CAPPELLO & NOËL LLP
TRIAL LAWYERS

Lawrence J. Conlan

June 18, 2021

Via Email

Chair Nelson and Members of the Board of Supervisors
c/o David Villalobos
Planning and Development Department
123 East Anapamu Street
Santa Barbara, CA 93101-2058
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Re: Appeal of SFS Farms 20APL-00000-00024 and 19LUP-00000-00312 by Melville Winery

Dear Honorable Members of the Board of Supervisors:

We are the attorneys for SFS Farms, which the Director approved for a Land Use Permit for outdoor cannabis cultivation on September 10, 2020. Melville Winery, which is located across Highway 246, and significantly to the northeast of the project, appealed the permit. The Planning Commission previously denied the appeal 4-1. Melville subsequently appealed to the Board. The appeal should again be denied, and the project should be approved. As set forth herein, as well as in the Staff recommendation to approve, the project fully complies with all legal requirements of the County's cannabis ordinance.

A. SFS Farms Proposes Cultivation in an Interior Portion of a 970 Acre Existing Farm and Ranch Property

The Director approved SFS Farms for a Land Use Permit for outdoor cannabis cultivation of approximately 82.62 acres and 4.18 acres of nursery operations. The grow area is set deep within a 970-acre property owned by the Campbell family, which has utilized it for farming and ranching for generations. It is distant from Highway 246, virtually invisible to anyone not traveling to the interior portion of the property, and more than 2 miles north of the Santa Ynez River. All nursery and cannabis cultivation will occur outdoors and there will be

no hoop structures used. The property is Ag II, not in or adjacent to any EDRN, and while no odor abatement plan is needed, the risk of odor is adequately mitigated for several reasons. There will be no on-site processing, and only two harvests per year contemplated, each of which will last no more than 3 weeks. In addition, the grow is remote and literally surrounded by existing agriculture and woodlands.

SFS Farms offers significant community benefits because the revenues it generates will help subsidize the legacy agricultural and ranching operations on the Campbell property, thereby preserving the rural nature of this area of the Santa Ynez Valley. From the outset of its project, SFS Farms made great efforts to work with neighbors and the community in order to address any potential opposition to its farm. SFS Farms took substantial steps to accommodate legitimate neighborly concerns.

On February 7, 2020, the Santa Barbara County Agricultural Preserve Advisory Committee (APAC) reviewed the project for consistency with the Agricultural Preserves and Farmland Security Zones (Uniform Rules). APAC voted 4 to 0 to find the farm consistent with the Uniform Rules.

SFS Farms will deploy best management practices for farming in general and for cannabis in particular that improve soil conditions and promote water efficiency. The farm is designed to adapt progressive farming methods developed and taught at California's leading university agricultural programs such as University of California Davis. The farm will be visually appealing and completely in harmony with the local community and existing nearby agricultural operations, with no new structures and a traditional, natural rural aesthetic. This farm will indisputably make a positive impact on the community by enriching the local economy and ensuring that the character of the Santa Ynez Valley is preserved.

B. The Melville Appeal is Factually Unsupported and Rehashes Generalized and Speculative Arguments that Have Been Rejected

When considering this appeal, the Board will not see a single argument against the project that it has not already reviewed in connection with other appeals opposing cannabis cultivation. Nor will it see any new evidence in support of the old arguments. Melville's recycled arguments have been systematically dismantled by the Superior Court in *Santa Barbara Coalition for Responsible Cannabis, Inc. v. County of Santa Barbara, et al.* #20CV01736 ("*Busy Bee*"). In *Busy Bee*, Judge Thomas P. Anderle rejected similar arguments by "the Coalition"

and upheld the County's Ordinance. Most notably, the Court held that the opponents of the ordinance failed to timely challenge it, and they failed to accurately address the tremendous efforts the County made in assessing impacts, including specifically in the area along Highway 246.

The relevant analysis for the Board here should begin with the significant findings and recommended approval by the Planning Department and Director that form the basis of the permit approval, along with the Staff recommendation to deny the appeal and grant de novo review of the project. As importantly, the Board can be confident that the County ordinance has been upheld in Court, and that it will withstand all varieties of arguments made by opponents of cannabis.

In approving the project, the Planning Department prepared a checklist pursuant to CEQA Guidelines to document the evaluation of the project and proposed operations. The checklist confirms that SFS Farms' project is within the scope of the PEIR certified by the Santa Barbara County Board of Supervisors on February 6, 2018, as well as the revised ordinance. The Department's findings and conclusions, and additional vetting of the issues raised on appeal, are critical guidance for a very important reason – Melville has presented zero competent factual evidence related specifically to this project that supports any of the issues on appeal. It is plain to see that there is no fair basis on which the Director's approval may be overruled, nor may it be modified in any way.

1. The Board has Already Addressed and Rejected Arguments Concerning Overconcentration of Cannabis in the Santa Rita Hills

Melville's first argument concerns the number of cannabis projects in an area concentrated with wine. This issue was squarely addressed when the County certified the PEIR. The PEIR analyzed the impacts of outdoor cultivation, indoor cultivation, and processing of cannabis products on AG-II zoned lots within the Santa Ynez region. The PEIR anticipated that certain areas in which cannabis activities historically have occurred, such as the Santa Ynez region, would continue to experience concentrated cannabis activities under the Program. After the PEIR was certified, the County capped at 1,575 acres the area of cannabis cultivation in the unincorporated area outside of Carpinteria. SFS Farms' proposed agricultural activities, like other traditional farms in the area, are standard agricultural practices in the Santa Ynez region and the AG-II zone district. There is nothing unusual about the SFS project site, and it has been used for cultivating row crops for generations.

Because of the project's location and traditional operations, there is no legitimate argument that the agricultural practices at this farm will cause undue impacts on traffic, agricultural resources, noise, or air quality beyond those already addressed in the PEIR, which was upheld by the court in *Busy Bee*. To be clear, growing cannabis is a land use for agricultural purposes and cannabis is an agricultural product. This farm, like others contemplated when the PEIR was certified, ensures that agricultural practices will continue to be carried out.

2. SFS Farms is Consistent with the Comprehensive Plan's Agricultural Element

The assertion by Melville that the project is not consistent with the agricultural element of the County's Comprehensive Plan lacks merit. There is no factual support for such a claim, and it is based on an incorrect reading of the Plan that improperly distinguishes cannabis from other agricultural products. That is not a legally supportable distinction.

This is not an appeal about a non-agricultural project set amidst a traditional farming community, as Melville implicitly suggests. Under State law and the County Ordinance, cannabis cultivation continues agricultural uses of properties like this one, and as the Board has already seen in numerous appeals, Melville lacks substantial evidence that farming cannabis is inconsistent with surrounding agriculture, or that it could compromise long term productivity of other farms in the area that grow grapes, tomatoes, or broccoli. SFS Farms has no processing and no manufacturing, nor will it have any construction or grading or other activities that could be considered "non-agricultural" and therefore this argument should be rejected.

3. SFS Farms Complies with the Williamson Act

Contrary to Melville's assertions on appeal, SFS Farms does comply with the Williamson Act and the APAC review of the project was appropriately done within the scope of APAC's responsibilities to ensure compatibility, to the extent that is even necessary for agricultural uses like cannabis. The site has been used for agriculture for generations. SFS Farms is simply a continuing agricultural use. Melville has not identified any evidence before APAC that SFS would significantly compromise the long-term productive agricultural capability of other parcels or displace or impair current or reasonably foreseeable agricultural operations on other parcels, or that it will result in significant removal of adjacent

contracted land from agricultural use. There is no evidence that terpene taint of grapes, even if it were shown to exist – and no such showing has been made - would lead to the conversion of vineyards to urban uses due to unprofitability. Likewise, there is no evidence that the threat of liability for pesticide overspray will lead to the conversion of agricultural land to urban uses.

In *Busy Bee*, the Coalition made similar arguments under the Williamson Act that Melville makes here. All of them were rejected for procedural reasons (failure to timely appeal) and for substantive reasons (inadequate evidence that the project would affect long-term productive agricultural compatibility). Like in *Busy Bee*, this argument has no merit, and it is not a competent or credible basis for reversing the Planning Department approval or the Planning Commission's decision.

4. The SFS Farms Project is Fully Compliant with CEQA

Melville asserts generally that there is no evidence that the County determined the project to be exempt from CEQA, or that County staff complied with CEQA. This is an unsupported claim that ignores the record and that disregards the substantial efforts made by the County. The Department determined that the environmental impacts of SFS Farms are within the scope of the PEIR, and that no new environmental document was required. When the Planning Department determines that any potential significant environmental effects of a project are mitigated or avoided, reliance on the PEIR is entirely appropriate and a sound basis for permit approval. The Planning Department staff addressed each and every mitigation measure necessary to confirm that a later EIR was not required.

The PEIR is plainly appropriate for SFS Farms. This is essentially a turn-key farm that will require no building construction, no grading, and no development whatsoever that is inconsistent with or out-of-character from the community. There is no demonstrable adverse impact to Melville or any other community member. The Melville argument regarding CEQA simply raises long-ago waived arguments against the findings and conclusions of the PEIR that the Board of Supervisors certified in early 2018.

SFS Farms is a simple, traditional farm, with no new structures, no hoop houses, a private water supply that will be used efficiently, and only two harvest periods per year. Any concerns about odor, waft, or drift are eliminated by the distant location and the progressive operational methods. After careful study, the

Planning Department concluded that the farm is within the scope of the PEIR, and that any significant effects are mitigated.

5. Melville May Not Rely on its Own Anticipated Illegal Pesticide Use to Deny Approval of SFS Farms

Both pesticide migration from neighboring agriculture onto cannabis crops and potential for "terpene taint" of grapes were considered in the PEIR. The PEIR contemplated land use conflicts, compatibility issues with businesses, including wineries, near outdoor and indoor cultivation sites due to odors. The PEIR describes the Program impacts to Agricultural Resources and proposed land uses under the proposed Project are potentially incompatible with existing zoning for agricultural uses and Williamson Act contracts. The PEIR explains that growing cannabis is a land use for agricultural purposes and cannabis products are agricultural products; utilizing a license to grow cannabis would ensure agricultural purposes are carried out.

Importantly, "agricultural land use conflicts" such as pesticide overspray, are not environmental impacts under CEQA. Rather, they are social and economic effects and they are not to be considered a significant environment effect and need be considered only to the extent that they are relevant to an anticipated physical change in the environment or, on the basis of substantial evidence, are reasonably likely to result in physical change to the environment.

Melville, like other grape growers who oppose cannabis, suggests that the threat of liability for pesticide drift will increase operating costs of other agricultural operations as they switch to less toxic pesticides or more precise application methods. But, as the Board is aware, it is Melville's responsibility to ensure that its pesticide use is legally compliant, and if its practices result in pesticide drift it is in violation of the law.

6. There Are No Legitimate Terpene or Odor Concerns that Melville Can Identify

SFS Farms is on property designated Ag II, it does not require a Conditional Use Permit, and it is not within an EDRN. For those reasons, and because SFS Farms is not proposing any onsite processing such as drying or trimming, it does not require a formal Odor Abatement Plan. The farm's location, furthermore, naturally mitigates risk of adverse odor impacts. Likewise, the project does not propose any activities that require a permit from the Air Pollution Control District.

Not surprisingly, the appeal does not even contend that odor from SFS Farms will be apparent at any nearby facility, neighborhood, school, or tasting room. Melville could not support such a claim with a scientific basis.

Instead, Melville relies on generalized and completely speculative assertions about the health and safety of SFS Farms employees and the public from VOCs. This demonstrates a profound misunderstanding of VOCs, which are generated by a wide variety of crops, including grapes grown at Melville Winery.

The odor issue is often conflated with allegations of terpene drift or waft in appeals of cannabis projects. After several years of cannabis appeals, there is still no reliable evidence that terpenes from cannabis cultivation impact to the quality or marketability of surrounding agricultural crops. In fact, terpenes are everywhere in agricultural and rural, wooded areas. The terpenes found in cannabis are similar to those found in roses, rosemary, orange trees, oak trees, and pine trees. Nevertheless, VOC and terpene risks were addressed in the PEIR and were considered as part of the analysis of air quality impacts. Melville did not raise any concerns about terpenes or "waft" during the PEIR process and therefore has waived its arguments about them now. In any event, Melville offers no evidence that its vineyards, or any other nearby vineyards, absorb cannabis terpenes and, if so, the affect it has on their quality.

C. Conclusion

Melville's appeal of the permit approved for SFS Farms is based on little more than conjecture and lack of understanding. The appeal is not well-reasoned, and there is no substantial or new evidence submitted in support. It is a fact and the law that cannabis is legal in the State of California. It is also a fact that Santa Barbara County has chosen to participate in this area, and has passed an ordinance with extensive community participation and input. The County's Ordinance was recently upheld by the Santa Barbara Superior Court, based on the County's thorough efforts in assessing and addressing potential impacts, as well as on the great deference that must be given to the County's ordinance under the law.

Cannabis will ensure strong and stable economic growth for Santa Barbara County; it has already enabled the County to weather the global pandemic that has knocked many communities to their knees.[1] As importantly, the cannabis

[1] The UCSB Economic Forecast Project, led by Dr. Peter Rupert, UCSB economics professor and former Chair of the Economics Department, has done a preliminary analysis of the positive monetary impacts of

ordinance, and farms like SFS Farms, will ensure that the rural character of our local community is preserved. Melville's appeal should be denied, and SFS Farms should be allowed to proceed with this project without further delay.

Respectfully,

CAPPELLO & NOËL LLP

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cannabis in Santa Barbara County. For more on the massive economic benefits of cannabis in Santa Barbara, see Initial Impact report at https://efp.ucsb.edu/Cannabis/implan_InitialAssessment.pdf

LAB TESTS AND STUDIES SHOWING NO IMPACT FROM CANNABIS TERPENES ON GRAPES

Certificate of Analysis

QA SAMPLE - INFORMATIONAL ONLY

ICAL ID: 20190731-055
Sample: 1907ICA3745.11010
PENCE ESTATE CHARDONNAY
Strain: PENCE ESTATE CHARDONNAY
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Moisture	Δ9-THC	CBD	Total Cannabinoids	Total Terpenes
NT	**NT**	**NT**	**NT**	**0.00 mg/g**
Water Activity				
NT				

Summary	SOP Used	Date Tested	
Batch			Pass
Terpenes	SOP:TERP.MS.Beverage1	08/01/2019	Complete
Pesticides	PEST.002 Edible	07/31/2019	Pass

Scan to see results

Cannabinoid Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g

Total THC=THCa * 0.877 + d9-THC;Total CBD = CBDa * 0.877 + CBD; NR= Not Reported, ND= Not Detected, *Reported by Dry Mass*; *analytical instrumentation used Cannabinoids:UHPLC-DAD, Moisture:Mass by Drying,Water Activity:Water Activity Meter, Foreign Material:Microscope*

Terpene Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g
α-Bisabolol	0.20	0.10	ND	ND	δ-Limonene	0.20	0.10	ND	ND
α-Humulene	0.20	0.10	ND	ND	Eucalyptol	0.20	0.10	ND	ND
α-Pinene	0.20	0.10	ND	ND	γ-Terpinene	0.20	0.10	ND	ND
α-Terpinene	0.20	0.10	ND	ND	Geraniol	0.20	0.10	ND	ND
β-Caryophyllene	0.20	0.10	ND	ND	Linalool	0.20	0.10	ND	ND
β-Myrcene	0.20	0.10	ND	ND	Ocimene	0.20	0.10	ND	ND
β-Ocimene	0.20	0.10	ND	ND	(-)-Guaiol	0.20	0.10	ND	ND
β-Pinene	0.20	0.10	ND	ND	(-)-Isopulegol	0.20	0.10	ND	ND
Camphene	0.20	0.10	ND	ND	p-Cymene	0.20	0.10	ND	ND
Caryophyllene Oxide	0.20	0.10	ND	ND	Terpinolene	0.20	0.10	ND	ND
cis-Nerolidol	0.20	0.10	ND	ND	trans-Nerolidol	0.20	0.10	ND	ND
δ-3-Carene	0.20	0.10	ND	ND	**Total**			**0**	**0**

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ), *analytical instrumentation used:HS-GC-FID-FID*

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Batch#:
Primary Size:
Total/Batch Size:
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Completed: 08/01/2019

Residual Solvent Analysis

Category 1	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) ,*analytical instrumentation used=HS-GC-FID-FID*

Heavy Metal Screening

	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:ICP-MS*

Microbiological Screening

	Result	Status

ND=Not Detected; *analytical instrumentation used:qPCR*

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Completed: 08/01/2019

Chemical Residue Screening

Category 1		LOQ	LOD	Status
	µg/g	µg/g	µg/g	
Aldicarb	ND	0.05	0.03	Pass
Carbofuran	ND	0.05	0.03	Pass
Chlordane	ND	0.1	0.05	Pass
Chlorfenapyr	ND	0.1	0.05	Pass
Chlorpyrifos	ND	0.05	0.03	Pass
Coumaphos	ND	0.05	0.03	Pass
Daminozide	ND	0.05	0.03	Pass
DDVP	ND	0.05	0.03	Pass
Dimethoate	ND	0.05	0.03	Pass
Ethoprophos	ND	0.05	0.03	Pass
Etofenprox	ND	0.05	0.03	Pass
Fenoxycarb	ND	0.05	0.03	Pass
Fipronil	ND	0.05	0.03	Pass
Imazalil	ND	0.05	0.03	Pass
Methiocarb	ND	0.05	0.03	Pass
Methyl Parathion	ND	0.1	0.05	Pass
Mevinphos	ND	0.05	0.03	Pass
Paclobutrazol	ND	0.05	0.03	Pass
Propoxur	ND	0.05	0.03	Pass
Spiroxamine	ND	0.05	0.03	Pass
Thiacloprid	ND	0.05	0.03	Pass

Mycotoxins	LOQ	LOD	Limit	Status

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Abamectin	ND	0.05	0.03	0.3	Pass
Acephate	ND	0.05	0.03	5	Pass
Acequinocyl	ND	0.05	0.03	4	Pass
Acetamiprid	ND	0.05	0.03	5	Pass
Azoxystrobin	ND	0.05	0.03	40	Pass
Bifenazate	ND	0.05	0.03	5	Pass
Bifenthrin	ND	0.25	0.1	0.5	Pass
Boscalid	0.167	0.05	0.03	10	Pass
Captan	ND	0.35	0.2	5	Pass
Carbaryl	ND	0.05	0.03	0.5	Pass
Chlorantraniliprole	ND	0.05	0.03	40	Pass
Clofentezine	ND	0.05	0.03	0.5	Pass
Cyfluthrin	ND	0.35	0.25	1	Pass
Cypermethrin	ND	0.35	0.2	1	Pass
Diazinon	ND	0.05	0.03	0.2	Pass
Dimethomorph	ND	0.05	0.03	20	Pass
Etoxazole	ND	0.05	0.03	1.5	Pass
Fenhexamid	ND	0.05	0.03	10	Pass
Fenpyroximate	ND	0.05	0.03	2	Pass
Flonicamid	ND	0.05	0.03	2	Pass
Fludioxonil	ND	0.05	0.03	30	Pass
Hexythiazox	ND	0.05	0.03	2	Pass
Imidacloprid	ND	0.35	0.1	3	Pass

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Kresoxim Methyl	ND	0.05	0.03	1	Pass
Malathion	ND	0.05	0.03	5	Pass
Metalaxyl	ND	0.05	0.03	15	Pass
Methomyl	ND	0.05	0.03	0.1	Pass
Myclobutanil	ND	0.05	0.03	9	Pass
Naled	ND	0.1	0.05	0.5	Pass
Oxamyl	ND	0.2	0.1	0.3	Pass
Pentachloronitrobenzene	ND	0.1	0.05	0.2	Pass
Permethrin	ND	0.25	0.1	20	Pass
Phosmet	ND	0.05	0.03	0.2	Pass
Piperonyl Butoxide	ND	0.25	0.1	8	Pass
Prallethrin	ND	0.05	0.03	0.4	Pass
Propiconazole	ND	0.05	0.03	20	Pass
Pyrethrins	ND	0.25	0.1	1	Pass
Pyridaben	ND	0.05	0.03	3	Pass
Spinetoram	ND	0.05	0.03	3	Pass
Spinosad	ND	0.05	0.03	3	Pass
Spiromesifen	ND	0.05	0.03	12	Pass
Spirotetramat	ND	0.05	0.03	13	Pass
Tebuconazole	ND	0.05	0.03	2	Pass
Thiamethoxam	ND	0.25	0.1	4.5	Pass
Trifloxystrobin	ND	0.05	0.03	30	Pass

Unknown Analyte(s):

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:LC-MSMS & GC-MSMS*

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08/01/2019

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Completed: 08/01/2019

Moisture	Δ9-THC	CBD	Total Cannabinoids	Total Terpenes
NT	**NT**	**NT**	**NT**	**0.00 mg/g**
Water Activity				
NT				

Summary	SOP Used	Date Tested	
Batch			Pass
Terpenes	SOP:TERP.MS.Beverage1	08/01/2019	Complete
Pesticides	PEST.002 Edible	07/31/2019	Pass

Scan to see results

Cannabinoid Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g

Total THC=THCa * 0.877 + d9-THC;Total CBD = CBDa * 0.877 + CBD; NR= Not Reported, ND= Not Detected, *Reported by Dry Mass*; *analytical instrumentation used Cannabinoids:UHPLC-DAD, Moisture:Mass by Drying,Water Activity:Water Activity Meter, Foreign Material:Microscope*

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α-Terpinene	0.20	0.10	ND	ND	Geraniol	0.20	0.10	ND	ND
β-Caryophyllene	0.20	0.10	ND	ND	Linalool	0.20	0.10	ND	ND
β-Myrcene	0.20	0.10	ND	ND	Ocimene	0.20	0.10	ND	ND
β-Ocimene	0.20	0.10	ND	ND	(-)-Guaiol	0.20	0.10	ND	ND
β-Pinene	0.20	0.10	ND	ND	(-)-Isopulegol	0.20	0.10	ND	ND
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cis-Nerolidol	0.20	0.10	ND	ND	trans-Nerolidol	0.20	0.10	ND	ND
δ-3-Carene	0.20	0.10	ND	ND	**Total**			**0**	**0**

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Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Residual Solvent Analysis

Category 1	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) ,*analytical instrumentation used=HS-GC-FID-FID*

Heavy Metal Screening

	LOQ	LOD	Limit	Status

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Microbiological Screening

	Result	Status

ND=Not Detected; *analytical instrumentation used:qPCR*

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Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Chemical Residue Screening

Category 1		LOQ	LOD	Status
	µg/g	µg/g	µg/g	
Aldicarb	ND	0.05	0.03	Pass
Carbofuran	ND	0.05	0.03	Pass
Chlordane	ND	0.1	0.05	Pass
Chlorfenapyr	ND	0.1	0.05	Pass
Chlorpyrifos	ND	0.05	0.03	Pass
Coumaphos	ND	0.05	0.03	Pass
Daminozide	ND	0.05	0.03	Pass
DDVP	ND	0.05	0.03	Pass
Dimethoate	ND	0.05	0.03	Pass
Ethoprophos	ND	0.05	0.03	Pass
Etofenprox	ND	0.05	0.03	Pass
Fenoxycarb	ND	0.05	0.03	Pass
Fipronil	ND	0.05	0.03	Pass
Imazalil	ND	0.05	0.03	Pass
Methiocarb	ND	0.05	0.03	Pass
Methyl Parathion	ND	0.1	0.05	Pass
Mevinphos	ND	0.05	0.03	Pass
Paclobutrazol	ND	0.05	0.03	Pass
Propoxur	ND	0.05	0.03	Pass
Spiroxamine	ND	0.05	0.03	Pass
Thiacloprid	ND	0.05	0.03	Pass

Mycotoxins	LOQ	LOD	Limit	Status

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Abamectin	ND	0.05	0.03	0.3	Pass
Acephate	ND	0.05	0.03	5	Pass
Acequinocyl	ND	0.05	0.03	4	Pass
Acetamiprid	ND	0.05	0.03	5	Pass
Azoxystrobin	ND	0.05	0.03	40	Pass
Bifenazate	ND	0.05	0.03	5	Pass
Bifenthrin	ND	0.25	0.1	0.5	Pass
Boscalid	0.167	0.05	0.03	10	Pass
Captan	ND	0.35	0.2	5	Pass
Carbaryl	ND	0.05	0.03	0.5	Pass
Chlorantraniliprole	ND	0.05	0.03	40	Pass
Clofentezine	ND	0.05	0.03	0.5	Pass
Cyfluthrin	ND	0.35	0.25	1	Pass
Cypermethrin	ND	0.35	0.2	1	Pass
Diazinon	ND	0.05	0.03	0.2	Pass
Dimethomorph	ND	0.05	0.03	20	Pass
Etoxazole	ND	0.05	0.03	1.5	Pass
Fenhexamid	ND	0.05	0.03	10	Pass
Fenpyroximate	ND	0.05	0.03	2	Pass
Flonicamid	ND	0.05	0.03	2	Pass
Fludioxonil	ND	0.05	0.03	30	Pass
Hexythiazox	ND	0.05	0.03	2	Pass
Imidacloprid	ND	0.35	0.1	3	Pass

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Kresoxim Methyl	ND	0.05	0.03	1	Pass
Malathion	ND	0.05	0.03	5	Pass
Metalaxyl	ND	0.05	0.03	15	Pass
Methomyl	ND	0.05	0.03	0.1	Pass
Myclobutanil	ND	0.05	0.03	9	Pass
Naled	ND	0.1	0.05	0.5	Pass
Oxamyl	ND	0.2	0.1	0.3	Pass
Pentachloronitrobenzene	ND	0.1	0.05	0.2	Pass
Permethrin	ND	0.25	0.1	20	Pass
Phosmet	ND	0.05	0.03	0.2	Pass
Piperonyl Butoxide	ND	0.25	0.1	8	Pass
Prallethrin	ND	0.05	0.03	0.4	Pass
Propiconazole	ND	0.05	0.03	20	Pass
Pyrethrins	ND	0.25	0.1	1	Pass
Pyridaben	ND	0.05	0.03	3	Pass
Spinetoram	ND	0.05	0.03	3	Pass
Spinosad	ND	0.05	0.03	3	Pass
Spiromesifen	ND	0.05	0.03	12	Pass
Spirotetramat	ND	0.05	0.03	13	Pass
Tebuconazole	ND	0.05	0.03	2	Pass
Thiamethoxam	ND	0.25	0.1	4.5	Pass
Trifloxystrobin	ND	0.05	0.03	30	Pass

Unknown Analyte(s):

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:LC-MSMS & GC-MSMS*

Infinite Chemical Analysis Labs
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San Diego, CA
(858) 623-2740
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Lic# C8-0000019-LIC

Josh M Swider

Josh Swider
Lab Director, Managing Partner
08/01/2019

Certificate of Analysis

QA SAMPLE - INFORMATIONAL ONLY

ICAL ID: 20190731-056
Sample: 1907ICA3745.11011
PENCE UNUM PINOT
Strain: PENCE UNUM PINOT
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Moisture	Δ9-THC	CBD	Total Cannabinoids	Total Terpenes
NT	**NT**	**NT**	**NT**	**0.00 mg/g**
Water Activity				
NT				

Summary	SOP Used	Date Tested	
Batch			Pass
Terpenes	SOP:TERP.MS.Beverage1	08/01/2019	Complete
Pesticides	PEST.002 Edible	07/31/2019	Pass

