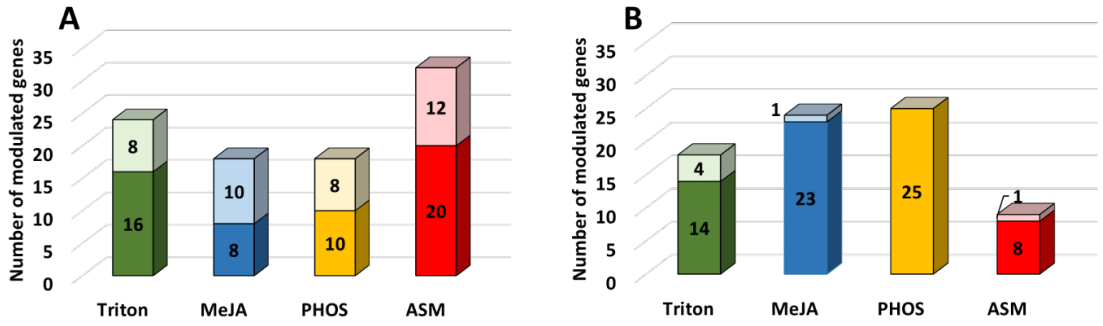


**FIGURE 2.** Number of genes significantly modulated at 6 days after treatment (A), and at 8 days and 2 days after inoculation (B). Triton, MeJA (methyl jasmonate), PHOS (phosphonate), ASM (acibenzolar-S-methyl). Dark = up-regulated, and light = down-regulated.

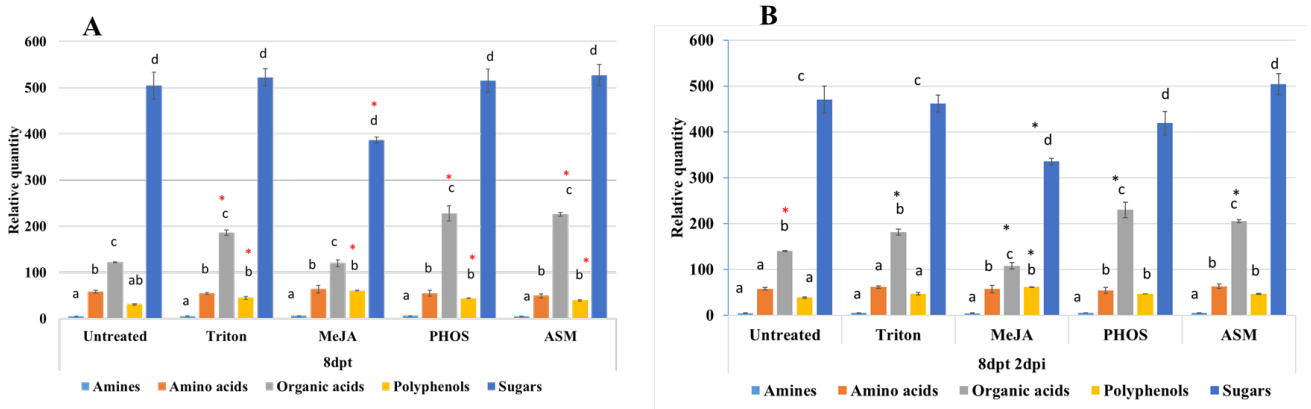


**FIGURE 3.** Relative abundance of the identified compounds by 1H-NMR spectroscopy at 6 dpt (A) and at 8dpt-2dpi (B). Asterisks denote significance difference ($p \leq 0.05$) with respective control (red: untreated un-incoculated and black: untreated inoculated control) and letters indicate significant differences among all the samples for one treatment.

In addition, MeJA always reduced sugars and increased polyphenols.

## 2. Specific effects on gene expression and metabolites at 6 dpt

### 2.1. Gene expression at 6 dpt

For simplicity, we use the terms 'up-regulated' or 'down-regulated' throughout this manuscript, even though we describe the abundance of the transcripts. Most of the selected genes involved in hormonal metabolism (ABA, auxin, cytokinin, gibberellins) and in ion homeostasis (aquaporin, iron, zinc, copper and calcium transporters) were modulated in the treated leaves compared to the control leaves. The Venn diagram (Figure 4A, S2) indicates that two genes were modulated in all treatments: the transcripts of *VvIRT,* an iron transporter gene, were highly abundant, while those of nitrate reductase (*VvNr*) were low in abundance. All treatments had a widespread effect on iron homeostasis, with up-regulation of the *VvFER* gene (chloroplast ferritin), except MeJA which down-regulated it and an associated iron transcription factor (*VvPYE*). The genes of iron transport and regulation were commonly up-regulated by ASM (*VvIRT, VvFER, VvYSL1, VvYSL7*), PHOS (*VvIRT*, *VvFER, VvFSD1*) and Triton (*VvIRT, VvFER, VvFSD1, VvYLS7*).

Zinc and copper transport genes were also modulated. Some copper transport genes (*VvCTR, VvPAA, VvCSD*) were commonly up-regulated, with the exception of MeJA. However, MeJA specifically up-regulated a copper-dependent superoxide dismutase gene (*VvCSD2*). *VvATX* and *VvATOX* genes (chaperone protein of copper transport) were down-regulated in PHOS and MeJA leaves, respectively. *VvZIP2* was up-regulated in PHOS and ASM leaves, and the calcium pathway genes were not significantly modulated.

Regarding aquaporins, *VvTIPs* genes were weakly modulated, except with the down-regulation of *VvTIP1-1* by ASM. The PIP genes were mainly up-regulated by MeJA and partially by Triton, but were more or less down-regulated by PHOS and ASM, with exceptions for *VvPIP1.1* and *VvPIP2.3*, respectively.

Concerning genes involved in hormonal pathways, three genes (*VvAA01, VvABCG40* and *VvHYD2*) involved in metabolism and the import of the ABA pathway were up-regulated in ASM leaves. Triton up-regulated ABA

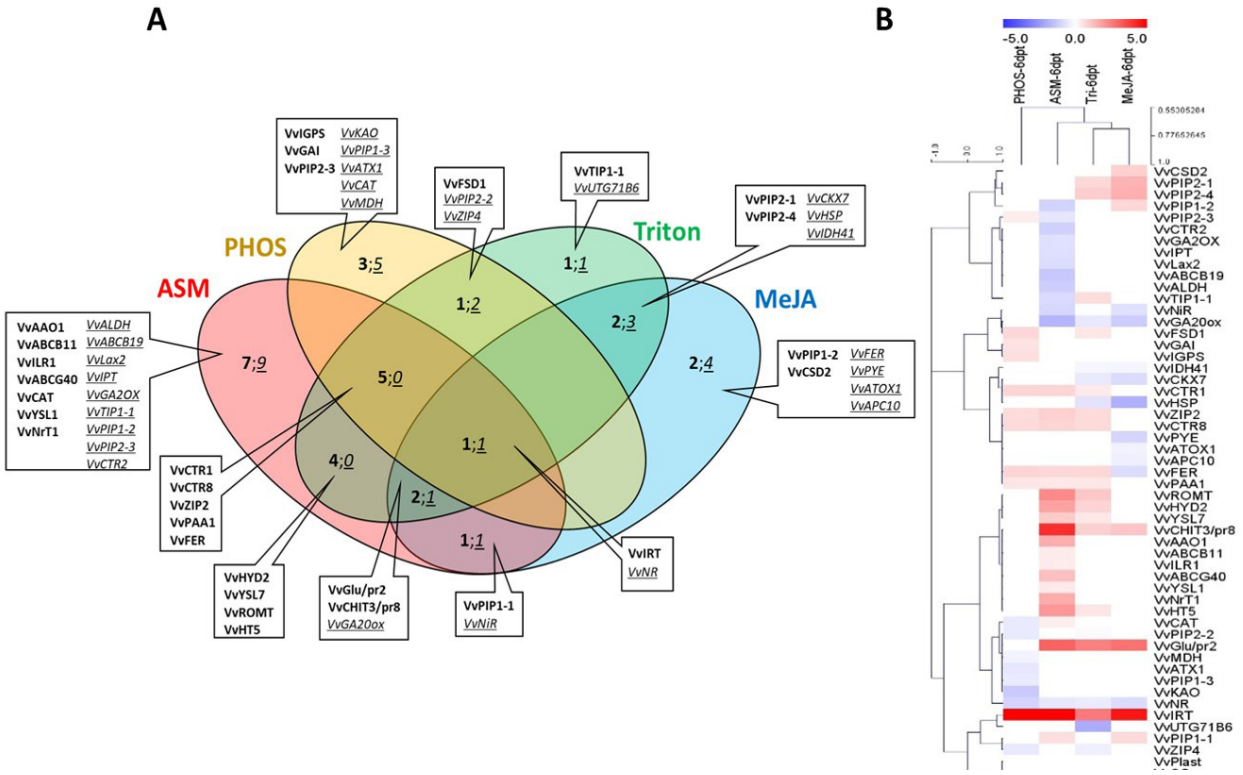


**FIGURE 4.** (A) Venn diagram with significantly modulated genes in grapevine leaves at 6 dpt with Triton (Tri), MeJA, PHOS, or ASM. Induced genes are represented in bold and down-regulated ones in underlined italic. Each treatment is represented by an ellipse, (B) Hierarchical clustering of relative expression of genes (log2-fold change relative to the untreated control). Each column represents the treatment and each line one gene. Colour scale bars indicate ratios corresponding to the mean of three experiments. Up-regulated and down-regulated genes are in shades of red and blue, respectively: bright red = higher than 5, and dark blue = lower than -5.

catabolism gene (*VvHYD*) and down-regulated *VvUTG25,* which is involved in ABA conjugation.

