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Research Article

Distribution of 3-Isobutyl-2-methoxypyrazine across Rachis Components of *Vitis vinifera* Shiraz and Cabernet Sauvignon

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Rootstock can significantly alter the concentration of methoxypyrazines (MPs) in the bunch stem (rachis) of *Vitis vinifera* L. cv. Cabernet Sauvignon and Shiraz, which has implications for winemaking and wine style. The distribution of MPs across the rachis is an important consideration, but such information was not available. This study aimed to address this research question by comparing MP concentrations in different rachis components throughout grape maturation and in the absence of ambient light. Shiraz and Cabernet Sauvignon bunches were sampled throughout development, segmented into four components (peduncle, top rachis, bottom rachis, and pedicel), and 3-isobutyl-2-methoxypyrazine (IBMP) was quantified in each. For both cultivars, IBMP showed a negative correlation with grape maturity, with concentrations in pedicel at harvest being significantly higher than other rachis components. Additionally, light exclusion significantly increased IBMP concentrations in all rachis segments. The concentration of IBMP varied significantly between different rachis components. The greatest concentrations were measured in the pedicel, which also contributed the largest proportion among the components to total rachis by weight. Due to elevated IBMP concentrations in rachis and the difficulties in excluding matter other than grape from a fermentor, the presence of pedicel during fermentation could produce Shiraz and Cabernet Sauvignon wines with higher concentrations of MPs, thereby potentially increasing vegetal sensory characteristics.

1. Introduction

The chemical composition of berries is heterogeneous within a vineyard, vine and bunch, and this variability could alter the sensory properties of a wine if heterogeneous grape parcels are harvested [1]. Asynchronous berry development contributing to heterogeneity can be attributed to aspects of *terroir*, which encompass geographical and climatic differences between grape growing regions, and the spatial variation of soil, sunlight, slope, and water availability within a vineyard [2].

Within bunch, berry developmental heterogeneity is dependent on seed content, which alters hormonal dynamics and sugar accumulation [3]. Furthermore, factors such as the location of a berry within a bunch [4], berry surface temperature, or berry proximity to leaves or stems are hypothesised to impact berry composition and maturity [5]. However, within vine and bunch level heterogeneity is not exclusive to berries; concentrations obtained from rachis for rotundone [6], amino acids [7], and methoxypyrazines (MPs) (e.g., 3-isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), and 3-*sec*-butyl-2-

methoxypyrazine (SBMP)) [8] also vary throughout the growing season. Despite this, the potential contribution of rachis to wine aroma might often be overlooked.

Winemakers may opt to include rachis during whole- or partial-bunch fermentation to produce desirable tannin [9, 10], colour [10, 11], pH, or ethanol changes to the wine [9, 12]. However, these techniques are avoided for varieties that are known to produce IBMP (an impact odorant with green capsicum character) in rachis and berry, such as Cabernet Sauvignon, because they can cause the perception of "stemmy" flavours in wine [13]. Even for varieties where a portion of whole bunches is favourable in producing quality wines, such as Pinot noir, stem addition exceeding 60% can produce wines with sensory characteristics associated with MPs [9]. Rachis can also be unintentionally present during fermentation as a by-product of harvesting and destemming practices, as a component of matter other than grape (MOG). Historically, MOG levels of approximately 5% w/w have been found in machine harvested fruit [14]; however, recent technological advancements can decrease these levels to around 1% [15]. Even so, there should still be concern, given the potential for MOG in the form of rachis to impart undesirable and nonvarietal "green" sensory characteristics to a variety like Shiraz [16], which otherwise lacks the genetic ability to produce IBMP in the berry [17].

Being characteristic of certain grape varieties, IBMP is a "varietal" aroma compound that can contribute notes of "green capsicum" and "grassy" to red wine at concentrations of 10–15 ng/L [18]. Such sensory characteristics are desirable when in balance with an overall wine bouquet, but at elevated concentrations, IBMP can contribute "herbaceous" and "vegetative" aromas that can dominate the sensory experience, decreasing both consumer liking and positive emotions associated with the wine [19]. Furthermore, even at concentrations below its sensory threshold, IBMP can alter wine aroma through synergistic interactions increasing perception of "smoky" and "tar" notes or antagonistic interactions that decrease desirable "red berries" and "floral violet" aromas [20]. As such, understanding how to control the concentration of IBMP (and other MPs) in wine is essential.

Recent research has shown Shiraz [8, 21] and Cabernet Sauvignon [22] vines grafted to rootstock can have significantly higher IBMP concentrations in rachis than those on own roots. This was attributed in part to rootstock-mediated vine vigour altering ambient light exposure of bunches [21, 22]. Furthermore, in Shiraz rachis the concentrations of IBMP, IPMP, and SBMP increased throughout berry maturity [8], contrasting the negative relationship between these variables observed in the berry [23].

With respect to the contribution of rachis to MOG and likelihood of contributing unwanted sensory characters, it can be expected that the pedicel component of rachis could most easily enter a fermentor, due to its small size or attachment to the berry. Although studies have addressed MP concentrations in rachis overall, the MP concentration of different parts of the rachis remained to be investigated. This study aimed to fill that knowledge gap by determining the concentration of IBMP, IPMP, and SBMP in different rachis components throughout grape maturation for *Vitis vinifera* L. cv Shiraz on Ramsey rootstock and own roots grown in the Barossa Valley, and Cabernet Sauvignon on 110 Richter rootstock grown in the Coonawarra. Trials exploring the exclusion of ambient light on MP distribution in Shiraz bunches were also undertaken. MPs were quantified by GC-MS/MS using an established stable isotope dilution assay and experimental data were analysed with linear mixed models (LMMs). Results from the study were intended to provide producers with an understanding of how the concentration of MPs across rachis components is influenced by grape maturity and light exposure, thereby giving information that helps to estimate their potential influence on wine sensory profiles.

