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Benzothiadiazole-primed defence responses and enhanced differential expression of defence genes in *Vitis vinifera* infected with biotrophic pathogens *Erysiphe necator* and *Plasmopara viticola*

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Benzothiadiazole (BTH), a salicylic acid analogue, strengthens plant defence mechanisms against a broad spectrum of pathogens. The role of pre-treatment with BTH in enhancing resistance against infection with various isolates of downy and powdery mildews (*Plasmopara viticola* and *Erysiphe necator*) was investigated in grapevine leaves. Tools were developed to better assess the defence status of the plant. In compatible interactions amongst a set of 19 genes, more than 57·2% of differentiated transcripts from *P. viticola* infected-leaves (*Pv*-infected leaves) and 90% from *E. necator*-infected leaves (*En*-infected leaves) were down-regulated at 24 h post-inoculation (hpi), indicating a manipulation of host responses by the pathogens. BTH treatment enhanced grapevine defences, with pathogen growth inhibited by 61–98%, depending on the pathogen isolate. Treatment also triggered up-regulation of pathogenesis-related protein genes such as *PR-1, PR-2, PR-3, PR-8* and *PR-10* in *Pv*-infected leaves, and *PR-3, PR-6* and *PR-10* in *En*-infected leaves. Treatment with BTH also led to regulation of indole pathway transcripts; in particular, anthranilate synthase was down-regulated at 24 hpi in all infected leaves, then strongly up-regulated afterwards according to the rate of pathogen development. Quantitation of polyphenols and stilbenes showed that pterostilbene was specifically accumulated in pre-treated leaves and associated with biological efficacy and significant increases in PR protein gene transcripts. The temporal evolution of defence-related genes in pre-treated infected leaves suggests that grapevine responses vary depending on the inter- or intra-species variability of pathogens.

Keywords: ASM, BTH, downy and powdery mildew, grapevine, plant defence genes, polyphenols

Introduction

Powdery (*Erysiphe necator*) and downy (*Plasmopara viticola*) mildew are important diseases of grapevine (*Vitis vinifera*). These two biotrophic pathogens, which are native to the United States, infect green vine tissues and cause significant economic losses as well as environmental damage through the repetitive applications of fungicides.

The biotrophic oomycete *P. viticola*, the causal agent of downy mildew, is a heterothallic endoparasite (Lafon & Clerjeau, 1988). Its development involves intercellular mycelial growth and the differentiation of haustoria, which penetrate parenchyma cells. Its population diversity in Europe is low, but it exhibits various phenotypes and genotypes (e.g. fungicide resistance; Chen *et al.*, 2007). In contrast, *E. necator* (grapevine powdery mildew) is an ascomycete and ectoparasitic biotrophic fungus. In Europe, its populations are divided into two distinct genetic groups, A and B, which have different ecological requirements (Amrani & Corio-Costet, 2006). Furthermore, group A isolates are significantly more sensitive to various fungicides than those in group B (Dufour *et al.*, 2011).

Plants possess the ability to defend themselves against pathogens, but the success of these defences depends on the speed and/or intensity of their response, in addition to activation of the defence mechanisms. If the defences are activated too late, the pathogen colonizes plant tissue. However, a part of these defences can be induced and confer protection against a broad spectrum of pathogens either locally to confine the pathogens at the infection site, or systemically to lead to the development of resistance within the whole plant (Walters *et al.*, 2007). Typically, this inducible resistance system can be associated with the accumula-

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tion of salicylic acid (SA), a molecule required for signal transduction in systemic acquired resistance (SAR; Pieterse & Van Loon, 2007), leading to the coordinated accumulation of pathogenesis-related proteins (PR proteins), the production of phytoalexins, and the reinforcement of plant cell walls. Recently, the application of inducers that mimic natural signalling compounds and have no direct antifungal activity, such as acibenzolar-S-methyl or benzothiadiazole (BTH), has been shown to be effective against a broad spectrum of pathogens in various plants (Sticher *et al.*, 1997; Brisset *et al.*, 2000; Bressan & Purcell, 2005). SAR induced by BTH has been clearly established as being dependent on the SA pathway (Friedrich *et al.*, 1996), with consequent regulation of the genes involved in primary and secondary metabolism, and also the accumulation of phenolic compounds (Lawton *et al.*, 1996; Brisset *et al.*, 2000; Iriti *et al.*, 2004; Hukkanen *et al.*, 2008). Amongst the PR proteins, *PR-1* protein transcripts are usually induced and represent a marker for SA signalling.

In the presence of a pathogen, grapevine triggers defence mechanisms with variable success depending on the degree of resistance of the varieties, thus suggesting the basal induction of defences (Fung *et al.*, 2008). Genes encoding cytoskeletal and phenylpropanoid pathways are primarily up-regulated during infection (Polesani *et al.*, 2008), as are genes encoding PR proteins such as β-1,3-glucanase (*PR-2*), chitinase (*PR-8*) and *PR-1, PR-10* and *PR-5* (Legay *et al.*, 2011). The success of a plant's defences depends on the final output resulting from the interactions of various factors, including the genetic and physiological characteristics of both partners in the host–pathogen interaction, as well as on environmental conditions.

Elicitors could be used to reinforce the inherent defences in susceptible cultivars. In *V. vinifera*, gene expression was documented after the use of inducers (Gomes & Coutos-Thevenot, 2009), in interactions between *Vitis* species and *P. viticola* (Hamiduzzaman *et al.*, 2005; Trouvelot *et al.*, 2008), and between *Vitis* and *E. necator* (Jacobs *et al.*, 1999; Belhadj *et al.*, 2008; Fung *et al.*, 2008). The efficacy of plant defence stimulation usually provides good reproducible results in the laboratory, but efficacy in the field is often disappointing (Campbell & Latorre, 2004; Perazzolli *et al.*, 2008).

The present study sought to elucidate the role played by BTH as an elicitor on infected grapevine leaves, and to identify defence markers specific or non-specific to pathogen variability (intra- or inter-species). The evolution of transcript profiles of selected defence-related genes during *V. vinifera*–biotrophic pathogen interaction was characterized, and the impact of pathogen diversity was investigated in the presence or absence of BTH elicitation. The relationship between transcript profiles, polyphenol contents and pathogen control should contribute to understanding the basic behaviour of grapevine after elicitation with BTH, and the ability of BTH to enhance its defences when subjected to various pathogens.

Materials and methods

Plant and fungal materials

Grapevine plants (*V. vinifera* cv. Cabernet Sauvignon) were propagated from wood cuttings in a greenhouse. After 3 weeks, rooted cuttings were potted in a sandy soil and were grown under controlled conditions at 25/20°C day/night temperature, with 75% relative humidity, and a 16 h day/8 h night photoperiod (0·51 W m^{-2}) with weekly fertilization (2 g L^{-1}, N-P-K 20% with trace elements). Two-month-old plants with 10–12 leaves were used for the experiments, and the third and fourth leaves below the apex were used for powdery and downy mildew inoculations, respectively.

Plasmopara viticola

A fungicide-sensitive isolate (Cou-15) and a fungicide-resistant isolate (Mic-128) from the laboratory monospore collection (Table 1) were multiplied on grapevine leaves and inoculated as described in Corio-Costet *et al.* (2011) with a single 15 μL drop per leaf of a 5000 sporangia mL^{-1} spore suspension, and incubated for 7 days at 22°C with a 16 h day/8 h night photoperiod.

Erysiphe necator

Powdery mildew isolates (one belonging to group A, Llu-41, and one to group B, Pv-74; Table 1) were inoculated under sterile conditions on decontaminated grape leaves, as previously described (Debieu *et al.*, 1995), by blowing spores from sporulating leaves (1000 conidia per cm^2 of leaf), and were incubated for 12–14 days at 22°C with a 16 h day/8 h night photoperiod.