The three genes of the gibberellin pathway were mainly down-regulated by MeJA, Triton and ASM (*VvGA20ox*). Only PHOS led to the up-regulation of a gibberellin regulatory gene (*Vv GAI*).

ASM essentially modulated the genes of the auxin pathway by inducing an auxin transporter (intracellular import, *VvABCB11*) and reducing those of export (*VvABCB19*) and import to leaf apex (*VvLAX*). *VvILR* coding for a IAA-Leu hydrolysis protein was also induced. PHOS up-regulated the gene *VvIGPS* (indol-3-glycerol phosphate).

MeJA and Triton modulated only few hormonal genes in common (down-regulation of *VvGA2ox* and *VvCKX7*), suggesting a weak effect of Triton at 6 dpt.

The genes associated with division (*VvAPC10, VvPDV1*) and those of of tricarboxylic acid cycle (TCA) (*VvMDH, VvCS, VvIDH*) were slightly modulated.

Of particular interest was the almost generalised transcript decrease, whatever the treatment, of nitrate and nitrite reductases (*VvNr, VvNir*). However, there was a significant increase in *VvNRT1* (nitrate/nitrite transporter) transcripts in ASM leaves, which was also associated with an up-regulation of a sugar transporter (*VvHT5*).

Several PR protein genes linked to stress responses (*VvPR2* and *VvPR8*) were up-regulated by all treatments, except PHOS. *VvROMT* (resveratrol-*O*-methyl transferase) was induced by Triton and ASM. *VvCAT* coding for a catalase was slightly up-regulated in ASM treated leaves and down-regulated in those treated by PHOS. Concerning two heat shock genes (*VvDnaj* and *VvHSP*), *VvHSP* was down-regulated in Triton- and even more so in MeJA-treated leaves. Triton was used as a co-formulant with MeJA and modulated several genes. The MeJA treatment enhanced the effects observed with Triton.

At 6 dpt, the homeostasis genes of iron, zinc, copper and aquaporins were strongly modulated. Among the PDS treatments, ASM had the greatest effect on hormone pathway genes. All treatments down-regulated nitrate and nitrite reductase genes.

## 2.2 NMR analysis at 6 dpt

Malic, acetic, tartaric and succinic acids increased the most in all treatments compared with the control plants (Figure 5A, Table S2). However, ASM increased the organic acids the most, the highest abundance being of fumaric acid.

Conversely MeJA led to the lowest fumaric acid content. Concerning sugars, only MeJA reduced significantly sucrose and fructose quantities (Figure 5B, Table S2). Phosphonate also had a negative impact on fructose. Despite a trend of increased sucrose and *myo*-inositol, the effect of ASM was not significant. It is noteworthy that *myo*-inositol increased (MeJA, ASM) or tended to increase (PHOS, Triton).

The increase in certain amino-acids was sometimes specific to the treatment (Figure 5C). PHOS increased glutamine (Gln), glutamic acid (Glu) and GABA contents, and MeJA raised proline and valine (Val) specifically. Triton, MeJA and ASM also increased threonine (Thr) and tyrosine (Tyr) levels. All the treatments resulted in one or more increases in amino acid content, with the exception of ASM, which significantly reduced the amount of alanine (Ala), but increased glutamine, threonine and tyrosine.

Regarding the amines in treated leaves, the quantities of trigonelline were greatly increased (3.6 to 6.1 times higher than in the control), except in ASM leaves (Figure 5D, Table S2). MeJA and PHOS had a positive impact on choline or adenine, respectively.

With regard to polyphenols, all treatments significantly increased the quantities of *trans*-feruloyl derivatives (from 1.37 to 2 times more) and quercetin-3-*O*-glucoside (from 1.6 to 2.48 times more) (Figures 3 and 5E, Table S2). Catechin was also increased by the treatments, except for the ASM. ASM doubled the content of shikimic acid, a compound upstream of polyphenol biosynthesis, which was also enhanced in Triton and MeJA leaves.

## 3. Specific effect at 8 days after treatment and two days after *P. viticola* inoculation (8dpt-2dpi)

### 3.1. Gene expression at 8dpt-2dpi

After *P. viticola* inoculation, the treated plants mainly responded with an up-regulation of genes involved in

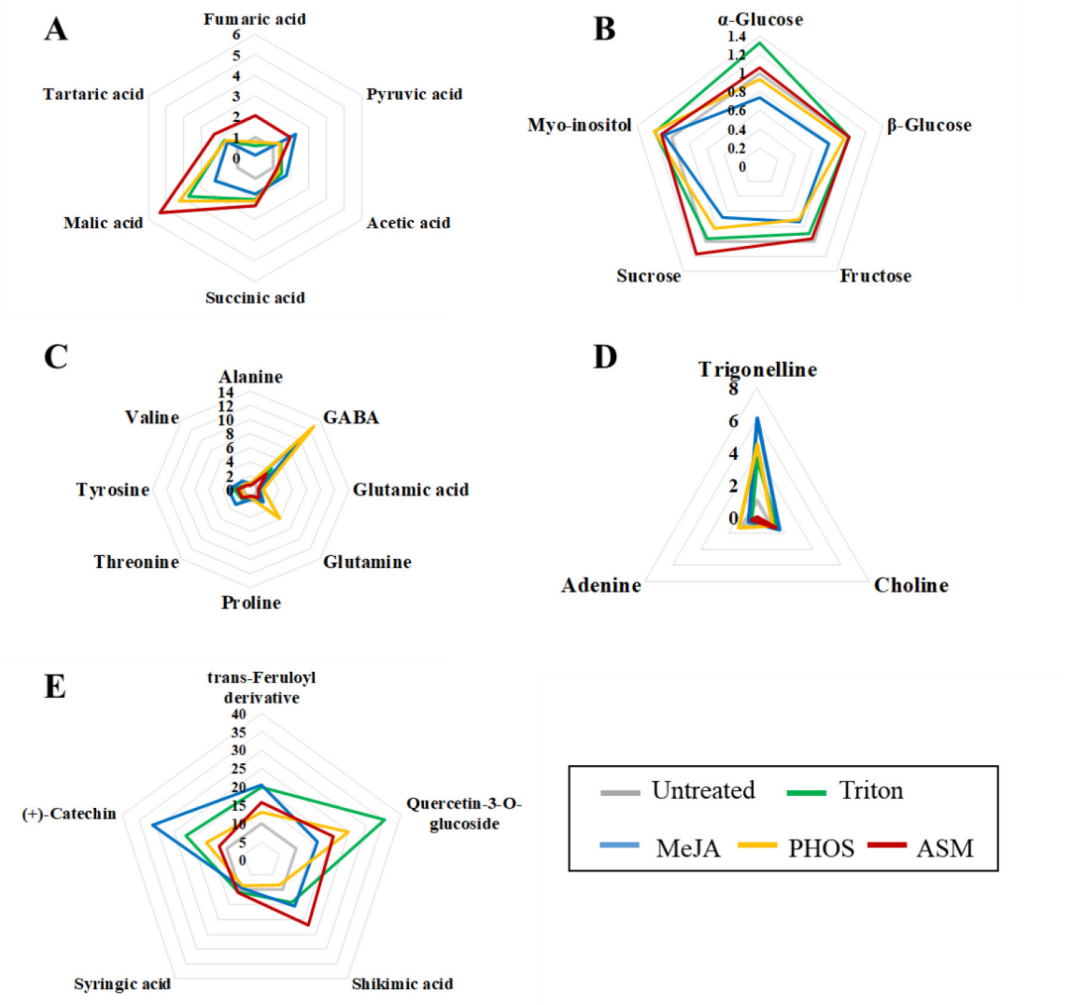


**FIGURE 5.** (A) Comparison of changes within organic acids, (B) sugars, (C) amino acids, (D) amines, and (E) polyphenols in grapevine leaves 6 days after treatment. Radar charts were generated from the fold change of the means of three replicates relative to the untreated control.

hormone pathways (Figures 6 and S2). Two genes were up-regulated by all treatments: *VvILR1,* involved in auxin remobilisation; and *VvGA20ox* (gibberellin activation). The transcripts *VvABCB11* (intracellular auxin import), *VvBGlu* (ABA ester hydrolysis) and *VvPIP1.3* (aquaporin) accumulated in all treated leaves, except in the ASM treatment (Figure 6A). Two transport genes, *VvLax* and *VvYSL7*, were up-regulated by PHOS and Triton. All PDS induced a gibberellin pathway signalling gene (*VvGAI*) and an ABA export gene (*Vv ABCG25*). The sugar transporter *VvHT5* gene was up-regulated by ASM and MeJA, and three genes were common to ASM, Triton and MeJA (*VvBTS, VvPR2* and *VvPip2.3*). In PHOS-treated leaves, the majority of specifically up-regulated genes were involved in regulating the auxin hormone pathway (auxin export, *VvABCB19*), cytokinin (*VvCKX3*), ABA (*VvUTG71B6*), ion homeostasis (*VvIRT*, activation-*VvPYE*), copper (*VvTPC1*), calcium transport (*VvGLR3. 4*), stress response (*VvDnaj, VvHSP, VvALDH*), and glycolysis (*VvPlast*).

