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Effect of Ochratoxin A-Producing *Aspergilli* on Stilbenic Phytoalexin Synthesis in Grapes

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Berries of *Vitis vinifera* L. cv. Barbera were infected, at veraison and during ripening, by a conidial suspension of *A. japonicus, A. ochraceus, A. fumigatus* and two isolates of *A. carbonarius* to control ochratoxin A production and stilbene induced synthesis. The experimental design provided also for intact and punctured berries and incubation temperature of 25 °C and 30 °C. All the tested fungi, except *A. fumigatus*, significantly increased *trans*-resveratrol synthesis over the control, while *trans*-piceid was not affected; only *A. ochraceus* significantly elicited the berries to synthesize piceatannol. The two isolates of *A. carbonarius* produced higher amounts of ochratoxin A than did the other fungi. A positive correlation between ochratoxin A and *trans*-resveratrol synthesis occurred. *trans*-Resveratrol and piceatannol showed fungicidal activity against *A. carbonarius*, being able to completely inhibit fungal growth at a concentration of 300 μg/g and 20 μg/g, respectively.

KEYWORDS: ***Vitis vinifera* L.; *Aspergillus* spp.; stilbenes; resveratrol; piceatannol; ochratoxin A.**

INTRODUCTION

Stilbenes are low molecular weight phenolics occurring in a number of plant species, including *Vitis vinifera* L. Grapevine stilbenes include many compounds such as *trans*- and *cis*-resveratrol, their glucosides (piceid or polydatin), viniferins, pterostilbene, piceatannol, astringin, and other resveratrol polymers (**Figure 1**). The *Vitaceae* stilbenes are biosinthetically derived from the shikimic-polimalonic acid pathway, as first proposed by Langcake and Pryce (*1*) and then proved by Fritzmeier and Kindl (*2*). Stilbene synthase (StSy), which is the key enzyme in the biosynthesis of stilbenes in various species, among which *Arachis hypogaea* and *Vitis* spp, converts one molecule of *p*-coumaroyl-CoA and three molecules of malonyl CoA into *trans*-resveratrol (*3*−*4*). Malonyl CoA is derived by elongation of acetyl CoA units, while *p*-coumaroyl-CoA from phenylalanine which, in plants, can be synthesized from sugars via the shikimate pathway. Nevertheless, pterostilbene would not be synthesized by the same stilbene synthase cited for resveratrol (*5*). Liswidowati et al. (*6*) found that in grapevine cells cultured in the presence of fragments of *Botrytis cinerea* cell walls or of a glucan elicitor, prepared from the same materials, there was a dramatic shut-off general protein synthesis and a selective formation of a small set of proteins involved in induced resistance; among them are stilbene synthase and phenylalanine ammonia-lyase (PAL). PAL is the first enzyme

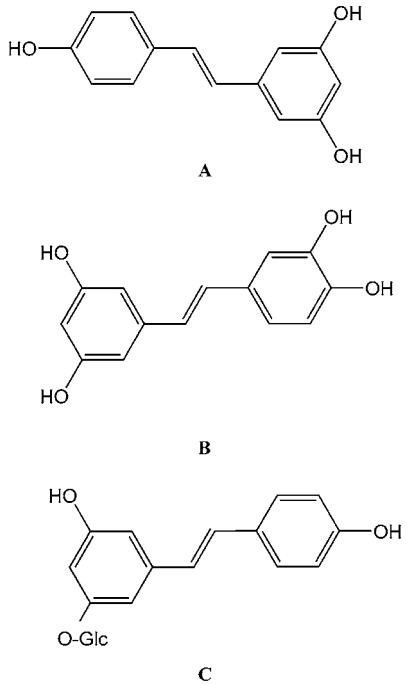

Figure 1. Structure of *trans*-resveratrol (A), piceatannol (B), *trans*-piceid (C).

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in the phenylpropanoid metabolism, while chalcone synthase (CHS) and StSty catalyze the first reactions of the general phenylpropanoid metabolism leading to the production of flavonoids and stilbenes. It is now accepted that both CHS and StSy have a common evolutionary origin, though they differ in the fact that the former is always constitutively expressed, while the latter is inducible (*7*).

Different cDNA sequences for grapevine stilbene synthase have been reported in the last 10 years. Sparvoli et al. (*8*) characterized the structural genes required for antocyanin and stilbene biosynthesis, among them are PAL, CHS, and StSy. Grapevine cDNA clones of genes coding for these enzymes, isolated by means of heterologous probes from snapdragon (*Anthirrinum majus*) and maize, were used as probes to describe the genomic organization of each gene. Southern blot analysis conducted on genomic DNA extracted from seedlings of *Vitis vinifera* cv Lambrusco to determine the copy number for each of the three genes showed that PAL and StSy genes are part of large multigene families, each consisting of 15−20 members, while for CHS a copy number of 3−4 genes was estimated. The authors also proposed that these gene families arose from a single ancestral gene and that duplication and molecular divergence might have contributed to the establishment of functionally distinct genes, characterized by different tissue specificities, developmental times of expression, and responses to environmental stimuli.