Leaf treatment with BTH and inoculation with pathogens

BTH (S-methyl benzo[1,2,3]thiadiazole-7-carbothioate, Bion®, 50WG, Syngenta) was dissolved in water and sprayed onto leaves at a concentration of 1·9 mM, 24 h before inoculation with the different pathogens. At each sampling time point (0, 24, 48 and 72 h post-inoculation,

Table 1 Characteristics of *Plasmopara viticola* and *Erysiphe necator* isolates

Pathogen	Isolates	Locality of vineyard	Sampling year	Fungicide sensitivity FAM	IPRO	MEF
P. viticola						
Pv-R	Mic-128	Bordeaux	2005	R	R	S
Pv-S	Cou-15	Bordeaux	2004	S	S	S
E. necator				Genetic group		
En-A	Llu-41	Pyrénées-orientales	2006		A	
En-B	Pv-74	Pays de l'Aude	2006		B	

FAM: Famoxadone; IPRO: Iprovalivarb; MEF: Mefenoxam; S: sensitive isolate; R: resistant isolate.

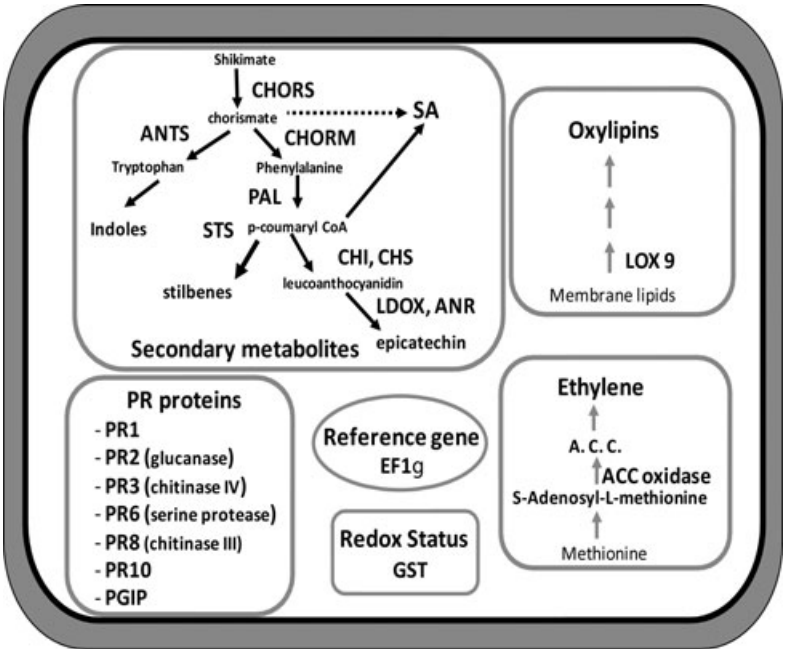

Figure 1 Schematic representation of pathways showing genes involved in transcript profile analysis.

hpi), six untreated leaves and six BTH-treated leaves, uninoculated or inoculated with each of the four pathogen isolates, were sampled. For each sample, six half leaves were used for biochemical analysis and the remaining six half leaves were used for gene expression. In addition, six other leaves treated or untreated with BTH were inoculated to confirm the biological efficacy of BTH treatment. The development of the disease was assessed 7 days after downy mildew inoculation or 12 days after powdery mildew inoculation. Disease intensity was estimated by measuring growth and intensity of fungal mycelium and sporulation, as described previously (Debieu *et al.*, 1995; Corio-Costet *et al.*, 2011), and was expressed as the mean ± standard deviation of the six replicates.

Table 2 Selected genes and corresponding primer sets used for analysis of transcript profiles from *Vitis vinifera* leaves

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)	GenBank no.	PCR primer efficiency
Elongation factor 1- γ chain (*EF1*γ)	GAAGGTTGACCTCTCGGATG	AGAGCCTCTCCCTCAAAAGG	AF176496	0·97
Phenylalanine ammonia lyase (*PAL*)	ACAACAATGGACTGCCATCA	CACTTTCGACATGGTTGGTG	X75967	1·08
Stilbene synthase (*STS*)	ATCGAAGATCACCCACCTTG	CTTAGCGGTTCGAAGGACAG	X76892	0·97
Leucoanthocyanidin dioxygenase (*LDOX*)	TGGTGGGATGGAAGAGCTAC	CCCACTTGCCCTCATAGAAA	X75966	0·84
Anthocyanidin reductase (*ANR*)	CCTGCCTCCAAGACACTAGC	GGCCATCAGAGTAGGGATGA	VVI000166	1·21
Chalcone isomerase (*CHI*)	AGAAGCCAAAGCCATTGAGA	CCAAGGGGAGAATGAGTGAA	X75963	1·10
Chalcone synthase (*CHS*)	CCAACAATGGTGTCAGTTGC	CTCGGTCATGTGCTCACTGT	X75969	1·12
PR protein class 1 (*PR-1*)	CCCAGAACTCTCCACAGGAC	GCAGCTACAGTGTCGTTCCA	AJ536326	0·88
PR protein class 10 (*PR-10*)	GCTCAAAGTGGTGGCTTCTC	CTCTACATCGCCCTTGGTGT	AJ291705	1·03
β-1,3-glucanase (*PR-2*)	GGGGAGATGTGAGGGGTTAT	TGCAGTGAACAAAGCGTAGG	AF239617	1·18
Chitinase class IV (*PR-3*)	TATCCATGTGTCTCCGGTCA	TGAATCCAATGCTGTTTCCA	VVU97521	1·14
Serine protease inhibitor (*PR-6*)	ACGAAAACGGCATCGTAATC	TCTTACTGGGGCACCATTTC	AY156047	1·23
Chitinase class III (*PR-8*)	AATGATGCCCAAAACGTAGC	ATAAGGCTCGAGCAAGGTCA	Z68123	1·05
Polygalacturonase inhibitor protein (*PGIP*)	CCGGGAAAATCCCATATTCT	AAGGTCCAACGACGTCAAAC	AF305093	0·96
Lipoxygenase 9 (*LOX-9*)	GACAAGAAGGACGAGCCTTG	CATAAGGGTACTGCCCGAAA	AY159556	0·89
Glutathione S- transferase (*GST*)	GGGATCTCAAAGGCAAAACA	AAAAGGGCTTGCGGAGTAAT	AY156048	0·99
1-aminocyclopropane, 1-carboxylic acid oxidase (*ACC*)	GAAGGCCTTTTACGGGTCTC	CCAGCATCAGTGTGTGCTCT	AY211459	0·86
Anthranilate synthase (*ANTS*)	AAAAATCCAAGAGGGGTGCT	AAGCTTCTCCGATGCACTGT	XM002281597	0·84
Chorismate mutase (*CHORM*)	TCATTGAGAGGGCCAAATTC	AGGAGGCAGAAAAAGCATCA	FJ604854	1·05
Chorismate synthase (*CHORS*)	GCCTTCACATGCAGATGCTA	CTGCAACTCTCCCAATGGTT	FJ604855	1·00

For biological, biochemical and gene expression assays, two independent experiments were carried out over the study period. All data were subjected to analysis of covariance by general linear model using the statistical program SYSTAT 11 (Systat Software, Inc.) and significant differences were determined by Tukey's Honestly Significant Difference (HSD) test at the level of $P \leq 0{\cdot}05$.

Expression experiments and qRT-PCR

A series of 20 genes was monitored by quantitative real-time polymerase chain reaction (qRT-PCR), including the γ-chain elongation factor 1 gene (*EF1-*γ), which was used as the internal standard to normalize the starting template of cDNA (Table 2). This gene was used because it is not involved in the plant response to infections, unlike α-tubulin or actin genes (Polesani *et al.*, 2008), and because it is very stable with mean C_q values of $20{\cdot}32 \pm 0{\cdot}061$. Treatment and/or infection had no effect on the C_q values of the *EF1-*γ gene. The gene set used included PR proteins, some genes involved in the phenylpropanoid pathway, others involved in the oxido-reduction system, in the ethylene or oxylipin pathways, or in the indole pathway (Table 2; Fig. 1).

Sprayed leaves in each experimental condition were removed at all time points and frozen at −80°C. Each sample consisted of six half leaves from which the mRNA was extracted, and technical duplicates or triplicates were performed. The data are the average of duplicates of at least two independent experiments for the 20 genes.

