06-pagay_05b-tomazic 04/04/13 17:25 Page35

THE INFLUENCE OF BIRD NETTING ON YIELD AND FRUIT, JUICE, AND WINE COMPOSITION OF *VITIS VINIFERA* L.

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Abstract

Aims: To investigate the impact of semi-permanent bird netting and timing of its application on Cabernet franc grapevine yield components and fruit, juice, and wine composition.

Methods and results: Semi-permanent bird netting was installed over Cabernet franc grapevines at various times – post-bloom, bunch closure, and veraison – of the 2004 growing season in the Niagara Peninsula of Canada. At harvest, vine yield components were measured followed by berry and must compositional analysis of soluble solids, pH, titratable acidity (TA), color, and polyphenols. Wines made from these grapes were also analyzed (pH, TA, color, and polyphenols). It was found that installation of bird netting over grapevines had minimal effect on yield components and berry composition regardless of when the nets were installed. Must composition revealed significant decreases in soluble solids, pH, and color as a result of the netting, the least impact being when the nets were applied at post-bloom. Wine composition was similar to the must data with the netted treatments resulting in lower pH, higher TA, and decreased color. Total anthocyanins and polyphenols were slightly reduced as a result of the netting.

Conclusions: Minimal impact of bird netting on yield, fruit, must and wine quality is a positive finding since netting is becoming more prevalent in vineyards worldwide due to changing migratory patterns of birds. It is recommended that netting be applied around post-bloom for the ease of application, to minimize shading effects, which could lead to decreased fruit quality, and to maintain yield.

Significance and impact of the study: Use of bird netting is becoming more prevalent by grape growers worldwide due to changing migratory patterns of birds that feed on grapes. This study shows that bird netting is not detrimental to yield and fruit and wine quality especially when applied early in the growing season.

Key words: bird netting, depredation, HPLC, anthocyanins, polyphenols

Résumé

Objectifs: Étudier l'impact d'un filet de protection semi-permanent contre les oiseaux et de sa période de pose sur le rendement et la composition des raisins, du moût et des vins du cépage Cabernet franc.

Méthodes et résultats: Un filet de protection semi-permanent contre les oiseaux a été installé sur des vignes de Cabernet franc à différentes périodes – après floraison, fermeture des grappes, et véraison – durant la saison 2004 dans la péninsule du Niagara au Canada. À la vendange, les composantes du rendement ont été analysées ainsi que la composition des baies et du moût telle que les sucres solubles, le pH, l'acidité totale (AT), la couleur et les polyphénols. Les vins réalisés à partir de ces raisins ont aussi été analysés (pH, AT, couleur et polyphénols). L'installation de filets de protection contre les oiseaux a eu un faible impact sur le rendement et la composition des raisins indépendamment de la période de pose du filet. La composition du moût a révélé une diminution significative des sucres solubles, du pH et de la couleur, conséquence de la pose du filet, lorsque celui-ci a été installé après la floraison. La composition des vins a été semblable à celle du moût avec un pH plus bas, une AT plus élevée et une diminution de la couleur chez les variantes avec filets de protection. Une légère réduction des anthocyanes et des polyphénols totaux a résulté de l'installation des filets de protection.

Conclusions: Le faible impact causé par l'installation de filets de protection contre les oiseaux sur le rendement et la qualité des raisins, du moût et des vins représente un résultat positif en regard de l'utilisation de plus en plus répandue des filets de protection dans les vignobles du monde entier, due au changement des déplacements migratoires des oiseaux. Il est recommandé d'installer les filets autour de la période après floraison pour la facilité de sa mise en place, pour minimiser les effets d'ombrage qui conduirait à une diminution de la qualité des raisins et pour assurer un rendement.

Signification et impact de l'étude: L'utilisation de filets de protection contre les oiseaux est devenue de plus en plus répandue chez les producteurs à l'échelle mondiale en raison du changement des flux migratoires des oiseaux se nourrissant de raisins. Cette étude montre que l'utilisation de filets n'est pas dommageable pour le rendement et la qualité des raisins et des vins lorsque ceux-ci sont installés tôt dans la saison.

Mots clés: filets de protection contre les oiseaux, déprédation, CLHP, anthocyanes, polyphénols

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06-pagay_05b-tomazic 04/04/13 17:25 Page36

INTRODUCTION

Bird damage to tender fruit can be quite extensive (Himelrick, 1985 ; Fraser *et al.*, 1998) and is of serious concern to the grower, particularly of high-value crops such as wine grapes. The rapid growth of agricultural areas for the production of tender fruit crops has made these crops the preferred source of plentiful high quality food that is easily accessible and of a convenient size for birds to feed on. Changes in migratory patterns due to altered regional climatic conditions have also resulted in greater numbers of birds arriving in farm lands of northeastern United States and Canada during the grape growing season. While other forms of bird exclusion (acoustic and visual deterrents, biosonics, chemical repellents) exist, these are generally not entirely effective at preventing birds from eating grapes. A physical barrier, as provided by the nets, is the only effective means of bird exclusion and additionally offers the grower the benefit of higher yields, saved labor costs from shoot tucking operations during the season, and reduced neighbor conflicts as acoustic deterrents would no longer be necessary.

