Manipulating light in the fruit zone improves WINE QUALITY

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Modified light and temperature in the fruit zone can have a positive role on the cluster microclimate, which consequently affect berry composition and therefore may improve wine quality or style.

However, it is important to remember the following:

• The relationships between fruit composition and wine quality or style are not direct. In other words, fruit analyses may give results on the concentration of a specific compound which will not reflect in the wine composition or in wine sensory analysis. Wine is a complex matrix within which numerous chemical compounds are interacting together and in addition, aromatic precursors present in the fruit are transformed during fermentation.

• The positive role of increased light on fruit composition has been well described by numerous authors. It should be noted that this abiotic factor has to be managed very carefully to avoid damage to the fruit (sunburn, berry shriveling, etc.), negatively affecting the fruit composition. In this regard, the thresholds of light and temperature's effect on the fruit composition are not well known and need further investigations.

• Fruit and canopy microclimates have to be managed to respect a specific site, canopy architecture and row orientation.

To demonstrate the positive role of light and temperature at the fruit level and to show the complexity of the relationships between fruit composition and wine style (wine composition and wine sensory description by a trained panel), green/herbaceous and tropical flavors have been chosen as sensory attributes for Sauvignon Blanc.

South Africa offers a great variety of Sauvignon Blanc wine styles, ranging from tropical to greener, herbaceous to the diversity of the climate and soil of the Western Cape region. The greenness in Sauvignon Blanc could be considered a wine style up to the point where the green attributes dominate, resulting in a one-dimensional wine. In red wines, mainly Merlot and Cabernet Sauvignon, the perception of green attributes may be considered an undesirable aroma.[3]

The green aroma descriptors of Sauvignon Blanc, Merlot, Cabernet Sauvignon, Cabernet Franc and Carmenere grapes and wines originate from 3-alkyl-2-methoxypyrazines (MPs). The most important MP found in grapes and wine is 3-isobutyl-2-methoxypyrazine (IBMP), whereas 3-isopropyl-2-methoxypyrazine (IPMP) is rarely present in grapes at harvest.

IBMP contributes to the green pepper, pea, and asparagus aromas while IPMP imparts earthier aromas.[1,11] The sensory detection threshold for IBMP was found to be very low; 2 ng/L in water and white wines and around 15 ng/L in red Bordeaux wines.[1,14]

In some Merlot and Cabernet Sauvignon wines, aromatic attributes related to eucalyptus and mint are found, which results in the wine sometimes being described as green. These attributes are, in fact, not linked to IBMP, although further research is needed.

IBMP in the grape berry is located in the skins (95%), seeds (4%) and pulp (1%).[14] IBMP biosynthesis in the grape berry starts after flowering, reaching the maximum concentration two to three weeks before *véraison*, after which it declines during maturation, not only in concentration, but also on a per berry basis.[13,16]

Recent studies suggest that IBMP found in the berry is synthesized by the berry

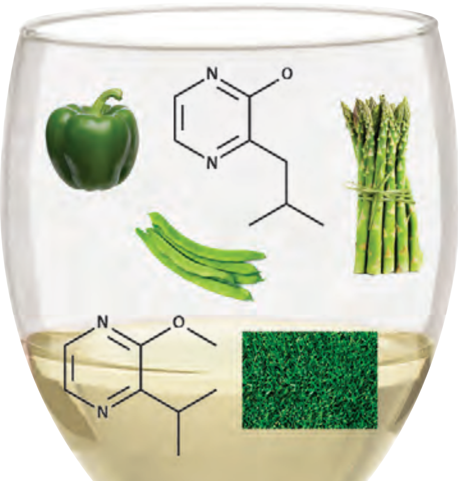

Figure 1: Attributes commonly used to describe methoxypyrazines present in Sauvignon Blanc wine: green pepper, asparagus and green beans.

