JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Lime-Induced Leaf Chlorosis on Ochratoxin A, *trans*-Resveratrol, and ε-Viniferin Production in Grapevine (*Vitis vinifera* L.) Berries Infected by *Aspergillus carbonarius*

LUIGI BAVARESCO,*,† SILVIA VEZZULLI,† SILVIA CIVARDI,† MATTEO GATTI,† PAOLA BATTILANI,‡ AMEDEO PIETRI,§ AND FEDERICO FERRARI#

Istituto di Frutti-Viticoltura, Istituto di Entomologia e Patologia Vegetale, Istituto di Scienze degli Alimenti e della Nutrizione, and Istituto di Chimica Agraria e Ambientale, Università Cattolica del Sacro Cuore, 29100 Piacenza, Italy

Berries of *Vitis vinifera* L. cv. Merlot, grown on a neutral or calcareous soil, were infected, at phenological phases of veraison and ripening, by a conidial suspension of *Aspergillus carbonarius* to control ochratoxin A production and *trans*-resveratrol- and ε-viniferin-induced synthesis as affected by the soil lime content. Chlorosis occurrence was evaluated by a visual rating scale at veraison, and the leaves from vines growing on the calcareous soil showed the typical yellowing, whereras those grown on the neutral soil were dark green. Berry mineral element yield was recorded at veraison and ripening. Infection symptoms on berries were more severe at ripening in bunches collected from vines grown in calcareous soil. Ochratoxin A concentration increased at phenological phase of veraison in berries harvested from vines cultivated in calcareous soil. *A. carbonarius* enhanced *trans*-resveratrol and ε-viniferin production in infected berries more than in the control samples. Moreover, at veraison their concentration in the berries collected from vines grown in calcareous soil was greater than that recorded from berries collected from vines grown in the neutral soil. The lowest symptom severity was observed on berries containing the highest copper concentration.

KEYWORDS: ***Vitis vinifera* L.; iron chlorosis; soil; *Aspergillus carbonarius*; ochratoxin A; resveratrol**

INTRODUCTION

Many cultivated perennial plants, including grapevine, do not tolerate calcareous soils because of their low degree of lime resistance (*1*). High lime levels in the soil are responsible for iron deficiency in the plant, usually recognized by yellow interveinal areas (chlorosis) in young leaves. The responses of sensitive fruit trees, including grapevines, to lime stress conditions include also shoot and root growth reduction and yield shortage (*2–4*). In Italy, lime-induced chlorosis is an abiotic disease that affects 50% of the viticultural area (~350,000 ha), the soil total carbonate concentration being >10%, especially in the southern part of the country where ochratoxin A-producing aspergilli are more widespread (*5*).

Since 1996, the occurrence of ochratoxin A has been detected in wine and grape juice (*6, 7*). The potential inoculum of ochratoxin A producers comes from the field mycoflora; aspergilli are dominant with respect to penicillia, and among these aspergilli section Nigri. *Aspergillus carbonarius* plays the main role because of the high percentage of positive strains that produced high amounts of ochratoxin A (*5, 8–11*). Ochratoxin A is a carcinogenic toxin in rodents, and it elicits teratogenic, immunotoxic, neurotoxic, nephrotoxic, hepatotoxic, and potentially cancerogenic properties. Jeswal (*12*) 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, an action possibly related to stilbene (such as *trans*-resveratrol) presence.

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. Stilbenes can be constitutive (in the woody parts of the plant) or induced (in soft tissues such as leaves and fruit) compounds; in the latter case they act like phytoalexins, being induced by biotic and abiotic elicitors (*13*). Stilbenes, as induced compounds, are related to vine resistance against pathogens such as *Botrytis cinerea* (*14–16*), *Plasmopara viticola* (*17*), *Uncinula necator* (*18*), and *Rhizopus stolonifer* (*19*); according to the

* Corresponding author (telephone +39 0523 599267; fax +39 0523 599268; e-mail luigi.bavaresco@unicatt.it).
† Istituto di Frutti-Viticoltura.
‡ Istituto di Entomologia e Patologia Vegetale.
§ Istituto di Scienze degli Alimenti e della Nutrizione.
# Istituto di Chimica Agraria e Ambientale.

