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Cluster shading effects on fruit quality, fruit skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid)

by

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S u m m a r y : The effects of 55 and 95 % cluster shading on fruit quality, fruit color, anthocyanin content, the contents of individual anthocyanins, and percentages of individual anthocyanins in the berry skins of cv. Reliance, were examined in detail in 1991. Juice soluble solids content was decreased significantly by 95 % shading in comparison to 55 % shading or unshaded control. Berry weight was decreased by cluster shading treatments following a linear relationship. 95 % shading produced fruit clusters that were lighter in color (CIE 1976 L^*), less red (CIE 1976 a^*), and more yellow (CIE 1976 b^*) compared to 55 % shading or unshaded control. Anthocyanin content as well as individual anthocyanins including delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, and acylated cyanidin derivative were decreased by 95 % shading. 95 % shading decreased the percentage of delphinidin-3-glucoside in comparison with 55 % shading and decreased the percentage of cyanidin-3-glucoside and increased the percentages of peonidin-3-glucoside, malvidin-3-glucoside, and acylated cyanidin derivative compared to 55 % shading or unshaded control.

K e y w o r d s : sunlight, cluster shading, table grape, berry, maturation, soluble solids, fruit color, anthocyanins.

Introduction

Sunlight has been found to be very critical for fruit color development in many grape cultivars (Kliewer 1970; Kliewer 1977; Crippen and Morrison 1986; Roubelakis-Angelakis and Kliewer 1986, Dokoozlian and Kliewer 1992). Cluster shading was shown to reduce total anthocyanin content in pigmented grape cultivars (Kakaota *et al.* 1984; Crippen and Morrison 1986; Rojas-Lara and Morrison 1989; Gao and Cahoon 1991 and 1992). Pigments responsible for the attractive red, blue, purple, and black color in grapes, are anthocyanins, a class of water soluble flavonoid pigments (Ribereau-Gayon 1982; Van Buren *et al.* 1970). Anthocyanins in De Chaunac are present in the vacuole of subepidermal cells in the berry skin (Moskowitz and Hrazdina 1981). The anthocyanin profile in a grape cultivar can be very complex. HPLC analyses of grape anthocyanin profiles have shown that a grape cultivar may have up to 20 different anthocyanins (Wulf and Nagel 1976; Hebrero *et al.* 1989). Detailed studies of individual anthocyanins in a given grape cultivar provided significant insight to the understanding of anthocyanin metabolism in grapes (Wicks *et al.* 1982; Roggero and Ragonnet 1986; Cacho *et al.* 1992).

Reliance (*Vitis* hybrid) is a red seedless grape with excellent taste, delicate aroma, early ripening, moderate disease resistance, and exceptional winter hardiness (Moore 1983). It was used in this study due to its economic importance as a red seedless table grape cultivar in Ohio (Cahoon 1983), a large variation in fruit color at maturity, and its relatively simple anthocyanin profile (Gao 1993).

Studies of sunlight exposure of fruit clusters in relation to red color development in Reliance can help justify the necessity of such cultural practices as selective leaf removal around fruit zone or shoot positioning to expose fruit clusters to sunlight for the best fruit pigmentation.

Studies of individual anthocyanins in Reliance grape can lead to a better understanding of the mechanism in which anthocyanin biosynthesis is regulated. Fruit cluster shading treatments can be used to test how the synthesis of different anthocyanins in Reliance grape is regulated by light. C_{18} reverse-phase HPLC analysis of the anthocyanins from Reliance grape showed 7 components (Gao 1993). With the joint use of HPLC, paper chromatography, thin layer chromatography, and spectral measurement, three components were identified as delphinidin-3-glucoside, cyanidin-3-glucoside, and peonidin-3-glucoside (Gao 1993). Four other anthocyanin components were tentatively identified as cyanidin-3,5-diglucoside, petunidin-3-glucoside, malvidin-3-glucoside, and an acylated cyanidin derivative.

The objective of this study was to investigate the effects of cluster shading on fruit color development in Reliance. Fruit quality, color, content of total anthocyanin and individual anthocyanins, and percentages of individual anthocyanins in the berry skin of the Reliance, were studied to gain a better understanding of the controlling mechanism in fruit color development.

