Vitis **46** (2), 57–61 (2007)

Role of the variety and some environmental factors on grape stilbenes

L. Bavaresco[1)], S. Pezzutto[1)], M. Gatti[1)] and F. Mattivi[2)]

[1)] Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Piacenza, Italy
[2)] Istituto Agrario di S. Michele all'Adige (IASMA), Centro Sperimentale, Dipartimento Qualità Agro-Alimentare, San Michele all'Adige, Italy

Summary

***V. vinifera* L. 'Barbera', 'Croatina', 'Malvasia di Candia aromatica', growing in the Piacenza viticultural area (North-West Italy) at four elevations (150, 240, 320, 420 m a.s.l.), were tested at harvest for grape stilbene (*trans*-resveratrol, *trans*-piceid, *cis*-piceid) synthesis over three years (2000-2002). Meteorological data were recorded, as well as vine production and fruit quality parameters. The most significant findings were: (i) *trans*-piceid was the most abundantly produced stilbene compound (103 µg·kg^{-1} berry FW), while *trans*-resveratrol was the least produced (57 µg·kg^{-1} berry FW); (ii) 'Barbera' and 'Croatina' had similar *trans*-resveratrol berry levels (71 µg·kg^{-1} berry FW and 76 µg·kg^{-1} berry FW respectively), higher than 'Malvasia di Candia aromatica' (24 µg·kg^{-1} berry FW); (iii) 'Barbera' had the highest *trans*-piceid and *cis*-piceid concentrations (235 and 136 µg·kg^{-1} berry FW, respectively) while 'Malvasia di Candia aromatica' had the lowest levels (13 and 1 µg·kg^{-1} berry FW, respectively); (iv) stilbenes increased with elevation up to 320 m, while decreasing at the highest altitude; (v) the vintage year only significantly affected the cis-piceid berry concentration which was positively related to the relative humidity at the end of ripening, and negatively related to degree-days at the end of ripening.**

K e y w o r d s : grape variety, climate, resveratrol, piceid, stilbenes.

Introduction

Stilbenes are natural compounds occurring in a number of plant families including *Pinaceae, Myrtaceae, Fagaceae, Liliaceae, Moraceae, Papilionaceae* and *Vitaceae.* Within *Vitaceae* they are synthesized in several species, including *Vitis vinifera* L., the most important species for table grapes, raisins and wine production. Stilbenes are low molecular weight phenolics that act like antifungal compounds, enabling the plant to overcome pathogen attack (Bavaresco and Fregoni 2001). These compounds are present in soft tissues (fruits, leaves, root tips and other herbaceous organs) as phytoalexins induced by biotic and abiotic stresses. Stilbenic phytoalexins have been identified in grapes as *trans*-resveratrol (*trans*-3,4',5-trihydroxistilbene), *trans*- and *cis*-piceid (*trans*- and *cis*-resveratrol-3-O-β-D-glucopyranoside) (Mattivi *et al*. 1995), ε-viniferin (*trans*-resveratrol dimer) (Bavaresco *et al.* 1997), pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxy-stilbene), piceatannol (*trans*-3,3',4,5'-tetrahydroxy-stilbene) or astringinin (Bavaresco *et al.* 2002), and pallidol (*trans*-resveratrol dimer).

Stilbenes are also present as constitutive compounds in the woody parts of the vine, such as stems and canes, roots, seeds (Ector *et al*. 1996), and in semi-herbaceous organs like cluster stems, where they help prevent decay.

Stilbenes (especially resveratrol) are claimed to have health functional properties when consumed as grapes or grape products, primarily due to antioxidant activity. Siemann and Creasy (1992) first detected resveratrol in wine, where it is extracted from berry skins during alcoholic fermentation and found as free and bound forms (3-β-glucoside of resveratrol, Mattivi *et al*. 1995). Small pieces of cluster stems immersed in the fermenting must may contribute to increasing resveratrol levels in wine.

