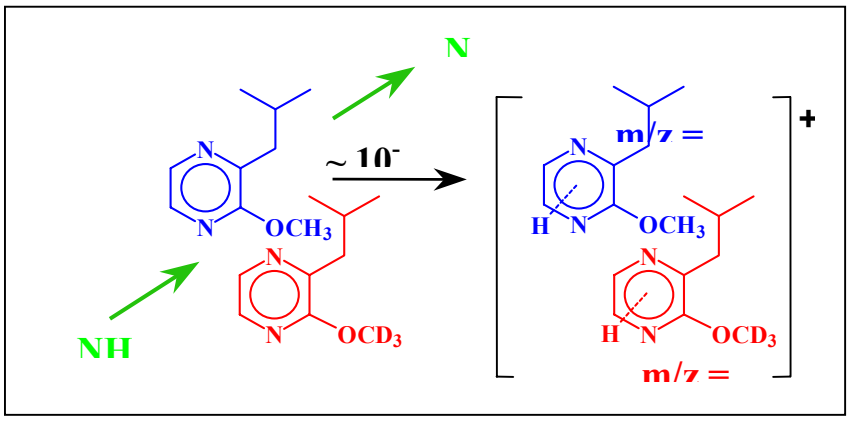
VITICULTURAL CONTROL OF GRAPE FLAVOUR IN CABERNET SAUVIGNON, SAUVIGNON BLANC AND SEMILLON VINES



FINAL REPORT to

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **UCS 92/1**

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Non-Technical Summary

Wine quality and wine price are dramatically influenced by grape-derived wine flavour. Such flavour contributes to varietal and regional distinctiveness. It is often crucial to wine flavour development with age, and it cannot be generated by winemaking practices. Knowledge of the compounds that contribute grape flavour is clearly important, but our knowledge of these flavour compounds is still poor.

The reason for our lack of knowledge is that these flavour compounds occur, but are of profound sensory importance, at remarkably low concentrations. In some cases a concentration as low as ng/L (or parts per trillion), the level of ultra-trace analysis. At these low concentrations, flavour compound detection, isolation and structural identification is a major challenge. In addition, study of how vine growing conditions or winemaking conditions influence their abundance, or their stability, requires that the flavour compounds be accurately quantified. For compounds at ultra-trace levels, the techniques to achieve this are few, the analyses are at the limits of equipment sensitivity, the equipment required is expensive, and the analytical time needed is considerable.

This research focuses on a family of grape-derived flavour compounds called methoxy-pyrazines. They are particularly important in grapes of *Vitis vinifera* cvs. Cabernet Sauvignon and Sauvignon Blanc. Methoxypyrazines provide wines of these grape varieties with a vegetative, herbaceous, grassy, or green aroma. This aroma can provide complexity and it is varietally distinctive, but at high levels it readily becomes overpowering. The aroma arises principally from isobutyl methoxypyrazine (IBMP), a flavorant that also contributes to the characteristic aroma of some vegetables such as peas and bell peppers. It has an extremely low odour detection threshold of 2 ng/L in water or white wine.

Earlier research by the principal researcher and coworkers was able to quantify the concentration of this methoxypyrazine in Sauvignon Blanc wines. That work indicated a strong dependence of IBMP concentration on the climate in the viticultural growing region and it showed that the grape IBMP concentration falls rapidly during ripening from a comparatively high level in the grape at the onset of ripening. This earlier research also examined the sensory impact of IBMP. The work established that IBMP, at only 2 ng/L, significantly affects white wine aroma and that wine IBMP concentration correlates well with perceptions of herbaceous/vegetative aroma in Sauvignon Blanc wines. Some work was also done with Cabernet Sauvignon grapes, which show a similar decrease of IBMP concentration during ripening. Also, with Cabernet Sauvignon grapes, the dependency of the grape IBMP concentration on the light exposure of the ripening fruit was quantified.

This project sought to take that knowledge further. One direction was to examine more closely the effect of some variables in the vineyard. This was approached with caution, as the cost, specialised equipment, and the time required for quantitative analysis at ultra-trace levels lowers the number of analyses that could be performed and limits the scope of the trial and the opportunities for replication. An unexpected difficulty appeared during the trial. It was the onset of unusually late rain combined with warm temperatures in the spring and summer of 1993. It led to a downy mildew infection that dramatically reduced the fruiting capacity of most of the vines, limiting the number of vines available to the trial and hindering replication. The outcome was a change of focus to an examination of some broad effects: the comparative effects of high and low crop yield, of irrigation and non-irrigation of vines, and of vines with and without nitrogen fertilisation. The trial results were not conclusive, suggesting that, at

least in that trial situation and in a season with little evidence of water stress, the influence of ripening stage and fruit exposure were more significant than the crop yield, irrigation and fertilisation effects.

In the 1994 ripening season, a follow-up trial was to be performed, together with a more detailed study of light exposure to compare exposed and shaded clusters on the same shoot and to compare exposed shoots with shaded shoots. However, the PhD student appointed to the program withdrew from his study in February 1994. The withdrawal was at a critical stage of measurement and sampling. In view of the imminent study leave of the principal researcher to the University of Bordeaux in 1994 for 6 months, it was decided to postpone those viticultural trials by 12 months.

Other viticultural trials did take place. A cooperative trial with the University of California, Davis, involving methoxypyrazine analysis at Charles Sturt University (CSU), studied the relationship of wine methoxypyrazine concentration to the vigour of the vines, the fruit cluster light level, and the wine flavour when assessed by sensory descriptive analysis. This clearly showed a link between high vine vigour, low light levels, perceived vegetative aroma and the measured methoxypyrazine concentrations.

A further trial examined the influence of fruit exposure and ripening on Sauvignon Blanc and Cabernet Sauvignon fruit under very cool viticultural conditions, in the Whitlands vineyard of Brown Bros in Victoria. It presented data that clearly support an influence for light exposure on methoxypyrazine production in both grape varieties. Aspects of the data also question the sometimes-presented belief that the decline of methoxypyrazine concentration during ripening is primarily a consequence of photodegradation of methoxypyrazines in the berry.

The difficulty of using warm areas for methoxypyrazine study was highlighted by analysis of the effects of different inter-row treatments (cultivation, mulched and sod) on the methoxy-pyrazine composition of Semillon grapes in an existing GWRDC trial in Griffith, NSW. The rapid decline of methoxypyrazine level during ripening lowered the concentration to a level below the odour detection threshold and too low for conclusions to be drawn.

