Stilbene Content of Mature *Vitis vinifera* Berries in Response to UV-C Elicitation

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A method using HPLC analysis has been used to compare the level of resveratrol and its derivatives, piceid, pterostilbene and ϵ-viniferin, in grapevine berries of three *Vitis vinifera* varieties. The concentration of these compounds has been evaluated in healthy and *Botrytis cinerea* infected grape clusters, both in natural vineyard conditions and in response to UV elicitation.

Keywords: *Grapevines; phytoalexins; stilbenes*

INTRODUCTION

Phytoalexins are defense compounds that grapevines—as other plants—synthesize in response to pathogens such as *Botrytis cinerea* or *Plasmopara viticola* (Langcake and Pryce, 1976; Langcake, 1981) and abiotic stresses such as UV light (Langcake and Pryce, 1977) or aluminum chloride (Adrian et al., 1996). They are stilbenes including resveratrol (3,5,4′-trihydroxystilbene) (Langcake and Pryce, 1976; Jeandet et al., 1991), pterostilbene (a dimethylated derivative of resveratrol) (Langcake et al., 1979), piceid (a resveratrol glucoside) (Waterhouse and Lamuela-Raventos, 1994), and viniferins (resveratrol oligomers) (Langcake, 1981).

After the pioneering work of Langcake and Pryce (1976), resveratrol has been the subject of numerous studies [for a review, see Soleas et al. (1997)]. Its potent benefits for the health of moderate red wine consumers also led to numerous works (Frankel et al., 1993; Jang et al., 1997). However, few studies are available concerning resveratrol derivatives, especially their analysis in the plant.

Recently, a method using HPLC has been developed to enable the simultaneous analysis of both cis and trans isomers of piceid, resveratrol, ϵ-viniferin, and pterostilbene (Jeandet et al., 1997). This method has first been used for the measurement of these stilbenes in wines (Adrian et al., 2000). Here, it is applied to the analysis of stilbenes in berries of three varieties of *Vitis vinifera*: Gamay, Pinot Noir, and Chardonnay. This study was carried out to describe the accumulation of trans and cis isomers of resveratrol, piceid, ϵ-viniferin, and pterostilbene in infected and noninfected grape berries, depending on several factors: distance from *B. cinerea* lesions and time after induction by UV light.

MATERIALS AND METHODS

Plant Material. Grape berries from Pinot noir and Gamay (black varieties) and Chardonnay (white variety) were used for this study. They were all collected at the experimental vineyard of the University of Burgundy at Marsannay la Côte, France.

Both healthy and *B. cinerea* infected mature grape clusters were sampled. Infected clusters were divided into three regions as previously described [see Jeandet et al. (1995)]: region I, infected site; region II, surrounding noninfected berries; and region III, berries far from the necrotic area (Figure 1).

For all series of samples, berries were either directly frozen until extraction or irradiated by UV light at 254 nm for 10 min (0.36 J/cm^{-2}) (Langcake et al., 1977; Jeandet et al., 1995) and incubated for 24 or 48 h before being frozen.

Stilbene Extraction. About 2 g of skins of berries was detached from flesh, weighed, and mixed in 20 mL of 80% methanol using an Ultra-Turrax. After filtration under vacuum, using a filtration funnel (porosity 3, 16−40 μm), the extract was dried under vacuum ($T \leq 40$ °C), redissolved in absolute ethanol (5 mL/g of fresh skin), and stored at −20 °C until HPLC analysis. The time of extraction did not exceed 15 min.

HPLC Analysis. HPLC analyses were carried out using a method coupling photodiode array detection and fluorometry as previously described [see Jeandet et al. (1997)].

Identification and Quantification of Stilbenes. Identification of each stilbene was achieved by comparison of their retention time and UV spectrum from 250 to 400 nm with those of standards (Jeandet et al., 1997).

As the fluorometric characteristics are the same for all stilbenes evaluated (Ex = 330 nm and Em = 374 nm), they were quantified using a calibration curve of *trans*-resveratrol. Indeed, all of the stilbenes analyzed here contain a *trans*-stilbene moiety with the same fluorometric characteristics (Jeandet et al., 1997).