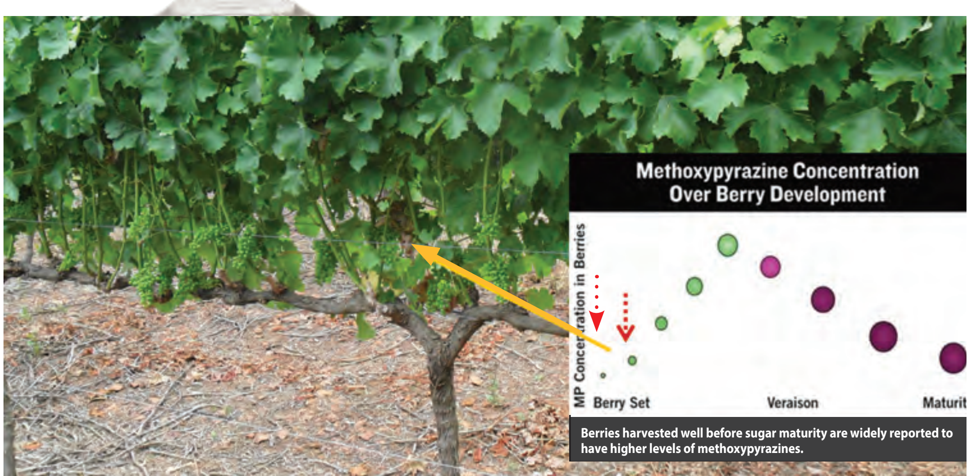

Figure 2: Opening the canopy at pea size (red arrow) affects the biosynthesis of IBMP in the berry, which in turn results in a reduced IBMP concentration in the wine (curve adopted from Roujon de Boudee, 2000).[14]

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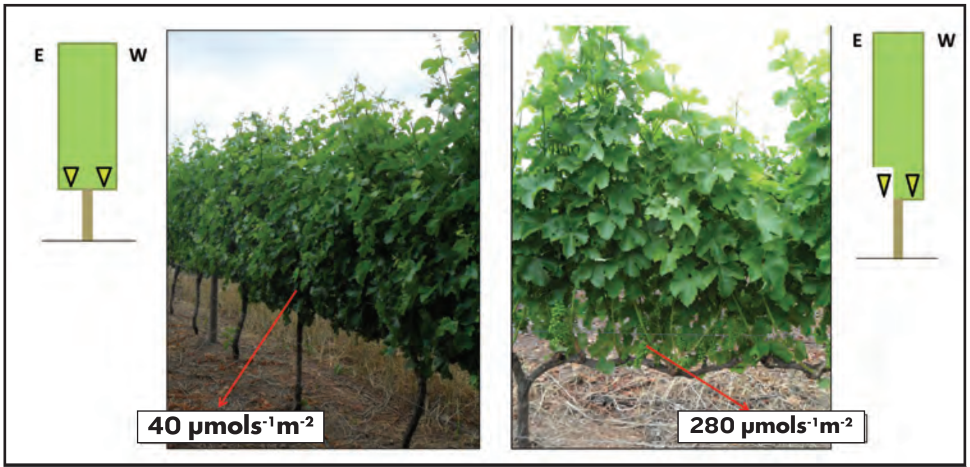

Figure 3: Leaf removal performed on Sauvignon Blanc vines at phenological stage berry pea size. Left: The shaded treatment (no leaf and lateral shoot removal) with a mean daily PAR (photosynthetically active radiation) of 40 μmols^{-1}m^{-2}. Right: The morning side exposed treatment with a mean daily PAR of 280 μmols^{-1}m^{-2}.

itself and not transported to the berry from either the leaves or shoots.[9] This is not in agreement with D. Roujou de Boubée who has reported that deuterated analogue of IBMP was translocated from the leaves to grape clusters.[14]

Abiotic factors such as light and temperature in the fruit zone and the vine water status, and various viticultural practices such as leaf removal, irrigation and vine training system can influence the concentration of IBMP in the berry and wine.[2,14,17] It has been shown that grapes and wines from cooler climatic regions contain higher concentrations of IBMP than grapes grown in warmer regions.

Recent studies have shown the importance of the timing and severity of leaf removal on the final concentration of IBMP in the mature grapes. J.J. Scheiner *et al.*, have shown that early leaf removal, performed 10 days after flowering, significantly reduced the IBMP concentration in Cabernet Franc grapes, while the same treatment, applied 40 and 60 days after flowering, had a less significant effect.[18]

Leaf removal performed after veraison had little or no effect on the IBMP concentration in grape berries. Bunch exposure pre-veraison is therefore crucial for reducing IBMP concentration in grape berries at harvest, affecting mainly the synthesis. However, light exposure after veraison does not influence IBMP degradation.[16,18,19]

To understand the effect of sunlight exposure to bunches on the IBMP concentration in grape berries during maturation and the resulting concentration in the wine, leaves and lateral shoots were removed on Sauvignon Blanc and Merlot vineyards in the Overberg and Stellenbosch regions (South Africa).

MATERIALS and METHODS

Sauvignon Blanc

The experiment was performed in a *Vitis vinifera* L. cv. Sauvignon Blanc (clone 316, grafted onto 101-14 rootstock) vineyard in the Overberg region of the western coastal area, South Africa (34°S; 19°E). The row orientation is northwest to southeast with

2.5 m x 1.8 m vine spacing. The training system is VSP (vertical shoot positioning) with a bilateral cordon and six two-bud spurs per running meter of cordon.

The treatments were randomly located within a greater experimental layout to exclude the natural heterogeneity of the vineyard and consisted of eight panels (four vines per panel) each.

