JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Ultraviolet-C and Induced Stilbenes Control Ochratoxigenic *Aspergillus* in Grapes

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This study investigated the efficacy of ultraviolet-C (254 nm) and induced stilbenes to inhibit *Aspergillus carbonarius* and *Aspergillus tubingensis* and control ochratoxin A production in grapes. In addition, the stilbene synthesis as a response to UV-C treatment and to infection of ochratoxigenic *Aspergillus* was compared. The initial microbial inactivation by a previously optimized UV-C illumination protocol for increasing *trans*-resveratrol content in grapes (50 W/m^2, 40 cm, 60 s) was similar on undamaged and damaged grapes, achieving 1.2 and 1.3 log conidia/100 g reductions, respectively. After 5 days of storage at 22 °C, UV-C treatment and the stilbenes induced by UV-C inhibited ochratoxigenic *Aspergillus* growth in undamaged grapes. UV-C elicited the biosynthesis of *trans*-resveratrol, while microbial infection and tissue damage triggered the biosynthesis of *trans*-piceid. *trans*-Resveratrol was not synthesized as a consequence of ochratoxigenic *Aspergillus* contamination. However, when *trans*-resveratrol was synthesized by UV-C, it contributed to inhibiting the development of ochratoxin A producing aspergilli. Furthermore, UV-C treatment also contributed to decrease ochratoxin A production by ochratoxigenic aspergilli. Therefore, UV-C is a promising emerging technology either for reducing the potential ochratoxigenic risk in grapes, which is of particular interest to the wine industry, and also for increasing *trans*-resveratrol content of grapes, which would provide an added value to the wine.

KEYWORDS: *Aspergillus carbonarius*; *Aspergillus tubingensis*; black aspergilli; mycotoxin; ochratoxin A; piceid; Resveratrol; Stilbenes.

INTRODUCTION

Ochratoxin A is a mycotoxin with nephrotoxic, carcinogenic, immunotoxic, genotoxic, and teratogenic effects (*1*). *Aspergillus* species belonging to section *Nigri*, commonly known as black aspergilli, have been identified as the main fungi responsible for ochratoxin A contamination in grapes and wine (*2*). *Aspergillus carbonarius* is considered the principle agent responsible for ochratoxin A contamination in grapes because the reported proportion of ochratoxin A producing strains in this species are higher than those reported for members of the *Aspergillus niger* aggregate. Nevertheless, the black aspergilli most frequently isolated from grapes are species belonging to the *A. niger* aggregate, mainly *A. tubingensis* and *A. niger* (*2, 3*).

In the European diet, grape products have been identified as the second major source of human exposure to ochratoxin A, after cereals (*4*). As a consequence, the European Commission has considered the need to impose regulatory limits and has established 2 ng/g as the maximum level of ochratoxin A in grape products (*5*). On the other hand, stilbenes in general, and *trans*-resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) in particular, which naturally occur in grapes (*6*) and wine (*7*), have been reported to be responsible for several beneficial effects related to their antioxidant (*8*), antimutagenic (*9*), anti-inflammatory (*10*), antiestrogenic (*11*), antiarrhythmic, cardioprotective (*12*), and cancer chemopreventive (*13*) properties. However, the content of *trans*-resveratrol in grapes is rather low (176 ± 235 μg/100 g fw) ranging from 0.1 to 667 μg/100 g fw depending on the variety (*14*).

Stilbenes can be constitutive compounds in the woody part of the plant or induced metabolites in soft tissues such as fruits and leaves. These phytochemical compounds, which act as part of the plant's defensive arsenal (phytoalexins), can be induced by biotic and abiotic elicitors (*15*). Previous studies have described the induction of resveratrol-related compounds in grapes as a response to infection of *Botrytis cinerea* (*16*), *Rhizopus stolonifer* (*17*), and ochratoxigenic aspergilli (*18*)

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10.1021/jf8018062 CCC: $40.75
Published on Web 10/09/2008

including *A. carbonarius* (*19*). Stilbenes can also be induced by abiotic elicitors such as ultraviolet-C (UV-C) illumination (*14, 20, 21*), ozone (*22*), methyl jasmonate as well as other chemicals (*23*). In particular, postharvest illumination with UV-C light has been proposed as a valuable method to increase the *trans*-resveratrol content of table grapes (*14, 20, 21*), wine grapes (*24*), and red wines (*7*) by induction of stilbenoid biosynthesis. Moreover, UV-C illumination has been extensively used in a broad range of antimicrobial applications, including surface disinfection of vegetable commodities (*25*).

The objective of this study was to investigate the efficacy of UV-C and induced stilbenes to inhibit ochratoxigenic *Aspergillus* and control ochratoxin A production in grapes. In addition, the induction of stilbene biosynthesis, as a response to abiotic stress such as UV-C and biotic stress (ochratoxin A producing strains) was studied.

MATERIALS AND METHODS

Fungal Strains and Growth Conditions. *A. carbonarius* CECT 2086 and *A. tubingensis* CECT 20543 were provided by the Spanish Type Culture Collection (CECT, Valencia, Spain). They were grown in potato dextrose agar (PDA, Scharlau Chemie S.A., Barcelona, Spain) plates at 25 ± 1 °C for 5−7 days. These two strain were selected after testing OTA production (*26*).