Resveratrol concentrations of grapes (and wines) analysed worldwide vary widely, depending on viticultural and enological conditions. Viticultural factors on grape stilbene levels have been poorly investigated, which led to the establishment of the field trial presented here examining the variation of stilbene levels in the grapes as affected by grape variety and the environment.

Materials and Methods

E x p e r i m e n t a l d e s i g n a n d p l a n t m a t e r i a l : A three-year-trial (2000-2002) was carried out in Tidone valley, Piacenza province, Italy (44° 54' Lat. N.), which is characterized by the following climatic parameters (Mennella 1967, 50 years data): average temperature, 12.2 °C; maximum temperature, 16.5 °C; minimum temperature, 8.4 °C; and rainfall, 794 mm. The viticultural area, some 3,500 ha wide, belongs to the DOC (Denominazione di Origine Controllata - Denomination of Wines by Origin) "Colli Piacentini". The main meteorological parameters and bioclimatic indices of the tested years, averaged from four representative meteorological stations of the valley, are reported in Tab. 1. The three most commonly cultivated *V. vinifera* L. varieties of this region were tested: two red wine cultivars, 'Barbera' and 'Croatina', and one white wine cultivar, 'Malvasia di Candia aromatica' (Malvasia C.a.). 'Malvasia C.a.' is normally vinified alone, producing a sweet aromatic wine, while 'Barbera' and 'Croatina' are

Correspondence to: Prof. L. Bavaresco, Istituto di Frutti-Viticoltura, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84, 29100 Piacenza, Italy. Fax: +39-0523-599-268. E-mail: luigi.bavaresco@unicatt.it

Table 1

Yearly meteorological data and bioclimatic indices of Tidone Valley (Piacenza province, Italy)

	Average T (°C)	Minimum T (°C)	Maximum T (°C)	D.D.[a] (°C)		Rainfall (mm)		T.V.I.[d]	F.s.I.[e]	R.H.[f]
				Seasonal[b]	Monthly[c]	Annual	Seasonal[b]			
2000	12.2	7.8	17.6	1570	356	827	386	32.7	1154	68
2001	12.0	7.8	17.4	1526	323	715	436	32.7	4139	73
2002	12.3	8.1	17.3	1486	284	868	427	31.0	2300	80

[a] Degree days (base 10 °C).
[b] From 1 April to 30 September.
[c] Last month (30 days) before harvest.
[d] Temperature variability index (∑ [(TDmax-TDmin) + (TMmax-TMmin)]) from 1 April to 30 September (Gladstones 1992).
[e] Fregoni simplified index ((∑Tmax-Tmin)*(n° d < 10°)) from 1 to 30 September (Fregoni and Pezzutto 2000).
[f] Relative Humidity of the last month before harvest.

processed together giving a wine suitable for aging called "Gutturnio". Each variety was grown in four commercial vineyards, located at 150, 240, 320, 420 m above sea level (a.s.l.). The soils are a fertile clay-calcareous flysch, and they were tilled to control weed growth. The vineyards were located on an eastern slope, and were Guyot trained (cane pruning with vertical shoot positioning) with an average number of 20 buds/plant. Plant density ranged from 2000 to 2600 vines/ha.

Yield components and fruit composition: At harvest 12 vines per variety and per vineyard (elevation) were chosen and all the clusters of each plant were counted and weighed, and the basal cluster of two medial shoots were sampled (2 clusters per vine) for fruit quality analyses. The following yield and fruit parameters were measured: grape yield ($kg \cdot plant^{-1}$); mean cluster weight (g); juice total soluble solids (°Brix) by a temperature-compensating refractometer; juice titratable acidity ($g \cdot l^{-1}$) by titration with NaOH 0.1 N; juice pH; berry *trans*-resveratrol (*trans*-3,4',5-trihydroxi-stilbene), *trans*- and *cis*-piceid (*trans*- and *cis*-resveratrol-3-O-β-D-glucopyranoside).