To study the impact/role of light and temperature, all leaves and lateral shoots were removed from the fruit zone on the morning side of the canopy to a height of 0.3 to 0.4 m above the cordon. The 100% morning side exposed bunches were compared with a control canopy (100% shaded bunches where no leaves or lateral shoots were removed). Leaf and lateral shoot removal was performed at the phenological stage of berry pea size on Dec. 19, 2011.

Stem water potential (Ψ_{SWP}) was used to determine vine water status.[7] The vines did not experience any water constraint during the growing and ripening period with a mean SWP of Ψ_{SWP} -450 kPa at *véraison*.

Micro-vinification of the two treatments was performed in triplicate in the Stellenbosch University experimental cellar with standardized methods. For the exposed treatment, only exposed bunches were harvested, whereas the complete canopy was harvested for the shaded treatment.

Sensory analyses were performed five months after bottling using descriptive analyses. The wines were tasted in triplicate, randomized per taster and presented in black glasses. Aromatic descriptors, generated by the tasting panel during training, were scored on an uncalibrated line scale with the aromatic detection thresholds ranging from "none" to "intense."

Merlot

The Merlot vineyard is in the Helderberg area, Stellenbosch (33°S; 18°E) in the Western Cape, South Africa. The Merlot vines (clone 348A grafted onto US8-7 rootstock) were planted in 2003. The row direction is east to west with vines spaced 2.4 m x 1.2 m. The trellis system is a VSP on a bilateral cordon with five two-bud spurs per running meter of cordon.

Pre-dawn leaf water potential (Ψ_{PD}) and stem water potential (Ψ_{SWP}) were used to determine vine water status and to manage irrigation, maintaining the vine water status at around -400 to -500 kPa for the Ψ_{PD} values.[7]

To study the effect of light and temperature on the berry IBMP concentration, leaves and lateral shoots were removed at the berry pea size phenological stage. All leaves and lateral shoots were removed on both sides of the canopy to the height of the first wire (100% exposed bunches). No leaves or lateral shoots were removed in the shaded treatment (100% shaded bunches) as a control. The grapes were harvested according to the sugar loading model at the fresh and mature fruit stages.[6,8]

Figure 4: Leaf removal as performed on Merlot vines at the phenological stage of berry pea size. Left: The shaded treatment (no leaf and lateral shoot removal). Right: Both sides of fruit zone exposed. This extreme treatment has been done for experimental purposes only. The site, row orientation, and irrigation management made it possible to avoid berry sunburn or berry shriveling. Normally, opening the canopy on one side of the fruit zone is sufficient to achieve lower IBMP concentrations in wine.

RESULTS and PERSPECTIVES

Sauvignon Blanc

The Sauvignon Blanc vineyard is exposed to the positive effect of the sea breeze, coming from the Atlantic Ocean, on the bunch microclimate. Microclimatic data was collected in the fruit zone, whereas climatic data collected from above the canopy was considered as mesoclimatic data.

The sea breeze allowed for the canopy to be opened in the fruit zone, and second to partially separate the effect of light and temperature on berry composition due to the cooling effect of the sea breeze.[4] The coolest temperature in both treatments was measured at 6 AM. The mean hourly temperature over the growing and ripening season for both treatments did not exceed 30.5° C, where 30° C is the upper limit of the temperature threshold for optimal vine functioning.

Due to the vineyard location, it was possible to open the canopy without experiencing any sunburn due to the occurrence of the sea breeze (see Figure 5). An increase in wind speed was observed from 10 AM onward, reaching a maximum speed between noon and 6 PM. The increase in wind speed resulted in a decrease in the ambient (mesoclimatic) and bunch temperatures, mainly from 1 PM onward (Figure 5).

Wind direction measurements (data not shown) confirmed that the wind direction was predominantly from the south (Walker Bay) and from the west

Treatment	Total soluble solids (°Brix)	Titratable acidity (g/L)	pH	Berry fresh mass (g)	Sugar per berry (mg)
Morning side exposed	24.4	6.53	3.39	1.95	475.81
Shaded	23.4	7.38	3.31	1.97	460.43

Table I: Basic Sauvignon Blanc grape berry maturity parameters for shaded and morning side exposed treatments at harvest (3/13/2012).

Merlot	Sampling dates	Harvest stages	°Brix	Titrable acidity	pH	Berry fresh mass of one berry (g)	Sugar per berry (mg)
Expo-both sides	2012/02/10	fresh fruit	23.4	5.74	3.46	1.58	370.83
	2012/02/20	mature fruit	25.2	4.94	3.51	1.61	406.23
100% Shaded	2012/02/10	fresh fruit	23.1	6.22	3.40	1.61	373.03
	2012/02/20	mature fruit	25.0	4.83	3.44	1.61	402.88

Table II: Basic grape berry maturity parameters for shaded and both sides exposed treatment for grapes harvested at fresh fruit stage (2/10/2012) and mature fruit stage (2/20/2012).