Stilbenes can be constitutive (in the woody parts of the plant) or induced (in soft tissues such as leaves and fruit); in the latter case, they act like phytoalexins, being induced by biotic and abiotic elicitors (*9*). Stilbenes, as induced compounds, are related to vine resistance against pathogens such as *Botrytis cinerea* (*10*−*12*), *Plasmopara viticola* (*13*), *Uncinula necator* (*14*), and *Rhizopus stolonifer* (*15*); no other fungi have been found to induce stilbene phytoalexin synthesis in grapevines. Stilbenes can also be triggered by bacteria (*16*) and abiotic elicitors such as UV irradiation, aluminum chloride, fosetyl-Al, ozone, and other chemicals (*9*). The stilbene synthesis is a genetic feature of the plants, being high in disease-resistant genotypes and low in disease-susceptible genotypes; nevertheless, the synthesis can be affected by environmental and cultural factors, such as fertilizer supply (*17*−*19*). As constitutive compounds, stilbenes are present in lignified organs such as stem, canes, seeds, roots, and cluster stems, and they are likely involved in the mechanisms of wood resistance to decay (*20*). During alcoholic and malolactic fermentation, stilbenes are extracted from the berry skins into the wine, and this process is affected by oenological practices (*21*, *22*).

Since 1996, occurrence of ochratoxin A contamination has been detected in wine and grape juice (*23*, *24*). Mycoflora potentially responsible for ochratoxin A in grapes are present in the field; *Aspergilli* are dominant with respect to *Penicillia*, and among these *Aspergilli* section *Nigri* is dominant, even though *A. ochraceus* and *A. fumigatus* ochratoxin A-producers are occasionally isolated. *A. carbonarius* probably plays the main role because of the high percentage of positive strains and the amount of ochratoxin A produced (*25*−*27*). Ochratoxin A is a carcinogenic toxin in rodents, and it possesses teratogenic, immunotoxic, and possibly neurotoxic and genotoxic properties. Punam Jeswal (*28*) has pointed out that concurrent administration of berry and leaf juice of common grape to mice together with ochratoxin A significantly reduces the hepatic and renal damage caused by ingestion of this mycotoxin, action possibly related to *trans*-resveratrol synthesis.

The aim of this work was to investigate the possibility for some ochratoxin A-producing fungi to elicit stilbenic phytoalexin synthesis in grape berries.

MATERIALS AND METHODS

Inoculation of Berries. At the beginning of August 2001, about 50 days after fruit set corresponding to veraison phenological stage, when the berry skin turns from green to red color, and in early October, during ripening, 15 bunches of *Vitis vinifera* cv. Barbera were collected from plants grown in a vineyard located in Piacenza viticultural area (Northern Italy). Groups of 30 berries were prepared by detaching randomly, from each bunch, berries without visible damage on their skin or visible fungal growth. These berries were used in an experiment of artificial inoculation, according to a four factor randomized complete block design with factors B, C, and D as split plots on factor A: (A) growth stage (1-veraison, 2-ripening); (B) fungus (1, *A. carbonarius* [MPVP-A566]; 2, *A. carbonarius* [MPVP-A372]; 3, *A. japonicus* [MPVP -A295]; 4, *A. ochraceus* [MPVP− A163]; 5, *A. fumigatus* [MPVP-A646]; 6, control); (C) berry status (1, intact; 2, punctured); (D) temperature of incubation (1−25 °C; 2−30 °C). Fungi used in this trial belonged to the fungal collection of the Institute of Entomology and Plant Pathology of the Catholic University of Piacenza.

All the berries were disinfected with a 2% sodium hypochlorite solution for 2 min and rinsed twice with sterile water before inoculation. Half of the berries of each group were punctured with a medical needle, while the others were used intact. Each group of berries was dipped into 100 mL of a conidial suspension for 5 min. Inoculum was prepared by growing fungi in Petri dishes (diameter 6 cm) with Czapek Yeast Agar (*29*) for 7 days at 25 °C, and by washing each dish with 10 mL of sterile distilled water. The suspensions obtained were adjusted to a concentration of 10^7 conidia/mL. Each fungus was used separately. After inoculation, berries were transferred on to a sterile grate into a box prepared as a moist chamber with paper abundantly wet on the bottom of the box that was put into a plastic bag; the box was incubated for 7 days at the appropriate temperature.

At the end of incubation, berries with growing moulds were counted to quantify the incidence of visible fungal colonisation; results were represented as percentage of berries with visible moulds. Afterward, all the berries of each treatment were disinfected with a 2% sodium hypochlorite solution for 2 min and rinsed twice with sterile water to eliminate fungi from their surfaces. Berries were divided into 2 subgroups and used for the analysis of *trans*-resveratrol and its related compounds and for the detection of ochratoxin A, respectively.