Stilbene extraction and measurement: About 20 g of fresh berries (without seeds) were ground in a mortar with 30 ml methanol 95 %, and vigorously shaken for 20 min at room temperature, according to Bavaresco *et al.* (1997). After filtration through GF/A Whatman filters, the liquid was evaporated under vacuum at 40 °C and the aqueous fraction was extracted twice with 5 ml ethylacetate and 5 ml $NaHCO_3$ (5 %). The organic phase was evaporated to dryness and stilbene compounds were recovered by 2 ml plus 1 ml methanol 100 % and stored in adiactinic vials at -18 °C. An HP 1090 series HPLC (Agilent, Palo Alto, CA) with a gradient system and diode array detector was used for RP-HPLC analyses on a ODS Hypersil 200 x 2.1 (5 µm) column with a guard ODS Hypersil 20 x 2.1 mm (5 µm) (Agilent, Palo Alto, CA) cartridge. The mobile phases consisted of 0.001 M phosphoric acid (A) and acetonitrile (B). Separation was carried out at 40 °C under the following conditions: linear gradients from 0 - 50 % B in 25 min, to 100 % B in 1 min. The column was equilibrated with 100 % A for 5 min before each analysis. The flow rate was 0.6 $ml \cdot min^{-1}$ and the injection volume 6 µl. The UV spectra were recorded from 200 to 400 nm. Detection occurred at 310 nm for *trans*-resveratrol and *trans*-piceid and at 282 nm for *cis*-piceid. All compounds were identified on the base of their UV spectra and retention time, compared to authentic standards. Samples were quantified by the external standard method. The *trans*-piceid was expressed as *trans*-resveratrol equivalents.

Statistical analysis: A three-way-ANOVA with interactions was used for all the tested parameters, and the means were compared by LSD at the 5% level.

Results

Weather: Meteorological conditions for the experimental sites are summarized in Tab. 1. During the three experimental years the annual mean temperature and rainfall were average as compared with the 50-year-data. However, a higher range of temperatures was noticed during the three experimental years. Comparison of various growing season indices found that degree days decreased from 2000 to 2002 while relative humidity increased; the other parameters did not show a linear trend.

Yield components and fruit composition (Tab. 2): All the tested parameters were significantly ($p < 0.001$) affected by the variety, the year, and the elevation. 'Malvasia C.a.' produced the heaviest fruit load (10.3 $kg \cdot vine^{-1}$), while 'Croatina' yield was lowest (6.5 $kg \cdot vine^{-1}$) even though it had the largest cluster (370 g); 'Barbera' had the smallest clusters (304 g). 'Barbera' and 'Croatina' accumulated the same level of soluble solids (20.2 °Brix), higher than 'Malvasia C.a.' (17.6 °Brix). Acidity was the highest (11.8 $g \cdot l^{-1}$) and pH the lowest (3.08) for 'Barbera', confirming known characteristics of that variety. Acidity of 'Croatina' and 'Malvasia C.a.' fruit was similar, while pH was quite different. The soluble solids and acidity levels over the years seemed to be related to degree days and temperatures during the last month (30 d) before harvest, which decreased from 2000 to 2002. The highest soluble solid concentration and the lowest acidity of the year 2000 were related to the lowest crop load and cluster weight. Increasing elevations were correlated with increasing acidity and decreasing pH, however no relationship of elevation with soluble solids, fruit load and cluster size could be observed.