2. Materials and Methods

2.1. Chemicals. Solvents and reagents were of analytical reagent (AR) grade or higher and were purchased from Sigma–Aldrich (Castle Hill, NSW, Australia). Labelled and unlabelled MPs used as analytical standards were previously synthesised [22].

2.2. Climate Data. Monthly average, minimum, and maximum temperatures and winter rainfall values were sourced from the Bureau of Meteorology's automatic weather stations for Barossa Valley (Australian BOM Station 023373 at 34.47°S, 139.00°E) and Coonawarra (Australian BOM Station 026091 at 37.29°S, 140.83°E). The Huglin index was calculated according to [24] with the value of 1.00 used for the length of day coefficient. Rainfall and Huglin index data are summarised in Table S1 of the Supplementary Material.

2.3. Vineyard Sites. Samples from the Barossa Valley region were collected from the Department of Primary Industries and Regions site in Nuriootpa, South Australia (34°28′34.4″S, 139°00′26.8″E). The vineyard was established in 2001 and consists of *Vitis vinifera* L. cv. Shiraz clone 1654 on own roots and Ramsey rootstocks. Further details have been reported previously [25].

Samples from the Coonawarra region were collected from a premium commercial vineyard (37°15′47.4″S, 140°49′58.7″E). The vineyard was established in 2012 and consists of *Vitis vinifera* L. cv. Cabernet Sauvignon Reynella and SA125 clones on 110 Richter rootstock.

The parentage of rootstocks present in the trials is summarised in a recent publication [21]. No significant pest or disease pressures were observed during the experimental seasons.

2.4. Maturity Variation Experiment. In 2019/20, Shiraz samples were taken at flowering (80% cap fall) on the 26th of November, 50% veraison on the 8th of January, and harvest on the 9th of March. Sampling locations at each time point were chosen to provide a representative sample from the southern, centre, and northern regions of the vineyard. At flowering, twenty-four bunches were collected from each sampling location and used to create six biological replicates for each rachis

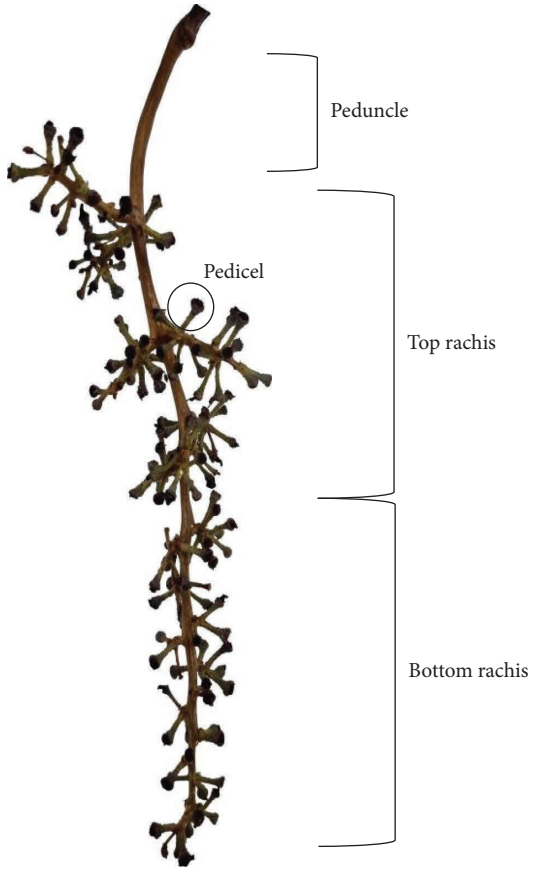

Figure 1: Schematic outlining the components that the rachis material was segmented into prior to extraction and analysis of methoxypyrazines.

component (peduncle, top rachis, and bottom rachis). No pedicel material was retained at flowering due to its small size at this phenological stage and sampling limitations. At veraison and maturity, twelve bunches were collected from each sampling location and used to create six biological replicates of each rachis component (pedicel, peduncle, top rachis, and bottom rachis) (Figure 1). Shiraz sampled in 2021/22 was taken from the same locations within the vineyard as for 2019/20 at flowering (80% cap fall) on the 25th of November, 50% veraison on the 27th of January, and harvest on the 15th of March. In 2021/22, each sampling location was further divided into two six-vine subregions designated east and west. At all time points, twenty-four bunches (twelve from each east and west subregion) were collected from each vineyard location and used to create eight biological replicates (four from each east and west subregion) of each rachis component (peduncle, top rachis, bottom rachis, and pedicel) (Figure 1). As for 2019/20, pedicel material was only collected at veraison and maturity. All rachis material was transported to the laboratory on ice where berry material was removed, and rachis was segmented into components, cut into approximately 1 cm pieces, frozen in liquid nitrogen, and stored at −80°C until analysis. An overview of the sampling methodology for Shiraz from Barossa Valley can be found in Figure S1 of the Supplementary Information.

Cabernet Sauvignon was sampled in the 2019/20 season at 80% veraison on 13th February and at harvest on the 20th of March. Sampling occurred prior to commercial harvest (15th of April) due to complications arising from the COVID-19 pandemic. Sampling locations were the same for each time point and were chosen to provide a representative sample from the southern, centre, and northern regions of the vineyard. From each sampling location, six bunches were chosen at random and used to create two biological replicates of each rachis component (peduncle, top rachis, bottom rachis, and pedicel) (Figure 1) per vineyard location. All rachis material was transported to the laboratory on ice where berry material was removed, and rachis was segmented into components, cut into approximately 1 cm pieces, frozen in liquid nitrogen, and stored at −80°C until analysis.

