

present on samples taken approximately a month before harvest. However, no other mould species were positively identified in those samples, so it is not known which fungi were the cause of the sooty mould infection of the grapes used for this trial.

The harvested fruit was stored at 0°C for three days prior to processing. The individual bunches of sooty mould-affected grapes were individually assessed for severity of infection, with the overall batch assessed as having approximately 50% infection. The fruit had been harvested selectively to exclude moulds other than sooty mould, and each bunch was reassessed prior to processing, with any bunches showing signs of insect damage or other fungal infection being discarded. The fruit was then de-stemmed and crushed, with 50mg/L of sulfur dioxide (SO₂) added during crushing, and the must homogenised. The bunches not affected with sooty mould were also destemmed and crushed, with the same SO₂ addition made during crushing and the must homogenised.

Following homogenisation, the unaffected batch of must was divided into six smaller containers for fermentation. Two containers each holding 28kg of must were left as 'controls'. Into two more of the containers, 25.2kg of unaffected must, and 2.8kg of the sooty mould-affected must was added, creating two replicates of a 5% sooty mould-affected treatment; and in the remaining two containers 14kg each of unaffected and affected must was added, creating two replicates of a 25% sooty mould-affected treatment.

The six fermentations were initiated by the addition of 250ppm Maurivin PDM active dried wine yeast, and were

conducted according to WIC Winemaking Services' small-scale red winemaking protocol. That protocol included fermentation on skins at 15-18°C, with all ferments plunged 20 times, and in the same pattern, twice a day. On day two, 3g/L of tartaric acid was added to each ferment, and they were inoculated with 10ppm of Lallemand VP41 *Oenococcus oeni* bacteria. The fermentations were pressed at approximately 2Bé using a 130-litre Diemme airbag press and an 18-minute press cycle, with the pressings immediately added back to the free-run. At pressing, 200mg/L of Mauriferm Activator yeast nutrient was added to encourage completion of the fermentations. The wines were stored in stainless steel containers at 20°C until malolactic fermentation (MLF) was complete, at which point 80mg/L of sulfur dioxide (SO₂) was added. The wines were then stored below 5°C for 10 weeks before being allowed to warm to approximately 15°C. The wines were then racked using a syphon-hose and gravity, and the day before bottling were filtered using a cross-flow filter. A copper fining trial was conducted post-filtration, and the three wines were subsequently fined with between 0.01 and 0.015ppm Cu²⁺ ions. The wines were bottled into 750mL bottles which were closed with screwcaps. Sensory evaluation was conducted approximately four weeks after bottling.

GRAPE AND MUST PROCESSING AND FERMENTATION

It can be difficult to visually identify sooty mould on ripe red grapes, and while every effort was made to eliminate

sooty mould-affected grapes from the 'unaffected' or 'control' ferments, it is possible that a small amount of sooty mould was present.

When the affected and unaffected batches of grapes were destemmed and crushed, no difference in odour was detected, and no aerial dust or spores were observed with the sooty mould-affected portion. However, at crushing, more colour was evident in the sooty mould-affected batch of must compared with the unaffected must, and that difference remained evident in the 25% sooty mould-affected ferments for approximately 48 hours. However, when post-crushing samples of the two batches of must were centrifuged, no visual colour difference was evident between the supernatants, and no differences in absorbance at 520nm or the concentration of anthocyanins were revealed by laboratory analysis (Table 1). Nevertheless, the clear visual difference at crushing does suggest that the organism(s) responsible for the sooty mould had to some extent infiltrated the grape skins, apparently resulting in greater fragmentation of the skins during destemming and crushing.

Results of must analysis prior to fermentation are provided in Table 1. No consistent differences were evident in the post-crushing analysis of the musts, except for a small reduction in sugar concentration with increasing sooty mould infection. No effect was evident on nitrogen concentration (alpha amino nitrogen, ammonia and YAN), pH or malic acid.

Importantly, issues related to scale, mealy bug, and sooty mould are known

Table 1. Must analysis prior to yeast inoculation (means of two replicates of each treatment).

	Control	5%	25%
Alpha amino nitrogen (mg/L)	180	177	182
Ammonia (mg/L)	35	36	32
°Brix	24.0	23.9	23.3
Malic acid (mg/L)	1.6	1.6	1.5
pH	3.85	3.85	3.82
Free sulfur dioxide (mg/L)	14	12	13
Total sulfur dioxide (mg/L)	26	22	21
Titrateable acid pH 8.2 (g/L)	3.4	3.34	3.5
Yeast assimilable nitrogen (mg/L)	209	207	207
Laccase activity (µ/ml)	-	0.0	0.0
Laccase 24-hour qualitative test	-	Negative	Negative
Ochratoxin A (µg/L)	-	<0.03	<0.03
Absorbance 520 nm (absorbance units)	0.4	-	0.4
Anthocyanin (mg/L)	22.9	-	22.0

to extend beyond any effect on wine composition. The observed reduction in carbohydrate in the sooty mould-affected musts might logically be linked to two factors:

- that scale and mealy bug extract sap from the vine, and excrete carbohydrate in the form of honeydew, thereby reducing the amount of sugar available for transport to the grapes
- a loss of photosynthetic activity when leaves become coated with sooty mould.