Knowledge of the desired concentration of methoxypyrazine compounds in the fruit is needed if flavour compound research is to produce better fruit flavour quality. This is particularly true with methoxypyrazines because they can readily overpower other flavour characteristics. While analysis of Sauvignon Blanc wines had shown the desirable concentration of IBMP in those wines, the desired concentration in Cabernet Sauvignon wines was not known. To answer this question, a range of Australian, New Zealand and French wines was investigated, including Bordeaux Cabernet Sauvignon wines. The outcomes confirm that IBMP is typically present in red wines of Bordeaux at about 10 ng/L. This level is attained in Australian wines produced from grapes of regions with a mean January temperature of ca. 20^{o}C, such as McLaren Vale (SA), Seymour (Vic) and Frankland (WA).

In 1995, a progressive loss of sensitivity of the university's Trio-2 gas chromatography-mass spectrometry (GC-MS) equipment made further methoxypyrazine analysis impossible. The instrument's manufacturer made several attempts, over the following two years, to identify and rectify the problem. However, it proved impossible to restore adequate instrumental sensitivity for successful methoxypyrazine analysis. To attempt to overcome this, the project studied the methoxypyrazine isolation process, increasing the recovery of material about 5-fold. However, the improvement was not sufficient to offset the approximately 80-fold loss of sensitivity of the instrument. During the study leave at Bordeaux, the feasibility of using a widely used alternative GC-MS system was examined, but its sensitivity was found to be too low for successful analysis.

The National Wine & Grape Industry Centre has now installed a GC-MS system at the Centre. However, the timescale of purchase and installation prevented use of that instrument in this project.

Background to the Research

The potential of a methoxypyrazine, 2-methoxy-3-isobutylpyrazine (IBMP), to contribute a vegetative/herbaceous aroma to Cabernet Sauvignon wine flavour was first reported by Bayonove et al. in 1975. Later work by Augustyn et al. (1982) implicated this compound in Sauvignon Blanc flavour also. However, in neither case was it possible to directly confirm the chemical structure of the odour active compound as IBMP because the concentration of the compound was below the level available to their instrumentation. For the same reason, quantification of the compound in either grapes or wine was not possible.

The first definitive, quantitative analysis of methoxypyrazines in wine was achieved by the principal investigator of this project, in conjunction with Dr Roger Harris and Dr Michael Lacey, at CSIRO Division of Plant Industry and CSIRO Division of Entomology, respectively, in Canberra. This work quantified three methoxypyrazines in a Sauvignon Blanc wine: IBMP, a structural isomer, 2-methoxy-3-*sec*-butylpyrazine (SBMP), and 2-methoxy-3-isopropylpyrazine (IPMP) in a Sauvignon Blanc wine. The research used an isotope-dilution technique in which stable isotope-labelled internal standards of IBMP and IPMP are added to the sample before analysis by gas chromatography-mass spectrometry (GC-MS). The technique showed that these three methoxypyrazines occur at the low part per trillion (ng/L) level (Harris et al., 1987).

Later work by these researchers quantified the occurrence of these compounds in a range of Australian and New Zealand Sauvignon Blanc wines. A significant regional effect was demonstrated, with significantly higher levels of IBMP in New Zealand Sauvignon Blanc wines (mean concentration 25.9 ng/L) by comparison with the level in Australian wines (mean concentration 6.8 ng/L) (Lacey et al., 1991). The work also confirmed that these methoxypyrazines occur in Sauvignon Blanc juice, that their concentration decreases markedly with ripening, and that cool ripening conditions can lead to enhanced methoxypyrazine concentrations, consistent with winemaking observations.

Further studies showed that IBMP and IPMP significantly affected the odour of a neutral white wine when the compounds were added at 1 ng/L and 2 ng/L, respectively, and that the perception of vegetative aroma became evident at 8 ng/L or above. Quantitative analysis of wines that had also been scored for intensity of vegetative aroma showed that there was an excellent correlation between the perceived odour and the determined IBMP concentration (Allen et al., 1991)

Application of the analytical technique to Cabernet Sauvignon grape extracts confirmed that all three methoxypyrazines were present and that IBMP was usually above its odour threshold. A study of Cabernet Sauvignon grape extracts was the focus of Project RMI3GW. That project showed that IBMP occurred in all samples and that much lower levels of IPMP and SBMP occurred. The level of IBMP consistently decreased during fruit ripening, and the ripest fruit had about 5% of the level of the least ripe fruit. Significantly lower iBMP levels occurred in fruit of minimal pruned vines than in spur pruned vines.

The difference between minimal-pruned and spur-pruned vines led to a study of the possible effect of light on grape berry methoxypyrazine concentration. The IBMP concentration in the most shaded fruit (at 3-9% of incident light) had a higher iBMP concentration by a factor of 2.0 (spur-pruned fruit) or 3.1 (minimal pruned fruit) over the exposed fruit (at 51 - 60% of incident light). However, when the light intensity at the sampled fruit clusters was measured in the spur-pruned vines and in the minimally pruned vines, the light intensity alone was not sufficient to explain the lower IBMP concentration in the minimally pruned fruit compared to the spur-pruned fruit. Furthermore, the measured methoxypyrazine concentrations showed that the decrease of IBMP concentration with ripening in the shaded fruit was insufficient for the increased IBMP level in the more shaded fruit to be explained by a ripening delay.

There was a need to learn more about the mechanism of the decrease of methoxypyrazine concentration in ripening, the extent to which other viticultural factors may be important, and the desirable level of methoxypyrazines in Cabernet Sauvignon grapes and wines. Those directions were the aim of this project.

Objectives of the Research Project

The project objectives are:

1. To identify viticultural variables that require control for effective methoxypyrazine flavour analysis.

The methoxypyrazine level in the grape berry is clearly responsive to many factors. This project seeks to learn more fully what they are, so that those that most significantly affect methoxypyrazine concentration can be controlled in trials that seek to elucidate the role of other factors.

2. To identify viticultural management that will improve grape methoxypyrazine flavour characteristics.

The project seeks knowledge of what grape methoxypyrazine levels are desired, and how these might be achieved. In view of the earlier quantitative analysis being performed mainly with Sauvignon Blanc, it was anticipated that Cabernet Sauvignon would be the principal focus of the study.

3. To understand the effect of vine management on grape flavour in terms of grapevine physiology.

A longer-term objective is to understand methoxypyrazine fruit flavour development in terms of grapevine physiology, that is in terms of factors influencing the biochemical behaviour of the vine.

Introductory Technical Information

Flavour compounds are invariably low molecular weight, volatile compounds. So their analysis is most successfully achieved with capillary column gas chromatography. However, flavour compounds such as methoxypyrazines are important in sensory terms at vanishingly low (parts per trillion, or ng/L) concentrations. To distinguish the trace levels of these compounds from the many other more abundant compounds requires the analytical sensitivity and selectivity of a mass spectrometer as the chromatography detector. In addition, as it is virtually impossible to introduce an exact amount of a sample into the mass spectrometer ion source, quantitative analysis is best performed with an internal standard that is added to the sample before analysis.