Shiraz and Cabernet Sauvignon grape bunches were collected at harvest from each sampling location and total soluble solids (TSS) (°Brix) were measured from two biological replicates of homogenate prepared by manually crushing 50 fresh berries randomly selected from bunches.

2.5. Exclusion of Sunlight on the Distribution of Methoxypyrazines in Grape Rachis. Experiments were performed over the 2021/22 growing season in the Barossa Valley and utilised opaque boxes designed to eliminate ambient light from the grape bunches while preventing temperature and humidity changes [26]. The experiment comprised boxed (box) and nonboxed (control) vines, with the treatment vines in each vineyard plot chosen to be representative samples of the southern, centre, and northern regions of the vineyard. Control bunches were those used in the maturity variation experiment and were sourced from vines located next to experimental vines. The box treatment was applied at 1 week postflowering (wpf) on the 23rd of November to whole bunches on own roots ($n = 24$) and Ramsey ($n = 20$). Samples were harvested at 14 wpf on the 15th of March and processed as described previously [22]. For box and control samples, TSS (°Brix) were measured from a homogenate prepared by manually crushing 15 fresh berries randomly selected from each box. TSS values for control were taken as outlined above.

2.6. Weight of Individual Rachis Components in Shiraz. Shiraz material ($n = 26$) from Wrattonbully, South Australia was sampled at commercial harvest in 2022, frozen at −20°C, transported in Styrofoam boxes on ice, and stored at −30°C for 4 months until processing. Rachis material from each bunch was segmented into peduncle, top rachis, bottom rachis, and pedicel, and individual weights of each component were determined.

2.7. Measures of Canopy Architecture. A surrogate measure of vine vigour was obtained in the Barossa Valley on the 17th of January 2022 using a LICOR LAI-2200C Plant Canopy Analyser. For every six-vine subregion (east and west) at each sampling location, one above-canopy reading (ambient light), and four below-canopy readings were taken to provide an estimate of leaf area index (LAI).

2.8. Quantitation of Methoxypyrazines. MPs were quantified in grape and rachis tissue using a stable isotope dilution assay with headspace SPME-GC-MS/MS [22] with modifications to sample preparation. Briefly, the modifications involved frozen rachis tissue (0.5–2 g, dependent on component) being ground to a fine powder with a cryomill (Retsch, Germany) in liquid nitrogen. Approximately 200 mg of rachis from the sunlight exclusion experiment and 500 mg of rachis from the maturation trials was accurately weighed for extraction and analysis as per the previous report [22]. The respective limit of detection (LOD) and limit of quantitation (LOQ) values (ng/kg) were 0.13 and 0.44 for IBMP, 0.11 and 0.37 for IPMP, and 0.15 and 0.48 for SBMP [22].

2.9. Statistical Analysis. Data were analysed using R (version 4.1.2, R Foundation for Statistical Computing, Vienna, Austria) in RStudio (version 2022.07.1, RStudio Inc., Boston, MA). Mixed-effect linear regression models (linear mixed model, LMM) were used to determine treatment effects on log-transformed MP concentrations with the "lmerTest" package. For the Shiraz maturity variation experiment, rootstock, vintage, berry maturity, and rachis component were set as fixed effects, and vineyard block was set as a random factor. For the light exclusion trials, rachis component, light, and rootstock were set as fixed effects, and vineyard row and °Brix were included as random factors. For the Cabernet Sauvignon maturity variation experiment, rootstock, berry maturity, and component were set as fixed effects and vineyard row was set as a random factor. Estimated marginal means (statistically modelled variable mean response for each level of a predictor variable) and standard error (SE) values of the models were calculated on back-transformed values using the "emmeans" package and compared using Bonferroni-adjusted pairwise comparisons. Summary statistics for variables with measurements below the LOD and/or LOQ were calculated using the Kaplan–Meier technique with Efron's bias correction using the "NADA" package. Summary plots were produced using "ggplot2".

3. Results and Discussion

3.1. Concentration and Distribution of 3-Isobutyl-2-Methoxypyrazine in Shiraz Rachis Components throughout Berry Maturation. Shiraz bunches were collected at harvest in 2022 from the Wrattonbully wine region to determine the proportion (% w/w) of individual rachis components (peduncle, top rachis, bottom rachis, pedicel) of the total rachis fresh weight. Timing of sampling was an important consideration; rachis reaches its definite size by veraison [27] but dehydration of the peduncle continues until harvest [28], which would decrease its fresh weight and cause a concentration effect of IBMP within this component. In addition, Shiraz rachis grown on Ramsey rootstock and own roots has been shown to increase in IBMP concentration through berry maturation, reaching its maximum at harvest [8].

Shiraz rachis fresh weight at harvest was calculated as 6.5% of the total bunch weight, which falls within the range of 3–7% for *Vitis vinifera* rachis [27]. The proportion (% w/w) of individual rachis components of the total fresh rachis weight was calculated for peduncle (10%), top rachis (18%), bottom rachis (9%), and pedicel (63%) (Figure S2 of the Supplementary Material). These values were used throughout this publication to estimate the contribution from each rachis component to the total IBMP concentration present within an average Shiraz rachis. To the best of our knowledge, this is the first published segmentation data for rachis material, with a single previous study showing that peduncle was approximately 20% of Shiraz rachis fresh weight [28].