RESULTS AND DISCUSSION

cis-Piceid was found at the same concentrations in Gamay and Chardonnay berries. Surprisingly, it has never been detected in Pinot noir grapes, although it is present in wines in high amounts (Goldberg et al., 1995). It is the first time that the occurrence of the cis form of a stilbene has been reported in grape berries. In addition, no other *cis*-stilbene could be detected in the grape extracts.

The trans isomer of piceid was found in higher amounts (reaching 40-fold more) than *cis*-piceid. Curiously, this compound was not detected in the Pinot noir variety, whereas it has previously been detected in Pinot noir wines.

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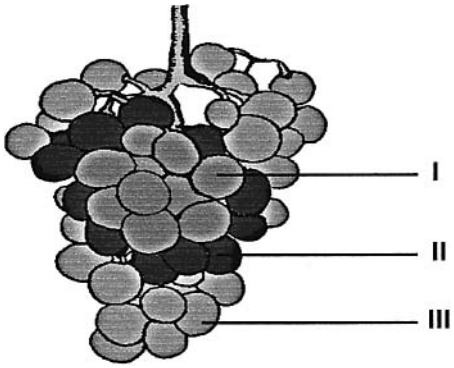

Figure 1. Zonation of grape clusters: I, *B. cinerea* infected site; II, noninfected berries surrounding zone I; III, berries located far from the necrotic area.

Table 1. Stilbene Contents (Micrograms per Gram of Fresh Weight of Skins) in Non-UV-Induced Berries of Gamay, Chardonnay, and Pinot Noir Varieties

	trans-piceid	*cis*-piceid	*trans*-resveratrol	ϵ-viniferin	pterostilbene
Gamay					
noninfected[a]	37.7	6.7	5.7	8.5	0
zone I	0	0	0.7	0	0.2
zone II	35.5	9.4	9.4	15.6	1.5
zone III	77.9	11.9	3.4	13	1.3
Chardonnay					
noninfected	26.2	5	6.7	2.2	0
zone I	0	0.1	0.7	3	0.2
zone II	70.2	10	48	12.2	4.7
zone III	41.4	9.6	21.8	4.6	0.2
Pinot Noir					
noninfected	0	0	0	0	0
zone I	0	0	1.8	5.9	0
zone II	0	0	11.4	13.1	0
zone III	0	0	0	0	0

[a] Noninfected, berries with no symptoms of *B. cinerea* attack; zones I−III, clusters as defined in Figure 1.

Table 1 shows the occurrence of resveratrol [5.7 and 6.7 μg/g of fresh weight (FW)] in non-irradiated berries from, respectively, the Gamay and Chardonnay varieties, indicating that they have already been stressed. As no symptoms could be detected on berries or on leaves, this may be due to latent *B. cinerea* inside berries or this may reflect the penetration of the fungus into the inner tissues through cracks on the cuticle surface (Bessis, 1972; Blaich et al., 1984).

Table 2 shows that for all of the conditions tested, UV light was always able to induce the production of resveratrol. This signifies that all berries are responsive to UV treatment, except those seriously attacked by *B. cinerea* (zone I). In zone I, berries were indeed necrosed—numerous cells have been killed by the fungus (Mansfield and Hutson, 1980; Jeandet et al., 1995).

In zones II and III, the resveratrol concentration was 2−30-fold higher in induced berries as compared to the noninduced ones (Tables 1 and 2). Resveratrol concentration is in the range of that previously described in the literature (Jeandet et al., 1995).

The highest amounts of resveratrol were detected in healthy and UV-C induced berries, in particular those incubated for 48 h after UV light exposure, indicating that a period of incubation of 24 h may be too short, in some cases, to evaluate the maximal phytoalexin response. For example, about 38 and 123 μg/g of FW were found in berries of the Chardonnay variety, respectively, 24 and 48 h following induction.