Critical to analytical accuracy and precision is the choice of the internal standard. Towards all factors that influence the analyte's recovery (losses in sampling, extraction, evaporation and derivatisation; adsorption onto glass; decomposition during chromatography; adsorption to active sites on the column; and variation of mass spectrometer operating conditions) it must behave as identically as possible to the analyte. This requires that the internal standard must be at least of the same class of compound as the analyte. Better is if it is a close homologue of the analyte. Widely recognised as best is a stable isotope-labelled analogue of the analyte. Mass spectrometry is unique in that it can distinguish mass differences arising from stable isotope labelling, allowing use of the technique called isotope dilution-mass spectrometry (ID-MS), the technique that provides the closest approach yet to a definitive method.

Use of a suitable stable isotope-labelled analogue as internal standard ensures that there is no possibility of the labelled analogue occurring naturally in any of the samples. In addition,

quantitative recovery of the analyte during isolation is not required, and recovery studies are not necessary. Furthermore, the retention time of a stable isotope-labelled analogue is very close to that of the analyte, reducing the opportunity for variation of instrumental parameters such as ionisation yield. A drawback to the use of a stable isotope-labelled analogue is the cost, both financially and in time, of its synthesis. However, in this work the availability of previously synthesised trideuterated-IBMP and trideuterated-IPMP allowed the analytical rigour of isotope dilution-mass spectrometry to be easily used.

As the concentration of the analyte in the sample decreases, the problem of intrusion of other components into the signal of interest increases. Analytical sensitivity and selectivity can be improved by using selected ion monitoring (SIM) in which only one or a few ions of the analyte and internal standard are detected. Chemical ionisation (CI) also increases selectivity by concentrating the ion mass profile into molecular parent ions such as $(M+H)^+$ ions. Careful choice of reagent gas proton affinity also improves the selectivity of ionisation.

This work used isotope dilution-mass spectrometry (ID-MS) with trideuterated-IBMP and trideuterated-IPMP as internal standards for the quantitative analysis of IBMP, ISMP and IPMP. The work was performed with a VG Trio-2 GC-MS instrument.

At the commencement of this research programme, much had been elucidated about the role of IBMP, IPMP and SBMP in Sauvignon Blanc wines and grapes and in Cabernet Sauvignon grapes. However, little was known about their concentration in Cabernet Sauvignon red wines.

The occurrence of IBMP in wines of Cabernet Sauvignon had been indicated by determination of 20 ng/L of IBMP in Cabernet Sauvignon grape must immediately after alcoholic fermentation (Allen et al., 1990a, 1990b). However, that concentration, in the low ng/L range, contrasts with an earlier study of a single Bordeaux red wine, by less rigorous isolation and analytical techniques, which estimated the concentration of IBMP to be 500 (± 70) ng/L (Boison and Tomlinson, 1990). This research therefore included a program to study the methoxypyrazine concentration in both Australian and French red wines, including Bordeaux red wines.

Research Methodology

Methoxypyrazine analysis

Grape extract preparation

Berries were separated from grape cluster stems, cooled to 4^{o}C, passed through a juicer (National MJ65-N) and the resulting juice treated with sulfur dioxide (100 mg/L) and centrifuged (3000 rpm, 10 min.). The clarified juice was treated with further sulfur dioxide (100 mg/L) and samples (typically 240 mL) stabilised by addition of sodium chloride (10% w/v) and sodium fluoride (0.25% w/v) then stored at -20°C in the dark under carbon dioxide headspace.

Methoxypyrazine Isolation.

An internal standard mixture of trideuterated-IBMP (d_3-IBMP) and trideuterated-IPMP (d_3-IPMP) (2.065 ng/µL d_3-IBMP in dichloromethane, 12.0 µL) was added to wine or juice samples (typically 240 mL) by calibrated microlitre syringe (Hamilton, 25 µL). After allowing time for equilibration (1-2 h), the sample was adjusted to pH 6 (10% NaOH) then distilled at atmospheric pressure, collecting 50 mL of distillate. The distillate was stirred with ion-exchange resin (Bio-Rad, AG50WX4 AR, 200 mg, 1 h, in the dark), filtered (glass sinter), and the filtered resin washed with water (2 x 1 mL). A suspension of the washed resin in water (1 mL) was adjusted to approx. pH 10 by dropwise addition of 10% NaOH solution.

After five minutes, the supernatant solution was removed, the resin was washed with water (0.5 mL) and the aqueous solutions were combined. The resin was then washed with dichloromethane (0.5 mL) and the aqueous solution extracted consecutively with the dichloromethane wash then a further portion of dichloromethane (0.5 mL). If not immediately evaporated, the combined dichloromethane extracts were sealed into glass ampoules and stored at -20°C in the dark. The dichloromethane extracts (1 mL) were concentrated to ca. 20 μL by slow evaporation, in the dark at room temperature under nitrogen, in tapered Reacti-vials (Pierce, 1 mL, then 100 μL). Concentrates were transferred into glass capillaries that were sealed then stored in the dark at -20°C until mass spectrometric analysis.

Mass Spectrometry.

Methoxypyrazine isolates were analysed with a VG Trio-2 capillary GC-quadrupole MS system using a HP5890 Series II gas chromatograph. Chromatography was performed with fused silica capillary columns, either dimethylsilicone (J&W, DB-1, 60 m x 0.32 mm i.d., 1.0 μm film thickness), 6% phenyl and 6% cyanopropyl dimethylsilicone (J&W, DB-1701, 30 m x 0.32 mm i.d., 1.0 μm film thickness) or carbowax (J&W, DB-Wax, 30 m x 0.32 mm i.d., 0.5 μm film thickness). Columns were directly coupled to the ion source. Injections were made by a syringe with silica needle into a HP 19245C temperature-programmable cool on-column capillary inlet with electronic pressure control, at 30°C, using a deactivated fused silica retention gap (J&W, 3 m x 0.32 mm i.d.) to reconcentrate components at the beginning of the stationary phase. The oven was programmed to provide a 2 min delay at 30°C followed by a temperature rise of either (i) 25°C/min to 90°C then 5°C/min to 200°C (DB-1), or (ii) 25°C/min to 110°C, 5°C/min to 150°C, 25°C/min to 210°C, then held at 210°C for 5 minutes (DB-1701 and DB-Wax). Helium was used as carrier gas at a constant (69 kPa, DB-Wax and DB-1701) or programmed (69 kPa for 0.5 min, then 138 kPa/min to 138 kPa, DB-1) inlet pressure.