Anecdotal reports suggest that heavy infestations of scale and mealybug can cause medium- to long-term declines in vine vigour, resulting in lower yields as well as more pronounced reductions in grape sugar than were apparent in this trial.

The sooty mould-affected musts were analysed for the presence of the enzyme laccase, which is associated with the fungus *Botrytis cinerea*, and for the presence of ochratoxin A, which is associated with certain *Aspergillus* and *Penicillium* species of fungi, but neither was detected (Table 1). No differences were observed between the treatments during fermentation, except for the previously noted increased apparent colour in the 25% sooty mould-affected ferments up to 48 hours post-yeast inoculation. All six fermentations progressed at the same rate, and were complete at approximately the same time, and this was also the case with the MLFs which were all complete within 20 days of inoculation with *Denococcus oeni* bacteria.

WINE ANALYSIS

After pressing, a suite of common wine analyses was performed (Table 2, see page 23). No differences in wine composition were identified, except for lower alcohol concentrations in the sooty mould-affected wines, likely due to the decreased sugar concentrations previously noted in the musts. Subsequently, a similar suite of analyses was performed periodically, including post-bottling (Table 2). Marked consistency is apparent for most analytes, both between analysis dates, and between the three treatments at each date. An increase in pH and fall in the concentrations of malic acid and titratable acidity was seen between the results obtained at pressing and subsequent analysis, consistent with the progression of malolactic fermentation. No differences in the concentrations of volatile acidity (expressed as acetic acid) were seen between the treatments on any of the four occasions on which that



Sooty mould on Chardonnay grapes.

variable was analysed.

Despite the same quantity of SO₂ having been added to all the treatments at each stage, the concentrations of free and total SO₂ were found to be consistently lower in the 25% sooty mould-affected treatment compared with the control. While unintended additional aeration of the 25% sooty mould-affected wine may have occurred during racking, resulting in a reduction of free SO₂, the pre- and post-bottling analysis appears to indicate a trend of a decreasing ratio of free to total SO₂ with increasing sooty mould infection. Although it should be noted that the ratios of free to total SO₂ in all three treatments are relatively high compared with commercially bottled Australian red wines (Godden *et al.* 2015), the apparent trend of lower ratios of free to total SO₂ with increasing sooty mould infection might be an indication that sooty mould can result in a greater proportion of SO₂ becoming bound to other compounds in the wine.

The wines were also analysed for

a suite of phenolics measurements on three occasions, the first being approximately two weeks after SO₂ had been added to the wines at the end of malolactic fermentation, and approximately monthly thereafter, with the final set of analysis being performed three weeks after bottling (Table 3, see page 24). While some differences between treatments were seen, many of these were within the error of the analytical methods used. The most notable difference was the increase in non-bleachable pigments in wines made from sooty mould-infected grapes, with consequent falls in the concentration of anthocyanins; the presumption being that the anthocyanins had been incorporated into the more colour-stable, non-bleachable pigments. These observations are consistent with an apparent increase in the hue, and what appears to be a slight increase in chemical age index 1. While these observations might indicate that the wines made with sooty mould-affected fruit were slightly more advanced in

Table 2. Wine analysis, pressing to post-bottling (means of two replicates of each treatment).