B. cinerea infected berries always contained lower amounts of resveratrol than the healthy ones. It can be hypothesized that either resveratrol is metabolized by *Botrytis* (Hoos and Blaich, 1990; Pezet et al., 1991; Sbaghi et al., 1996; Adrian et al., 1998; Breuil et al., 1998) or UV elicitation is less efficient on "preinduced" berries (that is to say, biotically stressed by the fungus). It could then mean that "overinduced" berries are less responsive to phytoalexin induction and, consequently, may be more susceptible to pathogen attacks. This is the first time, to our knowledge, that such a fact is reported.

All of the results presented show that mature berries remain able to produce stilbenes at significant concentrations (∼123 μg/g of FW 48 h after UV elicitation of healthy Chardonnay berries). Resveratrol concentration following UV light exposure of mature berries has thus been underestimated. In previous studies, the phytoalexin potential was measured <24 h after irradiation (Jeandet et al., 1991), finding quite almost undetectable values. The maximal phytoalexin response may then occur later in mature berries. This may explain the higher susceptibility of mature berries to *B. cinerea*. However, these results reinforce the role played by resveratrol—and more generally stilbenes—in the grapevine/pathogen interactions.

trans-ϵ-Viniferin was constantly detected in berries, whereas the cis isomer was undetectable. ϵ-Viniferin synthesis follows almost the same pattern as resveratrol (Douillet-Breuil et al., 1999).

Table 2. Stilbene Contents (Micrograms per Gram of Fresh Weight of Skins) in Berries 24 h (UV24) and 48 h (UV48) after UV Light Treatment

	trans-piceid		*cis*-piceid		*trans*-resveratrol		ϵ-viniferin		pterostilbene	
	UV24	UV48	UV24	UV48	UV24	UV48	UV24	UV48	UV24	UV48
Gamay										
noninfected[a]	79.7	75.4	5.8	16.4	15.9	91.4	16.4	43.8	0.5	1.4
zone I	4.3	0.0	0.0	0.0	2.7	0.0	1.5	0.0	0.0	0.0
zone II	30.2	57.2	7.0	7.9	51	56.5	19.0	43.2	0.0	1.4
zone III	33.5	60.4	7.2	13.6	51.8	66.1	14.9	47.5	0.8	1.9
Chardonnay										
noninfected	46.5	13.4	3.0	5.0	37.2	122.9	6.4	12.6	1.0	0.0
zone I	0.0	0.0	0.0	0.0	0.3	3.4	2.2	2.9	0.0	0.0
zone II	57.5	19.8	12.1	6.0	77.8	71.7	19.8	27.9	1.8	2.5
zone III	73.3	43.9	0.4	6.9	25.1	47.4	4.9	23.6	1.5	20.0
Pinot Noir										
noninfected	0.0	0.0	0.0	0.0	33.3	30.2	2.5	3.0	3.0	0.0
zone I	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
zone II	0.0	0.0	0.0	0.0	14.7	24.7	20.0	22.9	0.0	1.1
zone III	0.0	0.0	0.0	0.0	13.9	24.4	9.5	38.4	0.0	1.3

[a] Noninfected, berries with no symptoms of *B. cinerea* attack; zones I−III, clusters as defined in Figure 1.

As previously described (Bavaresco et al., 1997; Douillet-Breuil et al., 1999), pterostilbene production generally remains low and inconstant, particularly in mature berries. It has only been detected in infected berries of Chardonnay and unripe berries of Pinot noir at concentrations generally <5 μg/g of FW. According to Pezet et al. (1988), this compound certainly plays a role in the higher resistance of unripe berries to pathogens.

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LITERATURE CITED

Adrian, M.; Jeandet, P.; Bessis, R.; Joubert, J. M. Induction of phytoalexin (resveratrol) synthesis in grapevine leaves treated with aluminum chloride ($AlCl_3$). *J. Agric. Food Chem.* **1996**, *44*, 1979−1981.

Adrian, M.; Rajaei, H.; Jeandet, P.; Veneau, J.; Bessis, R. Resveratrol oxidation in *Botrytis cinerea* conidia. *Phytopathology* **1998**, *88* (5), 472−476.