Primers were designed at 60°C T_m to amplify fragments from 75 to 150 bp using PRIMER 3 software (http://frodo.wi.mit.edu/primer3/; Table 2). RNAs were extracted according to the method of Reid *et al.* (2006). Only high quality RNA samples were selected, with an absorbance ratio at 260/280 nm ranging from 1·82 to 2·06. Two micrograms of RNA treated by DNAse I (RQ1 RNase-free DNase, Promega) were reverse-transcribed using 2 μM oligo-d$(T)_{15}$, ribonuclease inhibitor and M-MLV reverse transcriptase (Promega), following the manufacturer's instructions.

The amplification specificity of each qRT-PCR was reinforced by a single peak in melt curve analysis, and no primer dimers were detected using agarose gel electrophoresis. Thereafter, the expression of the selected genes was assessed by using a CFX 96 system thermocycler (Bio-Rad) with SYBR® Green to detect dsDNA synthesis. For each reaction, 1 μL of each primer at 1 μM, and 7 μL of 2 × Blue SYBR Green fluorescein mix including Hot start DNA polymerase, dNTP and $MgCl_2$ (ABgene) and 5 μL of cDNAs, were used following the manufacturer's instructions. The cycling conditions were: denaturation cycle (94°C for 15 min); amplification and quantification cycle repeated 40 times (94°C for 10 s, 55°C for 10 s, 72°C for 20 s). Expression ratios for each cDNA were calculated for each time point relative to control leaves at the same time. Relative gene expression was obtained with the formula: fold induction = $2^{-[\Delta\Delta C_q]}$, where $\Delta\Delta C_q$ = [C_q GI (unknown sample) − C_q *EF1*γ (unknown sample)] − [C_q GI (reference sample) − C_q *EF1*γ (reference sample)]. GI is the gene of interest, and *EF1*γ is the grapevine elongation factor 1γ gene used as the internal control. The expression level of the gene of interest in the reference sample (e.g. untreated, uninoculated leaves) used for the relative expressions was considered to be 1. The mean values obtained by this calculation method varied from 0·01 to 109·7 (the $\Delta\Delta C_q$ corresponding means ranged from −6·65 to 6·78). The values of $\Delta\Delta C_q$ between 0 and 1 correspond to repressions. Each sample was assayed at least twice in each independent experiment. To ensure correct normalization in real-time PCR, amplification efficiencies should be similar. Each PCR system was tested with cDNA samples that were serially diluted 1:10 with distilled water. Amplification efficiencies are determined from the slope of the log-linear portion of the calibration curve. Specifically, PCR efficiency = $10^{-1/\text{slope}} - 1$, when the logarithm of the initial template concentration of known cDNA amounts (the independent variable) is plotted on the x-axis and C_q (the dependent variable) measured by the CFX 96 system software (Bio-Rad) is plotted on the y-axis. The PCR efficiency of each primer was between 0·84 and 1·23 (Table 2). The dissociation curves for each amplicon were then analysed to verify the specificity of each amplification reaction. The dissociation curve was obtained by heating the amplicon from 60 to 95°C (data not shown).

Means of relative expression obtained in control and other treatments and/or inoculations were compared with the analysis of variance using Tukey's post hoc test. Statistical analyses were carried out using the SYSTAT v. 11 program (Systat Software, Inc.).

Extraction and quantification of stilbenes

Stilbenes were extracted overnight with 20 mL methanol as described previously (Belhadj *et al.*, 2008) from each sample (80) of dried leaves (100 mg) obtained from the gene expression experiments (see below). Two independent experiments were performed. Analysis of stilbenes was carried out by HPLC on a 250 × 4 mm Prontosil Eurobond C18 reverse-phase column (4·0 ID × 250 mm, 5 μm; Bischoff Chromatography). Separation was carried out at a flow rate of 1 mL min^{-1} with a modified mobile phase composed of (A) H_2O: 1% TFA (97·5: 2·5 vol: vol) and (B) acetonitrile: A (80: 20 vol: vol). The run was set as follows: 0–5 min, from 10 to 20% B; 5–19 min, from 20 to 25% B; 19–20 min, from 25 to 30% B; 20–35 min, from 30 to 40% B; 35–50 min, 40% B; 50–55 min, from 40 to 60% B; 55–56 min, from 60 to 100% B; 56–60 min, 100% B; 60–62 min, from 100 to 10% B. Fluorimetric detec-

tion was performed at λ_{ex} = 300 nm and λ_{em} = 390 nm, and stilbene contents were estimated from specific calibration curves as described in Belhadj *et al.* (2008). Data were expressed as the means (± standard deviations) of at least four quantifications. Statistical analysis was carried out using Newman–Keuls or Student's *t*-tests at the level of $P \leq 0{\cdot}05$.

Results

BTH induction of grapevine resistance

Activation of grapevine defences by BTH treatment (1·9 mM) 24 h before inoculation inhibited growth of the four mildew isolates (Table 1) possessing different phenotypes and genotypes. Growth inhibition ranged from 62·0 to 98·3% (Fig. 2), with the downy mildew isolate Mic-128 (*Pv*-R) showing inhibition significantly better than expected (98·3%) ($P = 0{\cdot}005$). This isolate, which has good fitness (Corio-Costet *et al.*, 2011), has the peculiarity of being resistant to fungicides inhibiting cellulose synthase and mitochondrial cytochrome *b*, suggesting that the BTH effect on grapevine may promote the effectiveness of the plant's defences with regard to this phenotype of *P. viticola*.

Transcript profiles

Vitis vinifera is especially susceptible to mildew. To explore compatible interactions with different obligate pathogens, a comparison over time of transcript profiles of selected genes from leaves infected with two isolates of *P. viticola* (*Pv*-R and *Pv*-S) and two isolates of *E. necator* (*En*-A and *En*-B) was carried out (Table 2). The grapevine defences were measured by transcript evolution at 24, 48 and 72 hpi and complemented by biological efficacies at 8 or 12 dpi.

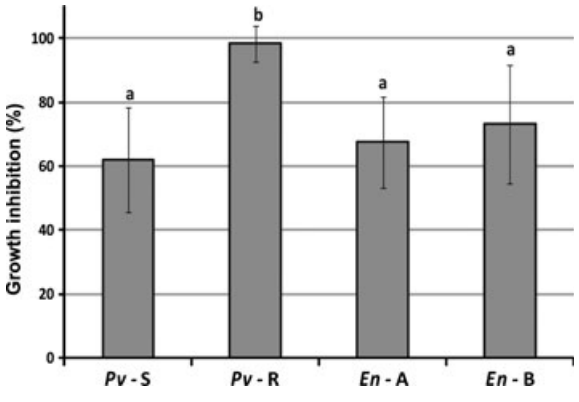

Figure 2 Growth inhibition of two isolates of *Plasmopara viticola* (Cou-15, *Pv*-S; Mic-128, *Pv*-R) and two isolates of *Eryisphe necator* (Llu-41, *En*-A; Pv-74, *En*-B) after pre-treatment with BTH at 1·9 mM. Different letters above columns show significant differences at $P \leq 0{\cdot}05$.

At 24 hpi, the transcript analysis showed mostly a down-regulation of genes, with 57·2–58·4% of the differentially-expressed transcripts significantly down-regulated in *Pv*-infected leaves, and 90% in *En*-infected leaves (Fig. 3a; Table 3). In *Pv*-infected leaves, some transcripts were up-regulated in the presence of both *P. viticola* isolates, such as *PR-3* and *PR-10*. However, transcript profiles also varied depending on the isolate and the genes under consideration (e.g. *PR-1, PR-6, CHI, CHS*). Indeed, in leaves infected with *Pv*-S, genes involved in the phenylpropanoid pathway (*CHI, CHS*) were strongly repressed, whereas in *Pv*-R infected leaves, it was the *LDOX* gene coding for an enzyme situated further downstream in the pathway which was specifically repressed. In *En*-infected leaves, the PR protein transcripts (*PR-1, PR-2, PR-3, PR-8, PGIP*) were mostly significantly down-regulated.