The rationale for using bird exclusion netting are : virtually 100 % effective bird exclusion strategy for crop protection resulting in higher realized yields to the grower ; decreased use of acoustic deterrents that may cause neighbor conflicts ; labor cost savings associated with one or more manual shoot tucking operations ; and a more upright, two-dimensional canopy resulting in reduced leaf and cluster shading. To-date, no studies have reported on the effects of long-term bird netting on vine yield and fruit composition, which we have addressed in this paper using the cultivar Cabernet franc. The goal of our study was to determine whether bird netting affected vine productivity and grape, juice, and wine composition as well as whether compositional differences, if any, warrant the expense of using semi-permanent netting as a viable bird exclusion strategy.

MATERIALS AND METHODS

1. Bird netting application

"Pro Guard" heavy-duty bird netting (Gintec Shade Technologies Inc., Windham Centre, ON) made of high-density UV-stabilized polyethylene of width 1.1 m was installed in 2004 in a commercial vineyard block (Whitty Farms, St. Catharines, ON) of six-year-old *Vitis vinifera* L. cv. Cabernet franc vines grafted on to 101-14 Mgt rootstock (*V. riparia* x *V. rupestris*) at a spacing of 1.2 m x 2.4 m (vine x row) and trained to the 'pendelbogen' system. The nets were attached to the first set of catch wires of the trellis and rolled down to the lower fruiting wire at the following Eichorn-Lorenz (E-L) phenological stages ("treatments") : post-bloom (E-L stage 27 ; 28 June 2004), bunch closure (E-L stage 33 ; 5 August 2004), and approximately veraison (E-L stage 35 ; 19 August 2004). An un-netted (control) treatment was also used in this study.

2. Experimental design

The experiment was set up in a single vineyard block as one vineyard row for each of four treatments (total of four rows in a single block), and 10 randomly selected vines per treatment row. In the winter preceding the growing season (2003-4), the vines were balance-pruned to approximately 15 nodes per meter of canopy with an extra trunk retained as per standard practice in Niagara. One shoot-tucking operation was done in the spring prior to dropping the nets for the first (post-bloom) treatment. Canopy light level or photosynthetically active radiation (PAR) was measured using an AccuPAR PAR-80 linear PAR/LAI ceptometer (Decagon Devices, Pullman, WA). Measurements were made of incident radiation (above the canopy) and light interception in the canopy interior by placing the linear probe horizontally along the canes and centered at the trunk. Light readings were taken at solar noon (1000h to 1400h) when light levels were greater than the saturating value of 1080 $\mu E/m^2/s$ and at temperatures ranging from 25 °C to 33 °C.

3. Harvest

Cabernet franc grapes were hand-harvested on 22 October 2004. Yield and cluster counts per vine were obtained and the ratio of these two numbers was used to determine the average cluster weight per vine. Skins were separated off a small sample of fruit (ca. 30 berries) from each treatment and kept at -40 °C for future analyses. Samples of 50 berries from each treatment were weighed and homogenized using a Waring blender and the juice was kept at -40 °C for later analysis of fruit composition. The remainder of the fruit was crushed and destemmed using a commercial crusher-destemmer. Approximately 300 L of grape must were obtained from crushing. Approximately 500 mL of juice sample was kept separately for must analysis while the remainder was vinified as per the method below (see '5. Winemaking').

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06-pagay_05b-tomazic 04/04/13 17:25 Page37

4. Berry composition

The frozen homogenized juice samples were thawed in a water bath (80 °C, 45 min) to re-dissolve precipitated tartrates and then clarified by centrifugation using an IEC Centra CL2 laboratory centrifuge (International Equipment Co., Needham Heights, MA) at 4500 rpm for 10 minutes. Soluble solids of the clarified samples were measured using an Abbé digital bench-top refractometer (American Optical Company, Buffalo, NY) with temperature correction. Titratable acidity (TA) and pH of the musts and wines were measured using a calibrated PC-Titrate automated titration system (Man-Tech Associates, Guelph, ON) with standardized 0.1 N NaOH. Color analysis of skins, musts and wines was based on a modified version of the method provided by Mazza *et al.* (1999). Using a 1-mm glass cuvette, absorbance measurements were made on all prepared samples at 420 nm and 520 nm wavelengths against a blank using an Ultrospec 2100 pro UV/Vis spectrophotometer (Biochrom, Cambridge, England). Anthocyanin measurements were completed on all prepared samples using the 'pH shift' method by measuring the differential absorbance at 520 nm between wines at pH 1.0 and pH 4.5 as per Metivier *et al.* (1980). Total anthocyanin concentration was expressed in malvidin chloride-3-glucoside equivalents. Phenolic analysis was done by the 'phenolics reduction method' as per Slinkard and Singleton (1977) using Folin-Ciocalteu phenol reagent (Folin's phenol reagent; Sigma-Aldrich, St. Louis, MO) on samples diluted 1:10 and absorbance readings taken at 765 nm. Total phenol concentrations in the samples were expressed in gallic acid equivalents (GAE). Grape skins were prepared for color and hue analysis as per a procedure obtained from Tibor Fuleki (Horticultural Research Institute of Ontario, Vineland Station, ON). Frozen Cabernet franc grape skins were taken and excess moisture and ice removed using a paper towel. Approximately 10 g of the skin sample was weighed in a 100 mL beaker and 10 mL of extraction buffer (95 % ethanol, concentrated hydrochloric acid, and distilled water in the ratio 85: 12:3) added. Skins and extraction buffer in the covered beaker were heated in a conventional microwave on 'high' for one minute. An additional 20 mL of extraction buffer was added and reheated for one minute. The resulting extract was decanted and filtered through a 0.45 µm filter for analysis.