(False Bay) during the noted hours. The main effect of this sea breeze was to cool down the berry and canopy temperature without directly affecting stomatal conductance.

The treatments did not significantly affect the berry fresh mass and main grape berry maturity parameters at harvest (see Table I).

Fifty percent flowering occurred Nov. 20, 2011 (from here on referred to as the date of flowering), *véraison* (50% of berries softened) was Feb. 2, 2012, and the grapes were harvested on March 13, 2012. The concentration of IBMP was analyzed in whole grape berries, twice before *véraison* (53 and 59 days after flowering), around *véraison* (73 days after flowering), during ripening (94 days after flowering) and at harvest (113 days after flowering).

For the morning-exposed treatments, only exposed berries were collected for MPs analyses and shaded treatment berries were sampled randomly within the entire canopy.

Leaf and lateral shoot removal from the fruit zone on the morning side resulted in lower IBMP concentrations during ripening (Figure 6). Significantly higher concentrations of IBMP were observed in the shaded treatment compared to the morning-exposed treatment for samples at 51, 59 and 73 days after flowering. At 94 days after the date of flowering and at harvest, no significant differences were noticed between the treatments.

A rapid decrease in the IBMP concentration in grape berries for the first three sampling dates of the morning-exposed treatment was observed, that could be a result of a lower IBMP synthesis in the grape berry which is most probably a consequence of early leaf and lateral shoot removal (berry pea size phenological stage).[17]

From 94 days after the date of flowering to 113 days after flowering (harvest), little change was noticed in the IBMP concentration of the morning-exposed treatment. Whereas the concentration of IBMP in the shaded treatment decreased slowly up to harvest for the same time period.

The final IBMP concentration in the grape berry at harvest can differ significantly between harvest seasons, being dependent on both leaf removal (direct or indirect effect of light in the fruit zone) and the climate (effect of temperatures: average, maximum and minimum including temperature differences between day and night).[10]

The interaction between light and temperature in the fruit zone is complex and

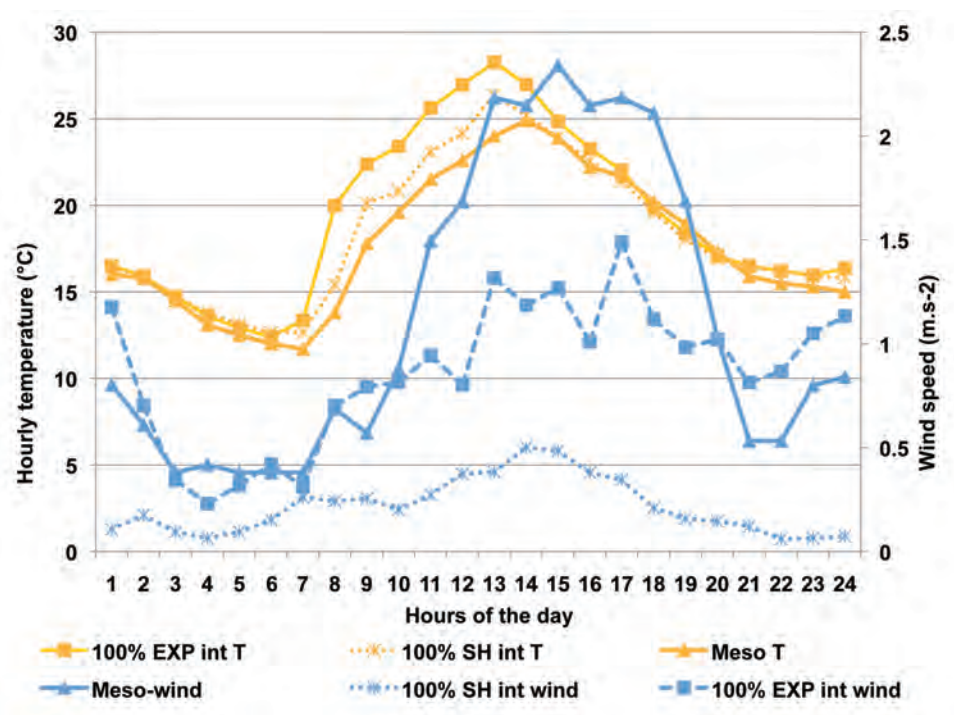

Figure 5: An example of sea breeze and temperature evolution in 24 hours. Figure 5 represents wind speed at the mesoclimatic level (Meso-wind), in the fruit zone in the morning side-exposed treatment (100% EXP interior wind), and in the fruit zone in the shaded treatment (100% SH interior wind). Temperature evolution has been recorded at the mesoclimatic level (meso-T), in the fruit zone for the morning side exposed treatment (100% EXP interior-T) and shaded treatment (100% SH interior-T). Climatic data collected above the canopy were considered as the mesoclimatic data, whereas microclimatic data were collected at the fruit zone. The mesoclimate could be defined as the climate at the vineyard level.

the goal of this study was to clearly show the direct effect of light intensity on fruit IBMP concentration and the very complex and indirect relationship between fruit and wine composition and wine sensory description.

