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Grape production, technological parameters, and stilbenic compounds as affected by lime-induced chlorosis

L. Bavaresco[1)], S. Civardi[1)], S. Pezzutto[1)], S. Vezzulli[1)] and F. Ferrari[2)]

[1)] Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italia
[2)] Istituto di Chimica agraria ed ambientale, Università Cattolica del Sacro Cuore, Piacenza, Italia

Summary

***Vitis vinifera* L. cv. Merlot clone R3, grafted on 3309 C (lime-susceptible) rootstock, was grown in pots on a non-calcareous and a calcareous soil. The aim of the experiment was to check the effect of lime stress conditions on chlorosis, grape yield, technological parameters and stilbene (resveratrol, piceid, piceatannol, ε-viniferin) concentrations in grapes. Lime-induced chlorosis decreased grape yield per plant to a very high extent, as a result of a reduction of cluster and berry size. Technological grape parameters such as soluble solids, pH, anthocyanins, increased under lime stress over the control, whilst titratable acidity was not affected. All the tested stilbenes, being stress compounds, increased in the grapes of chlorotic vines.**

Key words: *Vitis vinifera*, lime-induced chlorosis, grape yield, grape quality, stilbenes.

Introduction

Many cultivated perennial plants, including some *Vitis* spp., shows low degree of lime tolerance. High bicarbonate levels in the soil induce iron chlorosis (Mengel and Malissiovas 1981). While there is some evidence that iron chlorosis negatively affects fruit size and/or composition of a variety of fruits there are only very few literature data on fruit composition of wine grapes.

The aim of the experiment was to study the effect of lime stress conditions on grape production and fruit composition of cv. Merlot, emphasizing the berry concentration of stilbenes which are stress-related compounds, primarly acting as phytoalexins (Bavaresco and Fregoni 2001).

Material and Methods

Soil and plant material: *Vitis vinifera* L. cv. Merlot, clone R3, grafted on the lime suceptable rootstock 3309 C (*V. riparia* Michx. x *V. rupestris* Scheele) was potted (pot volume = 45 l) in a non- calcareous and a calcareous soil. The non-calcareous soil was prepared by mixing a natural neutral soil (70 %) with sand (10 %) and acidic peat (20 %). The calcareous soil was collected in a vineyard near Piacenza and utilized without further amendments. The main soil characteristics (Cottenie 1980) were as follows (non-calcareous vs. calcareous soil) sandy-clay-loam texture (for both soils), pH 7.3 and 8.2, 3 % and 67 % total carbonates, 1.5 % and 16.5 % active lime, 38 mg·kg^{-1} and 6 mg·kg^{-1} Olsen P, 164 mg·kg^{-1} and 87 mg·kg^{-1} exchangeable K_2O (extracted by $BaCl_2$), 80 mg·kg^{-1} and 14 mg·kg^{-1} available Fe (extracted by DTPA 0.005 *M* + $CaCl_2$ 0.01 *M* + triethanolamine 0.1 *M*). Basic nutrients were added, as follows (on pot basis): 4.5 g N, 1.5 g P_2O_5, 6.6 g K_2O, 0.5 g MgO, 2.5 mg B, 0.5 mg Cu, 5 mg Fe, 2.5 mg Mn, 0.25 mg Mo, 0.5 mg Zn. Fifteen pots per soil type with one plant each were placed outside on a platform covered with a hail-protection net. Water was supplied by drip irrigation, to maintain the soil near field capacity. At the end of the second growth year, each vine was cane-pruned, leaving one cane (8 buds) and one spur (2 buds). Data reported in this paper were recorded during the third year of growth.

Chlorosis assessment: Chlorosis was visually rated at fruit set and at veraison on the 4th and 5th leaf (from the shoot tip) of all shoots, using the scale of Pouget and Ottenwälter (1978), which ranks from 0 (no symptoms) to 5 (severe chlorosis, more than 10 % of the leaf blade with necrosis).

Grape analyses: At harvest time (155 d after bud burst) the following parameters were checked: number of clusters·vine^{-1}; grape yield (kg·vine^{-1}); cluster weight and berry weight. Fifteen replicates per soil type (corresponding to the 15 plants) were available.