5. Winemaking

Soluble solids of grape musts from each of the four treatments were initially measured using a Abbé digital bench-top refractometer (American Optical Company, Buffalo, NY) with temperature correction. Grape musts less than 22°Brix were adjusted to 22°Brix by chaptalizing with commercial cane sugar. Musts from each treatment were then divided into two equal batches as fermentation replicates and inoculated with Lalvin D-21 (Selection ICV) *Saccharomyces cerevisiae* yeast (Lallemand Corp., Montreal, QC) at a rate of 20 g/hL. 0.1 g of Fermaid K (Lallemand Corp., Montreal, QC) was also added to the yeast starter as a nutrient supplement. Fermentations were carried out on the skins at 24 °C for a period of five days with a twice-daily punch down of the caps until dryness. Maceration was allowed to continue until the caps had fallen, an additional two days. Wines were then pressed off the skins in a pneumatic press (Enoveneta, Piazzola sul Brenta, Padova, Italy) to a maximum pressure of 1.5 bar (22 psi), settled overnight, then racked into 6 L glass carboys. The young wines were inoculated with Lalvin EQ54 *Oenococcus oeni* lactic acid bacteria (Lallemand Corp., Montreal, QC) to initiate malolactic fermentation, which completed in approximately three weeks. The wines were racked, cold stabilized, filtered, bottled, and stored at 16 °C.

6. Must and wine composition

Must and wine compositional analyses were performed using the methods used for berry composition. In addition, finished wines were analyzed by HPLC to determine their polyphenolic composition (see '7. Anthocyanin and phenolic profiling').

7. Anthocyanin and phenolic profiling

Wines were analyzed by HPLC in order to obtain a profile of individual anthocyanin and phenolic compounds and to assess differences in their concentrations between the different netting treatments. A modified method based on that of Ibern-Gómez *et al.* (2002) was used using Agilent 1100 Series HPLC and ChemStation software (Agilent Technologies, Palo Alto, CA). Separation was carried out using an Agilent Zorbax Stablebond SB-C18 reverse-phase column (50 mm x 4.6 mm, 3.5 µm) with a Phenomenex SecurityGuard™ C-18 (4 mm) guard cartridge. The binary mobile phase consisted of 0.2 % trifluoroacetic acid (TFA) (solvent A) and HPLC-

J. Int. Sci. Vigne Vin, 2013, **47**, n°1, 35-45
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06-pagay_05b-tomazic 04/04/13 17:25 Page38

grade acetonitrile and 0.2 % TFA (solvent B). Flow rate was 1.0 mL/min. The gradient was as follows: 5 % B (0 min), 35 % B (15 min), 100 % B (16-25 min), 5 % B (26 min). Post-run time was 10 minutes. The wine samples and column temperatures were maintained at 30 °C. Sample injection volume was 5 µL. UV/Vis diode array detector wavelengths selected were 280, 320, 365, and 525 nm for hydroxybenzoic acids and flavan-3-ols, hydroxycinnamic acids, flavonols, and anthocyanins, respectively. Reference bandwidth for all detection wavelengths was 650 nm (bandwidth 5 nm). Anthocyanin standards were obtained from Extrasynthese SA (Genay, France) and Polyphenols Laboratories AS (Sandnes, Norway). Standards analyzed were delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and malvidin-3-*O*-glucoside. Identification of compounds was carried out by comparing their retention times with the standards. Acylated and coumaroylated anthocyanins were identified by comparison of peak retention times with those in the literature (Ibern-Gómez *et al.*, 2002). These anthocyanins were quantified based on relative concentrations of their equivalent non-acylated anthocyanins assuming equal absorptivities. Phenolic standards of gallic acid, (-)-epicatechin, caffeic acid, and quercetin were prepared at various concentrations typically found in wine in order to generate a standard curve, which was used for quantification of the individual phenolic compounds. (+)-catechin and caftaric acid concentrations were represented as (-)-epicatechin and caffeic acid equivalents, respectively, assuming equal absorptivities. All eight wine samples (two fermentation replicates per treatment) were prepared and analyzed in triplicate.

8. Statistical analysis

Statistical analysis was performed using the SAS statistical software package (PC version 8.1; SAS Institute, Cary, NC) using the GLM procedure. All yield components, berry, skin, must, and wine composition data was analyzed by analysis of variance (ANOVA) for each treatment. The three netting treatments were compared to the control by using Dunnett's *t*-test at a significance of 5 % ($p < 0.05$).