3.1.1. Rootstock and Stage of Grape Development. The concentration of IBMP in different rachis components from Shiraz grown on Ramsey and own roots in the Barossa Valley (Table S3 of the Supplementary Material) was dependent on rootstock and berry maturity ($P < 0.001$, Figure 2).

According to the LMM, concentrations of IBMP ranged at veraison from 2.51 ng/kg rachis for peduncle from own roots at harvest to 142 ng/kg rachis for Ramsey pedicel. At veraison, IBMP concentrations were significantly higher in Ramsey than own roots for pedicel (142 and 54.3 ng/kg rachis, respectively), top rachis (18.8 and 7.74 ng/kg rachis), and bottom rachis (12.9 and 3.58 ng/kg rachis), although this difference was no longer significant for any rachis component at harvest. This was somewhat surprising considering that own roots had a higher measure of vine vigour than Ramsey ($P < 0.001$, Table S2), and vigour has previously been positively correlated with rachis IBMP concentrations at harvest [21]. However, that prior research also showed no significant difference between IBMP concentrations in own roots and Ramsey Shiraz rachis at harvest for three vintages [21] that were sampled from the same vineyard used in the current work.

For both Ramsey and own roots, the concentration of IBMP in top rachis, bottom rachis, and peduncle significantly decreased between flowering and harvest ($P \leq 0.05$) with a downward trend evident as grape maturity increased (Figure 2). For pedicel, Ramsey was significantly lower at harvest (63.9 ng/kg rachis) than veraison (142 ng/kg rachis), and while own roots appeared to trend upwards from veraison (54.3 ng/kg rachis) to harvest (81.4 ng/kg rachis), there was no significant difference.

In contrast, previous research reported Shiraz rachis from the Murray Darling region of Victoria increased in MP concentration throughout the 2017/18 growing season, with IBMP, IPMP, SBMP being above the LOD by 8 wpf [8]. In the current study, however, SBMP remained below the LOD throughout both studied vintages, and IPMP was detected only sporadically and at low concentrations (data not shown). As rachis is composed of approximately 55 to 80% water [29], increased dehydration of rachis due to climate differences between the regions may have elevated the concentrations of MPs in Murray Darling rachis relative to

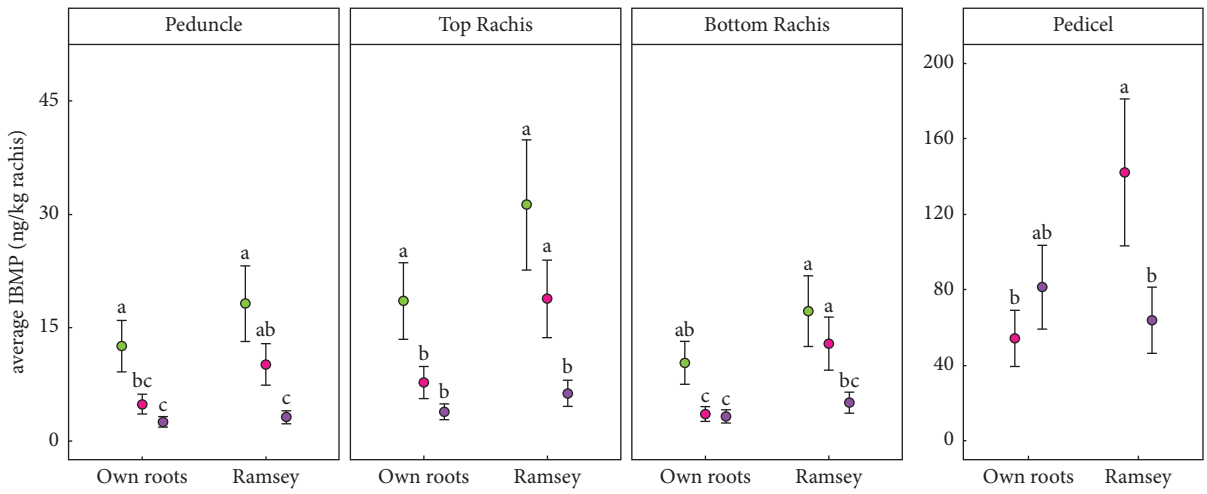

FIGURE 2: Estimated marginal means of IBMP (ng/kg of rachis ± SE) in the peduncle, top rachis, bottom rachis, and pedicel from Shiraz rachis sampled during the 2019/20 and 2021/22 vintages from the Barossa Valley, considering the interaction effect between rootstock (own roots and Ramsey) and berry maturity (flowering (• green), veraison (• red), and harvest (• purple)). Bars sharing the same letter within a component are not significantly different (linear mixed model, $\alpha = 0.05$, Bonferroni-adjusted). *Note.* Pedicel material was not sampled at flowering. Concentrations (ng/kg rachis) were calculated from ng/kg of component values (Figure S3 of the Supplementary Material) by considering component contribution (% w/w) to total rachis weight (Figure S2 of the Supplementary Material). Note the different *y*-axis scale for pedicel.

that from the Barossa Valley. As calculated by the Huglin index, the Murray Darling region was classified as very warm (3181) for the 2017/18 season, whereas Barossa Valley was classified as temperate warm for both 2019/20 (2379) and 2021/22 (2252).

Overall, the close agreement in trends and concentrations for Ramsey and own root rachis seen herein suggest that rootstock may have no bearing on the way that IBMP is distributed within rachis. Alternative explanations for the distribution of IBMP in different rachis components could relate to differences in light environment due to berry or vine shading.