Only four genes behaved in a similar way regardless of the pathogen considered: *LDOX* (polyphenol pathway), *ACC* (ethylene pathway) and *GST* (oxidative stress response system) transcripts which were all repressed, and the *CHORM* (indole pathway) transcript which was up-regulated.

At 48 h hpi (Fig. 3a; Table 3), most of the grapevine transcripts were not affected significantly in *Pv*-S and *En*-A-infected leaves (26·3 and 31·6% of differentiated genes, respectively). In contrast, in *En*-B and *Pv*-R-infected leaves, 42·1–57·9% of transcripts, respectively, were either down- or up-regulated. In *Pv*-infected leaves, some PR protein transcripts were up-regulated such as *PR-1, PR-2, PR-6*, *PR-8*, depending on the isolate under consideration, while in *En*-infected leaves only *PR-3* was over-expressed. A peculiarity of *En*-infected leaves was the accumulation of *STS* transcripts and the repression of ANR and CHORM transcripts (Table 3). In contrast, an up-regulation of *ANR* and *ANTS* transcripts was specific to *Pv*-infected leaves and, to a lesser extent, *ANR* was also slightly up-regulated in *En*-B-infected leaves.

At 72 hpi, the majority of differentiated transcripts (83·4–100%) in *Pv*-infected leaves were down-regulated, with significant down-regulation of transcripts of the phenylpropanoid pathway (*STS, CHI, CHS*), PR proteins, *LOX, GST* and *ACC* (Fig. 3a; Table 3). In *En*-infected leaves, the down-regulation was also high, with 62·5–83·4% of differentiated transcripts affected, with *CHI, PR-1* and *PR-2* commonly repressed. The *LDOX* and *CHI* genes were simultaneously over-expressed in *En*-infected leaves, suggesting the possibility of metabolic flow towards flavonoid biosynthesis.

Effect of BTH treatment on transcript profile of uninoculated leaves

To decipher the effect of BTH in grapevine, transcripts were monitored for 4 days in uninfected leaves. To simplify the gene expression data resulting from transcripts extracted from the third or fourth leaves, the averages obtained are presented in Table 4 and in Figure 3b (first

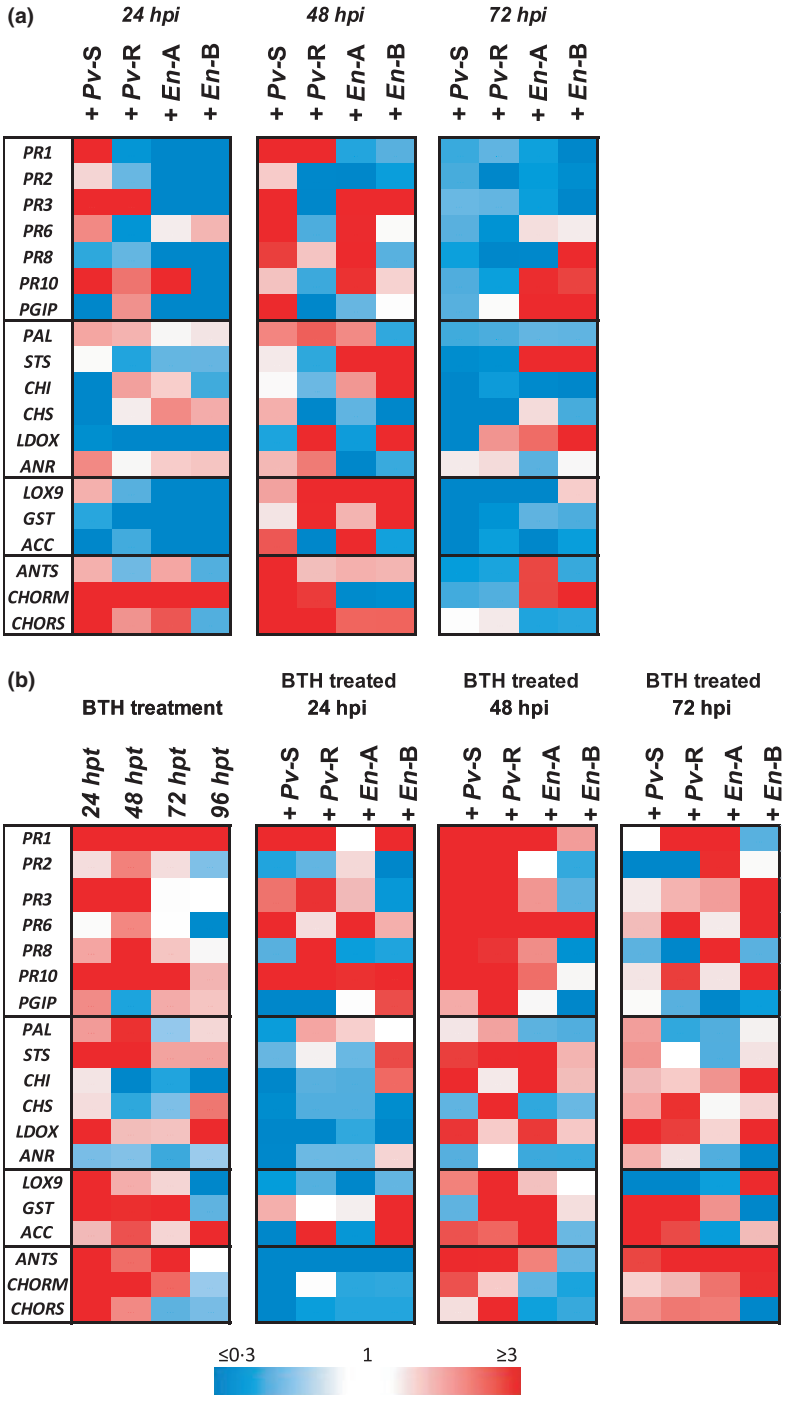

Figure 3 Relative gene expression in infected leaves with different isolates of *Plasmopara viticola* (*Pv*) and *Erysiphe necator* (*En*), without (a) or with (b) BTH pre-treatment. Each column represents the time point after pathogen inoculation (24, 48 or 72 hours post-inoculation, hpi) and each line corresponds to one gene represented by a single row of boxes. The colour scale bars represent the ratio values corresponding to the mean of two independent experiments. Genes over-expressed appear in shades of red, with expression level higher than 3 in bright red, while those repressed appear in shades of blue, with intensity lower than 0·3 in dark blue (white = no change in gene expression compared to control). In (b), the columns corresponding to BTH treatment alone represent the mean of the relative gene expression of the different foliar stages of two independent experiments.

column). No significant difference in gene expression according to the foliar stage was found except for the expression of the *ANR* gene at 96 h post-treatment (hpt) (P = 0·016, data not shown). Over time, 21–36·8% of transcripts were affected (Fig. 3b; Table 4), with up-regulation of PR protein transcripts mainly at 24 and 48 hpt, with a significant over-expression of *PR-1, PR-10* and *PR-3*. Stilbene synthase (*STS*), *GST* and *LOX-9* transcripts were up-regulated at 24 hpt as well as the chorismate mutase and chorismate synthase genes (*CHORS, CHORM*), which points to a possible role in lignin or SA synthesis required for *PR-1* gene expression (Slusarenko & Schlaich, 2003). Amongst the down-regulations, the chalcone isomerase gene (*CHI*) was generally repressed over time after treatment with BTH. Four days after the treatment only the *PR-1* gene was still significantly up-regulated. Overall, BTH treatment triggered the up-regulation of differentiated transcripts, but this up-regulation progressively decreased from 100% at 24 hpt to 16% at 96 hpt (Table 4).