The direct effect of the vintage that was observed, compared to the effect of temperature, was due to the effect of the heat waves and time and duration of the heat waves during fruit development and ripening. This allowed demonstration of the overlapping effect of temperature irrespective of the light intensity at the fruit level, even in a site (*terroir* unit) where the sea breeze cooled down the fruit temperature during the hottest hours of a day.

IBMP concentrations in produced wines were lower than what was found in the grapes as was expected due to the decrease in IBMP after juice clarification as previously observed.[14] No significant difference in the IBMP concentration in grape berries between both treatments was observed at harvest, whereas the IBMP concentrations in the wines were higher in the shaded treatment when compared to the morning side exposed treatment (Figure 7).

Slightly higher, but not significantly, IBMP concentrations in grape berries from the shaded treatment could explain higher IBMP concentrations in the wines. Due to the natural heterogeneity within the vineyard that can counteract the effect of the treatments, the larger winemaking sample (at least 50 kg of grapes) may be more representative in comparison to 100 berries used for grape berry analyses. The IBMP concentration in wine from the shaded treatment was above the detection threshold (2 ng/L), corresponding with the attributes detected during sensory wine evaluation.

Figure 8 shows the separation in wine sensory perception between the shaded and the morning side exposed treatment, with green attributes mainly linked to the shaded treatment (no leaf removal) and tropical fruity attributes linked to the exposed treatment (morning side exposed bunches).

Using principal component analyses (PCA), descriptors such as green pepper, grassy and overall greenness were grouped with IBMP. Tropical descriptors (grapefruit, passion fruit and guava) were mainly grouped with varietal thiols such as 3-sulfanyhexan-1-ol (3SH), 3-sulfanyhexyl acetate (3SHA) and some

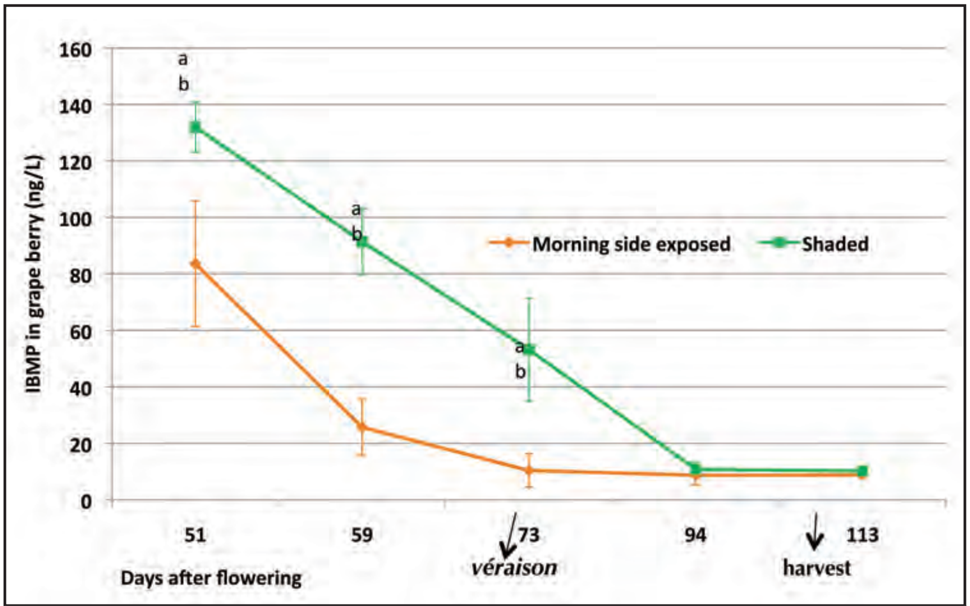

Figure 6: The concentration of IBMP (ng/L) in Sauvignon Blanc grape berries from 53 days after flowering (Jan. 12, 2012) to harvest, 113 days after flowering (March 13, 2012) for the shaded and the morning side exposed treatments.

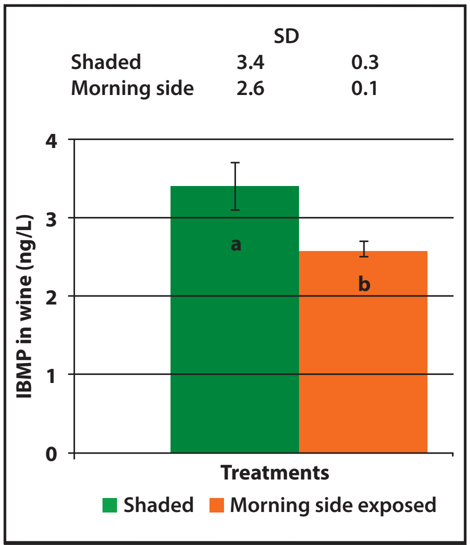

Figure 7: The concentration of IBMP (ng/L) in Sauvignon Blanc wines from shaded and morning side exposed treatments.

esters. By removing leaves and lateral shoots, a more complex (fruitier) style of Sauvignon Blanc was produced in comparison to the one-dimensional (greener) wine style produced from the shaded treatment.