Scan to see results

Cannabinoid Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g

Total THC=THCa * 0.877 + d9-THC;Total CBD = CBDa * 0.877 + CBD; NR= Not Reported, ND= Not Detected, *Reported by Dry Mass*; *analytical instrumentation used Cannabinoids:UHPLC-DAD, Moisture:Mass by Drying,Water Activity:Water Activity Meter, Foreign Material:Microscope*

Terpene Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g
α-Bisabolol	0.20	0.10	ND	ND	δ-Limonene	0.20	0.10	ND	ND
α-Humulene	0.20	0.10	ND	ND	Eucalyptol	0.20	0.10	ND	ND
α-Pinene	0.20	0.10	ND	ND	γ-Terpinene	0.20	0.10	ND	ND
α-Terpinene	0.20	0.10	ND	ND	Geraniol	0.20	0.10	ND	ND
β-Caryophyllene	0.20	0.10	ND	ND	Linalool	0.20	0.10	ND	ND
β-Myrcene	0.20	0.10	ND	ND	Ocimene	0.20	0.10	ND	ND
β-Ocimene	0.20	0.10	ND	ND	(-)-Guaiol	0.20	0.10	ND	ND
β-Pinene	0.20	0.10	ND	ND	(-)-Isopulegol	0.20	0.10	ND	ND
Camphene	0.20	0.10	ND	ND	p-Cymene	0.20	0.10	ND	ND
Caryophyllene Oxide	0.20	0.10	ND	ND	Terpinolene	0.20	0.10	ND	ND
cis-Nerolidol	0.20	0.10	ND	ND	trans-Nerolidol	0.20	0.10	ND	ND
δ-3-Carene	0.20	0.10	ND	ND	**Total**			**0**	**0**

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ), *analytical instrumentation used:HS-GC-FID-FID*

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08/01/2019

ICAL ID: 20190731-056
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PENCE UNUM PINOT
Strain: PENCE UNUM PINOT
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Residual Solvent Analysis

Category 1	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) ,*analytical instrumentation used=HS-GC-FID-FID*

Heavy Metal Screening

	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:ICP-MS*

Microbiological Screening

	Result	Status

ND=Not Detected; *analytical instrumentation used:qPCR*

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08/01/2019

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Strain: PENCE UNUM PINOT
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Chemical Residue Screening

Category 1		LOQ	LOD	Status
	µg/g	µg/g	µg/g	
Aldicarb	ND	0.05	0.03	Pass
Carbofuran	ND	0.05	0.03	Pass
Chlordane	ND	0.1	0.05	Pass
Chlorfenapyr	ND	0.1	0.05	Pass
Chlorpyrifos	ND	0.05	0.03	Pass
Coumaphos	ND	0.05	0.03	Pass
Daminozide	ND	0.05	0.03	Pass
DDVP	ND	0.05	0.03	Pass
Dimethoate	ND	0.05	0.03	Pass
Ethoprophos	ND	0.05	0.03	Pass
Etofenprox	ND	0.05	0.03	Pass
Fenoxycarb	ND	0.05	0.03	Pass
Fipronil	ND	0.05	0.03	Pass
Imazalil	ND	0.05	0.03	Pass
Methiocarb	ND	0.05	0.03	Pass
Methyl Parathion	ND	0.1	0.05	Pass
Mevinphos	ND	0.05	0.03	Pass
Paclobutrazol	ND	0.05	0.03	Pass
Propoxur	ND	0.05	0.03	Pass
Spiroxamine	ND	0.05	0.03	Pass
Thiacloprid	ND	0.05	0.03	Pass

Mycotoxins	LOQ	LOD	Limit	Status

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Abamectin	ND	0.05	0.03	0.3	Pass
Acephate	ND	0.05	0.03	5	Pass
Acequinocyl	ND	0.05	0.03	4	Pass
Acetamiprid	ND	0.05	0.03	5	Pass
Azoxystrobin	ND	0.05	0.03	40	Pass
Bifenazate	ND	0.05	0.03	5	Pass
Bifenthrin	ND	0.25	0.1	0.5	Pass
Boscalid	0.073	0.05	0.03	10	Pass
Captan	ND	0.35	0.2	5	Pass
Carbaryl	ND	0.05	0.03	0.5	Pass
Chlorantraniliprole	ND	0.05	0.03	40	Pass
Clofentezine	ND	0.05	0.03	0.5	Pass
Cyfluthrin	ND	0.35	0.25	1	Pass
Cypermethrin	ND	0.35	0.2	1	Pass
Diazinon	ND	0.05	0.03	0.2	Pass
Dimethomorph	ND	0.05	0.03	20	Pass
Etoxazole	ND	0.05	0.03	1.5	Pass
Fenhexamid	ND	0.05	0.03	10	Pass
Fenpyroximate	ND	0.05	0.03	2	Pass
Flonicamid	ND	0.05	0.03	2	Pass
Fludioxonil	ND	0.05	0.03	30	Pass
Hexythiazox	ND	0.05	0.03	2	Pass
Imidacloprid	ND	0.35	0.1	3	Pass

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Kresoxim Methyl	ND	0.05	0.03	1	Pass
Malathion	ND	0.05	0.03	5	Pass
Metalaxyl	ND	0.05	0.03	15	Pass
Methomyl	ND	0.05	0.03	0.1	Pass
Myclobutanil	ND	0.05	0.03	9	Pass
Naled	ND	0.1	0.05	0.5	Pass
Oxamyl	ND	0.2	0.1	0.3	Pass
Pentachloronitrobenzene	ND	0.1	0.05	0.2	Pass
Permethrin	ND	0.25	0.1	20	Pass
Phosmet	ND	0.05	0.03	0.2	Pass
Piperonyl Butoxide	ND	0.25	0.1	8	Pass
Prallethrin	ND	0.05	0.03	0.4	Pass
Propiconazole	ND	0.05	0.03	20	Pass
Pyrethrins	ND	0.25	0.1	1	Pass
Pyridaben	ND	0.05	0.03	3	Pass
Spinetoram	ND	0.05	0.03	3	Pass
Spinosad	ND	0.05	0.03	3	Pass
Spiromesifen	ND	0.05	0.03	12	Pass
Spirotetramat	ND	0.05	0.03	13	Pass
Tebuconazole	ND	0.05	0.03	2	Pass
Thiamethoxam	ND	0.25	0.1	4.5	Pass
Trifloxystrobin	ND	0.05	0.03	30	Pass

Unknown Analyte(s):

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:LC-MSMS & GC-MSMS*

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Josh M Swider

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Lab Director, Managing Partner
08/01/2019

Certificate of Analysis

QA SAMPLE - INFORMATIONAL ONLY

ICAL ID: 20190731-057
Sample: 1907ICA3745.11012
PENCE ROSA CHARDONNAY
Strain: PENCE ROSA CHARDONNAY
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Moisture	Δ9-THC	CBD	Total Cannabinoids	Total Terpenes
NT	**NT**	**NT**	**NT**	**0.00 mg/g**
Water Activity				
NT				

Summary	SOP Used	Date Tested	
Batch			Pass
Terpenes	SOP:TERP.MS.Beverage1	08/01/2019	Complete
Pesticides	PEST.002 Edible	07/31/2019	Pass

Scan to see results

Cannabinoid Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g

Total THC=THCa * 0.877 + d9-THC;Total CBD = CBDa * 0.877 + CBD; NR= Not Reported, ND= Not Detected, *Reported by Dry Mass*; *analytical instrumentation used Cannabinoids:UHPLC-DAD, Moisture:Mass by Drying,Water Activity:Water Activity Meter, Foreign Material:Microscope*

Terpene Profile

Analyte	LOQ	LOD	%	mg/g	Analyte	LOQ	LOD	%	mg/g
α-Bisabolol	0.20	0.10	ND	ND	δ-Limonene	0.20	0.10	ND	ND
α-Humulene	0.20	0.10	ND	ND	Eucalyptol	0.20	0.10	ND	ND
α-Pinene	0.20	0.10	ND	ND	γ-Terpinene	0.20	0.10	ND	ND
α-Terpinene	0.20	0.10	ND	ND	Geraniol	0.20	0.10	ND	ND
β-Caryophyllene	0.20	0.10	ND	ND	Linalool	0.20	0.10	ND	ND
β-Myrcene	0.20	0.10	ND	ND	Ocimene	0.20	0.10	ND	ND
β-Ocimene	0.20	0.10	ND	ND	(-)-Guaiol	0.20	0.10	ND	ND
β-Pinene	0.20	0.10	ND	ND	(-)-Isopulegol	0.20	0.10	ND	ND
Camphene	0.20	0.10	ND	ND	p-Cymene	0.20	0.10	ND	ND
Caryophyllene Oxide	0.20	0.10	ND	ND	Terpinolene	0.20	0.10	ND	ND
cis-Nerolidol	0.20	0.10	ND	ND	trans-Nerolidol	0.20	0.10	ND	ND
δ-3-Carene	0.20	0.10	ND	ND	**Total**			**0**	**0**

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ), *analytical instrumentation used:HS-GC-FID-FID*

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08/01/2019

Certificate of Analysis

QA SAMPLE - INFORMATIONAL ONLY

ICAL ID: 20190731-057
Sample: 1907ICA3745.11012
PENCE ROSA CHARDONNAY
Strain: PENCE ROSA CHARDONNAY
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Residual Solvent Analysis

Category 1	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status	Category 2	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) ,*analytical instrumentation used=HS-GC-FID-FID*

Heavy Metal Screening

	LOQ	LOD	Limit	Status

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:ICP-MS*

Microbiological Screening

	Result	Status

ND=Not Detected; *analytical instrumentation used:qPCR*

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QA SAMPLE - INFORMATIONAL ONLY

ICAL ID: 20190731-057
Sample: 1907ICA3745.11012
PENCE ROSA CHARDONNAY
Strain: PENCE ROSA CHARDONNAY
Category: Ingestible

Responsible AG Testing
Lic. #
None
San Diego, CA 92121

Lic. #

Batch#:
Primary Size:
Total/Batch Size:
Collected: 08/01/2019; Received: 08/01/2019
Completed: 08/01/2019

Chemical Residue Screening

Category 1		LOQ	LOD	Status
	µg/g	µg/g	µg/g	
Aldicarb	ND	0.05	0.03	Pass
Carbofuran	ND	0.05	0.03	Pass
Chlordane	ND	0.1	0.05	Pass
Chlorfenapyr	ND	0.1	0.05	Pass
Chlorpyrifos	ND	0.05	0.03	Pass
Coumaphos	ND	0.05	0.03	Pass
Daminozide	ND	0.05	0.03	Pass
DDVP	ND	0.05	0.03	Pass
Dimethoate	ND	0.05	0.03	Pass
Ethoprophos	ND	0.05	0.03	Pass
Etofenprox	ND	0.05	0.03	Pass
Fenoxycarb	ND	0.05	0.03	Pass
Fipronil	ND	0.05	0.03	Pass
Imazalil	ND	0.05	0.03	Pass
Methiocarb	ND	0.05	0.03	Pass
Methyl Parathion	ND	0.1	0.05	Pass
Mevinphos	ND	0.05	0.03	Pass
Paclobutrazol	ND	0.05	0.03	Pass
Propoxur	ND	0.05	0.03	Pass
Spiroxamine	ND	0.05	0.03	Pass
Thiacloprid	ND	0.05	0.03	Pass

Mycotoxins	LOQ	LOD	Limit	Status

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Abamectin	ND	0.05	0.03	0.3	Pass
Acephate	ND	0.05	0.03	5	Pass
Acequinocyl	ND	0.05	0.03	4	Pass
Acetamiprid	ND	0.05	0.03	5	Pass
Azoxystrobin	ND	0.05	0.03	40	Pass
Bifenazate	ND	0.05	0.03	5	Pass
Bifenthrin	ND	0.25	0.1	0.5	Pass
Boscalid	0.162	0.05	0.03	10	Pass
Captan	ND	0.35	0.2	5	Pass
Carbaryl	ND	0.05	0.03	0.5	Pass
Chlorantraniliprole	ND	0.05	0.03	40	Pass
Clofentezine	ND	0.05	0.03	0.5	Pass
Cyfluthrin	ND	0.35	0.25	1	Pass
Cypermethrin	ND	0.35	0.2	1	Pass
Diazinon	ND	0.05	0.03	0.2	Pass
Dimethomorph	ND	0.05	0.03	20	Pass
Etoxazole	ND	0.05	0.03	1.5	Pass
Fenhexamid	ND	0.05	0.03	10	Pass
Fenpyroximate	ND	0.05	0.03	2	Pass
Flonicamid	ND	0.05	0.03	2	Pass
Fludioxonil	ND	0.05	0.03	30	Pass
Hexythiazox	ND	0.05	0.03	2	Pass
Imidacloprid	ND	0.35	0.1	3	Pass

Category 2		LOQ	LOD	Limit	Status
	µg/g	µg/g	µg/g	µg/g	
Kresoxim Methyl	ND	0.05	0.03	1	Pass
Malathion	ND	0.05	0.03	5	Pass
Metalaxyl	ND	0.05	0.03	15	Pass
Methomyl	ND	0.05	0.03	0.1	Pass
Myclobutanil	ND	0.05	0.03	9	Pass
Naled	ND	0.1	0.05	0.5	Pass
Oxamyl	ND	0.2	0.1	0.3	Pass
Pentachloronitrobenzene	ND	0.1	0.05	0.2	Pass
Permethrin	ND	0.25	0.1	20	Pass
Phosmet	ND	0.05	0.03	0.2	Pass
Piperonyl Butoxide	ND	0.25	0.1	8	Pass
Prallethrin	ND	0.05	0.03	0.4	Pass
Propiconazole	ND	0.05	0.03	20	Pass
Pyrethrins	ND	0.25	0.1	1	Pass
Pyridaben	ND	0.05	0.03	3	Pass
Spinetoram	ND	0.05	0.03	3	Pass
Spinosad	ND	0.05	0.03	3	Pass
Spiromesifen	ND	0.05	0.03	12	Pass
Spirotetramat	ND	0.05	0.03	13	Pass
Tebuconazole	ND	0.05	0.03	2	Pass
Thiamethoxam	ND	0.25	0.1	4.5	Pass
Trifloxystrobin	ND	0.05	0.03	30	Pass

Unknown Analyte(s):

NR= Not Reported thus no analysis was performed, ND= Not Detected thus the concentration is less then the Limit of Quantification (LOQ) , *analytical instrumentation used:LC-MSMS & GC-MSMS*

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08/01/2019

AWRI
The Australian Wine Research Institute

Technical Review

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Highlights this issue

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The AWRI's annual report to Australian grapegrowers, winemakers and other stakeholders has been produced and will be distributed early in December.

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The John Fornachon Memorial Library will be closed from 12 noon Friday, 24 December 2010 until Friday, 7 January 2011.

TECHNICAL NOTES

Exploring the influence of pepper, eucalyptus and smoky flavour compounds on consumer preferences of red wines

Three naturally-occurring flavour components, rotundone ('peppery'), eucalyptol ('eucalyptus') and guaiacol ('smoky') were added to a Merlot cask wine at two different levels (low and high). Liking scores for these wines were collected from over 100 consumers. Overall, the smoky wine was less well-liked, while the wines with added eucalyptus flavour were more liked than the base wine for most consumers. The pepper flavour addition did not influence the preferences of most people.

How much oxygen gets into must during grape processing?

Measuring dissolved oxygen (DO) *in situ* for the first time at crushing during the 2010 vintage has thrown up some interesting figures. Using a technique which gives winemakers and scientists actual values of DO, researchers at the AWRI have identified that high DO values occur in must at crushing. Similar measurements made during pressing appear lower, indicating that crushing is the stage that can pick-up most oxygen.

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AWRI notes

Beyond ideas: our year in review

The AWRI's annual report to Australian grapegrowers, winemakers and other stakeholders has been produced and will be distributed early in December. An electronic copy of the report is available from our website. The AWRI's Managing Director will also present our annual report to the various state-based wine industry organisations over the next few months. We also published a review of our activities in the November issue of the *Australian and New Zealand Grapegrower and Winemaker*.

The activities at the AWRI benefit from collaborations with individuals from some 124 organisations from 12 different countries: Australia (Australian Capital Territory, New South Wales, Queensland, South Australia, Tasmania, Victoria, Western Australia), Belgium, Canada, Denmark, France, Germany, Italy, New Zealand, Slovenia, South Africa, Spain, United Kingdom and the USA. We gratefully acknowledge the assistance, cooperation and/or collaboration from our partners across the globe.

We are pleased to publish the highlights from our very rewarding year of activity below.

1. **A special wine tasting and technology showcase for key wine industry leaders were organised to celebrate the AWRI's 55th anniversary** of supporting Australian grape and wine producers.
2. **Breakthrough in smoke taint diagnostics**.(i) New multi-analyte methods for quantification of conjugated and free volatile phenols (including phenol, cresols, guaiacol, methylguaiacol, vinylguaiacol, syringol and methylsyringol) have been developed using HPLC-MS/MS and GC-MS, respectively. (ii) Aroma detection threshold values in a red wine base have been established for volatile phenol compounds implicated in bushfire smoke taint. (iii) Aided by synthesized glycosidic precursors the release of the volatile phenol guaiacol and its role in retro-nasal smoke flavor perception has been demonstrated.
3. **In a world-first, Australian producers of Pinot Grigio and Pinot Gris wines** have access to a simple labelling device which informs consumers the 'style' of the wine in the bottle at point of sale or before opening. Called the *PinotG Style Spectrum*, the label indicates to consumers whether the style of the Pinot Grigio or Pinot Gris wine is 'crisp' or 'luscious' or somewhere on the spectrum of possible styles in-between. Additionally, the labelling device will potentially help remove the confusion which results from the common use of the two names for the same variety, which are often difficult to relate to the style of the wine in the bottle.
4. **Improved understanding of the formation of tropical fruit aromas during winemaking** through the development and application of an HPLC-MS/MS method, which quantifies precursors to 3-mercaptohexanol (3-MH).

5. **Improved identification of compounds responsible for 'reductive' character**: compounds most likely associated with 'reductive' characters are hydrogen sulfide, methanethiol and dimethyl sulfide, while methyl thioacetate could act as a source of methanethiol over time. 'Struck flint' aroma in white wine may be linked to the compound benzyl mercaptan.
6. **Strong evidence links eucalyptol in red wine** to eucalyptus trees grown in close proximity to vineyards.
7. **Improved understanding of tannin** achieved indicates that (i) grape-derived cell wall materials have a stronger affinity for seed tannins than skin tannins; (ii) an increase in winemaker perception of quality is related to an increase in the concentration of tannins, particularly skin tannins in wine; and (iii) older tannins interact only weakly with proteins and this could explain the 'softening' effect that wines undergo with age.
8. **Tannin measurement went on-line** via a handy web portal, showing winemakers how to use tannin to their advantage and compare against regional and national measurements.
9. **Non-destructive analysis of wine in-bottle is now** possible through collaboration with the AWRI, Jeffress Engineering and Camo Software, using the BevScan. This technology could potentially be used to screen wine stocks to identify damaged from high quality wine due to bottling, packaging, storage or other variables.
10. **Yeast strain-derived sensory effects can be retained for long periods**. A sensory study on two sets of three-year-old Sauvignon Blanc wines showed that there were significant differences between wines made with different yeast strains, and these differences were retained for almost three years.
11. **Enhanced activity of two, previously uncharacterised, yeast genes** has been shown to increase the release of 3-mercaptohexanol during fermentation, increasing the pool of wine yeast genes available to improve wine flavour
12. **A proof-of-concept, GM, wine yeast prototype strain reduced ethanol concentration** from 15.5% (v/v) to 12% (v/v) in small-scale winemaking trials in both Chardonnay and Shiraz musts.
13. **AWRI-developed wine yeast wins award in Germany**. Maurivin Platinum, a low-H_2S yeast developed by the AWRI, won an award at Intervitis-Interfructa in Stuttgart, Germany, for Innovation in Processing for Wine.
14. **Genome sequences of five commercial wine yeast strains have been determined** and the data generated has highlighted what makes wine yeast different from other yeast.
15. **Alternatives to bentonite fining are gaining traction** with confirmation of the use of proteolytic enzymes to degrade haze-forming PR proteins, combined with heat treatment, can reduce the concentration of unstable grape proteins.
16. **Our understanding of red wine fruit flavours has significantly been improved** through establishment of relationships among compositional data and sensory properties from two large red wine sensory-consumer studies.

17. **Environmental web portal launched.** This allows users to search the AWRI's dedicated database of environmental articles; use the dedicated Environment Search Engine to search across multiple relevant websites related to environmental issues in one place; and to browse a range of specially-selected links clustered by topic.
18. **Confirmation that a high proportion of consumers prefer wines** with some 'green' capsicum-like flavour. Producers of Sauvignon Blanc have greater guidance regarding appropriate levels of this and the 'cat urine/sweaty' aroma.
19. **Sensory study shows split consumer preferences** for 'savoury' flavours in red wines.
20. **The WIC Winemaking Service was set up in January 2010** and has completed its first successful year of operation. The WIC Winemaking Service is a joint partnership between the AWRI and the University of Adelaide.
21. **AWRI staff members gave 320 oral presentations**, conducted 17 workshops and presented 20 posters.
22. **AWRI staff members presented 37 lectures** and coordinated the Grape Industry Practices, Policy and Communication six-week subject for undergraduate students.
23. **AWRI staff members supervised**/co-supervised 21 postgraduate students.
24. **Increased requests for information serviced.** AWRI staff members responded to 5,591 recorded requests for information during the 2009/2010 year. To put the statistics into perspective, 22 people contacted the AWRI seeking information on every working day of the year. This figure does not include the amount of problem solving samples investigated (1,000) or the number of Commercial Services analyses undertaken during the year.

Readers are encouraged strongly to read the AWRI's 2010 annual report in detail rather than relying on the brief details above for information.

Rae Blair, Communications Manager, *rae.blair@awri.com.au*

Library closure over Christmas and New Year

The John Fornachon Memorial Library will be closed from 12 noon Friday, 24 December 2010 until Friday, 7 January 2011. The library will reopen at 9:00 am Monday, 10 January 2011. Access to the library's online database, as well as access to all the exclusive online content available only to Australian levy payers, will continue to be available 24 hours a day, 7 days a week during this period via the AWRI website (www.awri.com.au).

Sean Boden, Systems Librarian, *infoservices@awri.com.au*

Technical notes

Exploring the influence of pepper, eucalyptus and smoky flavour compounds on consumer preferences of red wines

The Australian wine industry's ability to tailor winemaking techniques to produce wine styles that match expectations of winemakers and consumers is a key advantage in the current challenging times faced by the industry. The Australian Wine Research Institute has been leading research on grape and wine composition, defining the aroma and flavour impact of volatile wine components. In this context, it is essential to understand the importance of different compounds to consumer preferences of wines. This study reveals the reaction of consumers to different concentrations of naturally occurring flavour components found in some red wines to guide the industry in the production of highly accepted wines.

Previous consumer studies undertaken by the AWRI showed that different groups of consumers prefer distinct flavours such as red berry versus dark fruit flavour in red wines, or green flavour versus tropical fruit flavour in white wines (AWRI publications #1131, 1176, 1199). In addition, a proportion of consumers are very sensitive to faults or off-flavours such as TCA, bitterness or excessive development. This study explored consumer preferences and tolerances to naturally occurring flavour components in wines normally described as peppery, eucalyptus and smoky to understand desirable levels of these compounds in wines.

The compounds guaiacol, eucalyptol (1,8-cineole) and rotundone were added to a relatively low flavour bag-in-box Merlot base wine in two concentrations. The levels chosen represented typical levels observed in commercial wines. The base wine selected had very low levels of guaiacol and eucalyptol (5 and 0.18 µg/L respectively) and no detectable level of rotundone (less than 5 ng/L). Table 1 describes the six levels of the three flavour compound additions to the base wine.

Six spiked wines plus the Merlot base wine were profiled by 10 trained AWRI panellists who evaluated the wines in triplicate. The attributes *red berry, dark berry, vanilla, smoky, pepper,*

Table 1. Concentration of flavour compounds added to the Merlot base wine.

Flavour compound	Concentration added
Guaiacol Low	25 µg/L
Guaiacol High	50 µg/L
Eucalyptol Low	4 µg/L
Eucalyptol High	30 µg/L
Rotundone Low	25 ng/L
Rotundone High	125 ng/L

mint/eucalyptus, vanilla palate, smoky palate, pepper palate and *mint/eucalyptus palate* were significantly different among the samples ($P<0.05$).

The same seven wines were assessed by 104 consumers in Adelaide who were recruited based on their red wine consumption of at least one glass per week. All samples were served blind in ISO tasting glasses for both consumer testing and trained panel sensory evaluation. Wines were identified only with a three-digit code and were served in a randomised order to minimise any bias. Table 2 describes the demographic profile of the consumers, who rated each wine for overall liking and purchase intent, followed by a number of questions to explore their attitudes towards the wines. The assessments took place at the AWRI sensory booths in June 2010.

If we consider the overall liking scores averaged across all consumers, the wine with a high level of guaiacol was less well-liked, while the wines with added eucalyptol were liked slightly more than the base wine. Wines with added pepper flavour and low levels of added smoke flavour were moderately liked. The average liking scores for the seven wines are shown in Figure 1.

Consumers are not uniform in their preferences and different groups of consumers often respond differently to wine flavours. Three clusters of consumers with similar liking and disliking patterns were identified in this study. Figure 2 shows the association of the wines' sensory attributes with the three preference clusters, together with the total population average liking scores.

Table 2. Demographic information of 104 Adelaide consumers.

Demographic information	%
Gender	
Male	57
Female	43
Age	
18-25	11
26-35	18
36-45	22
46-55	26
> 56	23
Highest level of education achieved	
Secondary school	11
TAFE certificate/ apprenticeship/ diploma	23
University degree	43
Postgraduate	21
Household income (AUD)	
< $20,000	4
$20,000-$39,999	9
$40,000-$59,999	9
$60,000-$79,999	16
$80,000-$99,999	18
$100-$149,999	25
> $150,000	19
Years drinking wine	
< 2	1
2-5	7
6-10	18
11-20	18
>20	56
Amount typically spent on a bottle of wine (AUD)	
<$10	13
$10.00-$14.50	40
$15.00-$19.50	67
$20.00-$24.50	47
$25.00-$29.50	31
$30.00-$39.50	12
$40.00-$49.50	6
>$50.00	6

The first cluster had 29% of the participants, who most preferred the base wine with no additions. Looking at the sensory attributes, consumers in Cluster 1 were driven positively by *dark berry* and *pepper* and negatively by *mint/eucalyptus.* This group had a slightly higher proportion of males from 56 to 65 years of age who spend somewhat less per bottle of wine, and have a higher Shiraz consumption relative to the rest of the consumers in the study.

