Ross D. Sanders, Paul K. Boss, Dimitra L. Capone, Catherine Kidman, David W. Jeffery

Regional impact on rootstock/scion mediated methoxypyrazine accumulation in rachis

Proceedings of the XIIIth International Terroir Congress, 2020, pp.1-5

REGIONAL IMPACT ON ROOTSTOCK/SCION MEDIATED METHOXYPYRAZINE ACCUMULATION IN RACHIS

Ross D. Sanders[1,2,3], Paul K. Boss[1,3], Dimitra L. Capone[1,2], Catherine Kidman[4], David W. Jeffery[1,2]*

[1]Australian Research Council Training Centre for Innovative Wine Production, The University of Adelaide, PMB1 Glen Osmond, SA, 5064, Australia
[2]School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, PMB1 Glen Osmond, SA, 5064, Australia
[3]CSIRO Agriculture and Food, Locked Bag 2, Glen Osmond, SA 5064, Australia
[4]Wynns Coonawarra Estate, 77 Memorial Drive, Coonawarra, SA 5263, Australia

*Corresponding author: david.jeffery@adelaide.edu.au

Abstract

Aim: To investigate the impact of Geographical Indications (GI) of South Australia on the rootstock/scion-mediated methoxypyrazine accumulation within the rachis of Shiraz and Cabernet Sauvignon.

Methods and Results: Cabernet Sauvignon and Shiraz bunches were sampled at maturity from two South Australian GIs over the 2019 and 2020 harvest periods. From each region, a minimum of 18 bunches per rootstock/scion combination were sampled from across the vineyard and their rachis material was assessed for 3-isobutyl-2-methoxypyrazine (IBMP). Results indicated that region and rootstock choice significantly affect the concentrations of methoxypyrazines within the rachis material of both Shiraz and Cabernet Sauvignon varieties at harvest.

Conclusion: This research highlights the effect of regionality on the concentration of methoxypyrazines within the rachis material of Cabernet and Shiraz vines grown on common rootstock varieties. The outcomes will conceivably inform viticulturalists and winemakers of how methoxypyrazine characteristics of Shiraz and Cabernet Sauvignon rachis are impacted by common rootstock/scion combinations permitting informed rootstock selection and assisting in production of a target wine style.

Significance and Impact of the Study: The presence of rachis material during red must fermentation can confer methoxypyrazines to the wine. The presence of methoxypyrazines, and predominately 3-isobutyl-2-methoxypyrazine (IBMP), in red wine can impact the flavour and aroma profile due to their 'green' and 'earthy' characteristics. Interestingly, this phenomenon has been shown to impact the aroma profile of Shiraz wines, a variety that has not been shown to naturally produce methoxypyrazines within the berries. Furthermore, it appears that the concentration of methoxypyrazines within the rachis is mediated by rootstock/scion combination and the region in which the vines are grown. As rootstock uptake increases across Australia in response to biological threats and abiotic stresses, an understanding of the viticultural and regional influences on rootstock/scion mediated rachis composition is essential to facilitate the production of high-quality Australian wines under increasingly challenging conditions.

Keywords: Shiraz, Cabernet Sauvignon, *Vitis vinifera*, wine aroma

Introduction

3-Alkyl-2-methoxypyrazines (MPs) are grape-derived volatile compounds that are characteristic of certain grape varieties, including Cabernet Sauvignon and Sauvignon blanc. They can contribute distinctive 'green' aromas such as 'bell pepper', 'green bean' and 'asparagus' to wine (Bayonove *et al.,* 1975). The most prevalent MP in wine is 3-isobutyl-2-methoxypyrazine (IBMP), which has an aroma threshold in white wine between 1 to 2 ng/L (Allen *et al.,* 1991) and 10 to 15 ng/L in red varieties (Kotseridis *et al.*, 1998). IBMP concentration in berries at harvest is dependent on a multitude of climatic and viticultural factors including light exposure (Dunlevy *et al.*, 2013), temperature (Falcao *et al.,* 2007), water availability at key development stages and soil fertility (Mendez-Costabel *et al.,* 2014). The ease of extractability of MPs from grapes (Ryona *et al.*, 2009), and rachis (Capone *et al.*, 2018), their stability during fermentation, presence in different parts of the vine (Dunlevy *et al.,* 2010), and the general lack of effective remediation techniques (Liang *et al.,* 2018) means that care must be taken to prevent extraneous sources of MPs being present in the fermenter and producing wine with undesirable 'green' flavour attributes. The deliberate introduction of bunch stems into fermenters for the purpose of whole-bunch fermentation is increasing in popularity in Australia (Godden, 2018). In addition to deliberate inclusion of whole bunches, rachis material may enter the ferment through unintended pathways as a by-product of the harvesting, crushing, and destemming processes, and is a major component of matter other than grape (MOG) in ferments. Whilst whole-bunch ferments are generally associated with positive wine characteristics, the technique is often avoided with varieties known to produce MPs in their fruit, such as Cabernet Sauvignon.