RESULTS AND DISCUSSION

1. Vine yield components

In general, application of the netting had a negative effect on canopy light interception (Table 1). All netted vines with the exception of the mid-season treatment had lower light levels in the canopy interior than control vines, with the lowest light levels observed in the post-bloom treatment. By veraison, around mid-August, the post-bloom netted vines still had relatively lower light levels in the canopy than the control or mid-season vines. Mid-season vines had the highest canopy light interception possibly as a result of the longer shoots and consequently greater numbers of leaves. Light interception for the veraison treatment was marginally lower than the control treatment; this was possibly due to the fact that most of the shoot growth occurs early in the season and by veraison, little or no shoot growth occurs.

Fruit yield per vine was not significantly influenced by the bird netting except when the netting was applied around bunch closure (mid-season treatment); there was a 47 % increase in vine yield for the mid-season treatment compared to the control (Table 1). Cluster counts and weights of the mid-season treatment were also the highest among

Table 1 - Impact of bird netting on Cabernet franc light interception and yield components, 2004.

Treatment	Canopy light interception ($\mu E/m^2/s$)	Vine yield (kg)	Clusters per vine	Cluster weight (g)
Control	277	2.50 [a]	42	60.0
Post-bloom	156	2.65	38	71.8
Mid-season	322	3.68 *	51	72.5
Veraison	230	2.70	39	68.5

[a] Coefficient significance: * indicates significant difference from 'Control' at $p < 0.05$ according to Dunnett's t-test, otherwise not significant.

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06-pagay_05b-tomazic 04/04/13 17:25 Page39

treatments, which would have contributed to the highest total vine yield found in this treatment; however, the reason for these differences is unknown. Although not significantly different, cluster weights were approximately 14-21 % higher in the netted vines overall compared to the control vines, possibly owing to bird predation in the unnetted vines. The yield data was generally inconclusive likely owing to the fact that the netting was installed the same year (Spring 2004) as the fruit was harvested and, therefore, would not have affected inflorescence development that year

Table 2 - Impact of bird netting on berry, juice, and wine composition of Cabernet franc, 2004.

	Treatments			
	Control	Post-Bloom	Mid-season	Veraison
Berry composition				
Soluble solids (°Brix)	22.2 [a]	22.0	21.5	22.2
pH	3.51	3.48	3.48	3.44
TA (g/L)	6.42	5.20 *	5.52	5.33
Color (abs. units)	8.34	7.97	7.22	7.17
Hue (abs. units)	0.82	0.82	0.82	0.86
Total Anthocyanins (g/L)	0.48	0.43	0.37	0.37
Total Phenols (g/L)	1.38	1.25	1.13	1.93 *
Skin composition				
Color (abs. units)	7.31 [a]	8.06	8.26	7.2
Hue (abs. units)	0.45	0.46	0.46	0.47
Total anthocyanins (mg/g FW)	3.2	3.3	3.5	3.0
Total phenols (mg/g FW)	3.5	3.7	3.7	3.2
Juice composition				
Soluble solids (°Brix)	22.8 [a]	22.3 *	21.6 *	20.9 *
pH	3.56	3.57	3.53 *	3.55 *
TA (g/L)	7.95	8.15	8.51	8.58
Color (abs. units)	13.2	11.6 *	12.3 *	11.9 *
Hue (abs. units)	0.4	0.4	0.4	0.4
Total anthocyanins (g/L)	0.46	0.43 *	0.44	0.45
Total phenols (g/L)	2.36	2.2	1.73 *	2.62 *
Wine composition				
pH	3.52 [a]	3.49	3.43 *	3.47 *
TA (g/L)	5.7	5.62	5.96 *	6.05 *
Color (abs. units)	6.8	6.2 *	5.7 *	5.9 *
Hue (abs. units)	0.63	0.67 *	0.64 *	0.65 *
Total anthocyanins (g/L)	0.36	0.38	0.33	0.31
Total phenols (g/L)	2.00	1.87	1.83	1.98

[a] Coefficient significance: * indicates significant difference from 'Control' at $p < 0.05$ according to Dunnett's t-test, otherwise not significant.

J. Int. Sci. Vigne Vin, 2013, **47**, n°1, 35-45
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06-pagay_05b-tomazic 04/04/13 17:25 Page40

but perhaps the following season. Also, given the nature of the study, it was difficult to control bird predation in the un-netted (control) vines in order to isolate the ancillary effects of bird netting on fruit yield. Total cluster numbers were difficult to compare for the same reason. The higher cluster weights of the netted vines could be due to better fruit set conditions within the netting as the potentially detrimental effects of wind and rain on fruit set are reduced. The benefit of netting as a wind break has been suggested by several researchers in improving fruit set (Dry, 1993 ; Dry *et al.*, 1989 ; Ewart *et al.*, 1987 ; Kobriger *et al.*, 1984). It is thought that netting applied shortly after bloom may adversely affect flower cluster initiation and fruit set of the current year, as well as potential crop in the following year (Glen Creasy, 2005, personal communication). This could potentially explain the higher number of clusters per vine and higher cluster weights from the netted vines that we observed in the present study.