Mass spectra and selected ion monitoring were recorded in positive-ion mode at an electron energy of either 70 eV (EI) or 50 eV (CI), emission current of 200 μA (EI) or 700-800 μA (CI), and source temperature set to 180°C (EI) or 150°C (CI). Ammonia (anhydrous) was used as reagent gas at a source envelope pressure of 3×10^{-3} Pa. The ions monitored were *m/z* 167.1 and 170.1 (CI), or 124.1 and 127.1 (EI) with a dwell time of 0.10 s (DB-1) or 0.08 s (DB-1701, DB-Wax) and a span of 0.2 u. Scan spectra were recorded over the range 60 - 200 u, with scan time of 0.5 sec and interscan delay of 0.1 sec. Relative ion abundances were assessed from peak areas.

Standardisation.

Each day of analysis, the VG Trio-2 GC-MS system was mass calibrated in static (50-650 u) and dynamic (60-200 u) modes (EI, heptacosa) prior to methoxypyrazine determination. Source parameters were optimised in positive CI mode with *n*-amyl acetate (*m/z* 131: *m/z* 148 = 1:10) and the instrument response was determined with a standard solution (1 μL) of IBMP (200 pg/μL), IPMP (200 pg/μL) and SBMP (20 pg/μL). Quantitative analysis was performed only if the response with the standard solution was within 10% of the mean of previous satisfactory determinations. Solvent blank injections were made before and after standards to check system cleanliness and standard carryover.

Quantitative Analysis.

This was performed by GC-MS analysis in CI mode. Samples of methoxypyrazine concentrates (3 - 9 μL) were applied using, as necessary, multiple injections within a few minutes onto the cold column to allow the mass spectrometer vacuum system to cope with the injected solvent and to avoid the flooded zone exceeding the retention gap. Mole ratios ($^2H_0/^2H_3$) of components were calculated from the standard response determined that day, with correction for the trace of $^2H_0$ component in the $^2H_3$ internal standard that was measured during instrumental calibration.

Study of the effect of light and vine vigour on vegetative flavour in Californian Cabernet Sauvignon.

This work was pursued in conjunction with Prof. Ann Noble (Department of Viticulture and Enology, University of California, Davis) and Dr Deborah Ellliott-Fisk (Department of Land, Air and Water Resources, University of California, Davis). It combined the quantitative analysis of methoxypyrazines, at that time only achievable at Wagga Wagga, with the sensory descriptive analysis skills of Prof. Noble and the soil characterisation experience provided by Dr Elliott-Fisk. The study used a Cabernet Sauvignon vineyard in the Sonoma Valley. Soil analyses and soil horizon definitions were determined, and vine vigour was measured as winter pruning weights. Five sites of maximum soil diversity were selected. Light interception at the vine clusters was measured as photosynthetically active photon flux density, fruit was harvested, and the wine subjected to sensory descriptive analysis and methoxypyrazine quantitative analysis. Descriptive analysis was performed for the attributes 'rose aroma', 'chocolate aroma', 'berry aroma', 'berry flavour by mouth', 'astringency', 'vegetative aroma' and for 'vegetative flavour by mouth'. Partial least squares analysis was performed using soil variables, vine vigour, available light, grape and wine composition and methoxypyrazine concentrations.

Study of the effect of irrigation and nitrogen fertilisation on IBMP concentrations in Cabernet Sauvignon at Wagga Wagga

In November 1992, a postgraduate student, Mr Yuejun Jin, was appointed to pursue this part of the research as a program of study for a higher degree at CSU.

A vineyard trial was established in late 1992 at the University vineyard at Wagga Wagga to study, initially, the effect of irrigation, cropping level, and nitrogen availability on the methoxypyrazine concentration of Cabernet Sauvignon fruit during the 1993 ripening season. The trial used spur-pruned Cabernet Sauvignon vines (clone CS125) with adjustment of vine shoot number in November 1992 to provide uniform vine growing conditions amongst the selected vines.

Initially, selected vines in six rows were to be used in a vineyard block of 19 rows. However, unusual seasonal conditions provided high rainfall and high humidity in late 1992 and a relative lack of hot dry weather in late 1992/early 1993. This reduced the crop level, through downy mildew infection, and it markedly reduced the water stress available to the trial. Downy mildew particularly affected vines on the lower parts of the vineyard slope. For this reason, the trial was reduced to the less affected two upper rows (row 2, 8 selected vines; row 5, 8 selected vines) of those initially selected. Instead of a detailed study of crop yield and reduced deficit irrigation in 1993, the study examined broad effects, comparing vines with and without irrigation, and comparing vines with and without nitrogen fertiliser application, each being at two cropping levels..

Water availability in one row was monitored with tensiometers, with 4 vines irrigated and 4 vines not irrigated. Nitrogen was applied (January 1993) as ammonium nitrate at 70 g/vine (equivalent to 100 kg/ha) at 12-Jan-93 to 4 vines in the other row; the remaining 4 vines were not fertilised. Pre-dawn leaf water potential was measured with a pressure bomb by the standard procedure, for 3 leaves of irrigated vines and 3 leaves of non-irrigated vines, from 20th January. Cluster light exposure was determined as photosynthetically active photon flux density to select fruit of uniform exposure for flavour analysis. Measurement was made with a Li-Cor inc. model LI-185B with LI-1905B quantum sensor, providing values in $\mu E\ sec^{-1}\ m^{-2}$.

For each treatment (irrigated, non-irrigated, fertilised, non-fertilised) two of the four vines had the vine cluster number adjusted to 68 per vine and two were reduced to 34 per vine. Before cluster thinning, there were 64-79 clusters per vine.

Grape samples for methoxypyrazine analysis were taken as whole clusters at véraison (24-Feb-93) and at full maturity (2-Apr-93) with measurement of berry weight per cluster, berry number per cluster and berry volume. Leaf and stem samples were also taken for methoxypyrazine analysis at véraison.

Levels of IBMP in Cabernet Sauvignon and Sauvignon Blanc grapes with vines grown under cool conditions

Cabernet Sauvignon and Sauvignon Blanc whole clusters were collected in 1993 from vines at Brown Bros. Whitlands Vineyard, Victoria at véraison (22nd March) and shortly before normal harvesting (30th April). On each occasion, for each variety, 12 exposed and 12 shaded clusters were sampled with determination of the light level at each sampled cluster as photosynthetically active photon flux density (Li-Cor inc. model LI-185B with LI-1905B quantum sensor). Grapes from the 12 clusters were combined for methoxypyrazine determination.