However, emerging research has shown that whole-bunch ferments of Shiraz and Pinot noir varieties, which are genetically incapable of producing MPs in the grapes (Koch *et al.*, 2010), can produce wines with 'green' characteristics (Capone *et al.*, 2018). In recent years, research has highlighted that the concentration of MPs present in the rachis material could be influenced by the rootstock choice (Capone *et al.,* In preparation). Although rootstocks have previously been shown to impact the chemical composition of grapes, and in turn, the wine sensory profile (Olarte Mantilla *et al.*, 2018), rootstock influence on other vine organs remains largely unexplored. It is unknown how climatic variables that known to impact berry concentrations of MPs will impact rachis material.

In this study, rachis material from Cabernet Sauvignon and Shiraz vines from the terroirs of the Limestone Coast, Barossa Valley, and the University of Adelaide Waite Vineyard were analysed for IBMP concentration and the impact of region, rootstock, and *Vitis vinifera* variety was explored.

Materials and Methods

Cabernet Sauvignon vines grown in two regions on the Limestone Coast (LC1 and LC2) and the Barossa Valley (BV1), and Shiraz vines grown in the Barossa Valley (BV2) and the University of Adelaide Waite Campus (UAWC) vineyard, were utilised for the trial. Cabernet Sauvignon vines were on own roots, as well as on 1103 Paulsen, 110 Richter, M5512, M5489, and M6262 rootstocks in LC1 and 110 Richter, M5512, M5489, and M6262 rootstocks in LC2. Cabernet Sauvignon vines grown in the Barossa Valley (BV1) were grown on 110 Richter and 1103 Paulsen rootstocks. Shiraz vines in the Barossa valley (BV2) were grown on 140 Ruggeri and 1103 Paulsen rootstocks, and in UAWC, vines were grown on own roots and 140 Ruggeri, Schwarzmann, and Ramsey rootstocks.

Sampling during the 2018/19 harvest period was conducted as follows: for each treatment, six grape bunches were taken per sample location with three replicates, chosen to provide a representative overview of the vineyard. Sampling for the 2019/2020 harvest period was modified dependent on the vineyard. For Cabernet Sauvignon vines in LC1, one bunch was taken per vine for each rootstock location with 66 or 72 replicates. For Shiraz vines grown in BV1, six bunches were taken per sampling location with three replicates, chosen to provide a representative overview of the vineyard.

IBMP content of rachis material was determined using an adapted stable isotope dilution assay utilising solid-phase microextraction (SPME) coupled with a gas chromatograph–tandem mass spectrometry (GC-MS/MS) (Dunlevy *et al.*, 2010).

Results and Discussion

In the 2019 vintage, IBMP levels in rachis material from Cabernet Sauvignon grown in the Limestone Coast were not significantly different between regions (LC1 and LC2). Similarly, there was no difference in IBMP accumulation between own roots and rootstocks for these regions (Figure 1A). However, in 2020 when rachis from Cabernet Sauvignon grown in two different Geographical Indications (GIs) (LC1 and BV1) were compared, material from 1103 Paulsen and 110 Richter rootstocks grown in LC1 had significantly higher levels than those from BV1 (Figure 1B). Additionally, rachis from 1103 Paulsen grown in LC1 had significantly higher concentrations than 110 Richter grown in LC1.

The difference in the concentration of IBMP in the rachis material of the rootstocks grown in LC1 compared with BV1 suggested that terroir may be influencing the accumulation and retention of IBMP. In Cabernet Sauvignon, MP content in berries differs with changes in management practices and with vineyard variables such as water availability and soil fertility (Mendez-Costabel *et al.,* 2014); similar considerations may explain the variation in the current results.