Two representative clusters per vine were sampled for the technological analyses and the berries of those two clusters were detached from the rachis, mixed, and two sets of samples were prepared. The first set of berries was crushed by hand, in order to get the juice to be analysed as follows: soluble solids (°Brix) by a temperature-compensating refractometer (DBX-55, Atago Co, Ltd. Tokyo, Japan); titratable acidity (g·l^{-1}) by automatic titration with NaOH 0.1 N (Compact titrator, Crison, Alella, Spain); pH by a pH-meter coupled with the compact titrator Crison; tartaric acid (g·l^{-1}) by colorimetry (λ = 530 nm) according to the Rebelein method (Lipka and Tanner 1974). Total anthocyanins (mg·g^{-1} berry FW and mg·cm^{-2} skin) were determined after homogenization of the second set of berries by colorimetry (λ = 520 nm), according to Iland (1988).

The stilbenes *trans*-resveratrol, *trans*-piceid, piceatannol and ε-viniferin were extracted from the second set of berries according to Bavaresco *et al*. (1997). Berries corresponding to some 20 g of fresh weight were selected, their volume was calculated, seeds weighed and discarded and

Correspondence to: Prof. Dr. L. Bavaresco, Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy. Fax: +39-0523-599268. E-mail: luigi.bavaresco@unicatt.it

berries crushed in a mortar. The macerated berries were transferred to 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. 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 aqueous sodium bicarbonate. The organic phases were collected and evaporated *in vacuo* to dryness and redissolved in 2 x 1 ml of methanol (100 %) and stored in adiactinic glass vials at -18 °C, before HPLC analysis. Five replicates (including berries from 3 plants each) per soil type were available.

Standards and HPLC conditions: The *trans*-resveratrol (*trans*-3,4',5-trihydroxy-stilbene) and piceatannol (*trans*-3,3',4,5'-tertrahydroxy-stilbene) standards were puchased from Sigma (St. Louis, MO); *trans*-piceid (*trans*-resveratrol-3-*O*-β-D-glucopyranoside) was isolated from the roots of *Polygonum cuspidatum*; ε-viniferin (dimer of *trans*-resveratrol) was kindly supplied by G. HOOS (formerly BFA für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany). The purity of each stilbene was controlled by HPLC and the identity was confirmed according to MATTIVI *et al.* (1995).

For the stilbenic compounds, the HPLC system consisted of an Agilent HP 1100 series, Waldbronn, Germany, with an autosampler (50 µl injection volume) and a diode array detector set at 306 nm. A C18 Supelco column (Supelcosil ABZ plus 250 mm x 4.6 mm, 5 µm particle size) was used, eluting with a gradient of methanol (A) and 0.01 M KH_2PO_4 adjusted to pH 2.5 with phosphoric acid (B). The gradient was from 40 to 85 % of A, flow rate: 0.7 ml·min^{-1}. Detection limit was 0.1 mg·l^{-1}. Quantifications of stilbenes were done on the basis of peak areas using PC software. Data were expressed on the basis of berry fresh weight (µg·g^{-1}) and skin area (ng·cm^{-2}).

Statistical analysis: A one-way ANOVA was utilized and the effect of soil type on the tested parameters was assessed by the Fisher (F)-test.