Plant Material. Grapes of the variety Napoleon were harvested at commercial maturity during November of 2007 in Murcia (Spain), transported to the laboratory, and stored at 5 °C until sanitization. Twelve kg of grapes without visible damage on their skin or visible microbial infection were sanitized for 2 min with a 100 mg/L total chlorine solution prepared from sodium hypochlorite (Panreac, Montcada i Reixac, Barcelona, Spain) adjusted to pH = 6.50 with HCl. Afterward, grapes were rinsed with sterile distilled water for 1 min and air-dried for 30 min in a biohazard safety cabinet. Sanitized grapes were divided into 264 berry bunches consisting of five berries per bunch of approximately 45 g per bunch. Berry bunches were separated into two groups. In one group, each grape was five times punctured with a sterile needle (damaged grapes), while grapes of the other group were maintained without damage (undamaged grapes). These berries were used in the experiment of artificial fungal inoculation and UV-C treatment described as follows.

Artificial Fungal Inoculation. For grape inoculation, a conidia cocktail of *A. carbonarius* and *A. tubingensis* strains (CECT 2086, CECT 20543) was used because these species are considered two of the main black aspergilli responsible for ochratoxin A contamination in grapes and wine (*2, 3*). Conidia suspension was prepared by flooding three plates (6 days old) of PDA (Scharlau Chemie S.A.) with 9 mL of sterile nanopure water containing 0.05% Tween 80 (Fluka Biochemika, Steinheim, Germany) and rubbing the surface with a sterile glass rod. The conidial suspension was filtered through Whatman paper no. 1, diluted in sterile nanopure water, as necessary, and quantified by plate count on PDA (Scharlau Chemie S.A.), and by microscopy, using a Neubauer counting chamber. The two suspensions obtained were adjusted to a concentration of 9×10^5 conidia/mL and quantified using a Neubauer counting chamber. Five mL of each strain suspension were mixed together for the conidia of both strains being homogeneously distributed and, subsequently, 10 mL of sterile nanopure water were added. The resulting inoculum concentration (4×10^5 conidia/mL) was determined by microscopy, using a Neubauer counting chamber, and by plating appropriate serial dilutions of the inoculum onto PDA and incubating the plates for 5−7 days at 25 °C. Each grape of the berry bunch was inoculated with 20 μL of 4×10^5 conidia/mL. After inoculation, spots were air-dried for 30 min in a biohazard safety cabinet. Afterward, inoculated and noninoculated grape bunches were stored for 1 h at 15 °C before UV-C treatment.

UV-C Treatment. Grape bunches were UV-treated as previously described (*14*). Grape berries were placed in plastic trays and illuminated with 17 G30T8 germicidal lamps (Sylvania, USA) (30 W each lamp, peak output at 254 nm). The treatment chamber was covered with a protective reflecting inner layer, which enhanced homogeneous distribution of the emitted light and allowed indirect illumination of practically all sides of the chamber. To determine the UV-C illumination intensity of the lamps, a VLX 254 radiometer (Vilber Lourmat, Marne la Vallée, France) was used. The applied UV-C intensity was calculated as a mean of 18 UV-C readings in each side of the net and the average was 6.0 ± 0.1 kJ/m^2. The standard illumination parameters used for the present study were illumination power of 50 W/m^2, illumination distance of 40 cm, and two illumination times of 60 s, with the grapes turned over after the first illumination. UV-C illumination took place at 15 °C.

Packaging and Storage. Berry bunches were placed onto polypropylene trays (165 mm × 165 mm × 60 mm). Wet filter paper was placed on the bottom of the trays and then covered with aluminum foil. Subsequently, they were sealed in air conditions with a perforated plastic film (OPP multilayer films, 1255 perforations/m^2, 35 μm thickness) at a relative humidity of approximately 90−95% to avoid water loss and shriveling during storage. Trays were identified as "non-inoculated", "inoculated", "inoculated then UV-treated" and "UV-treated then inoculated".

All grapes were stored for up to 5 days at 22 °C in order to enhance the stilbenes induction (*7, 14*). The parameter "maximum day" (D_{max}) was defined as the elapsed number of days to achieve the maximum *trans*-resveratrol concentration (*20*). Induction capacity (*IC*) was calculated as the ratio between stilbene content of UV-C treated and untreated grapes at D_{max} of stilbene content.

Quality Indexes. Titratable acidity (TA), pH, and soluble solids content (SSC) of the samples were evaluated as quality indexes. The TA was determined by tritating 10 mL of juice with 0.1 mol/L NaOH to pH 8.1 (*27*). The pH was measured using a pH meter and SSC by a digital temperature compensated Atago N1 hand-held refractometer (Tokio, Japan).