2. Fruit and skin composition

Table 2 shows the composition data of Cabernet franc grape berries from 2004. No differences were observed in soluble solids between treatments. Lower levels of light in the canopy (leaves and clusters) are known to negatively impact photosynthesis and carbohydrate production and therefore sugar accumulation in the fruit (Reynolds *et al.*, 1986 ; Smart *et al.*, 1985 ; Smart, 1987). This also confirms evidence that leaf and cluster shading lowers both fructose and glucose concentrations in berries (Morrison, 1988). The mid-season treatment had the lowest soluble solids levels possibly owing to a sudden increase in canopy shading from the netting, directly and indirectly, at a time when sugar accumulation in the berries had not commenced. Once sugar accumulation commences at veraison, the process is irreversible (Coombe and Iland, 1987) although shading at this stage could affect the rate of sugar accumulation and final soluble solids level (Morrison, 1988).

pH values were slightly lower in the netted vines, as was TA. This could be attributed to lower light levels in the canopy, increased shade, and possibly higher malic acid concentrations in the fruit. Bergqvist *et al.* (2001) suggested that temperature may play a greater role in affecting pH than does sunlight exposure. It is interesting to note, however, that TA values were higher in the control treatment than the netted treatments, which confounds the literature mentioned prior. Color intensity was observed to be similar across treatments with intensity levels only slightly higher in the control treatment suggesting perhaps higher fruit exposure to sunlight. Hue or tint values were comparable across treatments, although the veraison treatment tended to have slightly higher yellow/brown pigments and less red/violet pigments, which is consistent with the color intensity value. Total anthocyanins and phenolics were generally lower in the netted treatments, which is an expected result, with the exception of the unusually high phenolic concentration in the veraison treatment. Similar results were obtained independently by Avenant (1994) and Smart *et al.* (1985), both of whom attributed lower phenolic concentrations to shading.

Compositional analysis of Cabernet franc grape skins are shown in Table 2. No differences were observed in color intensity, hue/tint, total anthocyanins, or total phenolics between treatments. The highest skin color values were observed in the post-bloom and mid-season treatments. Anthocyanin and phenolics concentrations were highest in the post-bloom and mid-season treatments, followed by the control and veraison treatments.

3. Juice composition analysis

Table 2 shows results of the juice composition analyses of Cabernet franc. Differences were observed in the soluble solids levels of the netted vines compared to the un-netted vines. Soluble solids were measured to be the highest in the control (un-netted) juice and decreased linearly the later netting was dropped in the growing season. Differences between the post-bloom and control treatments were the least among all the netting treatments. Earlier applications of netting created more shade early during active shoot growth of the vine ; the vines counteracted the shading effect by growing longer shoots with greater number of leaves thereby able to produce a greater amount of carbohydrate for the fruit. Shoot growth generally slows down before veraison so netting applied at this stage did not result in incremental shoot growth to compensate for the diminished canopy light environment. Also, shading tended to be greater when the nets were rolled down later in the season as the longer shoots and larger leaves were compressed by the nets. TA was not different between treatments although slightly higher in the shaded (netted) vines possibly owing to higher malic acid concentrations from the lower temperatures in the fruiting zone. However, fruiting zone temperatures were not specifically measured

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06-pagay_05b-tomazic 04/04/13 17:25 Page41

in this study. pH values were only slightly different between treatments ; later netted mid-season and veraison treatments showed some minor differences from control and post-bloom fruit. Coombe and Iland (1987) also reported similar findings to Bergqvist *et al.* (2001) of the role of temperature on must pH. Elevated potassium concentrations caused by leaf and cluster shading may play a role in determining grape juice pH. One study found that shading resulted in higher potassium concentrations and consequently higher pH (Morrison, 1988). Color and hue/tint were considerably lower in the netted treatments as a result of reduced light interception by the clusters and leaves, corroborating with results by Archer and Strauss (1989) and Coombe and Iland (1987). Phenolic pigments, or anthocyanins, were highest in the control must and lowest in the post-bloom. Total phenolic concentrations were highest in the veraison and control treatments and lowest in the post-bloom and mid-season treatments. There were no differences between the control and post-bloom juices, compared to the two later-netted treatments. Total anthocyanins and phenolics were in most cases slightly lower in the netted treatments, a likely consequence of reduced light interception, corroborating with other studies (Bergqvist *et al.*, 2001 ; Dokoozlian and Kliewer, 1996 ; Ristic *et al.*, 2007).

4. Wine composition analysis

Wine composition results for Cabernet franc are shown in Table 2, which closely matched the juice composition data (Table 2). pH was the highest in control wines and lower in the netted wines. Correspondingly, TA was lower in the control and post-bloom netted wines compared to the later-netted treatments. Lower pH and higher TA in the netted treatments was likely due to shading effects on clusters as well as lower cluster temperature. Higher TA has been attributed to higher malic acid concentrations resulting from lower grape berry temperatures (Lakso and Kliewer, 1975 ; 1978). The results also corroborate with a similar netting trial conducted in Marlborough, New Zealand in which netting decreased fruit zone temperature and increased TA (M. Trought, 2005, personal communication). As berry temperatures increase,

Table 3 - Impact of bird netting on select anthocyanin and polyphenol concentrations of Cabernet franc wine, 2004.