Table 3 Relative expression of defence-related genes that are significantly induced (bold) or repressed (underlined and italic) in leaves of *Vitis vinifera* after pathogen inoculation compared to uninoculated controls at $P \leq 0{\cdot}05$

	Plasmopara viticola isolate						*Erysiphe necator* isolate					
	Fungicide-sensitive			Fungicide-resistant			Group A			Group B		
Gene	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi
PR-1	**4·40**	**3·40**	*0·63*	*0·42*	**28·50**	*0·83*	*0·07*	–	*0·53*	*0·04*	–	*0·26*
PR-2	–	**1·50**	–	–	*0·34*	*0·13*	*0·04*	*0·19*	–	*0·03*	–	*0·38*
PR-3	**3·80**	–	–	**3·30**	*0·25*	-	*0·05*	**4·20**	–	*0·08*	**3·80**	*0·13*
PR-6	–	**4·40**	–	*0·42*	–	*0·40*	–	–	–	–	–	–
PR-8	–	–	*0·50*	–	**1·50**	*0·19*	*0·17*	–	*0·34*	*0·06*	–	–
PR-10	**4·60**	–	*0·71*	**2·30**	–	*0·50*	**7·40**	**2·90**	–	–	–	–
PGIP	*0·20*	–	*0·71*	**2·00**	*0·27*	–	*0·05*	–	–	*0·07*	–	–
PAL	–	–	–	–	–	–	–	–	–	–	–	–
STS	–	–	*0·36*	–	–	*0·38*	–	**6·80**	**10·80**	–	**9·80**	**39·00**
CHI	*0·01*	–	*0·11*	–	–	*0·45*	–	–	*0·34*	–	–	*0·01*
CHS	*0·02*	–	*0·28*	–	*0·21*	*0·25*	–	–	–	–	*0·20*	*0·67*
LDOX	*0·37*	*0·56*	*0·05*	*0·01*	**7·05**	**2·00**	*0·14*	*0·45*	**2·40**	*0·16*	**3·90**	**3·60**
ANR	**2·10**	–	–	–	**2·30**	**1·30**	**1·50**	*0·24*	–	**1·50**	*0·63*	–
LOX	**1·70**	–	*0·08*	–	**15·50**	*0·13*	*0·04*	–	*0·33*	*0·10*	**12·40**	**1·50**
GST	*0·57*	–	*0·14*	*0·26*	–	*0·40*	*0·20*	–	*0·77*	*0·09*	**6·40**	–
ACC	*0·01*	–	*0·19*	–	*0·31*	*0·50*	*0·08*	–	*0·18*	*0·11*	–	–
ANTS	–	**3·20**	*0·48*	–	**1·60**	–	–	–	–	–	**1·70**	–
CHORM	**9·10**	–	*0·67*	**6·00**	–	–	**12·70**	*0·36*	–	**10·40**	*0·37*	–
CHORS	–	–	–	–	**4·60**	–	–	–	–	–	–	–
Percentage of differentiated genes	63·1	26·3	68·4	36·8	57·9	63·1	52·6	31·6	31·6	52·6	42·1	42·1
Relative percentage of down-regulated genes	58·4	40	100	57·2	54·5	83·4	90	66·7	83·4	90	37·5	62·5

–: not significantly different.

Table 4 Relative expression of defence-related genes that are significantly induced (bold) or repressed (underlined and italic) in *Vitis vinifera* leaves pre-treated with BTH compared to uninoculated controls at $P \leq 0{\cdot}05$

Gene	24 hpt	48 hpt	72 hpt	96 hpt
PR-1	**81·60**	–	–	**28·10**
PR-2	–	–	–	–
PR-3	–	**7·90**	–	–
PR-6	–	–	–	*0·21*
PR-8	–	–	–	–
PR-10	**5·40**	–	–	–
PGIP	–	*0·30*	–	–
PAL	–	–	–	–
STS	**6·00**	–	–	–
CHI	–	–	*0·31*	*0·09*
CHS	–	*0·33*	–	–
LDOX	–	–	**2·10**	–
ANR	–	–	*0·34*	–
LOX-9	**16·40**	–	–	*0·12*
GST	**14·40**	**4·80**	–	*0·42*
ACC	–	–	–	–
ANTS	–	–	–	–
CHORM	**8·10**	**7·70**	–	–
CHORS	**9·10**	–	*0·42*	*0·53*
Percentage of differentiated genes	36·8	31·6	21	31·6
Relative percentage of up-regulated genes	100	50	25	16

Gene expression in BTH pre-treated grapevine leaves inoculated with *Plasmopara viticola* or *Erysiphe necator*

Inoculation of BTH pre-treated leaves with *P. viticola* isolates triggered over-expression of PR proteins which varied in time, depending on the isolates inoculated. At 24 hpi in *Pv*-infected leaves, down-regulation of genes involved in the phenylpropanoid and indole pathways was observed (Table 5; Fig. 3b). Transcript down-regulation was more marked in *Pv*-S-infected leaves than in *Pv*-R-infected leaves, perhaps due to the lesser efficacy of BTH treatment on the sensitive isolate (62% inhibition) than on the fungicide-resistant isolate (98·3% inhibition; Fig. 2). At 48 hpi, the majority of PR proteins, stilbene synthase (*STS*) and anthranilate synthase (*ANTS*) transcripts were commonly up-regulated in *Pv*-infected leaves. In *Pv*-R-infected leaves, *LOX-9, ACC* and *GST* genes were also up-regulated (Table 5, Fig. 3b), whereas in *Pv*-S-infected leaves, *CHI* and *PR-8* genes were more strongly up-regulated. At 72 hpi, the expression profiles were similar in *Pv*-infected leaves, with few genes regulated except for *PR-10* (up-regulated), and *LOX-9* and *PR-2* genes (down-regulated) (Table 5; Fig. 4b,c,i).

In *En*-infected leaves, PR protein transcripts were over-expressed later than in *Pv*-infected leaves (Table 5; Fig. 3b). At 24 hpi, *PR-10* was up-regulated and *LDOX* and *ANTS* were down-regulated, as in *Pv*-leaves (Table 5;

Table 5 Relative expression of defence-related genes that are significantly induced (bold) or repressed (underlined and italic) in *Vitis vinifera* leaves pre-treated with BTH after inoculation, compared to uninoculated controls at $P \leq 0.05$

	Plasmopara viticola						*Erysiphe necator*					
	Fungicide-sensitive			Fungicide-resistant			Group A			Group B		
Gene	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi	24 hpi	48 hpi	72 hpi
PR-1	–	**109·70**	–	**5·80**	**76·40**	–	–	**5·30**	–	–	–	–
PR-2	–	**6·84**	*0·03*	–	**4·60**	*0·49*	–	–	**2·90**	–	*0·63*	–
PR-3	–	**7·40**	–	**2·90**	**10·50**	–	–	–	**1·90**	–	–	**14·50**
PR-6	**3·60**	–	–	–	–	–	–	**9·10**	–	–	–	**12·20**
PR-8	–	**16·60**	–	**8·50**	–	*0·25*	–	–	**3·40**	–	*0·40*	–
PR-10	**6·30**	**6·80**	**1·20**	**6·20**	**11·60**	**2·80**	2·90	–	–	**3·80**	–	**3·30**
PGIP	*0·14*	–	–	*0·23*	–	–	–	–	*0·09*	–	*0·14*	–
PAL	*0·48*	–	–	–	–	*0·59*	–	–	–	–	–	–
STS	–	**2·77**	–	–	**2·90**	–	–	**18·20**	–	–	–	–
CHI	*0·01*	**11·40**	–	–	–	–	–	–	–	–	–	**7·00**
CHS	–	–	**1·80**	–	–	–	*0·71*	*0·59*	–	*0·36*	–	–
LDOX	*0·03*	–	–	*0·03*	–	–	*0·50*	**2·80**	–	*0·17*	–	–
ANR	*0·34*	–	–	–	–	–	–	–	–	–	–	*0·23*
LOX-9	–	**2·21**	*0·04*	–	**3·70**	*0·20*	*0·32*	–	*0·50*	–	–	**5·80**
GST	–	–	**3·88**	–	**15·50**	**4·97**	–	**7·20**	**2·00**	**4·20**	–	*0·32*
ACC	*0·20*	**2·63**	–	–	**2·50**	–	–	–	–	–	–	–
ANTS	*0·25*	**5·60**	–	*0·04*	**3·00**	–	*0·32*	–	**11·80**	*0·03*	–	**14·30**
CHORM	*0·18*	–	–	–	–	–	–	–	–	–	–	**2·90**
CHORS	–	–	–	–	–	–	–	–	–	–	–	*0·29*
Percentage of differentiated genes	52·6	31·6	21·1	36·8	47·4	26·3	15·8	31·6	31·6	26·3	15·8	52·6
Relative percentage of up-regulated genes	20	100	50	57·1	100	20	0	83·3	66·7	40	0	70

–: not significantly different.

