



HAL
open science

Response of Grapevine Defenses to UV-C Exposure

Alexandra Bonomelli, Laurence Mercier, Jérôme Franchel, Fabienne F. Baillieul, Émile Benizri, Marie-Claude Mauro

► **To cite this version:**

Alexandra Bonomelli, Laurence Mercier, Jérôme Franchel, Fabienne F. Baillieul, Émile Benizri, et al.. Response of Grapevine Defenses to UV-C Exposure. *American Journal of Enology and Viticulture*, 2004, 55 (1), pp.51-59. hal-02148288

HAL Id: hal-02148288

<https://hal.science/hal-02148288v1>

Submitted on 13 Jun 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Response of Grapevine Defenses to UV-C Exposure

Alexandra Bonomelli,¹ Laurence Mercier,¹ Jérôme Franchel,¹ Fabienne Baillieul,²
Emile Benizri,³ and Marie-Claude Mauro^{1*}

¹Laboratoire de Recherches Moët & Chandon, 6 rue Croix de Bussy, 51 200 Epernay, France

²Laboratoire de Biologie et Physiologie Végétales, équipe de Biochimie et Biologie Moléculaire des Plantes, UFR Sciences, URCA, BP 1039, 51 687 Reims 2, France

³Laboratoire Agronomie et Environnement, ENSAIA, 2 avenue de la forêt de Haye, BP 172, 54 500 Vandoeuvre les Nancy, France

*Corresponding author [Email: mmauro@moet.tm.fr]

Acknowledgments: The authors wish to thank Pr. Jean Guern and Dr. Michel Boulay for critical review of the manuscript.

Abstract: The defense potential of a tolerant American *Vitis rupestris* cultivar (Rupestris du Lot) and a susceptible European *Vitis vinifera* cultivar (Chardonnay) in response to UV-C irradiation was investigated. The expression of eight defense-related genes coding for enzymes of the phenylpropanoid pathway (phenylalanine ammonia lyase and stilbene synthase), the octadecanoid pathway (lipoxygenase), and pathogenesis-related proteins (class I and III chitinases, β -1,3-glucanase, class 6 pathogenesis-related protein, and class 10 pathogenesis-related protein) was followed by real-time reverse transcription polymerase chain reaction (RT-PCR). Phenolic compound accumulation was monitored by microscopic observation. Accumulation of resveratrol, a major grapevine phytoalexin, was evaluated by HPLC, and chitinase and β -1,3-glucanase enzyme activities were measured. Both grapevine species responded to UV-C treatment by enhancement of defense mechanisms. Intensity of some defense responses was correlated with tolerance to diseases, as previously described for stilbene accumulation: the tolerant species responded more intensely to UV-C exposure than the susceptible one. UV-C irradiation is a practical and reproducible method for inducing grapevine defense responses and can be useful in determining the defense potential of grapevine cultivars.

Keys words: grapevine, UV-C (200 to 280 nm), defense mechanisms, real-time RT-PCR

Abbreviations: class I chitinase (Chit1b); class III chitinase (CH3); lipoxygenase (LOX); phenylalanine ammonia lyase (PAL); class 6 pathogenesis-related protein (PR-6); class 10 pathogenesis-related protein (PR-10); stilbene synthase (StSy)

Structural and biochemical mechanisms are both involved in active and passive natural defenses of grapevine. Passive defenses include structural characteristics of leaves and fruits (thickness and composition of the cuticle, structure of the epidermis) and preformed antifungal compounds (anthocyanins and other phenolic substances). These passive defenses help to delay the infection process, allowing the establishment of active defense mechanisms such as enhanced lignification, synthesis of antimicrobial compounds, and pathogenesis-related (PR) protein accumulation (Jeandet et al. 1995). Phytoalexins are low molecular weight antimicrobial compounds produced by plants in response to fungal infection or abiotic stresses. In grapevines, stilbene phytoalexins accumulate in leaves and berries subjected to biotic stresses (fungal infection by *Botrytis cinerea*, *Plasmopara viticola*, or *Phomopsis viticola*) or abiotic stresses (UV irradiation, chemical treatments such as aluminium chloride, wounding, and ozone exposure) (Langcake and Pryce 1976, Dercks and Creasy 1989, Jeandet et al. 1995, Adrian et al. 1996, 2000, Douillet-Breuil et al. 1999). Furthermore, nitrogen or potassium fertilizers can influence phytoalexin production (Bavaresco et al. 1994). PR proteins have also been detected in leaves and berries following wounding, exposure to pathogens or elicitors (Busam et al. 1997, Robinson et al. 1997, Derckel et al. 1998, Giannakis et al. 1998, Jacobs et al. 1999, Bézier et al. 2002, Robert et al. 2002), and during berry development (Tat-

tersall et al. 1997, Salzman et al. 1998). Among them, chitinases and β -1,3-glucanases are the most studied proteins.

UV light is an abiotic stress that induces defense-related gene expression, phytoalexins, and PR protein synthesis in tobacco, rice, and *Arabidopsis* (Green and Fluhr 1995, Rakwal et al. 1999, Brosche et al. 2002, Nawrath et al. 2002). In grapevine, UV-C light is known to strongly induce accumulation of resveratrol (a major grape phytoalexin) and viniferins (oligomers of resveratrol) in leaves and berries (Langcake and Pryce 1976, Douillet-Breuil et al. 1999). In addition, the ability of grapevine cultivars to synthesize phytoalexins is correlated with resistance to downy mildew and gray mold (Dercks and Creasy 1989, Douillet-Breuil et al. 1999). However, there are no data available on other grapevine defenses induced following UV-C exposure, and no global comparisons been made between different grapevine species.

The objective of this research was to further characterize defense reactions elicited in grapevine in response to UV-C irradiation. Defense responses were analyzed in two grapevine species with different disease susceptibility: a tolerant *Vitis rupestris* cultivar, Rupestris du Lot, and a susceptible *V. vinifera* cultivar, Chardonnay. Histochemical characteristics and biochemical and molecular properties of some induced defense responses were measured. Accumulation of phenolic

compounds was followed by microscopy. Expression of varied defense-related genes coding for phenylpropanoid synthesis (PAL and StSy), octadecanoid synthesis (LOX), and PR proteins (class I and III chitinases, β -1,3-glucanase, PR-6, and PR-10) was followed by real-time RT-PCR in elicited leaves of Chardonnay and Rupestris du Lot. Chitinase and the β -1,3-glucanase activities and resveratrol accumulation were quantified. The possible involvement of these defense responses in disease resistance is discussed.