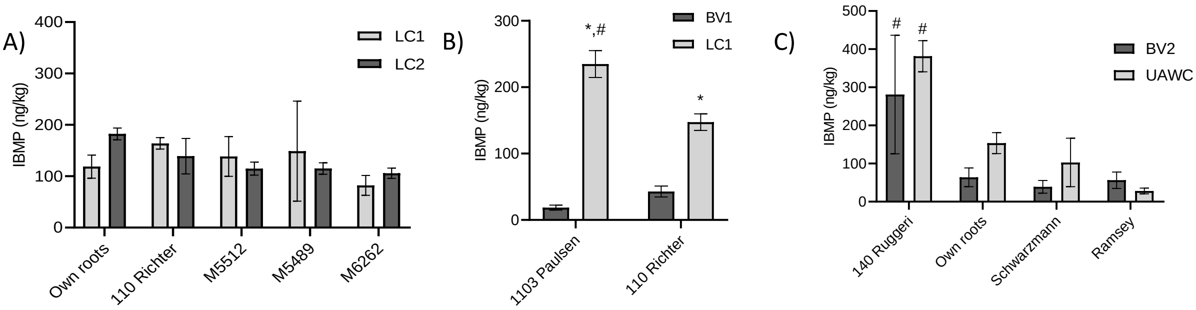

Figure 1. A) Intra-Geographical Indications: Concentration of IBMP in the rachis (mean ± SEM, n = 3) of Cabernet Sauvignon from Limestone Coast grown on 110 Richter, M5512, M5489, and M6262 rootstocks, and own roots at harvest in 2019. B) Inter-Geographical Indications: Concentration of IBMP in the rachis (mean ± SEM) of Cabernet Sauvignon grown in LC1 (n = 36) and BV1 (n = 6) at harvest in 2020. Vines were grown on 1103 Paulsen and 110 Richter rootstocks. * indicates significance (P ≤ 0.05) in rootstocks grown in different Geographical Indications and $^{\#}$ indicates significance (P ≤ 0.05) in rootstocks from same vineyard. C) Distribution of IBMP in the rachis (mean ± SEM, n = 3) of Shiraz grown on own roots and 140 Ruggeri, Schwarzmann, and Ramsey rootstocks at harvest in 2019. The rachis material was harvested from BV2 and the UAWC during the 2019 harvest period. $^{\#}$ indicates significance in rootstocks from the same vineyard.

In 2019, the IBMP concentration in the rachis of Shiraz grown in BV2 was not significantly different to Shiraz grown in UAWC, although the rachis from bunches grafted to 140 Ruggeri in both GIs had significantly higher concentrations of IBMP when contrasted with those on own roots (Figure 1C). The mechanism for this is unclear and requires further investigation.

When considering all data sets, it was apparent that 140 Ruggeri and 1103 Paulsen rootstocks, independent of scion, appeared to accumulate higher levels of IBMP in the rachis than other rootstocks. It was hypothesised that this difference may be driven by the parentage of these rootstocks and their associated attributes. 140 Ruggeri and 1103 Paulsen rootstocks are hybrids of American *Vitis* species, namely *V. berlandieri* and *V. rupestris*, which are known to produce rootstocks with high vigour characteristics (Jones *et al.,* 2009). Increases in vigour will lead to an increase in canopy size (Jones *et al.,* 2009) and water use (Gambetta *et al.*, 2012), decreasing rachis exposure to light, and increasing water flow throughout the vine. In the berry, decreasing light exposure (Dunlevy *et al.,* 2013) and increasing water availability (Mendez-Costabel *et al.,* 2014) have been positively correlated with increases in IBMP concentration at harvest. When considering high vigour, it is important to note that although Ramsey is also known to produce vines with high vigour, it does not appear to have the same increase in IBMP accumulation in the rachis in these GIs (Figure 1C).

Conclusions

With commercial uptake of rootstocks, having increased understanding of their impact on wine quality and flavour is essential. This investigation has highlighted that rootstock variety and terroir can influence the accumulation of IBMP in the rachis. The mechanisms driving this phenomenon are currently unknown and remain an important consideration for the future development of this work.

Acknowledgements

This research was supported by funding from University of Adelaide RTP Scholarship, Wine Australia, Commonwealth Scientific and Industrial Research Organisation (CSIRO), and Treasury Wine Estates (TWE). We acknowledge CSIRO staff, particularly Sue Maffei and Emily Nicholson, and University of Adelaide staff and students who assisted with this project. The University of Adelaide and CSIRO are members of the Wine Innovation Cluster.