Cluster 2 (33% of the consumers) preferences were positively related with the total population mean liking scores (r=0.88, $P<0.05$). The most important characteristic of this group was the strong

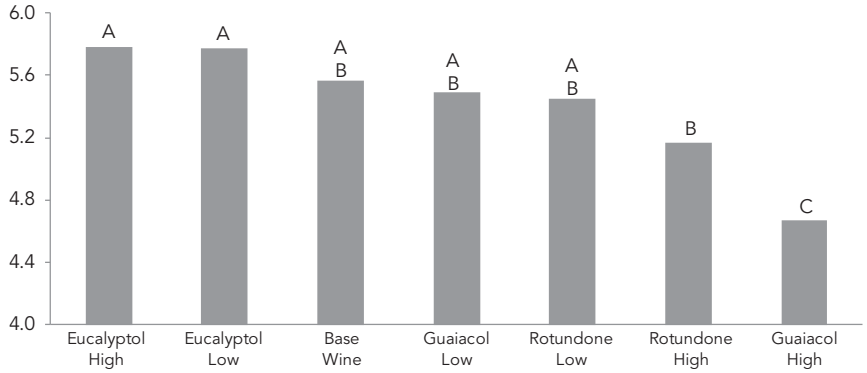

Figure 1. Mean overall liking scores for the seven wines (base wine plus low and high levels of flavour compound added to the base wine) from 104 Adelaide consumers. Bars showing the same letters are not significantly different from each other ($P<0.05$).

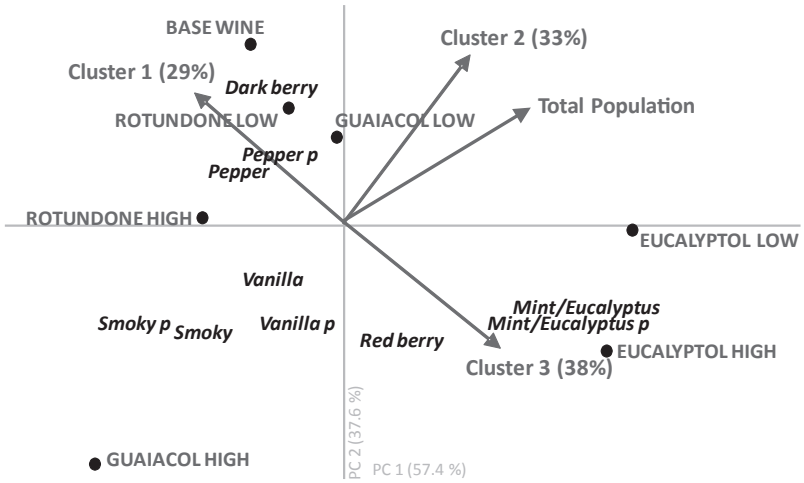

Figure 2. Preference map of the mean liking scores for three segments of red wine consumers and the total population. The sensory attributes generated by the AWRI trained panel are superimposed.
p = palate attributes.

negative influence of high levels of *smoky* flavour. Consumers in this second cluster preferred the wine with low levels of *eucalyptus,* and there were slightly more females with higher income in this group.

The third cluster's preferences were the opposite of the preferences from Cluster 1. Cluster 3 consisted of 38% of the consumers who strongly preferred *mint/eucalyptus* attributes and particularly liked the sample with the highest concentration of eucalyptol. These consumers were also positively driven by *red berry* aroma in the wine and liked less the base wine and the peppery wines.

Regarding the addition of guaiacol, this compound is known to be a component of oak wood, especially in barrels that are highly toasted, and is also involved in bush-fire smoke affected wines. For bush-fire smoke, it is known that other compounds can be involved (AWRI publication #1085) and further consumer studies will assess additional smoke-related components in combination.

In summary, consumers were affected by the levels of flavour compounds added to the base wine. High levels of guaiacol negatively affected the majority of consumers and eucalyptol was positively associated with liking for some consumers at both low and high concentrations. Rotundone addition was positive for a third of the consumers and fairly neutral to the rest. Preferences and tolerances for the different flavours thus vary considerably among consumers with distinct niches of consumers preferring specific flavours.

By determining the concentration of these compounds in a wine we can then indicate its perceived quality from a consumer perspective, although further work is required. The AWRI can accurately measure the levels of these components using instrumental analyses, and these are available on a commercial basis.

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How much oxygen gets into must during grape processing?

Introduction

Measuring dissolved oxygen (DO) *in situ* for the first time at crushing during the 2010 vintage has thrown up some interesting figures. Using a technique which gives winemakers and scientists actual values of DO, researchers at the AWRI have identified that high DO values occur in must at crushing. Similar measurements made during pressing appear lower, indicating that crushing is the stage that can pick-up most oxygen.

Why would a winemaker be interested in knowing about oxygen pick-up during grapes processing? A lot is known about dissolved oxygen pick-up during bottling and ageing in bottle, but little is known during vinification. Two major groups of compounds in must are affected by exposure to oxygen: phenolics and aroma compounds. The browning that occurs during traditional oxidative handling of must is caused by quinones of caftaric acid and flavanols generated through the action of polyphenol oxidases and oxygen on these compounds (Cheynier et al. 1990). Quinones are highly reactive radical species which can create cascades of further oxidative damage. Aroma compounds are also potentially exposed to this oxidative damage although the extent and mechanisms are not widely known, especially whether aroma precursors are involved. However, the effect of pressing and oxidative damage on thiol aromas has been studied on Sauvignon Blanc wines (Patel et al. 2010). Protection from oxygen for certain grape varieties during pressing is considered essential by some. The use of a range of techniques to stop this happening, such as the use of dry ice or other inert gases, is common. In fact, membrane presses that can be protected by inert gas have now been available for a number of years from several manufacturers. Anecdotal evidence shows that wines produced this way are fresher and more vibrant (Osicka 2010).

But how much oxygen *is* involved and if we are protecting the press, what is the impact at the crusher? Are winemakers 'locking the stable once the horse has bolted?'

Methodology

Measurements were made using Nomasense equipment at the Josef Chromy winery in Tasmania (JCW) and the University of Adelaide's Hickinbotham-Roseworthy Wine Science Laboratory (HRWSL) on both destemmer-crushers and membrane presses. Nomasense DO measurement works when light from a blue LED, shone down a fibre optic cable, excites an O_2 sensor spot to emit back a red-coloured fluorescent signal. When oxygen is present, the fluorescence signal is 'quenched' in proportion to the amount of oxygen in the juice (Anon 2010b). In these experiments, the oxygen-sensitive dot was placed inside a sight glass so this can then be placed anywhere along a pumping line. The sight glass was orientated so that the dot was at the bottom to maximize contact

with flowing liquid. The potential of altering the sample when taking a sample for analysis in the laboratory is, therefore, avoided by making readings *in situ*.

A Bucher-Vaslin 'Delta' destemmer-crusher was used at the JCW where the must is fed into an Enoveneta peristaltic pump by a short closed screw with the DO being measured at the output of the peristaltic pump. At the HRWSL, two crushers were used: a Diemme destemmer-crusher and a Demoisy 7EP crusher-destemmer. The sight glass was placed just after the screw drives below the crusher rollers before the must pump.

More than a dozen batches of several varieties of white grapes and Pinot Noir were measured during the 2010 vintage from both hand-picked and machine-harvested fruit.

Results and discussion

(i) Crushing

The average DO value during crushing measured over 12 batches of grapes was 6 mg/L with a standard deviation of 2 mg/L. The DO values of three batches of Riesling crushed at the JCW was over 9 mg/L which is close to the saturation level in grape juice. Measurements were taken only as the must pump was operating (Figure 1) or, for smaller loads at the HRWSL, when a reasonably steady state had been achieved. The DO values measured on two small-scale crushers at the HRWSL appeared more consistent and were lower than at the JCW. Although there are only a small number of mean values, the crusher size and design do appear to influence the amount of oxygen that can be taken up by the must. The Pinot Noir must had a lower DO which could be explained in the different sized grapes but, without proper replication and diversity of varieties, it is not possible to draw such conclusions.

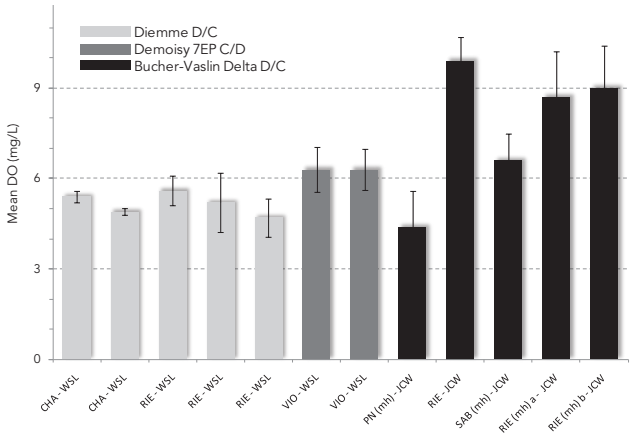

Figure 1. Mean DO values during crushing operations. Error bars = 1 sd
(mh: machine harvested D/C: destemmer-crusher C/D: crusher-destemmer)

Actual real-time DO profiles during crushing with a Bucher-Vaslin Delta destemmer-crusher are depicted in Figure 2. The DO values oscillated around a mean value probably due to variations in the supply of grapes into the hopper of the crusher. When the must pump stopped, the DO values dropped quickly showing that the oxygen dissolved in the must around the sensor was quickly consumed. Typically, the rate of localised oxygen consumption around the sensor spot was around 0.5 mg/L/min (results not shown). In the bottom right graph of Figure 2, the fast increase in DO is due to water push-through. On the time frame taken to fill a press and with little additional oxygenation the must DO will be near zero when pressing begins. This can explain why DO values measured after pressing appear much lower. Because of this, DO readings after the press do not indicate to how much oxygen a must has been exposed. For grapes that can be very sensitive to oxidative spoilage, such as Sauvignon Blanc, inerted commercial crushing equipment is now available (Anon 2010a).

Obviously, this situation of grape 'damage' activating enzymatic oxidation can also occur during machine harvesting. Depending on the equipment used and the degree of mechanical shear involved, oxidation is likely to occur in an uncontrolled manner.

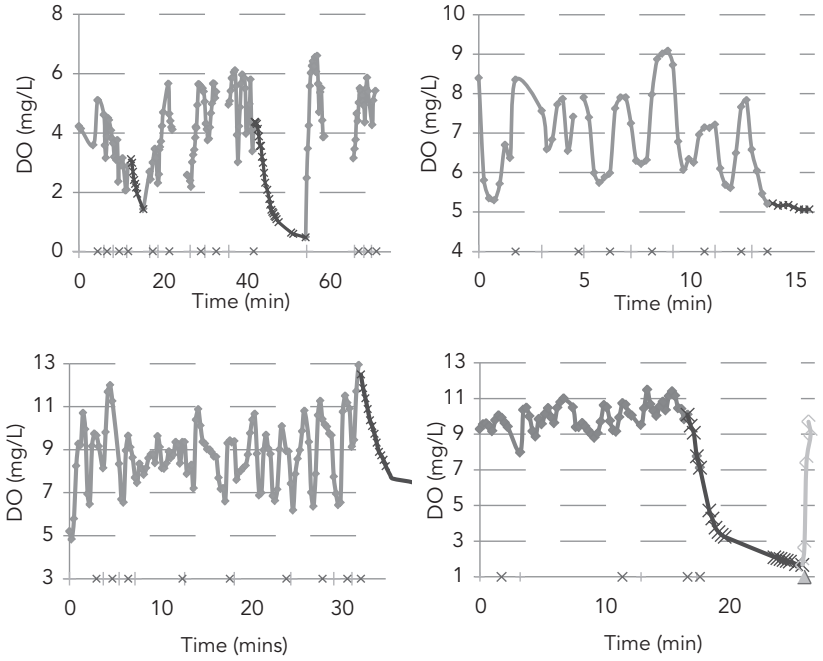

Figure 2. Real-time DO profiles during crushing at the JCW. DO (dynamic) light grey, DO (static) dark grey. Clockwise from top left: machine harvested (MH) Pinot Noir; MH Sauvignon Blanc; MH Riesling; hand-picked Riesling. Vertical bars on the time scale indicate pump on, crosses pump off.

(ii) Pressing

In both wineries, the sight glass used to measure press DO was placed after a buffer tank. For the smaller Willmes press at the HRWSL, a shallow 1000 L press tray (with dimensions the same as the foot print of the press) below the drum was protected with dry ice. At the JCW, a smaller press tray (250 L) integrated in the Bucher XPert 250 fed into a larger buffer tank (1,000 L) not protected with inert gas. When the press pump operates to transfer the juice to tank, the DO values represents a dynamic reading but when the pump is off, the DO value can be described as static and allows the measurement of oxygen consumption of the juice at that particular composition in the sight glass.

A typical DO profile in a commercial-scale press is shown in Figure 3. The DO value starts at 3 mg/L although the values during crushing were around 9 mg/L. The initial decrease on measured dynamic DO is the oxygen sensor spot acclimatising to the low DO environment. Intuitively, as there is a considerable air intake into the press upon press bladder deflation, a subsequent increase in DO should be observed. However, in Figure 3, the first deflate and crumble occurs at 60 minutes (draining time included), before which there had been four rapid increases in DO. This can most probably be explained by splashing in the empty buffer tank. The triangles along the time axis indicate when this occurred, immediately after which the DO increased.

Again, as during crushing, when the juice is static, the DO decreases due to enzymatic consumption of oxygen to oxidize the phenolic material present. Typically, the rate of decrease calculated from several press cycles ranges from 0.13 to 0.350 mg/L/min. As shown in the last third of the cycle in Figure 3, the rate of oxygen consumption (dotted line) decreases as the press cycle progresses. This behaviour was seen for several press runs.

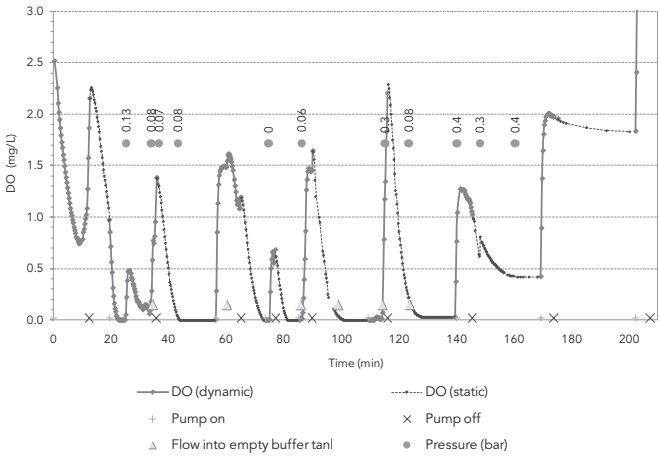

Figure 3. DO profile during pressing of crushed machine-harvested Riesling (Bucher Xpert 250 with ORTAL program)

(iii) Inerted pressing

Several manufacturers supply standard tank presses that can be inerted using carbon dioxide or nitrogen. The press is filled with inert gas before filling and inert gas is used when the bladder is deflated. Recycling of this gas has been incorporated into the design by at least one manufacturer. In the experiment at the JCW, Chardonnay was whole-bunch pressed using a Willmes 'Sigma' press operated using nitrogen supplied from a nine-bottle manifold. The press was not inerted before charging but all subsequent juice collection and bladder deflation occurred under protection of nitrogen.

Initially, the DO of the juice reached a maximum value of 2 mg/L during the dejuicing step. For the remainder of the press cycle, the DO never exceeded 0.18 mg/L. The emergent juice appeared a more vibrant yellow-green colour and clearer. Unfortunately for practical reasons, this was the only press run that could be measured with inert gas cover.

(iv) In-tank DO values post-pressing

The DO of the pressed juice was measured immediately post-pressing with a dipping probe containing the same sort of oxygen sensor used in the sight glass. Several of the tanks were re-measured on subsequent days during cold settling. The DO values and position of the probe are summarised in Table 1. Storage volumes of the tanks measured ranged from 1,000 L to 20,000 L.

It can be seen from these data that the DO in juice post-pressing is very low due to the rapid nature of enzymatic oxidation which will consume oxygen picked up during processing. The limited option

Table 1. Dissolved oxygen immediately following pressing and at various later time points

Juice	Date crushed	Time post pressing	Mean DO (mg/L)	Position of DO probe
Sauvignon Blanc	30/03/10	Immediately	0.15	middle of tank
Sauvignon Blanc	30/03/10	+ 12 hours	0.01	middle of tank
Sauvignon Blanc	30/03/10	+ 19 hours	0.01	middle of tank
Semillon	31/03/10	+ 8.5 hours	0.85	top 10 cm of tank
Semillon	31/03/10	+ 8.5 hours	0.04	bottom half tank
Chardonnay WBP no SO_2	01/04/10	+ 8.5 hours	0.03	top 10 cm of tank
Chardonnay WBP no SO_2	01/04/10	+ 8.5 hours	0.02	bottom 10 cm
Riesling 30' maceration	01/04/10	+ 4 hours	0.03	top 10 cm of tank
Riesling 30' maceration	01/04/10	+ 4 hours	0.02	bottom 10 cm
Riesling WBP inert press	01/04/10	Immediately	0.02	top 10 cm of tank
Riesling WBP inert press	01/04/10	Immediately	0.02	middle of tank
Chardonnay WBP	03/04/10	Immediately	0.02	top 10 cm of tank
Chardonnay WBP	03/04/10	Immediately	0.02	bottom 10 cm

WBP – whole bunch pressing

for oxygen pick-up across the juice processing chain compared to the total oxidative potential of the must and juice means that the oxygen actually dissolved will be the limiting factor, explaining the very low levels observed after pressing.

Conclusions

This limited survey of DO in must and juice has finally given actual numerical values for DO during juice processing. In doing so, the crushing step has been identified as a potential source of oxidative damage for aromatic grape varieties. It also indicated that the care taken in juice handling after the press can also be a factor for oxygen uptake, adding these areas as potential critical control points in the winemaking process. Ultimately, the wine style is chosen by the winemaker and the processing decisions used. To achieve fresh, vibrant wines by reductive techniques, protection of the whole juice processing chain needs to be considered.

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Current literature

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Oenology

General

189.01

Tracy, R., Skaalen, B. Wine microbe susceptibility is variable. Pract. Winery Vineyard 31(3), 41–45; 2010.

Currently, the wine industry adds a variety of chemical antimicrobial agents during the winemaking process to effectively manage the growth of spoilage microbes in product (juice, must, wine). Wine spoilage microbes can also be physically removed by methods such as filtration, but this column will only focus on chemical additives to control spoilage populations. Selecting the correct antimicrobial agent(s) and the appropriate concentration is complicated, because environmental conditions of wine vary, wine chemistry varies, the antimicrobial activity of chemical additives is strongly dependent on environmental and chemical conditions, and the susceptibility of wine microbes to each antimicrobial agent can be quite variable. This column discusses the most common antimicrobial agents currently used to manage spoilage microbes in wines (sulfur dioxide, dimethyl dicarbonate, sorbic acid, and lysozyme) and the variability of inhibitory properties associated with each preservative/sterilant.

189.02

Charlton, A.J., Wrobel, M.S., Stanimirova, I., Daszykowski, M., Grundy, H.H., Walczak, B. Multivariate discrimination of wines with respect to their grape varieties and vintages. Eur. Food Res. Technol. 231(5), 733–743; 2010.

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189.03

Howard, C. Oak barrels – looking after your investment. Aust. N.Z. Grapegrower Winemaker 559, 99–100; 2010.

Purchasing oak barrels is a major financial investment, as well as a major wine quality investment. You have made a purchasing decision on items that you will be using for the next four years, or perhaps longer, and in that time these barrels will be influencing the flavour and structure of four or more vintages of your wines. It is therefore essential that you look after your barrels properly during this time so that they remain sound and free of spoilage issues.

189.04

Phillips, C. Increasing number of US wineries turn to multiple closures. Aust. N.Z. Grapegrower Winemaker 559, 87–92; 2010.

Natural corks remain by far the most frequently used closure in the United States, and while technical corks remain the most widely used alternatives, screwcaps continue to make healthy gains to supplant synthetic closures as the second most widely used alternative to natural cork, according to results from *Wine Business Monthly*'s 2010 Closure Survey.

189.05

Berna, A.Z., Linton, G., Mahon, D., Trowell, S. Metal oxide sensors for grape and wine aroma analysis progress and prospects. Aust. N.Z. Grapegrower Winemaker 560, 86–92; 2010.

CSIRO has been investigating off-the-shelf E-nose technology for objective measurement of wine and grape aroma. In theory, E-noses have the ability to discriminate slight variations in complex mixtures, making this technique suitable for online process diagnostics and screening in many application areas. The two main components of E-nose are a sensing system and an automated pattern recognition system. The sensing system is an array of several different sensing elements. When the mixture of gases that occurs above a wine or crushed grapes is passed over the sensor array, the volatile organic compounds (VOCs) that are present produce a signature or pattern that is characteristic of the vapour. Until very recently, the most readily available electronic nose sensors based on metal oxide semi-conductors (MOS), could not be used with any alcoholic beverage. Furthermore, E-noses have limited discriminating power and cannot match the performance of a human nose. In this article the authors describe some recent developments in electronic nose technology and their applications to the Australian grape and wine industry.

189.06

He, J., Oliveira, J., Silva, A.M.S., Mateus, N., De Freitas, V. Oxovitisins: a new class of neutral pyranone-anthocyanin derivatives in red wines. J. Agric. Food Chem. 58(15), 8814–8819; 2010.

A new class of stable yellowish pigments with similar unique spectral features, displaying only a pronounced broad band around 370 nm in the UV-vis spectrum, was detected in an aged Port wine fraction obtained by a combination of chromatography on TSK Toyopearl HW-40(s) and Polyamide resins. These compounds were identified by liquid chromatography-diode array detector/electrospray ionization mass spectrometry (LC-DAD/ESI/MS) and shown to be direct oxidative derivatives of carboxy-pyranoanthocyanins (vitisins A) by synthesis experiments performed in a wine model solution. Their structures were fully characterized by MS and NMR spectroscopy (^{1}H, gCOSY, gHSQC, and gHMBC) and found to correspond to R-pyranone-anthocyanins (lactone or pyran- 2-one-anthocyanins). Their formation involves first the nucleophilic attack of water into the positively charged C-10 position of vitisins, followed by decarboxylation, oxidation, and dehydration steps, yielding a new and neutral pyranone structure. The occurrence of these novel pigments in aged wines points to a new pathway involving anthocyanin secondary products (vitisins A) as precursors of new pigments in subsequent stages of wine aging that may contribute to its color evolution.

189.07

Schubert, M., Glomb, M.A. Analysis and chemistry of migrants from wine fining polymers. J. Agric. Food Chem. 58(14), 8300–8304; 2010.

Fining of wine is prerequisite for the long-term stability of wine. Methods used are based not only on natural products, for example, proteins, but also on synthetic polymers such as polyvinylpolypyrrolidone (PVPP). Recently, new materials have been developed to overcome the disadvantageous use of traditional bluefining. These include polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/ PVP) to combine the benefits of PVPP with selective binding of metals such as copper or iron. This work developed a HPLC-MS^2 method to monitor the potential migration of monomers and respective degradation products *N*-vinylimidazole, *N*-vinyl-2-pyrrolidone, imidazole, and 2-pyrrolidone in wine. Use of 0.5 g/L PVPP led to <83 μg/L 2-pyrrolidone in a wine model solution within 30 min, whereas PVI/PVP resulted in nondetectable quantities of 2-pyrrolidone and 18 μg/L imidazole. Unexpectedly, the analysis of 140 wines revealed 2-pyrrolidone as a natural constituent. Independent model incubations verified 4-aminobutyramide and 4-aminobutyric acid as the immediate precursors.

189.08

Allen, M. Just say no to GMOs. WBM September, p. 34; 2010.

This extract from the author's book, *The Future Makers: Australian Wines for the 21st Century*, argues against using genetically modified organisms in wine and suggests that the Winemakers' Federation should strengthen its position on this issue, not weaken it.

Juice and wine handling

189.09

Howard, C. Selecting a pump for your winery. Aust. N.Z. Grapegrower Winemaker 560, 116–117; 2010.

Before selecting a specific type of pump to purchase you need to consider what type of fluids you want to move around, both in and out of vintage. There are several types of pumps which can be used to move wine and juice, however only a few types of pumps are suitable to move must and lees. This article guides the reader through the various issues which need to be addressed when selecting a pump.

189.10

Jones, L. (ed) Varietal report: Cabernet Franc. Aust. N.Z. Wine Ind. J. 25(4), 63–86; 2010.

McLaren Vale Cabernet Franc grower and maker Martin Lightfoot introduces this varietal report, labelling the grape as 'one of the great red wine wonders'. Like the winemakers that succeed Martin in the later pages of this report, he champions Cabernet Franc as a stand-alone varietal, saying it need not always be hidden away in a blend. Other vignerons reporting on their experience with Cabernet Franc are Stephen Doyle of Bloodwood Wines; Matt Carter of Bulong Estate Winery; John Cruickshank of Cruickshank Wines; John Barnier of Goona Warra Vineyard; Paul Drogemuller of Paracombe Premium Wines; Neil Tuffield and Mark Standish of Swooping Magpie Wines; Paul Batten, Folkert Janssen and Luke Surman of Wild Dog Winery and Ian Northcott and Mark Swann of Howard Vineyard. A tasting of 13 Cabernet Franc wines follows the report.

189.11

Liger-Belair, G., Bourget, M., Villaume, S., Jeandet, P., Pron, H., Polidori, G. On the losses of dissolved CO_2 during champagne serving. J. Agric. Food Chem. 58(15), 8768–8775; 2010.

Pouring champagne into a glass is far from being consequenceless with regard to its dissolved CO_2 concentration. Measurements of losses of dissolved CO_2 during champagne serving were done from a bottled champagne wine initially holding 11.4 ± 0.1 g L^{-1} of dissolved CO_2. Measurements were done at three champagne temperatures (i.e., 4, 12, and 18 °C) and for two different ways of serving (i.e., a champagne-like and a beer-like way of serving). The beer-like way of serving champagne was found to impact its concentration of dissolved CO_2 significantly less. Moreover, the higher the champagne temperature is, the higher its loss of dissolved CO_2 during the pouring process, which finally constitutes the first analytical proof that low temperatures prolong the drink's chill and helps it to retain its effervescence during the pouring process. The diffusion coefficient of CO_2 molecules in champagne and champagne viscosity (both strongly temperature-dependent) are suspected to be the two main parameters responsible for such differences. Besides, a recently developed dynamic-tracking technique using IR thermography was also used in order to visualize the cloud of gaseous CO_2 which flows down from champagne during the pouring process, thus visually confirming the strong influence of champagne temperature on its loss of dissolved CO_2.

189.12

Patterson, T. Concrete ideas for winemaking. Wines Vines 91(7), 50–53; 2010.

Concrete fermentors are increasingly popular among artisan winemakers due to their micro-oxygenation and thermal inertia. Winemakers say that concrete fermentors impart no flavors of their own (unlike oak), but add richness and volume (unlike stainless steel). Three suppliers are delivering concrete tanks to California wineries. Small sizes cost several thousand dollars. While long lasting, concrete requires more care than steel, but it can represent cost savings over time when compared to new barrels.

189.13

Bellot, E., Chauffour, E., Gregoire, P., Maritaud, C. Productions of Rosé wines in the world. Prog. Agric. Vitic. 127(13-14), 296–306; 2010.