Materials and Methods

Plant material. Hardwood cuttings of two *Vitis* species, *Vitis vinifera* cv. Chardonnay and *V. rupestris* cv. Rupestris du Lot, were collected in the vineyard during winter and kept at 4°C. After surface sterilization with Cryptonol (Novartis Agro SA, Rueil-Malmaison, France) at 25 mL/L, cuttings were grown in pots containing a soil (BF-4-fin, Tref, Coevorden, Netherlands)/hydrophobic rock wool mixture (1/1, v/v) in a growth chamber at 23°C with a 16-hr photoperiod. Plants were fertilized twice monthly with a NPK fertilizer solution (28-14-14, Plant Products Company, ON, Canada).

UV-C irradiation. Abiotic stress was achieved by irradiating the underside of detached leaves placed on wet paper in petri dishes with UV-C light (400 μ W/cm²) at 254 nm for 8 min at 9 cm distance. Dishes were then sealed with Parafilm and placed in a growth chamber.

Histochemistry. The autofluorescence of discs (1 cm diam) removed from induced leaves and mounted with the underside up in a lactic acid, glycerol, and water mixture (1/1/1, v/v/v) on glass slides was observed by epifluorescence microscopy (Labophot, Nikon, Champigny sur Marne, France) using a UV filter set (365 nm excitation wavelength and 400 nm barrier filter).

Chitinase and β -1,3-glucanase extraction and quantification. Grapevine chitinases and β -1,3-glucanases were extracted by grinding 150 mg of leaves into 2 mL of 50 mM sodium acetate buffer (pH 5) containing 0.25% Triton-X100 (v/v). This crude extract was centrifuged at 3000 g for 10 min (4°C). The supernatant was either used directly for measurement of chitinase activity, or precipitated with 90% (w/v) ammonium sulfate. The protein pellets were then dissolved in 50 mM sodium acetate buffer (pH 5) and used to assay β -1,3-glucanase activity. Chitinolytic activity was assayed with chitin substrate (CM-chitin-RBV, Loewe Biochemica, Sauerlach, Germany) as described by Derckel et al. (1996). One unit of activity is defined as the hydrolysis of 1 mg of chitin substrate per min per g fresh weight. β -1,3-glucanase activity was measured according to Derckel et al. (1996). An end-product calibration curve was established using glucose as a standard. One unit of β -1,3-glucanase activity is defined as the release of 1 mg sugars per min per g fresh weight. Each time point was determined as the average from data obtained from triplicate and is representative of three independent experiments.

Resveratrol quantification. Leaves were ground in liquid nitrogen and resveratrol was extracted with 1 mL methanol per 100 mg fresh weight. Cell fragments were removed by centrifugation (10 min, 4°C, 12,000 g) and the supernatant was mixed with water (1/9, v/v). In order to remove chlorophylls, the supernatant was passed through a Sep-Pack C-18 cartridge (Waters, St Quentin en Yvelines, France). After elution with methanol 90% (v/v), the sample was passed through a 0.22- μ m filter. Resveratrol was quantified with an HPLC system (Thermo Separation Products, Les Ulis, France) coupled to an automatic injector, as described by Coutos-Thévenot et al. (2001). Results were expressed as μ g resveratrol per g dry weight. Each time point was the average from data obtained from triplicate and is representative of three independent experiments.

RNA extraction. Total RNA was extracted from 200 mg of greenhouse leaves. Leaves were ground in liquid nitrogen to a fine powder and homogenized in extraction buffer (0.1 M Tris-HCl pH 8, 1% SDS, 0.1 M LiCl, 0.01 M EDTA). The suspension was purified by two phenol/chloroform/isoamyl alcohol (25/24/1, v/v/v) extractions, and the RNA was then purified by a LiCl (2 M final) differential precipitation. After centrifugation, the RNA pellet was resuspended in 20 μ L of RNase-free water and quantified by absorbance at 260 nm. Messenger RNA was extracted with magnetic beads (Dynal, Compiègne, France) according to the manufacturer's instructions.

Real-time RT-PCR analysis. Reverse transcriptions were done in a volume of 20 μ L consisting of 1 μ g mRNA, 5 μ M random hexamer, 5 μ M DTT, 0.5 mM dNTPs, 10 units/ μ L reverse transcriptase (Superscript II, Gibco, Cergy Pontoise, France), 1x buffer, and 2 units/ μ L of RNase inhibitor. RNase inhibitor, reverse transcriptase, DTT, and 1x buffer were added after the samples were held at 65°C for 10 min. Synthesis of cDNA fragments was then performed at 30°C for 10 min, 42°C for 50 min, and 95°C for 5 min on a Mastercycler gradient thermocycler (Eppendorf, Le Pecq, France).

PCR reactions were carried out in 96-well plates (25 μ L per well) in a reaction buffer containing 1x SYBR Green (Applied Biosystems, Courteboeuf, France) (including Taq polymerase, dNTPs, MgCl₂, and SYBR Green dye), 0.3 μ M primers, and cDNA. PCR conditions were 95°C for 15 sec (denaturation) and 60°C for 1 min (annealing/extension) for 40 cycles on a GeneAmp 5700 sequence detection system (Applied Biosystems). To distinguish the specific amplicons from nonspecific PCR products and primer dimers, melting analysis was done immediately after PCR by heating PCR products from 60 to 95°C.

Grapevine actin (ACT) (Bézier et al. 2002) was used as the internal positive control. Expression of eight defense-related genes was tracked (see Table 1): genes coding for enzymes of the phenylpropanoid pathway, phenylalanine ammonia lyase (PAL) and stilbene synthase (StSy) (primers were defined according to gene sequences published by Sparvoli et al. 1994); genes coding for an enzyme of the

Table 1 Genes analyzed by real-time RT-PCR.