The leaves treated with PHOS and MeJA underwent modulations of genes related to the ABA pathway (*VvAAO1, VvABCG40*), gibberellin regulation (*VvGA2ox*), and the activation of the chloroplastic ferritin gene (*VvFER*) and a nitrate/nitrite transporter (*VvNRT1*). This suggests that inoculation has an important effect on hormonal regulation and iron resources. MeJA specifically induced genes involved in isopentenyladenine biosynthesis (*VvIPT*), one superoxide dismutase (*VvCSD2*, already noted at 6 dpt), a malate dehydrogenase (*VvMDH*), and a chitinase (*VvPR8*). The only gene specifically induced by ASM was an ABA inactivation gene (*VvHYD2*). Clearly the inoculation of *P. viticola* resulted in the strong modulation of treated plants, specifically ABA and gibberellin pathways, with a potential remobilisation of auxin. In addition, sugar transport was activated in the ASM and MeJA treatments, suggesting sugars were required. Clustering (Figure 6B) indicates that ASM gene modulations were much similar to the control modulations than those of the other PDS treatments. The MeJA and PHOS treatments led to the largest modulations of monitored genes. Triton contributed slightly to the MeJA effect with only common up-regulations of aquaporin genes (*VvTIP* and *VvPIP*).

## 3.2. NMR analysis at 8dpt-2dpi

Regarding organic acids, there was a sharp drop in fumaric acid after MeJA treatment, as well as in PHOS treated leaves (Figure 7A, Table S3), similar to the results at 6 days. MeJA specifically decreased succinic and malic acids, while PHOS and ASM increased malic and tartaric acids (as well as Triton).

Regarding sugars at 8dpt-2dpi, MeJA significantly decreased α glucose and sucrose contents as at 6 dpt (Figure 7B,

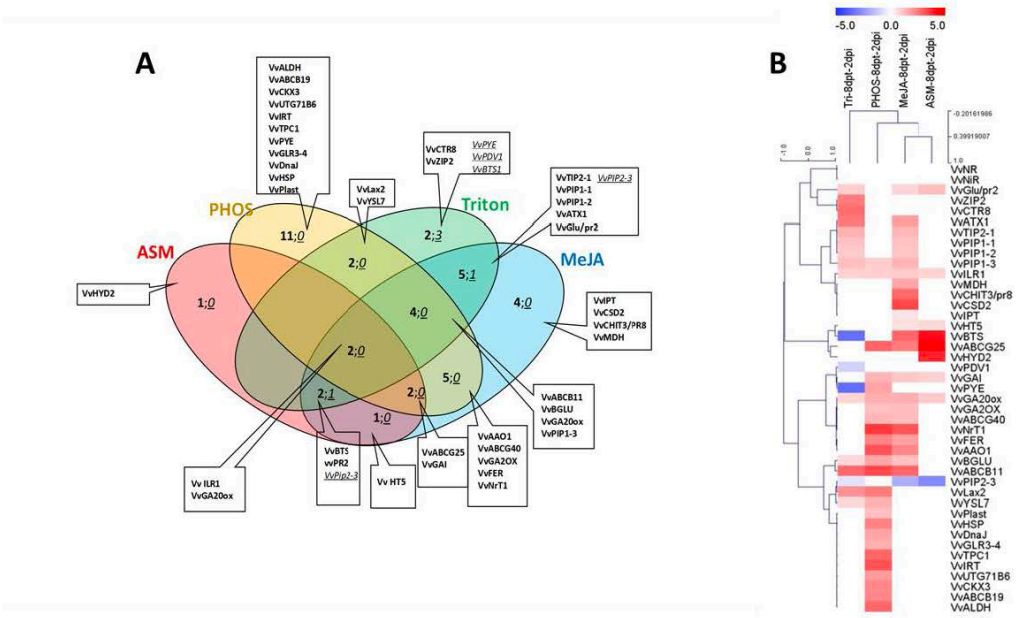


**FIGURE 6.** (A) Venn diagram with significantly modulated genes in grapevine leaves at 8dpt-2dpi with Triton (Tri), MeJA, PHOS, or BTH treatments. Induced genes were represented in bold and down-regulated ones in underlined italic. (B) Hierarchical clustering of relative expression of genes (log2-fold change relative to the untreated and inoculated control). Each column represents a treatment and each line one gene. Colour scale bars indicate ratios corresponding to the mean of three experiments. Upregulated and downregulated genes are in shades of red and blue, respectively.

Table S3). PHOS tended to have a similar profile, but with a significant increase in *myo*-inositol content.

Regarding amino acids, Glu decreased in all treated leaves, particularly after ASM treatment (Figure 7C, Table S3). Conversely, ASM-treated leaves showed a specific increase in Gln. Alanine levels also fell in the MeJA- and PHOS-treated leaves. Furthermore, threonine was increased by MeJA and tyrosine increased significantly in MeJA, ASM and PHOS treated-leaves.

There was little change in the relative abundance of amines. Nevertheless, we observed strong trigonelline increases from 1.6 (PHOS) to 13.7 (MeJA) after 6 days for all treatments except ASM, (Figures 7D and S3).

In terms of polyphenol abundance, catechin and quercetin-3-*O*-glucoside increased in all treated leaves, and particularly in those treated with MeJA and PHOS (between 3- and 4-fold in the case of catechin). MeJA also increased the *trans*-feruloyl derivative abundance and decreased that of shikimic acid (Figures 7E and S3).

In untreated and inoculated leaves (8dpt-2dpi), organic acids increased slightly but significantly (1.14 fold), as did catechin (1.76-fold) compared with uninoculated control leaves (Tables S2 and S3).

The effect of *P. viticola* was to enhance the expression of primary metabolic genes, consistent with the increase in specific metabolites (Figure S3).

## 4. Anti-downy mildew efficacy

Eight days after all PDS treatments under our experimental conditions, the mildew growth on leaves was strongly inhibited. Across all PDS treatments, *P. viticola* percent inhibition was very similar (97.63 ± 0.84; 97.30 ± 1.17 and 90.87 ± 2.23, respectively), except in the Triton treatment (39.76 ± 6.21).

## 5. Gene expression and NMR analysis combined

Combining two molecular approaches (q-RT-PCR of targeted genes and NMR) revealed differences between the treatments. Gene expression clearly distinguished the different treatments, with the exception of the Triton treatment and

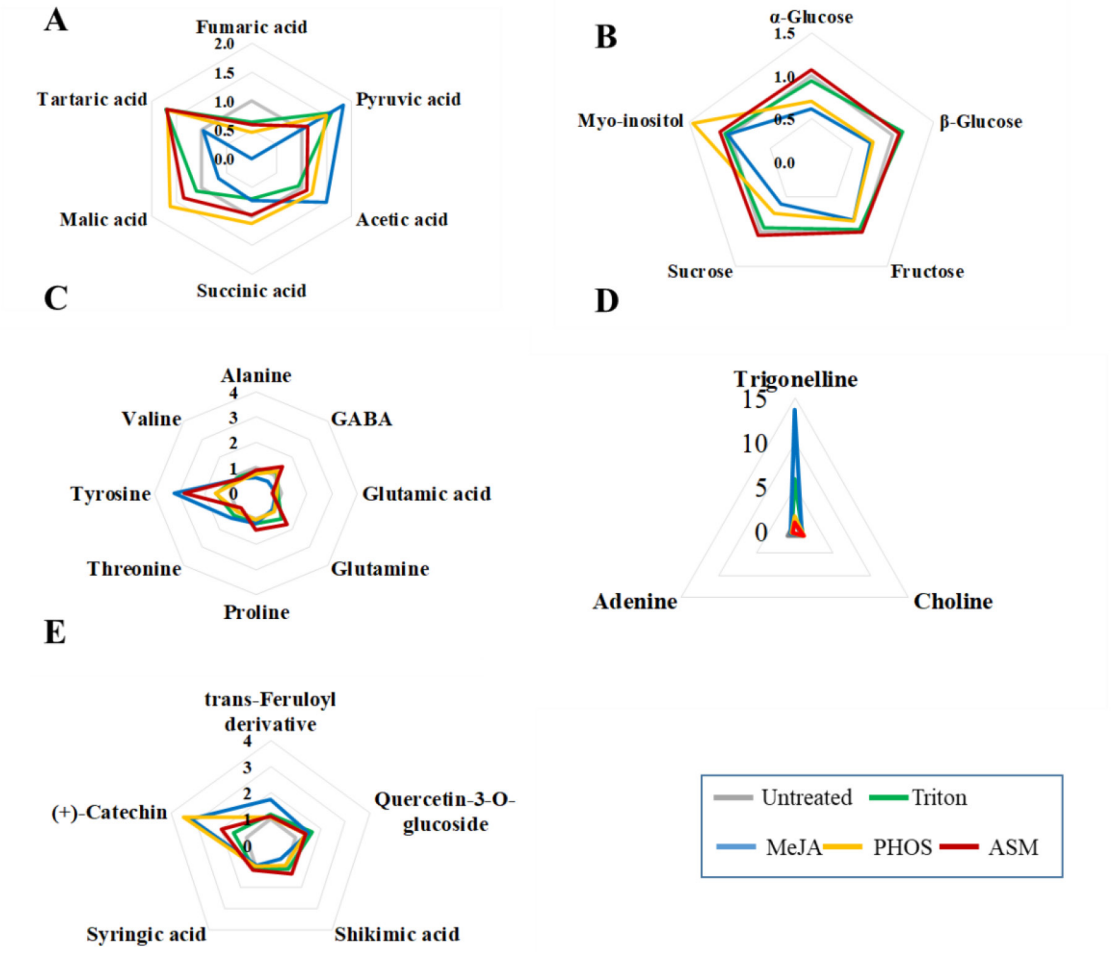


**FIGURE 7.** Radar charts of (A) relative quantity of organic acids, (B) sugars, (C) amino acids, (D) amines, and (E) polyphenols in leaves at 8days-2dpi. Radar charts were generated from the fold change of the means of three replicates relative to the untreated control.