Materials and methods

G r a p e v i n e s a n d t r e a t m e n t s : Own rooted Reliance grapevines in the experiment were planted in 1985 at Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio, U.S.A. Grape-

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vines were trained to the Single Curtain Cordon System. Row and vine spacings were 3.1 m x 2.4 m. Vines were pruned to 60 buds per vine. Shoot number was then adjusted to 50 per vine one week after full bloom. Cluster shading treatments applied were unshaded (control), 55 % shading, and 95 % shading when berries reached about 2-3 mm in diameter. Clusters were shaded with shading cages constructed of 55 or 95 % polypropylene shade cloth (Pak Unlimited, Norcross, Georgia, U.S.A). Shoots and leaves surrounding control or treated clusters were manipulated so that these leaves and shoots did not shade control or treated clusters. Two clusters were selected per treatment on each experimental vine. Each vine served as a replicate. The experimental design was a randomized complete block with 12 replicates.

Temperature around fruit clusters: Temperature readings were measured with thermocouples that were placed beside control and treated clusters on August 5, 1991, two weeks before harvest. A 21X Micrologger (Campbell Scientific Inc, Logan, Utah, U.S.A.) was used to continuously record air temperature readings beside fruit clusters over a period of 2 weeks.

Fruit quality and color: Fruit juice was obtained by pressing 100 berries per sample through a Garden-Way Squeezo strainer (Lamra Products, Boca Raton, Florida, U.S.A.). Soluble solids of the fruit juice was measured with an ABBE-3L Refractometer (Baush & Lomb Inc, Rochester, New York, U.S.A.). Juice pH was measured with a Beckman pH meter (Model PHI 45, Beckman Instruments Inc, Fullerton, California). Titratable acidity (TA %) was measured by a titration of 5 ml of juice with 0.1 N NaOH to pH 8.2. Fruit skin color was measured with a Minolta Chroma Meter (Model CR-100, Minolta Camera Co., Ltd, Higashi-Ku, Osaka, Japan) as CIE (Commission Internationale de I'Eclairage translated as the Intern. Commission of Illumination) 1976 L^*, a^*, and b^*. CIE 1976 L^* represents black to white as CIE 1976 L^* values increase from negative to positive. CIE 1976 a^* represents green to red color as CIE 1976 a^* values increase from negative to positive. CIE 1976 b^* represents blue to yellow color as CIE 1976 b^* values increase from negative to positive. Fruit skin color readings were taken from top, middle, and bottom portion of east-facing side of each treated cluster. The mean of these 3 readings was used.

Sample preparation for anthocyanin analysis: Fruit cluster samples were collected on August 18, 1991, and immediately placed in 4 °C storage. They were weighed and berry number per fruit cluster counted. Clusters were then frozen and stored at -20 °C for future analyses. Berry skins were removed and collected by first thawing the frozen berries in a refrigerator at 4 °C for 20 min. The berry skin was then peeled with tweezers, and kept in an ice chilled beaker. Finally, these berry skins were freeze dried and ground with a coffee mill.

Anthocyanin extraction and concentration: 1 g of ground berry skin was placed in 100 ml of 1 % 12 N HCl in methanol. The anthocyanin extraction was carried out overnight in a refrigerator at 4 °C. These anthocyanin extracts were then filtered through Whatman No.1 filter paper in a Buchner funnel. 20 ml of deionized distilled water was added to each anthocyanin extract. The anthocyanin extracts were concentrated with a rotary evaporator under vacuum at 30 °C. Each anthocyanin concentrate was transferred to a 25 ml volumetric flask and then brought to volume with deionized distilled water. 5 ml of each anthocyanin concentrate was then passed through a 0.2 µm syringe membrane filter which was equilibrated with 1 ml of respective anthocyanin concentrate to avoid anthocyanin dilution by syringe membrane filters. Each anthocyanin filtrate was stored at room temperature for less than 20 min in a screw capped sample vial before HPLC analysis.

HPLC analyses were performed on a Model SP4000 pump (Spectra-Physics, San Jose, California, U.S.A.) equipped with a 20 µl Rheodyne sample loop. The analytical column was a pH stable RP-18 Spherisorb (Merck, Darmstadt, Germany) (150 mm x 4.6 mm I.D.) packed with 5 µm particles by Alltech (Deerfield, Illinois, U.S.A.). A Spectra-Physics UV1000 variable wavelength detector and Spectra-Physics Model 4600 integrator were used.

