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Seeing through smoke

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Managing director Dan Johnson

As a result of bushfires and controlled burning, winemakers and grapegrowers are seeking more information about the effects of smoke on ripening grapes, wine composition and sensory qualities. What is the relationship between smoke taint and smoke exposure? How does smoke interact with grape and vine development? Research at the AWRI has led to the development of strategies to identify smoke exposure and a deeper understanding of smoke taint. This new insight is being used to provide advice and assistance to grape and wine producers.

At a glance

In a country with frequent bushfires and controlled burns, grapegrowers and winemakers are worried about the possible consequences of smoke exposure on their grapes. Can this lead to an undesirable smoke taint?

Research into volatile phenols and non-volatile phenolic glycosides suggests that phenolic glycosides do play a part in the ashy aftertaste by releasing free volatile phenols in the mouth.

The AWRI is investigating the best methods for detecting smoke exposure in grapes, and quantifying phenolic glycosides as smoke marker compounds is proving advantageous.

Analysis of phenolic glycosides and measurement of volatile phenols in grapes and wines is leading to improvements in the ability to distinguish between smoke and non-smoke affected wines. Collaboration between the AWRI and the Victorian Department of Primary Industries (DPI) and the DPI's Centre of Expertise in Smoke Taint Research (CESTR) has improved the knowledge base of compounds in smoke-affected wine and will enhance the response in support of grapegrowers and wine producers in the advent of a bushfire.

THE SMOKING GUN

Previous research into bushfire smoke and other contaminants has led AWRI scientists, working alongside researchers at The University of Adelaide and Victorian Department of Primary Industries, to identify various volatile phenols that are now known to be significant in the development of smoke taint characteristics. In recent reports[1,2], the AWRI discussed how volatile phenols are converted to non-volatile glycosides (phenolic glycosides) in the vine.

These phenolic glycosides play a key role as precursors for volatile phenols in wines: they can be released by enzymatic or acid-catalysed hydrolysis, causing smoke taint characteristics.

How, exactly, the volatile phenols in smoke are absorbed into the grapevine in the first place is yet to be determined. As a result, it is not possible to prevent the uptake of volatile phenols from smoke during bushfires or other smoke events.

There have been various attempts to manage smoke taint[3]. Concentrations of free volatile phenols from smoke have been reduced in wines by using reverse osmosis[4] and fining agents such as activated carbon[5].

Although these strategies have reduced the impact of smoke taint, they have not been able to solve the problem: they cannot remove smoke taint characters completely. Also, these processes are not selective and, in some instances, they can affect desirable aroma compounds. Moreover, the non-volatile glycoside-bound phenols remain in the wine.

WHAT IS THE ROLE OF VOLATILE PHENOLS IN SMOKE TAINT?

Using wine made from grapes exposed to bushfire smoke between 7 February and 14 March 2009 in the Yarra Valley, Victoria, AWRI researchers investigated the relationship between smoke-derived volatile phenols and negative sensory characters[6].

Table 1 shows that the control wines from non-smoke affected grapes had low or undetectable volatile phenol concentrations.

In wines made from grapes exposed to smoke, the concentration of guaiacol (a compound found in many oaked wines) was above its reported odour detection threshold value, as was *p*-cresol. *o*- and *m*-cresols (also found in oaked wines), present at concentrations approaching threshold. These results concluded that guaiacol and the cresols were indicators of smoke exposure and could be major contributors to smoke taint.

The three control wines were made from non-smoke affected grapes grown in the Coonawarra, in South Australia, the Yarra Valley, in Victoria, and the Adelaide Hills, in South Australia, respectively.

The other red wines were smoke-affected wines from the Yarra Valley, in Victoria.

SENSORY EFFECT

The next stage was sensory evaluation. To corroborate the sensory significance of guaiacol and the cresols, the AWRI's sensory panel determined their odour thresholds in a red wine, and also compared the odour (perceived by the nose) and 'taste' thresholds (i.e., the perceived flavour) of guaiacol additions to the same wine.