10.1021/jf073456+ CCC: $40.75
Published on Web 02/22/2008

findings of Bavaresco et al. (*10*) also some ochratoxin A-producing aspergilli can induce stilbene synthesis at berry level. Stilbenes can also be induced by bacteria (*20*) and abiotic elicitors such as UV irradiations, aluminum chloride, fosetyl-Al, ozone, methyl jasmonate, and other chemicals (*21, 22*). 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 (*23–26*). During alcoholic and malolactic fermentation, stilbenes are extracted from the berry skins into the wine, and this process is affected by enological practices (*27, 28*). Within the stilbenes, resveratrol is deserving of interest because it is claimed to be the active principle in red wines shown to reduce heart disease (*29*) and to have other beneficial biological functions (*30, 31*).

The aim of this work was to investigate the effect of lime-induced chlorosis on the severity of *A. carbonarius* infection and on the production of ochratoxin A and two stilbenes (*trans*-resveratrol and ε-viniferin) in berries collected at two phenological phases.

MATERIALS AND METHODS

Soil and Plant Material. *V. vinifera* L. cv. Merlot, clone R3, grafted on the hybrid rootstock *Vitis riparia* Michx. × *Vitis rupestris* Scheele 3309 C (a lime-sensitive rootstock) was potted (pot volume = 45 L) in a noncalcareous and a calcareous soil. Before the addition of basic nutrients (5 g of N/pot; 1.7 g of P_2O_5/pot; 7.2 g of K_2O/pot; 0.6 g of MgO/pot), the main soil characteristics (*32*) were as follows (noncalcareous vs calcareous soil, respectively): pH of 7.3 and 8.2, 3 and 67% total carbonates, 1.5 and 16.5% active lime, 38 and 6 mg/kg Olsen P, 80 and 14 mg/kg Fe. The pots were placed outside, on a platform covered with a hail-protection net, and water was supplied by drip irrigation, to keep the soil near field capacity. The experimental plan was provided for 15 plants per soil type. At the end of the second growth year, each vine was pruned with shoot positioning (nine shoots per vine). Data reported were recorded during the third year of growth.

Leaf Chlorosis Rating. This rating was evaluated according to the scale of Pouget and Ottenwälter (*33*), ranking from 0 (dark green leaves) to 5 (serious symptoms with blade necrosis), about 100 days after bud burst, corresponding to the phenological phase of "veraison", which is the time when the berry skin color turns red, on the fourth and fifth leaf from the shoot tip of all the shoots per each vine. Those leaves were chosen because iron chlorosis affects the young leaves, whereas the old ones (placed in the basal part of the shoot) are not affected.

Berry Analyses. At veraison and ripening phenological phases, 15 bunches from vines growing in each of the two soils were collected. The main constituent parameters of the berries, before inoculation, were as follows: at veraison, pH 2.87, soluble solids 12.0 °Brix in the neutral soil, and pH 2.67, soluble solids 11.1 °Brix in the calcareous soil; at ripening, pH 3.46, soluble solids 23.4 °Brix in the neutral soil, and pH 3.65, soluble solids 23.7 °Brix in the calcareous soil. The berries were also analyzed to detect macronutrients and trace elements, after wet digestion (H_2SO_4/H_2O_2) of the oven-dried material (70 °C, 3 days), by colorimetry (N, P, B), flame photometry (K, Ca), and atomic absorption spectrometry (Mg, Fe, Mn, Cu, Zn), according to the method of Cottenie (*32*).

Inoculation of Berries. Twelve groups of 30 berries (per each phenological phase) were prepared detaching randomly, from 30 bunches, berries without visible damage on their skin or visible microbial infection. These berries were used in an experiment of artificial inoculation, according to a three-factor randomized complete block design with factors B and C as split plots on factor A: (A) phenological phase (1, veraison; 2, ripening); (B) soil type (1, neutral; 2, calcareous); (C) fungal infection (1, control; 2, *A. carbonarius* [MPVP-A566]). The fungus used in this trial belongs to the fungal collection of the Institute of Entomology and Plant Pathology of the Università Cattolica del Sacro Cuore.