	Pressing			Four weeks post-completion of MLF and addition of SO ₂			Post-racking			Pre-bottling			Post-bottling		
	7 April 2017			24 May 2017			21 Jun 2017			5 July 2017			7 July 2017		
	Control	5%	25%	Control	5%	25%	Control	5%	25%	Control	5%	25%	Control	5%	25%
Alcohol (% v/v)	14.2	14.2	13.8	14.1	14.1	13.9	14.3	14.3	14.0	-	-	-	14.2	14.2	13.9
Glucose + Fructose (g/L)	0.35	0.2	0.15	0.7	0.6	0.5	0.65	0.60	0.50	-	-	-	0.6	0.5	0.5
Malic acid (g/L)	1.1	1.05	0.98	<0.05	<0.05	<0.05	-	-	-	-	-	-	-	-	-
pH	3.46	3.47	3.44	3.61	3.62	3.60	3.62	3.63	3.60	-	-	-	3.62	3.63	3.60
Specific gravity	-	-	-	0.993	0.993	0.993	0.992	0.992	0.993	-	-	-	0.993	0.992	0.993
Free sulfur dioxide (mg/L)	-	-	-	60	54	51	-	-	-	55	55	46	49	53	39
Total sulfur dioxide (mg/L)	-	-	-	101	88	84	-	-	-	94	98	86	81	92	71
Ratio free:total sulfur dioxide	-	-	-	0.59	0.61	0.61	-	-	-	0.59	0.56	0.53	0.60	0.58	0.55
Titratable acid pH 7.0 (g/L)	7.0	6.8	6.9	5.1	5.2	5.2	5.0	5.0	5.0	-	-	-	4.9	4.9	4.9
Titratable acid pH 8.2 (g/L)	7.5	7.2	7.3	5.7	5.8	5.8	5.6	5.6	5.6	-	-	-	5.5	5.5	5.5
Volatile acidity as acetic acid (g/L)	0.14	0.15	0.15	0.27	0.30	0.28	0.17	0.18	0.16	-	-	-	0.25	0.25	0.24
Turbidity (NTU)	-	-	-	-	-	-	21 ^a	6.4 ^a	2.7 ^a	-	-	-	0.16	0.16	0.15
Filterability index	-	-	-	-	-	-	1.3 ^a	2.0 ^a	3.3 ^a	-	-	-	-	-	-

^aAnalysis was performed on 26 June 2017

their development than the control, a greater proportion of stable colour pigments at this stage in the wines' life might be also considered a positive attribute. In any case, the phenolic differences between the wines were very small, and are considered to have little or no oenological or commercial relevance.

FILTRATION, SETTLING, WINE YIELD AND EXAMINATION OF LEES

Following racking, the three wines were analysed for turbidity, and for filterability according to the method described by Bowyer and Edwards. While an increase in the filterability index measurement was evident with increasing sooty mould content, the scores for all three wines were considered low, and there was no correlation between the filterability index score and the turbidity of the wines (Table 2). In practice, winemaking staff considered the wines easy to filter compared with most other red wines they had made using the same winemaking protocol during the 2017 vintage.

Following the first racking, the lees of each of the six fermentations were resettled in 2.5-litre measuring cylinders to investigate whether the presence of sooty mould influenced wine yield, lees settling or the quantity or nature of the lees. The lees were allowed to settle for approximately four weeks, and the clear wine was then racked ('second racking' in Table 4) and the quantities measured. The lees were then centrifuged for 10 minutes at 8000rpm, the quantity of supernatant wine was measured, and the lees solids were weighed. The lees solids were then desiccated, and reweighed.

While there was no clear trend to indicate that sooty mould infection affected wine yield after two rackings (Table 4), the data suggest that the presence of sooty mould inhibited settling, as demonstrated by an increase in the percentage of lees after the second racking of approximately 13% ('wet lees' in Table 4). In addition, an increase in lees solids after centrifugation

of approximately 10%, and in the dry weight of the lees of approximately 19% compared with the unaffected control, was also seen, but there was little difference in these measurements between the 5% and 25% sooty mould treatments. The higher lees content with the presence of sooty mould might be consistent with the observation of more apparent colour when the sooty mould-affected grapes were crushed, if that was attributable to greater fragmentation of the skins during crushing, subsequently resulting in a greater proportion of grape solids in the lees.

Analysis of a portion of the lees from each treatment was conducted following centrifugation to determine their yeast and plant-derived polysaccharide content (Table 5). For all the measurements presented in Table 5, there was an increase in the mass and percentage of both yeast and plant-derived polysaccharides with increasing sooty mould infection. While an increase in plant-derived polysaccharides might be explained by a greater proportion of grape solids in the must and lees resulting from the presence of sooty mould, the increase in yeast-derived polysaccharides is more difficult to explain. One possible explanation is that a portion of the 'yeast-derived' polysaccharides actually resulted from the sooty mould itself, given that polysaccharides of similar composition are a component of the cell walls of all microorganisms. However, the amount of polysaccharide quantified in the lees does not account for the overall quantum of lees solids, or the differences in lees volume or mass between the treatments presented in Table 4. The published literature on the composition of wine lees is minimal, and does not appear to contain information which can be used to explain the balance between the portion of lees composition which was quantified, and the total mass. However, based on previous observations at the AWRI, the remaining lees material is considered most likely to be a combination of plant-derived 'fibre', lipids and proteins. It is logical that more pathogenesis-related (PR) proteins would be formed in grapes

suffering sooty mould infection, and it is also possible that yeast could have assimilated some of those proteins during fermentation to build greater biomass, thereby accounting for a portion of the additional yeast-derived polysaccharides seen in the lees.