The base red wine had low levels of volatile phenols, with no detectable concentrations of *o*-, *p*- or *m*-cresols. Figure 1 provides the panel's best estimate thresholds for the volatile phenols. *m*-Cresol has the lowest aroma threshold value in red wine (20µg/L), comparable to that of guaiacol. The 'taste' (flavour) threshold for guaiacol is 27µg/L, which is similar to its aroma threshold (23µg/L). Comparing the thresholds of these volatile phenols with their actual concentrations suggested that guaiacol and *m*-cresol were likely to be important contributors to the aroma of smoke-affected red wine.

Table 1. Concentration of free volatile phenols in red wine from 2009 bushfires (µg/L, adapted from Parker *et al.* 2012[6]), in comparison to previously reported sensory thresholds.

Variety	guaiacol	Syringol	4-methyl syringol	4-methyl guaiacol	phenol	o-cresol	p-cresol	m-cresol
Control wines								
Cabernet Sauvignon	3	4	nda	nd	2	nd	nd	1
Pinot Noir	6	15	4	6	6	6	2	4
Pinot Noir	6	10	2	1	nd	2	nd	1
Smoke-affected wines								
Pinot Noir	12	6	3	3	17	8	5	6
Pinot Noir	18	21	8	6	18	10	5	8
Pinot Noir	8	11	3	4	17	8	5	5
Shiraz	36	20	12	9	26	6	3	3
Pinot Noir	15	18	4	3	15	6	4	7
Pinot Noir	23	22	5	3	52	11	6	9
Pinot Noir	10	16	3	1	22	5	4	5
Pinot Noir	16	18	7	2	33	11	6	8
Pinot Noir	7	14	4	2	1	6	4	3
Cabernet Sauvignon	16	23	5	5	29	6	3	7
Shiraz	35	23	3	4	17	6	2	2
Chardonnay	7	10	4	4	13	6	4	6
Pinot Noir	55	26	9	10	44	26	6	13
Cabernet Sauvignon	31	16	10	5	40	9	4	8
Shiraz	27	15	3	1	43	3	1	2
Sensory Detection thresholds	9.5[b]	570[b]	10000[c]	21[d]	7100[e]	31[e]	3.9d,10[e]	15d, 68[e]

Table 1: Notes
[a]not detected. [b]Odour detection threshold in aqueous 10% alcohol at pH 3.27. [c]Taste detection threshold in water[8] [d]Odour detection threshold in water[9]. [e]Odour detection threshold in aqueous 10% alcohol[10]

Further analysis revealed that guaiacol and *m*-cresol were indeed significant volatile phenols associated with both 'ashy' aftertaste and smoke aromas, as were the compounds 1-methylsyringol, 1-methylguaiacol, phenol and *o*-cresol.

This confirmed the discovery that guaiacol and the cresols were the most likely contributors to smoke taint and suggested that other phenols present below their individual thresholds could offer enhancing effects.

Further verification was achieved by spiking a base red wine with volatile phenols at concentrations found in a smoke-affected wine. The spiked red wine was described as similar to the actual smoke-affected wine, but it was reported to lack intensity in lingering ash flavour and aftertaste. This suggested that there are additional compounds that create smoke taint in red wine which are responsible for the production of the 'ash' flavour and aftertaste.

THE ROLE OF GRAPE METABOLITES, PHENOLIC GLYCOSIDES

Research into smoke-affected grapes and wine has also revealed significantly elevated levels of phenolic glycosides.

These phenolic glycosides are grapevine metabolites formed by glycosylation (enzymatic addition of various sugars) of volatile phenols from smoke; these glycosides can be cleaved during fermentation and wine ageing to release free volatile phenols. As non-volatile compounds they do not have a direct effect on aroma properties in their own right but, as outlined below, recent work has shown they can have a surprising role by releasing flavour during tasting.

