INFLUENCE OF LIGHT EXCLUSION ON ANTHOCYANIN COMPOSITION IN 'CABERNET SAUVIGNON'[1]

Pietro SCAFIDI[1]*, Maria Gabriella BARBAGALLO*[1]*, Mark O. DOWNEY*[2]
[1] University of Palermo – Dipartimento di Colture Arboree – Viale delle Scienze 11 - 90128 Palermo, I. E-mail: p.scafidi@unipa.it
[2] Department of Primary Industries Victoria, PO Box 905, Mildura, Vic. 3502, AUS.

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1. INTRODUCTION

In previous studies, the effect of bunch shading has been investigated by using of opaque polypropylene boxes (Downey *et al*., 2004; 2007) from fruit set to harvest. These boxes did not significantly alter berry development or ripening. Artificially shaded bunches had similar levels of total anthocyanins and tannins in the skins compared to sun-exposed fruit, although the composition of both anthocyanins and tannins were changed (Downey *et al*., 2004). In this study, differently from previous studies, the influence of artificial shading by applying of boxes over three different developmental stages was evaluated in 'Cabernet sauvignon'.

2. MATERIALS AND METHODS

2.1. Field experiments

The study was carried out in a E-W orientated 'Cabernet sauvignon' vineyard, located in Mildura, Australia (34°25' 28.70" S/ 142° 17' 02.02" E), trained to a two-wire vertical trellis. The boxes, designed by Downey *et al*. (2004) maintain airflow, exclude light and minimise changes in temperature and humidity, were made from a polypropylene sheet (0.6 mm), and were white outside and black inside. The dimensions were 0,25x0,20x010 m. Boxes were applied to bunches over three different developmental stages: 1) from fruit-set to harvest **(BF_SH)**; 2) from fruit-set to veraison **(BF_SV)**: 3) from veraison of exposed bunches to harvest **(BVH)**. Each trial was randomly replicated three times on three adjacent rows. All vines were defoliated in the bunch zone after fruit set to nullify the shading by leaves and boxed treatments were compared to the exposed fruit.

2.1.1 Berry sampling and analysis

Five bunches were sampled from each field replicate. Berries were removed and 30 berries were randomly sampled and weighed. For each 30-berry sample, the flesh was separated from the skins and then was crushed, centrifuged and juice total soluble solids (°Brix) measured using a refractometer. Each 30-berry sample represented one field replicate. The skin weight of each sub-samples was recorded and then, skins were ground to a fine powder in liquid nitrogen, and their total anthocyanin concentration and anthocyanin

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pattern of accumulation were determined by HPLC (Downey *et al.*, 2007; Downey, Rochfort, 2008).

3. RESULTS AND DISCUSSION

The berries boxed at fruit-set had delayed growth and ripening. When exposed bunches were fully coloured (14 January), boxed clusters had only 20 % of berries coloured. On February 4, the percentage of coloured berries in the boxed clusters (BF_SH: 93 % BVH: 91 %) was still significantly lower than that of exposed berries.

Berries, boxed until veraison (14 January) and exposed after this phenological stage, stopped their growth, while at harvest no difference in berry weight was found between the other treatments (tab. 1). It is uncertain what the mechanism for this sunlight shock would be.

Tab. 1 - Berry weight (g) during ripening. Values represent mean ± SE (n=3).

Treatment	**16/12**	**14/01**	**04/02**	**25/02**
Exposed	0.45±0.03	0.72±0.02	1.04±0.07	1.04± 0.01
BF_SH	0.38±0.02	0.53±0.02	0.82±0.02	1.03± 0.02
BF_SV	0.38±0.02	0.53±0.02	0.89±0.03	0.86±0.08
BVH	0.45±0.03	0.72±0.02	0.99± 0.05	1.12±0.03

At harvest similar values were found in total soluble solids concentration among treatments, except for the grapes shaded from veraison to harvest (BVH) (2). Sugar accumulation is independent from bunch shading, being due to photosynthetic activity of leaves, as previously reported by Haselgrove *et al*. (2000) and Downey *et al*. (2004).

Tab. 2 - Total soluble solids (°Brix) during ripening. Values represent mean ± SE (n=3).