All of the berries were disinfected with a 2% sodium hypochlorite solution for 2 min, rinsed twice with sterile distilled water, and punctured with a sterile needle before inoculation. The berries to be inoculated were dipped into 100 mL of a conidial suspension for 5 min. Inoculum was prepared by fungal development in Petri dishes (diameter = 6 cm) with Czapek yeast agar (*34*) for 7 days at 25 °C, 12 h of day light, 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. After inoculation, berries were transferred onto a sterile grate into a box prepared as a moist chamber (paper abundantly wet with sterile distilled water on the bottom of the box that was put into a sterile plastic bag). The box was incubated for 7 days at 25 °C. Punctured but not inoculated berries were utilized as control.

At the end of incubation, berries with infection symptoms were counted to quantify the incidence of visible fungal colonisation; results were represented as percentage of berries with visible molds. Afterward, all of the berries 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 two subgroups to detect *trans*-resveratrol and ε-viniferin and ochratoxin A concentrations.

Standards. The *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene) and ochratoxin A purchased from Sigma (St. Louis, MO) were used as standards; ε-viniferin (dimer of *trans*-resveratrol) was kindly supplied by G. Hoos (formerly BAZ, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany). The purity of each stilbene was controlled by HPLC, and the identity was confirmed according to the method of Mattivi et al. (*27*). 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 (*35*). After calibration of the ochratoxin A solution, working standards were prepared by evaporating an exact volume under a stream of nitrogen and dissolving again the residue in the mobile phase.

Extraction of Ochratoxin A. Five grams of berries was crushed in a polyethylene bag, and then 20 mL of a water solution containing PEG 8000 (10 g/L) and sodium hydrogen carbonate (50 g/L) was added. The mixture was homogenized for 30 min in a rotary shaker, filtered through a no. 595 folded filter paper (S&S) into a 50 mL graduated cylinder, and the volume was measured. The extract was centrifuged at 6400*g* for 10 min and immediately filtered again through a Millipore HATF 0.45 μm filter placed on a vacuum flask. 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. Ochratoxin A extraction from the berries was performed just before inoculation and 7 days later.

Extraction of Stilbenes. *trans*-Resveratrol and ε-viniferin were extracted from the berries according to the method of Bavaresco et al. (*16*). About 10 g of fresh berries were crushed in a mortar and poured in a 250 mL flask. Thirty milliliters of methanol/water (95:5; v/v) was added, and the mixture was vigorously shaken for 20 min by mixer, at room temperature. Seeds were discarded before maceration to avoid extraction of constitutive stilbenes. A filtration through GF/A Whatman filters followed, 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 sodium hydrogen carbonate (5%) by phase partitioning. The organic phases were collected and evaporated in vacuo to dryness and recovered by 2 × 1 mL of methanol (100%) and stored in adiactinic glass vials at −18 °C. Stilbene extraction from the berries was done just before inoculation and 7 days later.

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 470 nm emission. The system was controlled by 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/acetic acid (2%) for ochratoxin A (43:57; v/v) and for ochratoxin A methyl ester (55:45; v/v), at 1.2 mL/min. The injection volume was 30 μL. For the stilbenic compounds, the HPLC

Table 1. Effect of Soil Type, Fungal Infection, and Phenological Phase on the Berry Tested Parameters[a]

		symptoms (%)	ochratoxin A (μg/kg of FW)	*trans*-resveratrol (μg/g of FW)	ε-viniferin (μg/g of FW)
soil type	neutral	29 a	30.5 a	1.24 a	0.60 a
	calcareous	34 b	94.9 b	1.46 a	0.98 a
fungal infection	control	0 a	0.0 a	0.29 a	0.04 a
	A. carbonarius	86 b	125.5 b	2.41 b	1.55 b
phenological phase	veraison	20 a	90.3 a	1.50 a	1.36 a
	ripening	45 b	35.2 b	1.21 a	0.23 b

