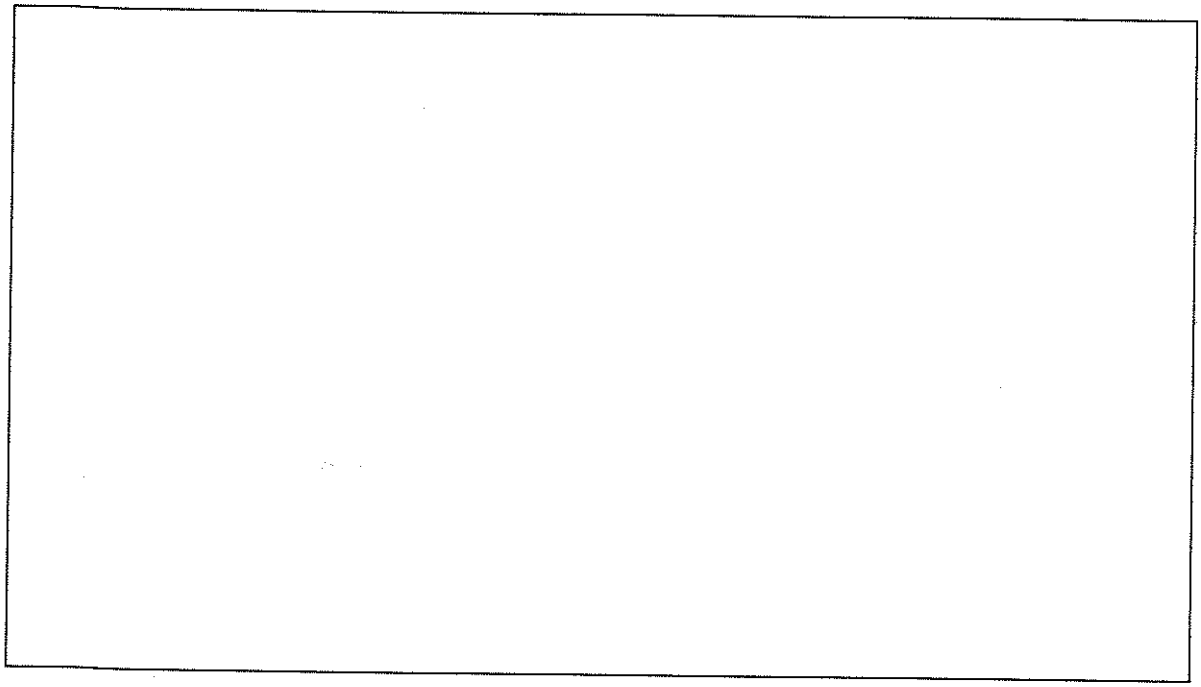
CHARLES STURT
UNIVERSITY

Grape and Wine
Research and
Development Corporation

Influence of viticultural practices on Cabernet Sauvignon varietal aroma



FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **RM1 3GW**

Principal Investigator: **Dr M Allen**

Research Organisation: **Charles Sturt University**

Project Title:	Influence of viticultural practices on Cabernet Sauvignon varietal aroma
Project Number:	RMI 3GW
Organisation:	Charles Sturt University
Location:	Wagga Wagga
Supervisor:	Associate Professor M Allen
Time Span:	1989-1990

EXECUTIVE SUMMARY

Objectives

- Identify the individual methoxypyrazine components of Cabernet Sauvignon fruit and compare their concentration with those already found for Sauvignon blanc fruit in the same vineyard.
- Examine the influence of ripening on the methoxypyrazine level of Cabernet Sauvignon fruit.
- Investigate the extent to which fruit exposure and pruning system influence the level of methoxypyrazine grape flavour components during ripening.
- Ascertain whether any variation that arises from fruit exposure or pruning system is best interpreted in terms of the effect of these aspects on fruit ripeness.

Methodology and Achievements

At the commencement of this work, a technique had been developed, in conjunction with CSIRO Division of Entomology, Canberra, to quantify methoxypyrazines rigorously at the low part per trillion (ng/L) level in grapes and wines using stable isotope dilution analysis by mass spectrometry. In this technique, synthetically prepared deuterium-labelled methoxypyrazines are added as internal standards prior to the analytical work-up. Using a magnetic sector-based gas chromatography–mass spectrometer (GC-MS) at CSIRO, the 'herbaceous/vegetative' flavour role of isobutyl methoxypyrazine (iBMP) in Sauvignon blanc wines had been confirmed, and effects of climate and ripening examined. This project extended that analysis to the grape variety Cabernet Sauvignon and investigated the extent to which fruit exposure and the pruning system might influence the methoxypyrazine level.

Grapes from spur-pruned and minimal pruned Cabernet Sauvignon vines of the same block at the CSU vineyard, Wagga Wagga, were sampled twice during ripening, soon after veraison (Jan 25th) and again closer to normal maturity (February 25/26th). In each case samples were taken at four leaf layers within the vine canopy (leaf layers 0, 1, 2 and '3 or more') with measurement of the light level at each removed cluster with a photosynthetic flux meter. For each pruning system, additional samples were taken of the most shaded layer at two one-week intervals after the main sampling to examine the possible influence of later ripening arising from shading.