	Treatments			
	Control	Post-Bloom	Mid-season	Veraison
Anthocyanins		(mg/L)		
Delphinidin-3-*O*-glucoside	12.5 [a]	12.6	10.8 *	11.4 *
Cyanidin-3-*O*-glucoside	0.6	0.6	0.5	0.6
Petunidin-3-*O*-glucoside	14.9	15.5	13.0 *	13.7 *
Peonidin-3-*O*-glucoside	9.6	9.1	8.2 *	8.1 *
Malvidin-3-*O*-glucoside	119.9	124.1	107.3 *	108.4 *
Malvidin-3-*O*-acetylglucoside	48.9	48.7	45.0 *	45.1 *
Malvidin-3-*O*-p-coumaroylglucoside	16.9	17.3	16.3	16.0 *
Polyphenolics				
Gallic acid	47.4	47.2	46.2 *	50.1
(+)-Catechin	45.4	48.0 *	50.7 *	53.7 *
(-)-Epicatechin	61.9	67.1 *	58.7	62.9
Caftaric acid	2.0	1.2	2.4	1.7
Caffeic acid	24.4	23.0 *	22.1 *	24.0
Quercetin	1.1	1.1	1.3 *	1.3 *

[a] Coefficient significance : * indicates significant difference from 'Control' at $p < 0.05$ according to Dunnett's t-test, otherwise not significant.

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06-pagay_05b-tomazic 04/04/13 17:25 Page42

malic acid precursor enzymes phosphoenol-pyruvate (PEP) carboxylase and malate dehydrogenase are inactivated followed by a concomitant increase in malic enzyme responsible for malic acid degradation. Premature senescence of basal leaves may have also contributed to lower pH in the fruit and wine, concurring with observations by Trought (M. Trought, 2005, personal communication). Color intensity was negatively affected by the netting ; all netted treatments resulted in lower color intensity values than the control. Conversely, hue/tint values were higher in the netted treatment wines with the post-bloom treatment having the highest value. Total anthocyanins were observed to be higher in the control and post-bloom treatments compared to the later-netted treatments indicating that shading caused by the nets and canopy when netting is applied late in the season can be detrimental to wine color. Morrison (1988) observed similar effects of shading, particularly when clusters were shaded. Similar results were obtained in Ohio with the hybrid cultivar Reliance (Gao and Cahoon, 1994). Total phenolics were higher in the un-netted (control) treatment than the netted treatments, likely the result of shading caused directly by the nets as well as mutual shading from the canopy.

Individual anthocyanins in the Cabernet franc wine were quantified by HPLC ; the results are presented in Table 3. Malvidin-3-glucoside was the predominant anthocyanin of all the anthocyanins analyzed. Un-netted and post-bloom wines had higher concentrations of this particular anthocyanin and these were different from the later-netted treatments. Malvidin-3-glucoside concentration correlated with total anthocyanin concentration measured spectrophotometrically (Table 2). This trend has been confirmed by other researchers (Gao and Cahoon, 1994 ; Spayd *et al.*, 2002). Control and post-bloom wines had higher concentrations of this anthocyanin compared to the later-netted treatments owing possibly to improved canopy light microclimate from the vertically positioned shoots and less canopy crowding from reduced vine vigor and shoot growth. Other major anthocyanins included the monoglucosides delphinidin, petunidin and peonidin, all which were higher in the control and post-bloom wines. Acylated pigments of malvidin and peonidin were higher in the control and post-bloom treatments, consistent with the trend observed in the monoglucoside pigments. Coumaroylated anthocyanins, which represented less than 9 % of all anthocyanins detected, were also predominantly of the malvidin form. A similar trend was observed for the two other acylated B-ring anthocyanins – petunidin-3-glucoside and peonidin-3-glucoside. Cyanidin concentrations were relatively unaffected as a result of the netting. Differences were observed to be least between the control and the post-bloom treatments, with the exception of some coumaroylated anthocyanins, likely the result of higher canopy temperature rather than decreased light levels (Downey *et al.*, 2004). Differences in coumaroylated anthocyanins between netted and un-netted treatments showed fewer differences in concentration than did non-acylated anthocyanins although this confounds a report that found that increased shading resulted in a greater proportion of coumaroylated anthocyanins even though total anthocyanin concentration was unchanged (Haselgrove *et al.*, 2000). Anthocyanin concentrations in red grape varieties are affected not only by the direct effect of shading caused by the netting but also indirectly, influenced by sugar concentration in the epidermal cells of berries (Pirie and Mullins, 1977). Expression of flavonoid-3-*O*-glucosyl transferase (UGFT), a key gene responsible for anthocyanin synthesis, greatly increases after veraison in both shaded and exposed fruit (Downey *et al.*, 2004), suggesting that application of netting earlier in the season should not negatively impact anthocyanin concentration in the fruit. Later netting application would, however, impact pigment biosynthesis given the greater shading that would result from the nets lowered at those times.