3.1.2. Vintage and Stage of Grape Development. The concentration of IBMP in Shiraz rachis components from the Barossa Valley was shown to be dependent on the simple main effect of vintage ($P < 0.001$) according to the LMM, with an average IBMP concentration of 103 ng/kg and 57.6 ng/kg in the 2019/20 and 2021/22 growing seasons, respectively. Grape maturity at harvest (as a surrogate measure of rachis maturity) varied significantly ($P < 0.001$) between vintages, with 2019/20 (27.4° Brix) being significantly lower than 2021/22 (28.9° Brix). Broadly these results suggest a negative relationship between IBMP concentration in rachis and grape maturity, similar to that observed for Cabernet Sauvignon berries [23].

In addition, the concentration of IBMP in rachis was dependent upon a three-way interaction between component, vintage, and berry maturity ($P = 0.03$) according to the LMM (Figure 3). IBMP concentrations ranged from a high of 110 ng/kg rachis for pedicel at veraison in 2019/20 down to 0.87 ng/kg rachis for peduncle at harvest in the same season. IBMP concentrations in pedicel were higher than all other rachis components at veraison and harvest, but pedicel did not significantly differ at either maturity time point in 2019/20 or 2021/22 (Figure 3). In 2021/22, top rachis (18.4 ng/kg rachis) and bottom rachis (9.59 ng/kg rachis) were different ($P < 0.05$) at veraison, which may suggest that the regulation of IBMP distribution in these organs is somewhat variable. Light exposure can significantly alter MP accumulation in Shiraz rachis material [21], so it was theorised that top rachis could experience higher levels of ambient light than bottom rachis throughout the growing season, due to its more exposed position in the bunch. As pedicel was not separated by bunch position, it is feasible that pedicel material could also differ in IBMP content based on its location within a bunch. However, due to shading from the berry, the variability in pedicel light exposure across the bunch should be lower than for top versus bottom rachis, although this aspect could be evaluated in future.

While the average IBMP concentration was significantly higher in 2019/20 (103 ng/kg) than 2021/22 (57.6 ng/kg), all components at harvest except for pedicel were significantly higher in IBMP concentration in 2021/22 than 2019/20. Vintage effects in rachis IBMP are attributed to differences in growing season temperature, with a negative correlation between temperature and IBMP in rachis proposed for Shiraz [21] and Cabernet Sauvignon [22]. However, the vintage effects seen within the current results were not readily explained due to climatic variables. The Huglin index values of 2379 and 2252 the 2019/20 and 2021/22 growing seasons, respectively, were similar and both were classified as "temperate warm," suggesting minimal temperature variation overall.

Notably, the IBMP concentration in pedicel material at harvest from either vintage was significantly higher than all other rachis components on a ng/kg of rachis basis (Figure 3), which suggests that pedicel is the most substantial source of rachis IBMP. As concentrations of IBMP in pedicel

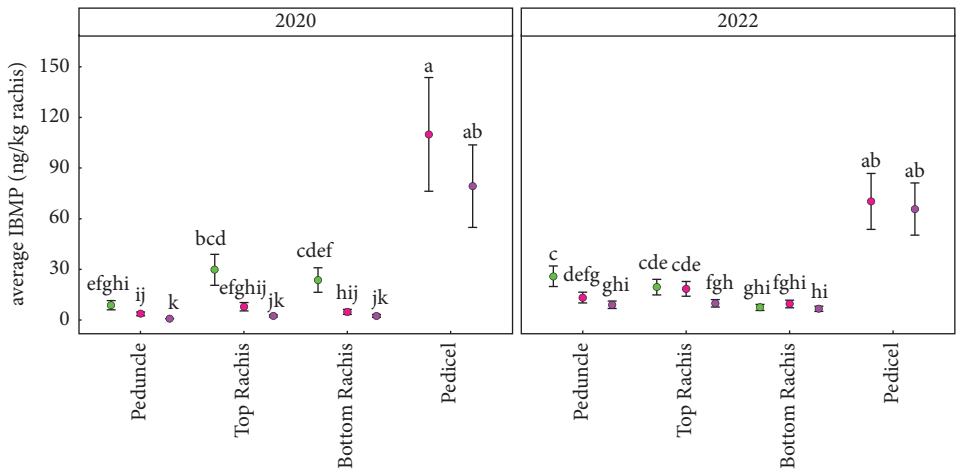

FIGURE 3: Estimated marginal means of IBMP (ng/kg rachis ± SE) in the peduncle, top rachis, bottom rachis, and pedicel of Shiraz rachis sampled during the 2019/20 and 2021/22 vintages from the Barossa Valley at (flowering (• green), veraison (• red), and harvest (• purple)) considering the three-way interaction effect between component, vintage, and berry maturity. Bars sharing the same letter between the plots are not significantly different (linear mixed model, $\alpha = 0.05$, Bonferroni-adjusted). *Note.* Pedicel material was not sampled at flowering. Concentrations (ng/kg rachis) were calculated from ng/kg of component values (Figure S4 of the Supplementary Material) by considering component contribution (% w/w) to total rachis weight (Figure S2 of the Supplementary Material).

are not significantly different between veraison and harvest, testing the pedicel at veraison could provide valuable information about potential wine sensory outcomes for winemakers who are considering partial- or whole-bunch fermentation or mechanically harvesting fruit without any sorting.