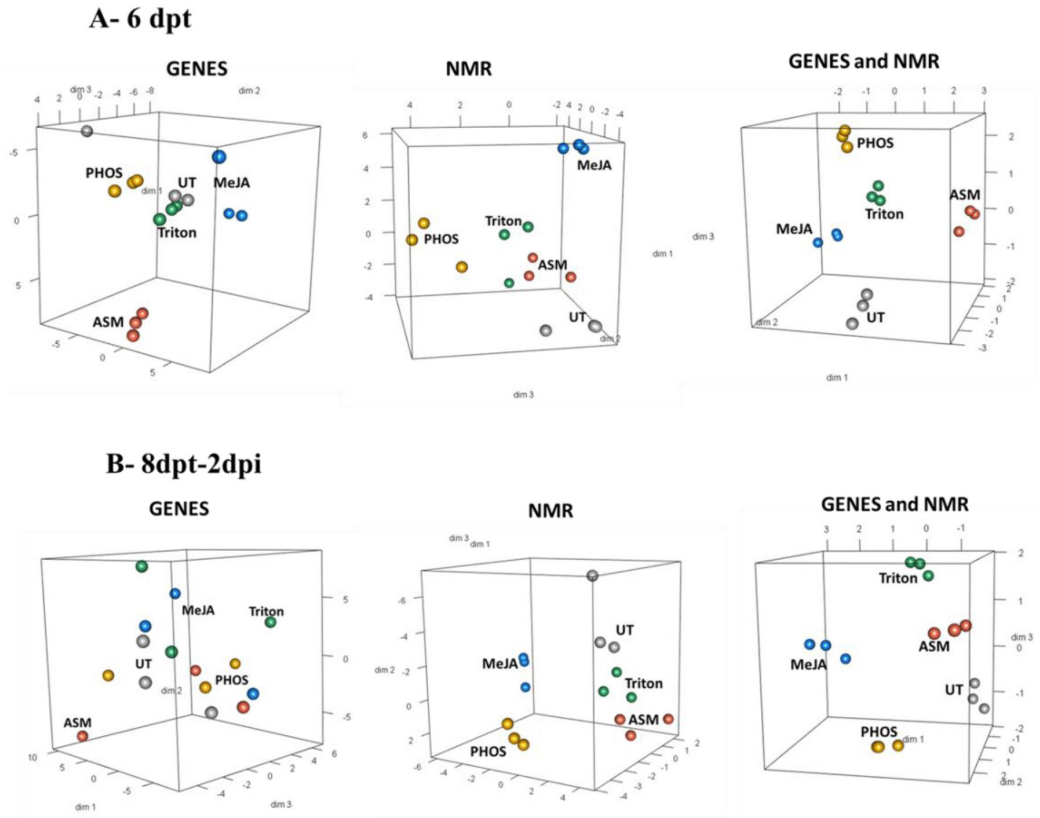


**FIGURE 8.** Principal component analysis (PCA) of gene expression and NMR data and multiple factor analysis (MFA), (A) at 6 dpt, and (B) at 8dpt-2dpi. Points represent values from samples depending on the treatment (red = ASM, yellow = PHOS, green = Triton, blue = MeJA, and grey = untreated/UT).

the control (very similar) (Figure 8A). NMR analysis alone differentiated the different treatments, MeJA being the most highly differentiated, and PHOS, Triton and ASM treatments close behind. Unlike at 6 dpt, the gene expression at 8dpt-2dpi was less effective when differentiating treatments than NMR data (Figure 8B). Irrespective of the time of analysis, the combination of approaches provided a clearer picture of the effect of each treatment than each approach alone.

## DISCUSSION

### 1. The importance of multi-approach analyses for grapevine

After PDS treatments, the grapevine responds by inducing a wide range of changes at the molecular level associated with defence mechanisms (Rojas *et al.*, 2014; Guttierrez-Gamboa *et al.*, 2017; Héloir *et al.*, 2018; Krzyzaniak *et al.*, 2018; Bodin *et al.*, 2020; Lemaître-Guillier *et al.*, 2017; Burdziej *et al.*, 2019). These responses require a set of primary metabolic regulatory processes that allow the plant to better allocate its resources. Some studies have investigated the effects of PDS (e.g., ASM, MeJA, PHOS, ethylene and oligosaccharides) using multiple approaches, which combine biological, enzymatic, metabolomic or transcriptomic analyses (Burdziej *et al.*, 2021; Krzyzaniak *et al.*, 2018). These have been applied to primary vine metabolism, especially over long periods (more than 4 days), and even to making comparisons between PDSs (Heloir *et al.*, 2018; Krzyzaniak *et al.*, 2018; Bodin *et al.*, 2020; Burdziej *et al.*, 2019). Information is available on grapevine cell cultures (e.g., Almagro *et al.*, 2022; Kryzaniak *et al.*, 2018), but not on grapevine plants, except for sulphated laminarin (PS3) (Heloir *et al.*, 2018; Lemaitre-Guiller *et al.*, 2017) and ASM (Bodin *et al.*, 2020). The results of studies on PS3 and derivatives indicate no effect on growth, but they have found nitrogen and sugar content to be modulated depending on leaf age, duration and number of treatments (Heloir *et al.*, 2018; Lemaitre-Guiller *et al.*, 2017). ASM has been reported to have no effect on root biomass and to modulate the expression of genes involved in different metabolic pathways (hormones, nitrogen) (Bodin *et al.*, 2020). In line with these studies, we described a variation in sugar and organic acid contents and numerous gene modulations, particularly for ion homeostasis and hormone pathways, depending on the treatment. The multi-approach enhanced the ability to accurately distinguish the effect of different treatment treatments.

### 2. Similar profile of grapevine responses to PDS treatments

Following the PDS treatments, several genes and metabolites were modulated at 6 dpt. Ion homeostasis (transport) genes were generally up-regulated in treated plants, while nitrate and/