Time Course of Berry Stilbenes Synthesis. A preliminary trial was managed in order to study the time course of stilbene synthesis in grapes artificially inoculated with *A. carbonarius* [MPVP-A566], according to the method described in the previous paragraph. Only punctured berries from ripe grapes were considered, incubated at 25 °C for 5, 8, 13, 15, and 18 days.

Effect of Stilbenes on in Vitro Growth of *A. carbonarius*. *trans*-Resveratrol and piceatannol were added to a synthetic must medium (*30*), reproducing conditions at veraison, at concentrations of 30 μg/g and 300 μg/g (*trans*-resveratrol) and 2 μg/g and 20 μg/g (piceatannol). The lowest values for both stilbenes were chosen depending on the average content that was detected in the present work in the whole berries (*trans*-resveratrol about 3 μg/g fresh weight; piceatannol about 0.2 μg/g fresh weight, corresponding to about 30 μg/g berry skin for *trans*-resveratrol and about 2 μg/g berry skin for piceatannol); in fact, stilbenes are located in berry skin, which is about 10% of the whole berry (w/w). Media were inoculated with *A. carbonarius* [MPVP-A566] and incubated at 20 °C and at 25 °C, and after 8 days, fungus growth rate (as mm of radial growth of colony per day) and ochratoxin A production (as μg/g of colony per day) were recorded. The experimental design also provided for a control (treatment without stilbenes addition). Each average value is the mean of three replicates.

Standards. The *trans*-resveratrol (*trans*-3,4′,5-trihydroxy-stilbene), piceatannol (*trans*-3,3′,4,5′-tetrahydroxy-stilbene) and ochratoxin A standards were purchased from Sigma (St Louis, MO); *trans*-piceid (*trans*-resveratrol-3-*O*-β-D-glucopyranoside) was isolated from the roots of *Polygonum cuspidatum*; the purity of each stilbene was controlled

Table 1. Effect of Growth Stage, Fungus Species, Berry Status, and Incubation Temperature on the Berry Tested Parameters

		symptoms (%)[c]	ochratoxin A (μg/Kg FW)	*trans*-resveratrol (μg/g FW)	*trans*-piceid (μg/g FW)	piceatannol (μg/g FW)
growth stage[a]	at veraison	32 B	4.80	2.24	0.53	0.00 A
	during ripening	68 A	2.06	1.86	0.96	0.18 B
fungus species[b]	*A. carbonarius (I)*	73 A	9.30 A	2.45 B	0.95	0.05 B
	A. carbonarius (II)	76 A	8.65 A	1.68 B	0.56	0.05 B
	A. japonicus	63 B	0.06 B	4.26 A	0.93	0.09 B
	A. ochraceus	57 B	2.31 B	1.97 B	0.90	0.26 A
	A. fumigatus	32 C	0.02 B	1.40 BC	0.84	0.08 B
	control	0 D	0.00 B	0.49 C	0.27	0.06 B
berry status[a]	intact	43 B	2.16 B	1.50 A	0.73	0.09
	punctured	65 A	4.69 A	2.59 B	0.75	0.09
incubation temp[a]	25 °C	45 B	3.00	2.05	0.68	0.09
	30 °C	55 A	3.85	2.05	0.81	0.10

[a,b] Each value is the mean of 84 (*a*) or 24 (*b*) data. [c] Values in each column without the same letters are significantly different ($P < 0.05$).

by HPLC, and the identity was confirmed according to Mattivi et al. (*21*). Working standards of stilbenes were prepared by dissolving them in methanol and diluting the solution with acetonitrile/water (40:60; v/v). A solution of ochratoxin A (40 μg/mL in benzene/acetic acid 99:1; v/v) was calibrated spectrophotometrically at 333 nm, using the value 5550 for the extinction coefficient and stored at −20 °C when not in use (*31*); after calibration of the ochratoxin A solution, working standards were prepared by evaporating an exact volume under a stream of nitrogen and dissolving the residue again in the mobile phase.

Extraction of Ochratoxin A. Five grams of berries were crushed in a polyethylene bag, then 20 mL of a water solution containing PEG 8000 (10 g/L) and sodium hydrogen carbonate (50 g/L) were added. The mixture was homogenized for 30 min, filtered through a No. 595 folded filter paper (S&S) into a 50 mL graduated cylinder, and the volume was recorded. The extract was centrifuged at 6400*g* for 10 min and immediately filtered again through an HATF 0.45-μm filter (Millipore) under vacuum. An aliquot of the solution was diluted 2−10 times with the mobile phase, depending on ochratoxin A concentration, and filtered through a Millex HV, 0.45-μm syringe filter (Millipore) before HPLC analysis.