[French] Most of Rosés wines have a fruity character, with 'fresh fruit' and a good acidity allowing a sensation of freshness in the mouth. The color is an important element strongly linked to the ACT of purchase, so it has to be controlled. To combine all these criteria, several requirements are necessary in the vineyard, as the choice of varieties, the management of the vigor, the control of the irrigation or the choice of harvest date. The Rosé, often considered as a technological wine, can be a real 'terroir'

wine, like in Provence. During all the technical practices in the vineyard, the wine grower decides the profile of grapes. The Rosé winemaking is made by different ways. Most of the world production of Rosé wine is obtained by skin maceration of red grapes. The rest of the production is made by blending a red must/wine and a white must/wine. The quality of grapes and the Rosé winemaking influence the color, the aromatic constitution and the chemical composition of the final product. The control of all the winemaking process allows the producer to make the Rosé wine wished by the consumer. For some years, the Rosé wine seduces more and more producing countries of wines in the five continents. Because of the variability of the winemaking process and the lack of international definition of Rosés, this market becomes difficult to encircle. The USA, France, Italy and Spain concentrate about 85% of the world production and represent 70% of the world's wines consumption.

A translation of the above article is available at a charge of $5 per page.

Microbiology

189.14

Andujar-Ortiz, I., Pozo-Bayon, M.A., Garcia-Ruiz, A., Moreno-Arribas, M.V. Role of specific components from commercial inactive dry yeast winemaking preparations on the growth of wine lactic acid bacteria. J. Agric. Food Chem. 58(14), 8392–8399; 2010.

The role of specific components from inactive dry yeast preparations widely used in winemaking on the growth of three representative wine lactic acid bacteria (*Oenococcus oeni*, *Lactobacillus hilgardii* and *Pediococcus pentosaceus*) has been studied. A pressure liquid extraction technique using solvents of different polarity was employed to obtain extracts with different chemical composition from the inactive dry yeast preparations. Each of the extracts was assayed against the three lactic acid bacteria. Important differences in the effect of the extracts on the growth of the bacteria were observed, which depended on the solvent employed during the extraction, on the type of commercial preparations and on the lactic acid bacteria species. The extracts that exhibited the most different activity were chemically characterized in amino acids, free monosaccharides, monosaccharides from polysaccharides, fatty acids and volatile compounds. In general, specific amino acids and monosaccharides were related to a stimulating effect whereas fatty acid composition and likely some volatile compounds seemed to show an inhibitory effect on the growth of the lactic acid bacteria. These results may provide novel and useful information in trying to obtain better and more specific formulations of winemaking inactive dry yeast preparations.

189.15

Boss, P., Dennis, E. Grapes, the essential raw material determining wine volatile composition: it's not just about varietal characters. Aust. N.Z. Grapegrower Winemaker 560, 78–82; 2010.

In some cases, certain grape compounds can be produced by yeast via the metabolism of sugars and nitrogenous precursors, for example the amino acid valine. Therefore, the combination of the grape and yeast pools of these compounds will contribute to the volatile profiles of the wine. In several viticultural studies the authors found that, even with controlled winemaking procedures, the levels of many of these fermentation-derived volatile compounds varied, suggesting the different grape samples could alter their production. Therefore, the authors set out to identify those wine components, produced by yeast, that could be influenced by grape composition and report their findings in this paper.

189.16

Verginer, M., Leitner, E., Berg, G. Production of volatile metabolites by grape-associated microorganisms. J. Agric. Food Chem. 58(14), 8344–8350; 2010.

Plant-associated microorganisms fulfill important functions for their hosts. Whereas promotion of plant growth and health is well-studied, little is known about the impact of microorganisms on plant or fruit flavor. To analyze the production of volatiles of grape-associated microorganisms, samples of grapes of the red cultivar 'Blaufraenkisch' were taken during harvest time from four different vineyards in Burgenland (Austria). The production of volatiles was analyzed for the total culturable microbial communities (bacteria, yeasts, fungi) found on and in the grapes as well as for single isolates. The microbial communities produced clearly distinct aroma profiles for each vineyard and phylogenetic group. Furthermore, half of the grape-associated microorganisms produced a broad spectrum of volatile organic compounds. Exemplarily, the spectrum was analyzed more in detail for three single isolates of *Paenibacillus* sp., *Sporobolomyces roseus*, and *Aureobasidium pullulans*. Well-known and typical flavor components of red wine were detected as being produced by microbes, for example, 2-methylbutanoic acid, 3-methyl-1-butanol, and ethyl octanoate.

Wine and health

189.17

Kypri, K., Jones, C., McElduff, P., Barker, D. Effects of restricting pub closing times on night-time assaults in an Australian city. Addiction doi: 10.1111/j.1360-0443.2010.03125.x, 1–8; 2010.

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Potenza, M.N., de Wit, H. Control yourself: alcohol and impulsivity. Alcohol. Clin. Exp. Res. 34(8), 1303–1305; 2010.

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Beulens, J.W.J., Algra, A., Soedamah-Muthu, S.S., Visseren, F.L.J., Grobbee, D.E., van der Graaf, Y. Alcohol consumption and risk of recurrent cardiovascular events and mortality in patients with clinically manifest vascular disease and diabetes mellitus: the Second Manifestations of ARTerial (SMART) disease study. Atherosclerosis 212(1), 281–286; 2010.

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189.26

Patra, J., Taylor, B., Irving, H., Roerecke, M., Baliunas, D., Mohapatra, S., Rehm, J. Alcohol consumption and the risk of morbidity and mortality for different stroke types – a systematic review and meta-analysis. BMC Public Health 10, 258–269; 2010.

Background: Observational studies have suggested a complex relationship between alcohol consumption and stroke, dependent on sex, type of stroke and outcome (morbidity vs. mortality). We undertook a systematic review and a meta-analysis of studies assessing the association between levels of average alcohol consumption and relative risks of ischemic and hemorrhagic strokes separately by sex and outcome. This meta-analysis is the first to explicitly separate morbidity and mortality of alcohol-attributable stroke and thus has implications for public health and prevention. **Methods:** Using Medical Subject Headings (alcohol drinking, ethanol, cerebrovascular accident, cerebrovascular disorders, and intracranial embolism and thrombosis and the key word stroke), a literature search of MEDLINE, EMBASE, CINAHL, CABS, WHOlist, SIGLE, ETOH, and Web of Science databases between 1980 to June 2009 was performed followed by manual searches of bibliographies of key retrieved articles. From twenty-six observational studies (cohort or case-control) with ischemic or hemorrhagic strokes the relative risk or odds ratios or hazard ratios of stroke associated with alcohol consumption were reported; alcohol consumption was quantified; and life time abstention (manually estimated where data for current abstainers were given) was used as the reference group. Two reviewers independently extracted the information on study design, participant characteristics, level of alcohol consumption, stroke outcome, control for potential confounding factors, risk estimates and key criteria of study quality using a standardized protocol.

Results: The dose-response relationship for hemorrhagic stroke had monotonically increasing risk for increasing consumption, whereas ischemic stroke showed a curvilinear relationship, with a protective effect of alcohol for low to moderate consumption, and increased risk for higher exposure. For more than 3 drinks on average/day, in general women had higher risks than men, and the risks for mortality were higher compared to the risks for morbidity. **Conclusions:** These results indicate that heavy alcohol consumption increases the relative risk of any stroke while light or moderate alcohol consumption may be protective against ischemic stroke. Preventive measures that should be initiated are discussed.

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189.33

Liu, M., Liu, R.-H., Song, B.-B., Li, C.-F., Lin, L.-Q., Zhang, C.-P., Zhao, J.-L., Liu, J.-R. Antiangiogenetic effects of 4 varieties of grapes *in vitro*. J. Food Sci. 75(6), 99–104; 2010.

The purpose of this study was to investigate the inhibitory effects of grapes on the human umbilical vein endothelial (HUVE) cells' capillary tube formation and matrix metalloproteinase-2 (MMP-2) expression secreted into the medium. Four different grape varieties (Concord, Niagara, Chardonnay, and Pinot Noir) were extracted using 80% acetone and the extracts were stored at −80°C. The total amount of phenolics and flavonoids for each of the 4 grape varieties were determined by spectrophotometry. Grape extracts were co-cultured with HUVE cells on Matrigel and inhibitory effects on tube formation were observed under a microscope. The inhibitory effects of grape extracts on MMP-2 expression were examined by zymogram. All 4 grape varieties inhibited the tube formation of HUVE cells in a dose-dependent manner on Matrigel. Except for Chardonnay, the other 3 grape varieties completely inhibited secretion of MMP-2 at 20 mg/mL. There was a significant positive relationship between the total phenolics and flavonoids and antiangiogenetic activities. The grapes tested have the potential to inhibit angiogenesis mainly by their phenolics and flavonoids contents, which partly contribute to their cancer chemopreventive efficacy.

189.34

Li, C.I., Chlebowski, R.T., Freiberg, M., Johnson, K.C., Kuller, L., Lane, D., Lessin, L., O'Sullivan, M.J., Wactawski-Wende, J., Yasmeen, S., Prentice, R. Alcohol consumption and risk of postmenopausal breast cancer by subtype: the Women's Health Initiative Observational Study. J. Natl. Cancer Inst. 102(18), 1422–1431; 2010.

Abstract available online from http://jnci.oxfordjournals.org/content/102/18/1422.abstract

189.35

Kerr, W.C., Ye, Y. Relationship of life-course drinking patterns to diabetes, heart problems, and hypertension among those 40 and older in the 2005 U.S. National Alcohol Survey. J. Stud. Alcohol Drugs 71(4), 515–525; 2010.

Abstract available online from http://www.jsad.com

189.36

Skov, S.J., Chikritzhs, T.N., Li, S.Q., Pircher, S., Whetton, S. How much is too much? Alcohol consumption and related harm in the Northern Territory. Med. J. Aust. 193(5), 269–272; 2010.

Abstract available online at http://www.mja.com.au/public/issues/193_05_060910/sko10932_fm.html

189.37

Kupersmidt, J.B., Scull, T.M., Austin, E.W. Media literacy education for elementary school substance use prevention: study of media detective. Pediatrics 126(3), 525–531; 2010.

Abstract available online from http://pediatrics.aappublications.org/cgi/content/abstract/126/3/525

189.38

O'Leary, C.M., Nassar, N., Kurinczuk, J.J., de Klerk, N., Geelhoed, E., Elliott, E.J., Bower, C. Prenatal alcohol exposure and risk of birth defects. Pediatrics 126(4), 843–850; 2010.

Abstract available online at http://pediatrics.aappublications.org/cgi/content/abstract/peds.2010-0256dv1

189.39

Maxwell, J.R., Gowers, I.R., Moore, D.J., Wilson, A.G. Alcohol consumption is inversely associated with risk and severity of rheumatoid arthritis. Rheumatology 49(11), 2140–2146; 2010.

Abstract available online from http://rheumatology.oxfordjournals.org/content/49/11/2140.abstract

189.40

Mostofsky, E., Burger, M.R., Schlaug, G., Mukamal, K.J., Rosamond, W.D., Mittleman, M.A. Alcohol and acute ischemic stroke onset: the stroke onset study. Stroke 41(9), 1845–1849; 2010.

Abstract available online at http://stroke.ahajournals.org/cgi/content/full/41/9/1845

CL

Viticulture

General

189.41

Ahrens, W. Case study: using layers to rejuvinate old vines. Aust. N.Z. Grapegrower Winemaker 559, p. 30; 2010.

Aged vines represent a unique and valuable resource. Eutypa is a cronic disease of grapevines that is common in South Australia. The majority of aged vineyards have at least some affected vines, with some carrying a high prevalence of infection. Use of layering techniques can maintain clonal integrity, average vine age and productivity of the vineyard, while reducing the impact of Eutypa infection.

189.42

Hoare, T. What next for Australia's grapegrowers? Aust. Vitic. 14(4), 27–29; 2010.

Drawing on his experience in vineyard management, including use of 'alternative' varieties, Tony Hoare crystal ball-gazes to suggest what the future might look like for Australia's winegrape growers. In this issue's column, Tony makes the following points: what next for Australian vineyards? – pull out, mothball, persist or fine tune?; respected *Decanter* magazine wine columnist Andrew Jefford recommends that the Australian wine industry changes its direction after spending 14 months in the country; terroir – the future focus of Australian fine wine; natural wines – achieving them through the right varieties and vineyard sites and management strategy; alternative varieties – predicting the future consumer trends and experimenting to achieve something special; and innovate or perish – keeping ahead of the competition. New clones, technology and accessing information.

189.43

Jones, L. Author of frost user's guide draws on international experience for redraft. Aust. Vitic. 14(4), 38–39; 2010.

Winner of the Limestone Coast Wine Industry Council's inaugural Emerging Leaders Award, Hans Loder, assistant vineyard and technical manager of Coonawarra-based Katnook Estate, recently visited vineyards in the Marlborough region of New Zealand, the Sonoma Valley, Napa Valley, Washington State and Yakima Valley regions of the US, and Canada to learn more about frost protection methods. One of the outcomes from the knowledge gathering undertaken during the tour will be for Loder to redraft his frost protection user's guide, titled Frost protection in viticulture: a user's guide for south-east irrigators, which was first developed in late 2006 in response to the severe frost events in South Australia's south-east during spring of the same year.

189.44

Carbonneau, A. La viticulture tropicale mondiale: le point de son évolution au IIème symposium international des vins tropicaux, Petrolina, Brésil (25–28 Mai 2010). Prog. Agric. Vitic. 127(13–14), 281–283; 2010.

[French] Abstract not available for reproduction

A translation of the above article is available at a charge of $5 per page.

189.45

Carbonneau, A., Ojeda, H., Escudier, J.-L. Innovative production systems in sustainable viticulture, facing the climate and consumption changes. Prog. Agric. Vitic. 127(13–14), 284–289; 2010.

[French] Following series of researches on training systems (foldable Lyre, Minimal Pruning or simplified Pruning), disease resistant varieties, technologies for wine or new products processing, a synthesis of selected innovations which take in account the changes due to climate or consumption behaviours is proposed. An experimental design of different models of production systems which integrates the previous innovations is necessary in the new context of a sustainable viticulture defined for the Mediterranean zone of France.

A translation of the above article is available at a charge of $5 per page.

Vine propagation and improvement

189.46

Gregutt, P. Clonal diversity improves quality: Washington vineyard trials prove the point with Cabernet Sauvignon. Vineyard Winery Manage. 36(5), 25–28; 2010.

Clonal trials are critical to improving wine quality. In Washington, numerous clones of Cabernet Sauvignon are being tried. Milbrandt's trials at Northridge tested five clones over three vintages. No single clone is best, but a mix of clones is a blending advantage.

Pests and diseases

189.47

Austin, C.N., Wilcox, W.F., Wicks, T. Influence of sunlight on powdery mildew severity in vineyards. Aust. N.Z. Grapegrower Winemaker 560, 58–63; 2010.

The author carried out studies on powdery mildew in South Australia from 2007–2009. The results of some of these studies on the effect of sunlight on the development of powdery mildew conducted in the USA and South Australia are described in this article.

189.48

Barnes, A.M., Sandhu, H.S., Wratten, S.D. Biodiversity in vineyards: worth the bother? Aust. N.Z. Grapegrower Winemaker 560, 25–33; 2010.

'Weeds' can be good, as can native Australian plants, as long as it's the right plant in the right place in the right number – and with the right economics as part of the package. Such noncrop plants can deliver a range of benefits to the vineyard, ranging from reducing variable costs to supporting wine tourism. In fact, all ecosystems, 'natural' or highly modified, provide a multitude of functions that benefit humankind. When values to mankind are assigned to these ecosystem functions they are known as ecosystem services (ES) or nature's services. Valuable ES include pollination, soil formation, flood mitigation, carbon capture, biological control, tourism and aesthetics. This paper reports on several projects in New Zealand which promote biodiversity in agriculture.

189.49

Godfrey, D., Scott, E.S., Grbin, P.R., Wicks, T.J., Crisp, P., Bruer, D. Control of grapevine powdery mildew using milk, oils and other natural materials. Aust. N.Z. Grapegrower Winemaker 559, 26–29; 2010.

The authors report on experiments to examine the efficacy of spray programs involving milk, whey and vegetable oils in a commercial organic vineyard in Langhorne Creek, South Australia, and a conventionally managed vineyard at the Waite Campus at Urrbrae, SA. The effects of selected treatments on juice and wine quality are also reported. A new project initiated in 2009, with funding from the Australian Research Council, provides the opportunity to investigate the effects of milk on powdery mildew. The principal constituents of bovine milk are water (88.3%), lactose (4.6%), fat (3.2%) and proteins (3.2%). Milk also contains biologically active components to protect the suckling young from infection. However, the antimicrobial activity of milk components against plant pathogens is poorly understood. This research, being conducted by Dr Dale Godfrey, examines the mechanisms by which selected milk components affect the fungus.

189.50

Monis, J., Constable, F., Habili, N. Highlights of the 16th meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (Dijon, France, 2009). Aust. N.Z. Grapegrower Winemaker 560, 46–51; 2010.

The 16th Meeting of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG) was held in Dijon, France between August 31 and September 4, 2009. The ICVG meeting is held once every three years to promote collaboration and interaction amongst grapevine pathologists who specialize in viruses, viroids, and phytoplasmas. There were 10 main sessions in which a broad range of research was presented. These included: introductory keynotes; detection, plant material and virus sources; Fanleaf, Fleck and other spherical viruses; epidemiology – survey of vineyards; phytoplasmas; molecular biology – new technologies; virus effects – Control-Crop performances; viruses of the Leafroll Disease Complex; Rugose Wood Complex viruses; emerging diseases and diseases of unclear etiology. This article is a summary of the research presented during each session.

189.51

Radford, S. Gearing up (again) for powdery mildew control – some practical tips. Aust. N.Z. Grapegrower Winemaker 559, 42–44; 2010.

This article offers some practical advice on controlling powdery mildew.

189.52

Radford, S. Choosing the best bird control options for your vineyard. Aust. N.Z. Grapegrower Winemaker 560, 64–66; 2010.

This article discusses the various devices which can be used to control birds in vineyards.

189.53

Walker, G. Stimulation of vine growth from incorporation of organic materials in root-knot nematode infested vineyard soil. Aust. N.Z. Grapegrower Winemaker 560, 19–20; 2010.

The effects of applying organic materials to soil were evaluated in a vineyard with stunted, unthrifty vines carrying high populations of root-knot nematode (RKN). Lucerne pellets incorporated into soil produced the highest numerical increases in vine shoot length, scion girth and trunk diameter, equivalent to 29% and 14% increases respectively in scion girth and trunk diameter over a single season compared with untreated vines. Shoot elongation was also stimulated following incorporation of an organic compost into soil, but not when it was applied as a mulch. RKN populations declined in soil adjacent to the organic materials (especially organic compost) six weeks after their incorporation, but did not decline in untreated or mulched soil. Lucerne pellets and compost provide nutrients both for vines and for beneficial soil organisms, and their growth stimulatory effects are due only in part to reductions in nematode numbers. These results are encouraging, and suggest that further work needs to be done to confirm that organic materials stimulate vine growth when incorporated into soil in existing vineyards, and to reduce plant parasitic nematode populations.

189.54

Wicks, T. Powdery mildew fungicides and fungicide resistance management programs. Aust. N.Z. Grapegrower Winemaker 560, 54–56; 2010.

In Australia, there is no shortage of options on the chemicals to use to control powdery mildew as over 50 products are registered. This includes a range of fungicides with active ingredients that cover six different modes of activity. This diverse range of products, each with different active ingredients and many with similar modes of action, can be confusing when deciding on the appropriate chemicals to apply throughout a growing season. This article reviews the various activity groups of fungicides.

189.55

Mehofer, M., Hanak, K., Diwald, M., Regner, F. Investigations into botrytis control in organic-biological viticulture. Mitt. Klosterneuburg Rebe Wein 59(4), 189–198; 2009.

Abstract not available for reproduction

Nutrition, soil and water

189.56

Edelstein, M., Plaut, Z., Ben-Hur, M. Water salinity and sodicity effects on soil structure and hydraulic properties. Adv. Hort. Sci. 24(2), 154–160; 2010.

Abstract available online from http://digital.casalini.it/15921573

189.57

Rieger, T. Vine sensor technologies: measuring grapevine water use via sap flow. Vineyard Winery Manage. 36(5), 46–50; 2010.

Grapevine water use can be highly variable, and differs by grape variety and rootstock. Vine sensor technologies that directly measure vine water status and sap flow are being tested and used in commercial vineyards. Sap flow sensors offer the potential to make better irrigation decisions that improve grape quality and avoid unnecessary irrigations. Researchers are using vine sap flow sensors to understand differences in water use between different grape varieties and rootstocks.

Canopy management

189.58

Winter, E., Lowe, S., Bulleid, N., Braybrook, D., Aldridge, M. Monitoring fruit zone temperatures for optimum grape and wine quality. Pract. Winery Vineyard September/October, 26–36; 2010.

This article presents data regarding fruit zone temperatures.

189.59

Bubner, R.M., Moran, M.A., Sadras, V.O. Effects of elevated daytime temperature on berry sensory attributes of Shiraz, Cabernet Franc, Semillon and Chardonnay. Aust. N.Z. Grapegrower Winemaker 560, 41–44; 2010.

The balance of grape sensory properties is important to achieve desired outcomes in finished wine. Indirect evidence suggests that warming trends over the last two decades might have decoupled berry composition, and that this decoupling has had direct consequences for wine attributes. Here we report direct measurements of sensory attributes of four grapevine varieties associated with a sustained daytime increase in temperature between 1 and 4°C.

Varieties

189.60

Jones, L. Mediterranean varieties finding their place under the Australian sun. Aust. N.Z. Wine Ind. J. 25(4), 17–18; 2010.

The *Wine Industry Journal* attended several workshops at the recent Australian Wine Industry Technical Conference, including the half-day event held on 3 July titled 'Emerging varieties from the Mediterranean and their potential for Australia', convened by Peter Dry, of The Australian Wine Research Institute, and Nick Dry, of Yalumba Nursery. This article reports on some of the wine-focussed elements of the workshop.

189.61

O'Keefe, G. Teroldego brings royal rewards. Aust. N.Z. Wine Ind. J. 25(4), 24–26; 2010.

Known as the 'royal wine of Trentino', Teroldego is an indigenous variety of the Trentino Alto Adige region, in northern Italy. In this article the author shares the viticultural and winemaking experiences of Michelini Wines who have been making wines from Teroldego since 1851.

189.62

Brook, S. Vagaries of Viognier. Decanter 35(12), 58–61; 2010.

Abstract not available for reproduction

189.63

Gregutt, P. Abacela Winery: putting Tempranillo on the map. Vineyard Winery Manage. 36(5), 32–38; 2010.

Abacela Winery in the US state of Oregon has specialised in the production of Tempranillo wines. This article provides an in-depth look at the winery and discusses the planting of the Tempranillo vineyards and the various clones used.

189.64

Spring, J.-L., Zufferey, V., Verdenal, T., Viret, O. Water supply and behaviour of Pinot Noir vines in the vineyard of Chamoson (VS). Rev. Suisse Vitic. Arboric. Hortic. 42(4), 258–266; 2010.

[French] Four plots have been planted with homogeneous material of Pinot Noir cultivar on typical soils of Chamoson area (Valais, Switzerland). Conducted from 1997 to 2000, this research allowed pointing out the influence of water nutrition on agronomical and oenological potential of Pinot Noir. In a situation without water restriction, vigour was clearly higher and budburst was delayed. In the must, soluble solids content was lower in absence of water constraint, while malic acid and nitrogen contents were higher. Meanwhile, pH remained relatively constant due to higher potassium content. In fact, any situation inducing regular and moderate water restriction during ripening did lead to wines with more polyphenols and more qualitative tannins, which were preferred by the panel of tasters.

A translation of the above article is available at a charge of $5 per page.

189.65

Bulleid, N. It's time for Tempranillo. WBM September, 28–29; 2010.

This article shares the enthusiasm demonstrated by TempraNeo, a group dedicated to the promotion of Tempranillo in Australia.

189.66

Yes GUR, that's my baby. WBM September, 52–54; 2010.

Adelaide Hills winery Hahndorf Hill has just released its debut vintage of Grüner Veltliner. In this article part-owner Marc Dobson talks about the variety that is starting to make waves in the Australian wine industry.

AWRI publications

1222

Holt, H.E., Birchmore, W., Herderich, M.J., Iland, P.G. Berry phenolics in Cabernet Sauvignon (*Vitis vinifera* L.) during late-stage ripening. Am. J. Enol. Vitic. 61(3), 285–299; 2010.

Abstract available online at http://ajevonline.org/cgi/content/abstract/61/3/285

1223

Bartowsky, E.J., Stockley, C.S. Histamine in Australian wines—a survey between 1982 and 2009. Ann. Microbiol. doi 10.1007/s13213-010-0070-z, 1–6; 2010.

Biogenic amines are found in a range of fermented foods and beverages, including wine. Absorption of these compounds in elevated concentrations may induce headaches, gastro-intestinal and respiratory distress. The main biogenic amines found in wine are histamine, tyramine, cadaverine and putrescine. Even though concentrations of histamine in wine are generally ten-fold lower than found in some fresh and other fermented foods, their presence may contribute to an adverse reaction when consumed in combination with other histamine-containing foods. It is well established that the main contribution of biogenic amines in wines is from lactic acid bacteria metabolism, especially during or after malolactic fermentation (MLF). A survey for histamine content of Australian red and white wines produced during 1982–1990 demonstrated a wide range of concentrations (mean 1.58 and 0.21 mg/L, respectively). A second survey of histamine content in red and white wines produced during 2003–2009 (mean 1.75 and 0.59 mg/L, respectively) showed that there were minimal changes in the mean histamine concentration over the period of the two sets of wines. All 238 Australian wines from 1982–1990 and 99 of 100 wines from 2003–2009 were below the former regulatory recommended limit of 10 mg/L for histamine in wine and were low compared to other wine-producing countries. Seven other biogenic amines measured in the Australian wines from 2003–2009 also had low means compared to other wine-producing countries.

1224

Borneman, A.R., Bartowsky, E.J., McCarthy, J., Chambers, P.J. Genotypic diversity in *Oenococcus oeni* by high-density microarray comparative genome hybridization and whole genome sequencing. Appl. Microbiol. Biotechnol. 86(2), 681–691; 2010.

Abstract available online at http://www.springerlink.com/content/w8gvu080336666jt/

1225

Ristic, R., Bindon, K., Francis, L.I., Herderich, M.J., Iland, P.G. Flavonoids and C13-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. Aust. J. Grape Wine Res. 16(3), 369–388; 2010.

Background and Aims: This study investigated flavonoid composition and C13-norisoprenoids (β-damascenone and β-ionone) in Shiraz grapes and wines, their relationships and links to wine sensory properties. **Methods and Results:** Differences in the grape berry flavonoid profile were created by exposing bunches to varying levels of sunlight intensity through canopy manipulation. Grapes were harvested at similar maturity and three replicate wines were made for each treatment in both vintages. Grapes produced under shaded canopy conditions had reduced anthocyanins and skin tannins, but little effect on seed tannins was observed. Pigmented polymers and tannins in wines were related to berry flavonoid composition (anthocyanins, skin and seed tannins, and their ratios). In grapes and wines, no significant effects were observed in response to canopy manipulation for two hydrolytically released C13-norisoprenoids, β-damascenone and β-ionone. Relationships were established for wine flavonoid composition, wine colour density, sensory perception of the astringency-related mouth-feel attributes and a quality scale. A positive relationship between wine quality score and hydrolytically released β-damascenone in both berries and wines was found, but not for free β-damascenone or any quantified forms of β-ionone. **Conclusion:** Higher concentrations of anthocyanins and skin tannins in berries, coupled with a lower concentration of seed tannins were associated with higher wine quality. The ratio anthocyanins*skin tannins/seed tannins is proposed as an indicator of wine flavonoid composition, wine colour and wine quality. Excessive canopy shade was detrimental to berry and wine composition and intensified sensory detection of 'straw' and 'herbaceous' characters in the wines. **Significance of the Study:** This study increases the understanding of the balance and composition of flavonoid compounds and C13-norisoprenoids in berries and their relationship with wine composition and wine sensory properties, but also highlights the importance of a canopy microclimate assessment.