3.1.3. Ambient Light Exclusion throughout Maturation. As with berry, light exclusion is known to yield significantly higher concentrations of IBMP, IPMP, and SBMP in Shiraz [21] and Cabernet Sauvignon [22] rachis, but the effect of light on MP distribution across the rachis was unknown. Addressing the hypothesis that differences in natural light exposure could be responsible for the observed differences in IBMP concentration between rachis components (peduncle, top and bottom rachis, and pedicel), light exclusion boxes were applied to Shiraz grape bunches at 1 wpf on Ramsey rootstock and own roots (box) with nonboxed bunches (control) taken from nearby vines at harvest. There was no significant difference in maturity (°Brix) for Shiraz berries obtained from Ramsey (28.2 ± 1.53) and own roots (29.7 ± 1.41) box samples at harvest.

The concentration of IBMP in Shiraz rachis at harvest (Table S4 of the Supplementary Material) for box and control samples was significantly dependent upon the simple main effect of rootstock ($P < 0.001$) (Figure 4) according to the LMM. The estimated marginal means were 72.2 ng/kg rachis (own roots) and 143 ng/kg rachis (Ramsey) for control bunches, and 780 ng/kg rachis (own roots) and 1219 ng/kg rachis (Ramsey) for box bunches. Although slightly lower, these values were reflective of previous research involving light exclusion trials on Shiraz, with box bunches being substantially higher than controls [21]. IPMP and SBMP were not detected in any control samples but were above the LOD for 100% and 96% of Ramsey box

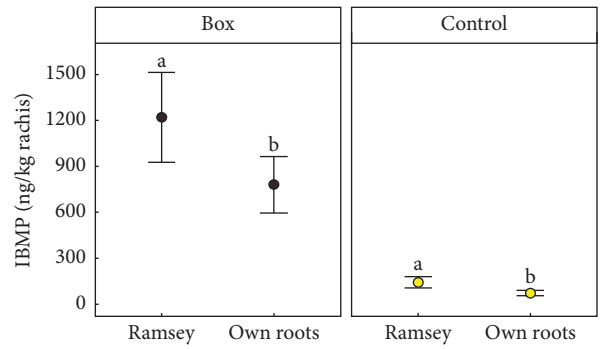

FIGURE 4: Estimated marginal means for IBMP concentration (ng/kg rachis ± SE) in Shiraz rachis for control (• yellow) and box (• charcoal) treatments at harvest (2022) considering the simple main effect of rootstock (Ramsey and own roots). Bars sharing the same letter within the same plot are not significantly different (linear mixed model, $\alpha = 0.05$, Bonferroni-adjusted). *Note.* IBMP concentrations (ng/kg rachis) were calculated by adding the IBMP concentration of the respective components together for every biological replicate while considering component proportion (% w/w) (Figure S2 of the Supplementary Material).

samples (Table S4 of the Supplementary Material). However, due to their low concentrations, the data were not analysed further.

An interaction between rachis component and light ($P < 0.001$) significantly affected the concentration of IBMP according to the LMM. The impact of light exclusion on IBMP is visualised on a ng/kg rachis (Figure 5(a)) and a ng/kg component (Figure 5(b)) basis.

On a per kilogram of rachis basis (Figure 5(a)), the marginal means for IBMP concentration ranged from 6.58 ng/kg rachis (bottom rachis, control) to 597 ng/kg rachis (pedicel, box). Pedicel box was significantly higher in IBMP concentration than all other components,

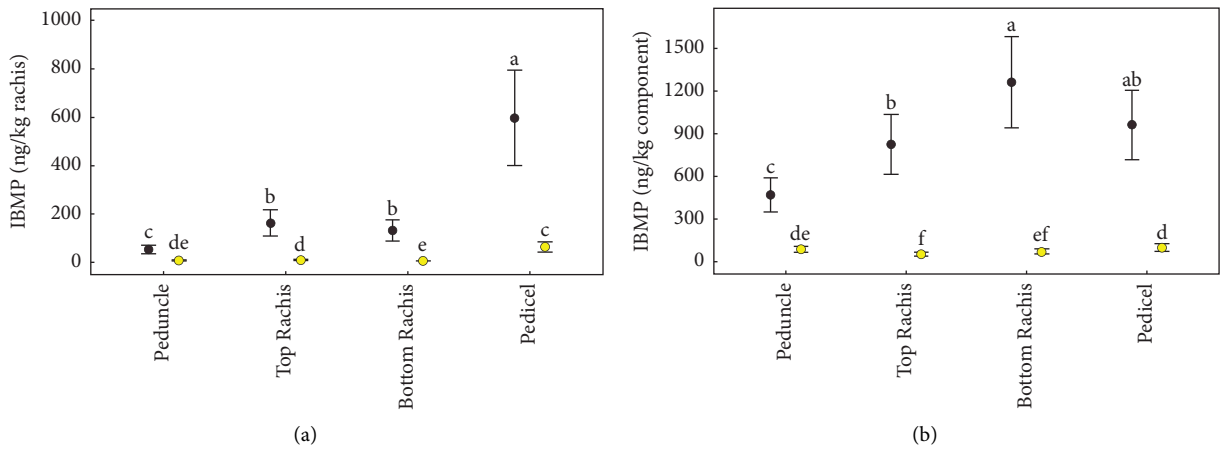

Figure 5: Estimated marginal means of IBMP concentration (a) (ng/kg rachis ± SE) and (b) (ng/kg component ± SE) in different Shiraz rachis components at harvest from control (• yellow) and box (• charcoal) grape bunches grown in the Barossa Valley (2022) considering the interaction between rachis component and light. Bars sharing the same letter within the same plot are not significantly different (linear mixed model, $\alpha = 0.05$, Bonferroni-adjusted). Values for Figure 5(a) were calculated from Figure 5(b) by considering the proportion (% w/w) of individual rachis components to total rachis fresh weight (Figure S2 of the Supplementary Material).

independent of light conditions. IBMP concentrations in pedicel control (64.5 ng/kg rachis) were equivalent to peduncle box (53.98 ng/kg rachis), and higher ($P \leq 0.05$) than top rachis control (9.85 ng/kg rachis), peduncle control (8.95 ng/kg rachis), and bottom rachis control (6.58 ng/kg rachis). Box and control samples trend in a similar manner, suggesting that bunch light exposure is an important consideration for controlling IBMP within a vineyard.