Study of the feasibility of the use of a Griffith, NSW, Semillon trial of cultivation, mulch and sod treatment between rows

Marked differences of petiole and total nitrogen had been found to occur in a GWRDC trial of Semillon at the Horticultural Research & Advisory Station (HRAS), of NSW Agriculture, Griffith, that used under-vine flood irrigation and which compared (amongst other effects) cultivation, mulch and sod treatment between the vine rows. This study sought to identify the IBMP concentration in the fruit close to harvesting maturity to evaluate whether the levels were suitable for a more detailed study of the effect of nitrogen availability on IBMP concentration.

Each HRAS treatment used 4 rows of 14 vines. Of these, two rows were selected for IBMP analysis, each row providing one of the two sampling replicates. Each replicate included 5 clusters, one from each of 5 vines in the row, taking fruit from leaf layer 0-1, with light measurements at each sampled cluster (Li-Cor inc. model LI-185B with LI-1905B quantum sensor).

Study of the methoxypyrazine level in Australian, New Zealand and French red wines

With one exception, the Australian and New Zealand wines analysed were commercial wines and the grape variety Cabernet Sauvignon contributed not less than 80% of the grape origin of each wine. One additional wine, selected for its particularly intense herbaceous/vegetative aroma, was a 100% Cabernet Sauvignon wine from young vines in a cool, maritime region of Australia (Mornington Peninsula). The wines were 2-4 years old at the time of analysis. The eight French wines were selected from the teaching wine cellar, Charles Sturt University. Prior to analysis, wines were either stored in unopened bottles at 10-14°C or a quantity (240 mL) transferred to a glass bottle with teflon-lined cap (250 mL, Schott) and stored in the dark at -20°C under carbon dioxide headspace.

Detailed Results

Study of the effect of light and vine vigour on vegetative flavour in Californian Cabernet Sauvignon.

The principal methoxypyrazine, IBMP, was found in the wines of all five sites at a level ranging from 2.8 to 37 ng/L, which is above its odour detection threshold. There was no detectable IPMP, and SBMP was below its odour detection threshold. The relationship of the IBMP level to wine vegetative aroma and to vine vigour and light level in the fruiting zone is given below (Table 1). The highest vigour was provided by site 12UP (a very old., leached soil with low pH (3.9) and the highest overall water holding capacity). Intermediate in vigour

were sites 25BUP, (high in pebbles), and site 25BLSW (a bouldery soil). Low in vigour were sites 12LO (shallow, well-drained, bouldery) and 25BLNE (sandy clay loam with good drainage).

Table 1. Concentration of IBMP, vegetative aroma by descriptive analysis, vine vigour and light exposure at five sites in the Sonoma vineyard.

Site	12UP	12LO	25BUP	25BLNE	25BLSW
IBMP concentration (ng/L)	37	8.9	6.5	2.8	8.5
Vegetative aroma	5.8	1.6	1.8	1.1	1.7
Vine vigour (Pruning Wt, kg/vine)	3.3	1.0	2.3	0.95	1.6
Light fraction in fruiting zone	0.17	0.28	0.57	0.55	0.36

Study of the effect of irrigation and nitrogen fertilisation on IBMP concentrations in Cabernet Sauvignon at Wagga Wagga

Although all three methoxypyrazines IBMP, IPMP and SBMP were determined in the grape extracts of this trial, data is presented only for IBMP as the other methoxypyrazine concentrations were invariably below their odour detection threshold (1 ng/L).

Table 2. Concentration of IBMP (ng/L) in Cabernet Sauvignon grapes at Wagga Wagga. Bold font indicates values above the odour detection threshold

Sampling	Crop yield	Irrigated	Non-irrigated	Fertilised	Non-fertilised
Véraison	High	**29.6**	**190.3***	**17.8**	**21.6**
	Low	**23.6**	**12.2**	**58.4**	**27.2**
Normal maturity	High	**2.5**	**3.9**	1.1	1.3
	Low	**2.5**	**2.2**	1.6	1.1

* Mean of two separate determinations (171.7 and 208.9 ng/L) of IBMP concentration in the grape extract.

The value of 190.3 ng/L for the concentration of IBMP in grapes of non-irrigated vines sampled at véraison appears to be an anomaly. It is the mean of two similarly high GC-MS analyses of the methoxypyrazine isolate. Unfortunately, the time delay of sample processing and analysis prevented re-sampling of the relevant vines. The most likely reason for such an anomalous result is an error in the addition of the internal standard to the grape extract. The addition requires a microsyringe to accurately measure out and add 12.0 μL of a dichloromethane solution of the trideuterated IBMP and IPMP to the sample. Dichloromethane is a low boiling point, highly volatile solvent and inadvertent volatilisation of some of the solution from the barrel of the syringe, during syringe and vial handling, will decrease the quantity of internal standard added and increase the determined IBMP concentration.

Levels of IBMP in Cabernet Sauvignon and Sauvignon Blanc grapes with vines grown under cool conditions

In several of these grape extracts, the two minor methoxypyrazines IPMP and SBMP were well above their odour detection threshold. Accordingly, data for all three methoxypyrazines are indicated below.

Table 3. Concentration of IBMP, IPMP and SBMP (ng/L) in Cabernet Sauvignon grapes at Whitlands, Victoria. Bold font indicates values above the odour detection threshold

	At 22-March-1993			At 30-April-1993		
	Exposed (E)	Shaded (S)	Ratio S/E	Exposed (E)	Shaded (S)	Ratio S/E
IBMP	**95.2**	**152**	1.60	**48.7**	**62.9**	1.29
IPMP	**5.1**	**10.1**	1.98	1.2	1.8	1.50
SBMP	1.4	1.5	1.07	1.3	2.3	1.77
Light exposure[1]	31.4%	0.53%		24.8%	5.5%	

[1] Mean of twelve measurements, one at each sampled cluster, expressed as a percentage of full light exposure

Table 4. Concentration of IBMP, IPMP and SBMP in Sauvignon Blanc grapes at Whitlands, Victoria. Bold font indicates values above the odour detection threshold

	At 22-March-1993			At 30-April-1993		
	Exposed (E)	Shaded (S)	Ratio S/E	Exposed (E)	Shaded (S)	Ratio S/E
IBMP	**69.6**	**109.2**	1.57	**40.7**	**59.6**	1.46
IPMP	**12.2**	**24.3**	1.99	**8.5**	**14.1**	1.66
SBMP	**14.8**	**19.0**	1.28	**3.6**	**3.1**	0.86
Light exposure[1]	28.0%	0.53%		16.6%	6.5%	

[1] Mean of twelve measurements, one at each sampled cluster, expressed as a percentage of full light exposure

Study of the feasibility of the use of a Griffith, NSW, Semillon trial of cultivation, mulch and sod treatment between rows

In all cases, the concentrations of IBMP, IPMP and SBMP were below their odour detection threshold and too low for meaningful comparison of methoxypyrazine level and nitrogen availability to be made.