[a] Each value is the mean of 12 data. Values in each column without the same letters are significantly different ($P \leq 0.05$)

system consisted of an Agilent HP 1100 series (Waldbronn, Germany) with an autosampler and diode array detector (DAD) set at 306 and 325 nm. A 250 × 4.6 mm i.d., 5 μm, C 18 Supelco Supelcosil ABZ plus column was used, eluting with a gradient of methanol (A) and 0.01 M potassium phosphate monobasic adjusted to pH 2.5 with phosphoric acid (B). The gradient was from 40 to 85% of A at a flow rate of 1.0 mL/min. The instrumental limit of detection was 0.05 mg/L. The injection volume was 50 μL.

Quantification of Ochratoxin A and Stilbenes. 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 (*7*). Amounts of *trans*-resveratrol and ε-viniferin standard between 1 and 500 ng were injected. For both 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. Ochratoxin A and stilbene data reported in the tables and figure are the difference between the values recorded 7 days after the inoculation and the value (if any) recorded just before the treatment.

Statistical Analysis. A three-way ANOVA with interactions was utilized (Statgraphics, Statistical Graphics Corp.). Soil type, fungal infection, and phenological phase were considered to be main effects. Means were compared by using the LSD test (5% level). Before analysis, arcsin transformation was applied to percentage data.

RESULTS

The soil significantly ($P \leq 0.05$) affected the chlorosis occurrence in the leaves, being dark green (rating = 0) in the neutral soil and light yellow (rating = 1.42) in the calcareous soil. Visible symptoms on grape berries inoculated with *A. carbonarius* were significantly influenced by all of the factors considered in the study. Berries were more susceptible to fungal infection when grown on calcareous soil, when infected as compared to the control, as expected, and when they were ripe (**Table 1**). The relationship among the three main effects is represented in **Figure 1A**; the effect of the soil type was more evident at veraison than at ripening.

Ochratoxin A content varied with soil type, fungal treatment, and phenological phase (**Table 1**). The calcareous soil induced significantly higher ochratoxin A production than the neutral, whereas no ochratoxin A was detected, as expected, in the uninoculated berries, being 125.5 μg/kg of fresh weight (FW) in those infected. At veraison more toxin was produced than at ripening. The relationship among the three main effects is represented in **Figure 1B**; the effect of the soil was significant only at veraison, the toxin content in the calcareous soil being much higher than in the other one.

Trans-Resveratrol berry concentration was significantly affected by the fungal infection (**Table 1**), being 0.29 μg/g of FW in the control and 2.41 μg/g of FW in the infected berries. The effect of the soil type in the inoculated berries (**Figure 1C**) was different depending on the phenological phase: at veraison the calcareous soil induced a higher level of the compound than the neutral soil, whereas during ripening the opposite occurred.

The berry concentration of ε-viniferin was significantly affected by the fungal infection and the phenological phase (**Table 1**), being higher in the infected berries than in the control and at veraison higher than at ripening. At ripening no significant differences were observed (**Figure 1D**), whereas at veraison the calcareous soil induced higher ε-viniferin levels than the neutral soil.

Low amounts of *trans*-resveratrol and ε-viniferin were also recorded in the uninoculated berries.

Mineral element berry concentrations as affected by the soil type and the phenological phase are reported in **Table 2**. The calcareous soil significantly reduced, at ripening, the berry concentrations of phosphorus and potassium, whereas at veraison the berry concentration of copper was reduced. The maturation degree did not significantly affect the macronutrient concentrations in both soil types, whereas copper level significantly decreased from veraison to ripening in the berries of the vines grown in the neutral soil.

DISCUSSION

This paper reports for the first time the role of a calcareous soil on the degree of *A. carbonarius* berry infection and ochratoxin A synthesis, whereas evidence has already been obtained on the role of the soil lime (*24*) and *A. carbonarius* (*10*) on stilbene concentration in grapes. The highest symptoms of the fungal colonization and the lowest ochratoxin A concentration at ripening confirm previous findings (*10*).