Microbial Analysis. Growth of inoculated ochratoxigenic aspergilli was followed during the experiment. Grapes were transferred into stomacher bags (Seeward Medical, London, UK) and homogenized using a stomacher (IUL Instrument, Barcelona, Spain) during 90 s. Inoculated aspergilli were enumerated by the standard plate count method using PDA (Scharlau Chemie S.A.) after incubation at 30 ± 1 °C for 5−7 days. Microbial counts were expressed as log conidia/100 g grape.

Extraction of Phenolic Compounds. Grapes were peeled with a sharp knife, and the skins were protected from exposure to light and frozen at −70 °C and then freeze-dried. The freeze-dried samples (0.5 g) were homogenized with an Ultraturrax T-25 (Janke and Kunkel, Ika-Labortechnick, Germany) with 10 mL of HPLC grade MeOH:formic acid (97:3, v/v) at 24000 rpm for 3 min. The extract was centrifuged at 5000 *g* for 5 min to obtain the supernatant and the pellet. The supernatant was filtered through a Millex-HV130 0.45 μm membrane filter (Millipore Corp., USA), and then analyzed by HPLC-DAD. The pellet obtained after centrifugation was extracted with 5 mL HPLC grade MeOH:formic acid (97:3, v/v) and sonicated in an ultrasonic bath for 15 min. The extract was centrifuged and filtered as described above and then analyzed by HPLC-DAD. Extracts were prepared in triplicate.

Identification and Quantification of *trans*-Resveratrol and *trans*-Piceid. Samples (50 μL) of the clear filtered extracts (supernatant and pellet described above) were analyzed by HPLC (Merck−Hitachi) equipped with a pump (model L-7100) and a photodiode array UV/vis detector (L-7455). Separations were achieved on a 250 mm × 4 mm i.d., 5 μm, reversed-phase LiChrocart C_{18} column (Teknokroma, Barcelona, Spain). The mobile phase was water with 5% formic acid (solvent A) and HPLC grade acetonitrile (solvent B) at a flow rate of 1 mL/min. Elution was performed with a gradient starting with 2% B in A to reach 32% B at 30 min, 40% B at 40 min, and 95% B at 50 min and then became isocratic for 5 min. Chromatograms were recorded at 320, 360, and 280 nm. *trans*-Resveratrol and *trans*-piceid were identified by their UV spectra recorded with a diode array detector by their MS spectra recorded with an ion trap MS-MS, and by chromatographic comparisons with resveratrol (Sigma, St. Louis, MO). Pure resveratrol (*m/z* 228) was used as stilbene standards and quantified at 320 nm (*14*). *trans*-Resveratrol and *trans*-piceid content was expressed

Table 1. Soluble Solids Content (SSC), Titratable Acidity (TA), and pH of UV-C Treated and Untreated Grapes at Day 0 and After 5 Days of Storage at 22 °C[a]

	Treatment	SSC (°Brix)	TA (%)	pH
Day 0	untreated	17.9 ± 2.8	0.41 ± 0.05	3.79 ± 0.12
Day 5	untreated	18.3 ± 1.2	0.34 ± 0.02	4.03 ± 0.10
	UV-C treated	17.6 ± 0.9	0.31 ± 0.01	4.04 ± 0.05
		ns	ns	ns

[a] Values are the mean of three replicates ± standard deviation. ns: no significant differences at $P < 0.01$.

as μg/100 g of fresh weight (fw) by considering the amount of these compounds in both supernatant and pellet.

Chromatographic separation was carried out as detailed above. The HPLC system equipped with DAD and mass ion trap detectors in series consisted of a HPLC binary pump (G1312A), an autosampler (G1313A), a degasser (G1322A), and a photodiode array detector (G1315B) controlled by software (v. A08.03) from Agilent Technologies (Waldbronn, Germany). The mass detector was an ion trap mass spectrometer (G2445A, Agilent Technologies) equipped with an electrospray ionization (ESI) system and controlled by software (v. 4.0.25). The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and daughter (MS-MS) spectra were meassured from *m/z* 100 to 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionization mode.

Extraction and Quantification Method of Ochratoxin A. Grapes were transferred into sterile filter stomacher bags (Seeward Medical) and crushed in a stomacher (IUL Instrument) during 120 s. A procedure described by Almela et al. (*28*) was followed for purification and isolation of ochratoxin A from grape juice. Briefly, 5 mL of juice sample was mixed with 5 mL of extraction buffer (5% $NaHCO_3$, 1% PEG). The mixture was vortexed for 3 min and then diluted with 40 mL of phosphate buffered saline (pH 7.2). Diluted extract (50 mL) was passed through an OtaCLEAN immunoaffinity column (LCTech GmbH, Dorfen, Germany) by a three-channel peristaltic pump. An Acrodisc glass fiber membrane (1 μm) (Pall Life Sciences, MI) was placed into each channel for removing precipitations. Afterward, the column was washed with 10 mL of distilled water and the residual water was removed from the column. Finally, ochratoxin A was eluted with 1 mL of methanol and directly analyzed by HPLC. The HPLC system consisted of a Shimadzu (series LC-10AD *vp*) equipped with an RF-10A spectrofluorimetric detector (Shimadzu Europe, Duisburg, Germany) and a chromatographic software. A 250 mm × 4.6 mm i.d., 5 μm, Supelcosil LC-18 column (Supelco, Bellefonte, PA) was used. One hundred μL of the purified ochratoxin A extract was injected and eluted using an isocratic mobile phase consisting of acetonitrile−water−acetic acid (55:44:1) at a flow rate of 1 mL/min. The detector was set at excitation and emission wavelength of 333 and 460 nm, respectively. Ochratoxin A (Sigma-Aldrich Quı´mica, Madrid, Spain) was used as standard, and quantification was on the basis of peak areas using the respective PC software (*28*).