The following conditions were used for the analyses of anthocyanins. Solvent A was 10 % formic acid in water. Solvent B was high purity acetonitrile. These solvents were filtered through 0.20 µm membrane filters and sparged with helium. Solvent flow rate was 1 ml/min. The solvent program used for anthocyanins was 95 % A initially, decreased from 95 % A to 72 % A in 20 min following a linear curve. Detection was carried out at 520 nm. Detector was set at 1 absorption unit full scale.

Determination of anthocyanin content: In order to convert the peak areas into pigment concentration per gram of dry berry skin, a solution of cyanidin-3-glucoside in 0.1 N HCl was prepared to establish a standard curve. The solution was filtered through a 0.2 µm membrane filter. Its absorbance was read at 520 nm. The concentration (g/100 ml) of this solution was calculated based on the extinction coefficient of cyanidin-3-glucoside in 0.1 N HCl. A series of dilutions was then made. 20 µl of diluted samples was injected into HPLC under identical analytical condition as for the Reliance samples.

A standard curve was established between the concentration of diluted cyanidin-3-glucoside solutions and their peak areas. The concentration of cyanidin-3-glucoside was calculated based on the following equation where the correlation coefficient was 0.998:

mg/100 ml = peak area x 0.00000391.

Since 1 g of dried berry skin was dissolved in a final volume of 25 ml, the concentration of cyanidin-3-glucoside was calculated as:

mg/g dried berry skin = (peak area x 0.00000391 x 25 ml)/100 ml.

The content of each individual anthocyanin was expressed as cyanidin-3-glucoside equivalent based on their respective peak area since cyanidin-3-glucoside was present at the highest concentration in Reliance anthocyanin profile (GAO 1993). The percentage of each individual anthocyanin was calculated as:

Percentage = (the content of individual anthocyanin x 100)/ anthocyanin content.

Anthocyanin content was calculated as the sum of the contents of all the individual anthocyanins.

Results and discussion

Temperature readings were taken to verify that differences created by the shading treatments were not from a secondary warming effect created by the shade cloth. The air temperature of one daily cycle beside the grape clusters was shown as trends were similar over the two week period. Air temperature readings beside control and shaded fruit clusters on August 8, 1991 are shown in the Figure. Air temperature beside 95 % shaded clusters was lower than that of control from 1 a.m. to 6 a.m., higher from 6 a.m. to 10 p.m., similar or equal from 10 p.m. to midnight. The air temperature of 55 % shaded clusters was similar to that of control except at noon when 55 % shading provided a cooling effect.

In studies with potted Carbernet Sauvignon grapes, pigmentation was enhanced by low day temperature of 20 °C in comparison with high day temperature of 30 °C (Buttrose *et al.* 1971). Kliewer and Torres (1972) reported that fruits ripened at cool night temperature (15 °C) had much greater coloration than at warm night temperature (25 °C) and a 10 °C decrease in day temperatures under high light improved fruit coloration by about 40 % (Kliewer 1970). Weaver and McCune (1960) found 1.1 °C difference between control clusters and clusters enclosed in brown bags. Weaver and McCune (1960) found that bagged clusters warmed up more slowly, but cooled off more slowly and that there was a shift in temperature within the bags as compared to control clusters. They concluded that temperature difference was very small and of questionable significance. The maximum temperature differences in this study between 95 % shaded and full sun control were observed to be 2.8 °C. Possible effects of temperature differences in this study on fruit color should be minimal.

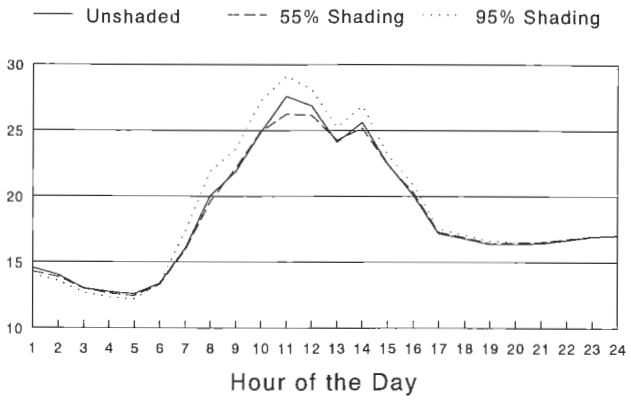

Figure: Air temperature readings beside fruit clusters under unshaded (control), 55 % or 95 % shading, were taken on August 5, 1991.