Plant material was processed immediately and stabilised with NaCl/NaF addition. Further isolation work to isolate methoxypyrazine concentrates, previously done at CSIRO, was delayed by changes of research priorities arising from a more stringent funding environment. To overcome this, further isolation work began at CSU in 1991 and the potential of using a newly installed quadrupole-type GC-MS instrument at CSU for quantitative analysis was studied. Very adequate sensitivity was gained with positive-ion chemical ionisation and on-column, large-volume GC injection. Instrument calibration took place early in 1992, using two synthetic stable isotope-labelled methoxypyrazines, iBMP and isopropylmethoxypyrazine (iPMP).

Methoxypyrazine analysis confirmed that iBMP occurred in all samples and that it was the major methoxypyrazine. In two samples, a component believed to be ethylmethoxypyrazine (EMP) dominated. However, as the latter has a much higher sensory detection threshold than iBMP, its occurrence has a much lower sensory impact, and it was not investigated further at that time.

Levels of iPMP and, *sec*-butylmethoxypyrazine (sBMP), an isomer of iBMP, were comparable. The levels were always less than 3.1 ng/L, and they were typically less than 0.2 ng/L in the riper fruit. This behaviour is similar to that found with Sauvignon blanc grapes, although it appears that the iPMP

level in Sauvignon blanc fruit may exceed that of Cabernet Sauvignon grapes under otherwise similar conditions. The levels found here indicate that neither iPMP nor sBMP will contribute to methoxypyrazine aroma in wine from this fruit, suggesting that Cabernet Sauvignon fruit and wine is even more dominated by iBMP than is wine from Sauvignon blanc fruit.

The effect of fruit ripening was measured over six sampling times (from 10.4 to 21.6 °Brix), during which the level of iBMP consistently decreased. The most ripe fruit had about 5% of the level in the least ripe fruit, regardless of the pruning system. This again is a situation comparable to that seen for Sauvignon blanc grapes. At all six sampling dates, and at all four light exposure levels, significantly lower iBMP levels occurred in fruit of minimal pruned vines (3.1 – 111 ng/L) than in spur pruned vines (10.9 – 189 ng/L).

The effect of light exposure on iBMP concentration was consistent across both sampling times and both pruning systems. The most shaded fruit (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 outermost leaf layer (51 - 60% of incident light). At the same leaf layer, the measured light intensity at the sampled fruit clusters was not significantly different between the spur-pruned vines and the minimally pruned vines. So differences of iBMP concentration in the spur-pruned and the minimally pruned fruit cannot be interpreted simply in terms of differences of canopy light distribution.

Although the more shaded fruit was generally less ripe, the relationship was not always true. With the spur-pruned vines, fruit at leaf layer 1 (19% of incident light) was more ripe than fruit of leaf layer 0 (58% of incident light), yet fruit at leaf layer 1 showed a higher iBMP concentration. Sampling of the most shaded layer, at two weekly intervals after the two principal sampling dates, showed that the fall of iBMP level that accompanied the increasing ripeness was insufficient for ripening delay to fully explain the increased iBMP level in the more shaded fruit.

Outcomes

The methoxypyrazine content of Cabernet Sauvignon fruit was dominated by iBMP, which was above its sensory detection threshold in all samples. Both iPMP and sBMP were very minor contributors with no sensory significance. Three factors, ripening, fruit exposure and pruning system, influenced the iBMP concentration. Over the range of parameters encountered in this work, these three factors appear to act independently. The most significant factor was grape ripening; it contributed to a twenty-fold fall in iBMP level. Less important, but still significant, was the light exposure of the fruit and the pruning system of the vine which, for the range of variation encountered in this work, contributed a 2-3 fold variation to the iBMP level.

Outputs

Allen, MS (1996). Methoxypyrazines of grapes and wines: differences of origin and behaviour. In Proceedings of the Ninth Australian Wine Industry Technical Conference, Adelaide, 16-19 July 1995, CS Stockley, AN Sas, RS Johnstone and TH Lee. (Eds) Winetitles, Adelaide: 83-86.

Allen, MS and Lacey, MJ (1993). Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. Viticultural and Enological Sciences 48: 211-213.

Allen, MS and Lacey, MJ (1997). Stable isotope dilution gas chromatography-MS for determination of methoxypyrazines ("green" aroma) in wine. In: Modern methods of plant analysis, Vol.19. Plant Volatile Analysis. H-F. Linskens and JF. Jackson (Eds), Springer-Verlag, Heidelberg: 193-210.

Allen, MS and Lacey, MJ (1998). Methoxypyrazines of Grapes and Wines. In Chemistry of Wine Flavor, AL Waterhouse and SE Ebeler (Eds), ACS Symposium Series 714, American Chemical Society, Washington, D.C.: 31-38.

Allen, MS, Lacey, MJ, and Boyd, SJ (1997). Methoxypyrazines: new insights into their biosynthesis and occurrence. In Proceedings of the Fourth Cool Climate Viticulture and Enology Symposium, 16-20 July 1996, Rochester, NY, USA, CD-ROM paper 66, Vitis Information Resources, http://vitis-ir.com. Printed proceedings: Paper V-36, Communication Services, New York State Agricultural Experimental Station, Geneva, New York.

Allen, MS, Lacey, MJ, Brown, WV, Harris, RLN and Tassie, E. (1990). Influence of pruning methods on methoxypyrazine flavour components of Cabernet Sauvignon grapes. Proceedings, Seventh Australian Wine Industry Technical Conference, Adelaide, 13-17 August 1989, Winetitles, Adelaide: 247.