1226

Blair, R. Key messages inspire AWITC delegates. Aust. N.Z. Grapegrower Winemaker 559, 55–61; 2010.

The 14th Australian Wine Industry Technical Conference was held in Adelaide from 3 to 8 July. This article provides a detailed synopsis of many of the papers presented at the conference.

1227

Coulter, A. Post-bottling spoilage – who invited Brett? Aust. N.Z. Grapegrower Winemaker 559, 78–86; 2010.

Post-bottling production of 4-EP can occur in wines that contain viable Brett cells. Even very small levels of residual sugar, levels that most winemakers would consider 'dry', can be utilised by Brett to produce levels of 4-EP that are well above the sensory threshold. If microbiological analysis reveals that a wine contains viable Brett cells, then the wine should be subjected to sterile, 0.45/µm membrane filtration before bottling. If the wine is sterile filtered and the bottling equipment downstream from the filtration unit is sterile, then it is extremely unlikely that you will be getting any visits from unwelcome guests.

1228

O'Brien, V., Forsyth, K. Technology: empowering your inner artisan. Aust. N.Z. Grapegrower Winemaker 560, 84–85; 2010.

This article puts forward an argument that suggests there are two ways to use process engineering or, more broadly, technology itself within the wine industry. Technology can help us make wine more reliably, efficiently and at lower costs, but the perceived hard edge of 'technology' is often associated with 'industrial' rather than 'artisan' winemaking. This is an unfair association. In addition to lowering costs, process engineering has enabled less intervention in the winemaking processes – therefore preserving more of the terroir and natural components in the grapes, removing the potential for bacterial and yeast contaminants and also minimising potential environmental impacts.

1229

Roget, W. Closing the debate: the AWRI 2010 red wine closure trial. Aust. N.Z. Grapegrower Winemaker 560, 106–107; 2010.

The Australian Wine Research Institute is about to embark on a new international closure benchmarking trial (AWRI 2010 red wine closure trial), designed to empower winemakers with technical information they can rely on to make informed decisions regarding their closure selection. This major trial will be conducted using a premium red wine matrix, with bottling scheduled to occur in late September 2010. The trial will utilise technically robust methodologies to ensure that both new and existing closure technologies are thoroughly and independently evaluated.

1230

Smart, R., Dambergs, B., Townsend, P., Pirie, A., Ravech, T., Sparrow, A. Pinot Noir clonal trials at Tamar Ridge. Aust. N.Z. Grapegrower Winemaker 559, 19–24; 2010.

This article presents a preliminary screening of Pinot Noir clonal trials conducted at Tamar Ridge in Tasmania.

1231

Blair, R. Delegates inspired by technical information in abundance at AWITC. Aust. N.Z. Wine Ind. J. 25(4), 11–16; 2010.

The 14th Australian Wine Industry Technical Conference was held in Adelaide from 3 to 8 July. This article provides a wrap-up of both the conference and Winetech 2010.

1232

Curtin, C., Chambers, P., Pretorius, S. Wine fermentation. Encyclopedio Biotechnol. Agric. Food , 689–694; 2011.

Abstract available online from http://www.informaworld.com/smpp/content~content=a924669178~db=all

1233

Stockley, C., Fox, A. Case study 3.2: Indigenous Australians and alcohol. In: Fox, A., MacAvoy, M. (eds.) Expressions of Drunkenness (Four Hundred Rabbits). Routledge, New York. pp. 100–119; 2010.

Abstract not available.

1234

Stockley, C., Saunders, J.B. The biology of intoxication. In: Fox, A., MacAvoy, M. (eds.) Expressions of Drunkenness (Four Hundred Rabbits). Routledge, New York. pp. 13–52; 2010.

Abstract not available.

1235

Mueller, S., Osidacz, P., Francis, I.L., Lockshin, L. Combining discrete choice and informed sensory testing in a two-stage process: can it predict wine market share? Food Qual. Pref. 21(7), 741–754; 2010.

An online discrete choice experiment was combined with a separate informed sensory hedonic test in a two-stage process to understand the interplay of wine sensory characteristics and extrinsic attributes such as packaging, price and brand awareness. This approach simulated the process of a consumer choosing a product from the shelf, tasting the product, and making a repurchase decision. The response measures were validated by relating them to market sales data. Twenty-one commercial Australian Shiraz red wines were characterised by a trained sensory panel. Four hundred and twenty-six regular wine consumers chose a wine for a dinner with friends from simulated shelves of the wines represented by photographs. Their choices were mainly a result of extrinsic wine attributes and the frequency of choice was found to be highly related to a wine's market share. The same consumers evaluated liking and made a repurchase decision in a central location tasting in an incomplete design, which included photos of each of the 21 wines. Price was found to be a strong positive driver of informed liking, and liking did not relate to the sales volume or to the initial choice in the online experiment. In contrast, the previously measured online choice was a strong predictor for repurchase with tasting, confirming that both product expectations at the initial purchase and intrinsic sensory attributes during product consumption substantially influence the repurchase decision. A number of common sensory characteristics were also positively and negatively related to both liking and repurchase intent. The study provided an insight into the relative importance of product expectation and actual sensory experience on informed repurchase intent. The combination of the discrete choice methodology with sensory descriptive data and consumer sensory testing shows promise.

1236

Bindon, K.A., Smith, P.A., Holt, H., Kennedy, J.A. Interaction between grape-derived proanthocyanidins and cell wall material. 2. Implications for vinification. J. Agric. Food Chem. 58(19), 10736–10746; 2010.

Proanthocyanidins (PAs) were isolated from the skins, seeds and flesh of commercially ripe grapes, and from wine and marc produced from the same source. In the grape berry, skin PAs accounted for 54% of the total extractable PA, while seed and flesh-derived PA accounted for 30% and 15% of the total, respectively. Following fermentation, 25% of the fruit PA was found in the wine, while 27% was found in the pericarp isolated from marc, and 48% was unaccounted for (either remaining in the seed or adsorbed to lees). To investigate the role that cell wall material (CWM) has on PA extraction during fermentation, CWM isolated from skin and flesh were combined with PA in model suspensions. In general, the affinity of flesh CWM for PA increased with increasing PA molecular mass (MM); however, this relationship was not observed for the interaction of skin CWM with skin PA. Subsequent experiments suggest that

the differences in the interaction of flesh and skin CWM with PA of higher MM (>15000 g/mol) may be limited by the structure of the CWM. Observed variations in the composition between skin and flesh CWM may explain the differences in PA interaction at high MM. Among wine-derived PA, no higher MM material was detected, suggesting that, during vinification, higher MM PA are nonextractable and/or are removed from the wine by interaction with CWM.

1237

Siebert, T.E., Solomon, M.R., Pollnitz, A.P., Jeffery, D.W. Selective determination of volatile sulfur compounds in wine by gas chromatography with sulfur chemiluminescence detection. J. Agric. Food Chem. 58(17), 9454–9462; 2010.

Volatile sulfur compounds can be formed at various stages during wine production and storage, and some may impart unpleasant 'reduced' aromas to wine when present at sensorially significant concentrations. Quantitative data are necessary to understand factors that influence the formation of volatile sulfur compounds, but their analysis is not a trivial undertaking. A rapid and selective method for determining 10 volatile sulfur-containing aroma compounds in wine that have been linked to 'offodors' has been developed. The method utilizes static headspace injection and cool-on-column gas chromatography coupled with sulfur chemiluminescence detection (GC-SCD). Validation demonstrated that the method is accurate, precise, robust, and sensitive, with limits of quantitation around 1 μg/L or better, which is below the aroma detection thresholds for the analytes. Importantly, the method does not form artifacts, such as disulfides, during sample preparation or analysis. To study the contribution of volatile sulfur compounds, the GC-SCD method was applied to 68 commercial wines that had reductive sensory evaluations. The analytes implicated as contributors to reductive characters were hydrogen sulfide, methanethiol, and dimethyl sulfide, whereas carbon disulfide played an uncertain role.

1238

Ugliano, M., Kolouchova, R., Henschke, P.A. Occurrence of hydrogen sulfide in wine and in fermentation: influence of yeast strain and supplementation of yeast available nitrogen. J. Ind. Microbiol. Biotechnol. doi 10.1007/s10295-010-0786-6, 1–7; 2010.

Abstract available online at http://www.springerlink.com/content/9056473hn052u176/

1239

Pretorius, S. Beyond value. WBM August, 66–67; 2010.

Value comes in many forms – some of which are tangible and some of which are not – but no less valuable to the wine sector. This article examines the forms of value and value drivers and cites work by experts in the field such as Göran Roos and Edward De Bono.

1240

Pretorius, S. Beyond leadership. WBM September, 50–51; 2010.

The idea that true leadership rejects hierarchy, and requires equality instead of authority is now considered sound corporate practice. In the wine business, this has particular importance. This article asks how can the wine sector work with each other sustainably and profitably to make new ideas a reality.

AWRI extension and roadshow calendar

December 2010

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AWRI Roadshows:

South West Aust Zone, Great Southern, WA – Mt Barker

- AWRI Packaging Workshop – 8 December 2010
- AWRI Seminar – 6 December 2010

South West Aust Zone, Great Southern, WA – Pemberton

- AWRI Packaging Workshop – 9 December 201
- AWRI Seminar – 7 December 2010

South West Aust Zone, Great Southern, WA – Margaret River

- AWRI Packaging Workshop – 10 December 2010
- AWRI Seminar – 8 December 2010

Greater Perth Zone, WA – Swan Valley

- AWRI Packaging Workshop – 6 December 2010
- AWRI Seminar – 9 December 2010

February 2011

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April 2011

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May 2011

AWRI Roadshows:

- Hunter Valley (1 seminar), Hunter Valley NSW
- Riverland (Renmark) & Sunraysia (2 workshops and 2 seminars), Lower Murray SA & Murray Darling VIC
- Rutherglen (1 seminar), North East Victoria VIC

June 2011

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AWRI Roadshows:

- Adelaide Hills & Langhorne Creek (1 seminar), Mount Lofty Ranges & Fleurieu SA
- McLaren Vale (1 seminar), Fleurieu SA
- Rutherglen (1 workshop), North East Victoria VIC

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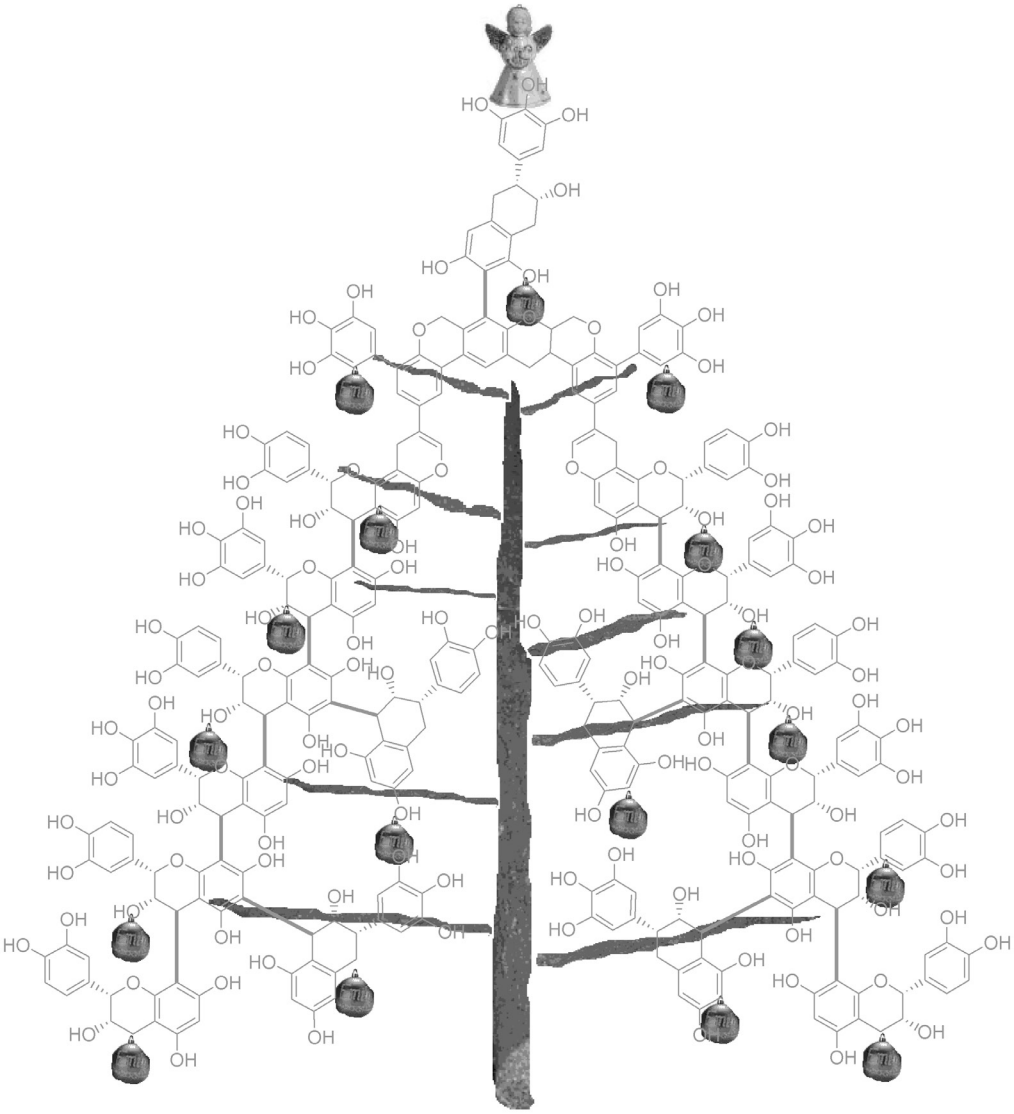

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Terpene Compounds as Possible Precursors of 1,8-Cineole in Red Grapes and Wines

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While the contribution of 1,8-cineole to the aroma of wine has been reported, it is a matter of controversy that the vineyards producing such wines are surrounded by *Eucalyptus* trees, which may contribute their essence to the grapes. However, experimental information presented in this paper suggests that 1,8-cineole can be produced by chemical transformation of limonene and α-terpineol, and this process may be responsible for the occurrence of *Eucalyptus*-like aroma in Tannat wines from vines not grown in the vicinity of *Eucalyptus* trees. A mechanism for the chemical transformation of these aroma compounds is proposed.

KEYWORDS: ***Eucalyptus*** **aroma; 1,8-cineole; limonene degradation; Tannat red wine**

INTRODUCTION

Wine aroma depends on numerous factors, with special importance being given to the variety of grape, vinification, maturation, and aging (*1*−*3*). It is well-known that the secondary metabolites of grapes are responsible for the principal aroma compounds in grape must and provide the basis of varietal character (*1*, *2*, *4*). Numerous studies on the volatile compounds of *Vitis vinifera* wines, as reviewed by Strauss (*5*), Versini (*2*), and Rapp (*3*), helped to elucidate the basic flavor chemistry in this field of special interest. Fermentation increases the chemical and aroma complexity of wine by assisting in the extraction of compounds from solids present in the grape must, modifying some grape derived compounds, and producing a substantial amount of yeast metabolites (*6*).

Enormous efforts have been focused on the topic of varietal characterization (*3*), for which is necessary to understand the influence of specific compounds on the total flavor impression. A good example of this approach is the identification of monoterpenoids, compounds with strong sensory qualities and present in a diverse range of plants such as *Vitis vinifera* varieties. Monoterpenols, particularly linalool, geraniol, and nerol, are responsible for the characteristic floral aroma in grapes and wines of *V. vinifera* cultivars such as Muscat, Gewürztraminer, and Riesling (*7*). Acid-catalyzed rearrangements during wine processing and aging can also result in changes in concentration and formation of new compounds that were not present in the original grapes and young wines (*8*, *9*). Moreover, in grapes, terpenoids exist in both free and glycosidically bound forms (*10*), and some of the bound terpenoids may be released either chemically (*11*, *12*) or by natural glycosidase activities of the grape or of yeast and bacteria during the vinification phases (*13*).

In wine tasting, the term "*eucalyptus*" describes a spicy, mintlike aroma of certain red wines. The typical eucalyptus odor (fresh, camphoraceous, cool) usually is related to the monoterpene compound 1,8-cineole (1,3,3-trimethyl-2-oxabicyclo-[2.2.2]octane), commonly known as eucalyptol (*14*). It was recently reported that vineyards producing such wines are surrounded by *Eucalyptus* sp. trees, which may contribute their essence to the grapes (*14*). In this paper, we provide evidence suggesting that the presence of 1,8-cineole in wines can arise from precursors typical of the grape itself, like limonene. Thus, we may be able to explain the eucalyptus-like scent also in wines coming from vineyards far away from *Eucalyptus* tree cultivation as observed in the case of Tannat wines, described frequently as mint-like-flavored.

1,8-Cineole, and chemically related compounds, have been quantified by GC/MS/SIM in monovarietal Tannat wines and single varietal grape samples of this variety harvested from southern Uruguayan vineyards. We also report the results of deuterium-labeling experiments that identify the sequence of chemical rearrangements that convert limonene to 1,8-cineole through reaction steps which involve the cyclization of *trans*-1,8-terpine catalyzed by the temperature and acidic conditions that can be encountered by grapes and wines.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Limonene, α-terpineol, 1,8-terpine, and 1-heptanol were purchased from Aldrich (Milwaukee, WI).

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Table 1. Changes during Thermal Conditioning in Synthetic Wines Fortified with Limonene or α-Terpineol, and in Tannat Wine with No Added Limonene or α-Terpineol

	limonene μg/L (±SE)[a]	α-terpineol μg/L (±SE)[a]	*trans*–1,8-terpine μg/L (±SE)[a]	*cis*–1,8-terpine μg/L (±SE)[a]	1,8-cineole μg/L (±SE)[a]
synthetic wine added with limonene (0.5 mg/L)	4.6 ± 0.3	4.4 ± 1.1	7.5	57.8 ± 12.3	24.1 ± 3.2
synthetic wine added with α-terpineol (0.5 mg/L)	3.4 ± 0.9	23.5 ± 2.2	50.8 ± 2.9	245.8 ± 52.3	8.4 ± 0.3
wine	2.3 ± 1.3	6.7 ± 0.8	21.4 ± 4.8	41.6 ± 16.1	13.7 ± 4.7

[a] SE = standard error around the mean ($n = 2$).

1,8-Cineole (99.8%) was purchased from the Center of Agroindustrial Technology (Cochabamba, Bolivia). Analytical grade solvents, dichloromethane (HPLC quality) and deuterium oxide (D_2O), were purchased from Sigma-Aldrich (Milwaukee, WI).

Sample Preparation. Tannat grapes and wines, of different vintages, were sourced from experimental and estate vineyards, mostly located in the region of the southern part of Uruguay away from the influence of *Eucalyptus* trees.

Chemical Transformation Models. Samples of 60 mL of each Tannat and synthetic wine (12.9% v/v hydroalcoholic solution containing 3.5 g/L of tartaric acid, 2.5 g/L of malic acid, 60 mg/L of sodium metabisulfite, adjusted to pH 3.2 with sodium hydroxide, with 0.5 mg/L of limonene or α-terpineol), sealed under nitrogen in vials, were heated at 45 °C for 20 days. Afterward, each sample was extracted and analyzed by GC. All experiments were performed in duplicate. An oven at controlled temperature monitored by a standard thermometer was used.

Isolation of Volatiles. *(a) Volatiles in Grapes.* Fifty grams of grape berries was harvested, at different ripening stages, from several grapevines selected randomly in the vineyards studied. Grape seeds were carefully removed from the frozen berries, and the pulp and the skins were extracted by sonicating for 5 min an aqueous solution of $CaCl_2$ (4%), then adding with 0.10 mL of internal standard (1-heptanol at 274 ppm in a 95% v/v ethanolic solution) and extracting by hand-shaking with 3 × 15 mL of dichloromethane. The organic phases were then separated, dried over sodium sulfate, and concentrated as described below.

(b) Volatiles in Wines and Synthetic Wines. Samples of wine and synthetic wine (60 mL containing 0.12 mL of the internal standard solution) were extracted as previously described. The organic phases were dried over sodium sulfate and then concentrated to 1.5 mL on a Vigreux column, stored at −10 °C, and, immediately prior to GC-MS analysis, further concentrated to 100 μL under a gentle nitrogen stream.

Identification. GC-MS analyses were performed using a Shimadzu GC-17 gas chromatograph coupled with a Shimadzu QP 5050 mass spectrometer (70 eV; acquisition mass range: 40−400 amu) supported by reference libraries (*15*, *16*) and equipped with a polar BP 20 (SGE, Australia) bonded fused-silica capillary column (25 m × 0.25 mm i.d. × 0.25 μm film thickness) with the following working parameters: injector temperature: 250 °C; interface temperature: 250 °C; carrier gas He: 92.6 kPa (55.9 cm/s); oven conditions: 8 min at 40 °C, 3 °C/min to 180 °C and 10 °C/min to 220 °C, 20 min at 220 °C; injection mode: split 1:40; injection volume, 1.0 μL. The identification of the compounds was confirmed by injection of pure standards and comparison of their retention index and relevant MS-spectra, while in SIM analysis by considering different typical fragments in a specific relevant ratio.

Quantitative Analysis. *(a) Thermal Conditioning Experiments in Wines and Synthetic Wines.* Volatile compounds (limonene, α-terpineol, *cis*- and *trans*-terpine, 1,8-cineole) were quantified by GC in the same experimental conditions as previously described for GC/MS, by the internal standard method using 1-heptanol (added as 2 mL/L of a 95% v/v ethanolic solution of 0.274 g/L) without consideration of calibration factors, that is, $F = 1.00$ for all compounds.

(b) Grapes and Wines. 1,8-Cineole was quantified by GC/MS/SIM comparing the area of the ion peak at *m*/*z* 81 with that of internal standard, *m*/*z* 70. A linear calibration curve ($r^2 > 0.96$; three replicate analyses at each concentration) was obtained when synthetic wine was treated with a 1,8-cineole standard solution (final concentration between 0.2 and 6.0 μg/L) and submitted to the same extraction and analysis procedures.

Degradation Mechanism of Limonene and α-Terpineol. Samples of 20 mL of synthetic wines, prepared as previously described but with 10% (v/v) D_2O in place of water and with 0.5 mg/L of limonene or α-terpineol, were heated at 45 °C under nitrogen for 20 days. The percentage of deuterium incorporated into limonene and 1,8-cineole was determined by GC/MS/SIM following the ion peaks at *m*/*z* 121/122 and 136/137 for limonene/limonene-*9*-*d*, and *m*/*z* 154/155 for 1,8-cineole/1,8-cineole-*2*-*d* or 1,8-cineole-*9*-*d*. The analysis of 1,8-cineole-*2*,*9*-*d* (*m*/*z* 156) was not performed because of the low concentration of this species.

Determination of Olfaction Thresholds. The odor threshold value for 1,8-cineole was determined by triangle test (*17*) using a Tannat wine sample with added 1,8-cineole. The samples were presented in order of increasing concentrations (1.0, 2.0, 4.0, and 8.0 μg/L) to individual panel members (10 trained judges whose ages ranged from 23 to 40 years) for assessment on the basis of possible odor differences. The wines were presented in individual testing booths, and 60 mL of samples were served at 20 ± 1 °C in approximately 250 mL, clear, tulip-shaped wine glasses (ISO 3591, 1977) identified with two-digit random codes and covered with a watch glass. The lowest concentration corresponding to 50% correct identification by the panelists determined the threshold value (*18*, *19*).

RESULTS AND DISCUSSION

Mechanism of 1,8-Cineole Formation. The volatile compounds identified in Tannat wine samples and their concentration ranges have been reported by Boido et al. (*20*). The levels of the monoterpene compounds, particularly mono-oxygenated monoterpenes, were all under their sensory thresholds, as is usually found for wines from neutral cultivars; however, rather high contents of limonene were found (up to 250 μg/L) (*20*).

Synthetic wines and a Tannat wine (1,8-cineole or terpine isomers not detected) were fortified with limonene or α-terpineol (0.5 mg/L), then heated to 45 °C for 20 days. The results, as reported in **Table 1**, show the presence of 1,8-cineole and both isomers of 1,8-terpine.

Previous studies have shown that α-terpineol can be formed from limonene under acidic conditions (*21*, *22*). Furthermore α-terpineol can be transformed into 4-(2-hydroxypropyl)-1-methylcyclohexanol (terpin hydrate or 1,8-terpine) (*23*) and this latter compound to 1,8-cineole (*24*). The reactions promoted by the temperature and acidic conditions are summarized in **Figure 1**. Hydration of limonene to form α-terpineol is followed by cyclization of *trans*-1,8-terpine, but not *cis*-1,8-terpine, to 1,8-cineole which may explain the higher content of the cis form shown in **Table 1**. The data in **Table 1** also show that the synthetic wine with added α-terpineol contains limonene even after 20 days, consistent with the reversible character of the reaction steps involved. Furthermore, other pathways involving epoxidation of the double bonds of limonene and α-terpineol (*25*) could be hypothesized, leading to a complex mixture of

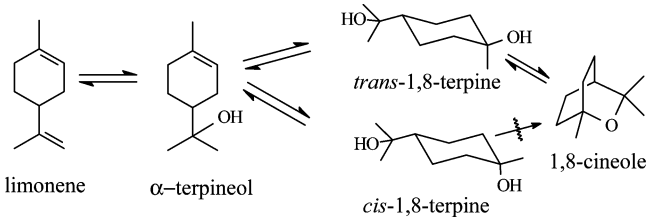

Figure 1. Mechanism proposed for 1,8-cineole synthesis from limonene in wines.

Table 2. Deuteration-Labeling Experiments for Limonene and α-Terpineol

synthetic wine		limonene added (0.5 mg/L)		α-terpineol added (0.5 mg/L)	
compound	*m/z*	% deuteration	±SE[a]	% deuteration	±SE
limonene	121/122	4.94	0.66	0.35	0.11
limonene	136/137	4.40	0.88	0.57	0.45
1,8-cineole	154/155	12.79	2.44	6.79	0.39

[a] SE = standard error around the mean ($n = 2$).

minor compounds (α-terpinolene, 1,4-cineole, 1-terpineol) detected in the synthetic wine in our experimental conditions.

Deuteration Experiments. To verify the production of 1,8-cineole and the reversible nature of the reactions involved, 20 mL samples of synthetic wines, prepared with 10% (v/v) D_2O in place of water, and with 0.5 mg/L of limonene or α-terpineol, were heated at 45 °C under nitrogen during 20 days.

The results of the deuterium-labeling experiments are reported in **Table 2**, showing values which were lower than those expected, indicating that the isotopic equilibrium was not attained. The extent of deuterium labeling is consistent with a sequence of chemical rearrangements from limonene to 1,8-cineole, through reaction steps which involve the cyclization of *trans*-1,8-terpine promoted by the temperature and acidic conditions that can be reached by grapes and wines.