On a per kilogram of component basis (Figure 5(b)), the marginal means of IBMP varied from 53.8 ng/kg component (top rachis, control) to 1260 ng/kg component (bottom rachis, box). Concerning light excluded components, bottom rachis was significantly higher than top rachis (827 ng/kg component) and peduncle (469 ng/kg component), but not significantly different to pedicel (961 ng/kg component). Pedicel was equivalent to bottom and top rachis but higher than peduncle. Notably, the pattern of distribution across the rachis was dissimilar to the control samples, although there were still significant differences between rachis components (Figure 5(b)), suggesting that bunch light exposure might contribute to the regulation of IBMP movement, biosynthesis, and/or storage in various components of rachis, perhaps in unison with other regulatory processes.

IBMP biosynthesis from the precursor hydroxypyrazine (IBHP) is regulated in berry by the *VvOMT* gene family, primarily through the activity of the methyltransferase enzyme VvOMT3 [30, 31]. Expression of*VvOMT*3 is upregulated in the berry when ambient light is excluded, significantly increasing concentrations of IBMP [23]. The exclusion of light also increases the concentration of IBMP in rachis [21, 22], but the molecular basis remains unknown. A study of Shiraz rachis found that IBMP biosynthesis throughout the growing seasons was not correlated with the levels of *VvOMT*3 expression [8]. Instead, those researchers proposed that translocation from other vines organs, particularly the roots where there are elevated concentrations of IBMP, could explain IBMP concentrations in the rachis. However, while the expression of genes in the *VvOMT* family has been shown to vary between vine organs [32], the differential expression of *VvOMT*3 across and within vine components remains unexplored. The significantly elevated concentrations of IBMP in the bottom rachis under light exclusion conditions (Figure 5(b)) may suggest that a targeted approach measuring gene expression in the different rachis components should be a consideration for future work that aims to elucidate IBMP biosynthesis in rachis.

Although light does not affect IBMP distribution in rachis in a uniform manner, the clear relationship between light and absolute IBMP concentration, established herein and elsewhere [21, 22], highlights that bunch light exposure might remain an important tool for grapegrowers to regulate IBMP not only in berry but also in rachis.

3.2. Methoxypyrazine Distribution in Cabernet Sauvignon Rachis. Preliminary experiments were conducted over the 2019/20 growing season to quantify IBMP distribution in the rachis of Cabernet Sauvignon clones (Reynella and SA125) grown on 110 Richter rootstock in Coonawarra (Table S5 of the Supplementary Material). The concentration of IBMP in Cabernet Sauvignon rachis was significantly different between rachis components ($P < 0.001$) according to the LMM. The estimated marginal means ranged from 52.4 ng/kg component for peduncle to 229 ng/kg component for pedicel (Figure 6(a)). Pedicel had significantly higher concentrations than bottom rachis (140 ng/kg component) and top rachis (124 ng/kg component), which themselves were equivalent, and significantly higher than peduncle (52.4 ng/kg component). Additionally, IBMP concentrations in Cabernet Sauvignon rachis varied significantly with berry maturity ($P < 0.001$) (Figure 6(b)), from 148 ng/kg at veraison to 98 ng/kg at harvest. This suggests that IBMP concentrations in rachis are negatively correlated with berry maturity in a similar manner to IBMP in not only Cabernet Sauvignon

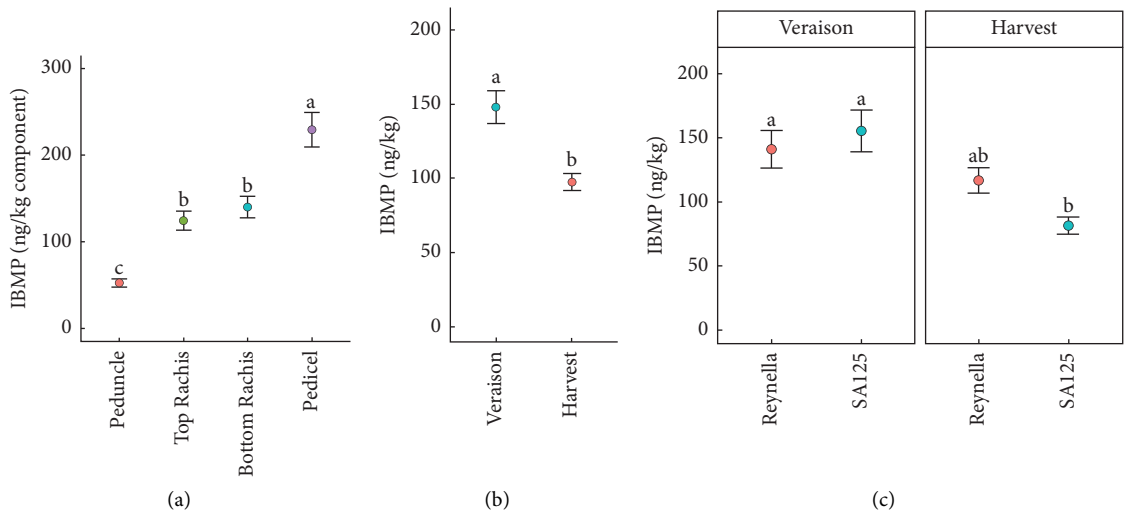

FIGURE 6: Estimated marginal means of IBMP concentration in rachis components (±SE) of Cabernet Sauvignon grown in Coonawarra over the 2019/20 season, considering the simple main effects of (a) component, (b) berry maturity, and (c) the interaction effect between berry maturity and clone. Bars sharing the same letter within the same plot are not significantly different (linear mixed model, $\alpha = 0.05$, Bonferroni-adjusted).

berries [31] but also Shiraz rachis, as described in an earlier section.