SENSORY EFFECT OF PHENOLIC GLYCOSIDES

The AWRI flavour chemistry team prepared glycosides of guaiacol, *m*-cresol and syringol and added them separately to a model wine.

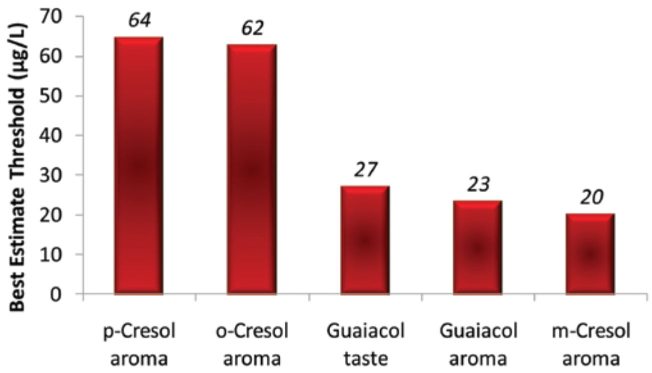

Figure 1. Odour and taste threshold values of guaiacol and cresols in red wine.

In a formal sensory assessment, guaiacol and *m*-cresol glycosides produced a marked 'ashy' aftertaste, or lingering ashy flavour compared with a control model wine (Figure 2, see page 44). Also, *m*-cresol glycoside produced a noticeable medicinal flavour, while the syringol glycoside produced no significant effect compared with the control wine.

In a further experiment, samples of expectorated wine were collected for chemical analysis, which confirmed the presence of the free volatile phenols and suggested that they had been released in the mouth.

The AWRI concluded that salivary enzymes in the mouth were hydrolysing the glycosidic bonds and releasing the free volatile phenols, which are then discernible retro-nasally. Phenolic glycosides do play a part in the residual 'ashy' aftertaste and

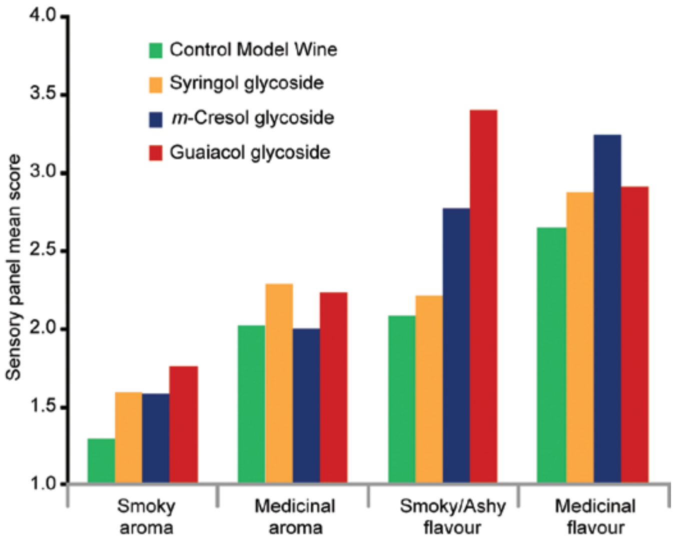

Figure 2. Aroma and flavour intensity scores from a trained sensory panel assessing glycosides of smoke-related phenols added to a model wine.

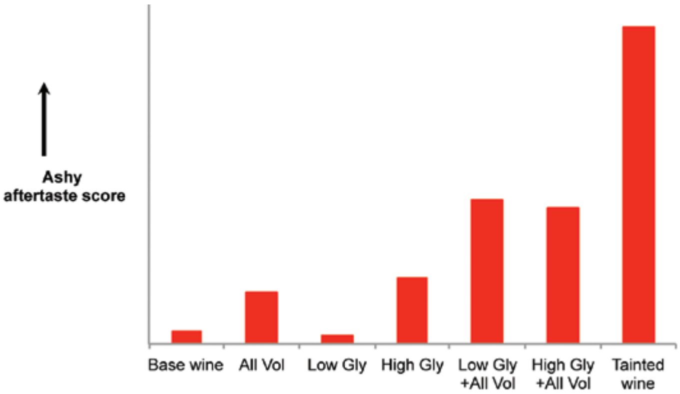

Figure 3. Burnt or ashy aftertaste rating of reconstituted wines.