Gene	Primer sequence	Amplicon (base pair)	Accession number
Actin (ACT)	Sense 5' AGC TGG AAA CTG CAA AGA GCA G 3' Antisense 5' ACA ACG GAA TCT CTC AGC TCC A 3'	94	AF369524
Phenylalanine ammonia lyase (PAL)	Sense 5' TCC TCC CGG AAA ACA GCT G 3' Antisense 5' TCC TCC AAA TGC CTC AAA TCA 3'	100	X75967
Stilbene synthase (StSy)	Sense 5' AGG AAG CAG CAT TGA AGG CTC 3' Antisense 5' TGC ACC AGG CAT TTC TAC ACC 3'	100	X76892
Lipoxygenase (LOX)	Sense 5' CTG GGT GGC TTC TGC TCT C 3' Antisense 5' GAT AAG CCG CAG ATT CAT GC 3'	98	AY159556
Class I basic chitinase (Chit1b)	Sense 5' ATG CTG CAG CAA GTT TGG TT 3' Antisense 5' CAT CCT CCT GTG ATG ACA TT 3'	609	Z54234
Class III chitinase (CH3)	Sense 5' AGA TGG CAT AGA CTT CGA CA 3' Antisense 5' GTA CTT TGA CCA CAG CAT CA 3'	427	AJ291507
β -1,3-glucanase (GLUC)	Sense 5' AAT TTG ATC CGC CAC GTC AA 3' Antisense 5' TGC GGC TCC TTC TTG TTC TC 3'	100	AF239617
Class 6 PR protein (PR-6)	Sense 5' AGT TCA GGG AGA GGT TGC TG 3' Antisense 5' GCA CTA GGG TCC GTG TTT GGG TCG ACG 3'	131	AY156047
Class 10 PR protein (PR-10)	Sense 5' ACC ACA CCA AGG GCG ATG TA 3' Antisense 5' CGT AGG CTT CGA TAG CCT TGA A 3'	100	AJ291705

octadecanoid pathway, lipoxygenase (LOX) (A. Bézier 2002, personal communication); genes coding for PR proteins, class I and III chitinases (Chit1b and CH3), β -1,3-glucanase, class 6 pathogenesis-related protein (PR-6) (A. Bézier 2002, personal communication), and class 10 pathogenesis-related protein (PR-10) (primers were defined according to the gene sequence published by Robert et al. 2001).

Data analysis. Quantification was achieved according to Bézier et al. (2002) by comparison with pure standards (Qiaquick Purification Kit, Qiagen, Courtaboeuf, France). Transcript level was calculated using the standard curve method (User's Manual, Abi Prism 7700 Sequence Detection System, Applied Biosystems). Standard curves were generated by performing real-time PCR on dilutions of specific purified DNA, which consisted of a PCR product amplified from genomic DNA. The copy number of these standards was calculated from concentration, measured by absorbance at 260 nm. Standard curves were constructed by plotting the Ct (Ct, or threshold cycle, is defined as the PCR cycle number at which fluorescence increases over background levels) versus the logarithm of the copy number of specific PCR products. The absolute copy number for each sample was calculated from standard curves using their Ct value. A corrected copy number was then calculated for each sample by normalization against grapevine actin as an endogenous control and untreated leaves as a reference sample. Subsequently, for each gene, the reference sample was referred to as the 1x expression level and results were expressed as the fold increase of mRNA level over the reference sample. Relative gene expression was obtained with the following formula: (gi mRNA copy number of sample/

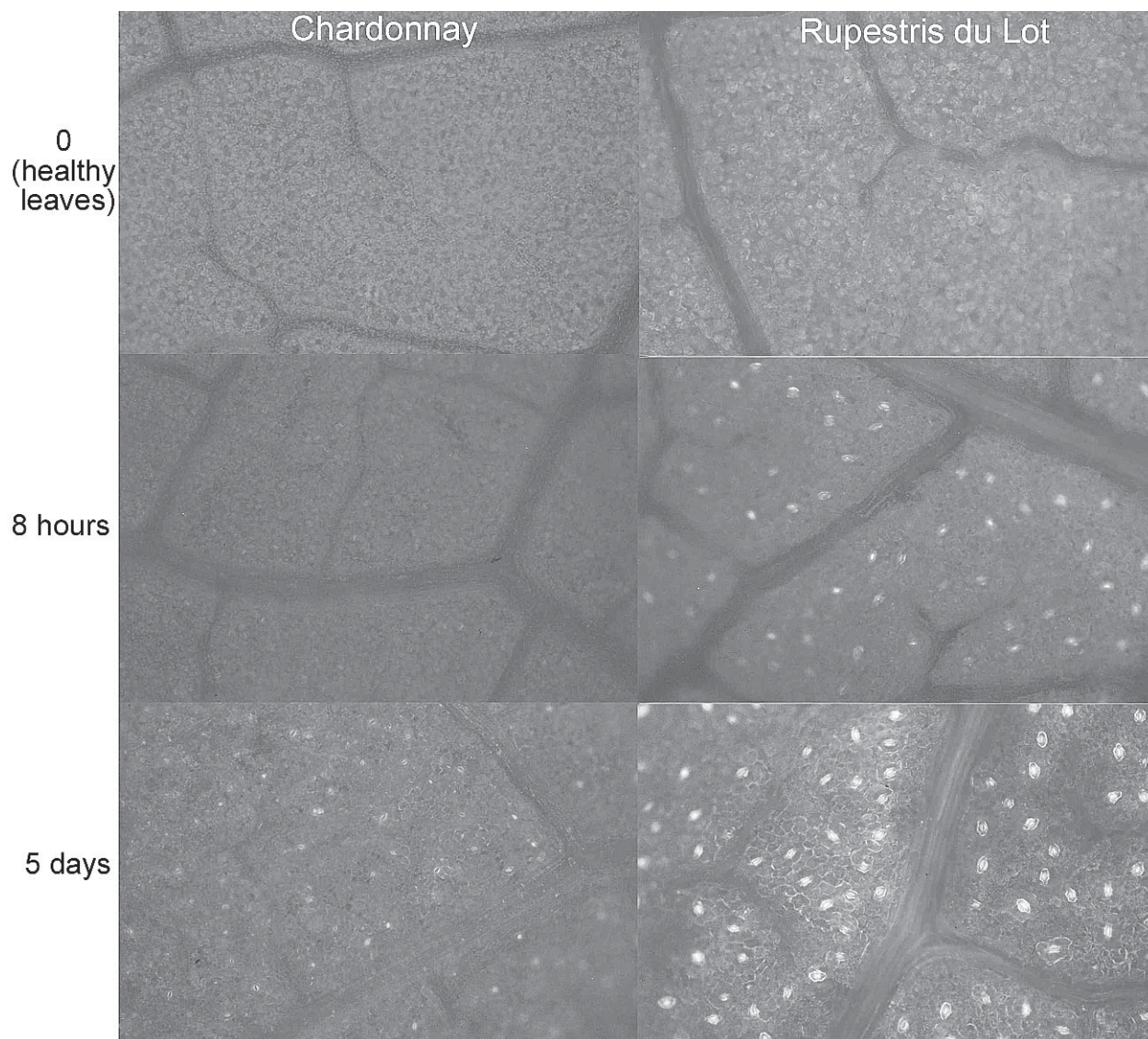
ACT mRNA copy number of sample)* ACT mRNA copy number of reference sample, where gi is the gene of interest (defense-related gene) and ACT is the actin grapevine gene used as an internal control. The reference sample was chosen to represent 1x expression of the gene of interest (such as untreated leaves). Each time point was determined as an average from data obtained from duplicate and is representative of two independent experiments.

