JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Methyl Jasmonate Induces Defense Responses in Grapevine and Triggers Protection against *Erysiphe necator*

ASSIA BELHADJ,† CASSANDRINE SAIGNE,† NADÈGE TELEF,† STÉPHANIE CLUZET,† JÉRÔME BOUSCAUT,‡ MARIE-FRANCE CORIO-COSTET,‡ AND JEAN-MICHEL MÉRILLON*,†

Laboratoire de Mycologie et Biotechnologie Végétale, EA 3675, Université Victor Segalen Bordeaux 2, UFR Sciences Pharmaceutiques, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France, and UMR Santé Végétale, INRA/ENITAB, 71 Avenue Edouard Bourleaux, BP 81, 33883 Villenave d'Ornon Cedex, France

Grapevine is subject to a number of diseases that affect yield and wine quality. To limit the excessive use of phytochemicals in the vineyard, alternative strategies have to be developed. Plant treatment with signaling molecules like elicitors stimulates their natural defense mechanisms. To improve grapevine tolerance against fungal pathogens, *Vitis vinifera* plants were treated with a natural exogenous elicitor, methyl jasmonate (MeJA). MeJA-treated leaves (Cabernet Sauvignon foliar cuttings) reacted by increasing transcript levels coding pathogenesis-related proteins (acidic class IV chitinase, serine protease inhibitor, polygalacturonase-inhibiting protein, and β-1,3-glucanase) and coding enzymes involved in phytoalexin biosynthesis (one phenylalanine ammonia lyase and one stilbene synthase). This was correlated with the accumulation of stilbenes (antimicrobial compounds). The eliciting activity of MeJA was confirmed by enhanced tolerance of grapevine foliar cuttings and vineyard against powdery mildew (75% and 73%, respectively). On the basis of these original results, MeJA can therefore act as an efficient elicitor in an alternative strategy of grapevine protection.

KEYWORDS: Elicitor; powdery mildew; stilbenes; PR proteins; *Vitis vinifera*

INTRODUCTION

Fungal diseases are a major problem in grapevine cultivation around the world. Grapevine (*Vitis vinifera* L.) is susceptible to many fungi, such as *Botrytis cinerea* (gray mold), *Plasmopara viticola* (downy mildew), *Erysiphe necator* (powdery mildew), and *Eutypa lata* (dieback). Fungal infection reduces fruit quality and yield, either by direct infection of berries themselves or through a reduction in plant vigor. Disease control is currently achieved by intensive use of fungicides. Although these chemicals are relatively effective when applied as part of a strategic spray program, the cost to the grower and the environmental impact of the residues remain undesirable. Alternative strategies involving genetic manipulation of host defense mechanisms have been shown to increase the resistance of a number of crop species to fungal pathogens (*1*−*4*), but such practices are forbidden in French vineyards (*1*).

Another approach consists of the induction of natural plant defenses by using elicitors. A variety of molecules can act as elicitors, including oligo- and polysaccharides, peptides, proteins, and lipids (*5*, *6*). Elicitor perception triggers various signaling pathways: ion fluxes, oxidative burst, and synthesis of signal molecules such as salicylic acid, jasmonic acid, and ethylene. Defense-related genes are induced leading to reinforcement of plant cell walls, accumulation of antimicrobial compounds such as phytoalexins, and synthesis of proteins with hydrolytic or inhibitory activity toward microbes (*7*).

Owing to their involvement in the signal transduction cascade leading to defense responses, jasmonic acid (JA) and its more active derivative, methyl jasmonate (MeJA), have been used as inducers of defense mechanisms in a number of systems (*8*).

Thus, jasmonates induce genes encoding pathogenesis-related (PR) proteins such as chitinases and β-1,3-glucanases in tobacco and tomato (*9*−*11*). They also stimulate proteinase inhibitor gene expression in tobacco (*12*). JA induces the production of a wide range of plant secondary metabolites, such as alkaloids, terpenoids, flavonoids, phenolic compounds, and phytoalexins in various cell cultures (*Taxus cuspidata*, *Rubia tinctorium*, *Glycine max*, etc.) and in Arabidopsis plants (*13*−*16*).

In grapevine, MeJA has been shown to stimulate deposition of callose and the accumulation of PR-proteins and to induce production of salicylic acid in leaves and in suspension-cultured cells (*17*, *18*).

The present study sought to determine whether exogenous

* Author to whom correspondence should be addressed. Tel.: +33 5 57 57 46 89. Fax: +33 5 57 57 46 88. E-mail: jean-michel.merillon@phyto.u-bordeaux2.fr.
† Université Victor Segalen Bordeaux 2.
‡ UMR Santé Végétale.

10.1021/jf0618022 CCC: $33.50
Published on Web 10/28/2006

Table 1. Gene Accession Numbers and Sequences of Gene Primers Used for Real-Time Quantitative Polymerase Chain Reaction

names	NCBI[a] accession no. or TC TIGR[b]	forward primer (5'–3')	reverse primer (5'–3')
PAL	X75967	TGCTGACTGGTGAAAAGGTG	CGTTCCAAGCACTGAGACAA
STS	AF274281	GTGGGGCTCACCTTTCATT	CTGGGTGAGCAATCCAAAAT
CHIT4c	AY137377	GGCGACGAATCCATTTATGTTA	CGGAACAAGGGTTTCATAATTC
PGIP	AF305093	ACGGAACTTGTTCCAGTTTGAT	CGATTGTAACTCACGTTCAGGA
PIN	AY156047	GCAGAAACCATTAAGAGGGAGA	TCTATCCGATGGTAGGGACACT
GLU	AF239617	TACCTCTTTGCCATGTTTGATG	AGTACTTCGGCTGTTTGTTTGG
ACT	TC30205	TCAGCACTTTCCAGCAGATG	TAGGGCAGGGCTTTCTTTCT

[a] NCBI, National Center for Biotechnology Information. [b] TC TIGR, tentative consensus no. according to The Institute of Genome Resarch.

application of methyl jasmonate (MeJA) on vineyard (*V. vinifera* L.) is able to induce defense responses and lead to protection toward pathogens. After treatment of plants with MeJA, expression of defense-related genes encoding enzymes involved in the phenylpropanoid pathway (*PAL*, *STS*) and encoding PR proteins (*CHIT4c*, *PIN*, *PGIP*, and *GLU*) was monitored by real-time quantitative polymerase chain reaction. We performed quantitative analysis of stilbenes, the major antimicrobial compounds of grapevine, to check for a correlation between the level of expression of the genes involved in the biosynthesis of stilbenes and the accumulation of these products. Grapevine protection with MeJA was evaluated toward downy (*P. viticola*) and powdery (*E. necator*) mildew on detached leaves of MeJA-treated plants (Cabernet Sauvignon) and in vineyard (Merlot).