Sample name	Description
Base wine	No addition
All vol	Volatile phenols matching the concentration of phenols of an actual tainted wine (Shiraz from 2007 bushfires)
Low gly	Low glycoside-isolate addition (equivalent to 500μg/L syringol gentiobioside)
High gly	High glycoside-isolate addition (equivalent to 1500μg/L syringol gentiobioside)
Low gly + All vol	Low glycoside-isolate and all volatile phenols addition
High gly + All vol	High glycoside-isolate and all volatile phenols addition
Tainted wine	Actual smoke tainted wine: 2009 Shiraz, Yarra Valley

contribute to 'smoky' flavours by releasing free volatile phenols in the mouth.

HOW VOLATILE PHENOLS AND NON-VOLATILE GLYCOSIDES IMITATE SMOKE TAINT

To test the significance of phenolic glycosides for 'ashy' aftertaste and 'smoky' flavour in red wine, a base wine was spiked with volatile phenols and phenolic glycosides to simulate a real smoke-affected wine (see Figure 3).

It was confirmed that phenolic glycosides contribute to the taint characters: a 'smoky' aroma and a 'burnt' and 'ashy' aftertaste. The combination of different volatile phenols, together with their non-volatile glycosides, imitated smoke taint the best and produced the most significant effect on the sensory properties of the reconstituted smoke-tainted wine.

SMOKE DETECTION

The AWRI has concentrated its efforts on developing strategies to detect and measure smoke exposure in grapes.

These strategies include providing advice and assistance to grapegrowers and winemakers regarding harvesting after a smoke event. This is important since it is not currently possible to prevent the uptake of smoke compounds in grapes and vines; it is also not possible to fully remove key phenols and their glycosides from smoke-affected wines.

PHENOLIC GLYCOSIDES USED AS SMOKE MARKERS

Based on the following observations, phenolic glycosides complement existing smoke markers, free phenols:

- in a model experiment, after a grapevine is exposed to smoke, the amount of volatile phenols taken up by grapes is related to the intensity and duration of that smoke exposure[11]
- the volatile phenols in grapes are rapidly metabolised into their more stable and non-volatile glycosidic forms (phenolic glycosides)[1,12]. Smoke-induced glycosides persist and accumulate in grapes until harvest[12]
- phenolic glycosides are easily extracted into wine and act as a pool of precursors to release volatile phenols over time, during fermentation, ageing and storage[1,13]
- the presence of phenolic glycosides in wine is specific to smoke exposure of grapes and has not been attributed to barrel ageing, unlike free volatile phenols in wine which are also extracted from barrels.

CAN WE REDUCE THE RISK OF PRODUCING SMOKE-AFFECTED WINE?

The AWRI undertook a comprehensive survey of baseline levels of volatile phenols and their phenolic glycosides in samples of control grapes and un-oaked laboratory-scale wines.

In collaboration with industry partners, samples were collected from 11 grapegrowing regions and five major varieties: Cabernet Sauvignon, Chardonnay, Pinot Noir, Riesling and Shiraz.

Volatile phenols were measured over two vintages to statistically determine upper limits of their natural abundance. It was possible to determine whether the samples were smoke-affected if the concentrations of volatile phenols or phenolic glycosides present were higher than their upper limits.

Figure 4 illustrates the guaiacol and total phenolic glycoside concentrations (sum of six selected glycosides) of grapes and wine samples suspected of smoke exposure.