Statistical Analysis. Two repetitions were carried out. Results from one of the experiments are presented, although similar tendency was observed in both cases. Microbial data represent the mean of five replicates per treatment and sampling date, while phenolic and ochratoxin A content data represent the mean of three replicates per treatment and evaluation period, respectively. Each replicate consists of a five berry bunch. Analysis of variance (ANOVA), followed by Tukey's test with a significance level of $P \leq 0.05$ was carried out on these data using SPSS (Windows 2000, Statistical Analysis).

RESULTS AND DISCUSSION

Quality Indexes. Quality indexes such as SSC, pH, and TA were analyzed to determine the initial maturity, quality level, and to detect possible changes resulting from the UV-C treatment and the storage conditions (**Table 1**). SSC, pH, and TA of untreated grapes were well preserved after storage. UV-C treatment did not influence negatively the quality characteristics of the grapes when compared to untreated berries.

Effect of UV-C on Survival and Growth of Ochratoxigenic *Aspergillus* Inoculated on Grapes. Black *Aspergillus* infection was not observed in the grapes before inoculation. However, after inoculation with *A. carbonarius* and *A. tubingensis* cocktail, black *Aspergillus* population was 3.4 and 3.8 log conidia/100 g for undamaged and damaged grapes, respectively. In inoculated grapes, the level of black *Aspergillus* was maintained during 5 days of storage in undamaged grapes (**Figure 1A**) while it was increased in damaged grapes, achieving counts of 5.9 log conidia/100 g (**Figure 1B**). These results support the importance of a good preharvest and postharvest management to avoid injury on the grape surface, preventing the internalization and growth of ochratoxigenic *Aspergillus.* These observations are in agreement with the results of a previous study in which development of *A. carbonarius* was higher in damaged grapes than in undamaged ones (*29*).

In inoculated then UV-treated grapes, counts of black *Aspergillus* immediately after UV-C treatment were 2.2 and 2.5 log conidia/100 g for undamaged and damaged berries, respectively (**Figure 1**A,B). It was clearly shown that the initial microbial inactivation by UV-C treatment was similar on undamaged and damaged grapes, achieving 1.2 and 1.3 log conidia/100 g reductions, respectively, when compared to the inoculated grapes. However, after 5 days of storage, growth of ochratoxigenic *Aspergillus* on inoculated then UV-treated grapes was only observed on damaged berries, which allowed *Aspergillus* penetration and development, achieving levels of 5.6 log conidia/100 g (**Figure 1B**). These results are in agreement with previous studies that shown black *Aspergillus* spp. appear to be secondary invaders infecting grapes damaged by preharvest factors such as rain, other fungal species, insects, and mechanical impact (*29, 30*). Furthermore, the higher availability of nutrients in damaged grapes would be expected to enhance the survival and growth of ochratoxigenic *Aspergillus.* In undamaged grapes, the level of ochratoxigenic *Aspergillus* on inoculated then UV-treated grapes was reduced during storage, achieving levels of 0.6 log conidia/100 g after 5 days at 22 °C (**Figure 1A**). This reduction indicated the existence of a UV-C sublethal damage on the treated conidia, which were progressively inactivated during 5-day storage at 22 °C. Therefore, the reduction of inoculated fungal levels on UV-C treated grapes could be the consequence of an inability to repair DNA damage.

Illumination of grapes before inoculation was also carried out to investigate the potential of UV-C-induced resistance to ochratoxigenic *Aspergillus.* In undamaged grapes, levels of ochratoxigenic *Aspergillus* were maintained initially and during storage in those UV-treated then inoculated grapes (**Figure 1A**). These results could suggest that induced *trans*-resveratrol and *trans*-piceid were not effective against ochratoxigenic *Aspergillus* inoculated in grapes just after treatment and stored at 22 °C for 5 days. However, we can not be sure of their ineffectiveness against ochratoxigenic *Aspergillus* if contamination occurs two or more days after *trans*-resveratrol and *trans*-piceid induction. Indeed, in a previous work, it has been shown that the fungal resistance of UV-C treated tomatoes stored at 13 °C was developed gradually from 3 days after treatment, reaching a maximum at around 10−12 days after treatment and remaining effective throughout the storage period of 35 days (*31*). UV-C induced compounds could be effective at higher concentrations as