MATERIALS AND METHODS

Plant Material. Plants of cultivated grapevine (*V. vinifera* L. cv Cabernet Sauvignon) were propagated from wood cuttings in a greenhouse (INRA, Villenave d'Ornon, France). They were grown under controlled conditions at 25/20 °C day/night temperature, with 75% relative humidity and a 16-h photoperiod (350 μmol/m^2/s). Two-month-old plants with 10−12 leaves were used for the experiments.

Experiments in vineyard were conducted in commercial vineyards in Arbis (southwest region of France) on *V. vinifera* L. cv Merlot healthy plants. During the experiments, the average relative humidity was 50−70% and temperature was 24−28 °C.

MeJA Treatment on Foliar Cuttings. MeJA (Sigma) was dissolved in 1% EtOH to the final concentration of 5 mM and added to an aqueous solution containing the wetting agent Triton X-100 at 0.1% (v/v) (Sigma). Ten milliliters of this solution was sprayed per foliar cutting. Control plants were sprayed with the Triton solution at 0.1%. Positive control plants were treated with Aliette (Fosetyl-Al, Fertiligène, France) (3 g/L), while negative ones were untreated.

For all experiments, 15 plants per treatment were entirely sprayed and each experiment was repeated in triplicate. After 72 h of treatment, leaves were collected for fungal inoculation or stilbene quantification. For molecular analysis, leaves were collected 12, 18, 24, and 72 h after treatment, frozen in liquid nitrogen, and stored at −80 °C until analysis.

MeJA Treatment in Vineyard. MeJA was dissolved in 1% EtOH to a final concentration of 5 mM (0.67 kg/ha) or 15 mM (2 kg/ha) and added to an aqueous solution containing the wetting agent Triton X-100 (0.1%) (Sigma). Control plants were sprayed only with the Triton solution (0.1%). Other control plants were treated with Thiovit (Syngenta) (12.5 kg/ha). Treatments were replicated every 7−10 days from May to August (from the bloom to the beginning of veraison) on four adjacent vines arranged in a randomized complete block design.

RNA Extraction and Quantification of Gene Expression by Real-Time Quantitative Polymerase Chain Reaction (RT-PCR). The leaves were ground in presence of liquid nitrogen and the resulting powder was used for RNA extraction. Total RNA was extracted from leaf samples as described by Chang et al. (*19*). Contaminating DNA in RNA samples was removed by incubating with 20 units of RNase-free DNase I (Promega Corp.) for 30 min and stopped with a phenol/chloroform/isoamylic alcohol mixture (25/24/1, v/v/v). The integrity of the total RNA was checked by electrophoresis on 1.2% agarose gel stained with ethidium bromide (66 ng/L). DNase-treated RNA (2 μg) was reverse-transcribed with 3 μM of oligo(dT) using the ImProm-II reverse transcription system (Promega Corp.) according to the manufacter's instructions. To determine the mRNA copy number of genes studied, real-time quantitative RT-PCR was performed using the detection system MyiQ (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad). PCR reactions were carried out in triplicate in 96-well plates (25 μL per well) in a reaction buffer containing 1× iQ SYBR Green Supermix (including Taq polymerase, dNTPs, SYBR Green I dye, 6 mM $MgCl_2$), 400 nM forward and reverse primers, and a 1:10 dilution of reverse-transcribed RNA. After denaturation at 95 °C for 5 min, amplification was performed in a two-step procedure: 30 s of denaturation at 95 °C and 1 min of annealing and extension at 60 °C, with a total of 45 cycles.

To check the specificity of the PCR product, melting curves were analyzed for each data point. Transcript levels were calculated as described by Arrieta-Montiel et al. (*20*) using the standard curve method from triplicate data, with actin gene as internal control and control leaves (at time zero) as reference sample.

The standard curve was constructed using templates of known copy number for target sequence: serial dilutions of cloned target sequence in pGEM-T easy vector (Promega Corp.) were used. The number of copies in each dilution was calculated with the following formula: (number of moles) (6.02×10^{23}) = number of copies. All standard samples were assayed in triplicate. The copy number of the sample was estimated by plotting the threshold cycle (Ct values) against the logarithm of the starting copy number. The absolute copy number for each sample was calculated from standard curves using the Ct value and was normalized against grapevine actin gene as internal control and control leaves as reference sample.

The gene-specific primers are indicated in **Table 1**. Relative gene expression was obtained with the formula: fold induction = $2^{-[\Delta\Delta Ct]}$, where $\Delta\Delta Ct$ = [Ct GI (unknown sample) − Ct VACT (unknown sample)] − [Ct GI (reference sample) − Ct VATC (reference sample)]. GI is the gene of interest and VACT is the grapevine actin gene used as internal control. The reference sample is the sample chosen to represent 1 × expression of the gene of interest (e.g., control leaves) (*21*).

Inoculation Procedures and Disease Evaluation. Inoculations were performed 72 h after MeJA treatment on leaves. As previous authors had noted that older leaves from the bottom of the shoots are more resistant (*22*), inoculation experiments were therefore performed on young leaves from the upper part of the plant. Before inoculation, leaves were washed and dried. *P. viticola* and *E. necator* fungal strains were obtained from INRA, Villenave d'Ornon, France.

P. viticola (strain Cou100-Bordeaux) was maintained on grapevine leaf-disk at −22 °C and subcultured twice before the assay. Sporangia were collected with a paint brush and suspended in dematerialized water at 4 °C to obtain a final density of 5000 sporangia/mm^2. Thoroughly rinsed, cleaned, and dried leaves were placed upside down on moist filter paper in Petri dishes. The lower surfaces of the leaves were inoculated with freshly prepared sporangia suspension (15 10-μL droplets per leaf, one leaf per plant) and incubated for 16 h at 22 °C in the dark. The droplets were then gently removed with a pipet

connected to an air pump and leaves were reincubated for 7 more days at 22 °C under a 16-h photoperiod (25 μE/m^2/s) and 75% relative humidity.