In grapes, according to their total phenolic glycoside concentration, 36 out of 136 samples were classified as smoke-affected. According to their guaiacol concentration, only 17 of the samples were correctly identified as affected. Of six samples containing total phenolic glycosides at concentrations exceeding 100μg/kg, all were incorrectly diagnosed as 'not smoke-affected' by guaiacol alone, indicating how important phenolic glycoside analysis is as a means for detection of smoke exposure.

Meanwhile, 50 wines out of 150 samples were classified as smoke-affected according to their total phenolic glycoside concentration, while only 13 were classified smoke-affected by the free guaiacol measurement.

IDENTIFYING SMOKE EXPOSURE

By analysing phenolic glycosides and measuring volatile phenols in grapes and wines, rather than relying on the existing guaiacol and 4-methylguaiacol measures, the ability to diagnose smoke exposure in grapes and wine has been enhanced. This should lead to significant improvements in the Australian wine sector's ability to distinguish between non-smoked and smoke-affected samples.

INTO THE FUTURE

The method used for the quantification of phenolic glycosides in grapes and wine (HPLC-MS/MS method) has been validated and the AWRI is in the process of expanding its capacity to analyse larger numbers of grape and wine samples. For more information about the analysis of phenolic glycosides in grapes and wine, contact Randell Taylor at the AWRI on (08) 8313 6618 or Randell.Taylor@awri.com.au

The AWRI is also collaborating with the Victorian Department of Primary Industries (DPI) and the DPI's Centre of Expertise in Smoke Taint Research (CESTR). The aim is to use the analytical methods described here for the quantification of volatile phenol glycosides in smoke-affected grapes and wine. It is hoped that the collaboration will increase and enhance joint knowledge about compounds present in smoke-affected samples at a more rapid rate.

A symposium held in Melbourne in 2012 brought together researchers and industry members from Victorian grapegrowing regions (a summary of the presentations can be found on the AWRI website[14]). ▸

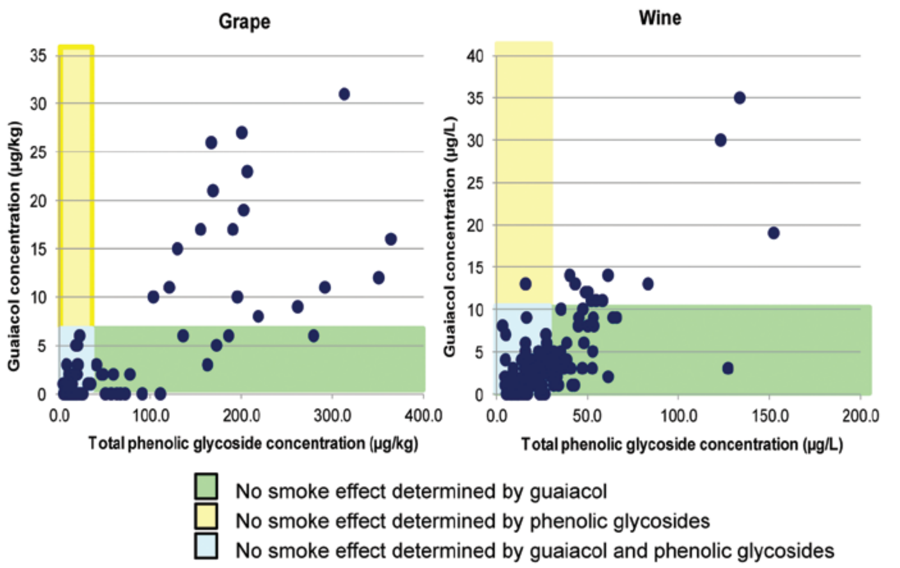

Figure 4. Guaiacol and total phenolic glycoside concentrations of grapes and wine samples suspected of smoke exposure.

In addition, the AWRI is working with DPI/CESTR to enhance a bushfire emergency response plan for the wine sector, which will ensure a rapid and co-ordinated response to support regional wine producers if there is a major fire and/or smoke event.

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