Results and Discussion

Chlorosis occurred at both sampling times on plants growing in the calcareous soil; while at fruit set a light chlorosis (pale green, 0.9 rating) was observed, the calcareous soil induced a more severe chlorosis at veraison (2.1 rating). Chlorosis negatively affected productivity, reducing yield per plant by 82 %, cluster weight by 68 %, and the berry weight by 47 % as compared to normal growth conditions. On the other hand, soluble solids and anthocyanins were increased under lime- stress conditions, while the organic acids were not affected, as was shown by BAVARESCO and PONI (2003) for table grapes. The high sugar concentration is likely a consequence of grape yield reduction. The percentage of anthocyanin increase, on the basis of berry fresh weight was 72 % over the control, on the basis of skin area 42 %. Therefore lime stress conditions may have somehow affected the biosynthesis of the compounds. The biochemical mechanisms involved are not clear, but it may be speculated that, iron being constituent of enzymes involved in lignin synthesis, iron deficiency may switch the shikimate pathway towards other phenolics including anthocyanins. The paper reports, for the first time, effect of lime-stress conditions on stilbene levels of ripe fruit (berries), which increase over the level of understressed plants (Figure). The most important compound was *trans*-resveratrol, followed by piceatannol, *trans*-piceid and ε-viniferin. The relative increase over normal conditions, on the basis of berry fresh weight, was 635 % for *trans*-resveratrol, 1609 % for *trans*-piceid, 550 % for piceatannol and 500 % for ε-viniferin. Considering stilbene concentration on the basis of berry skin area, the relative increase was 468 % for *trans*-resveratrol, 1003 % for *trans*-piceid, 406 % for piceatannol and 367 % for ε-viniferin. This means that the high levels of stilbenes are not only due to concentration, but above all to an enhanced synthesis in berry skins. The involved mechanisms are not clear and deserve further investigations, but we may deduce that, as for anthocyanins, stilbenes are products of the shikimate pathway increase accordingly. In a previous experiment BAVARESCO *et al.* (2001) were able to demonstrate that another nutrient stress (nitrogen shortage) led to an increase of *trans*-resveratrol concentration in Cabernet Sauvignon grapes.

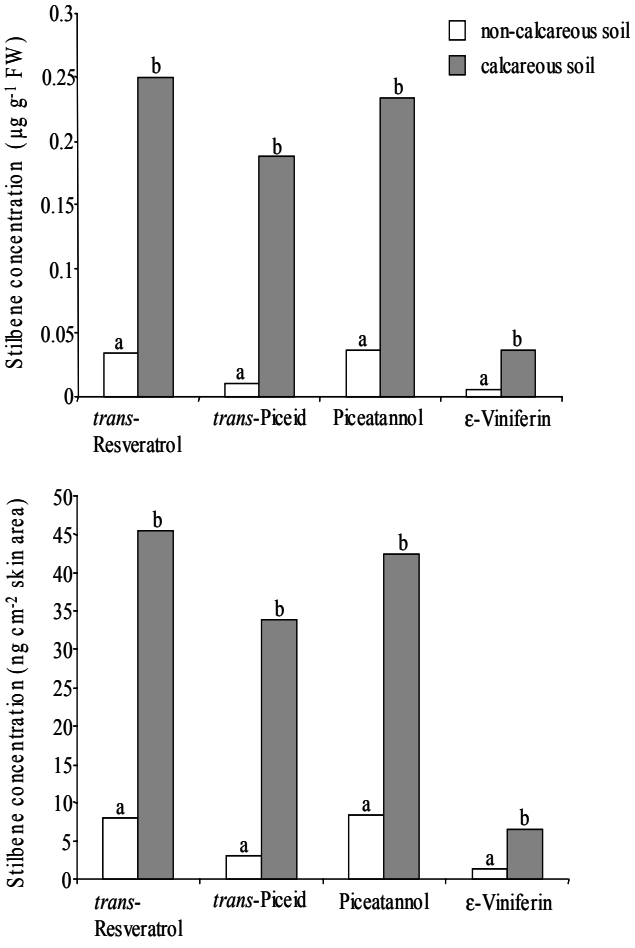

Figure: Stilbene concentrations in berries as affected by soil type: values, for each compound, followed by different letters are significantly different at $p \leq 0.05$.

T a b l e

Yield and quality at harvest as affected by soil type

	Clusters plant^{-1}	Grape yield (kg plant^{-1})	Cluster wt (g)	Berry wt (g)	Soluble solids (°Brix)	pH	Titratable acidity (g l^{-1})	Tartaric acid (g l^{-1})	Antho-cyanins (mg g^{-1} FW)	Antho-cyanins (mg cm^{-2})
Non-calcareous soil	17 a	2.8 a	171 a	1.5 a	20.8 a	3.44 a	5.17 a	7.31 a	0.57 a	0.180 a
Calcareous soil	11 b	0.5 b	54 b	0.8 b	22.1 b	3.58 b	4.97 a	7.29 a	0.98 b	0.256 b

Values in each column with different letters are significantly different ($p \leq 0.05$).

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