These reactions were accelerated by addition of acetic acid to synthetic wines (data not shown), thus indicating dehydration as the slowest reaction step (*26*). These results suggest the presence of two different kinetics, explaining, as reported in **Table 2**, the greater amount of deuterated limonene produced in synthetic wine added with limonene standard after 20 days, by comparison with the results obtained from α-terpineol in the same experimental conditions. As proposed in **Figure 2**, limonene-*9-d* (**9**) is produced from limonene (**1**) as a consequence of only one dehydration step, while limonene-*2-d* (**5**) and limonene-*9-d* (**9**) are both obtained from α-terpineol (**2**) through two slow dehydration steps.

Moreover, the obtained results also made it possible to explain the relationships between the deuterated forms of 1,8-cineole found in our experimental conditions (**Table 2**): a higher deuteration level, almost twice, for 1,8-cineole obtained from limonene (**1**) by the synthesis of 1,8-cineole-*2-d* (**8**) or 1,8-cineole-*9-d* (**12**), by comparison with the results obtained from α-terpineol (**2**), where only one molecule of 1,8-cineole-*2-d* (**8**) was produced through the faster step of 1,8-terpine formation (**Figure 2**).

Quantitative Analysis of 1,8-Cineole in Grape and Wine. Tannat wines from vineyards growing out of the influence of *Eucalyptus sp.*, located in the Southern part of Uruguay and with particularly intense eucalyptus aroma, were analyzed. Wine volatiles were isolated and concentrated by extraction with dichloromethane, followed by GC/MS/SIM analysis. The 1,8-

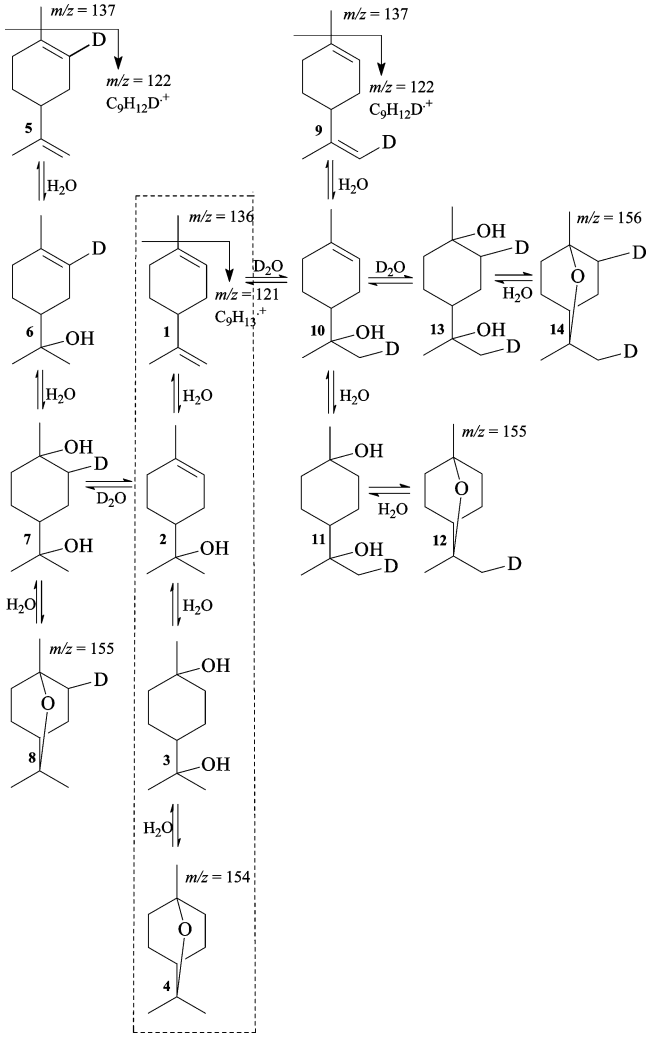

Figure 2. Equilibrium character of the reactions associated with the mechanism proposed for 1,8-cineole synthesis from limonene in wines. Deuterium labeling experiment.

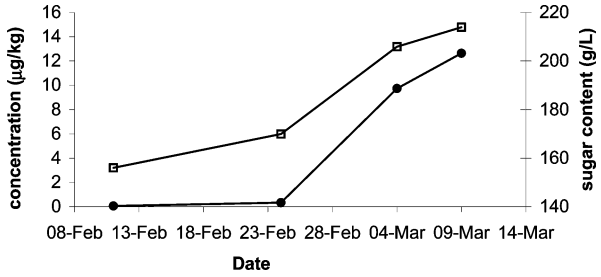

Figure 3. 1,8-Cineole contents in grape samples during ripening: (-●-) 1,8-cineole content; (-□-) sugar content.

cineole content in the samples of wine ranged from 1 to 5 μg/L. The analyses performed on grapes collected at different ripening stages showed higher contents of 1,8-cineole for samples at the end of the vintage (overripe fruit) as reported in **Figure 3**.

Determination of Olfaction Thresholds. Odor threshold value for the 1,8-cineole was determined by the triangle test (*17*) by using a Tannat wine sample added with 1,8-cineole as described under Materials and Methods. The sensory threshold value found for 1,8-cineole in Tannat wine was 1.3 μg/L, which is in agreement with literature data for a Merlot wine (*14*). This value was exceeded by the 1,8-cineole concentration found for many of the Tannat wines analyzed.

In conclusion, this study has identified a process by which "*eucalyptus*" aroma may develop in red wines of the Tannat variety, by formation of 1,8-cineole from limonene or α-terpineol. The explanation for these results can be found in the chemical rearrangements associated with limonene, which was shown to interconvert with 1,8-cineole at pH 3.2 and 45 °C. Furthermore, it was found that 1,8-cineole concentrations in grapes at the beginning of the ripening were very low, but showed an important increase throughout the ripeness. Finally, the studies with model wine showed that 1,8-cineole can be produced from limonene and α-terpineol under conditions related to those of red wine aging.

ABBREVIATIONS USED

GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; SIM, selected ion monitoring.

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Vineyard and Fermentation Studies To Elucidate the Origin of 1,8-Cineole in Australian Red Wine

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Supporting Information

ABSTRACT: Preliminary investigations revealed that the proximity of *Eucalyptus* trees to grapevines can directly influence the concentration of the aroma compound 1,8-cineole present in the corresponding red wines. For two different vineyards, the closer the grapevines were to the trees, the greater was the amount of 1,8-cineole in the wines elaborated from those grapes. This led us to carry out further studies to quantify the levels of 1,8-cineole found in grape berries, leaves, and stems at set distances from *Eucalyptus* trees over multiple vintages. Generally, the highest concentration of 1,8-cineole was found in the grapevine leaves, followed by grape stems and then grapes. In each sample type, we observed greater concentrations of 1,8-cineole in samples closer to the trees. Various fermentation treatments carried out with Shiraz grapes showed that matter other than grapes (MOG, e.g., *Eucalyptus* or grape leaves) could contribute significant amounts of 1,8-cineole to the finished wines. These studies confirmed that vineyard position and winemaking conditions can determine the 1,8-cineole concentration in red wine. The fermentation study also showed for the first time that the concentration of rotundone in red wine can be strongly influenced by grapevine leaves and stems in the ferment.

KEYWORDS: *1,8-cineole, GC−MS, Eucalyptus trees, rotundone, wine aroma, red wine*

INTRODUCTION

Australia is the native habitat of the *Eucalyptus* genus, but its home has expanded to many countries around the world, including China, India, and Brazil. Every continent apart from Antarctica has been populated by *Eucalyptus* trees.[1] There are over 850 species of *Eucalyptus* grown around the world, and they can prosper in diverse climates.[1] *Eucalyptus* trees have a multitude of uses in industries including cultivation of timber for construction, pulp, fuel, and essential oil production.[1] Most species of *Eucalyptus* contain volatile essential oils in their leaves, although the bulk of the world's *Eucalyptus* oil production comes from only six species.[2] Depending on the species, the main component (60−90%) of the oil from most of these *Eucalyptus* trees is 1,8-cineole, commonly known as eucalyptol.[2] *Eucalyptus* oils are present in numerous consumer goods, and 1,8-cineole has also been found as a component of red wine, where it has been described as "fresh", "cool", "medicinal", and "camphoraceous".[3]

The origin of 1,8-cineole in wine has not been verified, but several theories have been reported. Herve et al. proposed that the "eucalyptus" character in wines occurs when vineyards are adjacent to *Eucalyptus* trees,[3] whereas Farina et al. used hydrolytic studies to propose that terpene compounds such as α-terpineol and limonene were precursors of 1,8-cineole.[4] More recently, we showed that hydrolysis of limonene and α-terpineol at wine pH gave very low molar conversions into 1,8-cineole (<0.6%) over a 2-year period, which does not account for the concentration of 1,8-cineole in many young red wines.[5]

A study by Kalua and Boss[6] suggested that Cabernet Sauvignon grapes have a tendency to form 1,8-cineole, which was the major monoterpene found early in berry development but which decreased during ripening. This was contrary to the observations of Farina et al., who reported an increase in 1,8-cineole toward the end of berry ripening.[4] Kalua and Boss also found that 1,8-cineole was detected at similar levels in berries situated adjacent to *Eucalyptus* trees as at some distance from the trees,[7] which is in contrast with the proposal of Herve et al.[3] Kalua and Boss suggested that the existence of 1,8-cineole in berries may be attributable to the persistence of the compound from floral tissues, or, alternatively, the production of 1,8-cineole may be promoted by herbivore predation, as reported for other plant species (ref 7 and refs therein).

We recently conducted a survey of 190 commercially available Australian wines of mixed varieties, highlighting that 1,8-cineole was found in significant concentrations in red wines only.[5] We also showed that a continuous increase in the concentration of 1,8-cineole occurred during red wine fermentation but ceased once the wine was drained from the skins, indicating that the compound was extracted from the grape skins and/or matter other than grapes (MOG).[5] It was reasoned that the differences in winemaking techniques between red and white wines explained the absence of 1,8-cineole in the latter.[5]

A study by Saliba et al. indicated a consumer rejection threshold of 27.5 μg/L for 1,8-cineole in a red wine, and levels below this were deemed to be acceptable to consumers.[8] Another survey of consumers showed that on average the

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participants may have had a slight preference for a wine spiked with 1,8-cineole at 4 and 30 μg/L as compared to the unspiked wine containing 0.18 μg/L, with one cluster of consumers (38%) strongly preferring the wine spiked with 30 μg/L of 1,8-cineole.[9] Of more than 150 commercially available Australian red wines we had previously analyzed,[5] only two contained 1,8-cineole above 28 μg/L. Because 1,8-cineole is extremely stable in wine and is barely scalped by synthetic closures,[5] it would be advantageous to be able to clarify how this character arises in wine so 1,8-cineole profiles can be tailored to meet consumer demands.

Our previous results were the first demonstration of when 1,8-cineole was evolved during red winemaking,[5] but we also wanted to confirm the impact of vineyard variables to elucidate the origin of 1,8-cineole in red wine. Therefore, a detailed study of the relationship between grape composition and proximity to *Eucalyptus* trees was conducted over three vintages, and the impact of grape leaves/stems and *Eucalyptus* leaves contained in red wine fermentations was also investigated.

MATERIALS AND METHODS

Materials. Wine samples from Western Australia, Victoria, and Coonawarra were supplied by the producers. 1,8-Cineole was purchased from Sigma-Aldrich (Castle Hill, NSW, Australia), and $^2H_6$-1,8-cineole was synthesized as described in Capone et al.[5] Rotundone ((3*S*,5*R*,8*S*)-3,4,5,6,7,8-hexahydro-3,8-dimethyl-5-(prop-1-en-2-yl)-1(2*H*)-azulenone) was synthesized as described in Wood et al.,[10] and $^2H_5$-rotundone was synthesized as outlined in Siebert et al.[11] Stock solutions of standards were prepared volumetrically in redistilled ethanol and stored at −20 °C, and working solutions were stored at 4 °C until required. All chemicals were analytical reagent grade unless otherwise stated, and water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Merck solvents and sodium chloride (NaCl) were purchased from Rowe Scientific (Lonsdale, SA, Australia), and other chemicals were obtained from either Sigma-Aldrich (Castle Hill, NSW, Australia) or BDH (Kilsyth, VIC, Australia).

Grape Samples for Vineyard Study. Healthy Shiraz grapes (basic chemical data for each vintage appear in Supporting Information Table 1) were hand-harvested from the Padthaway region of South Australia one day prior to commercial harvest. This study was conducted over three vintages (2008, 2009, and 2011), in the same locations each year (±4 m using GPS). Triplicate samples were taken from three locations within four rows (i.e., 3 × 3 × 4 = 36 samples). Rows 1, 10, 20, and 60 were chosen, with row 1 being within about 5 m of a group of *Eucalyptus* trees and row 60 being the furthest away, around 125 m from the trees. Grape leaves were also collected from the same positions in 2009 and 2011, and *Eucalyptus* leaves were also taken in 2011 from the grapevine canopy in the first row for analysis and addition to ferment treatments. Polyethylene traps were installed in the vineyard in the same row sampling locations in 2008 and 2009.

Fermentation Treatments and Winemaking. Shiraz wines were prepared by a contracted research winemaker from grapes harvested from the first two rows (i.e., within 10−15 m of *Eucalyptus* trees) from the Padthaway vineyard. Hand-harvested fruit (approximately 550 kg) was collected and delivered to the winemaking facility and stored at 0 °C in a coldroom for 24 h. Fruit was randomized into 9 × 50 kg lots. Seven of these lots were crushed and destemmed, and duplicate batches were pressed to juice immediately through a 50 kg bag press under CO_2 (rosé treatment). The other five batches were used for the treatments with the addition of grapevine leaves and stems or *Eucalyptus* leaves and bark. Berries from the remaining two 50 kg lots were hand plucked from the stems and crushed to serve as duplicate controls (control). Each treatment replicate had 50 mg/L of SO_2 added as potassium metabisulfite (PMS) when crushed. The rosé treatment juices were transferred into 50 L stainless steel vessels in a 20 °C temperature controlled room, and the other 50 kg lots were transferred into 50 kg plastic drums with their skins. The duplicate control samples had no further additions prior to inoculation. Triplicate treatments had 500 g of grapevine leaves, which were obtained from the first row, and approximately 1.3 kg of grape stem (from the destemming process) added back into the ferments (grape leaf/stem treatment). The final duplicate treatments contained four *Eucalyptus* leaves (1 g total) and a small piece of *Eucalyptus* bark (3.5 g total) that were collected from within the grapevine canopy (eucalypt treatment). Because of the potential for *Botrytis* activity in vintage 2011, 200 mg/L of VR Supra tannin was added to the ferments (excluding rosé), and they were all supplemented with 100 mg/L diammonium phosphate and inoculated with 300 mg/L Maurivin EC1118 (PDM) wine yeast (Mauri Yeast Australia). All ferments were pressed and racked 4 days after inoculation and then put through malolactic fermentation (MLF, except the rosé). When MLF was complete the wine was racked off gross lees, 60 mg/L SO_2 was added as PMS, and the wines were cold stabilized at 0 °C in a coldroom for 72 h. The stable wine was adjusted to 80 mg/L of total SO_2 added as PMS and passed through a Z6 grade filter (polishing, nonsterile), then a 0.45 μm sterile membrane, and bottled under ROTE screwcap closures in 375 mL bottles (basic chemical data obtained after bottling can be found in Supporting Information Table 2).

Preparation of Samples for 1,8-Cineole Analysis. *Wines and Ferments.* An aliquot (50 μL) of an ethanol solution containing $^2H_6$-1,8-cineole (5.12 μg/mL) was added to the sample (10 mL) in a 22 mL amber glass screw cap SPME vial. A 5 mL aliquot of the sample was removed, and 5 mL of Milli-Q water was added to the vial. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand, then sealed and kept at 4 °C until GC−MS analysis. The ferment samples were placed in a water bath at 65 °C for 15 min before storage at 4 °C until GC−MS analysis.

Grapes. Approximately 1 kg of Shiraz grape berries from each replicate position was plucked from their stems and randomized into triplicate 200 berry lots, which were weighed and homogenized with a household stab mixer (Breville Wizz Stick). The homogenate was weighed out into 8 g lots in 22 mL glass screw cap vials with aluminum lined lids (Supelco, Australia). A 1 mL aliquot of redistilled ethanol was added to each vial along with an aliquot (50 μL) of an ethanol solution containing $^2H_6$-1,8-cineole (5.12 μg/mL), and vials were agitated on a shaker for up to 7 days (length of extraction time was found not to be critical). After shaking was complete, 9 mL of Milli-Q water was added to each vial, and shaking was continued for a further 3 h. Approximately 10 mL of the extract was removed into an amber 20 mL SPME vial, 2 g of NaCl was added, and samples were heated in a water bath at 65 °C for 15 min before storage at 4 °C until GC−-MS analysis.

Grape Stems. The stems from the destemmed grapes were weighed into approximately 50 g lots. The stems were finely cut using both secateurs and scissors and weighed out in triplicate 8 g lots in 22 mL glass screw cap vials with aluminum lined lids. A 1 mL aliquot of redistilled ethanol was added to each vial along with an aliquot (50 μL) of an ethanol solution containing $^2H_6$-1,8-cineole (5.12 μg/mL), and vials were agitated on a shaker for between 5 and 7 days. When shaking was complete, 9 mL of Milli-Q water was added to each vial, and shaking was repeated for 3 h. A 5 mL aliquot of the sample was removed into a 20 mL amber screw cap SPME vial, and 5 mL of Milli-Q water was added. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand, then sealed and stored at 4 °C, ready for GC−MS analysis.

Grape Leaves. Samples were collected at each row and position in the vineyard. Approximately 30 g of grape leaves was weighed out from each of the three positions within a row. The leaves were finely cut using both secateurs and scissors, and triplicate 8 g lots from each position were weighed into 22 mL glass screw cap vials with aluminum lined lids. A 2 mL aliquot of redistilled ethanol was added to each vial along with an aliquot (50 μL) of an ethanol solution containing $^2H_6$-1,8-cineole (5.12 μg/mL) and agitated on a shaker for between 5 and 7 days. When shaking was complete, the samples were transferred into 40 mL glass screw cap vials, an aliquot of Milli-Q water (18 mL) was

added to each vial, and shaking was repeated for 3 h. A 5 mL aliquot of the sample was removed into a 20 mL amber screw cap SPME vial, and 5 mL of Milli-Q water was added. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand, then sealed and stored at 4 °C, ready for GC−MS analysis.

Polyethylene Traps. Food grade polyethylene sheets were cut into 20 cm × 30 cm rectangles and placed between wire mesh and sewn in

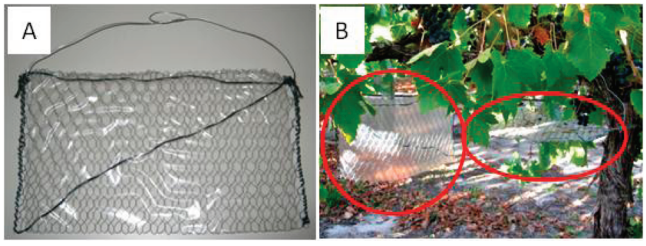

Figure 1. Vineyard trap for airborne 1,8-cineole fashioned out of 20 cm × 30 cm polypropylene sheet and wire mesh (A) and traps installed in the grapevine canopy (B) in vertical (left) and horizontal (right) positions.

place using fine wire (Figure 1A). A handle was fashioned using wire, and the traps were installed in the vineyard (triplicate positions in rows 1, 10, 20, and 60 at each of the grape sampling positions) in a vertical configuration (vintage 2008) and both a vertical and a horizontal direction (vintage 2009, Figure 1B). The traps were erected in early January and removed approximately 3 months later, one day prior to commercial harvest. The polyethylene sheets were removed from the wire mesh, carefully rolled, and placed into measuring cylinders equipped with glass stoppers. Redistilled ethanol was added to each cylinder to allow complete immersion of polyethylene sheet (130 mL), which was soaked for 4 days. A 1 mL aliquot of the ethanol extract was placed into an amber 20 mL screw cap SPME vial, and 9 mL of Milli-Q water was added along with 50 μL of $^2H_6$-1,8-cineole (5.12 μg/mL). After the sample was shaken, 5 mL was removed, and 5 mL of Milli-Q water was added. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand, then sealed ready for GC−MS analysis.

Skin and Flesh. Approximately 1 kg of Shiraz fruit from row 1 of the Padthaway vineyard was plucked and randomized, and triplicate 200 berry lots were weighed out. Each grape berry was individually squashed, and the pulp and the skins were separated. The seeds were removed from the pulp and discarded, and the skin and flesh samples were weighed. The separate samples were homogenized with a stab mixer, and then triplicate 8 g lots of both skin and pulp were weighed separately into 22 mL glass screw cap vials with aluminum lined lids. A 1 mL aliquot of redistilled ethanol was added to each vial along with an aliquot (50 μL) of an ethanol solution containing $^2H_6$-1,8-cineole (5.12 μg/mL), and the samples were then agitated on a shaker for 6 days. When shaking was complete, 9 mL of Milli-Q water was added, and samples were shaken for a further 3 h. A 5 mL aliquot of the sample was removed into a 20 mL amber screw cap SPME vial, and 5 mL of Milli-Q water was added, together with 2 g of NaCl, and the contents were shaken by hand, then sealed ready for GC−MS analysis.

GC/MS Analysis of 1,8-Cineole. Quantitative analysis of 1,8-cineole was carried out as described in Capone et al.[5]

Preparation of Samples and GC/MS Analysis of Rotundone. Wine samples were prepared for rotundone analysis using the same parameters as described in Siebert et al.,[11] except a Varian Factor Four VF-35 ms, 60 m × 0.25 mm × 0.25 μm film thickness fused silica capillary column (Agilent Technologies Australia, Forest Hill, VIC, Australia) was used. Grape leaf and grape stem extractions were carried out as detailed in Wood et al.[10] with the following modifications: finely cut up grape leaf and grape stem (2 g) from the vintage 2011 investigations were weighed into 20 mL glass screw cap vials with aluminum lined lids and soaked in 20 mL of redistilled ethanol for 48 h. The samples were filtered, and 10 mL of the ethanolic extracts was placed in a 100 mL volumetric flask and topped up to the mark with Milli-Q water and prepared for rotundone analysis.

Identification of *Eucalyptus* Species. The species of *Eucalyptus* located in the vicinity of the grapevines in Padthaway was identified by a botanist as *Eucalyptus leucoxylon* subsp. *pruinosa* (South Australian Blue Gum).

Statistical Analysis. The effects of the various treatments were analyzed using one-way analysis of variance (ANOVA) and Student's *t* comparison of means using unequal variance (JMP 5.0.1a, SAS Institute Inc., Cary, NC). Two-way ANOVAs with interactions with year and row number as factors were also conducted for grape berries, grape leaves, and grape stems. Other statistical data were obtained using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Preliminary investigations were carried out on wines derived from three different regions of Australia. Wine producers provided the wine samples after conducting fermentations on separate parcels of fruit from the associated vineyards. In the first two investigations, wines were made from batches of grapes harvested at set distances from *Eucalyptus* trees in single vineyards in Western Australia and Victoria. The results in Figure 2 clearly show that the greatest amount of 1,8-cineole

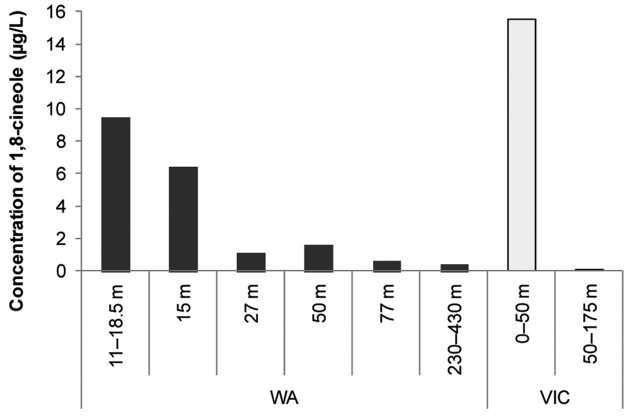

Figure 2. Concentration (μg/L) of 1,8-cineole in wines arising from single vineyards in Western Australia and Victoria located in close proximity to *Eucalyptus* trees. The *x*-axis indicates the distance of the grapevine rows to the *Eucalyptus* trees. For the WA wine, the 15 m samples were picked from row 2 and the samples 11−19 m were picked from rows 1, 3, and 4. The remaining WA samples were from individual rows at the distances specified in the figure.

was found in wines elaborated from grapes obtained from the rows closest to the *Eucalyptus* trees. For the wines from Western Australia, the highest concentration of 1,8-cineole (9.5 μg/L) was derived from fruit harvested within 20 m of the trees. The concentration of 1,8-cineole in the corresponding wines was lower the further away the fruit was harvested, and was almost negligible when fruit was obtained at 230−430 m away from trees (0.4 μg/L, Figure 2). The same trend was observed in the investigation of wines from Victoria, where grapes harvested within 50 m of the *Eucalyptus* trees afforded a wine 1,8-cineole concentration of 15.5 μg/L, and those harvested further away produced a wine with 0.1 μg/L (Figure 2). From these results, it appeared that harvesting fruit a distance of approximately 50 m from *Eucalyptus* trees was sufficient to minimize the concentration of 1,8-cineole in the corresponding wine. In a third investigation, wines from consecutive vintages were provided from the Coonawarra region where the vineyard was in close proximity to well-

established *Eucalyptus* trees. The wines contained relatively high amounts of 1,8-cineole, at 47 μg/L (2006 vintage) and 81.5 μg/L (2007 vintage), and were considered by the winemaker to display an obvious "eucalyptus" character. These wines were not sold commercially and were blended with wine made from other fruit, which is common practice to moderate and refine wine sensory characters. These preliminary investigations supported the theory by Herve et al. that the presence of 1,8-cineole is likely to be related to *Eucalyptus* trees.[3] Additional vineyard studies were therefore undertaken to examine possible modes of transmission of 1,8-cineole from *Eucalyptus* trees to the grapes and subsequently into the wine.

Vineyard Study. The relationship between grape composition and proximity to *Eucalyptus* trees was investigated, including evaluation of grape bunches, stems, and leaves. A vineyard was selected that had *Eucalyptus* trees in the vicinity of the vines and a history of producing wines with 1,8-cineole concentrations well above the recognition threshold of 3.2 μg/L in a red wine reported for this compound.[3] As part of these investigations, the location of 1,8-cineole within the grape berry was determined (Figure 3), using fruit collected from the row

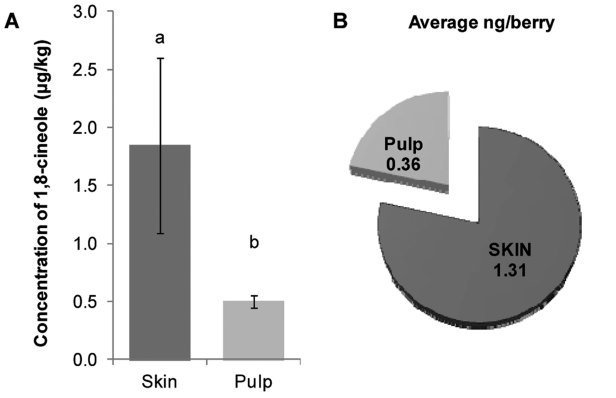

Figure 3. Concentration of 1,8-cineole in grape skin and grape pulp (A) as μg/kg and (B) as μg/berry. Error bars represent the standard deviation of three replicates. Different letters indicate significant differences between the means ($p < 0.05$).

closest to the *Eucalyptus* trees (within 5 m). There was a statistically significant difference between grape components ($p = 0.0403$), with skin containing approximately 4 times as much 1,8-cineole on a per kilogram basis as compared to the pulp (Figure 3A). As expected on the basis of its extraction during winemaking,[5] most of the 1,8-cineole was contained in the skin (approximately 80%) on a per berry basis (Figure 3B).