For inoculation with *E. necator* (strain BE3-Bordeaux), detached leaves (one per plant) were cleaned, decontaminated with NaOCl 5%, rinsed, and dried. Eight leaf disks (1.8 cm diameter) were taken from each leaf (four disks per side) of each tested plant using a cork borer. Disks were deposited lower side down on sterile agar plates and placed at the bottom of a Plexiglas settling tower (*23*). Conidia were blown in at the top from sporulating leaves (60−80 conidia per mm^2 of leaf). Inoculated leaves were incubated for 14 days at 22 °C under a 16-h photoperiod (25 μE/m^2/s).

Disease intensity was estimated by measuring the level of growth and intensity of fungal mycelium and sporulation on leaves. The contamination level was visually evaluated and expressed as a percentage of total leaf area according to a 0−100% scale (0 corresponding to the absence of pathogen development). The mycelium density and the aspect of spores were observed with a microscope.

In vineyard, the parcel in which the experiments were conducted is particularly susceptible to powdery mildew, and numerous disease symptoms are detected there each year. Powdery mildew (*E. necator*) contaminations were natural and promoted by rainfall. Disease intensity was evaluated visually by estimating pathogen attack frequency and percentage of infected leaf surface.

Quantification of Stilbenes. Stilbenes were extracted from freeze-dried leaves (100 mg) overnight with MeOH (10 mL) at 4 °C. After centrifugation (3500*g*, 10 min), the supernatant was concentrated and then prepurified on a Sep-Pak C18 cartridge (Waters) to remove chlorophylls. After elution with methanol 90% (v/v), the extract was evaporated to dryness (<40 °C). Leaf extracts were then dissolved in methanol 50% (v/v) (1 mL). Samples were then filtered through nylon membrane filters (Titan 2, 0.45 μm, ICS). During sample preparation, extracts were protected from light to avoid photochemical isomerization of *trans*-stilbenes to the less fluorescent *cis* forms. Analysis of stilbenes was performed by HPLC on a 250 × 4 mm Prontosil C18 (5 μm) reverse-phase C18 column (Bischoff Chromatography, Leonberg, Germany) protected by a guard column of the same material. Separation was performed at a flow rate of 1 mL/min with a mobile phase composed of (A) H_2O:TFA 1% (97.5/2.5, v/v) and (B) ACN:A (80/20, v/v). The run was set as follows: 0−13 min, from 14% B to 18% B; 13−15 min, 18% B; 15−34 min, from 18% B to 32% B; 34−36 min, 32% B; 36−40 min, from 32% B to 40% B; 40−49 min, from 40% B to 80% B; 49−50 min, from 80% B to 100% B; 50−56 min, 100% B. Fluorimetric detection was performed at λ_{ex} = 300 nm and λ_{em} = 390 nm. Stilbene contents were estimated from calibration curves prepared with pure standards.

trans-Resveratrol was purchased from Sigma Chemical Co. (St. Louis, MO) and *trans*-pterostilbene from Sequoia Research Products (Pangbourne, United Kingdom). *trans*-Piceid (*trans*-resveratrol 3-*O*-β glucoside) was purified from *V. vinifera* L. cell cultures as previously described (*24*). *trans*-δ-Viniferin was synthesized by horseradish peroxidase from *trans*-resveratrol (*25*). *trans*-ϵ-Viniferin was purified from woody material and characterized by NMR and MS as previously described (*26*).

Statistical Analysis. Data were expressed as the means ± standard deviations of 3−15 determinations. Statistical analysis was performed using Newman−Keuls or Student's *t*-tests and $P < 0.05$ was considered to be significant.

RESULTS

Effects of MeJA on Defense Gene Expression in Grapevine Foliar Cuttings. The expression pattern of six genes involved in defense was analyzed using RT-PCR. Two genes involved in the biosynthesis of polyphenol compounds were studied: one phenylalanine ammonia lyase (*PAL*) gene encoding the first enzyme of the pathway and one stilbene synthase (*STS*) gene encoding the enzyme responsible for the synthesis of stilbenes like piceids and resveratrol, the main phytoalexins produced by grapevine in response to biotic and abiotic stresses (*1*, *25*, *27*, *28*). The expression of four genes encoding PR proteins was also considered: an acidic class IV chitinase (*CHIT4c*) gene, a serine protease inhibitor (*PIN*) gene, a polygalacturonase-inhibiting protein (*PGIP*) gene, and a β-1,3-glucanase (*GLU*) gene.

In untreated leaves, no significant transcript accumulation of the studied genes was detected during the 72-h incubation period (**Figure 1**). In Triton- or MeJA-treated leaves, the expression of *PAL*, *STS*, and *CHIT4c* genes was rapidly and transiently up-regulated (**Figure 1A**−**C**). The induction by MeJA peaked at 18 h with a maximal intensity 44-, 11-, and 25-fold higher, respectively, than in untreated leaves. Triton treatment induced 2-fold less the expression of *PAL*, *STS*, and *CHIT4c* genes, compared to MeJA-treated leaves. Transcript accumulation of these genes slowly decreased until 72 h after treatment.

Serine protease inhibitor (*PIN*) gene was transiently induced (**Figure 1D**) and reached a maximal level after 18 h (43-fold higher than untreated and Triton control leaves). Then, the level of expression of PIN fell rapidly for 24 h and then slowly increased between 24 and 72 h post-treatment (21-fold induction).

The RT-PCR analysis also showed that the *PGIP* and *GLU* genes were up-regulated in grapevine leaves in response to MeJA (**Figure 1E**,**F**). PGIP accumulation began immediately after treatment, peaked at 12 h, and then decreased progressively for 72 h. The induction of *GLU* gene was greater than that of the *PGIP* gene, since it peaked at 18 h (48- and 8-fold induction, respectively) and then slowly decreased for 72 h.