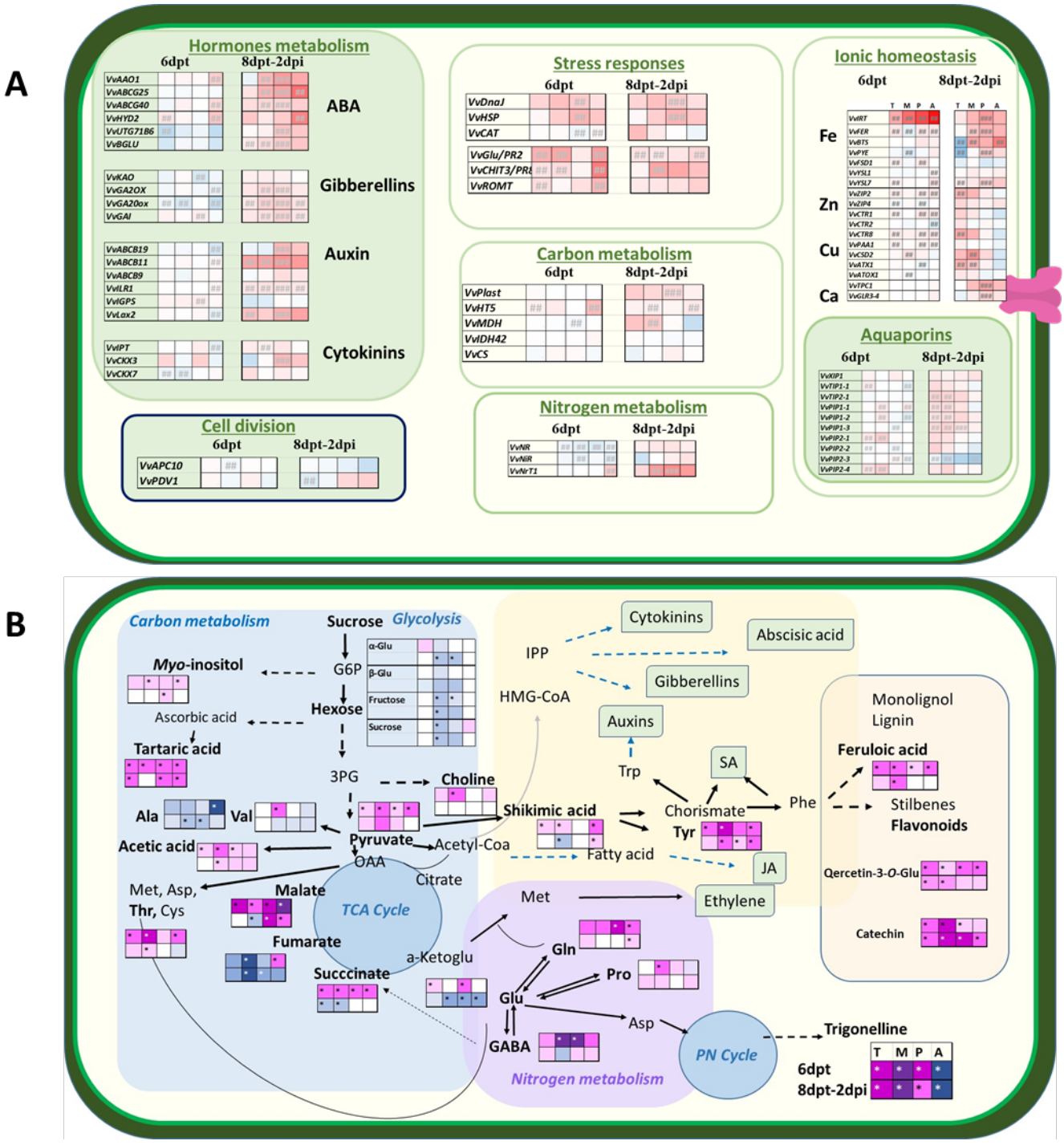


**FIGURE 9.** (A) Gene expression and (B) metabolite diagrams summarising the main effects of PDS treatments at 6 dpt (left-hand block) and 8dpt-2dpi (right-hand block). Each column represents a treatment in leaves treated with (from left to right) Triton (T), MeJA (M), PHOS (P) and ASM (A). The more intense the colours, the higher the content of transcripts or metabolites. The relative expression of genes is represented in log2-fold change relative to the untreated control at 6 dpt, and in log2-fold change relative to the untreated and inoculated control at 8dpt-2dpi. Regarding metabolites, the first line represents their abundance at 6 dpt and the line below the abundance at 8dpt-2dpi. The more abundant the metabolite, the more intense the purple-pink and the less abundant the metabolite, the more intense the blue. Asterisks indicate significance relative to the control. TCA = tricarboxylic acid; PN = pyridine nucleotide; 3PG: = 3-phopshoglycerate; G6P: = glucose-6-phosphate; a-Ketoglu = alpha-ketoglutarate; IPP = isopentenyl pyrophosphate.

or nitrite reductases were quite repressed, as well as genes of the gibberellin pathway (Figure 9). Ion homeostasis regulates numerous processes (development and stress responses) as it was reported in the case of iron in conjunction with other micronutrients (Zn, Mn) (Hanikenne *et al*., 2021). A major source of nitrogen is nitrate, and its uptake, assimilation, transport and remobilisation is important in plant response to abiotic and biotic stresses (Mur *et al*., 2017). Gibberellins play a role in many facets of plant development and responses to environmental stresses (Castro-Camba *et al*., 2022).

Eight days after treatment and two days after inoculation (8dpt-2dpi), iron homeostasis and gibberellin pathway genes were modulated, in addition to those of auxin (import and remobilisation) (Figure 9A). Although the biological material differed somewhat (leaves collected directly from whole plants and detached leaves from these plants, at 6 dpt and 8 dpt-2dpi, respectively), we noted that these modulations were similar at 6 dpt and 8dpt-2dpi, supporting their probable importance. Two genes involved in the gibberellin pathway (regulation/catabolism) were induced, indicating that a general effect of stress was the negative regulation of gibberellin biosynthesis or the accentuation of their degradation (Fukazawa *et al.*, 2023). Indeed, insensitive protein gibberellin protein (*VvGAi*) has been described as being associated with hormones (e.g., jasmonate), and is thought to act like a molecular switch between immunity and growth in many plants (Yang *et al.*, 2012). We suggest that the modulation of gibberellin pathway genes plays a role in grapevine defence.

After infection, the ABA pathway genes in treated plants were strongly induced (Figure 9A). These results corroborate those obtained after infection and treatment with phosphonate or ASM in the Moscato variety (Pagliarani *et al.*, 2020). In general, growth hormones (GA, ABA, auxins, cytokinins) synthesis is manipulated by pathogenic fungi (Han *et al.*, 2019).

When comparing gene expression analysis of untreated inoculated plants (8dpt-2dpi) with non-inoculated plants (8dpt), a strong decrease in the abundance of all transcripts encoding hormone pathways and ion homeostasis was noted (over 45 genes, data not shown). By contrast, the analysis of inoculated treated plants (8dpt-2dpi plants relative to uninoculated treated plants at 8 dpt) showed only a few down-regulations of expression (between 0 and 13), suggesting that the effect of the elicitors was poorly affected by the presence of the pathogen (at least until 48 h after inoculation).

Regarding metabolites, organic acids, *myo*-inositol and products of the shikimate pathway (e.g., quercetin-*O*-glucoside) had significantly accumulated at 6 dpt in all treated plants (Figure 9B). It is known that, in general, the TCA cycle and glycolysis are mobilised after elicitation to provide energy for the synthesis of defense molecules, such as flavonoids (Bolton *et al.*, 2009; Rojas *et al.*, 2014). In our study, the accumulation of organic acids after PDS treatments confirmed their role in the reprogramming of resources to be redirected to defence responses. Concerning polyphenols, our results highlight the greater abundance of flavonoids and compounds involved in lignin and parietal reinforcement (feruloyl derivatives), in particular catechin after downy mildew inoculation. They typically accumulate after PDS and/or stress (Jeandet *et al.*, 2023; Burdziej *et al.*, 2021) resulting in the activation of the shikimic pathway.

Tyrosine, which plays an important role in phenylpropanoid and lignification biosynthesis, significantly accumulated at 6 dpt and even more at 8dpt-2dpi in all PDS treatments, suggesting an intensification of this accumulation by *P. viticola.* By contrast, Glu was greatly reduced after all treatments. Once again, all these results indicate the occurrence of post-infection reprogramming in stimulated plants (Figure 9).

It is worthy of note that various gene expressions and the accumulation or absence of metabolites were specific to the treatment applied, whether in the absence of inoculation (6dpt) or 48 h after inoculation (8dpt-2dpi). This indicates that the effect of the pathogen on the plant's metabolism is weaker when the plant defence response has been induced beforehand.

## 3. Specific effects of MeJA versus other PDS

The sharp decreases in sucrose and fructose at 6 dpt in the MeJA treatment indicate that carbon metabolism was highly disrupted as a result of a drop in photosynthetic activity or the reallocation of carbon (Gould *et al.,* 2021). This disruption likely activated the pyruvate pathway, leading to the simultaneous accumulation of acetic acid (by reduction of fumarate (Savchenko *et al.,* 2019) and shikimic acid (precursor of a defensive substance; Burdziej *et al.*, 2021) (Figure 9B). Conversely, *myo*-inositol, which is involved in many processes (e.g., cell wall biogenesis, stress responses, membrane trafficking) (Loewus and Murthy, 2000), was higher in MeJA- and ASM-treated leaves, indicating a reallocation of sugars to *myo*-inositol.

MeJA modulated copper homeostasis genes as *VvATOX1* (involved in electron flow for photosynthesis and respiration), and *VvCSD2* (superoxide dismutase involved in oxidative stress) (Andresen *et al.*, 2018). Transcripts of aquaporin genes were generally more abundant after MeJA/Triton than PHOS or ASM. The PIP (plasmalemma intrinsic proteins) facilitate the transport of water and small molecules, and are associated with tolerance to stresses (drought and pathogens) (Afzal *et al.*, 2016). This is consistent with the role of jasmonate in response to drought or biotic stress and its impact on aquaporin gene expression (Wasternak and Hause, 2013).

MeJA modulated amino acid and amine production, often in combination with PHOS (e.g., GABA and trigonelline). GABA and Glu fluctuations can result from GABA biosynthesis by Glu decarboxylation (Qiu *et al.*, 2020). The high GABA content found (Figure 7B) may play a role in the restriction of ROS generation, in the equilibrium of the carbon/nitrogen balance, and in the GABA/malate ratio, thus regulating ion fluxes (Ramos-Ruiz *et al.*, 2019; Qiu *et al.*, 2020). In addition, MeJA specifically increased

the accumulation of proline, valine and threonine (at 6 dpt), as described at 24 hpt by Burdziej and colleagues (2019). Proline, involved in carbon-nitrogen storage, could also be a source of GABA (Ramos-Ruiz *et al.*, 2019).

Trigonelline, a nicotinic alkaloid, was found to have accumulated in MeJA- and PHOS-treated leaves (inoculated or not), but not in ASM-treated ones. This molecule usually accumulates in grapevine under stress (Lima *et al.*, 2017).

As well as the polyphenol accumulation common to all treatments, MeJA increased post-infection *trans*-feruloyl derivatives (Figure 9B). Similarly, MeJA has been found to promote the accumulation of antioxidants (e.g., ferulic acid, catechin) and superoxide dismutase activity under stress in Monastrell grapevine (Almagro *et al.*, 2022).

## 4. Specific effects of ASM versus other PDS

Like the PHOS treatment, the ASM treatment resulted in a significant abundance of malic and tartaric acids before and after inoculation (Figure 9B). Tartaric acid is involved in the catabolism of ascorbic acid, and contributes to stress tolerance (Burbidge *et al.*, 2021). As other PDS in absence of *P. viticola*, the abundance of most organic acids increased with ASM, except acetic acid, which was instead specific to MeJA.