Table 5. Concentration of IBMP, IPMP and SBMP in Semillon grapes, Griffith.[1]

	Cultivation		Mulch		Sod	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
IBMP	0.2	0.4	0.5	0.4	0.1	0.6
IPMP	0.2	0.2	0.1	ND	ND	ND
SBMP	0.3	0.1	0.3	ND	0.2	0.3

[1] ND = Not detectable

Study of the methoxypyrazine level in Australian, New Zealand and French red wines

Eight French wines were analysed. They were from the Médoc region of Bordeaux, in which Cabernet Sauvignon is important and often dominant; the St.Émilion and Pomerol region of Bordeaux, where Merlot, Cabernet Franc and, to a lesser extent, Cabernet Sauvignon are important; and Burgundy, where Pinot noir is the red grape variety. Reported Bordeaux vineyard compositions (Parker, 1991) are indicated in footnotes to Table 6.

Table 6. Concentration of IBMP and IPMP in French red wines [1,2]

Wine	Region	Methoxypyrazine (ng/L)		
		IBMP	IPMP	SPMP
1982 Léoville-Las Cases[3]	St. Julien	14.9	< 0.4	0.23
1983 Léoville-Las Cases[3]	St. Julien	10.6	< 0.4	0.18
1983 Cantemerle[4]	Haut Médoc	9.3	10.1	1.9
1982 Conseillante[5]	Pomerol	9.8	< 0.1	0.06
1982 Canon[9]	St.Émilion	7.1	< 0.4	0.05
1982 Magdelaine[7]	St.Émilion	7.1	< 0.5	ND
1983 Gevrey Chambertin[8]	Burgundy	< 0.7 [9]	ND	0.26
1983 Morey St. Denis[8]	Burgundy	< 0.4 [9]	ND	0.12

[1] ND = Not detectable. [2] superscripts 3 - 8 indicate vineyard compositions as reported by Parker (1991). [3] 67% Cabernet Sauvignon, 17% Merlot, 13% Cabernet Franc, and 3% Petit Verdot. [4] 40% Cabernet Sauvignon, 40% Merlot, 18% Cabernet Franc, and 2% Petit Verdot. [5] 45% Merlot, 45% Cabernet Franc, and 10% Malbec. [6] 55% Merlot, 45% Cabernet Franc. [7] 90% Merlot, 10% Cabernet Franc. [8] 100% Pinot noir. [9] upper limit due to coelution

Nine Australian and three New Zealand wines were analysed (Table 7). They were from a range of regions to examine the effect of climate. The latter is indicated by the mean January temperature (MJT). The Mornington Peninsula wine was included as a 100% Cabernet Sauvignon wine with a particularly intense herbaceous/vegetative aroma.

Table 7. Concentration of IBMP in Australian and New Zealand Cabernet Sauvignon-based wines and the mean January temperature (MJT) of the growing region

Growing region	IBMP (ng/L)	MJT (°C)
Hunter Valley, NSW	3.6	22.7
Griffith, NSW	6.2	23.6
Leeton, NSW	7.6	23.6
Seymour, Vic.	9.1	21.2
McLaren Vale, SA	11.2	19.8
Frankland, WA	12.3	20.0
Mudgee, NSW	17.1	22.7
Yarra Valley, Vic.	26.1	18.0
Hawkes Bay, NZ	27.6	18.8
Auckland, NZ	27.6	17.9
Hawkes Bay, NZ	28.6	18.8
Mornington Peninsula, Vic	56.3	18.0

Discussion of Results

Study of the effect of light and vine vigour on vegetative flavour in Californian Cabernet Sauvignon.

First, the determined IBMP concentration in the wine of each of the five sites (Table 1) showed very good correlation with the 'vegetative aroma' found by descriptive analysis ($r = 0.90$, $p < 0.05$).

The highest vigour site, 12UP, provided the wine with the highest IBMP concentration of the group (37 ng/L). This concentration is expected to be intensely, probably overly, vegetative.

Descriptive analysis indicated that this wine had the highest intensity ratings for 'vegetative aroma' and for 'vegetative flavour by mouth' but low intensity ratings for 'rose aroma', 'chocolate aroma', 'berry aroma', and 'berry aroma by mouth'. The site also provided the least light exposure in the fruiting zone.

In contrast, the wine from the lowest vigour site, 25BLNE, had the lowest concentration of IBMP (2.8 ng/L), a value only marginally above its odour detection threshold. Descriptive analysis indicated high intensity ratings for 'chocolate aroma', 'berry aroma', and 'berry aroma by mouth', the desirable descriptors of fully ripened fruit, and the lowest intensity ratings for 'vegetative aroma' and for 'vegetative flavour by mouth'. This site also had a high level of exposure in the fruiting zone.

The remaining three sites provided wine with similar IBMP levels (site 12LO, 8.9 ng/L; site 25BUP, 6.5 ng/L; site 25BLSW, 8.5 ng/L). These three sites showed a range of vine vigour (from 1.0 to 2.3 kg/vine pruning weight) and a range of light exposure in the fruiting zone (from 0.28 to 0.57 of full exposure), and it appears that increasing vine vigour offset increasing light exposure. This suggests that both vigour and light exposure are significant factors affecting methoxypyrazine levels. Importantly, the data suggest that vine vigour does not lead to increased IBMP levels simply through increased shading within the fruiting zone.

Study of the effect of irrigation and nitrogen fertilisation on IBMP concentrations in Cabernet Sauvignon at Wagga Wagga

Disregarding the anomalously high IBMP concentration found with grapes of the non-irrigated, high crop load vines at véraison, there are no clear trends apart from the decrease of berry IBMP concentration with grape maturity, which is already well established. In part, this lack of trend may arise from the unusually wet conditions which made the irrigation effect minor. During much of the fruiting and ripening period the tensiometer readings indicated only minor differences between the irrigated and non-irrigated treatments. The lack of an evident fertilisation, crop yield, or irrigation effect suggests that the vines either found none of these treatments limiting or berry methoxypyrazine concentrations are unaffected by these treatments.

Continuation of the trial into 1994, with tighter control of experimental treatments, was in progress. However the PhD student withdrew from study for the degree at the commencement of February 1994, at a crucial stage of the trial. Continuation of the trial was not immediately possible.