Effects of MeJA on Stilbene Accumulation. The *trans*-form content (the main form found in leaves) of five major stilbenic phytoalexins—resveratrol, piceid, ϵ-viniferin, δ-viniferin, and pterostilbene—was quantified. In Triton control leaves, the sole phytoalexin slightly detected was the *trans*-piceid (**Figure 2A**), whereas in MeJA-treated grapevine foliar cuttings, all the phytoalexins under study were produced (**Figure 2**).

During the 72-h analysis, *trans*-piceid (**Figure 2A**) was quantitatively the most prolific stilbene produced. Its production starts 12-h post-treatment, reaching a plateau at 48-h post-treatment (244 nmol/g DW). *trans*-Resveratrol (**Figure 2A**) accumulated with a similar profile but in slightly less quantity (176 nmol/g DW).

ϵ-Viniferin (**Figure 2A**) was produced after about 18 h and accumulated quantitatively to about 80 nmol/g DW and then decreased slowly to 25 nmol/g DW at 72 h.

Pterostilbene and δ-viniferin (**Figure 2B**) were also detected in MeJA-treated leaves, but at relatively lower levels compared to the quantity of the other phytoalexins. These compounds accumulated transiently, peaked at 18 h (10 and 8 nmol/g DW, respectively), and then decreased slightly until 72 h post-treatment, to reach 3 nmol/g DW.

Effects of MeJA on Grapevine Protection. *On Excised Leaves from Foliar Cuttings.* Degree of protection for powdery mildew was represented as a percentage of infected leaf surface (**Figure 3**). Test validity was checked by using the Aliette compound (Fosetyl-Al) (3 g/L) as a positive control.

Pretreatment with MeJA 3 days before inoculation induced a strong reduction of the infection by *E. necator* (75%) (**Figure 3**) compared to control leaves. Aliette protection rate was about 83% for *E. necator*. No significant decrease in the development of *P. viticola* was observed (14%) (data not shown).

In Vineyard. **Figure 4** shows the evolution of *E. necator* contamination on vineyard grapevine leaves. Disease intensity was estimated as a percentage of infected leaf surface. Test

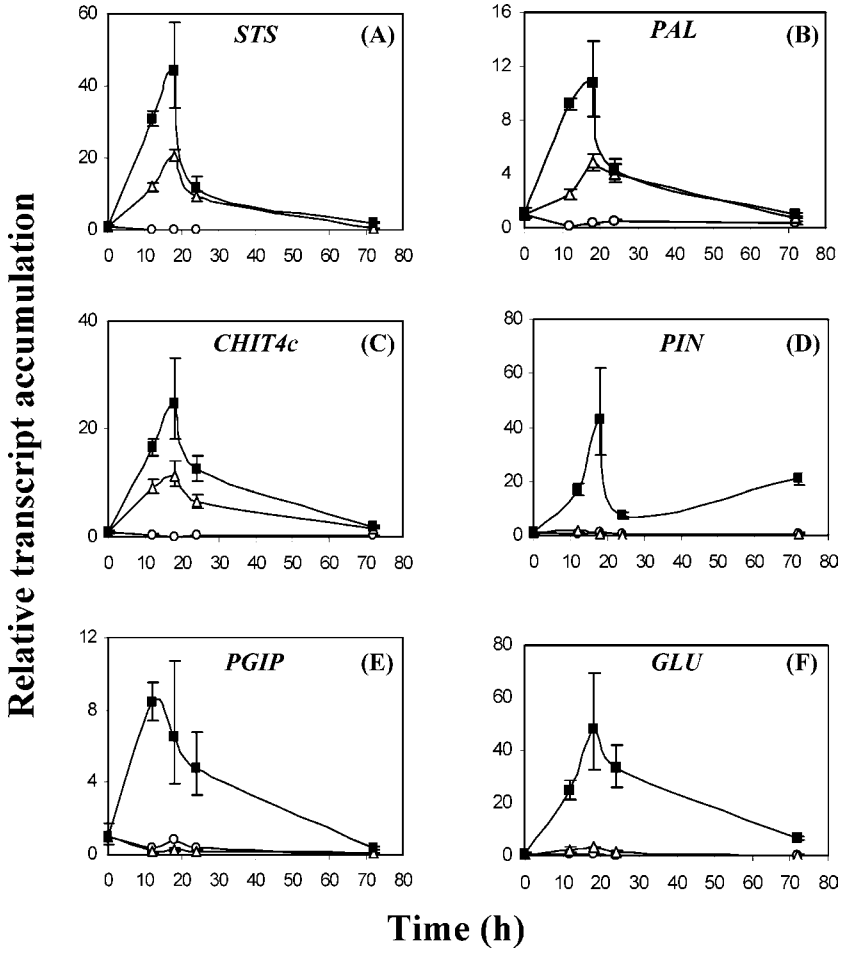

Figure 1. Transcript accumulation of defense genes in untreated (open circles), Triton-treated (open triangles), and MeJA-treated grapevine leaves (closed squares). Genes of interest encode the following proteins: (**A**) a stilbene synthase (*STS*), (**B**) a phenylalanine ammonia lyase (*PAL*), (**C**) an acidic class IV chitinase (*CHIT4c*), (**D**) a serine protease inhibitor (*PIN*), (**E**) a polygalacturonase-inhibiting protein (*PGIP*), and (**F**) a β-1,3-glucanase (*GLU*). Analyses were performed by real-time quantitative polymerase chain reaction. Transcripts levels were calculated by using the standard curve method from triplicate data, with grapevine actin gene as internal control and nontreated leaves (at time zero) as reference sample. Results represent the mean fold increase of mRNA level over untreated leaves, plotted against the 1× expression level. Absolute copy number of mRNA for each gene in the reference sample was 8.5 (*STS*), 29 (*PAL*), 1.3 (*CHIT4c*), 14 (*PIN*), 7.6 (*PGIP*), and 14 (*GLU*) $\times 10^5$ molecules/μg total RNA. Results are means ± standard deviations of three experiments.