Fruit soluble solids content was decreased significantly by 95 % shading in comparison to 55 % shading or the unshaded control (Tab. 1). There were no significant differences in fruit soluble solids between 55 % shading and unshaded control. Kliewer (1977) reported that 54 % full sun did not reduce soluble solids in Emperor grapes while 15 % full sun did. Cluster weight and juice pH were not affected by cluster shading. However, juice TA was increased significantly by 55 % shading in comparison to unshaded or 95 % shading. Berry weight was decreased by cluster shading treatments following a linear relationship. 95 % shading produced fruit clusters that were lighter in color, less red, and more yellow in comparison to unshaded control or 55 % shading.

Anthocyanin content in the berry skin measured with HPLC was decreased by 95 % shading in comparison to unshaded control or 55 % shading (Tab. 2). No difference in fruit anthocyanin content was found between 55 % shading and unshaded control. Kliewer (1977) reported that 85 % shading of Emperor grapes decreased fruit anthocyanin content whereas 46 % shading did not in comparison to 100 % full sun.

The contents of individual anthocyanins including delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-

Table 1

Effects of cluster shading on cluster weight, fruit qulaity and skin color (CIE 1976 L , a , and b) of Reliance grapes

Cluster shading treatment	Soluble solids °Brix	pH	TA (%)	Cluster weight (g)	Berry weight (g)	L	a	b
Unshaded control	20.7a	3.37a	0.47a	276.7a	2.2a	27.5b	9.1a	1.9b
55% Shading	20.3a	3.17a	0.51b	279.2a	2.1b	29.1b	7.8a	1.2b
95% Shading	19.1b	3.30a	0.48a	264.7a	1.9c	34.5a	4.9b	5.8a
LSD	0.74	0.23	0.02	39.7	0.1	1.7	1.4	1.2
Linear contrast	**	NS	NS	NS	**	**	**	**
Quadratic contrast	*	NS	NS	NS	NS	**	NS	**

Mean separation by least significant difference (LSD) at 0.05 level and orthogonal contrast at 0.01 or 0.05 level.
NS, *, ** Nonsignificant at 0.05 level or significant at 0.05 or 0.01 level, respectively.
Abbreviations: TA: titratable acidity; L represents light to dark color; a , red to green color; b , yellow to blue color.

Table 2

Effects of fruit cluster shading on content of total anthocyanin and individual anthocyanins in the berry skin of Reliance grapes

Cluster shading treatment	Dp-3-g (mg/g)	Cy-3-g (mg/g)	Pt-3-g (mg/g)	Pn-3-g (mg/g)	Mv-3-g (mg/g)	Acylated Cy (mg/g)	Anthocyanin Content (mg/g)
Unshaded control	0.98a	5.31a	0.11a	0.25a	0.04a	0.70a	7.21a
55% Shading	0.97a	4.69a	0.11a	0.25a	0.04a	0.61a	6.51a
95% Shading	0.20b	0.99b	0.02b	0.07b	0.01b	0.22b	1.46b
LSD	0.36	1.54	0.05	0.09	0.09	0.13	2.05
Linear contrast	**	**	**	**	**	**	**
Quadratic contrast	**	**	*	**	*	**	**

Mean separation by least significant difference (LSD) at 0.05 level and orthogonal contrast at 0.01 or 0.05 level.
*, ** see Tab. 1.
Abbreviations: Dp-3-g, delphinidin-3-glucoside; Cy-3-g, cyanidin-3-glucoside; Pt-3-g, petunidin-3-glucoside; Pn-3-g, peonidin-3-glucoside; Mv-3-g, malvidin-3-glucoside; Acylated Cy, acylated cyanidin derivative.