Table 3 also presents the major polyphenolic compounds detected by HPLC. Gallic acid concentrations tended to be the highest in the veraison treatment followed by the control and post-bloom treatments. This was more or less consistent with measurements of total phenolics using the phenolics reduction (“Folin”) method (Table 2). Catechin and epicatechin concentrations were generally higher in the netted treatments compared to the control indicating that light plays a role in tannin biosynthesis and/or degradation. Differences between the control and post-bloom treatments were least for catechin and highest for epicatechin. Making any general conclusions on tannin concentration differences would be conjecture at this time and further study is required. Caftaric acid concentrations were highest in the control and mid-season treatments. Caffeic acid, a product of the hydrolysis of caftaric acid, was highest in the control treatment and lowest in the mid-season treatment. Caftaric and caffeic acid concentrations are mediated by the hydrolysis agents that convert the former compound to the

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06-pagay_05b-tomazic 04/04/13 17:25 Page43

latter. Netting application had a substantial effect on caffeic acid concentration, which was lower in all the netting treatments than the control. Interestingly, caffeic acid concentration was higher than caftaric acid although differences between treatments were less pronounced. This suggests that hydrolysis of caftaric to caffeic acid may be light mediated, among other factors, and increased shading would result in lower caffeic acid concentrations. Quercetin, an important flavonol in grapes, is synthesized early in berry development and was highest in the later-netted vines compared to the early-netted or un-netted vines, possibly as a direct consequence of greater light levels in the canopy prior to the nets being applied. Quercetin synthesis is light-dependent and studies on Pinot noir showed elevated concentrations of quercetin with increased sunlight cluster exposure (Price *et al.*, 1995). This finding is consistent with work by Downey *et al.* (2004) who showed that flavonol synthase (FLS) gene expression greatly increased at bloom and during fruit ripening in exposed clusters but decreased in shaded clusters of Shiraz. The Downey *et al.* study also found that expression of *VvFLS1*, the gene encoding FLS, which initiates flavonol biosynthesis, was down-regulated considerably by shading and is therefore light-regulated. Similar findings in response to light exposure have been reported in other fruit including apple (Yuri *et al.*, 2010), tomato (Torres *et al.*, 2006), and strawberry (Josuttis *et al.*, 2010). Given that quercetin concentrations were higher in the later-netted treatments, which were subjected to greater shading, suggests that quercetin biosynthesis occurs early in the berry development process rather than during ripening, i.e. post-veraison. Measurement of quercetin in the developing grape berry through maturation would provide better insight into the relationship between canopy light environment and timing of its biosynthesis.

CONCLUSIONS

The long-term bird netting trial conducted in 2004 on Cabernet franc revealed some insightful results as it affected yield and fruit, juice and wine composition. Minimal, if any, differences in fruit composition were found between netted and un-netted treatments, which are a positive finding, despite higher vine yields in the netted vines. In some cases, juice composition was negatively affected as a result of the netting; many of these differences could be attributed to the direct effects of shading caused by the netting. There were also some differences in individual anthocyanins and phenolic compounds as affected by light and other variables due to the netting. While the use of netting resulted in higher yields, a direct consequence of physically excluding birds from the fruit, it was shown to have ancillary benefits, which has not been sufficiently researched or documented hitherto. With regards to timing of netting application, based on results of this study, it is recommended that netting be applied over the canopy shortly after bloom to minimize negative effects of the netting on fruit composition and to maintain yield.

Much work is still needed in order to better understand the implications of bird-netting application and its timing on grape and wine composition and quality. Future work in this area needs to delve further into how light quality within the nets affects the photosynthetic rate of leaves and consequently sugar accumulation and fruit composition. Although not specifically measured in this study, wind affects photosynthesis and consequently fruit composition, mainly by altering the relative humidity and temperature inside the canopy as well as fruit clusters; this would certainly be worth investigating. As the use of long-term bird netting becomes more prevalent due to increasing bird depredation, its effects on fruit quality will need to be better understood by grape growers in order to continue the production of high quality fruit.

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REFERENCES

Archer E. and Strauss H. C., 1989. Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. *S. Afr. J. Enol. Vitic.*, **10**, 74-77.

Avenant J. H., 1994. The effect of hail netting on the performance of table grapes in the summer rainfall regions of South Africa. *In: Proceedings of the International Symposium on Table Grape Production*. J. M. Rantz (ed.), pp. 227-229.

Bergqvist J., Dokoozlian N. and Ebisuda N., 2001. Sunlight exposure and temperature effects on berry

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06-pagay_05b-tomazic 04/04/13 17:25 Page44

growth and composition of Cabernet-Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic*., **52**, 1-7.

Coombe B. G. and Iland P. G., 1987. Grape berry development. *In : Proceedings of the 6th Australian Wine Industry Technical Conference*. Australian Industrial Publishers, Adelaide.

Dokoozlian N. K. and Kliewer W. M., 1996. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hortic. Sci.,* **121**, 869-874.

Downey M. O., Harvey J. S. and Robinson S. P., 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.,* **10**, 55-73.

Dry P. R., Reed S. and Potter G., 1989. The effect of wind on the performance of Cabernet Franc grapevines. *Acta Hortic.,* **240**, 143-146.

Dry P. R., 1993. Exposure to wind affects grapevine performance. *Aust. Grapegrow. Winemak.,* **352**, 73-75.