Extraction of Stilbenic Phytoalexins. *trans*-Resveratrol, *trans*-piceid, and piceatannol were extracted from the berries according to Bavaresco et al. (*12*). About 10 g of fresh berries were crushed in a mortar and placed in a 250-mL flask; 30 mL of methanol/water (95:5; v/v) was added, and the mixture was vigorously shaken for 20 min at room temperature. Seeds were discarded before maceration to avoid extraction of constitutive stilbenes. After filtration through GF/A (Whatman) filters, the liquid was evaporated in vacuo at 40 °C, and the water residue was extracted twice with 5 mL of ethyl acetate and 5 mL of 5% aqueous sodium bicarbonate. The organic phases were collected and evaporated in vacuo to dryness and redissolved in 2 × 1 mL of methanol (100%) and stored in adiactinic glass vials at −18 °C.

HPLC Conditions. For ochratoxin A, the HPLC system consisted of a Perkin-Elmer 200, equipped with an ISS 200 sampling system and a Jasco FP-920 fluorescence detector set at 333 nm excitation and set at 470 nm emission. The system was controlled by a Perkin-Elmer Turbochrom PC software. A 150 × 4-mm i.d., 4-μm, Select B RP-8 column (Merck) was employed at ambient temperature, with a mobile phase of acetonitrile/2% acetic acid (43:57; v/v) for ochratoxin A and (55:45; v/v) for ochratoxin A methyl ester, at 1.2 mL/min. The injection volume was 30 μL. For stilbenic phytoalexins, the HPLC system consisted of a Perkin-Elmer 200, equipped with an ISS 200 sampling system and a Jasco FP-1520 fluorescence detector set at 330 nm excitation and set at 374 nm emission. Borwin 1.5 software was used for data storage and evaluation. A 125 ×-4 mm i.d., 5-μm, Lichrospher RP-18 column (Merck) was employed at ambient temperature, using gradient elution with acetonitrile/aqueous 2% acetic acid, from 8 to 52% of acetonitrile in 15 min, at 1 mL/min. The injection volume was 20 μL.

Quantification of Ochratoxin A and Stilbenic Phytoalexins. Amounts of ochratoxin A standard between 2 and 60 pg were injected. Derivatization of ochratoxin A through methylation of the extracts with subsequent HPLC analysis was used for qualitative confirmation of positive samples (*24*). Amounts of *trans*-resveratrol standard between 8 and 100 pg were injected, while for piceatannol and *trans*-piceid the amounts injected were between 0.4 and 10 pg. Both for ochratoxin A and stilbenic phytoalexins, quantification was on the basis of peak areas using the respective PC software. Each value is the mean of three replicates.

Statistical Analysis. A four-way-ANOVA (Factors Randomized Complete Block Design with factors B, C, and D as split plots on factor A) with interactions was utilized (experiment model No. 33 in MSTAT). Growth stage (A), fungus species (B), berry status (C), and incubation temperature (D) were considered as main effects. Means were compared by using the Tuckey's test (5% level) (*32*). Before analysis, arcsin transformation was applied to percentage data. Linear regressions between the tested parameters were calculated.

RESULTS

Visible symptoms on grape berries artificially inoculated with ochratoxin A-producing fungi were significantly influenced by all the factors considered in the study. Berries were more susceptible to fungal infection when they were ripe, when damage was visible on their skin, and if incubated at 30 °C instead of at 25 °C. *A. carbonarius* caused significantly higher disease incidence, followed by *A. japonicus* and *A. ochraceus*. *A. fumigatus* has been less aggressive than all the other fungi considered (**Table 1**).

Ochratoxin A content varied both with fungus and berry status. In particular, both the isolates of *A. carbonarius* considered produced the same high amount of toxin, while all the other fungi produced the same amounts of ochratoxin A of uninoculated berries (**Table 1**).

With respect to the stilbenes, *trans*-resveratrol was significantly affected by the fungus and the berry status and *trans*-piceid was not affected by any of the tested factors, while piceatannol berry concentration varied depending on the growth stage and the fungus. *trans*-Resveratrol decreased, while *trans*-piceid increased from veraison to ripening, although not in a significant way. Piceatannol, instead, increased along with berry growth in a significant way, being produced only during ripening (**Table 1**). Within the tested stilbenes, the highest values occurred for *trans*-resveratrol, the lowest for piceatannol. All the tested fungi, except for *A. fumigatus*, triggered *trans*-resveratrol production, while only *A. ochraceus* increased piceatannol berry concentration over the control (**Table 2** and **Figure 2**); no significant effect on *trans*-piceid concentration occurred with fungus. The highest level of *trans*-resveratrol was