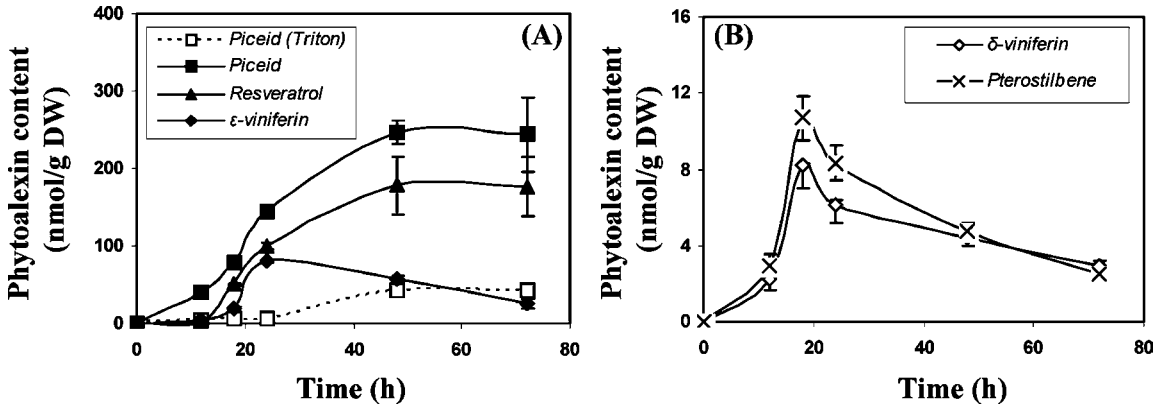

Figure 2. Time course of accumulation of several *trans*-stilbenes in *Vitis vinifera* (L.) cv. Cabernet Sauvignon leaves in response to MeJA treatment. (**A**) Piceid, resveratrol, and ϵ-viniferin and (**B**) δ-viniferin and pterostilbene. No stilbene except piceid was detected in Triton-sprayed leaves (open squares). Results represented are means ± standard deviations of three experiments.

validity was checked by monitoring the effects of THIOVIT, a protective and curative commercial extract containing 80% sulfur (12.5 kg/ha).

In our vineyard assays, a low degree of natural infection was detected on grapevine before July 20, 2005, owing to absence of rainfall at the beginning of the experiments. The temperature was high and the average relative humidity low.

From July 20 to August 9, powdery mildew progressed constantly and strongly, infecting from 3 to 43% of control leaf surfaces. In contrast, in MeJA-treated plants, the percentage of

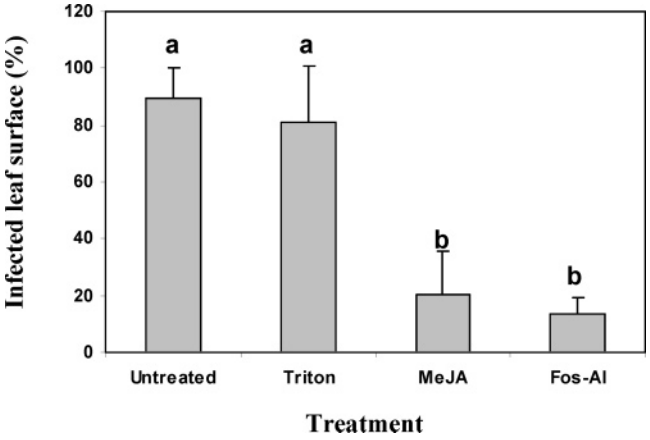

Figure 3. Protection of grapevine-detached leaves in response to MeJA treatment against *E. necator*. MeJA and the known active Aliette (Fosetyl-Al) were sprayed on plants 3 days before inoculation. Disease was assessed 14 days postinoculation and expressed a percentage of infected leaf surface. Fifteen plants were used per treatment. The experiments were repeated in triplicate with similar results. Different letters indicate values significantly different than control experiments ($P < 0.05$).

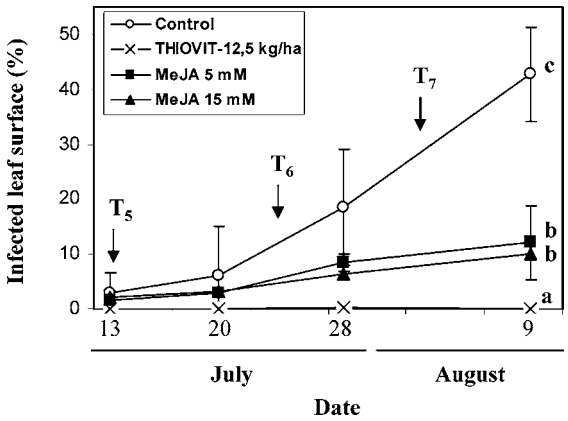

Figure 4. Protection of grapevine plants (Merlot) in response to MeJA against *E. necator* in vineyard. MeJA at two concentrations (5 and 15 mM) or Thiovit was sprayed on plants. Control plants were untreated. Four healthy vine stocks per treatment were sprayed every 7–10 days from May to August (from bloom to beginning of veraison). Only the period from July 13 to August 9 is represented. Disease assessment is expressed as a percentage of infected leaf surface. T_5, T_6, and T_7 correspond respectively to fifth, sixth, and seventh MeJA treatments. The experiment was performed once. Different letters indicate values significantly different than control experiments ($P < 0.05$).

leaf surface infection by *E. necator* was only 12.1% with 15 mM MeJA and 10.1% with 5 mM MeJA. Owing to its curative effect, THIOVIT-treated plants were completely protected (100%).

DISCUSSION

On grapevine foliar cuttings (**Figure 3**), a concentration of 5 mM MeJA is sufficient to trigger considerable protection against *E. necator*: i.e., 75% disease reduction. MeJA pretreatment of plants in vineyard (**Figure 4**) reduced the development of *E. necator* by approximately 73% compared to control plants, as shown mainly by a significant reduction in the infected leaf area.

Jasmonates have also been reported to induce local and systemic protection against *Phytophtora infestans* in potato and tomato plants (*29*), *Pythium ultimum* in Norway spruce (*30*), powdery mildew in barley seedlings (*31*), and downy mildew in grapevine plants (*18*).

The protection afforded by MeJA could be due to induction of defense-related proteins and enhanced production of antimicrobial compounds such as phytoalexins. Indeed to our knowledge, MeJA has no fungitoxic effect (*32*) and to prevent this possible effect, the foliar cuttings leaves were washed before fungal inoculations.