Data concerning the berry copper concentrations and the visible symptoms are very interesting to discuss, because the two parameters seems to be negatively related. Although resistance and tolerance are genetically controlled, they are considerably influenced by environmental factors such as mineral nutrition (*36–38*). According to Graham (*39*) copper can improve disease resistance as it is involved in lignin and other phenolic synthesis.

The increased incidence of infected berries under lime stress conditions might be related to an impaired plant secondary metabolism, making the vine more susceptible to diseases; the physiological mechanisms were not investigated in the present research, but we can assume that the nutritional disorder affects, by means of the mineral nutrion of the vine, the balance between primary and secondary metabolic pathways. The chlorosis occurrence significantly influences the fungus metabolism, the ochratoxin A increase being more than proportional to symptom enhancement.

With respect to the stilbenes, the effect of the soil on *trans*-resveratrol concentration of uninoculated berries was the same as reported by Bavaresco et al. (*24*); lime stress conditions increased, in fact, the level of the stilbenic compound of the control berries, especially at ripening. Infected berries reacted to the soil conditions in a different way, depending on the phenological phase. At veraison, the higher *trans*-resveratrol

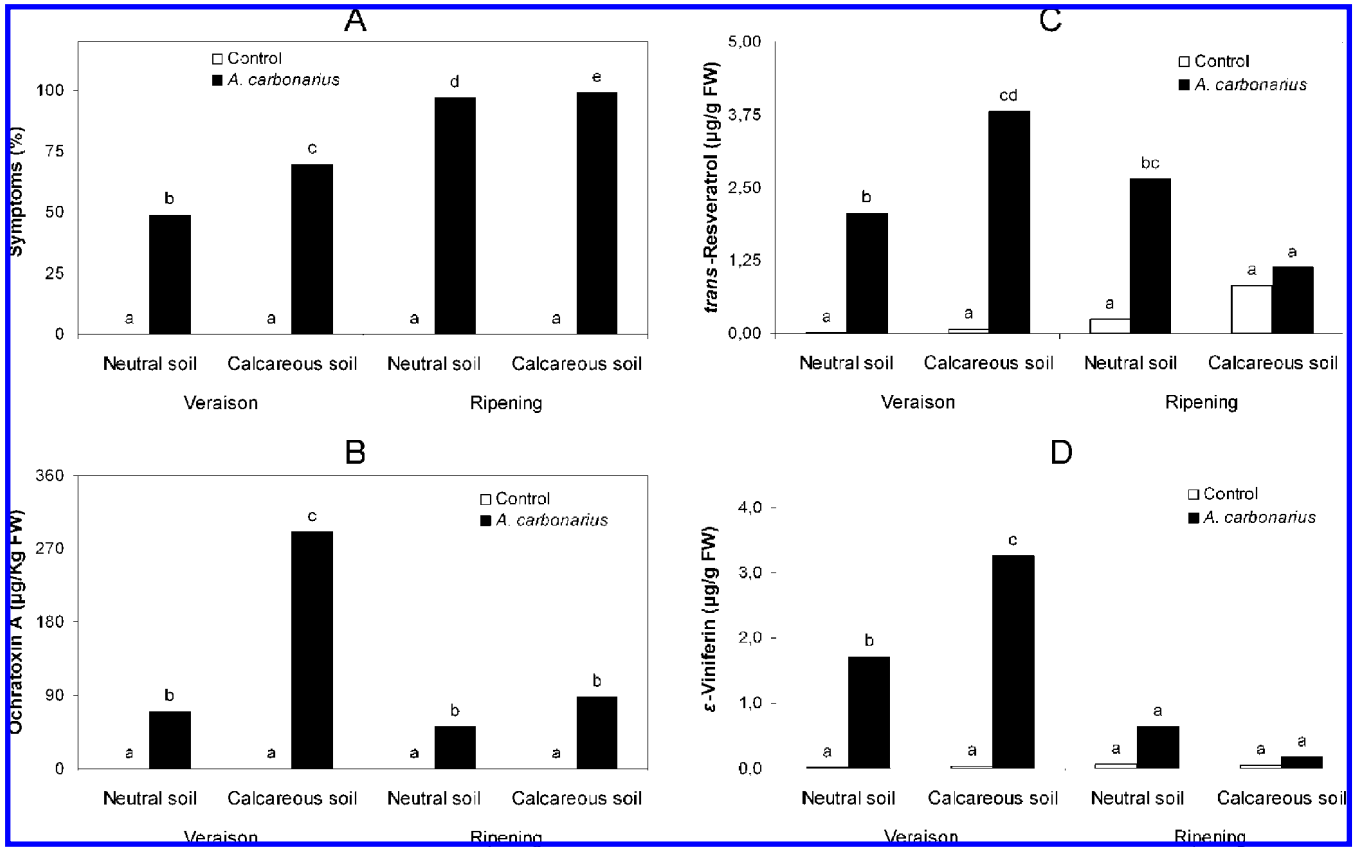

Figure 1. (**A**) Infection symptoms depending on the phenological phase, soil type, and berry infection. Values with the same letter are not significantly different ($P \leq 0.05$). (**B**) Ochratoxin A berry concentrations depending on the phenological phase, soil type, and berry infection. Values with the same letter are not significantly different ($P \leq 0.05$). (**C**) *trans*-Resveratrol berry concentrations depending on the phenological phase, soil type, and berry treatement. Values with the same letter are not significantly different ($P \leq 0.05$). (**D**) ε-Viniferin berry concentrations depending on the phenological phase, soil type, and berry treatement. Values with the same letter are not significantly different ($P \leq 0.05$).