Ewart A. J. W., Iland P. G. and Sitters J. H., 1987. The use of shelter in vineyards. *Aust. Grapegrow. Winemak.,* **280**, 19-22.

Fraser H. W., Fisher K. H. and Frensch I., 1998. *Bird Control on Grape and Tender Fruit Farms*. OMAFRA Factsheet Order No. 98-035. Ontario Ministry of Agriculture, Food and Rural Affairs, Vineland Station, Ontario.

Gao Y. and Cahoon G. A., 1994. Cluster shading effects on fruit quality, fruit skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid). *Vitis*, **33**, 205-209.

Haselgrove L., Botting D., van Heeswijck R., Høj P. B., Dry P. R., Ford C. and Iland P. G., 2000. Canopy microclimate and berry composition : The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. *Aust. J. Grape Wine Res.,* **6**, 141-149.

Himelrick D., 1985. Battling the birds : The war without Mesurol. *Eastern Grape Grower & Winery News,* **11**, 22-25.

Ibern-Gómez M., Andrés-Lacueva C., Lamuela-Raventós R. M. and Waterhouse A. L., 2002. Rapid HPLC analysis of phenolic compounds in red wines. *Am. J. Enol. Vitic.,* **53**, 218-221.

Josuttis M., Dietrich H., Treutter D., Will F., Linnemannstons L. and Kruger E. 2010. Solar UVB response of bioactives in strawberry (*Fragaria* x *ananassa Duch*. L.) : A comparison of protected and open-field cultivation. *J. Agric. Food Chem*., **58**, 12692-12702.

Kobriger J. M., Kliewer W. M. and Lagier S. T., 1984. Effects of wind on water relations of several grapevine cultivars. *Am. J. Enol. Vitic*., **35**, 164-169.

Lakso A. N. and Kliewer W. M., 1975. The influence of temperature on malic acid metabolism in grape berries. I. Enzyme responses. *Plant Physiol*., **56**, 370-372.

Lakso A. N. and Kliewer W. M., 1978. The influence of temperature on malic acid metabolism in grape berries. II. Temperature responses of net dark CO_2 fixation and malic acid pools. *Am. J. Enol. Vitic*., **29**, 145-149.

Mazza G., Fukumoto L., Delaquis P., Girard B. and Ewert B., 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *J. Agric. Food Chem*., **47**, 4009-4017.

Metivier R. P., Francis F. J. and Clydesdale F. M., 1980. Solvent extraction of anthocyanins from wine pomace. *J. Food Sci*., **45**, 1099-1100.

Morrison J. C., 1988. The effects of shading on the composition of Cabernet Sauvignon grape berries. *In : Proceedings of the Second International Symposium on Cool Climate Viticulture and Oenology*. R. E. Smart *et al.* (eds.), N.Z. Soc. for Viticulture and Oenology, pp. 144-146.

Pirie A. and Mullins M. G., 1977. Interrelationships of sugars, anthocyanins, total phenols and dry weight in the skin of grape berries during ripening. *Am. J. Enol. Vitic.,* **28**, 204-209.

Price S. F., Breen P. J., Valladao M. and Watson B. T., 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic*., **46**, 187-194.

Reynolds A. G., Pool R. M. and Mattick L. R., 1986. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis*, **25**, 85-95.

Ristic R., Downey M. O., Iland P. G., Bindon K., Francis I. L., Herderich M. and Robinson S. P., 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Aust. J. Grape Wine Res*. **13**:53-65.

Slinkard K. and Singleton V. L., 1977. Total phenol analysis : Automation and comparison with manual methods. *Am. J. Enol. Vitic*. **28** : 49-55.

Smart R. E., Robinson J. B., Due G. R. and Brien C. J., 1985. Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. *Vitis*, **24**, 119-128.

Smart R. E., 1987. Influence of light on composition and quality of grapes. *In : Symposium on Grapevine Canopy and Vigour Management, XXIInd International Horticultural Congress*. W. M. Kliewer (ed.), *Acta Hortic.*, **206**, 37-47.

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06-pagay_05b-tomazic 04/04/13 17:25 Page45

Spayd S. E., Tarara J. M., Mee D. L. and Ferguson J. C., 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic*., **53**, 171-182.

Srinivasan C. and Mullins M. G., 1976. Reproductive anatomy of the grape vine (*Vitis vinifera* L.) : Origin and development of the Anlage and its derivatives. *Ann. Bot*., **38**, 1079-1084.

Srinivasan C. and Mullins M. G., 1981. Physiology of flowering in the grapevine – a review. *Am. J. Enol. Vitic*., **32**, 47-63.

Torres C. A., Andrews P. K. and Davies N. M., 2006. Physiological and biochemical responses of fruit exocarp of tomato (*Lycopersicon esculentum* Mill.) mutants to natural photo-oxidative conditions. *J. Exp. Bot.*, **57**, 1933-1947.

Yuri J. A., Neira A., Quilodran A., Razmilic I., Motomura Y., Torres C. and Palomo I., 2010. Sunburn on apples is associated with increases in phenolic compounds and antioxidant activity as a function of the cultivar and areas of the fruit. *J. Food Agric. Env.* **8** : 920-925.

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