Treatment	**14/01**	**04/02**	**25/02**
Exposed	12.30±0.93	18.10±0.21	22.95±0.18
BF_SH	9.67±0.47	16.53±0.50	23.03±0.24
BF_SV	9.67±0.47	18.33±0.37	23.63±0.19
BVH	12.30±0.93	16.60±0.40	21.30±0.35

Bunch shading delayed anthocyanin accumulation, as already found by Dokoozlian and Kliewer (1996), Barbagallo *et al.* (2007), Chorti *et al*. (2010). Light was crucial for anthocyanin synthesis from veraison, but not before this stage (tab. 3).

Tab. 3 - Total skin anthocyanins (mg g^{-1} of skin)during ripening. Values represent mean ± SE (n=3).

Treatment	16/12	14/01	04/02	25/02
Exposed	0.08±0.01	0.80±0.21	2.47±0.26	3.48±0.13
BF_SH	0.07±0.01	0.18±0.02	0.84±0.05	2.11±0.17
BF_SV	0.07±0.01	0.18±0.02	3.04±0.73	4.57±0.52
BVH	0.08±0.01	0.80±0.21	1.08±0.19	1.60±0.22

However, berries shaded from fruit set to harvest also produced anthocyanins, so light was not the unique factor affecting anthocyanin biosynthesis, consistent with the observations of Downey *et al.* (2004).

At harvest (25 February), concentration (mg g^{-1} of skin) of non-acylated anthocyanins was higher in the skin of berries exposed after veraison (Exposed, BF_SV). In these treatments, malvidin-3-glucoside was lower, while delphinidin-3-glucoside and petunidin-3-glucoside were higher, indicating a decrease of the methylating process. The higher content of acylated anthocyanins in boxed berries was due to a higher level of acetyl-glucoside anthocyanins (tab. 4). The shift in hydroxylation pattern with shading reported by Downey *et al*. (2004) was not observed in 'Cabernet sauvignon' (tab. 4).

Tab. 4 - Anthocyanin composition at harvest (25/02/2008) expressed as %
Values represent mean ± SE (n=3)

Treatment	Non-acylated Glucoside	3'-hydroxylated	3',5'-hydroxylated	Acylated glucoside	Acetyl glucoside
Exposed	56.6±0.33	8.5±0.16	91.5±0.16	43.4±0.55	31.7±0.37
BF_SH	47.7±0.33	5.6±0.19	94.4±0.19	52.3±0.37	41.0±0.11
BF_SV	58.1±0.26	9.5±0.25	90.5±0.25	41.9±0.39	32.1±0.23
BVH	45.3±1.47	6.6±0.35	93.4±0.35	54.7±1.52	38.9±0.76

4. CONCLUSION

Bunch shading delayed ripening and reduced anthocyanin concentration (Dokoozlian, Kliewer, 1996). Light was decisive from veraison for anthocyanin accumulation. In other plant species, eg. apple and petunia, where light is essential for anthocyanin accumulation; tissues grown in the dark do not accumulate anthocyanins (Dong *et al*., 1998, Katz, Weiss, 1999). In our trial in the absence of light, there was an accumulation of anthocyanins.We concluded that light was not the only determining factor for anthocyanin accumulation in 'Cabernet sauvignon'.

Bunch shading after veraison (BVH) reduced anthocyanins synthesis (Barbagallo *et al*., 2007; Chorti *et al*., 2010). As found by Takeda *et al.* (1988), after anthocyanin synthesis began, light was important for holding maximum activity of enzymes involved in the production of these compounds. The light conditions after veraison changed anthocyanin pattern. The light exclusion in the last stage of ripening caused esterification processes that led to the production of the acetate form, as reported by Ristic *et al*. (2007) in 'Shiraz'.

Abstract

The aim of this study was to determine how artificial shading influenced berry development and anthocyanin accumulation in 'Cabernet sauvignon'. Opaque polypropylene boxes were applied to grape bunches over three different developmental stages. The vines were defoliated in the bunch zone to nullify the shading by leaves. Bunch shading before veraison delayed growth and ripening. Light exposure after veraison influenced anthocyanin components and pattern.

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