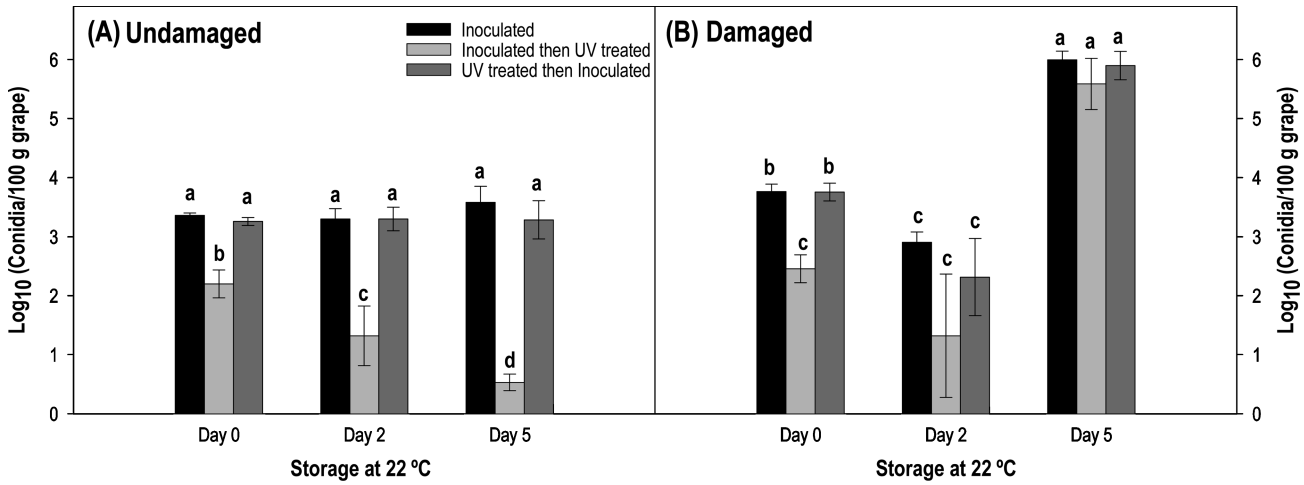

Figure 1. Effect of UV-C on survival and growth of ochratoxigenic *Aspergillus* inoculated on undamaged (A) and damaged (B) grapes during 5 days of storage at 22 °C. Values are the mean of five replicates of five grapes, and error bars represent the standard deviation. Significant differences at $P < 0.01$ are represented by different letters.

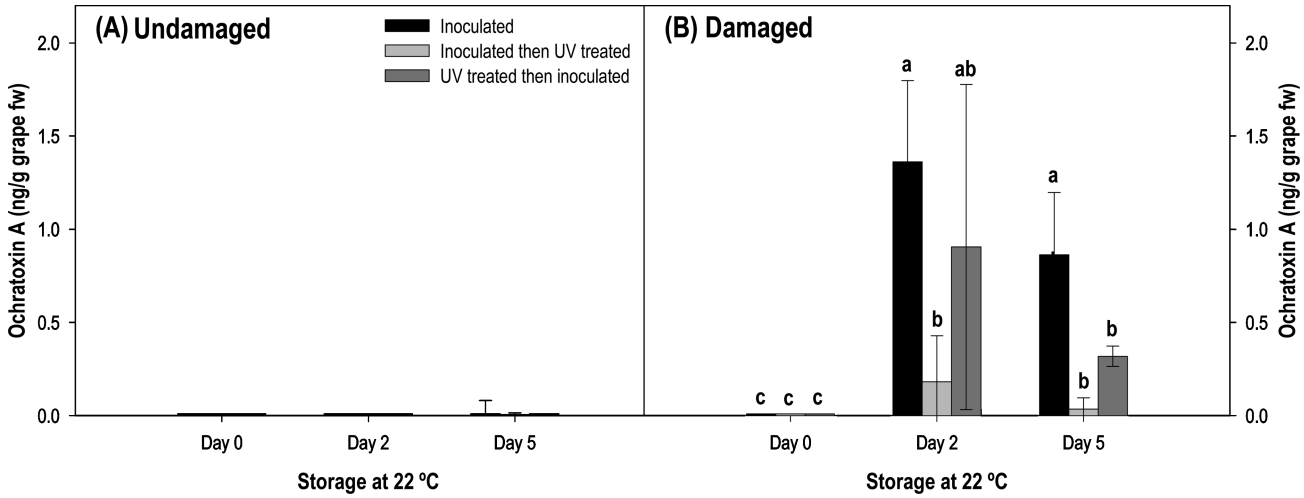

Figure 2. Effect of UV-C for controlling ochratoxin A production on undamaged (A) and damaged (B) grapes during 5 days of storage at 22 °C. Values are the mean of three replicates of five grapes, and error bars represent the standard deviation. Significant differences at $P < 0.01$ are represented by different letters.

has previously been indicated in other fruits (*31, 32*). More research should be carried out to investigate the effectiveness of *trans*-resveratrol induction in grapes for fungal inhibition.