In our experiment, MeJA treatment on grapevine leaves induces expression of several classes of PR proteins. For example, transcript levels of an acidic chitinase gene, *CHIT4c*, increased rapidly after MeJA treatment and peaked after 18 h (**Figure 1C**). *CHIT4c* was similarly induced by Triton treatment but at a 2-fold less level. Grapevine chitinase genes are known to be inducible under stress conditions such as fungal challenge, elicitor treatment, or exposure to phytohormones such as ethylene, jasmonic acid, and salicylic acid (*33*, *7*). Chitinase accumulated in plants to degrade chitin, a major cell wall component of most fungi (*34*, *35*). However, this induction varies according to the stress applied, the organ studied, and the grapevine cultivar (*36*−*38*). On the basis of our results, the induction by MeJA treatment might be to a sufficient level to trigger grapevine protection against *E. necator*. Triton treatment is not efficient.

Grapevine *GLU* gene expression (**Figure 1F**) was highly up-regulated after MeJA treatment. The class I β-1,3-glucanases are antifungal vacuolar proteins involved in plant defense and their production was enhanced in response to developmental, hormonal, and pathogenesis-related conditions (*39*). The antifungal activity of plant β-1,3-glucanases is thought to hydrolyze the structural β-1,3-glucan present in fungal cell walls (*37*). Moreover, the combination of chitinase and β-1,3-glucanases is believed to potentiate the antifungal activity and was shown experimentally to inhibit the growth of many pathogenic fungi (*39*). Giannakis et al. (*40*) reported a correlation between the combined activities of chitinase and β-1,3-glucanase in a range of grapevine cultivars and observed field resistance to powdery mildew. They also demonstrated that chitinase and glucanase proteins purified from the leaves of a resistant cultivar inhibited the growth of powdery mildew in an in vitro bioassay.

PIN and *PGIP* mRNA transcripts also accumulated in MeJA-treated leaves. The inhibitors of serine proteases (PIN) belong to the class of antifungal PR-6 proteins, which have a potent activity against plant and animal pathogens (*41*). Farmer and Ryan (*42*) showed that airborne MeJA induces the expression of PIN proteins in tomato leaves. Polygalacturonase inhibitor proteins (PGIPs) act to thwart fungal penetration of the plant cell wall as an early induced plant defense to fungal attack (*43*). PGIPs are ubiquitous cell wall proteins that specifically inhibit the activity of fungal polygalacturonases (PGs), some of which are important fungal pathogenicity factors. PGIP levels have been shown to correlate in several cases with an increased resistance of plants to fungi (*44*, *45*). Therefore, a putative role of PGIP and PIN could partly explain the reduced infection of grapevine leaves infected by *E. necator*.

In leaves of Triton- or MeJA-treated plants, the phenylpropanoid pathway was also up-regulated. This up-regulation in Triton-sprayed leaves is not sufficient (2-fold less than MeJA treatment) to induce stilbene accumulation. In the case of MeJA treatment, *PAL* and *STS* gene induction (**Figure 1A**,**B**) led to a noteworthy increased production of resveratrol and its derivatives piceid, viniferins, and pterostilbene (**Figure 2A**). The activation of this biosynthesis pathway is one of the most important resistance reactions in many plants (*46*). Resveratrol

and its derivatives such as viniferins (dimerization), pterostilbene (methylation), and piceid (glycosylation) represent the major forms of phytoalexins in grapevine (*1*, *47*, *48*) and are produced by grapevine in response to elicitor and pathogens attacks (*24*, *26*, *27*). Piceid and resveratrol were quantitatively the major stilbenes produced, a finding in accordance with our previous study (*49*). Resveratrol is synthesized by a stilbene synthase after a stress (*48*) and has been shown to confer a tolerance to powdery mildew and downy mildew (*50*). Its accumulation in leaves of MeJA-treated plants before inoculation by *E. necator* could also explain the reduction in disease development. Concerning piceid, it could be a form of storage or resveratrol transport in the plant (*51*). In response to a stress, the presence (of basal piceid levels could constitute a pool of immediately usable resveratrol, which can rapidly be mobilized as a primary defense response. We also noted a transient accumulation of dimers of resveratrol (ϵ- and δ-viniferins) in elicited leaves. Both compounds are highly fungitoxic, and the presence of both viniferins in stressed grapevine leaves has been correlated with enhanced protection against downy mildew (*25*, *47*). Nevertheless, Dercks and Creasy (*52*) reported that ϵ-viniferin added to a sporangial suspension of *P. viticola* was 86 and 91% degraded in suspensions of 4.5×10^4 and 9×10^4 sporangia/mL after 5 h of incubation. These results suggest that *P. viticola* zoospores are able to degrade ϵ-viniferin and might explain why mycelium development with *P. viticola* infections on MeJA-treated leaves is not reduced.

Our data showed that pterostilbene is also detected in leaves of MeJA-pretreated plants, even if the amounts seemed low compared to the other stilbenes analyzed. Pterostilbene is indeed a biologically very active phytoalexin that is usually found in low quantities in grapevine (*53*). In the majority of grapevine cultivars, pterostilbene levels remain very low or undetectable and few studies have reported its presence in response to elicitation.

Under grapevine field conditions, MeJA also triggered a significant protection against *E. necator*, the causal agent of powdery mildew. This raises the hypothesis that all the defense mechanisms elicited by MeJA treatment on grapevine foliar cuttings also occurred in vineyard, thus accounting for the protection observed against *E. necator*.

To our knowledge, this is the first report that describes the efficiency of MeJA in vineyard. In routine agronomic use, management programs integrating MeJA might reduce the intensive use of fungicides against *E. necator*.

ACKNOWLEDGMENT

We thank Jérôme Jolivet and Xavier Capdevielle for plant care and technical assistance and Dr. Ray Cooke for revising the English manuscript.

LITERATURE CITED

(1) Coutos-Thévenot, P.; Poinssot, B.; Bonomelli, A.; Yean, H.; Breda, C.; Buffard, D.; Esnault, R.; Hain, R.; Boulay, M. *In vitro* tolerance to *Botrytis cinerea* of grapevine 41B rootstock in transgenic plants expressing the stilbene synthase *Vst1* gene under the control of a pathogen-inducible PR 10 promoter. *J. Exp. Bot.* **2001**, *358*, 901−910.