Levels of IBMP in Cabernet Sauvignon and Sauvignon Blanc grapes with vines grown under cool conditions

At either sampling date, the Whitlands Cabernet Sauvignon fruit extracts had a higher concentration of IBMP than the Whitlands Sauvignon Blanc extracts (Table 3 and Table 4). This was not the case for IPMP nor SBMP which, although usually at or below their odour detection threshold in warm viticultural areas, were well above the odour detection threshold with Sauvignon Blanc. Moreover, the IPMP/IBMP concentration ratio and the SBMP/IBMP concentration ratio for the Whitlands Sauvignon Blanc fruit was much higher than has been found in Sauvignon Blanc fruit from warmer areas. Thus, although the cool conditions increased the IBMP concentration, the conditions led to a proportionately greater increase of concentration of IPMP and SBMP than of IBMP. The slightly different odour of IPMP and SBMP, compared to IBMP, would be expected to provide wine from this cool area Sauvignon Blanc with a different nuance of flavour (more musty-earthy, less herbaceous-fragrant) to that of the more IBMP-dominant fruit of warmer areas.

With both varieties, the extracts of Whitlands fruit, when sampled close to normal harvesting, contained a much higher concentration of the three methoxypyrazines than fruit of these varieties in warmer areas. This was expected. However, the extracts of the fruit sampled close to véraison contained little more IBMP than véraison fruit from warmer areas. Thus,

cool growing conditions do not appear to increase the IBMP level throughout fruit development but rather diminish the rate of decrease of IBMP from véraison onwards.

There has been speculation, by some researchers, that the decrease of IBMP level during ripening is an outcome of methoxypyrazine instability to light (photodegradation), a process known to occur with methoxypyrazines after isolation from grape tissue. The data of Table 3 and Table 4 appear not to support that speculation. The Whitlands fruit shows a lower rate of loss of IBMP during ripening than is found in warmer areas. However, the Whitlands fruit is unlikely to be subjected to lower light exposure than fruit in warmer areas. Thus, it would seem that the lower temperature in the high elevation of the Whitlands vineyard is more likely to be the factor of importance in influencing the rate of loss of IBMP.

This hypothesis may also be supported by the lesser difference between the IBMP level of the exposed and shaded fruit at April 30th, in this Whitlands fruit, than in the same fruit at March 22nd, and by the smaller ratio of the IBMP concentration in the shaded fruit relative to the unshaded fruit (Ratio S/E, in Tables 3 and 4). Clearly, as ripening progressed, the difference between the IBMP levels of the shaded and unshaded fruit decreased, a situation that is not expected if the decrease of IBMP with ripening is driven by light exposure. Some caution is necessary with this interpretation as the canopy appears to have become more open during the six week interval between the sampling, leading to a decrease with time of the exposed/shaded light level ratio from 59 to 4.5 (Cabernet Sauvignon) or from 53 to 2.6 (Sauvignon Blanc). Consequently, further work would be needed, with sampling at a wide range of light levels, for rigorous conclusions to be drawn.

Study of the feasibility of the use of a Griffith, NSW, Semillon trial of cultivation, mulch and sod treatment between rows

Methoxypyrazine analysis indicated that the concentrations of IBMP, IPMP and SBMP were all below their odour detection threshold and too low for meaningful comparison of methoxypyrazine level and nitrogen availability to be made. The findings confirmed the propensity for the IBMP level to fall below its odour detection threshold even prior to normal harvesting maturity in very hot regions such as the MIA.

Study of the methoxypyrazine level in Australian, New Zealand and French red wines

In the two Pinot noir wines of Burgundy (Table 6, wines 7 and 8), the concentration of IBMP could be given only as an upper limit, due to co-elution, however this upper limit was below its odour detection threshold. The relative absence of IBMP in these two wines and its occurrence in all other wines agrees with a dependence of the occurrence of IBMP on grape cultivar.

The concentration of IBMP in the remaining 18 Cabernet Sauvignon containing wines (3.6-56.3 ng/L; Table 6, wines 1-6; Table 7, all wines) is similar to that previously determined in French, Australian and New Zealand Sauvignon Blanc wines (Lacey et al., 1991).

It is recognised that Cabernet Sauvignon wines from warm areas usually have lower vegetative/herbaceous aroma than wines of this grape variety from cool areas. If IBMP is responsible for this aroma, a corresponding variation of its concentration would be expected. Table 7 relates its determined concentration in the Australian and New Zealand wines to the mean January temperature (MJT) of the growing region, an indicator of average temperature conditions in the southern hemisphere growing season (Smart and Dry, 1980; Dry and Smart, 1984). Although other factors are also likely to be important, such a relationship is evident (linear regression: $R = 0.754$, $p < 0.005$) confirming behaviour similar to that found previously with Sauvignon Blanc grapes and wines

It seems apparent that the previous report of the concentration of IBMP to be 500 (± 70) ng/L in a Bordeaux red wine, by less rigorous isolation and analytical techniques (Boison and

Tomlinson, 1990), is incorrect. Through absence of a suitable internal standard, those researchers appear to have considerably overestimated the level of IBMP, particularly as the wine with the highest level in this study (Table 7, final wine) was far more intensely vegetative and herbaceous than is normally found in Bordeaux red wines.

In all wines, except the two Pinot noir wines (Table I, wines 7 and 8) which contained very low levels of both IBMP and IPMP, and one Bordeaux wine (Table I, wine 3), the abundance of IPMP was typically only 2% of that of IBMP and its concentration was less than 1 ng/L. As the sensory detection threshold for IPMP (1 ng/L) is close to that for IBMP (2 ng/L) these low concentrations of IPMP suggest that the component will not contribute significantly to the sensory character of these wines.

However, the concentration of IPMP in one wine (Table 6, wine 3) slightly exceeded that of IBMP. The following evidence rigorously confirmed the assignment of the former component as IPMP. First, the signal for the component displayed a symmetrical peak shape and the expected peak width (established by the internal standard) across two GC stationary phases of widely differing polarity (DB-1 and DB-1701) and across both EI and CI modes of ionization. This diminishes the likelihood of the signal intensity being augmented by co-elution with another compound. Secondly, the retention time of the component was identical (± 0.01 min) to that of synthetic IPMP on each of the contrasting stationary phases with, in each case, selected ion monitoring in both CI and EI modes to capitalize on the different mass channels of the base peaks (CI, *m/z* = 153.1; EI, *m/z* = 137.1). Third, the difference between the elution time of the component and that of the internal standard **3** was identical (± 0.01 min) to that determined with synthetic IPMP. Fourth, a relative lack of coeluting impurities was demonstrated by the consistency of the determined concentration across the four combinations of stationary phase and ionization mode (DB-1/CI, 9.7 ng/L; DB-1/EI, 9.7 ng/L; DB-1701/CI, 10.6 ng/L; DB-1701/EI, 10.5 ng/L). Finally, the EI mass spectrum (*m/z* 60-200) of the component, although weak, displayed the characteristic fragmentation pattern of isopropyl methoxypyrazine [wine component, DB-1 column, *m/z* (relative intensity):152 (37), 137 (100), 124 (29); synthetic IPMP, *m/z* (relative intensity):152 (30), 137 (100), 124 (25)].