Table 2

Effect of the variety, vintage year, and elevation on production and fruit quality parameters

	Crop yield ($kg \cdot vine^{-1}$)	Cluster weight (g)	Soluble solids (°Brix)	Titratable acidity ($g \cdot l^{-1}$)	pH
Variety					
Barbera	7.3	304	20.3	11.8	3.08
Croatina	6.5	370	20.2	7.2	3.22
Malvasia C.a.	10.3	341	17.6	6.7	3.09
$LSD_{0.05}$	0.44	12.3	0.12	0.09	0.015
Year					
2000	7.2	316	20.7	7.3	3.19
2001	9.0	362	18.6	8.4	3.14
2002	7.8	337	18.7	10.0	3.06
$LSD_{0.05}$	0.44	12.3	0.12	0.09	0.015
Elevation					
150 m	8.2	340	18.9	7.7	3.20
240 m	7.9	361	20.0	7.8	3.17
320 m	9.3	323	19.4	9.4	3.06
420 m	6.6	329	19.0	9.3	3.09
$LSD_{0.05}$	0.51	14.3	0.14	0.10	0.017

Stilbene composition: *Trans*-resveratrol concentration in the berry was significantly affected by the variety ($p < 0.01$), by the elevation ($p < 0.01$), but not by the year (Tab. 3). 'Barbera' and 'Croatina' had similar values which were higher than that of 'Malvasia C.a.'. Levels of *trans*-resveratrol increased up to elevation 3 (320 m) and then decreased somewhat. *Trans*-piceid berry concentration was significantly affected by the variety ($p < 0.001$), by the elevation ($p < 0.001$), but not by the year (Tab. 3). The stilbene berry concentration ranged from 13 $\mu g \cdot kg^{-1}$ ('Malvasia C.a.') to 235 $\mu g \cdot kg^{-1}$ ('Barbera') and it was enhanced up to elevation 3 and then substantially reduced at elevation 4 (420 m). *Cis*-piceid was significantly ($p < 0.001$) affected by the variety, the year and the elevation (Tab. 3). The effect of the variety and the elevation was the same as observed for *trans*-piceid, however the compound increased each year; it was not detected in 2000, but was 80 $\mu g \cdot kg^{-1}$ in 2001 and 107 $\mu g \cdot kg^{-1}$ in 2002.

Berry concentrations of *trans*-resveratrol did not significantly change for any variety over the three years; 'Barbera' and 'Croatina' levels were higher than in 'Malvasia C.a.' (Tab. 3). The stilbene level of 'Barbera' and 'Croatina' significantly increased at elevations up to 320 m. As concerning *trans*-piceid, every variety behaved differently depending on the year examined, however 'Barbera' always had highest values, while 'Malvasia C.a.' had the lowest values (Tab. 3). The stilbene level of 'Barbera' significantly increased up to the 320 m elevation and then decreased; *trans*-piceid level of 'Croatina' and 'Malvasia C.a.' did not change significantly in relation to elevation (Tab. 3). 'Barbera' *cis*-piceid increased significantly with years while 'Croatina' *cis*-piceid levels were significantly different only in 2000. The stilbene level of 'Barbera' and 'Croatina' significantly increased up to 320 m and then decreased. No significant effects of year and elevation were noticed for 'Malvasia C.a.' The year x elevation interaction of 'Bar-

Table 3

Effect of the variety, vintage year, and elevation on stilbene grape concentration

	Trans-resveratrol ($\mu g \cdot kg^{-1}$)				*Trans*-piceid ($\mu g \cdot kg^{-1}$)				*Cis*-piceid ($\mu g \cdot kg^{-1}$)			
	Barbera	Croatina	Malvasia C.a.	average	Barbera	Croatina	Malvasia C.a.	average	Barbera	Croatina	Malvasia C.a.	average
Year												
2000	60	55	21	45	252 b	25	18	98	0 a	0	0	0 a
2001	64	107	8	60	197 a	80	8	95	154 b	82	4	80 b
2002	90	66	43	66	255 b	77	13	115	254 c	67	0	107 c
Elevation												
150 m	20 a	10 a	6	12 a	141 a	48	7	65 a	47 a	11 a	0	19 a
240 m	12 a	110 b	55	59 b	182 a	54	22	86 a	104 b	57 ab	5	55 b
320 m	156 b	82 b	23	87 b	459 b	85	4	186 b	291 c	77 b	0	123 c
420 m	98 b	101 b	12	70 b	157 a	56	10	74 a	103 b	55 ab	0	53 b
Av./cvs	71 b	76 b	24 a	57	235 c	61 b	13 a	103	136 c	50 b	1 a	62