Additionally, four grapevine rows were selected in the same vineyard at set distances from the *Eucalyptus* trees, and grapes were sampled over three vintages. Row 1, 10, 20, and 60 were chosen, with row 60 being the furthest from the trees (around 125 m) and selected as a control row, because it was presumed to be far enough from the *Eucalyptus* trees to be unaffected by them. Triplicate sampling was conducted at each of the three positions within each row, with the results presented in Figure 4. We again observed a clear trend with greater concentrations of 1,8-cineole found for grapes in the rows closest to the *Eucalyptus* trees. Fruit sampled from row 1 had 1,8-cineole concentrations that were 2−10 times higher than fruit from row 10. A significant interaction between year and row number was found ($p = 0.015$). However, 1,8-cineole concentration decreased monotonically with row number in all three years,

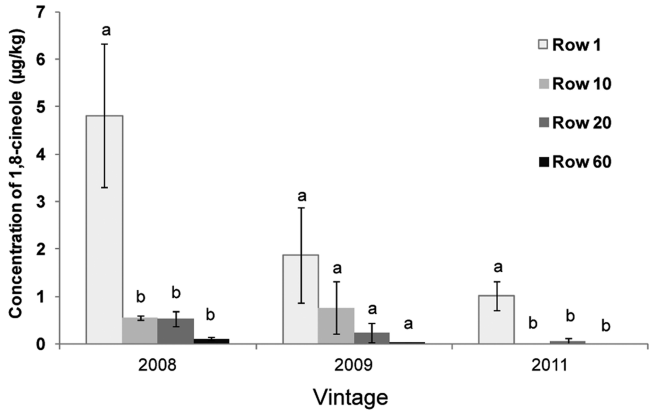

Figure 4. Concentration of 1,8-cineole (μg/kg) in grapes from different rows at set distances from the *Eucalyptus* trees over three vintages. Error bars represent the standard error of the mean for three replicates. Different letters indicate significant differences between the means ($p < 0.05$).

and the interaction was due to a much larger decrease from row 1 to 10 in 2008 as compared to the other years. There were vintage variations in overall 1,8-cineole concentration, with 2008 being the highest and 2011 the lowest. The error bars (standard error of the mean) in Figure 4 show that large variation exists within rows, and there may be a number of factors that could influence this variability including the vigor of the canopy, degree of grape exposure, or position and size of the berries. These results tended to indicate the airborne transfer of 1,8-cineole, which was pronounced within 5 m of the *Eucalyptus* trees and was seemingly limited to within 20 m of them. This concept of airborne transfer of volatile organic compounds is not surprising as it has been shown to occur in other studies, including those involving plants.[12−15]

The concentration of 1,8-cineole was much greater on a per weight basis in the grape leaf and stem samples taken from the same position as the berries. This is possibly due to the large surface area of the grape leaf or composition of leaf or stem epidermis in comparison to the grape berry or the fact that leaves can obscure the grape bunches, minimizing airborne transfer onto berries. Figure 5 presents the concentration of 1,8-cineole in the grape leaves over vintage 2009 and 2011, showing higher levels in rows closer to the trees. Grape leaves were not analyzed in vintage 2008 but were included in subsequent vintages after we observed a large number of grape leaves and stems in a commercial fermentation. The implication of grape leaves being able to affect the concentration of 1,8-cineole in wine was therefore considered, particularly for machine-harvested fruit. The row position for the grape leaves at set distances from the *Eucalyptus* trees had a significant effect in each vintage ($p < 0.0001$ across both vintages), again indicating the possibility of airborne transfer. Interestingly, while the impact on grape berries was restricted to rows that were close to the trees, grape leaves as far away as row 60 revealed measurable 1,8-cineole concentrations. Figure 5 also shows that grape stem 1,8-cineole concentrations were similar to those obtained for the grape leaves, and also followed the same trend, with greater concentrations of 1,8-cineole found in grape stems harvested closest to the *Eucalyptus* trees. We again found that row position was highly significant ($p < 0.0001$ across both vintage 2009 and 2011). In the grape stem, there was greater variability within rows, particularly in vintage 2011, but similar to the results for grape leaves, 1,8-cineole could be

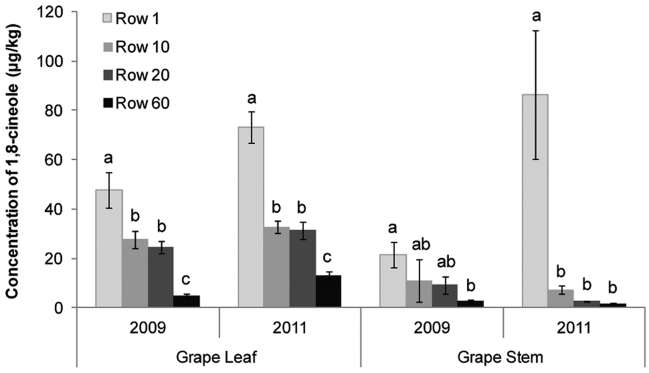

Figure 5. Concentration of 1,8-cineole (µg/kg) in grape leaves and stems from different rows at set distances from the *Eucalyptus* trees over two vintages. Error bars represent the standard error of the mean of three replicates. There were statistically significant differences ($p < 0.0001$) for the grape leaves in the various rows across both 2009 and 2011 vintages. There were statistically significant differences ($p < 0.0001$) for the grape stems in the various rows across both 2009 and 2011 vintages. Different letters indicate significant differences between the means ($p < 0.05$).

found at greater distances from the trees as compared to grape berries.

To verify that airborne transmission of 1,8-cineole was possible, as first suggested by Herve et al.,[3] we designed volatile traps for use in the vineyard (Figure 1A). The traps comprised polyethylene sheets sewn between wire mesh and installed in the same rows as the fruit collected for the study. Polyethylene sheets were chosen because our previous work on 2,4,6-trichloroanisole[16] and flavor scalping[17] showed polyethylene to be a good adsorber of nonpolar volatile compounds. We confirmed that 1,8-cineole could be adsorbed onto the polyethylene prior to installation of the traps in the vineyard (data not shown). In vintage 2008, the traps were installed in a vertical position only, whereas in vintage 2009, they were installed in both horizontal and vertical positions (Figure 1B). The traps installed in the vertical orientation in both vintages showed similar trends, where the greater concentrations of 1,8-cineole were found in the samples closest to the *Eucalyptus* trees (Table 1). This highlighted that aerial transfer of 1,8-cineole was possible and distance was a potential factor. The traps installed in the horizontal position showed a similar trend after the first row. In general, the horizontal traps adsorbed more 1,8-cineole than the vertical traps despite the greater exposure of the former to sunlight. This indicated the possibility that 1,8-cineole can be transferred as an aerosol as well as or instead of in the vapor phase. The anomalous result for row 1 may result from greater exposure of the horizontal traps to sunlight as the canopy growth was visibly less vigorous in this row.

Table 1. Average Concentration of 1,8-Cineole (µg/trap) and Standard Deviation (SD) Determined for Triplicate Polyethylene Traps Suspended in the Grapevine Canopy in Different Rows, Which Were at Increasing Distance from *Eucalyptus* Trees

	trap position and vintage					
row position	vertical 2008	SD	vertical 2009	SD	horizontal 2009	SD
row 1	1.0	0.3	0.65	0.3	0.9	0.3
row 10	0.6	0.2	nd[a]		2.3	0.1
row 20	0.5	0.1	nd		1.7	0.9
row 60	0.4		nd		nd	

[a]nd < 0.05 µg/trap.

During collection of vineyard samples, we noticed *Eucalyptus* twigs, bark, and leaves lodged within the grapevine canopy. We collected and analyzed some of this material and determined that if the 67.5 g collected from the canopy was harvested and totally extracted in a 1 tonne fermenter, it could contribute around 210 µg/L of 1,8-cineole in the corresponding wine. This theoretical amount, being considerably higher than in any wine we have so far analyzed, led us to carry out a range of fermentation experiments that included the addition of *Eucalyptus* material.

Determination of the Effect of MOG in Ferments. Grape leaves and stems can be found in fermentations, and *Eucalyptus* leaves and twigs can lodge in the grapevine canopy in the vicinity of the trees. While at least some 1,8-cineole in wine can arise from aerial transfer to grapes, there could be an even more important contribution from MOG (i.e., *Eucalyptus* and grape leaves). We therefore performed a study on the effect of MOG using grapes picked from the Padthaway vineyard. Hand-harvested fruit from the first two rows closest to a stand of *Eucalyptus* trees was collected. The fruit was delivered to the winery where it was completely randomized and sorted into multiple 50 kg lots for replicate fermentations. One treatment (rosé) involved first crushing and destemming grapes and then immediately subjecting them to a bag press, so that skin contact was minimized. This wine was then made similar to a rosé style. Another treatment (control) that involved hand plucked grape berries was chosen to eliminate any traces of MOG in the ferment. A third treatment (grape leaf/stem) involved passing the grapes through the crusher/destemmer and adding back the stems into the ferments along with grape leaves collected from row 1 to create a grapevine-based MOG effect. A final treatment (eucalypt) was performed by passing the grape bunches through the crusher/destemmer and adding a mix of *Eucalyptus* leaves and bark into the ferments to create a *Eucalytpus*-based MOG effect.

Each of these treatments was analyzed daily throughout fermentation to determine the evolution of 1,8-cineole (Figure 6). The rosé style wine was not included in Figure 6 as the concentration of 1,8-cineole was ≤0.4 µg/L and did not change throughout fermentation. This verified that maceration with skins and/or MOG is needed to contribute to 1,8-cineole concentration in wine, and further explains why we did not find 1,8-cineole in a range of white wines,[5] because these are generally made without skin contact. The evolution of 1,8-cineole during fermentation of the other treatments was consistent with the commercial scale fermentations assessed previously.[5] The controls exhibited a small increase in the concentration of 1,8-cineole (to 1.8 µg/L), which provided confirmation that 1,8-cineole is extracted from grape skins and can increase in concentration during fermentation[5] (when compared to the rosé treatment). For grape leaf/stem treatments, the concentration of 1,8-cineole (around 6.0 µg/L) can be seen to increase several-fold relative to the control samples, finishing with levels above the reported odor difference threshold of 1.1 µg/L for 1,8-cineole.[3] This was consistent with the higher amounts of 1,8-cineole determined in grape leaves and stems as compared to the berries. The eucalypt treatments were even more informative, revealing

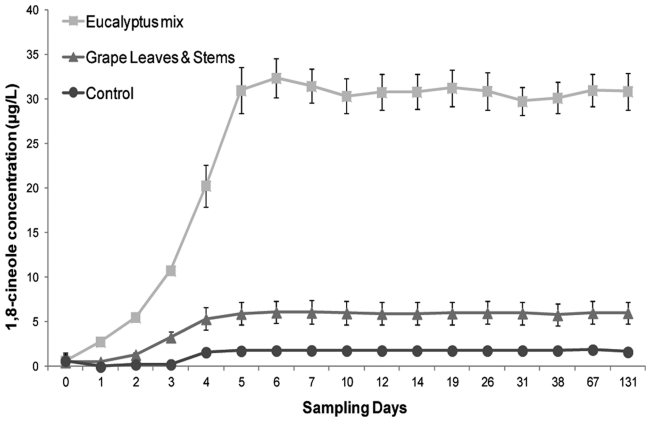

Figure 6. Mean concentrations of 1,8-cineole (μg/L) for the various replicated treatments determined during fermentation and storage. Error bars represent the 95% confidence interval (i.e., 2 × standard error of the mean) of the replicate ferments. Where error bars are not visible, the standard error was zero. Day 0 = crushed and cold soaked, day 1 = inoculated with yeast, day 6 = pressed, day 8 = racked, day 12 = inoculated for MLF, day 38 = racked, day 67 = prior to bottling, and day 131 = 64 days postbottling.

substantially increased concentrations of 1,8-cineole (approximately 30 μg/L). These relatively high levels could easily be achieved, depending on how the vineyard parcels are allocated, as in our study we removed 33 *Eucalyptus* leaves lodged within the grape bunches during hand sorting and randomizing of the 550 kg of hand-harvested fruit. Considering this vineyard and many others are normally harvested by machine, it would be reasonable to expect there would be a noticeable contribution to 1,8-cineole concentration in the wine as a result of such MOG beyond what is extracted solely from the grapes.

In our experiments, the presence of *Eucalyptus* leaves and to a lesser extent grapevine leaves and stems in the harvested grapes was determined to be the main contributor to 1,8-cineole concentrations in the wine. While there were apparent differences between vintages for the grapevine material examined, there was a clear effect of proximity to *Eucalyptus* trees, and the impact of MOG was obvious. Winemakers can heed these results and base decisions on them, fermenting fruit that grows near *Eucalyptus* trees separately and using it for blending, or ensuring minimal MOG is included from grapes that are machine-harvested in the vicinity of *Eucalyptus* trees. Such an effect from 1,8-cineole may also be evident in other viticultural regions around the world where *Eucalyptus* trees are a part of the natural landscape.

Following the production of these experimental fermentation treatments, six assessors informally evaluated the finished wines to assess their 1,8-cineole aroma. These rudimentary assessments revealed an obvious "eucalyptus" aroma to all of the assessors for the wines produced with the addition of *Eucalyptus* leaves. Surprisingly, the wines with the addition of the grape leaves and stems seemed to exhibit a strong "peppery" aroma that was less evident in the other treatments. This raised our curiosity about the nature of the compound(s) responsible for this character.

Effect of MOG on Wine Rotundone Concentrations. The sesquiterpene rotundone, previously identified as being responsible for giving wine a pepper aroma, has an extremely low aroma detection threshold of 16 ng/L in red wine.[10] We therefore analyzed all of the finished wines from the MOG study to determine rotundone concentrations. Rotundone was found in high concentrations, above 200 ng/L in the grape leaf/stem treatments (Table 2), where it was about 13 times above the reported aroma detection threshold of this compound. This particular treatment exhibited up to 6 times more rotundone than the controls (around 36 ng/L) and contained the highest concentration of rotundone of all of the treatments. The fermentation with the addition of *Eucalyptus* leaves had slightly higher amounts of rotundone (around 54 ng/L) than the controls, most likely due to small amounts of stems that would have been present as the grapes were passed through a crusher/destemmer and not all of the stems were removed. As expected, the rosé style wine contained much lower concentrations of rotundone (around 7 ng/L), which is below its aroma detection threshold. The rosé and control results were in accord with the findings of Caputi et al.,[18] highlighting that the largest proportion of rotundone in grape berries is located in the skins.

Table 2. Concentration of Rotundone from Duplicate Measurements (Means ± SD) of Wines Arising from the 1,8-Cineole Investigations and Duplicate Grape Leaf and Stem Extractions

samples	rotundone
Fermentation Treatments	
rosé style 1	8.5 ± 0.7 ng/L
rosé style 2	≤5 ng/L
control (hand plucked) 1	34.5 ± 2.1 ng/L
control (hand plucked) 2	38 ± 0 ng/L
grape leaf and stem 1	221 ± 1.5 ng/L
grape leaf and stem 2	213.5 ± 0.7 ng/L
grape leaf and stem 3	205.5 ± 2.1 ng/L
eucalyptus mix 1	58 ± 0 ng/L
eucalyptus mix 2	49.5 ± 0.7 ng/L
Ethanolic Extracts	
grape leaf row 1	4.8 ± 2.7 μg/kg
grape leaf row 20	0.9 ± 0.3 μg/kg
grape stem row 1	12.4 ± 0.5 μg/kg
grape stem row 20	0.65 ± 0.5 μg/kg

To confirm the impact of MOG on wine rotundone concentrations, we also determined the amount of rotundone in both grape leaf and grape stems extracts, thereby showing these were the main contributors to elevated wine rotundone levels. Grape leaves contained an average of 2.8 μg/kg, whereas grape stems contained an average of 6.5 μg/kg (Table 2). We observed large variability between row positions, but this is not surprising as large vineyard variability has been previously shown for other volatile compounds.[19] This vineyard variability may be attributed to factors such as vine vigor, size of the canopy, or health status of the vines.

If we consider the average concentration of rotundone found in the stem sample (6.5 μg/kg) and assumed complete extraction into a 50 kg ferment, this could equate to approximately 280 ng/L of rotundone in the finished wine. Similarly, if we consider the leaf extracts (average 2.9 μg/kg), total extraction into the ferment could equate to approximately 50 ng/L of rotundone in the finished wine. As such, if there was complete extraction into the ferment from both the grape leaves and the stems, this would contribute approximately 330 ng/L of rotundone to the wine. This is consistent with the greater amounts in the finished grape leaf/stem wines as compared to the controls.

In addition to our findings regarding 1,8-cineole, we have also shown through our fermentation treatments that the presence of grape leaf and grape stem can considerably enhance the concentration of rotundone in a finished wine much more than the grapes themselves. This serendipitous result could provide an avenue for manipulating rotundone concentration in wine, which hitherto has eluded winemakers. This could be particularly important for red wines made with whole bunch pressing or for ferments containing grape leaf and stem. Overall, the results give winemakers practical options for having a level of control over both 1,8-cineole and rotundone concentrations through vineyard and winery operations. The proximity of grapevines to *Eucalyptus* trees has a conclusive effect on 1,8-cineole concentrations in wine, while the presence of MOG can not only impact 1,8-cineole levels, but also wine rotundone concentrations. These factors can lead to altered wine sensory characteristics and highlight that there is more to consider than grape composition alone when investigating vineyard effects of wine aroma.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables displaying basic analytical data for Shiraz juices and wines. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GC−MS, gas chromatography−mass spectrometry; MOG, matter other than grapes; MLF, malolactic fermentation

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Evolution and Occurrence of 1,8-Cineole (Eucalyptol) in Australian Wine

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A new method has been developed for the quantitation of 1,8-cineole in red and white wines using headspace solid-phase microextraction (SPME) combined with stable isotope dilution analysis (SIDA) and gas chromatography−mass spectrometry (GC-MS). An extensive survey of Australian wines (44 white and 146 red) highlighted that only red wines contained significant amounts of 1,8-cineole (up to 20 μg/L). Hydrolytic studies with limonene and α-terpineol, putative precursors to 1,8-cineole, showed a very low conversion into 1,8-cineole (<0.6%) over a 2 year period, which does not account for the difference between white and red wines. 1,8-Cineole was chemically stable in model wine solution over 2 years, and absorption from a Shiraz wine by bottle closures was most evident for a synthetic closure only (14% absorption after 1 year). Two commercial ferments at two different locations were monitored daily to investigate the evolution of 1,8-cineole throughout fermentation. Both ferments showed daily increases in 1,8-cineole concentration while in contact with grape solids, but this accumulation ceased immediately after pressing. This observation is consistent with the extraction of 1,8-cineole into the ferment from the solid portions of the grape berries.

KEYWORDS: Wine aroma; 1,8-cineole; eucalyptol; SPME; SIDA; GC-MS

INTRODUCTION

1,8-Cineole, correctly identified by Jahns in 1884 (*1*), was initially recognized as the major constituent of the essential oil from leaves of *Eucalyptus globulus* by Cloëz, who labeled it eucalyptol (*2*). Eucalyptus essential oil (containing up to 90% 1,8-cineole) has since been used at low concentrations as a flavoring agent in a diverse range of foods and beverages (*3*, *4*), as a constituent in fragrances, cosmetics, and aromatherapy (*3*), and as a therapeutic ingredient with a range of applications (see refs *5*−*7* and citations therein). In fact, the medicinal use of eucalyptus leaves by indigenous Australians dates back many millennia (*7*). 1,8-Cineole is generally recognized as safe (GRAS) and has been used as an additive in cigarettes (see ref *8* and citations therein), evidently to improve flavor properties, reduce throat irritation, or enhance the cooling effects of menthol.

1,8-Cineole has a characteristic aroma described as "eucalyptus", "fresh", "cool", "medicinal", and "camphoraceous" and was first reported in wine by Herve et al. (*9*). That study showed that 1,8-cineole played an important role in the occurrence of "eucalyptus" character in wine. They also determined the difference and recognition thresholds of 1,8-cineole in a California Merlot as 1.1 μg/L and 3.2 μg/L, respectively (*9*). Herve et al. proposed that the "eucalyptus" character in wines occurs due to vineyards being in the vicinity of eucalyptus trees (*9*), but the origin of 1,8-cineole in wine is still unclear.

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To explain the presence of 1,8-cineole in Tannat grapes and wines from Uruguay, Farina et al. suggested that terpene compounds such as α-terpineol and limonene were possible precursors (*10*). Their postulated pathway to the formation of 1,8-cineole involved the hydration of limonene, forming α-terpineol, which was further hydrated to give a mixture of 1,8-terpines, with cyclization of *trans*-1,8-terpine leading to 1,8-cineole. They also put forward other theories involving double-bond epoxidation to explain the formation of minor components arising under their experimental conditions (*10*). Their studies with model wine showed that 1,8-cineole can be produced from limonene and α-terpineol under accelerated aging conditions at wine pH, but they gave only semiquantitative data for the products. Moreover, they found that 1,8-cineole concentrations in their Tannat grape samples at the beginning of ripening were very low, but showed a significant increase throughout ripening, and they determined an odor threshold for 1,8-cineole in the Tannat wine similar to that reported for Merlot (*10*).

Further confounding matters, the results from Farina et al. contrast with the work of Kalua and Boss, who found that 1,8-cineole levels decrease during ripening of Australian Cabernet Sauvignon and Riesling grapes (*11*), whereas other Tannat wines from Uruguay were shown to contain terpenoids but not 1,8-cineole (*12*). It is interesting to note that both Tannat studies involved vineyards in southern Uruguay, which also happens to be an area where eucalyptus plantations are readily encountered (*13*). Nonetheless, the studies relating to 1,8-cineole indicated there are a number of possible explanations for its presence

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in wine, although the relative significance of each is yet to be examined.

This paper describes the development of an accurate analytical method for determining 1,8-cineole in wine using a deuterium - labeled analogue and headspace solid-phase microextraction combined with gas chromatography−mass spectrometry (SPME-GC-MS). The method was applied to 190 commercial Australian wines to determine to what extent 1,8-cineole is present in wine in significant concentrations. Several factors thought to influence the concentration of 1,8-cineole in wine were also investigated, including its evolution during fermentation, formation from potential precursors, and stability during storage.

MATERIALS AND METHODS

Materials. Nondeuterated standards including 1,8-cineole, (*S*)-(−)-limonene and α-terpineol were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Stock solutions of standards were prepared volumetrically in redistilled ethanol and stored at −20 °C, and working solutions were stored at 4 °C until required. All chemicals were of analytical reagent grade unless otherwise stated, and water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Merck solvents, sodium chloride (NaCl), and L-(+)-tartaric acid were purchased from Rowe Scientific (Lonsdale, SA, Australia), and other chemicals were obtained from either Sigma-Aldrich or BDH (Kilsyth, VIC, Australia). Supelco SPME fibers (Sigma-Aldrich) were polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm, carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm, polyacrylate coating (PA) 85 μm, polydimethylsiloxane (PDMS) 100 μm, and both a 1 cm and a 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm.

Wine and Juice Samples. A range of bottled commercial white wines (44 in total) comprising 12 Riesling, 10 Sauvignon blanc, 10 Semillon, and 12 Chardonnays and red wines (146 in total) comprising 43 Shiraz, 45 Cabernet Sauvignon, 25 Merlot, 17 Pinot noir, 10 blends of Cabernet Sauvignon and Merlot, and 6 Durif wines were obtained from retail outlets. An additional seven commercial Shiraz wines of differing vintages were all produced from a single vineyard in the Padthaway region of southeastern Australia. Shiraz juice and fermentation samples were supplied by Australian producers from fruit obtained from a single vineyard in the Padthaway region and a single vineyard in the McLaren Vale region for the fermentation experiments.

NMR Analysis. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded with Bruker spectrometers operating at 400 or 600 MHz for proton and at 100 or 150 MHz for carbon nuclei, respectively. Chemical shifts were recorded as δ values in parts per million (ppm). Spectra were acquired in $CDCl_3$ at ambient temperature, and resonances were assigned by routine 2D correlation experiments. For ^{1}H NMR spectra, the peak due to residual $CHCl_3$ (δ 7.26) was used as the internal reference. For ^{13}C NMR spectra, the central peak of the $CDCl_3$ triplet (δ 77.16) was used as the internal reference.

High-Resolution Mass Spectrometry (HRMS). Spectra were obtained on a Bruker micrOTOF-Q II instrument with electrospray ionization (ESI) in positive mode. Samples dissolved in methanol at concentrations of approximately 1−2 mg/L were analyzed by flow injection.

Preparation of d_6-1,8-Cineole (6). The synthetic route to d_6-1,8-cineole (**6**) is shown in **Figure 1**. Ethyl 4-methylcyclohex-3-ene-1-carboxylate (**3**) was prepared according to the method of Inukai et al. (*14*) from isoprene (**1**) and ethyl acrylate (**2**) (*15*) on a multigram scale. Spectroscopic data for ester **3** were in full accord with those reported by Fringuelli et al. (*16*).

To magnesium turnings (0.867 g, 35.7 mmol) and iodine (ca. several crystals) in dry Et_2O (20 mL) under N_2 was added d_3-methyl iodide (5.17 g, 2.22 mL, 35.7 mmol) in dry Et_2O (20 mL) dropwise at reflux. After complete addition of the iodide, the mixture was heated for 30 min, ester **3** (2.02 g, 12.0 mmol) in dry Et_2O (10 mL) was added, and heating was continued for a further 1 h. The solution was chilled in an ice bath and quenched with a saturated solution of NH_4Cl. The organic layer was concentrated in vacuo to yield d_6-α-terpineol (**4**) (1.85 g, 11.5 mmol, 96%) as a pale yellow oil. Spectroscopic data were in full accord with those reported for the unlabeled compound (*17*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum. Compound **4** was used without further purification in the next step.

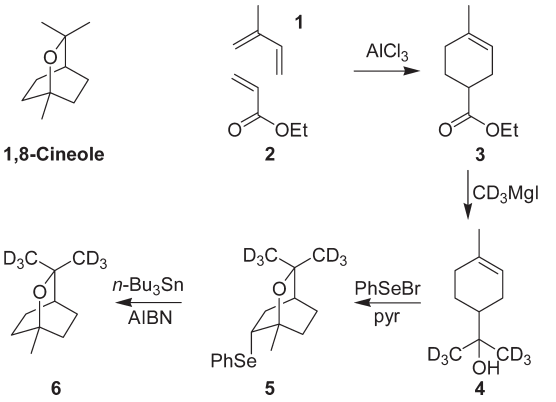

Figure 1. Structure of 1,8-cineole and synthetic route to d_6-1,8-cineole (**6**) used as an internal standard for GC-MS analysis.

ESI-HRMS, *m*/*z* calcd for $C_{10}H_{11}D_6^+$ ([M + H$^+$ − H_2O]), 143.1701; found, 143.1710.