Nitrogen and carbon metabolism was also induced, particularly nitrogen (*VvNrt1*) and sugar transporter genes (*VvHT5*) (Figure 9A). These genes have already been described as up-regulated by ASM in Cabernet-Sauvignon (Bodin *et al.*, 2020), Moscato and Nebbolio grape leaves (Pagliarani *et al.*, 2020). While ASM modulated the energy metabolism, it had no effect on sugar abundance, unlike MeJA.

ASM promoted the accumulation of amino acids, such as Gln, Thr and Tyr. Iriti *et al.* (2005) have also described an accumulation of different amino acids (Asp, Gly, Pro). Meanwhile, Burdziej and colleagues (2019) noted that at 2 dpt, the amino acid content was lower in PDS-treated leaves than in control leaves, indicating that there was a strong temporal and environmental modulation of these compounds. There is a link between protein levels, oxidative stress and amino acid balance, link which depends in part on glutamine synthetase activity (Liu *et al.*, 2010). Glu and Gln are signalling molecules for growth, development and defense (Liao *et al.*, 2022) and their homeostasis is important due to the strong link between Glu with GABA, carbon metabolism, and energy production. Thus the regulation of Gln/Glu/GABA biosynthesis was one of the major effects of the three PDS on primary metabolism, each with its own specificity (Figure 7B).

Another feature of ASM treatment was the absence of trigonelline (Figure 9B), unlike MeJA and PHOS treatments, in agreement with results obtained at 2 dpt by Burdziej *et al.* (2019).

Hormonal pathway genes were generally highly modulated by ASM. There was a high accumulation of the transcripts of genes responsible for ABA transport and metabolism, as was also reported by Pagliarani *et al.* (2020); ABA induces stomatal closure, thereby limiting the invasion of downy mildew zoospores (Allègre *et al.*, 2009). The *P. viticola* inhibition after ASM treatment and gene modulation indicates that there was synergistic interaction between SA and ABA as a defence response, in agreement with Liu *et al.* (2016). ASM also affected the auxin pathway by repressing two extracellular transporter genes (*VvABCB19; VvLax*) and activating intracellular import and remobilisation (*VvABCB11, VvILR*), in agreement with the results of Bodin and colleagues (2020).

At 6 dpt, ASM induced mostly homeostasis genes (iron, copper and zinc). However, with the exception of the *VvBTS* gene, which activates iron homeostasis in the case of deficiency, fewer effects were observed after inoculation. This could be a sign of iron deficiency, which has been linked to plant defence responses and the regulation of several hormones (auxin, ethylene, cytokinin, gibberellic acid), as reported in Arabidopsis (Verbon *et al.*, 2017). Regarding copper and zinc homeostasis genes, ASM and PHOS treatments overlapped more than MeJA. The up-regulation of genes (*VvCTR1* and *VvYLS*) was consistent with the findings of Bodin and colleagues (2020). ASM at 6 dpt also led to the down-regulation of aquaporins, except *VvPIP1.1* (common to MeJA), which is related to stomatal regulation and ABA metabolism (Sabir *et al.*, 2021).

## 5. Specific effects of potassium phosphonate (PHOS)

PHOS was characterised by the effect it had on the TCA cycle at 6 dpt, like the other PDS, and by an increase in organic acids, as was the case for ASM. As regards sugars, there was a particularly high increase in *myo*-inositol content (at 8dpt-2dpi), which may indicate the occurrence of phosphate mobilisation in grapevine, as described for Arabidopsis (Berkowitz *et al.*, 2013). However, PHOS had no effect on the other sugars, except for a slight decrease in alpha-glucose after inoculation.

Regarding the other PDS, ion homeostasis transcripts were abundant, with some specifities (chloroplastic iron superoxide dismutase, regulation trafficking of calcium, voltage-dependent copper transporter). The PHOS treatment modulated the ion homeostasis genes to the greatest extent after inoculation. Unlike MeJA, PHOS had little effect on, or even down-regulated, PIP genes.

PHOS modulated few genes of the hormonal pathway before inoculation. Overall, in conjunction with the modulated genes (*VvGAI*, *VvKAO*), the biosynthesis of gibberellins may have been suppressed or the molecules degraded. PHOS specifically increased the abundance of an auxin biosynthesis transcript (*VvIGPS*), consistent with the study of Esraghi *et al.* (2014). Similar to MeJA, after inoculation, the ABA pathway genes were up-regulated, particularly those involved in transport; this is consistent with work by Massoud *et al.* (2012) in Arabidopsis.

One of the peculiarities of PHOS was the up-regulation of *VvDnaj* (chaperone protein involved in development and heat

stress) and *VvHSp* (heat shock protein) genes, suggesting that these genes are strongly involved in stress response after PHOS treatment (Andrasi *et al.*, 2021) (Figure 9A).

## CONCLUSION

Plant metabolites are often divided into primary metabolites (basic activities) and specialised metabolites (environmental response), but this classification is not always obvious (Wang *et al.*, 2022): the upregulation of primary metabolism also occurs in plant-pathogen interactions and has been proposed as signal transduction leading to plant defense responses. (Rojas *et al.*, 2014). In our study, all the treatments stimulated a primary metabolism pathway (TCA cycle) with organic acid accumulation to a greater and/or lesser extent, depending on the PDS used and/or the presence of a pathogen. The strong effect (reduction) of MeJA on sugars (except for *myo*-inositol) was highlighted, in contrast to the PHOS and ASM treatments. Consistent with our results, several transcriptomic and metabolomic studies have documented that elicitors modulate the expression of glycolysis and the TCA cycle genes (e.g., Rojas *et al.*, 2014; Heloir *et al.*, 2018). Amino acid (e.g., Glu, Gln, Pro) biosynthesis, which allows nitrogen supply, was also strongly enhanced by PDS. The regulation and biosynthesis of hormones were modulated by the different treatments before and after infection. Their metabolites are the subject of debate, which is exacerbated by the increasingly blurred distinction between the two types of metabolism (Fabregas *et al.*, 2022). It appears that the so-called primary metabolism is strongly involved in the establishment and efficiency of plant defense systems and/or responses to various stresses.

The use of $^{1}$H NMR, coupled with the expression of primary metabolism genes, was a useful tool for analysing the effect of different PDS in various situations. This multidisciplinary approach (biology, metabolomics and transcriptomics) enabled us to identify common impacts and characteristics of PDS. Six to 8 days after a single treatment, *V. vinifera* is able to defend itself against downy mildew by re-allocating resources to the biosynthesis of compounds involved in stress responses (e.g., polyphenols, *myo*-inositol, GABA and trigonelline), depending on the PDS. We found modulations of ion homeostasis genes and hormone pathways that, together with an abundance of specific metabolites, were characteristic (thus a signature) of the treatment used.

The impact of a PDS on plants commonly depends on the dose applied, the number of treatments, the treated organ and its age, the grape variety and environmental factors. The cost to plant defence has often been described, as well as the fact that primary metabolism fuels it and can lead to a possible trade-off between growth and defence (Bolton, 2009). While ASM has been described as costly to berry growth and ripening (Dietrich *et al.*, 2005; Miliordos *et al.*, 2023), it does not affect the grapevine plant itself (Bodin *et al.*, 2020). A laminarin derivative has also been demonstrated to have no cost to grapevine (Héloir *et al.*, 2018). The trade-off between growth and defence is not so obvious, as the interaction between primary and secondary metabolism needs to be coordinated in order to achieve a balance depending on plant resources (Kliebenstein, 2016). Our study did not indicate that there was any cost to the plant's defences (except possibly in the case of the MeJA treatment). Taken together, this suggests that the cost of setting up defences is related to the intensity of the stimulation, the nature of the inducer and the environment of the plant.

The three PDS used here had significant effects on the genes involved in hormonal biosynthesis, as well as on ion homeostasis and metabolites, demonstrating the close interplay between primary and secondary metabolic regulation. This innovative combination of approaches brought to light responses common to different PDSs, as well as responses specific to individual PDS with mildew protection 6-8 days after treatment (long-term). This combined approach can be extended to laboratory or field studies; for example, to identify markers (PDS and stress status, etc.) or to understand the interaction of molecules, microorganisms and abiotic stress with vine physiology and defence.