Although higher, the concentration of IBMP in Cabernet Sauvignon rachis trended similarly to Shiraz pedicel (126 ng/kg component), bottom rachis (27.2 ng/kg component), top rachis (13.5 ng/kg component), and peduncle (8.73 ng/kg component) sampled from the Barossa Valley at harvest in 2019/20 (Figure S4 of the Supplementary Material). As the Cabernet Sauvignon and Shiraz samples were sourced from different regions, the difference in IBMP concentration in rachis seen herein was difficult to attribute solely to varietal differences. Previous work has shown large variations in IBMP concentration for Shiraz rachis across multiple regions in a single vintage [21] and a regional influence could not be discounted in the present study.

IBMP concentration in Cabernet Sauvignon rachis components was dependent on an interaction effect between clone and berry maturity ($P = 0.006$) with values for SA125 varying significantly ($P \leq 0.05$) from veraison (155 ng/kg) to harvest (81.5 ng/kg) (Figure 6(c)). In comparison, Reynella did not vary significantly ($P > 0.05$) in IBMP concentration from veraison to harvest (141 ng/kg and 117 ng/kg, respectively). There was no statistical difference in IBMP between SA125 and Reynella rachis at harvest. This contrasted with previous findings, in which IBMP concentrations in the berry of Carménère [33] and Sauvignon blanc [34] clones, and in the rachis of Shiraz clones 1654 and BVRC [21], varied significantly at harvest. Such variability is proposed to arise due to genetic variation between clones [34], but further research is required to understand the biological mechanism that leads to clonal variation in IBMP concentration in berry or rachis.

As vintage and rootstock have been shown to regulate MP accumulation in Cabernet Sauvignon rachis [22], further research encompassing these variables is necessary to support the preliminary trends in MP distribution observed in the present study with Cabernet Sauvignon rachis grown on 110 Richter over a single vintage. Furthermore, the elevated IBMP concentration in pedicel at harvest implies that the presence of Cabernet Sauvignon pedicels in a fermentor has the potential to increase the concentration of IBMP and alter wine sensory characteristics. Pedicel is the most likely MOG to enter a fermentor, so it would be interesting to determine the proportion of MOG attributable to pedicel as a result of different crushing and sorting techniques, to ascertain the likely impact on wine style.

4. Conclusion

This research significantly expands upon existing knowledge by showing that IBMP distribution throughout Shiraz rachis is not equivalent and can be significantly impacted by rootstock, stage of grape development, and vintage. As the concentration of IBMP was not significantly different between veraison and harvest in pedicel, the main contributor of overall rachis IBMP, quantification of IBMP in pedicel at veraison could inform winemakers about potential sensory outcomes related to rachis presence during fermentation. This trial also reinforced the importance of bunch light exposure throughout the growing season in mediating IBMP concentrations in rachis at harvest, providing knowledge that will be useful for managing IBMP in the vineyard or winery. It remains to be determined how the variation in IBMP across Shiraz rachis components occurs. Additional research considering the impact of berry shape and cluster compactness on pedicel light exposure throughout the growing season may help to further explain the elevated concentrations of IBMP in pedicel observed in the present study.

In addition, the impact of viticultural region remained unexplored and, in conjunction with assessing other rootstock and scion combinations, would be an opportunity for

future research. Furthermore, a preliminary trial suggested that IBMP concentrations in Cabernet Sauvignon rachis may be affected by grape maturity and clone, which has implications for wine quality and style. Additional work could be useful to confirm these results and determine the impact of vintage and rootstock on the distribution of MPs across the bunch stem.

Putting the work into a practical context, in a best-case scenario, if only 1% w/w of MOG is permitted in top grade fruit with a maximum of 50% being rachis, such unintentional inclusion of rachis during fermentation would be insufficient to exceed the detection threshold of IBMP in red wine based on the results of this study. A direct effect on the sensory profile of wine would therefore be unlikely, even in the worst-case scenario of complete bunch light exclusion throughout the growing season. However, subthreshold concentrations of IBMP can alter the sensory profile of wine, so the presence of rachis components during fermentation should remain a primary consideration for winemakers.

Data Availability

The data used to support the findings of this study are included within the article and the supplementary information file.

Conflicts of Interest

The authors declare that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper. R.D.S is a recipient of an Australian Government Research Training Program Scholarship and a CSIRO iPhD scholarship, funded by the Australian Government with additional support from Wine Australia (WA Ph1901) and Treasury Wine Estates.

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Supplementary Materials

Supplementary data include figures showing average weight of rachis components and estimated marginal means for IBMP in rachis components based on interactions; tables showing rootstock and climate information for the regions, leaf area index for Shiraz vines, average concentration of IBMP for rachis components of Shiraz sampled at different times, average concentrations of IBMP, IPMP, and SBMP in different rachis components of Shiraz in control and box experiments, and average concentration of IBMP for rachis components of Cabernet Sauvignon sampled at different times. (*Supplementary Materials*)

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