Results

Phenolic compound accumulation. Exposure to UV-C light induced phenolic compound accumulation, particularly in the tolerant species. Leaf discs of healthy or UV-treated Chardonnay and Rupestris du Lot leaves were observed by microscopy under UV light. Healthy Rupestris du Lot leaves emitted a blue fluorescence located in stomata and veins, which indicated the constitutive presence of phenolic compounds. In contrast, few autofluorescent stomata cells were observed in healthy Chardonnay leaves (time 0, Figure 1). No changes in fluorescence over time were observed in detached leaves. As soon as 8 hr after exposure to UV-C light, additional blue autofluorescence was detected in stomata and cell walls of Rupestris du Lot leaves. The fluorescence intensity increased over at least 5 days. Cell wall thickening was also observed (Figure 1). In Chardonnay leaves, the same phenomenon was observed, but autofluorescence intensity was weaker (Figure 1).

Resveratrol accumulation. UV-C light induced resveratrol accumulation in leaves of both cultivars, with different time course and level of accumulation. Resveratrol accumulation

Figure 1 UV-treated leaves of Rupestris du Lot and Chardonnay. The underside of detached *V. vinifera* cv. Chardonnay or *V. rupestris* cv. Rupestris du lot leaves placed on wet paper in petri dishes was irradiated by UV light at 254 nm for 8 min at a distance of 9 cm. The undersurface was then observed under UV light (365 nm) (x 100).



was monitored by HPLC in detached control leaves and UV-exposed leaves of Chardonnay and Rupestris du Lot. Healthy Chardonnay and Rupestris du Lot leaves did not produce detectable levels of resveratrol (time 0, data not shown). Traces of resveratrol were measured in detached control leaves over time. After UV-C treatment, leaves of both cultivars accumulated resveratrol at high levels (almost 400 $\mu\text{g/g}$ dry weight for Chardonnay and 800 $\mu\text{g/g}$ dry weight for Rupestris du Lot), with maximum accumulation during the first day after elicitation. However, resveratrol production in Chardonnay leaves was transient (between 8 hr and 3 days after exposure to UV-C light), whereas elevated concentrations of resveratrol were found in Rupestris du Lot leaves for at least 6 days (Figure 2).

Chitinase and β -1,3-glucanase activities. UV-C treatment induced chitinase and β -1,3-glucanase activities in

grapevine. Production of chitinases and β -1,3-glucanases were followed through enzymatic activity measurements in detached controls and UV-treated leaves. A basal constitutive level of β -1,3-glucanase activity was detected in Rupestris du Lot leaves (time 0, data not shown). The β -1,3-glucanase activity in Chardonnay leaves and chitinase activity in leaves of both species were very low (time 0, data not shown) and did not increase over time in detached leaves. A time-course study of chitinase and β -1,3-glucanase activities revealed that the PR proteins are elicited by UV-C light in both species (Figures 3 and 4). Chitinase and β -1,3-glucanase activities followed the same induction pattern in the two species: activity increased as soon as 1 day after UV-C exposure and accumulated for at least 6 days. β -1,3-glucanase activity was greater in Rupestris du Lot leaves (1.5-fold) while chitinase activity was about 2-fold greater in Chardonnay leaves.

Figure 2 Resveratrol accumulation in UV-treated leaves of Chardonnay (A) and Rupestris du Lot (B). Control and UV-exposed leaves were ground in liquid nitrogen, resveratrol was then extracted and quantified by HPLC. Results represent three independent experiments. Vertical bars indicate standard errors.

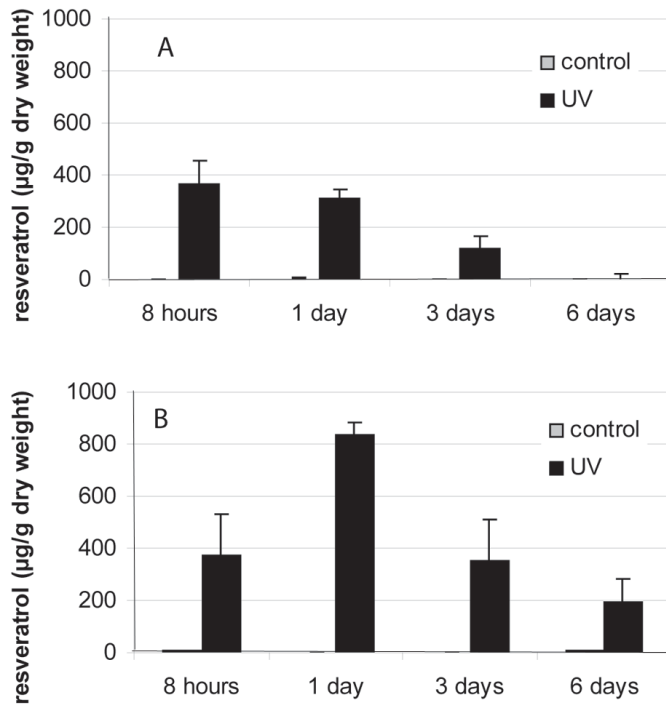
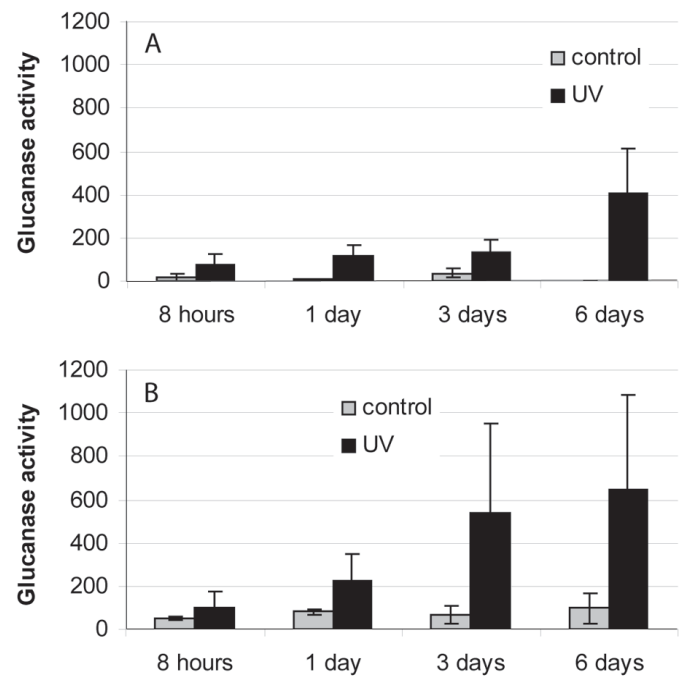