Previous attempts to identify IPMP in grapes and wines have been hampered *inter alia* by its very low concentration (Augustyn et al., 1982; Harris et al., 1987; Lacey et al., 1991), by lack of an internal standard and poor reproducibility of retention times (Augustyn et al., 1982), by use of only a single GC stationary phase (Augustyn et al., 1982; Harris et al., 1987; Lacey et al., 1991), and by use of only one ionization mode (Augustyn et al., 1982; Lacey et al., 1991). Thus, the data of the present study significantly strengthen the evidence for the occurrence of IPMP in wine.

As both IPMP and IBMP have a reported sensory detection threshold of ca. 2 ng/L in water and white wine, the influence of IPMP on the aroma of wine 3 of Table 6 may be comparable or even exceed that of IBMP.

Two further bottles of the same wine were analysed. Their IPMP concentration was well below the IBMP concentration and below the odour detection threshold IPMP (ca. 1-2 ng/L in water or white wine. This suggests that the unusually high level of IPMP in one bottle of this wine may arise from bottle-specific microbial contamination, perhaps associated with the cork. A high level of IPMP can sometimes be generated by some microorganisms (Leete et al., 1992; Gallois et al., 1988). Further work is needed to investigate whether IPMP can arise in wine in this way.

Implications and Recommendations

1. The perception of vegetative/herbaceous flavour in Cabernet Sauvignon wines arises from the occurrence of IBMP. Quantitative determination of IBMP can be used to measure this flavour contribution and to study factors that influence it (where the additional cost and

difficulty of analysis is justified by the ability to use the accuracy and precision that it provides).

2. The abundance of IPMP and SBMP in Cabernet Sauvignon wines is usually very small and less than in Sauvignon Blanc wines. In routine studies of vegetative flavour, particularly in Cabernet Sauvignon grapes or wines, measurement of IPMP or SBMP is not routinely required.
3. An unusually high level of IPMP may occur as an occasional contaminant in wine leading to bottle variation, possibly from the cork. If this is the case, then it may also occur in wines of other grape varieties that do not produce methoxypyrazines. It will generate a musty/vegetative odour in the wine, different to odours of chloroanisole origin, and there is anecdotal evidence of the occasional occurrence of this problem in the industry.
4. Further research is needed to identify the processes behind the decrease in IBMP concentration in Cabernet Sauvignon and Sauvignon Blanc grapes during ripening. The evidence to date does not fully support the hypothesis that photodegradation is responsible.
5. Within Australia and New Zealand, there is good correlation between the IBMP concentration in Cabernet Sauvignon wines and the climate of the region in which the grapes are grown, as indicated by MJT. This suggests that climate is of paramount importance in determining IBMP berry concentrations.

Technical Summary: Outcomes of the Research

The cooperative study with the University of California, Davis, showed that the concentration of IBMP in Cabernet Sauvignon wine correlated well with the perception of vegetative flavour in those wines ($r = 0.90$, $p < 0.05$). This is consistent with IBMP being above its odour detection threshold in such wines and with it being the source of that flavour. In the Cabernet Sauvignon fruit analysed in that trial and the other trials, IPMP and SBMP are usually very minor components, even more minor than in Sauvignon Blanc fruit. However, the occurrence of IPMP at a level above IBMP in one bottle of one wine identified that, in some circumstances, the impact of IPMP may exceed that of IBMP, perhaps from cork contamination.

The data from the cooperative study with the University of California also suggest that both decreased light exposure of the fruit and increased vigour of the vine increase the IBMP concentration, and that the effect of vine vigour is not simply due to a leaf shading effect. In the viticultural trial at Wagga Wagga, however, no significant effect on the methoxypyrazine concentration could be attributed to reduced crop load, to absence of irrigation, or to nitrogen fertilisation.

The decrease of berry IBMP concentration during ripening and with increased fruit light exposure has been attributed sometimes to a photodegradation of methoxypyrazines in the berry, analogous to that found for isolated methoxypyrazines in solution (Heymann et al., 1986). However, Cabernet Sauvignon and Sauvignon fruit in a cold, elevated Victorian vineyard showed a much reduced decline in IBMP, concentration compared to fruit in warm vineyards, even though both show very similar berry concentrations at véraison. This suggests that the decline in IBMP concentration may principally be a result of berry metabolism, and the influence of light and temperature on it, rather than photodegradation. In line with this is that the decline in IBMP concentration for Cabernet Sauvignon in the cold vineyard location was greater than that for Sauvignon Blanc fruit, despite the light absorption capacity of the anthocyanin skin pigments in Cabernet Sauvignon after véraison.

Analysis of a range of Australian and New Zealand wines showed that the IBMP concentration correlated well with the mean January temperature (MJT) of the region of

origin ($r = 0.75$, $p < 0.005$). The wines are from the fruit of vineyards with widely differing soils and viticultural practices that will influence crop yield, vine vigour and fruit exposure. Moreover, the climate in the year of production may not be well reflected in the mean temperature. However, the agreement between IBMP concentration and MJT is remarkably good, confirming that, after grape cultivar selection, the most important factor for methoxypyrazine production is the growing temperature. While exposure has been found to influence IBMP concentration, the maximum influence identified so far is a factor of about 3. This contrasts with the almost 10 to 20-fold variation between the warm and cold regions in this study.

In the analysed Cabernet Sauvignon wines of Bordeaux, the concentration of IBMP was 7.1 – 14.9 ng/L. This concentration is less than was found in the Australian wine at comparable heat summation. This may be an outcome of the varietal composition of Bordeaux red wines, particularly in St.Émilion and Pomerol where Merlot is usually the dominant variety. It may also be that the soil and/or viticultural practices in Bordeaux reduce the IBMP concentration below that expected for a climate in which the ripening of Cabernet Sauvignon can sometimes be marginal.

In one bottle of a Bordeaux wine, an unusually high level of IPMP was identified. This level has allowed the occurrence of IPMP in wine to be rigorously verified for the first time, including analysis by electron ionisation mass spectrometry in full scan mode.

Parts of this work have been reported in refereed papers (Allen et al., 1994; Allen et al., 1995), in a book chapter (Allen and Lacey, 1997), in conference proceedings (Allen, 1996; Allen et al., 1997; Noble et al., 1995) and in technical papers (Allen, 1995a,b). The refereed papers and book chapter are attached to the printed version of this report.

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