Effect of UV-C on Ochratoxin A Accumulation in Grapes Inoculated with Ochratoxigenic *Aspergillus*. As was expected, ochratoxin A was not detected in noninoculated grapes during 5 days of storage at 22 °C. However, after inoculation with the *A. carbonarius* and *A. tubingensis* cocktail, the ochratoxin A level increased during storage reaching the maximum level at day 2 and achieving a concentration of 1.4 ng/g in damaged grapes (**Figure 2**). Ochratoxin A content was higher in damaged grapes than in undamaged ones, in agreement with a previous study (*29*). In undamaged grapes, after 5 days of storage at 22 °C, ochratoxin A was not detected in treated berries (**Figure 2A**). In damaged grapes, production of ochratoxin A was almost totally inhibited in inoculated then UV treated berries, achieving levels lower than 0.04 ng/g after 5 days of storage (**Figure 2B**). Moreover, in UV treated then inoculated grapes, there was a reduction in OTA accumulation when compared to nontreated berries, reaching levels of 0.3 ng/g after 5 days of storage (**Figure 2B**). However, we can not confirm that this reduction was the consequence of trans-resveratrol induction rather than the metabolism of ochratoxin A by the fungi.

Effect of UV-C Treatment and Fungal Infection on Phenolic Content. The main interest of the polyphenol analysis was to compare the phenyl propanoid metabolism induction by abiotic (UV-C) and biotic elicitors (fungal infection) on undamaged and damaged grapes. The content of *trans*-resveratrol in noninoculated and inoculated grapes was similar and maintained at the same level during 5 days of storage (**Figures 3**, **4**). In addition, *trans*-resveratrol content did not increase in damaged grapes when compared to undamaged ones either at day 0 or after storage (**Figure 3**). Similarity in the content of *trans*-resveratrol between noninoculated and inoculated grapes was observed despite the ochratoxigenic *Aspergillus* levels in damaged berries (5.9 Log conidia/100 g). These results suggest that *trans*-resveratrol was not elicited by ochratoxigenic aspergilli. However, in a previous study, it has been shown that *trans*-resveratrol is accumulated in *A. carbonarius* inoculated grapes at veraison time but not during ripening (*18*). Moreover, *trans*-resveratrol formation decreases from veraison to ripening in berries elicited by biotic (ochratoxigenic aspergilli) and abiotic (UV-C) factors (*18, 33*). Despite the commercial maturity stage of the grapes, a significant increase in the peak corresponding to *trans*-resveratrol was observed as a consequence of the UV-C treatment without significant differences between inoculation before or after UV-C treatment (**Figure 3**). *trans*-Resveratrol was the main induced stilbene after UV-C

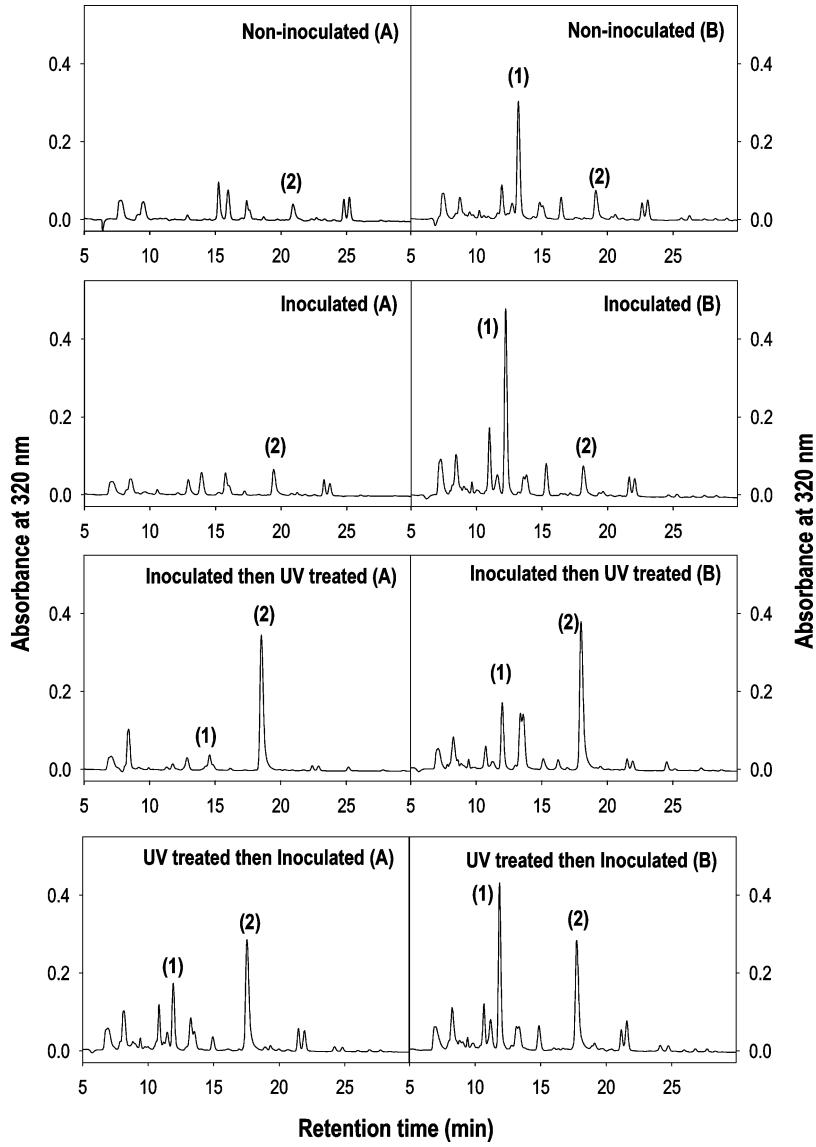

Figure 3. HPLC chromatograms at 320 nm of undamaged (A) and damaged (B) skin extracts of grapes stored 2 days at 22 °C. (1) *trans*-piceid; (2) *trans*-resveratrol.