Table 2. Effect of Incubation Temperature and Stilbene Compounds on *Aspergillus carbonarius* Growth and Ochratoxin A Production

incubation temp (°C)	active ingredient (μg/g)	growth rate (mm/d)	ochratoxin A/d (μg/kg)
20	*trans*-resveratrol (30)	0.95	359.06
20	piceatannol (2)	0.99	311.49
20	control	1.73	208.23
25	*trans*-resveratrol (30)	2.18	233.79
25	piceatannol (2)	1.88	427.12
25	control	2.48	183.43

induced by *A. japonicus* (4.26 μg/g fresh weight), while the highest level of *trans*-piceid was induced by *A. carbonarius* (I) (0.95 μg/g fresh weight). The punctured berries had a higher level of *trans*-resveratrol than the intact ones (2.59 μg/g fresh weight and 1.50 μg/g fresh weight, respectively); the puncture did not affect *trans*-piceid and piceatannol berry concentrations. The tested stilbenes were not affected by the incubation temperature (**Table 1**).

The effect of the puncture in increasing visible symptoms after fungus infection was evident both at veraison and during ripening. Intact and punctured berries showed higher susceptibility during ripening than at veraison. During ripening, differences among fungi and between intact and punctured berries were reduced as compared to veraison (**Figure 3**).

The symptoms of punctured berries were significantly higher than those of intact berries, at veraison, in the case of infection by *A. carbonarius* (I), *A. carbonarius* (II), *A. japonicus* and *A. ochraceus*, but not by *A. fumigatus* and for the control. The symptoms were not affected by the puncture, during ripening, for berries infected by *A. carbonarius* (I), *A. carbonarius* (II), *A. japonicus*, *A. fumigatus*, and the control, while the symptoms of punctured berries were significantly higher than those of intact ones under *A. ochraceus* infection (**Figure 3**).

At first sampling, the effect of the puncture in increasing ochratoxin A production was evident after just *A. carbonarius* (I) and *A. carbonarius* (II) infection. These two isolates increased ochratoxin A production in a significant way over the other fungi, confirming their higher ochratoxigenic property. During ripening, no significant differences occurred (data not shown).

The effect of the puncture in increasing *trans*-resveratrol after fungus infection was evident at the first sampling, while at the second one, no significant effect was observed. The punctured berries showed lower values during ripening than at veraison; on the other hand, intact berries showed higher values during ripening than at veraison (**Figure 4**).

Simple regressions were calculated regarding visible symptoms development, ochratoxin A, *trans*-resveratrol, *trans*-piceid, and piceatannol level in berries. When all data were considered together, a positive and significant correlation ($r = 0.22$) at the 1% level between visible symptoms and ochratoxin A occurred. When again visible fungal infection symptoms were considered, similar correlations were calculated with *trans*-resveratrol level ($r = 0.28$), *trans*-piceid level ($r = 0.23$), and piceatannol level ($r = 0.24$). An interesting and positive correlation ($r = 0.20$) was calculated between ochratoxin A production and *trans*-resveratrol level. Moreover, a positive and significant correlation ($r = 0.42$) at the 1% level between *trans*-resveratrol and *trans*-piceid occurred. A lower correlation, significant at the 5% level, was calculated between *trans*-resveratrol and piceatannol. Finally, an important and strong correlation ($r = 0.31$) was calculated between *trans*-piceid and piceatannol.

Time Course of Berry Stilbene Synthesis. This trial showed that *trans*-resveratrol concentration increased until day 18 (3.04 μg/g fresh weight), whereas *trans*-piceid increased until day 15 (0.62 μg/g fresh weight) and then decreased. Piceatannol was produced just at day 13 with a nonsignificant concentration (**Figure 5**).

Stilbene Effect on in Vitro Growth of *A. carbonarius*. *trans*-Resveratrol (30 μg/g) and piceatannol (2 μg/g) addition to the synthetic must medium decreased the growth rate of *A. carbonarius* below that of the control, showing an inhibitive effect of these phytoalexins (**Table 2**). On the other hand, the presence of phytoalexins triggered ochratoxin A production, confirming that this toxin is stimulated under stress conditions. The trial managed at 300 μg/g *trans*-resveratrol and at 20 μg/g piceatannol showed a complete inhibition of fungus growth, and therefore it was not possible to detect ochratoxin A content.