Means within columns followed by different letters differ significantly at $p \leq 0.05$.

bera' is presented in the Figure. The berry concentrations of *trans*-resveratrol increased up to the 420 m elevation in 2000, they increased up to 320 m and then decreased in 2001 and 2002. The highest *trans*-piceid grape concentrations were observed at 320 m in every year. *cis*-Piceid was not synthesized in 2000, while in 2001 and 2002 the vineyards located at 320 m had the highest values.

Discussion

The three sources of variation (grape variety, vintage year, vineyard elevation) contributed, at different extents, to concentrations of stilbenes. Observations of higher *trans*-resveratrol levels in the two red-wine varieties ('Barbera' and 'Croatina') as compared to the white-wine grape, 'Malvasia C.a.', contradict previous data obtained in whole berries or berry skins (Romero-Perez *et al*. 2001) where no significant differences between white and red berries were recorded; interestingly even comparisons between bronze- and dark-skinned Muscadine berries resulted in similar *trans*-resveratrol concentrations (Ector *et al*. 1996). On the other hand, the higher *trans*- and *cis*-piced levels of 'Barbera' and 'Croatina' as compared to 'Malvasia C.a.' is consistent with the literature (Romero-Perez *et al*. 2001). When wines are considered, those obtained from white-skinned varieties have, as a rule, lower stilbene levels than those derived from dark skins (Fregoni *et al.* 1994; Goldberg *et al*. 1995 a). The effect of the genotype (grape variety) seems to be very important even though it is still unclear what physiological aspects control stilbenes. This difference among varieties was evident each year, especially in 2001. The three varieties differed in production and fruit quality parameters, confirming the known features of those genotypes in the Piacenza region (Fregoni *et al.* 1992). 'Barbera' had a high acidity level, while 'Croatina' had low acidity levels at the same sugar concentrations. An effect of the year is only evident for *cis*-piceid and it was related to the relative humidity (positively) and the degree days (negatively) of the last stage of ripening. This finding makes sense because under increasing humidity fungal pressure is more severe prompting the elicitation of phytoalexins (Jeandet *et al.* 1995). The effect of the vintage year was particularly significant for 'Barbera', which was most responsive to environmental conditions. It is well-known that under severe fungal pressure, induced by environmental conditions, including very high relative humidity (> 80 %), the balance between induced and degraded (by fungi) phytoalexins is shifted toward degraded phytoalexins resulting in a reduced net concentration at the berry level. The effect of temperature is more complex, since it affects both plant and fungal physiology. The result on fungi is indirect, and related to the negative effect of rising temperatures on relative humidity. The results presented here, indicating a positive effect of lower temperatures on stilbene synthesis, are consistent with studies by Goldberg