Fig. 4b,h). At 48 hpi, few genes were up-regulated and regulation seemed to depend on the isolate under consideration. There was no significant up-regulation in *En*-B-infected leaves, but in *En*-A-infected leaves, several genes were up-regulated (*PR-1, PR-6, STS, LDOX, GST*) (Table 5; Fig. 4a,e,g,j). At 72 hpi, most PR protein transcripts were up-regulated in either or both *En*-A and *En*-B-infected leaves, e.g. *PR-2, PR-3, PR-6, PR-8* and *PR-10*, as were transcripts involved in the tryptophan pathway (*ANTS*) (Table 5, Fig 3b).

The evaluation of the relative expression of 10 genes undergoing expression modulation over time after pre-treatment with BTH and pathogen inoculations revealed different responses of grapevine leaves according to the isolate under consideration. This was particularly the case for genes coding for PR proteins, where the plant responses were generally more marked in the presence of *P. viticola* than *E. necator* (Fig. 4a–f). Interestingly, transcripts of anthranilate synthase (*ANTS*; Fig. 4h) were down-regulated at 24 hpi and could be a common marker for grapevine responses to the presence of biotrophic pathogens, in addition to *PR-10* and *PR-1* genes, which were up-regulated in all infected leaves after treatment with BTH. Other genes could be specific markers of one pathogen species, such as *PR-3* and *PR-8* in *Pv*-infected leaves. Depending on the pathogen isolate, faster or slower plant defence responses were also noted (e.g. *PR-6* in *En*-treated leaves, or *PR-8* in *Pv*-leaves). For other genes such as *GST* or *STS*, plant responses manifested themselves in the same way whatever the pathogen, but with different intensities, while for other genes such as the *LOX-9* gene, the pattern was different depending on the isolate (Fig. 4g,i,j).

Effects of pathogens and BTH on stilbene accumulation

BTH treatment exhibited no significant effect on total polyphenol content in leaves over the 3 days of analysis (Fig. 5). No significant differences were found depending on the isolate, so data of four experiments were pooled for each mildew species. A significant increase in total content was obtained only in *Pv*-infected leaves (254 and 233 $\mu g\ g^{-1}$ of dry weight for *Pv*-S and *Pv*-R, respectively) and *En*-infected leaves (375 and 344 $\mu g\ g^{-1}$ of dry weight for *En*-A and *En*-B, respectively), without BTH treatment. A more precise quantification of three major stilbenoid phytoalexins (resveratrol, piceid and pterostilbene) showed that quantitatively *trans*-piceid produced the highest level of stilbene, with a high increase at 24, 48 and 72 hpi in *En*-infected leaves (Fig. 6a). *Trans*-resveratrol content was very low in uninfected leaves, often less than 2 $\mu g\ g^{-1}$ of dry weight (Fig. 6b). Its content increased strongly in *En*-infected leaves with or without

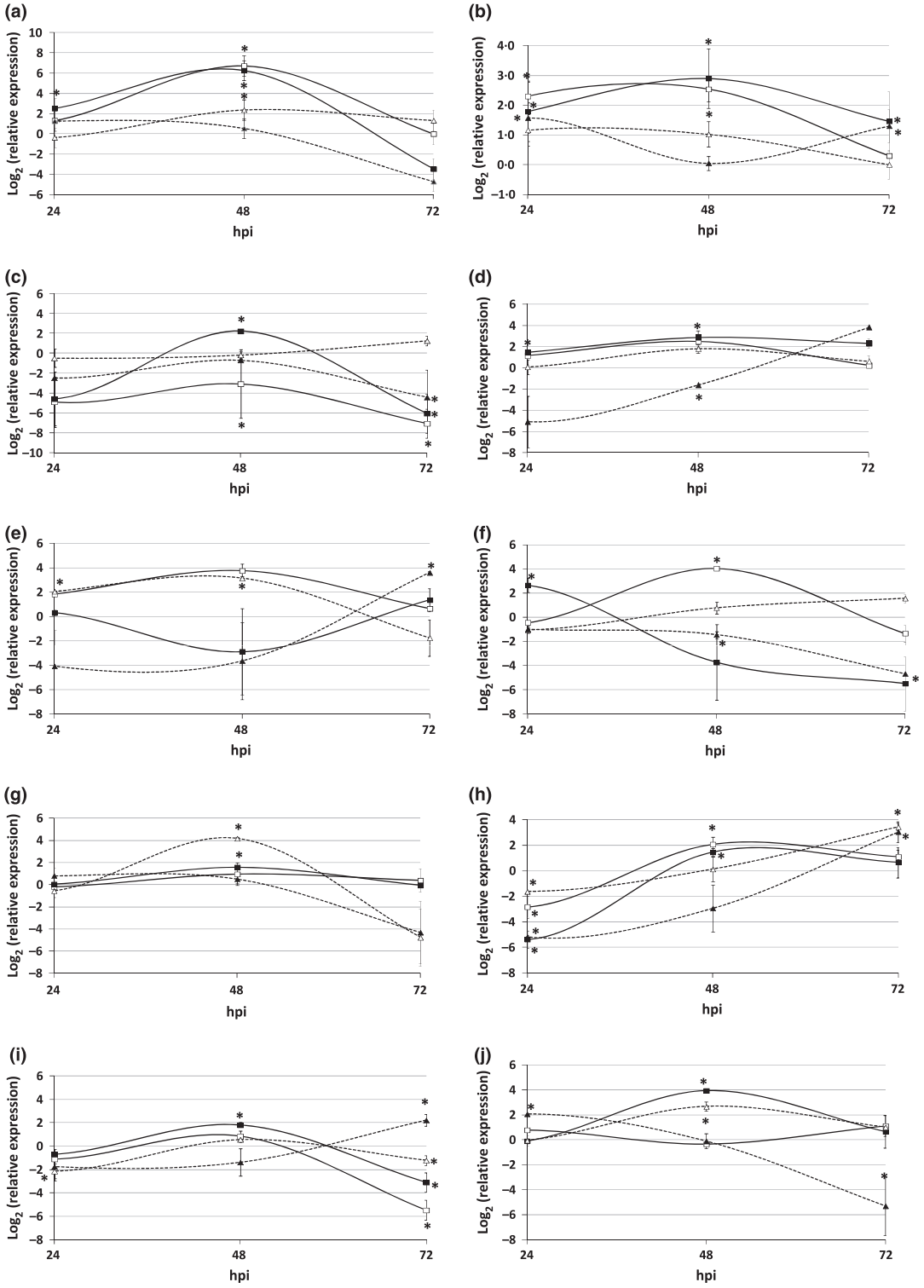

Figure 4 Comparative expression profiles of 10 genes (a, *PR-1*; b, *PR-10*; c, *PR-2*; d, *PR-3*; e, *PR-6*; f, *PR-8*; g, *STS*; h, *ANTS*; i, *LOX-9*; j, *GST*) over 3 days in infected leaves after BTH pre-treatment 24 h before inoculation. The $\log_2$ normalized expression values were plotted for each gene at three time points (24, 48, 72 hours post-inoculation, hpi). Data correspond to the mean of two independent experiments for each *Erysiphe necator* isolate (n = 2 *En*-A (white triangle) and n = 2 *En*-B (black triangle); dotted lines) and for each *Plasmopara viticola* isolate (n = 2 *Pv*-S (white square) and n = 2 *Pv*-R (black square); solid lines). Stars represent values significantly different from controls at $P \leq 0{\cdot}05$. The data is shown as the relative expression of genes compared to control samples (untreated and uninoculated). Bars represent a 95% confidence interval calculated from at least two replicates of two independent experiments.