Table 2. Effect of Soil Type and Phenological Phase on the Berry Macronutrient and Trace Element Concentrations (on Dry Weight Basis)[a]

soil type	phenological phase	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (µg/g)	Mn (µg/g)	Cu (µg/g)	Zn (µg/g)	B (µg/g)
neutral	veraison	0.77 a	0.26 abc	1.05 abc	0.08 a	0.05 a	27.36 a	7.62 a	29.51 a	4.58 a	21.38 a
	ripening	0.66 a	0.31 b	1.11 b	0.06 a	0.04 a	29.70 a	5.62 a	22.63 b	3.98 a	20.15 a
calcareous	veraison	0.80 a	0.17 ac	0.90 abc	0.08 a	0.04 a	27.47 a	7.94 a	22.68 b	5.74 a	24.98 a
	ripening	0.73 a	0.21 c	0.83 c	0.08 a	0.05 a	26.40 a	7.90 a	21.80 b	4.63 a	17.76 a

[a] Each value is the mean of three data. Values in each column without the same letters are significantly different ($P \leq 0.05$).

concentration observed under lime stress conditions than in the control can be the consequence of the eliciting activity of the fungus, the symptoms of which were worse in the chlorotic vines than in the normal ones. At ripening the calcareous soil reduced the stilbene concentration of the inoculated berries, whereas infection symptoms increased. Because the recorded stilbene concentration is the result of the balance between synthesis and oxidation (due to the fungus), under increasing fungal attacks the phytoalexin can undergo a stronger destruction process. The effect of the soil on ε-viniferin berry concentration is the same as observed for *trans*-resveratrol.

In conclusion, iron deficiency disorder due to calcareous conditions of the soil has a negative effect also on the fruit resistance toward *A. carbonarius* while eliciting ochratoxin A production. The stilbene contents, triggered by the fungal infection, are incresaed by lime stress conditions at veraison and depressed at ripening. It is therefore important for the grower to correct the deficiency status of the vines to better protect the plant against fungal diseases.

ACKNOWLEDGMENT

We are grateful to Giuseppe Bruzzi (Institute of Viticulture), Silvia Formenti (Institute of Plant Pathology), and Terenzio Bertuzzi (Institute of Feed Science and Nutrition) for their contribution to the project.