Figure 3 Induction of β -1,3-glucanase activity in UV-treated leaves of Chardonnay (A) and Rupestris du Lot (B). Control and UV-exposed leaves were ground in liquid nitrogen and β -1,3-glucanase activity was estimated. One unit of β -1,3-glucanase activity is defined as the release of 1 mg sugars per min per g fresh weight. Results represent three independent experiments. Vertical bars indicate standard errors.



Expression of defense-related genes. Expression of eight defense-related genes coding for enzymes of the phenylpropanoid pathway (PAL and StSy), the octadecanoid pathway (LOX), and PR proteins (an acidic chitinase, CH3, a basic chitinase, Chit1b, a β -1,3-glucanase, a PR-6, and a PR-10) was then analyzed in leaves of Chardonnay and Rupestris du Lot in response to UV-C exposure. Induction of these genes was monitored by real-time RT-PCR. Grapevine actin gene expression was used as an internal control. Values obtained for untreated leaves of Chardonnay were chosen to represent 1x expression of the different genes. Gene expression is considered enhanced when induction is over 5x that of untreated leaves.

A slight induction of expression was detected for all genes in detached control leaves of Chardonnay and Rupestris du Lot (C15, C24, and C48). Maintaining detached leaves on wet paper in petri dishes for 3 days seemed to cause weak stress. In UV-treated leaves (UV15, UV24, and UV48), expression of all defense-related genes was greatly enhanced as soon as 15 hr after exposure. Chit1b gene was induced only in Rupestris du Lot leaves. PR-6 gene expression was delayed and reached a maximum only 48 hr after elicitation. (Figure 5). Differences in induction between susceptible and tolerant species were observed. The increase of PAL, StSy, and PR-10 gene expression seemed to be equivalent between the two cultivars, while β -1,3-glucanase, CH3, and PR-6 appeared to be slightly more induced in Rupestris du Lot leaves. Furthermore, expression of LOX and Chit1b was clearly higher in Rupestris du Lot than in Chardonnay.

Figure 4 Induction of chitinase activity in UV-treated leaves of Chardonnay (A) and Rupestris du Lot (B). Control and UV-exposed leaves were ground in liquid nitrogen and chitinase activity was estimated. One unit of chitinase activity is defined as the hydrolysis of 1 mg chitin substrate per min per g fresh weight. Results represent three independent experiments. Vertical bars indicate standard errors.

