Vitis **50** (1), 43–44 (2011)

Research Note

Preliminary results on the effect of cluster thinning on stilbene concentration and antioxidant capacity of *Vitis vinifera* L. 'Barbera' wine

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K e y w o r d s : 'Barbera', cluster thinning, resveratrol, antioxidant activity, DPPH.

Introduction: Stilbenes are low molecular weight phenolics synthesized by several plants, including grapevine, with a constitutive or inducible origin. It has long been established that *trans*-resveratrol, the first to be discovered and the most studied compound, plays a significant antifungal role acting as a phytoalexin. Other experiments have shown its important antioxidant activity is positively involved on human health (Alarcón de la lastra *et al.* 2006), as well as a role in extending the lifespan of yeasts and other organisms by interacting with the sirtuin metabolism (Lamming *et al*. 2004). Stilbenes are synthesized following the activation of stilbene synthase (Vezzulli *et al.* 2007) by abiotic and biotic elicitors, including main fungal diseases (Jeandet *et al.* 1995; Bavaresco *et al.* 2008). The grape variety, the environmental conditions (Bavaresco *et al*. 2007, de Andres de Prado *et al.* 2007) and some cultural practices affect grape and the wine stilbene concentration, and therefore site selection and cultural practices including cluster thinning (Prajitna *et al.* 2007) can be managed in order to increase the grape and wine concentrations of these beneficial metabolites. This investigation studied the influence of cluster thinning on stilbene concentration and antioxidant activity in wines from *Vitis vinifera* L. 'Barbera'.

Material and Methods: The trial was carried out in 2009 on two *Vitis vinifera* 'Barbera' vineyards placed at 245 and 380 m asl, in Tidone Valley (Piacenza, Northern Italy), north exposed and planted in 1990. The vines were Guyot trained (vertical shoot positioning), leaving 11 nodes/vine, vine spacing of 2.3 m between rows and 2.5 m in the row, with coupled plants, corresponding to 3,500 vines/ha. At veraison (August 3) a cluster thinning treatment (CT) was applied removing the distal clusters and keeping only one cluster per shoot, corresponding to a 50 % severity, while unthinned vines were included as control (C). Treatments were compared in a 2 blocks design. At ripening (September 17) the main yield (grape yield per vine, buds and clusters number) and qualitative (soluble solids, pH, titratable acidity) variables were determined and, according to the field replicates, two 10-cluster samples (about 3 kg) each from 5 vines/block were harvested and vinified per each vineyard. Grapes were destemmed, crushed and treated with 0.1 $g \cdot L^{-1}$ of potassium metabisulfite, and stored according to the experimental design. Inoculation was made with *Saccharomyces cerevisiae* (Fermivin PDM®, DSM Delft, The Nederlands), hydrated in sucrose solution and, added at 0.2 g yeast/L of must. Fermentation was carried out in 2.5 L flasks at 25 °C during 13 d; cap was manually pushed down twice a day. After fermentation wines were racked and cold-stabilized at 2 °C for 30 d, bottled and stored at the same temperature. Wines were analyzed in order to determine its stilbene concentration (January 2010) and antioxidant activity (April 2010).

S t i l b e n e s : For each treatment, four wine samples (2 per vineyard by origin) were processed in triplicate. Wine was directly injected after a 5 min centrifugation at 3500 rpm. Analyses were performed by an HPLC (Agilent HP 1100 – Waldbronn, Germany) coupled to a diode detector (DAD) set at 325 nm to detect every stilbenes except *cis*-resveratrol which was set at 306 nm. A C18 (Supelco Supelcosil) column was adopted according to Bavaresco *et al.* (2008). Commercial standard of *trans*-resveratrol (Sigma chemicals) was utilized while piceid standard was kindly supplied by F. Mattivi, Fondazione Mach, S. Michele all'Adige, corresponding to a purity above 98 %. Quantitative analysis was performed by measuring peak areas.