DISCUSSION

This research identifies, for the first time, some ochratoxin A-producing fungi as elicitors of stilbenes in grapevine berries. The research was also successful in detecting piceatannol production as affected by a biotic elicitor such as *A. ochraceus*. Piceatannol has previously been detected in ripe berries of potted plants of cv. Cabernet Sauvignon (*33*). The effect of fungus inoculation on *trans*-resveratrol and *trans*-piceid berry concentration, at veraison, over the control was significant only for punctured berries and not for the intact ones. It seems mandatory for the fungus to find an entry to the berry in order to elicit the skin to produce the two stilbenes. The lack of piceatannol production at veraison is likely related to a genetic control, expressing piceatannol synthesis only at ripening time. Because evidence has been obtained that inducible stilbene synthase gene expression declines during ripening (*34*, *35*), an enzyme other than stilbene synthase and expressing only at ripening is likely involved in piceatannol biosynthesis, by adding one more −OH group to the molecule of *trans*-resveratrol. During ripening, *trans*-resveratrol was significantly elicited over the control only by *A. japonicus* infecting intact berries; this is likely to occur because, at ripening time, berry skin has some microlesions allowing fungi to penetrate the cuticle and therefore to elicit production in the skin. The data show a genetic control, as concerning the ochratoxin A-producing fungi, for the elicitation of stilbenes (*trans*-resveratrol and piceatannol) at ripening. *Trans*-resveratrol production of the punctured berries decreased from veraison to ripening, according to previous data (*12*, *36*, *37*) from trials with berries elicited by abiotic (UV rays) and biotic (*Botrytis cinerea*) factors.

Each fungus seems to have a different genetic capability to trigger stilbene synthesis and to induce symptoms. *A. japonicus* and *A. ochraceus*, for instance, show very different *trans*-resveratrol levels under the same pathogenicity.

It is difficult to explain the different capabilities of the tested fungi to elicit stilbene synthesis. There are very few data in the literature on the interaction between those fungi and grapevine (*25*−*27*, *30*), and we can therefore only speculate that some unknown mechanisms allow some *Aspergillus* species to trigger the biosynthesis of different stilbenes at different levels. Another factor to consider is that the concentrations of stilbenes that have been detected as induced by the fungi are the net balance of berry production and fungus degradation; the data we recorded cannot indicate to what extent the two phenomena occurred.

Data concerning the symptoms of fungal colonization on the berries are very interesting to discuss. During ripening, most

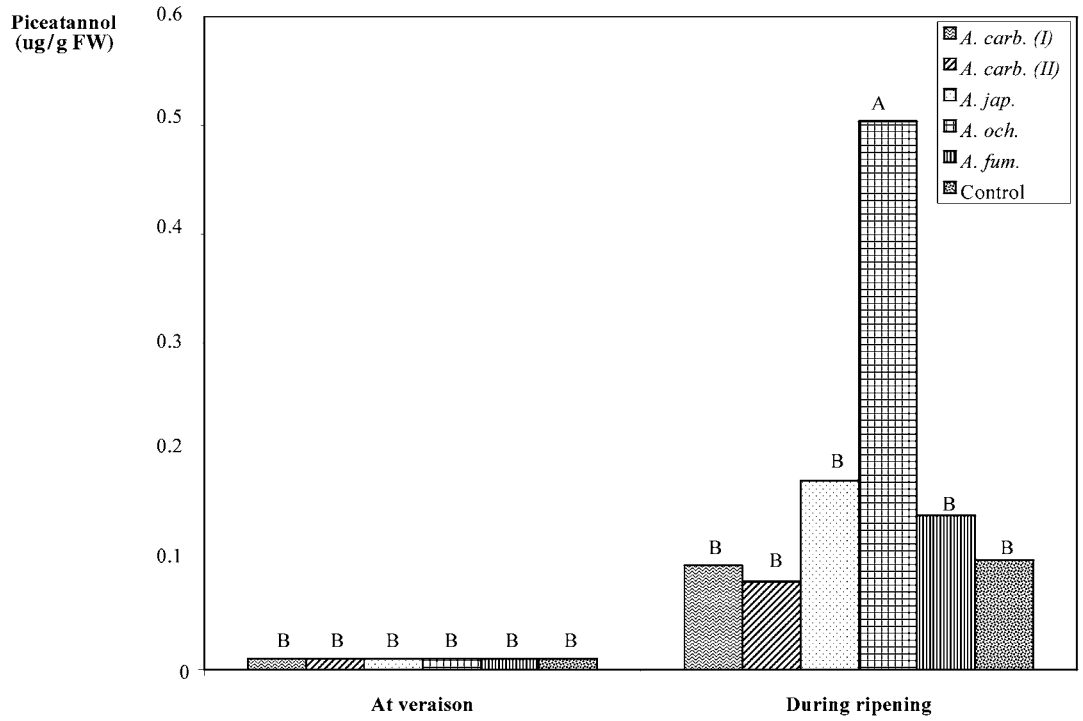

Figure 2. Piceatannol berry concentration depending on the growth stage and the fungus species. Values with the same letter are not significantly different ($P < 0.05$).