LITERATURE CITED

(1) Tagliavini, M.; Rombolà, A. D. Iron deficiency and chlorosis in orchard and vineyard ecosystems. *Eur. J. Agron.* **2001**, *15*, 71–92.

(2) Alvarez-Fernandez, A.; Paniagua, P.; Abadia, J.; Abadia, A. Effects of Fe deficiency chlorosis on yield and fruit quality in peach (*Prunus persica* L. Batsch). *J. Agric. Food Chem.* **2003**, *51*, 5738–5744.

(3) Bavaresco, L.; Poni, S. Effect of calcareous soil on photosynthesis rate, mineral nutrition and source-sink ratio of table grapes. *J. Plant Nutr.* **2003**, *26*, 2123–2135.

(4) Bavaresco, L.; Presutto, P.; Civardi, S. VR O43-43: a lime-susceptible rootstock. *Am. J. Enol. Vitic.* **2005**, *56*, 192–195.

(5) Battilani, P.; Pietri, A.; Bertuzzi, T.; Languasco, L.; Giorni, P.; Kozakiewicz, Z. Occurrence of ochratoxin A producing fungi in grape grown in Italy. *J. Food Prot.* **2003**, *66*, 633–636.
(6) Majerus, P.; Otteneder, H. Detection and occurrence of ochratoxin A in wine and grape juice. *Dtsch. Lebensm. Rundsch.* **1996**, *92*, 388–390.
(7) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Addit. Contam.* **1996**, *13*, 655–668.
(8) Da Rocha, C. A. R.; Palacios, V.; Combina, M.; Fraga, M. E.; De Oliveira Rekson, A.; Magnoli, C. E.; Dalcero, A. M. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. *Food Addit. Contam.* **2002**, *19*, 408–414.
(9) Sage, L.; Krivoboc, S.; Delbos, E.; Seigle-Murandi, F.; Creppy, E. E. Fungal flora and ochratoxin A production in grapes and musts from France. *J. Agric. Food Chem.* **2002**, *50*, 1306–1311.
(10) Bavaresco, L.; Vezzulli, S.; Battilani, P.; Giorni, P.; Pietri, A.; Bertuzzi, T. Effect of ochratoxin A-producing aspergilli on stilbenic phytoalexin synthesis in grapes. *J. Agric. Food Chem.* **2003**, *51*, 6151–6157.
(11) Battilani, P.; Giorni, P.; Bertuzzi, T.; Formenti, S.; Pietri, A. Black aspergilli and ochratoxin A in grapes in Italy. *Int. J. Food Microbiol.* **2006**, *111*, S53–S60.
(12) Jeswal, P. Antidotal effect of grape juice (*Vitis vinifera*) on ochratoxin A caused hepatorenal carcinogenesis in mice (*Mus musculus*). *Cytobios* **1998**, *93*, 123–128.
(13) Bavaresco, L.; Fregoni, C. Physiological role and molecular aspects of grapevine stilbenic compounds. In *Molecular Biology and Biotechnology of the Grapevine*; Roubelakis-Angelakis, K. A., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001; pp 153–182.
(14) Langcake, P.; McCarthy, W. V. The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. *Vitis* **1979**, *18*, 244–253.
(15) 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.
(16) Bavaresco, L.; Pettegolli, D.; Cantù, E.; Fregoni, M.; Chiusa, G.; Trevisan, M. Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *Botrytis cinerea*. *Vitis* **1997**, *36*, 77–83.
(17) 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.
(18) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Andrés-Lacueva, C.; de la Torre-Boronat, M. C. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food Chem.* **2001**, *49*, 210–215.
(19) Sarig, P.; Zutkhi, Y.; Monjauze, A.; Lisker, N.; Ben-Arie, R. Phytoalexin elicitation in grape berries and their susceptibility to *Rhizopus stolonifer*. *Physiol. Mol. Plant Pathol.* **1997**, *50*, 337–347.
(20) Paul, B.; Chereyathmanjiyil, A.; Masih, I.; Chapuis, L.; Benoît, A. Biological control of *Botrytis cinerea* causing grey mould disease of grapevine and elicitation of stilbene phytoalexin (resveratrol) by a soil bacterium. *FEMS Microbiol. Lett.* **1998**, *165*, 65–70.