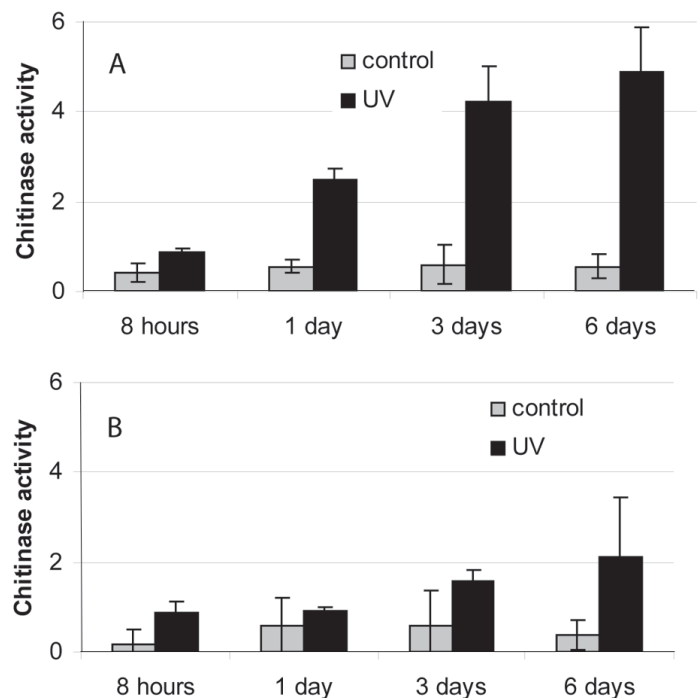
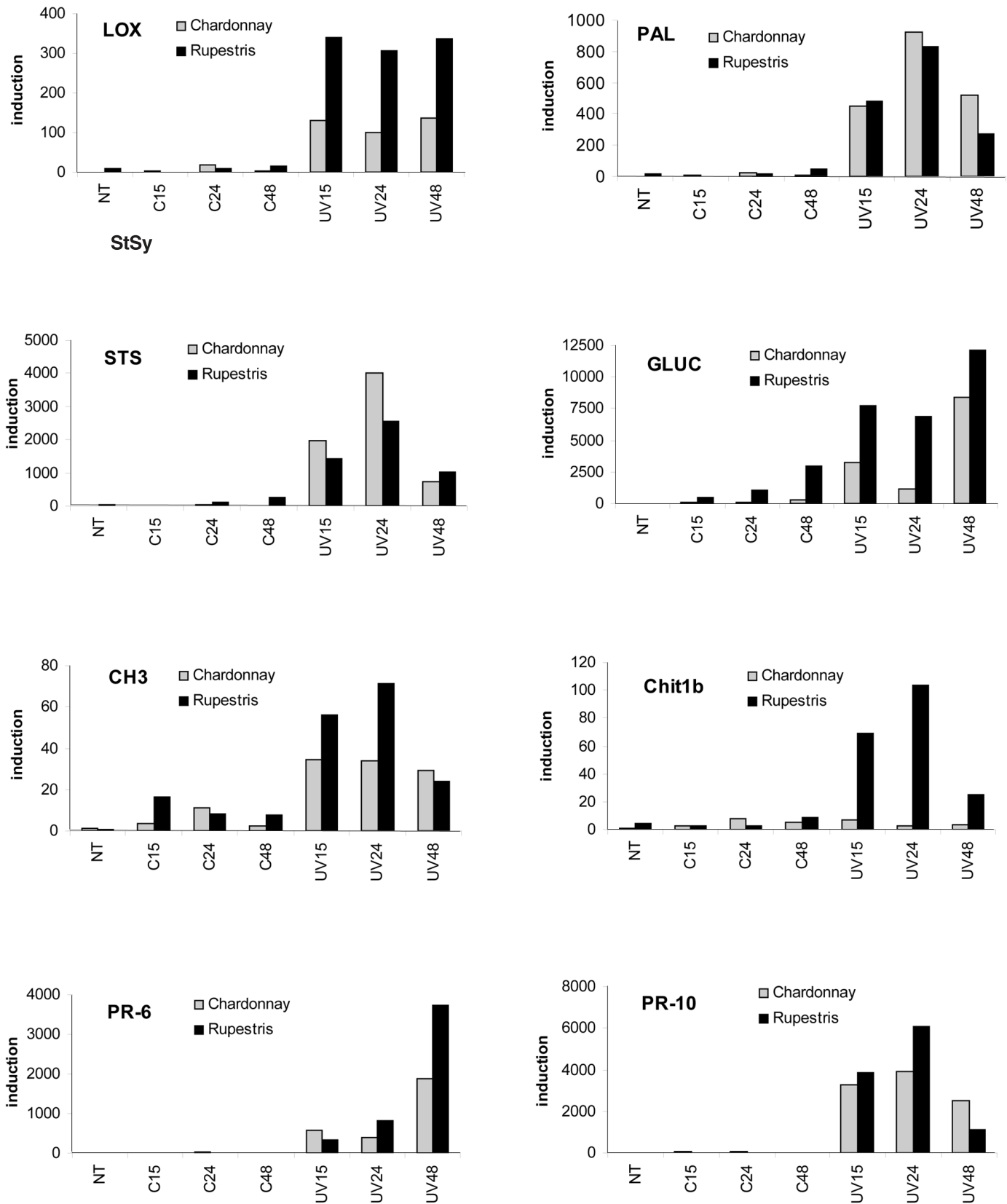


Figure 5 Defense-related gene expression in UV-treated leaves of Chardonnay and Rupestris du Lot: lipoxygenase (LOX), phenylalanine ammonia lyase (PAL), stilbene synthase (StSy), β -1,3-glucanase (GLUC), acidic chitinase (CH3), basic chitinase (Chit1b), PR-6, and PR-10. Control (C) and UV-exposed (UV) leaves were harvested 15, 24, and 48 hr after treatment, ground in liquid nitrogen, and gene expression was determined by real-time RT-PCR on cDNA samples. Actin gene expression was used as internal control and untreated Chardonnay leaves (NT) were defined as 1x expression level for each analyzed gene. Results represent two independent experiments.



Discussion

In grapevine, UV-C exposure was only known to elicit stilbene phytoalexin accumulation (Langcake and Pryce 1976, Adrian et al. 2000). Varied defense responses (gene expression, enzymatic activities) have previously been characterized in grapevine in response to diverse biotic and abiotic stresses, but their induction has not been studied in leaves exposed to UV-C light. No global comparison of varied grapevine defense responses had been made between grapevine species of different disease resistance.

This study demonstrated that both *Vitis* species respond to UV-C exposure by enhancement of a set of defense responses. First, phytoalexin accumulation was confirmed at the histochemical, molecular, and biochemical levels. Genes coding for enzymes of the phenylpropanoid pathway (PAL and StSy) were highly induced. As PAL and StSy are members of small gene families, we can assert that transcript accumulation is specific to their respective gene families, but it is unclear whether amplification is specific to one or several genes of each family. UV light has been described as an inducer of enzymes of the general phenylpropanoid pathway, and particularly of enzymes such as phenylalanine ammonia lyase, chalcone synthase and stilbene synthase (Fritzemeier and Kindl 1981, Sparvoli et al. 1994). PAL and StSy gene expression is also elicited in grapevine leaves infected with *Botrytis cinerea* (Bézier et al. 2002), and StSy transcripts accumulated in response to *Pseudomonas syringae* pv. *pisi*, a bacterium that induces an incompatible interaction (Robert et al. 2001). As a consequence of StSy mRNA accumulation, resveratrol accumulated in UV-exposed leaves of both cultivars, as observed by microscopic observations and HPLC analysis. The blue fluorescence observed in veins, stomata, and cell walls of elicited leaves is probably due, at least in part, to stilbenes. Resveratrol show bright blue fluorescence when examined under long wavelength UV light (Langcake and Pryce, 1976). Phytoalexin accumulation is inducible by numerous biotic and abiotic stresses (Langcake and Pryce 1976, Dercks and Creasy 1989, Jeandet et al. 1995, Adrian et al. 1996, 2000, Douillet-Breuil et al. 1999).