(2) Hain, R.; Reif, H. J.; Krause, E.; Langebartels, R.; Kindl, H.; Vornam, B.; Wiese, W.; Schmelzer, E.; Schreier, P. H.; Stocker, R. H.; et al. Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature* **1993**, *361*, 153−156.

(3) Jach, G.; Gornhardt, B.; Mundy, J.; Logemann, J.; Pinsdorf, E.; Leah, R.; Schell, J.; Maas, C. Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *Plant J.* **1995**, *8*, 97−109.

(4) Jongedijk, E.; Tigelaar, H.; van Roekel, J. S. C.; Bres-Vloemans, S. A.; Dekker, I.; van den Elzen, P. J. M.; Cornelissen, B. J. C.; Melchers, L. S. Synergistic activity of chitinases and β-1,3-glucanases enhances fungal resistance in transgenic tomato plants. *Euphytica* **1995**, *85*, 173−180.

(5) Boller, T. Chemoperception of microbial signals in plant cells. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1995**, *46*, 189−214.

(6) Côté, F.; Ham, K. S.; Hahn, M. G.; Bergmann, C. W. Oligosaccharide elicitors in host-pathogen interactions. Generation, perception, and signal transduction. *Subcell Biochem.* **1998**, *29*, 385−432.

(7) Kombrink, E.; Somssich, I. E. Defense responses of plants to pathogens. *Adv. Bot. Res.* **1995**, *21*, 1−34.

(8) Creelman, R. A.; Mullet, J. E. Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4114−4119.

(9) Ward, E. R.; Uknes, S. J.; Williams, S. C.; Dincher, S. S.; Wiederhold, D. L.; Alexander, D. C.; Ahl-Goy, P.; Metraux, J. P.; Ryals, J. A. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* **1991**, *3*, 1085−1094.

(10) Fukuda, Y.; Shinshi, H. Characterization of a novel *cis*-acting element that is responsive to a fungal elicitor in the promoter of a tobacco class I chitinase gene. *Plant Mol. Biol.* **1994**, *24*, 485−493.

(11) Enkerli, J.; Gisi, U.; Moesinger, E. Systemic acquired resistance to *Phytophthora infestans* in tomato and the role of pathogenesis related proteins. *Physiol. Mol. Plant Pathol.* **1993**, *43*, 161−171.

(12) O'Donnell, P. J.; Calvert, C.; Atzorn, R.; Wasternack, C.; Leyser, H. M. O.; Bowles, D. J. Ethylene as a signal mediating the wound response of tomato plants. *Science* **1996**, *274*, 1914−1917.

(13) Mueller, M. J.; Brodschelm, W.; Spannagl, E.; Zenk, M. H. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7490−7494.

(14) Mirjalili, N.; Linden, J. C. Methyl jasmonate induced production of taxol in suspension cultures of *Taxus cuspidata*: Ethylene interaction and induction models. *Biotechnol. Prog.* **1996**, *12*, 110−118.

(15) Gundlach, H.; Müller, M. J.; Kutchan, T. M.; Zenk, M. H. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2389−2393.

(16) Brader, G.; Tas, E.; Palva, E. T. Jasmonate-dependent induction of indole glucosinolates in arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. *Plant Physiol.* **2001**, *126*, 849−860.

(17) Repka, V.; Fischerová, I.; Šilhárová, K. Methyl jasmonate is a potent elicitor of multiple defense responses in grapevine leaves and cell-suspension cultures. *Biol. Plant* **2004**, *48*, 273−283.

(18) Hamiduzzaman, M. M.; Jakab, G.; Barnavon, L.; Neuhaus, J. M.; Mauch-Mani, B. β-Aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Mol. Plant Microbe Interact.* **2005**, *18*, 819−829.

(19) Chang, S.; Puryear, J.; Cairney, J. A simple and efficient method for isolating RNA from pine trees. *Plant Mol. Biol. Rep.* **1993**, *11*, 113−116.

(20) Arrieta-Montiel, M.; Lyznik, A.; Woloszynska, M.; Janska, H.; Tohme, J.; Mackenzie, S. Tracing evolutionary and developmental implication of mitochondrial stoichiometric shifting in the common Bean. *Genetics* **2001**, *158*, 851−886.

(21) Winer, J.; Jung, C. K. S.; Shackel, I.; Williams, P. M. Development and validation of real-time quantitative reverse transcriptase−polymerase chain reaction for monitoring gene expression in cardiac myocytes *in vitro*. *Anal. Biochem.* **1999**, *270*, 41−49.

(22) Reuveni, M. Relationships between leaf age, peroxidase and β-1,3-glucanase activity, and resistance to downy mildew in grapevines. *J. Phytopathol.* **1998**, *146*, 525−530.

(23) Délye, C.; Corio-Costet, M. F. Origin of primary infections of grape by *Uncinula necator:* RAPD analysis discriminates two biotypes. *Mycol. Res.* **1998**, *102*, 283−288.

(24) Waffo, Teguo, P.; Decendit, A.; Vercauteren, J.; Deffieux, G.; Mérillon, J. M. *Trans*-resveratrol-3-O-[beta]-glucoside (piceid) in cell suspension cultures of *Vitis vinifera*. *Phytochemistry* **1996**, *42*, 1591−1593.

(25) Langcake, P.; Pryce, R.J. The production of resveratrol and the viniferins by grapevines in response to ultraviolet radiation. *Phytochemistry* **1977**, *16*, 1193−1196.

(26) Pezet, R.; Perret, C.; Jean-Denis, J. B.; Tabacchi, R.; Gindro, K.; Viret, O. Delta-viniferin, a resveratrol dehydrodimer: One of the major stilbenes synthesized by stressed grapevine leaves. *J. Agric. Food Chem.* **2003**, *51*, 5488−5492.

(27) Adrian, M.; Jeandet, P.; Veneau, J.; Weston, L. A.; Bessis, R. Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. *J. Chem. Ecol.* **1997**, *23*, 1689−1702.

(28) Langcake, P.; Pryce, R. J. A new class of phytoalexins from grapevines, *Experientia* **1977**, *33*, 151−152.

(29) Cohen, Y.; Gisi, U.; Niderman, T. Local and systemic protection against *Phytophthora infestans* induced in potato and tomato plants by jasmonic acid and jasmonic methyl ester. *Phytopathology* **1993**, *83*, 1054−1062.