References

Allen, MS., Lacey, MJ., Harris, RLN., Brown, WV., 1991. Contribution of methoxypyrazines to Sauvignon Blanc wine aroma. American Journal of Enology and Viticulture, 42: 109-12.

Bayonove, C., Cordonnier, R., Dubois, P., 1975. Study of an aromatic characteristic fraction of Cabernet Sauvignon grape variety, identification of 2-methoxy-3-isobutyl-pyrazine. Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D, 281: 75-78.

Capone, D., Pearson, W., Bindon, K., Kassara, S., Solomon, M., Bey, L., Francis, L., Herderich, M., Johnson, D., 2018. What makes a red wine green? Wine & Viticulture Journal, 33: 32-35.

Capone, DL., Clingeleffer, PR., Maffei, SM., Francis, IL., Boss, PK., In preparation. Green flavour in Shiraz wine, evidence that Ramsey rootstock can alter the accumulation of methoxypyrazines in Shiraz bunch stems. Australian Journal of Grape and Wine Research.

Dunlevy, JD., Soole, KL., Perkins, MV., Dennis, EG., Keyzers, RA., Kalua, CM., Boss, PK., 2010. Two *O*-methyltransferases involved in the biosynthesis of methoxypyrazines: grape-derived aroma compounds important to wine flavour. Plant Molecular Biology, 74: 77-89.

Dunlevy, JD., Soole, KL., Perkins, MV., Nicholson, EL., Maffei, SM., Boss, PK., 2013. Determining the methoxypyrazine biosynthesis variables affected by light exposure and crop level in Cabernet Sauvignon. American Journal of Enology and Viticulture, 64: 450-58.

Falcao, LD., de Revel, G., Perello, MC., Moutsiou, A., Zanus, MC., Bordignon-Luiz, MT., 2007. A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C_{13}-norisoprenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. Journal of Agricultural and Food Chemistry, 55: 3605-3612.

Gambetta, GA., Manuck, CM., Drucker, ST., Shaghasi, T., Fort, K., Matthews, MA., Walker, MA., McElrone, AJ., 2012. The relationship between root hydraulics and scion vigour across *Vitis* rootstocks: what role do root aquaporins play? Journal of Experimental Botany, 63: 6445-55.

Godden, P., 2018. Ask the AWRI: Understanding whole-bunch fermentation. Australian & New Zealand Grapegrower & Winemaker, 652: 63.

Jones, TH., Cullis, BR., Clingeleffer, PR., Ruhl, EH., 2009. Effects of novel hybrid and traditional rootstocks on vigour and yield components of Shiraz grapevines. Australian Journal of Grape and Wine Research, 15: 284-92.

Koch, A., Doyle, CL., Matthews, MA., Williams, LE., Ebeler, SE., 2010. 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. Phytochemistry, 71: 2190-2198.

Kotseridis, Y., Beloqui, AA., Bertrand, A., Doazan, JP., 1998. An analytical method for studying the volatile compounds of Merlot noir clone wines. American Journal of Enology and Viticulture, 49: 44-48.

Liang C., Ristic R., Jiranek V., Jeffery DW., 2018. Chemical and sensory evaluation of magnetic polymers as a remedial treatment for elevated concentrations of 3-isobutyl-2-methoxypyrazine in Cabernet Sauvignon grape must and wine, *Journal of Agricultural and Food Chemistry*, 66, 7121-30. doi:10.1021/acs.jafc.8b01397

Mendez-Costabel, MP., Wilkinson, KL., Bastian, SEP., Jordans, C., McCarthy, M., Ford, CM., Dokoozlian, NK., 2014. Effect of increased irrigation and additional nitrogen fertilisation on the concentration of green aroma compounds in *Vitis vinifera* L. Merlot fruit and wine. Australian Journal of Grape and Wine Research, 20: 80-90.

Olarte Mantilla, SM., Collins, C., Iland, PG., Kidman, CM., Ristic, R., Boss, PK., Jordans, C., Bastian, SEP., 2018. Shiraz (*Vitis vinifera* L.) berry and wine sensory profiles and composition are modulated by rootstocks. American Journal of Enology and Viticulture, 69: 32-44.

Ryona, I., Pan, BS., Sacks, GL., 2009. Rapid measurement of 3-alkyl-2-methoxypyrazine content of winegrapes to predict levels in resultant wines. Journal of Agricultural and Food Chemistry, 57: 8250-8257.