We have demonstrated for the first time that UV-C exposure induces numerous and varied defense responses in grapevine leaves. Lipoxygenase (LOX), an enzyme of the octadecanoid pathway, was strongly induced in both cultivars. LOX is a 9-lipoxygenase-type enzyme induced by elicitor application, wounding, or pathogen attack in potato and tobacco (Rusterucci et al. 1999, Gobel et al. 2001). This gene is also induced in grapevine leaves infected with *Botrytis cinerea* (A. Bézier 2002, personal communication). Genes encoding PR proteins (class I and III chitinases, β -1,3-glucanase, PR-6, and PR-10) were also elicited in both *Vitis* species by UV-C exposure; Chit1b was not induced in Chardonnay leaves, however. CH3 was induced in both cultivars, whereas Chit1b was only elicited in Rupestris du Lot leaves. Grapevine chitinases are inducible by various biotic stresses and are known to be differentially induced accord-

ing to the stress applied, the organ studied, and the grapevine cultivar (Derckel et al. 1996, Busam et al. 1997, Jacobs et al. 1999, Robert et al. 2002). PR-6 transcripts accumulated in response to *Botrytis cinerea* infection (A. Bézier 2002, personal communication), and the PR-10 gene is induced in grapevine during the incompatible interaction with *Pseudomonas syringae* pv. *pisi* (Robert et al. 2001). Chitinase and β -1,3-glucanase activities were induced in the two cultivars by UV-C light. Enhanced enzymatic activities and/or protein accumulation has been described in grapevine leaves in response to biotic stresses such as *Botrytis cinerea*, *Uncinula necator*, and *Plasmopara viticola* infection (Derckel et al. 1996, Giannakis et al. 1998).

Induction of multiple defense responses in UV-exposed grapevine leaves is not surprising, as UV light has previously been shown to induce synthesis of phytoalexins and PR proteins in tobacco, rice, and *Arabidopsis* (Brederode et al. 1991, Green and Fluhr 1995, Brosche et al. 2002). Nawrath et al. (2002) recently showed that in *Arabidopsis*, signal-transduction pathways after UV light exposure and pathogen inoculation share common elements. However, several distinct transduction pathways are activated according to the stress applied (such as elicitors, necrotrophic or biotrophic pathogens), leading to the activation of different defense mechanisms (Thomma et al. 2001).

A few previous studies have examined differences in defense responses between cultivars of different disease resistance in response to pathogen inoculation. However, no such study has used an abiotic stress such as UV exposure. In our study, moderated differences were observed between the two *Vitis* species in response to UV-C exposure.

First, differences were noticed in healthy leaves. The tolerant species showed weak blue fluorescence in stomata and veins and a basal β -1,3-glucanase activity was measured. The nature, intensity, and accumulation of induced defense reactions also differed.

Phenolic compound accumulation (probably of stilbene) was higher in Rupestris du Lot leaves, as seen in microscopic observations. Furthermore, stilbene localization was different: blue fluorescence was observed in stomata and veins in both cultivars, while cell wall thickening was observed only in the tolerant cultivar leaves. Histochemical studies have previously shown that nature, localization, intensity, and accumulation of phenolic compounds (flavonoids, lignin, stilbenes) are correlated with susceptibility to downy mildew (Dai et al. 1995). In our study, resveratrol accumulation was low and transient in Chardonnay, while higher concentrations were found over a prolonged period in Rupestris du Lot. Studies have demonstrated that phytoalexin accumulation has been correlated with tolerance to gray mold (Douillet-Breuil et al. 1999) and downy mildew (Dercks and Creasy 1989, Dai et al. 1995) and was higher in American *Vitis* species. PAL and StSy transcript induction is equivalent between the two grapevine species, while resveratrol accumulation differs in intensity and timing.

Resveratrol may be differentially metabolized in the two species and is probably more rapidly glycosylated to piceid and/or oxidized into dimers and oligomers (viniferins) in Chardonnay (Douillet-Breuil et al. 1999).

PR proteins were also differentially induced. β -1,3-glucanase transcript accumulation and enzymatic activity were greater in Rupestris du Lot. The expression level of an acidic chitinase gene (CH3) was also greater in the tolerant species, and transcripts of a basic chitinase gene (Chit1b) increased in Rupestris du Lot only. In contrast, chitinase activity was greater in Chardonnay. We followed acidic chitinase activity and analyzed one acidic and one basic chitinase gene. These genes may not be representative of the activity measured, as a multitude of chitinase isoforms (Derckel et al. 1996) and genes (Jacobs et al. 1999, Bézier et al. 2002, Robert et al. 2002) have been characterized in grapevine and have been shown to be constitutive and/or inducible. However, as we observed for β -1,3-glucanase enzymatic activity, correlations have previously been established among chitinase, peroxidase, and β -1,3-glucanase enzymatic activities and tolerance to downy and powdery mildews (Giannakis et al. 1998, Reuveni 1998). Finally, LOX and PR-6 transcripts accumulate in the tolerant cultivar leaves. 9-Lipoxygenase is involved in the development of cryptogeiin-induced hypersensitive cell death in tobacco (Rusterucci et al. 1999) and may be implicated in grapevine disease tolerance. PR-6 are protease inhibitors that may inhibit pathogen proteases. These defense responses may play an important role in disease resistance and defense against pathogens.

As some differences were observed between species in response to abiotic stress, it would be interesting to conduct a similar study of defense responses after pathogen inoculation and to develop techniques to increase the defense potential of Chardonnay.

Conclusion

The study of numerous defense reactions demonstrated that grapevine responded to UV-C treatment by enhancement of defense responses. The nature, intensity, and time-course accumulation of some defense responses were correlated with disease tolerance. UV irradiation is a practical and reproducible tool for inducing grapevine defense mechanisms. Furthermore, it can be a useful indicator for determining the defense potential of grapevine cultivars.