Merlot

The treatments did not affect the berry fresh mass and classical ripening parameters irrespective of the harvest dates at fresh and mature fruit stages (Table II). The increase in Brix between fresh fruit and mature fruit stages can be attributed to an increase in sugar per berry.

The IBMP concentration in Merlot grape berries decreased during maturation. A higher concentration of IBMP in grape berries was observed in the shaded treatment (no leaves or lateral shoots removed), compared to the both sides exposed treatment, where leaves and laterals were removed at the berry pea size phenological stage (Figure 9).

At the last three sampling dates for both treatments, although no significant decrease in the IBMP concentration was observed, a lower IBMP concentration was noted for the both sides exposed treatment compared to the shaded treatment.

IBMP concentrations in the wines were higher for the shaded treatment than the both sides exposed treatment, that was also observed in the grape berries (Figure 9). No noticeable difference was seen in the IBMP concentration between wines for the two harvest stages of fresh and mature fruit (Figure 10).

The IBMP concentration in grapes remained relatively stable after reaching a certain concentration.[16] The both sides exposed treatment resulted in wines with an IBMP concentration of less than the detection threshold of 15 ng/L in red wines, whereas the IBMP concentration in the wines of the shaded treatment was 18.1 and 18.3 ng/L respectively for grapes harvested at the fresh and mature fruit stages (Figure 10).[13]

Conclusions

Increased light in the fruit zone has an effect on berry composition, generally

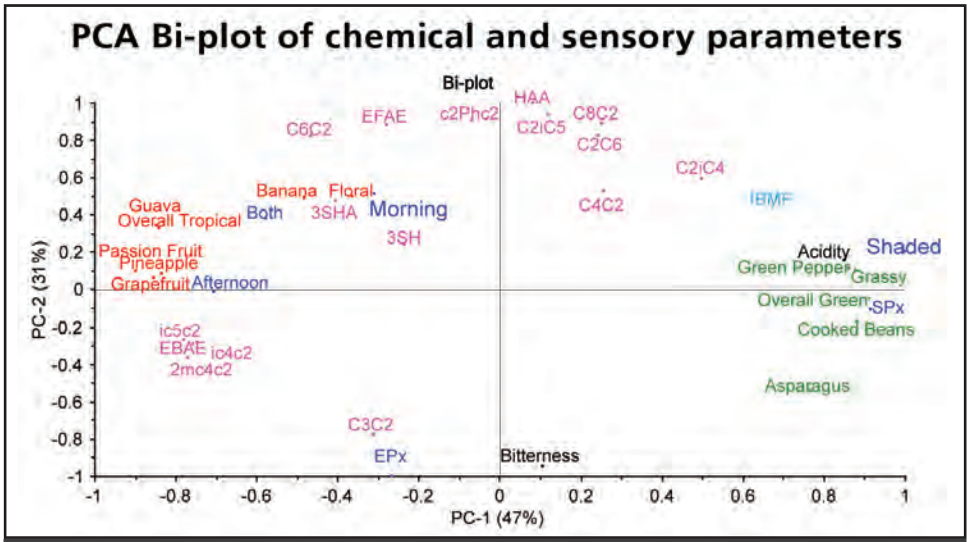

Figure 8: A PCA plot indicating the distribution of the treatments according to the sensory analyses results and chemical analyses where the morning side exposed treatment is indicated as "Morning" and the shaded treatments as "Shaded." PC-I represents 47% variability when PC-2 represents 3I% of variability. The figure shows a clear difference between wines from shaded and exposed treatments: a more one-dimension green-style wine, associated with IBMP, from shaded treatment, and a more complex wine from exposed-bunches treatment, associated with thiols.