(30) Kozlowski, G.; Buchala, A.; Métraux, J. P. Methyl jasmonate protects Norway spruce [*Picea abies* (L.) Karst.] seedlings against *Pythium ultimum* Trow. *Physiol. Mol. Plant Pathol.* **1999**, *55*, 53−58.

(31) Walters, D.; Cowley, T.; Mitchell, A. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. *J. Exp. Bot.* **2002**, *53*, 747−756.

(32) Prost, I.; Dhondt, S.; Rothe, G.; Vicente, J.; Rodriguez, M. J.; Kift, N.; Carbonne, F.; Griffiths, G.; Esquerre-Tugaye, M. T.; Rosahl, S.; Castresana, C.; Hamberg, M.; Fournier, J. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiol.* **2005**, *139*, 1902−1913.

(33) Graham, L. S.; Sticklen, M. B. Plant chitinases. *Can. J. Bot.* **1994**, *72*, 1057−1083.

(34) Schlumbaum, A.; Mauch, F.; Vogeli, U.; Boller, T. Plant chitinases are potent inhibitors of funga1 growth. *Nature* **1986**, *324*, 365−367.

(35) Mauch, F.; Mauch-Mani, B.; Boller, T. Antifungal Hydrolases in Pea Tissue: II. Inhibition of fungal growth by combinations of chitinase and beta-1,3-glucanase. *Plant Physiol.* **1988**, *88*, 936−942.

(36) Busam, G.; Kassemeyer, H. H.; Matern, U. Differential expression of chitinases in *Vitis vinifera* L. responding to systemic acquired resistance activators or fungal challenge. *Plant Physiol.* **1997**, *115*, 1029−1038.

(37) Jacobs, A. K.; Dry, I. B.; Robinson, S. P. Induction of different pathogenesis-related cDNAs in grapevine infected with powdery mildew and treated with ethephon. *Plant Pathol.* **1999**, *48*, 325−336.

(38) Aziz, A.; Poinssot, B.; Daire, X.; Adrian, M.; Bezier, A.; Lambert, B.; Joubert, J. M.; Pugin, A. Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Mol. Plant Microbe Interact.* **2003**, *16*, 1118−1128.

(39) Sela-Buurlage, M. B.; Ponstein, A. S.; Bres-Vloemans, S. A.; Melchers, L. S.; Van Den, Elzen, P.; Cornelissen, B. Only specific tobacco (*Nicotiana tabacum)* chitinases and [β]-1,3-glucanases exhibit antifungal activity. *Plant Physiol.* **1993**, *101*, 857−863.

(40) Giannakis, C.; Bucheli, C. S.; Skene, K. G. M.; Robinson, S. P.; Scott, N. S. Chitinase and β 1,3-glucanase in grapevine leaves: A possible defence against powdery mildew infection. *Aust. J. Grape Wine Res.* **1998**, *4*, 14−22.

(41) Van Loon, L. C.; Van Strien, E. A. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiol. Mol. Plant Pathol.* **1999**, *55*, 85−97.

(42) Farmer, E. E.; Ryan, C. A. Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7713−7716.

(43) Caprari, C.; Mattei, B.; Basile, M. L.; Salvi, G.; Crescenzi, V.; De Lorenzo, G.; Cervone, F. Mutagenesis of endopolygalacturonase from *Fusarium monoliforme:* Histidine residue 234 is critical for enzymatic and macerating activities and not for binding to polygalacturonase-inhibiting protein (PGIP). *Mol. Plant−Microbe Interact.* **1996**, *9*, 617−624.

(44) De Lorenzo, G.; Ferrari, S. Polygalacturonase-inhibiting proteins in defense against phytopathogenic fungi. *Curr. Opin. Plant Biol.* **2002**, *5*, 295−299.

(45) D'Ovidio, R.; Raiola, A.; Capodicasa, C.; Devoto, A.; Pontiggia, D.; Roberti, S.; Galletti, R.; Conti, E.; O'Sullivan, D.; De Lorenzo, G. Characterization of the complex locus of bean encoding polygalacturonase-inhibiting proteins reveals subfunctionalization for defense against fungi and insects. *Plant Physiol.* **2004**, *135*, 2424−2435.

(46) Dixon, R. A.; Paiva, N. L. Stress-induced phenylpropanoid metabolism. *Plant Cell* **1995**, *7*, 1085−1097.

(47) Pezet, R.; Gindro, K.; Viret, O.; Spring, J. L. Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. *Physiol. Mol. Plant Pathol.* **2004**, *65*, 297−303.

(48) Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from the vitaceae: Biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731−2741.

(49) Larronde, F.; Gaudillière, J. P.; Krisa, S.; Decendit, A.; Deffieux, G.; Mérillon, J. M. Airborne methyl jasmonate induces stilbene accumulation in leaves and berries of grapevine plants. *Am. J. Enol. Vitic.* **2003**, *54*, 60−63.

(50) Dai, G. H.; Andary, C.; Mondolot-Cosson, L.; Boubals, D. Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera*, *V. rupestris* and *V. rotundifolia* and the downy fungus, *Plasmopara viticola*. *Physiol. Mol. Plant Pathol.* **1995**, *46*, 177−188.

(51) Douillet-Breuil, A. C.; Jeandet, P.; Adrian, M.; Bessis, R. Changes in the phytoalexin content of various *Vitis* spp. in response to ultraviolet C elicitation. *J. Agric. Food Chem.* **1999**, *47*, 4456−4461.

(52) Dercks, W.; Creasy, L. L. The significance of stilbene phytoalexins in the *Plasmopara viticola*−grapevine interaction. *Physiol. Mol. Plant Pathol.* **1989**, *34*, 189−202.

(53) Jeandet, P.; Breuil, A. C.; Adrian, M.; Weston, L. A.; Debord, S.; Meunier, P.; Maume, G.; Bessis, R. HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. *Anal. Chem.* **1997**, *69*, 5172−5177.

Received for review June 27, 2006. Revised manuscript received September 20, 2006. Accepted September 21, 2006. This study was supported by the Conseil Interprofessionnel du Vin de Bordeaux (C.I.V.B.).

JF0618022