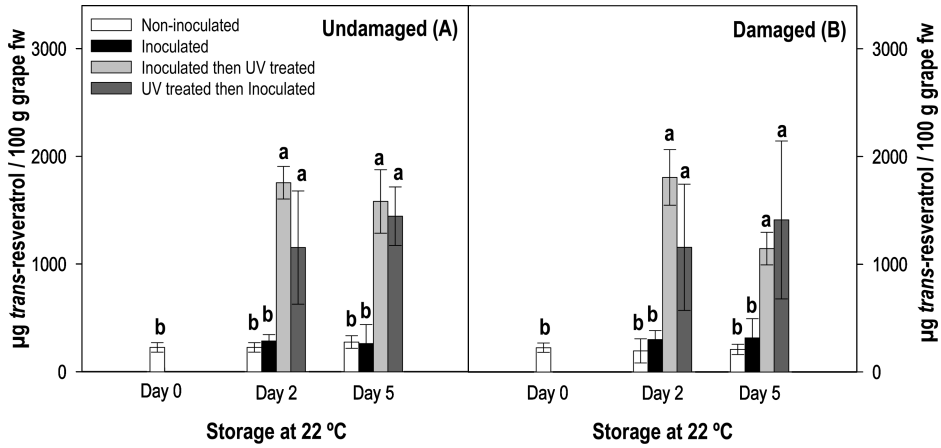

Figure 4. Effect of UV-C and fungal infection on the content of *trans*-resveratrol in undamaged (A) and damaged (B) grapes during 5 days of storage at 22 °C. Values are the mean of three replicates of five grapes, and error bars represent the standard deviation. Significant differences at $P < 0.01$ are represented by different letters.

illumination (**Figure 3**). This is in agreement with a previous study where UV-C induced stilbenes were assayed on red and white table grape varieties, including Napoleon (*14*). In the present study, content of *trans*-resveratrol induced during cultivation by sunlight was 226 μg/100 g in Napoleon grapes before UV-C treatment. Similar contents were shown in the previously mentioned study (*14*). However, the induction capacity of *trans*-resveratrol in the present study was 8-fold (**Figure 4A,B**) compared with 4.4-fold

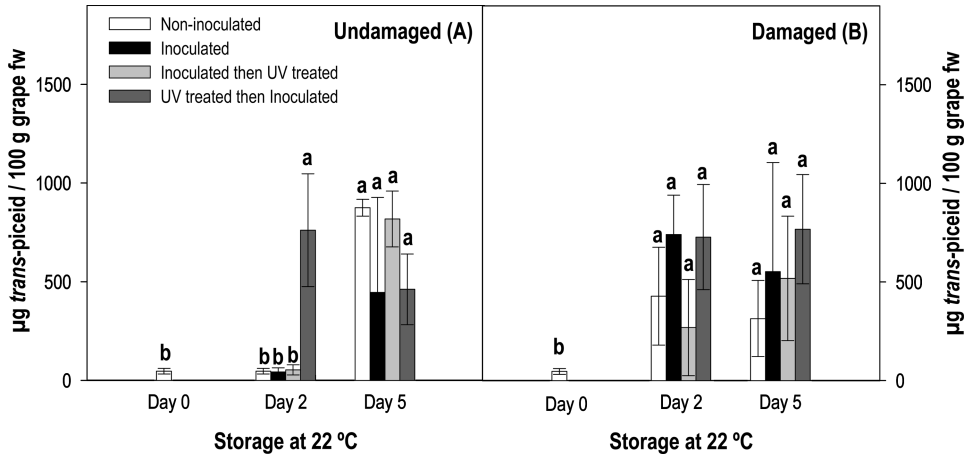

Figure 5. Effect of UV-C and fungal infection on the content of *trans*-piceid in undamaged (A) and damaged (B) grapes during 5 days of storage at 22 °C. Values are the mean of three replicates of five grapes, and error bars represent the standard deviation. Significant differences at $P < 0.01$ are represented by different letters.