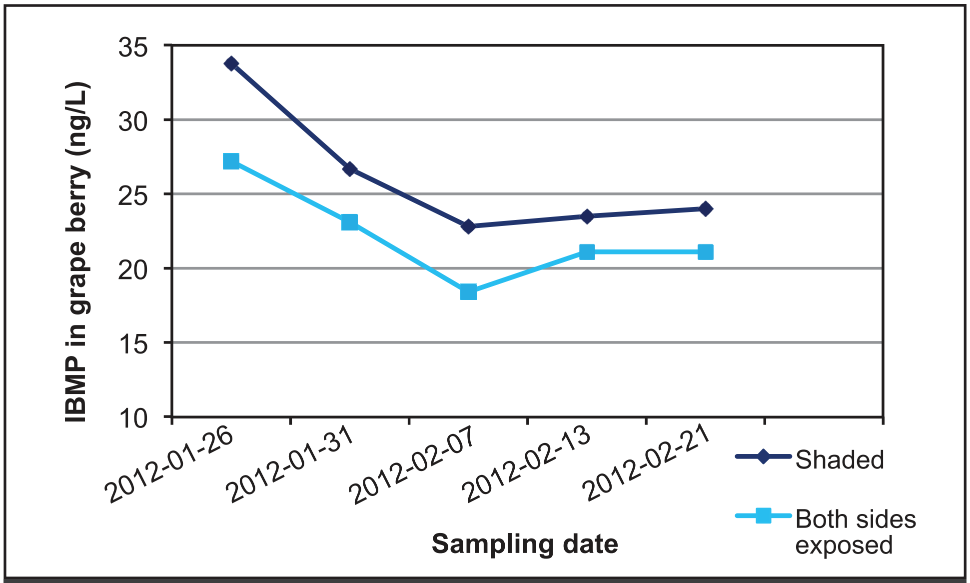

Figure 9: The concentration of IBMP (ng/L) in Merlot grape berries from Jan. 26, 20I2, to harvest (Feb. 2I, 20I2) for the shaded and both sides exposed treatments.

improving the resulting wine quality by decreasing IBMP concentration and increasing anthocyanins and polyphenols in the wine,[15] and enhancing the fruitiness of the wine.

IBMP synthesis and degradation patterns are complex and can be influenced by many environmental conditions and canopy management practices. Early leaf removal (at the berry pea size phenological stage) is crucial to reduce the IBMP concentration in the grapes.[9]

Temperature is as important for the IBMP concentrations in grapes and can counteract the effect of light intensity in the fruit zone.[10] Furthermore, high IBMP concentrations in Sauvignon Blanc wines may mask the fruitiness of the wine and may be considered a negative aroma, although mainly in red wines like Merlot and Cabernet Sauvignon.

To reduce the pyrazine level in the berries and consequently in the wine, early (berry pea size) canopy manipulation can be applied in the fruit zone by removing all leaves and lateral shoots at a height of 0.3 to 0.4 m above the cordon in a VSP training system.

In Sauvignon Blanc grape berries, no significant difference in the IBMP concentration at harvest was observed, whereas higher IBMP concentrations from the shaded treatment in resulting wines were found. IBMP degradation patterns can differ between vintages and can sometimes overcome the effect of bunch sun exposure, in combination with the average temperature of the vintage and number, time of occurrence and length of heat waves experienced in the South African grapegrowing region.[2,10]

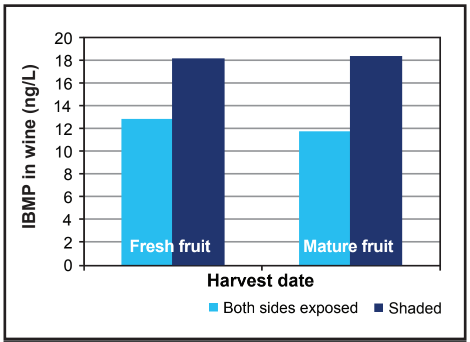

Figure 10: The concentration of IBMP (ng/L) in Merlot wines for the both sides exposed and shaded treatments, harvested at fresh and mature fruit stages. No decrease in IBMP concentration was observed during ripening from fresh to mature fruit stages, for both treatments. A noticeable difference in wine IBMP concentration was observed between the treatments: ~ 12ng/L for wine from exposed bunches and ~18 ng/L for wine from shaded bunches.

- Removing all leaves and lateral shoots in the fruit zone on one side of the canopy is an effective tool to reduce IBMP concentration in a wine. The choice of the canopy side to remove leaves has to be chosen properly according to row orientation and site location to prevent possible sunburn in warm climatic conditions. The timing of leaf removal is crucial to reduce IBMP concentrations in grapes. Leaf removal performed before *véraison* (at berry pea size) results in a significant decrease of the IBMP concentrations in grapes, whereas treatments applied after *véraison* had little or no effect on IBMP concentration in grapes.[10,14,16]
- By removing only laterals from the fruit zone and retaining basal adult leaves of the primary shoot intact, sufficient light will be allowed into the fruit zone. Selective opening of the fruit zone would however, be more time consuming than removing both laterals and basal leaves simultaneously.
- In a warm/hot climate, similar results can be obtained by indirectly increasing light in the fruit zone using a modified VSP trellis such as the Smart Dyson, the sprawling VSP (allowing light to penetrate the canopy and to reach bunches through the top of the canopy/diffuse light penetration) or implementing a new Lys training system.[5]