Adrian, M.; Jeandet, P.; Breuil, A. C.; Levite, D.; Debord, S.; Bessis, R. Assay of resveratrol and derivated stilbenes in wines by direct-injection high-performance liquid chromatography. *Am. J. Enol. Vitic.* **2000**, *51* (1), 37−41.

Bavaresco, L.; Petegolli, D.; Cantu, E.; Fregoni, M.; Chiusa, G.; Trevisan, M. Elcitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. *Vitis* **1997**, *36* (2), 77−83.

Bessis, R. Etude en microscopie électronique à balayage des rapports entre l'hôte et le parasite dans le cas de la pourriture grise. *C. R. Acad. Sci. Paris Ser. D* **1972**, *274*, 2991−2994.

Blaich, R.; Stein, U.; Wind, R. Perforationen in der cuticula von weinbeeren als morphologischer factor der *Botrytis*-resistenz. *Vitis* **1984**, *23*, 242−256.

Breuil, A. C.; Adrian, M.; Pirio, N.; Meunier, P.; Bessis, R.; Jeandet, P. Metabolism of stilbene phytoalexins by *Botrytis cinerea*: characterization of a resveratrol dehydrodimer. *Tetrahedron Lett.* **1998**, *39*, 537−540.

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

Frankel, E. N.; Waterhouse, A. L.; Kinsella, J. E. Inhibition of human LDL oxidation by resveratrol. *Lancet* **1993**, *341*, 1103−1104.

Goldberg, D. M.; Ng, E.; Karumanchiri, A.; Yan, J.; Diamandis, E. P.; Soleas, G. J. Assay of resveratrol glycosides and isomers in wine by direct-injection HPLC. *J. Chromatogr. A* **1995**, *708*, 89−98.

Hoos, G.; Blaich, R. Influence of resveratrol on germination of conidia and mycelial growth of *Botrytis cinerea* and *Phomopsis viticola*. *J. Phytopathol.* **1990**, *129*, 102−110.

Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Metha, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1997**, *275*, 218−220.

Jeandet, P.; Bessis, R.; Gautheron, B. The production of resveratrol (3,5,4′-trihydroxystilbene) by grape berries in different developmental stages. *Am. J. Enol. Vitic.* **1991**, *42* (1), 41−46.

Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P. Production of the phytoalexin resveratrol by grapes as a response to *Botrytis* attack under natural conditions. *J. Phytopathol.* **1995**, *143*, 135−139.

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

Langcake, P. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ϵ-viniferin, α-viniferin and pterostilbene. *Physiol. Plant Pathol.* **1981**, *18*, 213−226.

Langcake, P.; Pryce, R. J. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* **1976**, *9*, 77−86.

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

Langcake, P.; Cornford, C. A.; Pryce, R. J. Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. *Phytochemistry* **1979**, *18*, 1025−1027.

Mansfield, J. W.; Huston, R. A. Microscopical studies on fungal development and host responses in broad bean and tulip leaves inoculated with five species of *Botrytis*. *Physiol. Plant Pathol.* **1980**, *17*, 131−144.

Pezet, R.; Pont, V. Mise en évidence de ptérostilbène dans les grappes de *Vitis vinifera*. *Plant Physiol. Biochem.* **1988**, *26*, 603−607.

Pezet, R.; Pont, V.; Hoang-Van, K. Evidence for oxidative detoxication of pterostilbene and resveratrol by a laccase-like stilbene oxidase produced by *Botrytis cinerea* Pers. *Physiol. Mol. Plant Pathol.* **1991**, *39*, 441−450.

Sbaghi, M.; Jeandet, P.; Bessis, R.; Leroux, P. Degradation of stilbene phytoalexins in relation to the pathogenicity of *Botrytis cinerea* to grapevines. *Plant Pathol.* **1996**, *45*, 139−144.

Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. Wine as a biological fluid: history, production, and disease prevention. *J. Clin. Lab. Anal.* **1997**, *11*, 287−313.

Waterhouse, A. L.; Lamuela-Raventos, R. M. The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochemistry* **1994**, *37*, 571−573.

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