A n t i o x i d a n t a c t i v i t y : The antioxidant activity was determined by reduction of free radical DPPH method (Brand-Williams *et al*. 1995) modified by the authors as follows. A stock solution of diluted red wine (1:25 in methanol) was stored at 4 °C for 90 min and then centrifuged (7 min at 8000 rpm) to promote a precipitation and a separation of pectins, respectively. Afterwards the stock solution was further diluted to obtain 5 final dilutions (1:25, 1:50, 1:125, 1:250, 1:500). A 150 µL of diluted wine was added to 3 mL of a 1.64 M methanolic solution of DPPH (Carlo Erba, Milano), incubated for 60 min at room temperature in the darkness and then the absorbance of the reaction solution was measured at 517 nm. For each sample, a blank containing 150 µL diluted wine and 3 mL methanol was incubated to correct the wine interference on absorbance. A control containing 150 µL methanol and 3 mL of the same DPPH solution was incubated. The percentage of inhibited DPPH was calculated as follows: $\%inib = 100 - [(DO_{sample} - DO_{blanc})/DO_{control}] * 100$. A linear regression was obtained and the EC_{50} (the final wine dilution required to inhibit 50 % of the initial DPPH in the reaction solution) was calculated based on the line equation. Antioxidant capacity was reported as mmol Trolox equivalent per L of wine (TE $mmol \cdot L^{-1}$).

S t a t i s t i c a l a n a l y s i s : Data were analyzed using analysis of variance and the least significance differ-

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T a b l e

Impact of cluster thinning on anthocyanins, polyphenols, stilbene concentration and antioxidant activity of 'Barbera' wines

Treatment	Anthocyanins mg/L	Total Polyphenols mg/L	*trans*-Piceid mg/L	*trans*-Resveratrol mg/L	Trolox equivalent mmol/L
Cluster thinning	730	3535	0.40	1.41	10.0
Control	524	2698	0.26	0.98	6.6
F	*11.40*	*22.81*	*15.85*	*63.36*	*6.94*
Significance	*	**	**	***	*
$LSD_{0.05}$	*170.0*	*487.2*	*0.051*	*0.316*	*3.12*

*,**,***, ns: Significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively. Means were separated by the LSD test.

ence was calculated by LSD test ($\alpha = 0.05$). A SPSS 15.0 per Windows software was adopted.

Results and Discussion: Cluster thinning applied at veraison reduced grape yield by 51.7 %, and titratable acidity by 9.6 %, while increasing the soluble solids and pH by 8.2 % and 2.6 %, respectively (data not reported), consistent with the literature (Mazza *et al.* 1999, Reynolds *et al.* 2007). The wines made from treated vines showed higher anthocyanin and polyphenols concentrations (Table) compared to the control, ranging from 730 mg/L (CT) to 524 $mg \cdot L^{-1}$ (C) and from 3,535 $mg \cdot L^{-1}$ (CT) to 2,698 $mg \cdot L^{-1}$ (C), respectively. Cluster thinning enhanced the *trans*-piceid and *trans*-resveratrol wine concentration over the control, as well, ranging from 0.40 $mg \cdot L^{-1}$ (CT) to 0.26 $mg \cdot L^{-1}$ (C) and from 1.41 $mg \cdot L^{-1}$ (CT) to 0.98 $mg \cdot L^{-1}$ (C), respectively (Table). The TE was higher in wines from cluster thinning treatment (10.0 $mmol \cdot L^{-1}$) than the control (6.60 $mmol \cdot L^{-1}$), corresponding to a 52 % enhancement (Table). Wine stilbenes and antioxidant activity of thinned vines were ≈ 50 % higher than the control vines, corresponding approximately to the cluster thinning severity. Results suggest that the antioxidant activity is related to the polyphenols, anthocyanins and also stilbene content in spite of their lower concentration. Our results confirm previous findings on wines from hybrids (Prajitna *et al.* 2007) even though in the present experiment a later time of thinning was applied and the grape variety belongs to *Vitis vinifera*. Other investigations are required to confirm these preliminary data and further studies on the gene expression level are in progress.

This research is carried out by the financial support of Cantina Sociale di Vicobarone (Piacenza, Italy)

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Received May 27, 2010