Early leaf removal before *véraison* seems to be an efficient tool to reduce the concentrations of IBMP in grape berries. As proposed by other authors, the IBMP concentration in the grape berry is not only light- but also temperature-dependent, which could, according to the site and vintage details, counteract the effect of the light intensity. Further research on this topic is needed, mainly to understand the complex relationships between fruit and wine composition, and ultimately wine sensory description. PWV

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Bibliography

1. Allen, M.S., M.J. Lacey, R.L.N. Hars, W.V. Brown. 1991 "Contribution of methoxypyrazines to Sauvignon Blanc wine aroma." Am. *J. of Enol. & Vit.* 42, 109–112.
2. Belancic, A. and E. Agosin. 2007 "Methoxypyrazines in grapes and wines of *Vitis vinifera* cv. Carmenere." *Am. J. of Enol. & Vit.* 58 (4), 462–469.
3. Bogart, K., and L. Bisson. 2006 "Persistence of vegetal characters in wine grapes and wine." *Practical Winery & Vineyard*, March/April. 13–20 pp.
4. Bonnardot, V., V.A. Carey, O. Planchon, S. Cautenet,. 2001 "Sea breeze mechanism and observations of its effects in the Stellenbosch wine producing area." *Wynboer*, 147: 10–14.
5. Castro, R., G. Cargnello, C. Intrieri, A. Carbonneau. 1995 "A new training system proposed for experimentation: the LYS shape." *Le progres Agricole et Viticole*, 22, 493–497.
6. Deloire, A.J. 2012 "Ripening of the grape and aroma in wine." *Wynboer*, 269: 108–109.
7. Deloire, A.J., D. Heyns. 2011 "Leaf water potential – principles, methods and thresholds." *Wynboer*, 265: 119–121.
8. Deloire, A.J. 2011 "The concept of berry sugar loading." *Wynboer*, 257: 93–95.
9. Koch, A., C.L. Doyle, M.A. Matthews, L.E. Williams, S.E. Ebeler. 2010 "2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype." *Phytochemistry* 71, 2190–2198.
10. Koch, A., S.E. Ebeler, L.E. Williams, M.A. Matthews. 2012 "Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries." *Physiologia Plantarum* 145, 275–285.
11. Murray, K.E., and F.B. Withfield. 1975 "Occurrence of 3-alkyl-2-methoxypyrazine in raw vegetables." *J. of the Science of Food & Agriculture* 26, 973–986.
12. Razungles, A., C.L. Bayonove, R.E. Cordonnier, J.C. Sapis. 1988 "Grape carotenoids: changes during the maturation period and localization in mature berries." *Am. J. of Enol. & Vit.* 39 (1), 44–48.
13. Roujou de Boubée, D., C. Van Leeuwen, D. Doubourdieu. 2000 "Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening." *J. of Ag. & Food Chemistry* 48, 4830–4834.
14. Roujou de Boubée, D. 2000 "Researches on 2-methoxy-3-isobutylpyrazine in grapes and wines: analytical, biological and agronomic approaches." Thesis, University Bordeaux II.
15. Ristic, R., M.O. Downey, P.G. Iland, K. Bindon, I.L. Francis, M. Herderich, S.P. Robinson. 2007 "Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties." *Aus. J. of Grape & Wine Research* 13 (2), 53–65.
16. Ryona, I., B.S. Pan, D.S. Intrigliolo, A.N. Lakso, G.L. Sacks. 2008 "Effect of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (Vitis vinifera L. cv. Cabernet Franc)." *J. of Ag. & Food Chemistry* 56, 10838–10846.
17. Sala, C., O. Busto, J. Guasch, F. Zamora. 2004 "Influence of Vine Training and Sunlight Exposure on the 3-Alkyl-2-methoxypyrazines Content in Musts and Wines from the Vitis vinifera Variety Cabernet Sauvignon." *J. of Ag. & Food Chemistry* 52 (11), 3492–3497.
18. Scheiner, J.J., G.L. Sacks, B. Pan, S. Ennahli, L. Tarlton, A. Wise, S.D., L.J.E. Vanden Heuvel. 2010 "Impact of Severity and Timing of Basal Leaf Removal on 3-Isobutyl-2-Methoxypyrazine Concentrations in Red Winegrapes." *Am. J. of Enol. & Vit.* 61 (3), 358–364.
19. Šuklje, K., K. Lisjak, H.B. Česnik, L. Janeš, W.J. Du Toit, Z. Coetzee, A. Vanzo, A.J. Deloire. 2012 "Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazines, glutathione and hydroxycinnamates evolution during berry ripening of Sauvignon Blanc (*Vitis vinifera* L.)" *J. of Ag. & Food Chemistry* 60, 9454–9461.