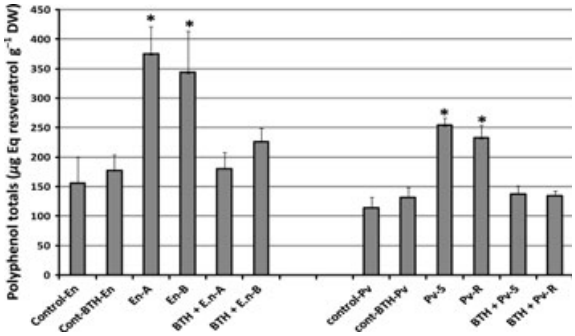

Figure 5 HPLC quantification of polyphenols in *Vitis vinifera* leaves in response to BTH treatment with or without pathogen inoculations. Results represent the means (± standard deviations) of four experiments. Stars represent the significant values at $P \leq 0{\cdot}05$.

pre-treatment with BTH, reaching 8·86 ± 0·75 μg g^{-1} of dry weight at 24 hpi, i.e. 6-fold more than in control leaves (1·42 ± 0·58 μg g^{-1} of dry weight). Accumulation was less in *Pv*-infected leaves. Only pterostilbene increased significantly in relation to BTH treatment, reaching 16·9–21 μg g^{-1} of dry weight in *En*-infected leaves after BTH treatment (Fig. 6d).

Discussion

Treatment of grapevine leaves with BTH led to a significant reduction in *P. viticola* and *E. necator* development and induced changes in the expression of transcripts and in pterostilbene contents, depending on the sampling time and the pathogen. Analysis of transcripts from grapevine leaves with or without pre-treatment with BTH over 3 days threw light on the potential defence pathway required during the SAR response. After the different mildew attacks, a large proportion of genes were repressed, indicating the suppression of defence responses in *Pv*- and *En*-infected grapevine leaves, as suggested by Polesani *et al.* (2008). Therefore, the real effect of BTH on gene expression may be underestimated. As gene expression levels are generally expressed relative to uninoculated and untreated controls, data was also expressed by calculating the relative gene expression, i.e. gene expression relative to the expression of untreated inoculated leaves instead of untreated uninoculated leaves (Fig. 7) to obtain the real effect of BTH after inoculation. Therefore, various transcripts (PR proteins, *STS, PAL*, *ACC*, *GST*) were strongly up-regulated in *Pv*-R-infected leaves treated with BTH, and at 24 hpi this correlated with better efficacy of grapevine defence against this isolate. The reference gene chosen to calculate the relative gene expression then becomes all important, showing that the pathogen can lead to significant down-regulation that may be compensated by the plant defence activator treatment.

The transcript pattern of *Pv*-infected leaves was significantly different from that of *En*-infected leaves, showing that grapevine possesses different defence systems to fight against these two biotrophic pathogens. This may be

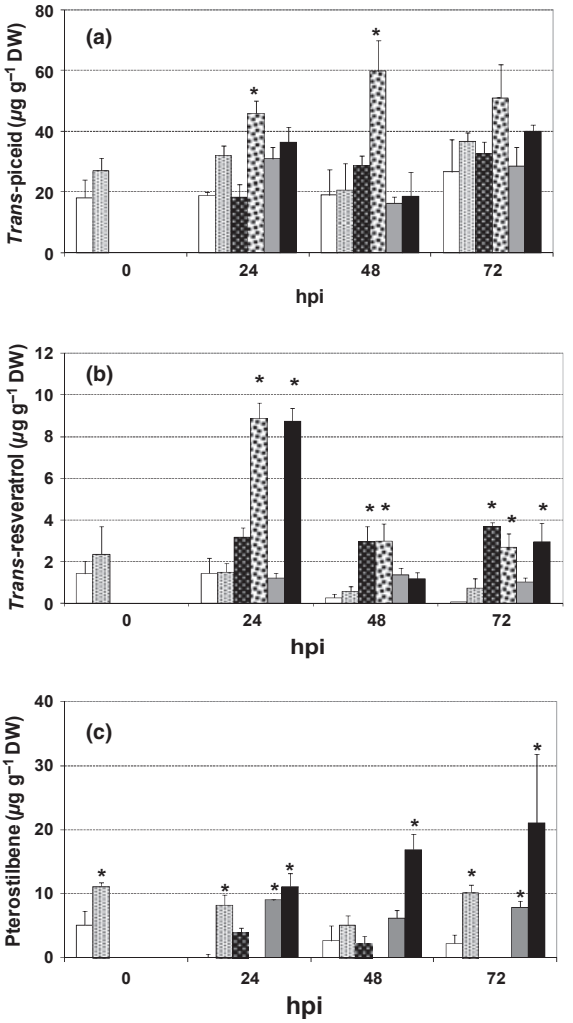

Figure 6 HPLC quantification of three stilbenes: (a) *trans*-piceid, (b) *trans*-resveratrol and (c) pterostilbene, in *Vitis vinifera* leaves in response to BTH treatment (1·9 mM), with or without *Plasmopara viticola* (*Pv*) or *Erysiphe necator* (*En*) inoculations. Results represent the means (± standard deviations) of four experiments. Stars represent the significant values at $P \leq 0{\cdot}05$. (□) control; (▨) BTH treatment; (▩) *Pv*-infected leaves; (▩) *En*-infected leaves; (▩) *Pv*-infected leaves and BTH treatment; (■) *En*-infected leaves and BTH treatment.

related to the ectoparasitic development of powdery mildew on leaves and the endoparasitic development of the downy mildew inside tissues, as well as with their speed of development and their biological specificity. One of the characteristics of responses associated with BTH is the induction of PR gene transcripts such as *PR-1*, β-1,3-glucanase (*PR-2*), chitinases and thaumatin-like proteins, which are known to accumulate mainly in the extracellular spaces (Van Loon & van Strien, 1999). Usually, PR proteins (PR-1, PR-10, chitinases) and enzymes of the phenylpropanoid pathway are modulated, but a broad range of transcripts seems to be dependent on the plant and on the experimental conditions (Lawton *et al.*, 1996; Brisset *et al.*, 2000; Hukkanen *et al.*, 2008).

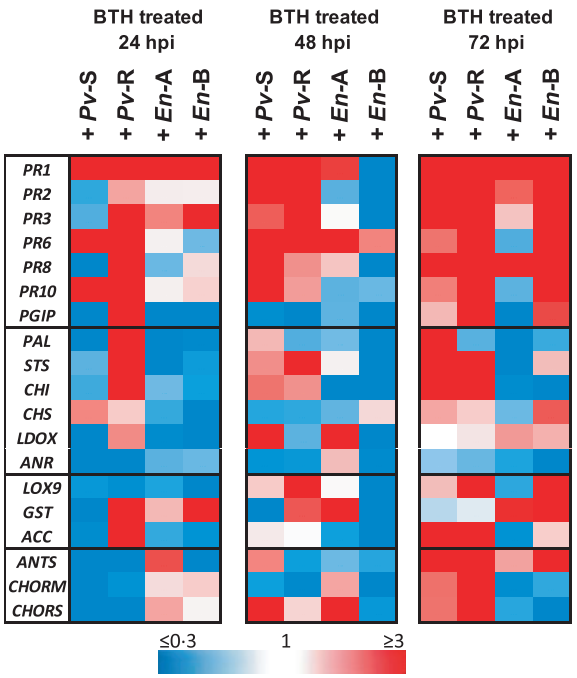

Figure 7 Relative gene expression in leaves infected with different isolates of *Plasmopara viticola* (*Pv*) and *Erysiphe necator* (*En*), after BTH treatment, compared to the gene expression found in untreated inoculated leaves. Each column represents the time point after pathogen inoculation (24, 48 or 72 hours post-inoculation, hpi) and each line corresponds to one gene represented by a single row of boxes as described in Fig. 3.