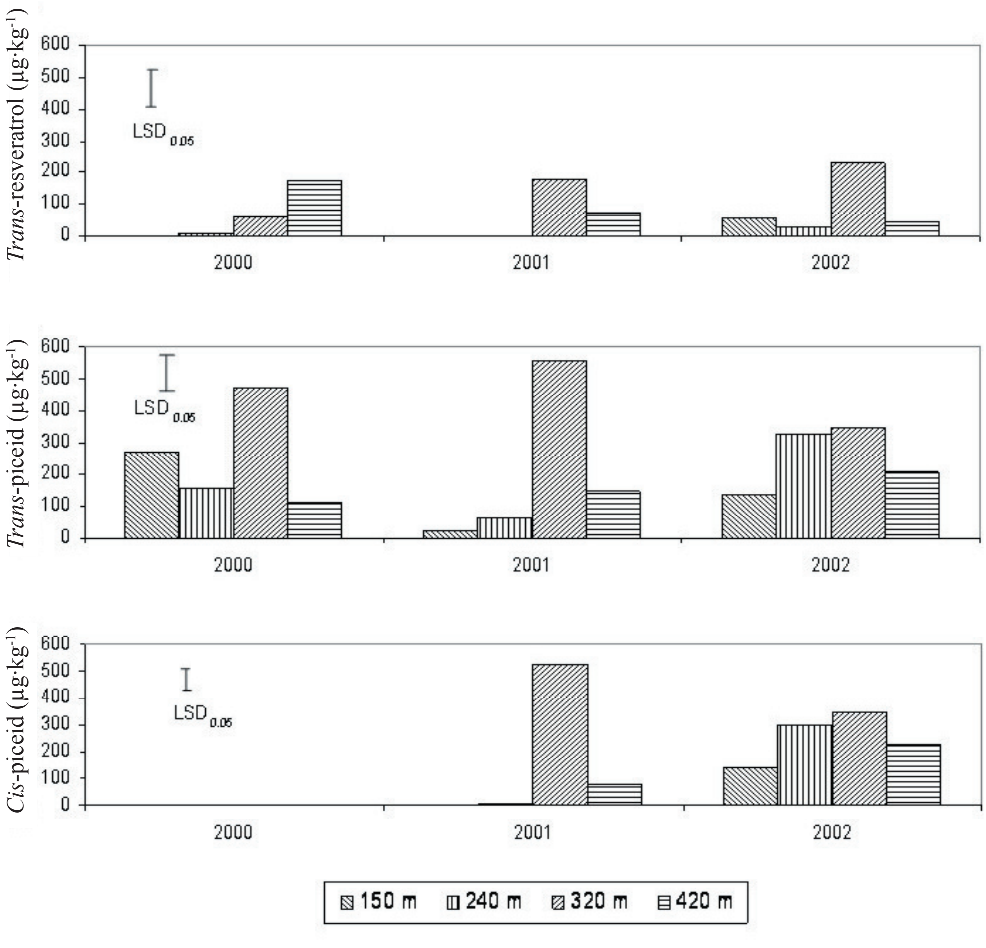

Figure: Stilbene grape concentration of 'Barbera' depending on the year and the elevation.

et al. (1995 b) on resveratrol of wines from different growing areas, but an additional (Goldberg *et al*. 1999) study found that the relationship of stilbene do not always increase with lower temperatures. It is difficult to explain why in 2000 no *cis*-piceid was synthesized, and why *trans*-resveratrol and *trans*-piceid were not significantly affected by the year, particularly when different annual meteorological conditions influenced fruit quality resulting in decreased soluble solids and increased acidity as expected from 2000 to 2002. The effect of elevation on stilbene grape content is very interesting because every compound increased from elevation 1 (150 m) to elevation 3 (320 m) and then decreased at elevation 4 (420 m). The general effect of elevation on stilbenes varied among varieties, and was only consistent with 'Barbera', demonstrating the important role of genotype in stilbene studies. The effect of different years was also important, especially for *trans*-resveratrol, as evidenced by different elevations having the highest values in different years. Meteorological factors other than UV-B irradiation are likely to be involved in stilbene synthesis when grapes are grown at different elevations.

Conclusions

The most significant finding of the trial were: (i) There is a strong genetic impact on stilbene synthesis, 'Barbera' produced the highest levels and 'Malvasia C.a.' the lowest. (ii) The relative humidity and the temperature in the last phase of ripening play an important role in *cis*-piceid levels, especially for 'Barbera'. (iii) Stilbene synthesis increased with elevation up to 320 m, and then decreased.

Acknowledgements

The research was supported by the CRPV (Centro Ricerche Produzioni Vegetali), Faenza (RA) and the MIUR (Ministero Istruzione Università e Ricerca). The authors wish to thank Mr. G. Bruzzi (Università Cattolica lab crew) for his contribution to the project, and the "Servizio Agrometeorologico" of Piacenza provincial administration for providing the meteorological data.