Literature Cited

- Adrian, M., P. Jeandet, R. Bessis, and J.M. Joubert. 1996. Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminium chloride (AlCl₃). *J. Agric. Food Chem.* 44(8):1979-1981.
- Adrian, M., P. Jeandet, A.C. Douillet-Breuil, L. Tesson, and R. Bessis. 2000. Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. *J. Agric. Food Chem.* 48(12):6103-6105.
- Bavaresco, L., M. Fregoni, and D. Petegolli. 1994. Effect of nitrogen and potassium fertilizer on induced resveratrol synthesis in two grapevine genotypes. *Vitis* 33:175-176.
- Bézier, A., B. Lambert, and F. Baillieul. 2002. Study of defense-related gene expression in grapevine leaves and berries infected with *Botrytis cinerea*. *Eur. J. Plant Pathol.* 108:111-120.
- Brederode, F.T., H.J.M. Linthorst, and B.J.C. Cornelissen. 1991. Differential induction of acquired resistance and PR gene expression in tobacco by virus infection, ethephon treatment, UV light and wounding. *Plant Mol. Biol.* 17:1117-1125.
- Brosche, M., M.A. Schuler, I. Kalbina, L. Connor, and A. Strid. 2002. Gene regulation by low level UV-B radiation: Identification by DNA array analysis. *Photochem. Photobiol.* 1(9):656-664.
- Busam, G., H.H. Kassemeyer, and U. Matern. 1997. Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. *Plant Physiol.* 115:1029-1038.
- Coutos-Thévenot, P., B. Poinssot, A. Bonomelli, H. Yean, C. Breda, D. Buffard, R. Esnault, R. Hain, and M. Boulay. 2001. *In vitro* tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase *vsr1* gene under the control of a pathogen-inducible promoter. *J. Exp. Bot.* 52(358):901-910.
- Dai, G.H., C. Andary, L. Mondolot-Cosson, and D. Boubals. 1995. Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera*, *V. rupestris* and *V. rotundifolia* and the downy mildew fungus, *Plasmopara viticola*. *Physiol. Mol. Plant Pathol.* 46(3):177-188.
- Derckel, J.P., L. Legendre, J.C. Audran, B. Haye, and B. Lambert. 1996. Chitinases of grapevine (*Vitis vinifera* L.): Five isoforms induced in leaves by salicylic acid are constitutively expressed in other tissues. *Plant Sci.* 119:31-37.
- Derckel, J.P., J.C. Audran, B. Haye, B. Lambert, and L. Legendre. 1998. Characterization, induction by wounding and salicylic acid, and activity against *Botrytis cinerea* of chitinases and β -1,3 glucanases of ripening grape berries. *Physiol. Plant.* 104:56-64.
- Dercks, W., and L.L. Creasy. 1989. The significance of stilbene phytoalexins in the *Plasmopara viticola*-grapevine interaction. *Physiol. Mol. Plant Pathol.* 34:189-202.
- Douillet-Breuil, A.C., P. Jeandet, M. Adrian, and R. Bessis. 1999. Changes in the phytoalexin content of various *Vitis* spp. in response to ultraviolet C elicitation. *J. Agric. Food Chem.* 47:4456-4461.
- Fritzemeier, K.H., and H. Kindl. 1981. Coordinate induction by UV light of stilbene synthase, phenylalanine ammonia lyase and cinnamate 4-hydroxylase in leaves of Vitaceae. *Planta* 151:48-52.
- Giannakis, C., C.S. Bucheli, K.G.M. Skene, S.P. Robinson, and N. Steele Scott. 1998. Chitinase and β -1,3-glucanase in grapevine leaves: A possible defense against powdery mildew infection. *Aust. J. Grape Wine Res.* 4:14-22.
- Gobel, C., I. Feussner, A. Schmidt, D. Scheel, J. Sanchez-Serrano, M. Hamberg, and S. Rosahl. 2001. Oxylinin profiling reveals the preferential stimulation of the 9-lipoxygenase pathway in elicitor treated potato cells. *J. Biol. Chem.* 276(9):6267-6273.
- Green, R., and R. Fluhr. 1995. UV-B induced PR-1 accumulation is mediated by active oxygen species. *Plant Cell* 7:203-212.
- Jacobs, A.K., I.B. Dry, and S.P. Robinson. 1999. Induction of different pathogenesis-related cDNAs in grapevine infected with powdery mildew and treated with ethephon. *Plant Pathol.* 48:325-336.

- Jeandet, P., R. Bessis, M. Sbaghi, and P. Meunier. 1995. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *J. Phytopathol.* 143:135-139.
- Langcake, P., and R.J. Pryce. 1976. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* 9:77-86.
- Nawrath, C., S. Heck, N. Parinthewong, and J.P. Métraux. 2002. EDS5, an essential component of salicylic acid-dependent signalling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. *Plant Cell* 14:275-286.
- Rakwal, R., G.K. Agrawal, and M. Yonekura. 1999. Separation of proteins from stressed rice (*Oriza sativa* L.) leaf tissues by two-dimensional polyacrylamide gel electrophoresis: Induction of pathogenesis-related and cellular protectant proteins by jasmonic acid, UV irradiation and copper chloride. *Electrophoresis* 20:3472-3478.
- Reuveni, M. 1998. Relationships between leaf age, peroxidase and β -1,3-glucanase activity, and resistance to downy mildew in grapevines. *J. Phytopathol.* 146:525-530.
- Robert, N., J. Ferran, C. Breda, P. Coutos-Thévenot, M. Boulay, D. Buffard, and R. Esnault. 2001. Molecular characterization of the incompatible interaction of *Vitis vinifera* leaves with *Pseudomonas syringae* pv. *pti*: Expression of genes coding for stilbene synthase and class 10 PR protein. *Eur. J. Plant Pathol.* 107:249-261.
- Robert, N., K. Roche, Y. Lebeau, C. Breda, M. Boulay, R. Esnault, and D. Buffard. 2002. Expression of grapevine chitinase genes in berries and leaves infected by fungal or bacterial pathogens. *Plant Sci.* 162:389-400.
- Robinson, S.P., A.K. Jacobs, and I.A. Dry. 1997. A class IV chitinase is highly expressed in grape berries during ripening. *Plant Physiol.* 114:771-778.
- Rusterucci, C., J.L. Montillet, J.P. Agnel, C. Battesti, B. Alonso, A. Knolls, J.J. Bessoule, P. Etienne, L. Sully, J.P. Blein, and C. Triantaphyllides. 1999. Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogin in tobacco leaves. *J. Biol. Chem.* 274:36446-36455.
- Salzman, R.A., I. Tikhonova, B.P. Bordelon, P.M. Hasegawa, and R.A. Bressan. 1998. Coordinate accumulation of antifungal proteins and hexoses constitutes a developmentally controlled defense response during fruit ripening in grape. *Plant Physiol.* 117:465-472.
- Sparvoli, F., C. Martin, A. Scienza, G. Gavazzi, and C. Tonelli. 1994. Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). *Plant Mol. Biol.* 24:743-755.
- Tattersall, D.B., R. Van Heeswijck, and P.B. Hoj. 1997. Identification and characterization of a fruit-specific thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. *Plant Physiol.* 114:759-769.
- Thomma, B.P.H.J., K.F.M. Tierens, I.A.M.A. Penninckx, B. Mauch-Mani, W.F. Broekaert, and B.P.A. Cammue. 2001. Different micro-organisms differentially induce *Arabidopsis* disease response pathways. *Plant Physiol. Biochem.* 39:673-680.