PR transcripts *PR-1, PR-10, PR-3, PR-2* and *PR-8* significantly increased in time after BTH treatment in infected leaves (Fig. 4a,b,d,f), as seen in other plant species (Van Loon & van Strien, 1999; Brisset *et al.*, 2000; Hukkanen *et al.*, 2008). The role of PR proteins in defence against *P. viticola* remains uncertain because *PR-1* was sometimes, but not always, induced in infected leaves of susceptible cultivars (Legay *et al.*, 2011). In the present study, *PR-1* was the most abundant transcript up-regulated after BTH induction of SAR and it was a common marker for SAR. As previously reported in grapevine (Hamiduzzaman *et al.*, 2005 Chong *et al.*, 2008), BTH treatments strongly induced *PR-1* expression before mildew inoculation, indicating the direct activation of defence processes.

As expected, *PR-10* transcripts increased in infected leaves after BTH induction, suggesting that PR-10 may also be a marker of grapevine defence against *P. viticola* and, to a lesser extent, against *E. necator*. The transcripts corresponding to chitinases (*PR-3, PR-8*), glucanase (*PR-2*) and *PR-6* exhibited increasing dependence on pathogen isolates. For example, a delay in the transcript over-expression of *PR-3* and *PR-6* was observed in *En*-B-infected leaves. It is suggested that genetic group A of *E. necator*, which develops more quickly and produces more spores than isolates of genetic group B (Montarry *et al.*, 2008), could trigger plant responses earlier in relation to pathogen development. Similarly, at 24 hpi, the transcript level of *PR-8* in *Pv*-R-infected leaves was significantly greater than in *Pv*-S-infected leaves, followed by a higher level of *PR-2* transcripts at 48 hpi. The earlier and more intense up-regulation combination could partially explain the better growth inhibition (98·3%) obtained after BTH treatment in *Pv*-R-infected leaves.

In this study, the *β*-1,3-glucanase (*PR-2*) or chitinase transcripts, corresponding to acidic class III endochitinase (*PR-8*) and class IV endochitinase (*PR-3*), were over-expressed more intensely and for a longer duration in infected leaves after BTH treatment and could be considered as markers of BTH induction. Correlation of *β*-1,3-glucanase (*PR-2*) and chitinase activities with pathogen resistance has been reported and both are thought to boost defence against downy and powdery mildew (Giannakis *et al.*, 1998).

PR proteins in grapevine appear to be part of the front line of defence due to rapid induction of their genes after infection (Gomes & Coutos-Thevenot, 2009), which continue over time up to 48 hpi. PR proteins also display synergisms, confirming that in most cases, an assortment of PR proteins belonging to diverse subclasses is induced, rather than one single PR protein (Brisset *et al.*, 2000).

BTH also elicited grapevine defences in infected leaves by triggering up-regulation of *GST* coding for an enzyme involved in the redox status of the plant, in accordance with glutathione-S-transferase induction activities that were enhanced by SA (Sappl *et al.*, 2004).

The case of ACC, an enzyme involved in the ethylene pathway produced in most tissues in response to stresses, was more interesting, because *ACC* transcripts were usually down-regulated in *Pv*-infected leaves. However, after BTH treatment in *Pv*-infected leaves, an up-regulation was observed, suggesting that BTH tended to modulate the *ACC* transcripts. Moreover, a proteomic analysis identified ACC after BTH treatment in arctic bramble (Hukkanen *et al.*, 2008), and Jacobs *et al.* (1999) reported the induction of *PR-3* and *PR-2* genes after ethephon treatment, two genes that were also found over-expressed after BTH treatment together with the *ACC* gene. Collective evidence now shows that a coordinated network prevails among SA, jasmonic acid and ethylene signalling pathways engaged in the establishment of resistance against a pathogen, and the possibility that the ethylene pathway is also involved in BTH elicitation seems to be confirmed by this study.

BTH also acted in infected leaves at 24 hpi by down-regulation of aromatic amino acid pathway transcripts (*ANTS, CHORM*) followed by up-regulation later on, indicating an effect of BTH either on the secondary salicylic pathway (isochorismate pathway), on lignol synthesis, or on potential indole alkaloid biosynthesis. It is suggested that the indole pathway might be involved in grapevine plant defence after elicitation. BTH treatment may act either directly on toxic indole alkaloid biosynthesis (tetrahydro-*β*-carbolines in the grapevine; Ali *et al.*, 2010), as does *Arabidopsis thaliana* with camalaxin production, or indirectly by dis-

rupting plant growth and the auxin signals, or yet again by promoting SA biosynthesis through the isochorismate pathway. This effect of BTH and its involvement in grapevine resistance would be worth investigating further.

An inducer such as BTH enhances the resistance of treated tissues associated with the rapid induction of two key enzymes in the phenylpropanoid pathway (PAL) and chalcone isomerase (CHI) (Lawton *et al.*, 1996). However, in the present study in grapevine leaves, *STS* transcripts were up-regulated in both untreated and *En*-infected leaves at 48 and 72 hpi, leading to an increase in total polyphenol content. This demonstrates that the accumulation of these compounds at this quantity was not able to interrupt pathogen growth. Commonly, stilbene levels can discriminate cultivars that are susceptible or resistant to powdery mildew and they have been used as a reliable resistance marker to assess resistance to mildews. However, studies have described resveratrol and piceid as not being toxic, or only slightly, to *E. necator* and *P. viticola*, and then only at high concentrations (Pezet *et al.*, 2004). In the present study, piceid quantification showed similar amounts to those found in grapevine leaves treated with methyl jasmonate or ethephon (Belhadj *et al.*, 2008), but the *trans*-resveratrol amount was three times lower than expected. Moreover, pterostilbene alone was found in high concentrations in BTH-treated leaves (whether inoculated or not), suggesting a role of this compound in growth inhibition of pathogens, as indicated by Slaughter *et al.* (2008) who suggested the involvement of pterostilbene in resistance against downy mildew after induction.

After BTH treatment, *STS* transcripts were slightly up-regulated at 48 hpi, without any increase in total polyphenol, suggesting that the increase in stilbene was delayed. On the other hand, it may be that the high BTH concentration used (1·9 mM) did not promote stilbene biosynthesis, as suggested by Dao *et al.* (2009) in *A. thaliana*, and that metabolization (methoxylation or oligomerization) of resveratrol occurred only in more toxic compounds, such as pterostilbene or viniferin. The low or non-accumulation of total polyphenols and the increase in pterostilbene found in this study suggests the methoxylation of resveratrol or piceid, or the reavailability of stored polyphenols. The early recognition of pathogens and the speed of synthesis or metabolization of stilbenes could be essential in defining the level of resistance against mildews.

Concerning flavonoids which have been described as being induced only in resistant and intermediate-resistant *P. viticola* cultivars (Dai *et al.*, 1995), *CHI* transcripts were rather repressed in time after mildew infections with different isolates, so it is hypothesized that the trend for up-regulation of this gene after BTH treatment in *Pv*-infected leaves may also play a role in inhibiting pathogen growth. In conclusion, the effect of BTH on the phenylpropanoid pathway seems rather complex because it involves up-regulation of key enzymes in plants, and possibly enhancement of flavonoid or specific stilbene accumulations.

The present findings provide insights into the potential use of transcripts and stilbenes as markers of the defence status of grapevine leaves with or without elicitation or infection. Altogether, they indicate that modulation of defence gene regulation is often more marked after inoculation with isolates that are less sensitive to fungicides (*Pv*-R or *En*-B). This could be due to the greater efficacy of grapevine defence on these phenotypes.

The different responses observed in infected leaves according to the isolates could be due to the aggressiveness of isolates and also to the effector-triggered susceptibility of the plant, which reduces its immune response to a resistance level that is insufficient to provide effective protection against the pathogen attack. Indeed, without BTH treatment, the up-regulation of the PR protein genes in infected leaves seemed insufficient, suggesting a threshold of expression necessary to protect the plant that is closely related to the activation of defence mechanisms, as indicated by the induction of defence markers e.g. PR proteins. It is likely that BTH primes over-expression of PR protein transcripts with the production of pterostilbene, and that this could be partially responsible for the efficacy of defence. Understanding the mechanisms and possessing markers of grapevine resistance status will be a prerequisite for developing inducers for integrated pest management in vineyards.

Acknowledgements

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