EI-MS, *m*/*z* (%) 160 (M$^+$, 0.1), 142 (68), 124 (61), 93 (66), 92 (25), 81 (41), 79 (11), 67 (15), 65 (100), 46 (23).

d_6-2-Phenylseleno-1,8-cineole (**5**) was prepared according to the method of Bugarčić et al. for the unlabeled compound (*18*). Briefly, reaction of d_6-α-terpineol (**4**) (0.479 g, 2.99 mmol), pyridine (0.237 g, 240 μL, 2.99 mmol), and phenylselenyl bromide (0.776 g, 3.29 mmol) afforded phenylselenoether **5** (0.700 g, 2.22 mmol, 74%) as a colorless oil after purification on silica gel with CH_2Cl_2 followed by solvent removal. Spectroscopic data were in full accord with those reported for the unlabeled compound (*19*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum.

Reduction of selenide **5** was performed according to the procedure of Nicolaou et al. (*20*). Accordingly, compound **5** (0.700 g, 2.22 mmol), tri-*n*-butyltin hydride (0.938 g, 867 μL, 3.22 mmol), and azobisisobutyronitrile (2.22 mL, 0.02 M in toluene, 0.044 mmol) gave title compound **6** (0.271 g, 1.69 mmol, 76%) as a colorless oil after purification on silica gel with CH_2Cl_2 followed by solvent removal. Spectroscopic data were in full accord with those reported for the unlabeled compound (*21*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum.

ESI-HRMS, *m*/*z* calcd for $C_{10}H_{11}D_6^+$ ([M + H$^+$ − H_2O]), 143.1701; found, 143.1692.

EI-MS, *m*/*z* (%) 160 (M$^+$, 100), 142 (79), 132 (12), 131 (19), 114 (78), 113 (94), 96 (48), 90 (85), 89 (38), 81 (98), 75 (46), 72 (89), 59 (30), 55 (26), 46 (57), 43 (84).

Method Optimization. "Bag-in-box" wine (200 mL) was spiked with 1,8-cineole at a concentration of 0, 5, or 100 μg/L, and the mixtures were shaken. Aliquots (10 mL) were transferred into 22 mL amber screw-cap vials for headspace SPME-GC-MS analysis. Various preconditioned SPME fibers were trialed on these samples. The fibers investigated were PDMS/DVB, CAR/PDMS, PA, PDMS, and DVB/CAR/PDMS at the recommended operating temperatures for each fiber. Once the best fiber was determined, different sampling parameters were investigated individually. The parameters were no dilution, no salt, and no mixing; diluting the sample by 10 and 50% with Milli-Q water (v/v); salting the sample with either 1 or 2 g of NaCl; and inclusion of stirring (500 rpm) or agitation (400 rpm, agitation on 99 s and off 1 s) during fiber extraction.

GC-MS Instrumentation. Samples were analyzed with an Agilent 6890N gas chromatograph (Santa Clara, CA) fitted with a Gerstel MPS2 autosampler (Lasersan Australasia Pty Ltd., Robina, QLD, Australia) and coupled to an Agilent 5973N mass spectrometer. The gas chromatograph was fitted with either a 30 or 60 m J&W DB-Wax fused silica capillary column (0.25 mm i.d., 0.25 μm film thickness) during method development. The carrier gas was helium (BOC gases, ultrahigh purity), and the flow rate was 1.7 mL/min. The oven temperature started at 50 °C, was held at this temperature for 4 min, then increased at 10 °C/min to 125 °C, then increased at 30 °C/min to 240 °C, and held at this temperature for 10 min. The injector was held at 240 °C throughout the run. Positive ion

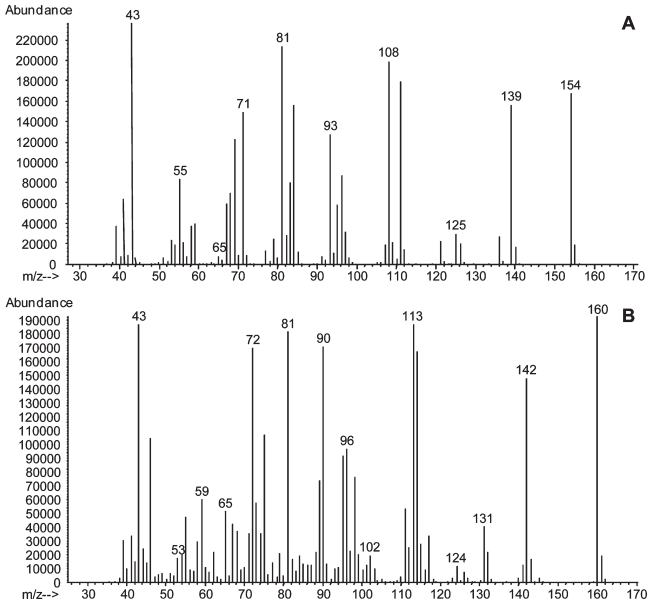

Figure 2. Electron ionization mass spectra of (**A**) unlabeled 1,8-cineole and (**B**) d_6-1,8-cineole (**6**).

electron impact spectra at 70 eV were recorded in the range m/z 35−350 for scan runs.

Optimized Method for Preparation of Juice and Wine Samples for Analysis. An aliquot (50 μL) of an ethanol solution containing d_6-1,8-cineole (**6**) (5.12 μg/mL) was added to white or red wine (10 mL) in a 22 mL glass screw-cap amber SPME vial. For red wine, 5 mL of the sample was removed and 5 mL of Milli-Q water was added to the vial. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand and then sealed prior to GC-MS analysis.

Quantitative GC-MS Analysis of 1,8-Cineole. Quantitation was carried out using the GC-MS system with a 60 m DB-Wax column as described above. The autosampler was fitted with a 2 cm, 50/30 μm DVB/CAR/PDMS SPME fiber. The sample headspace was extracted at 50 °C for 40 min with agitation at 400 rpm (99 s on, 1 s off) and desorbed in the inlet for 15 min. The splitter, at 42:1, was opened after 36 s. Injection was done in pulsed/splitless mode with an inlet pressure of 45.0 psi maintained until splitting. The injection liner was a Supelco injection sleeve made of deactivated borosilicate glass, 0.75 mm i.d. The oven temperature started at 50 °C, was held at this temperature for 2 min, then increased at 5 °C/min to 150 °C, then increased at 20 °C/min to 240 °C, and held at this temperature for 10 min. For quantitation, mass spectra were recorded in selected ion monitoring (SIM) mode. **Figure 2** displays the full-scan mass spectrum of each compound. The ions monitored in SIM runs were m/z 113, 114, 117, 132, 142, and 160 for d_6-1,8-cineole and m/z 108, 111, 126, 139, and 154 for 1,8-cineole. Selected fragment ions were monitored for 20 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise ratio and the least interference from other wine components, whereas the other ions were used as qualifiers.

Analytical Method Validation. The analytical method was validated by a series of duplicate standard additions of 1,8-cineole (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, and 100 μg/L) to a commercial young dry white "bag-in-box" wine (9.5% ethanol, pH 2.98) and a commercial young dry red "bag-in-box" wine (12.5% ethanol, pH 3.16). To determine the precision of the analysis, seven replicate samples were spiked with 1,8-cineole at two different concentrations (2 and 25 μg/L). For quantifying the analyte in batches of unknown samples, duplicate sets of standards were prepared at the same time as the juice and wine samples, by adding d_6-1,8-cineole standard solution (50 μL) to 10 mL of model wine (10% aqueous ethanol, saturated with potassium hydrogen tartrate, pH adjusted to 3.2 with tartaric acid) spiked with 1,8-cineole at concentrations of 0, 2, 10, 25, 50, and 100 μg/L (total of 12 standards). To ensure that the accuracy of the analysis was maintained, duplicate control wine samples, spiked with 1,8-cineole at concentrations of 0, 2, and 25 μg/L (total of six control wines), were included with every set of samples to be quantified. All validation samples were prepared and analyzed according to the optimized method.

Hydrolytic and Stability Studies. Model wines at pH 3 and 3.4 (10% ethanol, saturated with potassium hydrogen tartrate, adjusted to the required pH with tartaric acid) were used in each case. For the hydrolytic study, limonene and α-terpineol were separately spiked at 500 μg/L, and for the degradation study, 1,8-cineole was spiked at 50 μg/L (giving six spiked solutions in total). The solutions were divided into 25 mL glass ampules (54 for each, containing approximately 20 mL), sparged with nitrogen, and sealed. Thirty ampules of each spiked solution were stored at 25 °C, and the remaining 24 ampules of each were stored in an incubator at 45 °C (accelerated aging). Samples stored at 25 °C were analyzed for 1,8-cineole after 0, 4, 8, 16, 52, and 104 weeks, and those stored at 45 °C were analyzed for 1,8-cineole after 0, 1, 4, 8, and 16 weeks. Triplicate samples were analyzed for 1,8-cineole at each time point according to the optimized method.

Fermentation Study. Fermentations were followed every day from berry crush to the end of fermentation with two separate, commercially harvested Shiraz grape parcels at two independent wineries. Fruit from the McLaren Vale region, South Australia (SA), was fermented in an open fermentor (10 tonne), and fruit from the Padthaway region, SA, was fermented in a closed fermentor (19.33 tonne in a 20 tonne fermentor). Samples (100 mL) were collected in triplicate each day, spiked with 500 μL an ethanolic solution of d_6-1,8-cineole (5.12 μg/mL) immediately after collection, and then shaken by hand, sealed, and transported to the laboratory on ice. An aliquot of each sample (5 mL) was placed into a 22 mL amber screw-cap vial and diluted with 5 mL of Milli-Q water, and 2 g of NaCl was added. The samples were heated in a water bath at 67 °C for 15 min to terminate fermentation and then analyzed according to the optimized method.

Scalping Study. Sixty liters of Shiraz wine (14.1% ethanol, pH 3.15, titratable acidity = 7.4 g/L, SO_2 (free) = 27 mg/L, SO_2 (total) = 87 mg/L) were spiked with 1,8-cineole at approximately 100 μg/L. The wine was passed through a Z6 grade pad (nonsterile, 0.8 μm nominal pore size) and transferred into either 750 mL flint glass bottles or glass ampules. Bottles (24 of each) were sealed with Reference 2 natural cork (cork mouth bottle finish), Nomacorc synthetic closure (cork mouth bottle finish), and Stelvin screw cap (BVS bottle finish), and 48 ampules (50 mL) and 24 ampules (25 mL) were also sealed at the time of bottling. Bottles and ampules were stored in a climate-controlled cellar (between 18 and 20 °C) until analysis. Triplicate samples were analyzed for 1,8-cineole after 0, 3, 6, and 12 months according to the optimized method.

Statistical Analysis. The results reported for the calibration of the method were derived from the average of two replicate measurements for each concentration of analyte (and seven replicates for repeatability samples). The limit of detection (LOD) and limit of quantitation (LOQ) for 1,8-cineole were determined by multiplying the standard error of the y-intercept by 3.3 (for LOD) and 10 (for LOQ) and dividing these values by the slope of the calibration curve for each standard. Statistical analyses were performed with Microsoft Excel 2003, with the LINEST function used to obtain calibration function slopes and intercepts and their associated standard errors.

RESULTS AND DISCUSSION

Method Development and Optimization. For reliable determinations by headspace SPME-GC-MS, a deuterated analogue of 1,8-cineole was prepared for use as an internal standard. **Figure 1** depicts the synthetic route, which relied on a Lewis acid catalyzed Diels−Alder reaction (*14*) to form ester **3**, followed by Grignard addition to incorporate the deuterium labels, furnishing d_6-α-terpineol (**4**). Phenylselenoetherification in the presence of pyridine (*18*) afforded bicyclic ether **5**, and reduction of the selenide (*20*) gave several hundred milligrams of d_6-1,8-cineole (**6**), with an overall yield of 54% from ester **3**. Recently, Horst and Rychlik prepared small quantities of d_3-1,8-cineole with a comparable yield using a similar strategy (*22*). Ions used in the GC-MS method for quantitation and qualification were selected from the full-scan mass spectra of labeled and unlabeled 1,8-cineole (**Figure 2**).

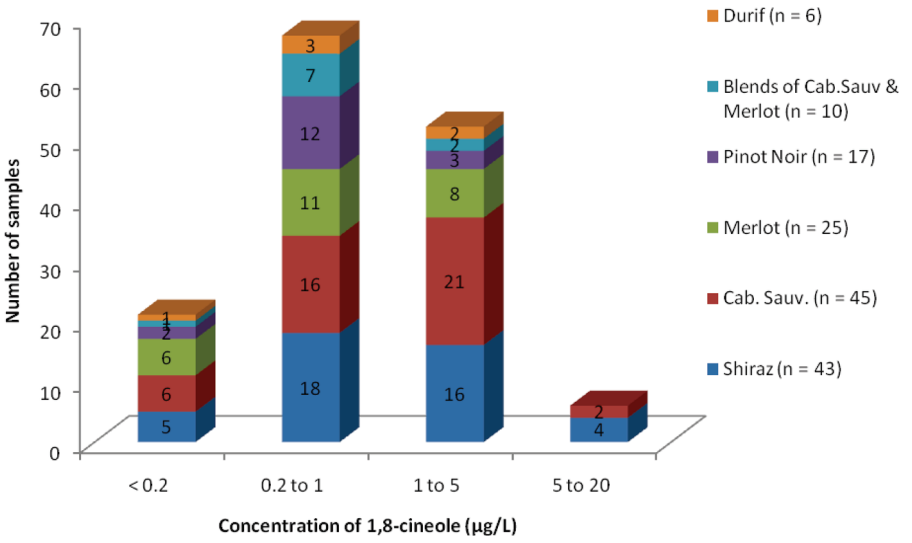

Figure 3. Concentration of 1,8-cineole (μg/L) in 146 commercially available Australian red wines of different vintages and varieties (analyzed in May 2007).

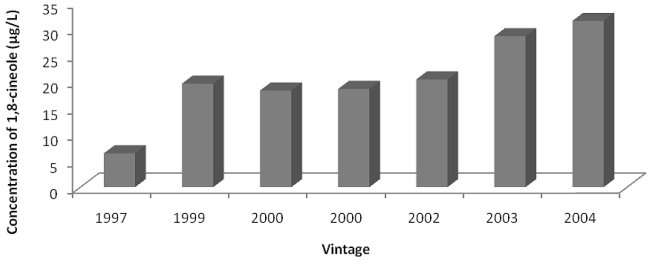

Figure 4. Concentration of 1,8-cineole (μg/L) in Australian commercial Shiraz wine produced from the same vineyard over different vintages (analyzed in July 2007).

Of the various fibers investigated for the extraction of 1,8-cineole from wine, the 2 cm DVB/CAR/PDMS fiber gave the strongest recovery (Supplementary Figure 1 in the Supporting Information). The PA fiber was the least effective at absorbing 1,8-cineole from wine. Evaluation of the various parameters trialed for white wine with the chosen fiber showed the addition of 2 g of salt and agitation gave the best extraction efficiency (Supplementary Figure 2 in the Supporting Information). Dilution of the samples to 50% with water had no effect on the white wine (9.5% alc/vol) (Supporting Information, Supplementary Figure 2) but increased the sensitivity by approximately 17% for a higher alcohol content red wine (12.5% alc/vol) (Supplementary Figure 3 in the Supporting Information). Method sensitivity relative to ethanol content when using headspace SPME has been demonstrated before (*23*,*24*), and the importance of diluting higher alcohol wines prior to analysis has been shown previously for red wine when using headspace SPME (*25*). Initially, using a 30 m DB-Wax column gave adequate sensitivity, but there was another peak coincident with 1,8-cineole. Changing to a 60 m column of the same phase with a slower temperature ramp separated the analyte from the coeluter. The method was then validated with the optimized sampling and chromatographic conditions.

Method Validation. The standard addition curves obtained were linear throughout the concentration range, with a coefficient of determination (R^2) of 0.999 for a white wine and 1.000 for a red wine. The method sensitivity was excellent, with calculated LOQs of 0.29 and 0.20 μg/L for the white and red wines, respectively, and calculated LODs of 0.09 and 0.07 μg/L for the white and red wines, respectively. The precision of the analysis was determined for seven replicate samples containing internal standard at two concentrations of 1,8-cineole. Spikes at 2 and 25 μg/L gave respective standard deviations of 0.07 and 0.48 μg/L for the white wine and 0.04 and 0.36 μg/L for the red wine. This equates to relative standard deviations of $<5\%$ in all cases. Furthermore, red, white, and model wines all gave identical calibration slopes, showing the quantitative analysis was not dependent on the matrix (data not shown).

Evaluation of 1,8-Cineole in Commercial Australian Wine. The method was applied to a survey of 190 commercially available bottled Australian wine samples. The wines were chosen randomly from different parts of Australia and comprised 146 red wines incorporating Shiraz (43), Cabernet Sauvignon (45), Merlot (25), Pinot noir (17), Durif (6), and red wine blends (10), along with 44 white wines made up of Riesling (12), Sauvignon blanc (10), Semillon (10), and Chardonnay (12). The results from the red wines are summarized in **Figure 3**. Of the red wines analyzed, 40% contained 1,8-cineole above the reported detection threshold, and several wines were substantially higher. Incidentally, the wine in this survey that contained the highest amount of 1,8-cineole (19.6 μg/L) was a Shiraz produced from a vineyard that had eucalyptus trees within a few meters of the nearest row of vines. In contrast to the situation for red wines, 1,8-cineole was not detected above 0.8 μg/L in any of the 44 white wines analyzed (data not shown). These results provided the basis for additional investigation into the occurrence and evolution of 1,8-cineole, which seemed to be important in red wine only.

Scalping and Stability Studies. Further examination of a number of commercial wine vintages produced over a number of years from a single Shiraz vineyard that had eucalyptus trees within several meters of the nearest row of vines showed various levels of 1,8-cineole and indicated an apparent trend toward increased 1,8-cineole concentrations in younger wines (**Figure 4**). This invoked a number of possibilities for the differences, such as the age of the vines, changes to winemaking practices, the instability of 1,8-cineole, or scalping of the compound by closures. The most feasible studies were to address the issues of stability and scalping. To this end we examined a Shiraz wine, spiked with 1,8-cineole, at various time points. Over a 12 month period no significant scalping was observed for wine stored under natural cork or Stelvin closure relative to the wine stored in glass ampules; the only closure that showed a moderate reduction (14%) was a synthetic closure (data not shown). The latter result is not surprising considering that 1,8-cineole is relatively nonpolar

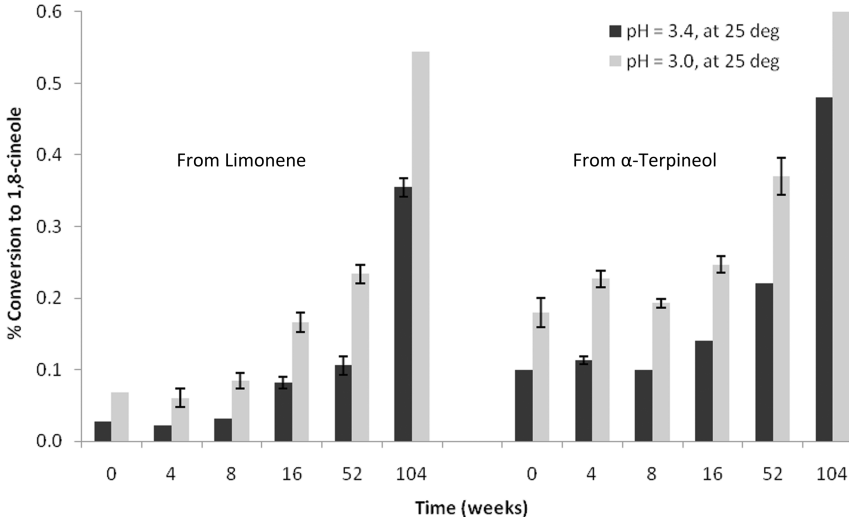

Figure 5. Hydrolytic study assessing percent molar conversion to 1,8-cineole of limonene and α-terpineol in model wine at pH 3.0 and 3.4 stored at 25 °C. Model wines were spiked separately with 500 μg/L of terpene precursors and assessed for 1,8-cineole at each time point. Error bars represent the standard deviation of three replicates. Where no error bars are shown, the standard deviation was zero.

and could be prone to scalping, particularly by synthetic closures (*26*). With regard to stability, 1,8-cineole was found to be very persistent in model wine (10% ethanol, saturated with potassium hydrogen tartrate, adjusted to the required pH with tartaric acid) when stored at different pH and temperatures. For samples stored at pH 3.0 or 3.4 and 25 °C, there was no discernible degradation of 1,8-cineole at either pH even after 2 years (data not shown). Additionally, samples stored at pH 3.0 and 3.4 under accelerated aging conditions (45 °C) showed no diminution of 1,8-cineole concentration after 16 weeks (data not shown), highlighting the stability of the compound under wine-like conditions. We can conclude from these scalping and stability experiments that 1,8-cineole is unlikely to suffer any substantial decline in concentration during aging of wine under ordinary storage conditions. Therefore, it appears that drivers of 1,8-cineole concentration in red wines may be associated with environmental factors or winemaking and viticultural practices.

Hydrolytic Studies. Farina et al. have suggested that significant quantities of 1,8-cineole could be generated from limonene and α-terpineol (*10*). To obtain precise data related to conversion of terpenoid precursors, experiments were carried out to determine if it was possible to generate significant quantities of 1,8-cineole from limonene and α-terpineol as suggested by Farina et al. (*10*). Monoterpenes such as these are more commonly associated with white grape varieties, yet the white wines we analyzed contained levels of 1,8-cineole well below its aroma detection threshold. Farina et al. proposed a mechanism for the formation of 1,8-cineole from either limonene or α-terpineol, which proceeds via the *trans*-isomer (*10*). However, it is unlikely that such a pathway would produce the product; the only arrangement likely to do so must arise from the *cis*-isomer adopting a boat conformation. Furthermore, their mechanism requires α-terpineol as an intermediate forming from hydration of limonene. However, contrary to expectation, if this was indeed true, their reported levels of cineole produced were 3-fold higher when limonene was the sole spiked compound than when α-terpineol was the sole spiked compound (*10*).

We therefore conducted precise hydrolytic experiments and analyzed for the production of 1,8-cineole in model wines spiked separately with limonene and α-terpineol. Samples were treated in the same way as the stability studies and examined over a period of time. The results were expressed as percent conversion to 1,8-cineole on a molar basis at both 25 °C (**Figure 5**) and 45 °C (data not shown). It was our observation, when the low levels of 1,8-cineole already present in the samples at $t = 0$ were subtracted from the total, that the amounts of additional 1,8-cineole generated were similar for both substrates. As expected, the production of 1,8-cineole at higher temperature (45 °C) was 2−4 times greater than the corresponding time points at room temperature (25 °C), with 16 weeks at 45 °C being similar to 2 years at 25 °C. Production of 1,8-cineole was also higher at the lower pH, consistent with the acid-catalyzed nature of the conversions. Overall, the amount of 1,8-cineole produced was low, however; even after 104 weeks at 25 °C, there was, at most, around 0.6% conversion (**Figure 5**), giving concentrations of 1,8-cineole close to its aroma detection threshold and about 10 times lower than those reported by Farina et al. (*10*). Furthermore, the results must be considered in the context of the high spiking levels of terpenoid precursors (500 μg/L). Under normal circumstances their conversion to 1,8-cineole would appear to be relatively unimportant to wines that are a few years old but might contribute to 1,8-cineole in older wines, that is, 10 years or older.

Fermentation Study. The results of our survey of commercial Australian wines (**Figure 3**) indicated that only in red wines was the concentration sufficiently high to have a possible sensory impact, as indicated by threshold data (*9*, *10*). This led us to examine the hypothesis that the compound accumulates in grape solids (skins, stalks, etc.) and is only extracted through maceration during winemaking. Therefore, two different commercial fermentations were sampled on a daily basis from crush to the end of fermentation with Shiraz grapes from two different commercial producers. Samples were heated after preparation to terminate the fermentation process prior to analysis. With both ferments there was a steady increase in 1,8-cineole concentration during fermentation on skins, which ceased after pressing (**Figures 6** and **7**). Also noteworthy is the difference in the concentrations of 1,8-cineole extracted during winemaking with these two parcels of fruit, and the variability in concentration of the replicates for the first 3 days (**Figure 7**) due to less homogeneous mixing during cold soaking. The minor decrease of 1,8-cineole observed after pressing is unexplained but might be due to loss of the compound during transfer between tanks. Nonetheless, these results strongly indicated that 1,8-cineole was extracted from grape solids with increases in ethanol as fermentation

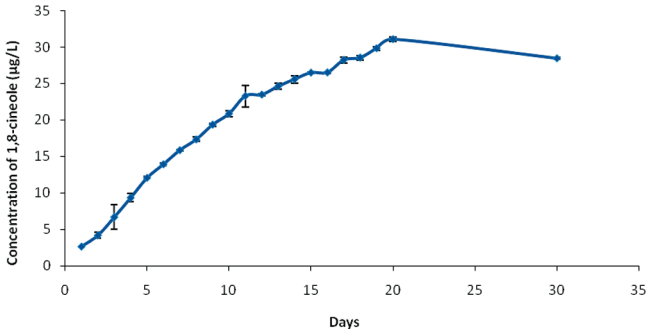

Figure 6. Concentration of 1,8-cineole (μg/L) extracted during fermentation with Padthaway fruit in a closed fermentor. The wine was pressed off skins on day 20. Error bars represent the standard deviation of three replicates.

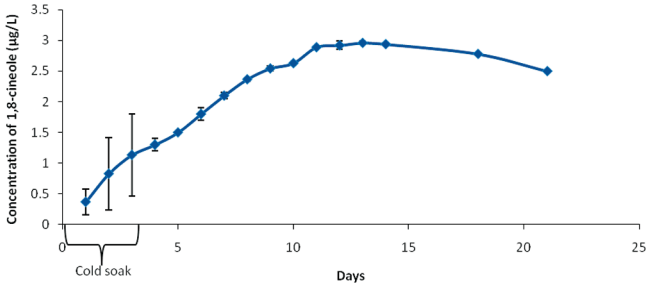

Figure 7. Concentration of 1,8-cineole (μg/L) extracted during fermentation with McLaren Vale fruit in an open fermentor. The wine was pressed off skins on day 11. Error bars represent the standard deviation of three replicates.

progressed, although at this point it cannot be ruled out that matter other than grapes (MOG) in the ferments (e.g., eucalyptus leaves) has also played a role.

Questions remain about whether 1,8-cineole is present in wines due to being biosynthesized in the grapevine or absorbed from the environment due to vineyard proximity to eucalyptus trees. We have provided further insight into the origin/occurrence of 1,8-cineole in wine by showing that it is a phenomenon chiefly associated with red wine, that the compound is stable during storage and barely scalped by closures, and that it is extracted during red winemaking in the presence of solids only. We have also discounted terpenoid precursors as being substantial contributors to 1,8-cineole concentrations in younger wine. Future work will focus closely on the effect of eucalyptus trees in an attempt to resolve the origin surrounding 1,8-cineole in wines.

ABBREVIATIONS USED

SIDA, stable isotope dilution analysis; SPME, solid-phase microextraction; GC-MS, gas chromatography−mass spectrometry; SIM, selected ion monitoring; LOD, limit of detection; LOQ, limit of quantitation; MOG, matter other than grapes.

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Supporting Information Available: Figures displaying SPME fiber performance and evaluation of parameters for 1,8-cineole extraction from white and red wine. This material is available free of charge via the Internet at http://pubs.acs.org.

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