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## REFERENCES

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*, 110*,* 101-166. https://doi.ord/10.1016/bs.abr.2024.02.013i

Afzal, Z., Howton, T.C., Sun, Y., & Mukhtar, M.S. (2016). The roles of aquaporins in plant stress responses. *Journal of Developmental Biology*, 4, 9. https://doi.org/10.3390/jdb4010009

Allègre, M., Héloir, M-C., Trouvelot, S., Daire, X., Pugin, A., Wendehenne, D., & Adrian, M. (2009). Are grapevine stomata involved in the elicitor- induced protection against downy mildew? *Molecular Plant-Microbe Interactions*, 22(8), 977–986. https://doi.org/10.1094/MPMI-22-8-0977

Almagro, L., Calderón, A.A., Pedreño, M.A., & Ferrer, M.A. (2022). Differential response of phenol metabolism associated with antioxidative network in elicited grapevine suspension cultured cells under saline conditions. *Antioxidants*, 11, 388. https://doi.org/10.3390/antiox11020388

Andrasi, N., Pettko-Szandtner, A., & Szabados, L. (2021). Diversity of plant heat shock factors: regulation, interactions and functions. *Journal of Experimental Botany,* 72(5), 1558-1575, https://doi.org/10.1093/jxb/eraa576

Andresen, E., Peiter, E., & Küpper, H. (2018). Trace metal metabolism in plants. *Journal of Experimental Botany*, 69(5), 909-954. https://doi.org/10.1093/jxb/erx465

Bellée, A., Cluzet, S., Dufour, M-C., Mérillon, J-M., & Corio-Costet, M-F. (2018). Comparison of the impact of two molecules on plant defense and efficacy against *Botrytis cinerea* in the vineyard: A plant defense inducer (Benzothiadiazole) and a fungicide (Pyrimethanil). *Journal of Agricultural and Food Chemistry,* 66(13), 3338-3350. https://doi.org/10.1021/acs.jafc.7b05725

Berkowitz, O., Jost, R., Kollehn, D.O., Fenske, R., Finnegan, P.M., O'Brien, P.A., Hardy, G.E., & Lambers, H. (2013). Acclimation responses of *Arabidopsis thaliana* to sustained phosphite treatments. *Journal of Experimental Botany*, 64(6), 1731-43. https://doi.org/10.1093/jxb/ert037

Bodin, E., Bellée, A., Dufour, M-C., Andre, O., & Corio-Costet, M-F. (2020). Grapevine stimulation: a multidisciplinary approach to investigate the effects of biostimulants and a plant defense stimulator. *Journal of Agricultural and Food Chemistry,* 68(51). 15085-15096. https://doi.org/10.1021/acs.jafc.0c05849

Bolton, M.D. (2009). Primary metabolism and plant defense-fuel for the fire. *Molecular Plant-Microbe Interactions*, 22(5), 487-497. https://doi.org/10.1094/MPMI-22-5-0487

Burbidge, C. A., Ford, C. M., Melino, V. J., Wong, D. C. J., Jia, Y., Jenkins, C. L. D., Soole, K. L., Castellarin, S. D., Darriet, P., Rienth, M., Bonghi, C., Walker, P., Famiani, F., & Sweetman, C. (2021). Biosynthesis and cellular functions of tartaric acid in grapevines. *Frontiers in Plant Science, 12*, 643024. https://doi.org/10.3389/fpls.2021.643024

Burdziej, A., Da Costa, G., Gougeon, L., Le Mao I., Bellée, A., Corio-Costet, M-F., Mérillon, J-M. Szakiel, A., Richard, T., & Cluzet, S. (2019). Impact of different elicitors on grapevine leaf metabolism monitored by 1H NMR spectroscopy. *Metabolomics*, 15,67. https://doi.org/10.1007/s11306-019-1530-5

Burdziej, A., Bellée, A., Bodin, E., Valls Fonayet, J., Magnin, N., Szakiel, A., Cluzet, S., & Corio-Costet, M.F. (2021). Three types of elicitors induce grapevine resistance against downy mildew via common and specific immune responses. *Journal of Agricultural and Food Chemistry,* 69 (6), 1781-1795. https://doi.org/10.1021/acs.jafc.0c06103

Castro-Camba, R., Sánchez, C., Vidal, N., & Vielba, J.M. (2022). Interactions of gibberellins with phytohormones and their role in stress responses. *Horticulturae*, 8, 241. https://doi.org/10.3390/horticulturae8030241

Corio-Costet, M., Dufour, M., Cigna, J., Abadie, P., & Chen, W. (2010). Diversity and fitness of Plasmopara viticola isolates resistant to QoI fungicides. *European Journal Of Plant Pathology*, 129(2), 315-329. https://doi.org/10.1007/s10658-010-9711-0

Delaunois, B., Farace, G., Jeandet, P., Clément, C., Baillieul, F., Dorey, S., & *et al*. (2014). Elicitors as alternative strategy to pesticides in grapevine? Current knowledge on their mode of action from controlled conditions to vineyard. *Environmental Science and Pollution Research*, 21, 4837-4846. https://doi.org/10.1007/s11356-013-1841-4

Dietrich, R., Ploss, K., & Heil, M. (2005). Growth responses and fitness costs after induction of pathogen resistance depend on environmental conditions. *Plant Cell and Environment,* 28, 211-222. https://doi.org/10.1111/j.1365-3040.2004.01265.x

Dufour, M-C., & Corio-Costet, M-F. (2013a). Variability in the sensitivity of biotrophic grapevine pathogens (*Erysiphe necator* and *Plasmopara viticola*) to acibenzolar-S-methyl and two phosphonates. *European Plant Journal of Plant Pathology,* 136(2), 247-259. https://doi.org/10.1007/s10658-012-0159-2

Dufour, M-C., Lambert, C., Bouscaut, J., Mérillon, J-M., & Corio-Costet, M-F. (2013b). Benzothiadiazole-primed defense responses and enhanced differential expression of defense genes in *Vitis vinifera* infected with biotrophic pathogens (*Erysiphe necator* and *Plasmopara viticola*). *Plant Pathology,* 62, 270-382. https://doi.org/10.1111/j.1365.3059.2012.02628.x

Dufour, M-C., Magnin, N., Dumas, B., Vergnes, S., & Corio-Costet, M-F. (2016). High-throughput gene-expression quantification of grapevine defense responses in the field using fluidigm microfluidics dynamic arrays. *BMC Genomics,* 1, 957. https://doi.org/10.1186/s12864-016-3304-z

Eichmann, R., & Schadfer, P. (2015). Growth versus immunity -- A redirection of the cell cycle? *Current Opinion in Plant Biology,* 26,106-112. https://doi.org/10.1016/j.pbi.2015.06.006

Eshraghi, L., Anderson, J.P., Aryamanesh, N., McCOMB, J.A., Shearer, B., & St Hardy, G.E. (2014) Suppression of the auxin response pathway enhances susceptibility to Phytophthora cinnamomi while phosphite-mediated resistance stimulates the auxin signalling pathway. *BMC Plant Biology*, 14, 68. https://doi.org/10.1186/1471-2229-14-68

Fabregas, N., & Fernie, A.R. (2022). The reliance of phytohormone biosynthesis on primary metabolite precursors. *Journal of Plant Physiology*, 268, 153589. https://doi.org/10.1016/j.jplph.2021.153589

Fukazawa, J., Mori, K., Ando, H., Mori, Y., Kanno, Y., Sea, M., & Takahashi, Y. (2023). Jasmonate inhibits plants growth and reduces gibberellin levels via microRNA5998 and transcription factor MYC2. *Plant Physiology*, 193(3), 2197-2214. https://doi.org/10.1093/plphys/kiad453

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gut Rella, M., Meier, B., Dincher, S., Staub, T., Uknes, S., Métraux J-P., Kessmann, H., & Ryals, J. (1996). A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *The Plant Journal*, 10(1), 61-70. https://doi.org/10.1046/j.1365-313X.1996.10010061.x

Gould, N., Thorpe, M.R., Taylor, J.T., Boldingh, H.L., Mackenzie, C.M., & Reglinski, T. (2021). Jasmonate-induced defense elicitation in mature leaves reduces carbon export and alters sink priority in grape (*Vitis vinifera* Chardonnay). *Plants*, 10, 20406. https://doi.org/10.3390/ plants1011240

Guest, D., & Grant, B.R. (1991). The complex action of phosphonates as antifungal agents. *Biological Review*, 66, 159-187. https://doi.org/10.1111/j.1469-185X.1991.tb01139.x

Gutierrez-Gamboa, G., Portu, J., Santamaria, P., Lopez, R., & Garde-Cerdan, T. (2017). Effects on grape amino acid in through foliar application of three different elicitors. *Food Research International*, 99(1), 688-692. https://doi.org/10.1016/j.foodres.2017.06.022

Han, X., & Kahmann, R. (2019). Manipulation of phytohormone pathways by effectors of filamentous plant pathogens. *Frontiers in Plant Science,* 10, 822. https://doi.org/10.3389/fpls.2019.00822

Hanikenne, M., Esteves, SM., Fanara, S., Rouached, H. (2021). Coordinated homeostasis of essential mineral nutrients: a focus on iron. *Journal of Experimental Botany*, 72(6):2136-2153. https://doi.org/10.1093/jxb/eraa483. PMID: 33175167

Harm, A., Kassemeyer, H.H., Seibicke, T., & Regner, F. (2011). Evaluation of chemical and natural resistance inducers against downy mildew *Plasmopara viticola* in grapevine. *American Journal of Enology and Viticulture,* 62(2), 184. https://doi.org/10.5344/ajev.2011.09054

Heloir, M-C., Khiook, I.L.K., Lemaitre-Guillier, C., Clément, G., Jacquens, L., Bernauid, E., Trouvelot, S., & Adrian, M. (2018). Assessment of the impact of PS3-induced resistance to downy mildew on grapevine physiology. *Plant Physiology and Biochemistry*, 133, 134-141. https://doi.org/10.1016/j.plaphy.2018.10.030

Iriti, M., Rossoni, M., Borgo, M., Ferrara, L., & Faoro, F. (2005). Induction of resistance to gray mold with benzothiadiazole modifies amino acid profile and increases proanthocyanidins in grape: primary versus secondary metabolism. *Journal of Agricultural and Food Chemistry,* 53, 9133-9139. https://doi.org/10.1021/jf050853g

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(9), 2225. https://doi.org/10.3390/agronomy13092225

Kliebenstein, D. J. (2016). False idolatry of the mythical growth versus immunity tradeoff in molecular systems plant pathology. *Physiological And Molecular Plant Pathology*, *95*, 5559. https://doi.org/10.1016/j.pmpp.2016.02.004