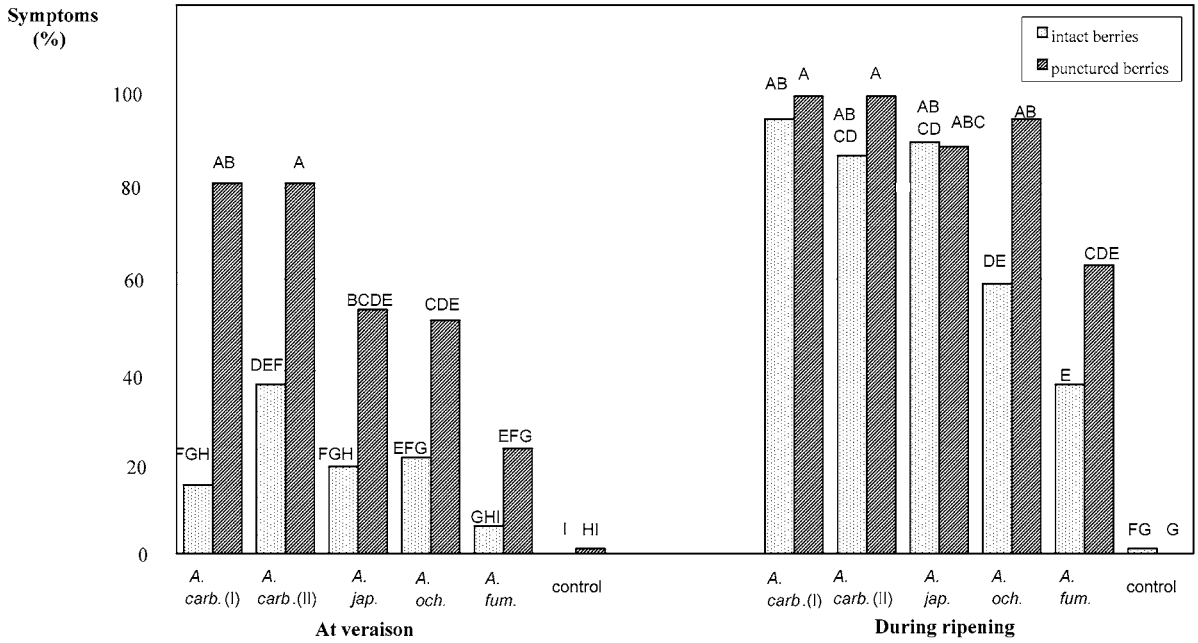

Figure 3. Infection symptoms depending on the growth stage, the fungus species, and the berry status. Values with the same letter are not significantly different ($P < 0.05$).

of the fungi were able to be aggressive also in intact berries (in addition to in those punctured), because it is likely that ripe berries are more easily attacked by fungi due to microlesion in the skin, allowing fungi to penetrate the cuticle; no data are available in the literature on the method of penetration of *Aspergilli* in grape berries.

The in vitro trial on the effect of stilbenic compounds on *A. carbonarius* showed a clear reduction of fungal growth due to *trans*-resveratrol and piceatannol. These two stilbenes therefore have a fungicidal activity, which completely kills the fungus when *trans*-resveratrol and piceatannol are supplied at a concentration 10 times higher (300 μg/g and 20 μg/g, respectively) than the values recorded in the present trial as a physiological concentration after elicitation by *A. carbonarius*. Piceatannol especially seems to be a powerful fungicide, because it is active at very low concentrations (2 and 20 μg/g), compared with literature data on the effect of other stilbenes such as resveratrol on mycelial growth and spore germination of *Botrytis cinerea* and *Rhizopus stolonifer* (*15*), where the complete inhibition of fungal growth was reached at a concentration of 500 μg/g. According to Langcake (*13*), a *trans*-resveratrol concentration higher than 200 μg/mL was necessary to reduce the release of zoospores from sporangia of *Plasmopara viticola* or the motility of the zoospores after their release by 50%. With