3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, and acylated cyanidin derivative were decreased by 95 % shading in comparison with 55 % shading or unshaded control (Tab. 2). There were no significant differences in individual anthocyanins between 55 % shading and unshaded control. The results demonstrated that cluster shading not only decreased total anthocyanin content but also the content of individual anthocyanins. WICKS *et al.* (1982) studied the influences of light and ethephon (an ethylene releasing compound) on anthocyanins determined by thin layer chromatography in Tokay, Emperor, Cardinal, and Ribier grapes. WICKS *et al.* (1982) found that cyanidin-peonidin pathway was induced by light, but light requirement can be overcome by an appropriate application of ethephon. Biosynthesis of anthocyanins in Reliance was not completely stopped by 95 % shading. This agrees with the results obtained by KLIEWER (1977) that total anthocyanin content in Emperor clusters under 3 or 0.8 % full sun (97 or 99.2 % shading) reached 34 % of that in clusters under full sun.

The percentages of most individual anthocyanins in the berry skin were also affected by 95 % cluster shading (Tab. 3). 95 % shading decreased the percentage of delphinidin-3-glucoside in comparison with 55 % but not unshaded control. The percentage of cyanidin-3-glucoside was decreased while the percentages of peonidin-3-glucoside, malvidin-3-glucoside, and acylated cyanidin derivative were increased by 95 % shading in comparison with 55 % shading or unshaded control. Cluster shading treatments had no effect on the percentage of petunidin-3-glucoside in comparison with unshaded control. The biosynthesis of peonidin-3-glucoside, malvidin-3-glucoside, or acylated cyanidin derivative seem to be much less light dependent than cyanidin-3-glucoside in Reliance. WICKS *et al.* (1982) reported that cyanidin-3-glucoside, the only anthocyanin in Tokay grape, appeared only in the presence of light with or without ethephon. This agrees with the finding that cyanidin-3-glucose in Reliance grape was more light dependent than other anthocyanins.

In a practical sense, results from this cluster shading study can help Reliance grape growers determine whether shoot positioning or selective leaf removal around fruit zone to expose fruit cluster to more sunlight is necessary to improve fruit color. Reliance clusters reach the full color potential if light levels are above 45 % full sunlight. However, when fruit clusters receive only 5 % full sunlight (95 % shading), cultural means such as selective leaf removal or shoot positioning would be beneficial to expo-

Table 3

Effects of fruit cluster shading on the percentages of individual anthocyanins in berry skin

Cluster shading treatment	Dp-3-g %	Cy-3-g %	Pt-3-g %	Pn-3-g %	Mv-3-g %	Acylated Cy %
Unshaded control	13.5ab	73.5a	1.3a	3.3b	0.5b	7.9b
55% Shading	15.5a	71.2a	1.6a	3.8b	0.6b	7.4b
95% Shading	11.3b	66.9b	1.6a	5.4a	0.8a	14.2a
LSD	3.0	4.3	0.5	1.0	0.2	2.9
Linear contrast	NS	**	NS	**	**	**
Quadratic contrast	**	NS	NS	NS	NS	**

Mean separation by least significant difference (LSD) at 0.05 level and orthogonal contrast at 0.01 or 0.05 level.
*, **, NS see Tab. 1. Abbreviations: see Tab. 2.

sure Reliance fruit cluster to enough sunlight for good red color development. Partial defoliation at veraison was shown to have the highest anthocyanin content in Cabernet Sauvignon grapes in comparison with partial defoliation at bud break, berry set, or pea size (HUNTER *et al.* 1991).

SMART *et al.* (1982) found that basal leaf illuminance was improved early in the season by canopy division and low shoot density; after flowering, the major effect was of shoot positioning. MORRIS *et al.* (1985) found that shoot positioning in Niagara grapes produced darker (more red color) fruit clusters than without shoot positioning. SHAULIS and MAY (1971) found that shoot crowding reduced yield while control of the shoot crowding with canopy dividing increased yield in Sultana grapes.

Reliance, with its relatively simple anthocyanin profile, was a good model for studying individual anthocyanin. Advances in HPLC made the simultaneous quantitative analyses of individual anthocyanins in a given grape cultivar possible. In future studies, light of different wavelengths with same intensity, should be used to determine how the light quality affects individual anthocyanin components and possible enzymes involved in anthocyanin biosynthesis of a given pigmented grape cultivar, i.e. Reliance. Light of different intensity at same temperature should also be studied in relation to fruit color development. Fruit color development could be improved effectively by the manipulation of light intensity or light quality around the fruiting zone, i.e. supplemental lighting, light reflectors, shoot positioning, and partial defoliation.

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