in the previous study (*14*). As **Figure 4** shows, the response intensity of grapes to UV-C treatment was time dependent. Indeed, the accumulation of *trans*-resveratrol was significantly enhanced by storage time ($P < 0.001$) and by UV-C treatment ($P < 0.001$) as well as by the combination of both ($P < 0.001$) (**Figure 4**). In a previous study, the *trans*-resveratrol content in Napoleon grapes starts to increase 2 days after UV-C treatment and reaches D_{max} by day 5 and declines thereafter (*14*). In contrast, in the present study, the induction of *trans*-resveratrol in Napoleon started after UV-C treatment achieving D_{max} at day 2 as occurred with table grapes of the variety "Superior" (*22*). In the present study, levels of *trans*-resveratrol content were maintained throughout 5 days of storage (**Figure 4**). As a result, *trans*-resveratrol content was 1755 and 1807 μg/100 g for undamaged and damaged grapes, respectively (**Figure 4A,B**). These *trans*-resveratrol contents were higher than that reported previously for Napoleon variety (1149 μg/100 g) (*14*). In damaged grapes, *trans*-resveratrol accumulation was not able to avoid black aspergilli development. In fact, in a previous work, it was reported that *trans*-resveratrol was only able to inhibit *A. carbonarius* growth at concentration 3×10^5 μg/100 g in grape must media (*18*).

Viniferin was detected in all the grape samples, but it was not quantified as it was only present in trace amounts in most of grape samples. When other stilbenes such as *trans*-piceid (*trans*-resveratrol-3-*O*-β-glucoside) were evaluated, no differences in their content were observed between noninoculated and inoculated grapes (**Figure 5**). Therefore, inoculation with ochratoxigenic *Aspergillus* did not specifically elicit the accumulation of the *trans*-piceid. However, there was a significant increase ($P < 0.001$) in the accumulation of *trans*-piceid during storage, particularly faster in damaged grapes ($D_{max} = 2$) than in undamaged berries ($D_{max} = 5$) (**Figure 5A,B**). Therefore, *trans*-piceid accumulation in UV-C untreated grapes could be a consequence of the total microbial contamination rather than a specific response to ochratoxigenic *Aspergillus.* This is probably the result of severe stress induced by both total microbial infection and tissue damage. In fact, high counts of total mesophilic bacteria as well as total molds and yeasts were observed independently of the treatment and the skin damage (data not shown). On the other hand, in undamaged grapes, *trans*-piceid increase as a response to the microbial infection was faster in UV-C-treated ($D_{max} = 2$) than in untreated ($D_{max} = 5$) grapes (**Figure 5A**). Therefore, the piceid accumulation was evident earlier in UV-C treated grapes than in nontreated berries. This agrees with a previous study in which rishitin accumulation as a response to *B. cinerea* was faster in UV-C treated tomatoes than in untreated ones (*31*). *trans*-Piceid has been detected in several grape varieties (*14*). However, its content was only induced by UV-C in some grape varieties such as Moscatel, Flame, Tempranillo, Syrah, Garnacha, and Cariñena, achieving a maximum concentration of 162 μg/100 g fw (*14, 24*). In the present study, *trans*-piceid concentration reached a maximum content of 1247 μg/100 g of grapes. This is in contrast with a previous study where *trans*-piceid contents in Napoleon variety were 236 and 330 μg/100 g fw before and after UV-C treatment, respectively (*14*). The induction of *trans*-piceid content in the present study in relation to the previous study could be the result of differences in cultivar conditions. Previous studies indicated that higher levels of piceid are found in grape berry skins of cultivars grown in warm and dry regions (*24*). The warm climatic conditions favor microbial development and ochratoxigenic fungi contamination (*34*). These results support our findings, which suggest that *trans*-piceid formation is related to the microbial infection but not specifically to ochratoxigenic fungi contamination. *trans*-Resveratrol was not accumulated as a consequence of black aspergilli contamination. However, when it was synthesized by other elicitors, it contributed to inhibiting the development of black aspergilli. It is difficult to explain the different capabilities of the biotic and abiotic stress to elicit *trans*-resveratrol synthesis in ripe grapes. We can only speculate that in ripe grapes, UV-C elicits the biosynthesis of *trans*-resveratrol as a result of an increased transcription of genes encoding the stilbene-synthase, while fungal contamination triggers stilbene-synthase but also the transcription of genes encoding the glycosyl-transferase which converts *trans*-resveratrol to *trans*-piceid. The antimicrobial activity of this glucoside is higher than its aglycone resveratrol (*35*). Therefore, stilbene biosynthesis elicitation could take place in the field during the grape cultivation due to abiotic elicitors such as sunlight exposure but also due to biotic elicitors such as microorganisms, especially in regions where grape cultivars are grown in warm climatic conditions. A primary focus for continuing research is the development of vineyard management strategies to reduce the incidence of black *Aspergillus* spp., in particular *A. carbonarius*, in grape bunches. Postharvest application of UV-C to grapes is a promising emerging treatment either for reducing the potential ochratoxigenic risk in grapes and also for increasing *trans*-resveratrol content of wine, which would provide an added value.

ACKNOWLEDGMENT

The technical assistance of Mercedes Almagro is gratefully acknowledged.

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Received for review June 12, 2008. Revised manuscript received September 2, 2008. Accepted September 4, 2008. We are grateful to Spanish CICYT (Comisión Interministerial de Ciencia y Tecnología) project AGL2007-65056 for financial support and M.V.S. to the CSIC (Spanish National Research Council) for the I3P contract (I3PDR-7-01).

JF8018062