References

Bavaresco, L.; Fregoni, C.; 2001: Physiological role and molecular aspects of grapevine stilbenic compounds. In: K. A. Roubelakis-Angelakis (Ed.): Molecular Biology and Biotechnology of the Grapevine, 153-182. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Bavaresco, L.; Fregoni, M.; Trevisan, M.; Mattivi, F.; Vrhovsek, U.; Falchetti, R.; 2002: The occurrence of the stilbene piceatannol in grapes. Vitis **41**, 133-136.

Bavaresco, L.; Petegolli, D.; Cantù, E.; Fregoni, M.; Chiusa, G.; Trevisan, M.; 1997: Elicitation and accumulation of stilbene phytoalexins in grapevine berries infected by *B. cinerea*. Vitis **36**, 77-83.

Ector, B. J.; Magee, J. B.; Hegwood, C. P.; Coign M. J.; 1996: Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds, and wine. Am. J. Enol. Vitic. **47**, 57-62.

Fregoni, M.; Bavaresco, L.; Petegolli, D.; Trevisan, M.; Ghebbioni, C.; 1994: Indagine sul contenuto di resveratrolo in alcuni vini della Valle d'Aosta e dei Colli piacentini. Vignevini **21**, 33-36.

Fregoni, M.; Pezzutto, S.; 2000: Principles et premières approches de l'indice bioclimatique de qualité de Fregoni. Prog. Agric. Vitic. **117**, 390-396.

Fregoni, M.; Zamboni, M.; Boselli, M.; Fraschini P.; Scienza, A.; Valenti, L.; Panont, C. A.; Brancadoro L.; Bogoni, M.; Failla, O.; Filippi, N.; Laruccia, N.; Nardi, I.; Lega, P.; Linoni, F.; Libè, A.; 1992: Ricerca pluridisciplinare per la zonazione viticola della Val Tidone (Piacenza, Italia). Vignevini **19**, 52-80.

Gladstone, J.; 1992: Viticulture and Environment, Winetitles, Adelaide.

Goldberg, D. M.; Karumanchiri, A.; Soleas, G. J.; Tsang, E.; 1999: Concentrations of selected polyphenols in white commercial wines. Am. J. Enol. Vitic. **50**, 185- 195.

Goldberg, D. M.; Karumanchiri, A.; Yan, E.; Ng, J.; Diamandis, E. P.; Soleas, G. J.; 1995 b: Direct gas chromatographic-mass spectrometric method to assay *cis*-resveratrol in wines: preliminary survey of its concentration in commercial wines. J. Agric. Food Chem. **43**, 1245-1250.

Goldberg, D. M.; Yan, J.; Diamandis, E. P.; Ng, E.; Karumanchiri, A.; Soleas, A.; Waterhouse, A. L.; 1995 a: A global survey of *trans*-resveratrol concentrations in commercial wines. Am. J. Enol. Vitic. **46**, 159-165.

Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P.; 1995: Resveratrol content of wines of different ages: Relationships with fungal disease pressure in the vineyard. Am. J. Enol. Vitic. **46**, 1-3.

Mattivi, F.; Reniero, F.; Korhammer, S.; 1995: Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. J. Agric. Food Chem. **43**, 1820-1823.

Mennella, C.; 1967: Il clima d'Italia nelle sue caratteristiche e varietà e quale fattore dinamico del paesaggio, EDART, Napoli.

Romero-Perez, A. I.; Lamuela Raventòs, R. M.; Andrés-Lacueva, C.; de la Torre-Boronat, M. C.; 2001: 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. **49**, 210-215.

Siemann, E. H.; Creasy, L. L.; 1992: Concentration of phytoalexin resveratrol in wine. Am. J. Enol. Vitic. **43**, 49-52.

Received October 25, 2006