Kushalappa, A.C., Yogendra, K.N., & Karre, S. (2016). Plant innate immune response: qualitative and quantitative resistance. *Critical Reviews in Plant Sciences,* 35(1), 38-55. http://dx.doi.org/10.1080/07352689.2016.1148980

Krzyzaniak, Y., Negrel, J., Lemaitre-Guillier, C., Clément, G., Mouille, G., Klinguer, A., Trouvelot, S., Héloir, M., & Adrian, M. (2018). Combined enzymatic and metabolic analysis of grapevine cell responses to elicitors. *Plant Physiology And Biochemistry*, 123, 141-148. https://doi.org/10.1016/j.plaphy.2017.12.013

Lemaitre-Guillier, C., Hovasse, A., Schaeffer-Reiss, C., Recorbet, G., Poinssot, B., Trouvelot, S., Daire, X., Adrian, M., & Heloir, M.C. (2017). Proteomics towards the understanding of elicitor induced resistance of grapevine against downy mildew. *Proteomics*, 156, 113-125. https://doi.org/10.1016/j.jprot.2017.01.016

Liao, H., Chung, Y., & Hsieh, M. (2022). Glutamate: A multifunctional amino acid in plants. *Plant Science*, 318, 111238. https://doi.org/10.1016/j.plantsci.2022.111238

Lima, M.R.M., Machado, A.F., & Gubler, W.D. (2017). Metabolic study of chardonnay grapevines double stresses with Esca-associated fungi and drought. *Phytopathology,* 107(6), 669-680. https://doi.org/10.1094/PHYTO-11-16-0410R

Liu, G., Ji, Y., Bhuiyan, N.H., Pilot, G., Selvaraj, G., Zou, J., & Wei, Y. (2010) Amino Acid Homeostasis Modulates Salicylic Acid–Associated Redox Status and Defense Responses in Arabidopsis. *The Plant Cell*, 2, 3845–3863, https:// doi.org/10.1105/tpc.110.079392

Liu, S., Wu, J., Zhang, P., Hasi, G., Huang, Y., Lu, J., & Zhang, Y. (2016). Response of phytohormones and correlation of SAR signal pathway genes to the different resistance levels of grapevine against Plasmopara viticola infection. *Plant Physiology And Biochemistry*, 107, 56-66. https://doi.org/10.1016/j.plaphy.2016.05.020

Loewus, FA., & Murthy, PP. (2000). Myo-inositol metabolism in plants. *Plant Science*, 150, 1-9. https://doi.org/10.1016/S0168-9452(99)00150-8

Massoud, K., Barchietto, T., Le Rudulier, T., Pallandre, L., Didierlaurent, L., Garmier, M., Ambard-Bretteville, F., Seng, J.M., & Saindrenan, P. (2012). Dissecting phosphite-induced priming in *Arabidopsis* infected with *Hyaloperonospora arabidopsidis*. *Plant Physiology,* 159, 286–298. https://doi.org/10.1104/pp.112.194647

Miliordos, D-E., Alatzas, A., Kontoudakis, N., Unlubayir, M., Hatzopoulos, P., Lanoue, A., & Kotseridis, Y. (2023). Benzothiadiazole affects grape polyphenol metabolism and wine quality in two Greek cultivars: Effects during ripening period over two years. *Plants*, 12, 1179. https://doi.org/10.3390/ plants12051179

Mohammadi, M.A., Cheng, Y., Aslamhara, M., Jakada, B.H., Wai, M.H., Ye, K., He, X., Luo, T., Ye, L., Dong, C., Hu, B., Priyadarshani, S.V.G.N., Wang-Pruski, G., & Qin, Y. (2021). ROS and oxidative response systems in plants under biotic and abiotic stresses: Revisiting the crucial role of phosphite triggered plants defense response. *Frontiers in Microbiology*, 12, 631318. https://doi.org/10.3389/fmicb.2021.631318

Mur, L. A. J., Simpson, C., Kumari, A., Gupta, A. K., & Gupta, K. J. (2017). Moving nitrogen to the centre of plant defence against pathogens. *Annals Of Botany*, mcw179. https://doi.org/10.1093/aob/mcw179

Pagliarani, C., Moine, A., Chitarra, W., Meloni, G. R., Abbà, S., Nerva, L., Pugliese, M., & Gullino, M.L. (2020). The molecular priming of defenses responses is differently regulated in grapevine genotypes following elicitor application against powdery mildew. *International Journal of Molecular Science,* 6776. https://doi.org/0.3390/ijms21186776

Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., & Pertot, I. (2008). Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. *Biological Control,* 47, 228-34. https://doi.org/10.1016/j.biocontrol.2008.08.008

Pesce, S., Mamy, L., Sanchez, W., Amichot, M., Artigas, J., Aviron, S., Barthélémy, C., Beaudouin, R., Bedos, C., Bérard, A., Berny, P., Bertrand, C., Bertrand, C., Betoulle, S., Bureau-Point, E., Charles, S., Chaumot, A., Chauvel, B., Coeurdassier, M.,. . . Leenhardt, S. (2023). Main conclusions and perspectives from the collective scientific assessment of the effects of plant protection products on biodiversity and ecosystem services along the land–sea continuum in France and French overseas territories. *Environmental Science And Pollution Research*. https://doi.org/10.1007/s11356-023-26952-z

Pinto, KMS., Cordeiro do Nascimento, L., Cintra de Souza Gomes, E., Da Silva, HF., & dos Reis Miranda, J. (2012). Effiency of resistance elicitors in the management of grapevine downy mildew *Plasmopara viticola*: epidemiological, biochemical and economic aspects. *European Jounral of Plant Pathology*, 134: 745-754. https://doi.org/10.1007/s10658-012-0050-1

Qiu, X-M., Sun, Y-Y., Ye, X-Y., & Li, Z-G. (2020). Signaling role of glutamate in Plants. *Frontiers in Plant Science*, 10-2019. https://dx.doi.org/10.3389/fpls.2019.01743

Ramos-Ruiz, R., Martinez, F., & Knauf-Beiter, G. (2019). The effects of GABA in plants, *Cogent Food and Agriculture*, 5:1, 1670553. https://doi.org/10.1080/23311932.2019.1670553

Reglinski, T., Havis, N., Rees, H.J., & de Jong, H. (2023). The practical role of induced resistance for crop protection. *Phytopathology*, 11, 719-731. https://doi.org/10.1094/PHYTO-10-22-0400.IA

Rojas, C.M., Senthil-Kumar, M., Tzin, V., & Mysore, K.S. (2014). Regulation of primary metabolism during plant-pathogen interactions and its contribution to plant defense. *Frontiers in Plant Science*, 5, 17. https://doi.org/10.3389/fpls.2014.00017

Sabir, F., Zarrouk, O., Noronha, H., Loureiro-Dias, M. C., Soveral, G., Gerós, H., & Prista, C. (2021). Grapevine aquaporins: Diversity, cellular functions, and ecophysiological perspectives. *Biochimie*, 188, 61-76. https://doi.org/10.1016/j.biochi.2021.06.004

Savchenko, T.V., Rolletschek, H., & Dehesh, K. (2019). Jasmonates-mediated rewiring of central metabolism regulates adaptative responses. *Plant Cell and Physiology*, 60(12), 2613-2620. https://doi.org/10.1093/pcp/pcz181

Shigenaga, A.M., Berens, M.L., Tsuda, K., & Argueso, C.T. (2017). Towards engineering of hormonal crosstalk in plant immunity. *Current Opinion in Plant Biology*, 38, 164-172. http://doi.org/10.1016/j.pbi.2017.04.021

Taibi, O., Salotti, I., & Rossi, V. (2023). Plant resistance inducers affect multiple epidemiological components of *Plasmopara viticola* on grapevine leaves. *Plants*, 12, 2938. https://doi.org/10.3390/plants12162938

Verbon, E. H., Trapet, P. L., Stringlis, I. A., Kruijs, S., Bakker, P. A., & Pieterse, C. M. (2017). Iron and Immunity. *Annual Review Of Phytopathology*, 55(1), 355-375. https://doi.org/10.1146/annurev-phyto-080516-035537

Walters, D.R., Newton, A.C., & Lyon, G.D. (2014). Induced resistance for plant defense, A sustainable approach to crop protection, second edition, Wiley–Blackwell, Chichester, UK, pages 352.

Wang, S., Li, Y., He, L., Yang, J., Fernie, A.R., & Luo, J. (2022). Natural variance at the interface of plant primary and specialized metabolism. *Current Opinion in Plant Biology,* 67, 102201. https://doi.org/10.1016/j.pbi.2022.102201

Wasternack, C., & Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. *Annals of Botany*, 111, 1021-1058, https://doi.org/10.1016/10.1093/aob/mct067

Wu, L., Gao, X., Xia, F., Joshi, J., Borza, T., & Wang-Pruski, G. (2019). Biostimulant and fungicidal effects of phosphite assessed by GC-TOF-MS analysis of potato leaf metabolome. *Physiology and Molecular Plant Pathology*, 106, 49-56. https://doi.org/10.1016/j.pmpp.2018.12.001

Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L.J., Li, Q., Xiao, L.T., Sun, T.P., Li, J., & Deng, X.W. et *al.* (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceeding of National Academy of Science USA*, 109, 1192–1200. https://doi.org/10.1073/pnas.120161610.