(21) Jeandet, P.; Bouillet-Breuil, A. C.; Bessis, R.; Delbord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731–2741.
(22) Vezzulli, S.; Civardi, S.; Ferrari, F.; Bavaresco, L. Methyl jasmonate as a trigger of resveratrol synthesis in cultivated grapevine. *Am. J. Enol. Vitic.* **2007**, *58*, 530–533.
(23) Bavaresco, L.; Pezzutto, S.; Ragga, A.; Ferrari, F.; Trevisan, M. Effect of nitrogen supply on *trans*-resveratrol concentration in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Vitis* **2001**, *40*, 229–230.
(24) Bavaresco, L.; Civardi, S.; Pezzutto, S.; Vezzulli, S.; Ferrari, F. Grape production, technological parameters, and stilbenic compounds as affected by lime-induced chlorosis. *Vitis* **2005**, *44*, 63–65.
(25) Bavaresco, L.; Pezzutto, S.; Gatti, M.; Mattivi, F. Role of the variety and some environmental factors on grape stilbenes. *Vitis* **2007**, *46*, 57–61.
(26) De Andrés-de Prado, R.; Yuste-Rojas, M.; Sort, X.; Andrés-Lacueva, C.; Torres, M.; Lamuela-Raventós, R. M. Effect of soil type on wine produced from *Vitis vinifera* L. cv. Grenache in commercial vineyards. *J. Agric. Food Chem.* **2007**, *55*, 779–786.
(27) Mattivi, F.; Reniero, F.; Korhammer, S. Isolation, characterization and evolution in red wine vinification of resveratrol monomers. *J. Agric. Food Chem.* **1995**, *43*, 1820–1823.
(28) Pezet, R.; Cuenat, P. Resveratrol in wine: extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *Am. J. Enol. Vitic.* **1996**, *47*, 287–290.
(29) Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523–1526.
(30) Frémont, L. Biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663–673.
(31) Lamming, D. W.; Wood, J. G.; Sinclair, D. A. Small molecules that regulate lifespan: evidence for xenohormesis. *Mol. Microbiol.* **2004**, *53*, 1003–1009.
(32) Cottenie, A. Soil and plant testing as a base of fertilizer recommendations. *FAO Soils Bulletin 32*; FAO: Rome, Italy, 1980.
(33) Pouget, R.; Ottenwälter, M. Etude de l'adaption de nouvelles variétés de porte-greffes à des sols très chlorosantes. *Connais. Vigne Vin* **1978**, *12*, 167–175.
(34) Pitt, J. I. *The Genus Penicillium and Its Teleomorphic States Eupenicillium and Talaromyces*; Academic Press: London, U.K., 1979.
(35) Association of Official Analytical Chemists. *Official Methods of Analysis*; AOAC: Arlington, VA, 1995; Vol. 49, p 38.
(36) Bavaresco, L.; Eibach, R. Investigations of the influence of N fertilizer on resistance to powdery mildew (*Oidium tuckeri*), downy mildew (*Plasmopara viticola*) and on phytoalexins syntesis in different grapevine varieties. *Vitis* **1987**, *26*, 192–200.
(37) Saber, S. M. Fungal contamination, natural occurrence of mycotoxins and resistance for aflatoxin accumulation of some broad bean (*Vicia faba L.*) cultivars. *J. Basic Microbiol.* **1992**, *32*, 249–258.
(38) Marschner, H. *Mineral Nutrition of Higher Plants*; Academic Press: London, U.K., 1995.
(39) Graham, R. D. Effects of nutrient stress on susceptibility of plant to disease with particular reference to the trace elements. *Adv. Bot. Res.* **1983**, *10*, 221–276.

Received for review November 27, 2007. Revised manuscript received January 10, 2008. Accepted January 10, 2008. Work supported by FPV, KAI, contract QLK1-CT-2001-01761 (WINE-OCHRA RISK).

JF073456+