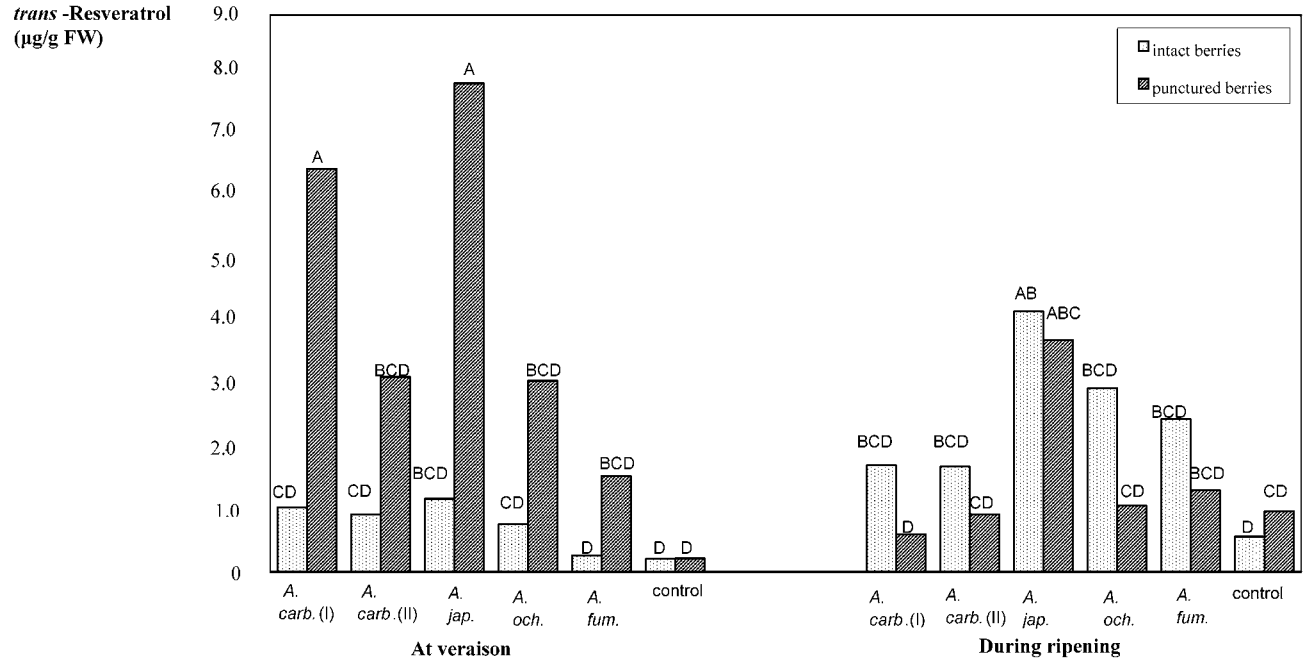

Figure 4. *trans*-Resveratrol berry concentrations depending on the growth stage, the fungus species, and the berry status. Values with the same letter are not significantly different ($P < 0.05$).

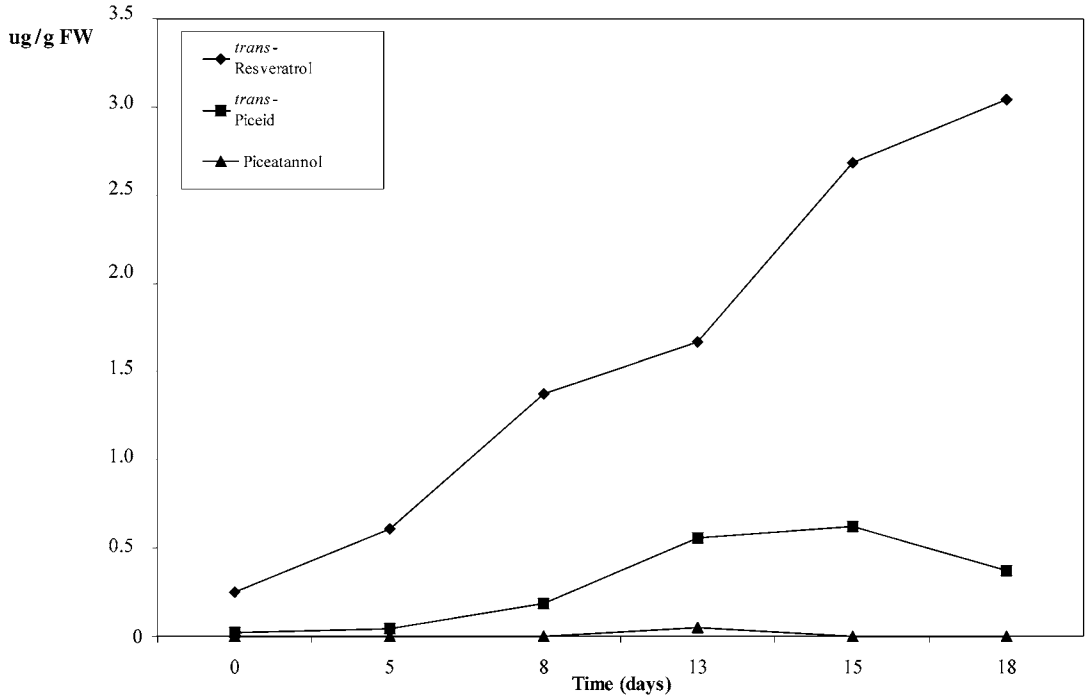

Figure 5. Time course of stilbene synthesis after berry elicitation by *A. carbonarius* [MPVP-A566].

respect to *Phomopsis viticola*, a mycelial growth reduction of 70% occurred under a *trans*-resveratrol concentration >100 μg/g (*38*).

The positive correlation between ochratoxin A and *trans*-resveratrol means that most likely grapes and wines with a high ochratoxin A contamination also have a high *trans*-resveratrol level. Nevertheless, this does not seem to be the case for ochratoxin A and *trans*-resveratrol induced by *A. japonicus*. If ochratoxin A is produced by *A. ochraceus*, piceatannol also occurs in the grapes.

The positive effect of stilbene treatments on ochratoxin A production by *A. carbonarius* is related to a stress reaction by the fungus, whose growth is impaired.

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