SENSORY ANALYSIS

Sensory analysis, consisting of a balanced reference triangle test, was conducted on the three wines approximately four weeks after bottling. Thirty-five experienced, screened and qualified assessors were asked to evaluate the wines for aroma and palate, selecting the sample in the set that was different from the other two.

No difference was identified between the control wine and the 25% sooty mould-affected treatment wine. However, a significant difference was identified

between the 5% treatment wine and the control wine ($P < 0.01$), and while the assessors were asked to comment on the nature of any differences they saw in the wines, no consistent trends were seen in the descriptions provided.

Consequently, a descriptive sensory analysis was conducted on the three wines by an expert panel. Significant differences ($P \leq 0.05$ or lower) were identified between the wines for two attributes, 'rubber/sulfide aroma' and 'fruit aftertaste', with the 5% treatment wine rated highest for rubber/sulfide aroma and lowest in fruit aftertaste. This suggests that reductive winemaking artefact may have been the main driving factor of the difference between the control wine and the 5% treatment wine, independent of the presence of sooty mould (Figure 1).

CONCLUSION

In this trial the presence of sooty mould on fruit had little effect on the sensory properties, chemical composition, or processing of the wines made from that fruit, notwithstanding the increased volume of lees produced in fermentations containing sooty mould. However, caution should be exercised when interpreting the results as it is possible that in many situations where sooty mould is present, other microorganisms will have also proliferated, which alone, or in combination with the sooty mould, could have a negative effect on the wine's sensory properties and composition.

With regard to the fruit used for this trial, low humidity and extended sunshine hours were experienced in the weeks before harvest, and no 'fresh'

Table 3. Results of phenolics analysis.

	Control			5%			25%		
	5 May 2017	21 June 2017	27 July 2017	5 May 2017	21 June 2017	27 July 2017	5 May 2017	21 June 2017	27 July 2017
Chemical age index 1	0.18	0.16	0.20	0.17	0.17	0.23	0.18	0.18	0.22
Chemical age index 2	0.05	0.05	0.05	0.04	0.05	0.06	0.05	0.05	0.06
Wine colour density (absorbance units)	12	14	13	10	12	13	11	14	13
Wine colour density SO ₂ corrected (absorbance units)	15	16	14	14	14	14	15	15	15
Hue	0.5	0.45	0.49	0.51	0.45	0.54	0.52	0.46	0.54
Degree of ionisation %	16	21	19	14	19	18	15	21	19
Total anthocyanins (absorbance units)	772	743	712	731	724	692	754	713	692
Total phenolics (absorbance units)	47	45	44	45	44	43	47	45	45
Non-bleachable pigment (absorbance units)	1.83	1.80	1.88	1.57	1.71	2.15	1.88	1.84	2.14
Tannin epicatechin concentration (mg/L)	825	863	768	794	710	652	879	750	683

Table 4. Wine yield and degree of settling of and quantity of lees (sum of two replicates of each treatment).

	Wine volume recovered after two rackings	Equivalent wine yield per tonne after two rackings	Volume of 'wet' lees after 2nd racking	'Wet' lees as % of wine volume after 2nd racking	Lees solids after centrifugation as % of wine volume after 2nd racking	Volume of wine removed by centrifugation	Wine removed by centrifugation as % of lees after 2nd racking	Dry weight of lees solids after desiccation	Dry weight of lees solids as % of wine volume after 2nd racking
	mL	L	mL	%	%	mL	%	g	%
Control	35720	638	2920	8.2%	3.2%	1787	61	545	18.7
5%	36920	659	3360	9.1%	3.5%	2120	63	663	19.7
15%	35490	634	3270	9.2%	3.5%	2043	62	647	19.8

Table 5. Polysaccharide content of the lees.

	Total polysaccharide in lees (% wine volume)	Total polysaccharide in lees (% dry weight)	Total polysaccharide in lees (g/L of wine)	Grape-derived polysaccharide in lees (% wine volume)	Grape-derived polysaccharide in lees (% dry weight)	Grape-derived polysaccharide in lees (g/L of wine)	Yeast-derived mannose+glucose in lees (% wine volume)	Yeast-derived mannose+glucose in lees (% dry weight)	Yeast-derived mannose+glucose in lees (g/L of wine)
Control	2.06%	11.00%	1.68	0.24%	1.31%	0.20	1.81%	9.69%	1.84
5%	2.30%	11.66%	2.09	0.27%	1.38%	0.25	2.03%	10.28%	1.85
25%	2.46%	12.42%	2.26	0.28%	1.40%	0.26	2.18%	11.02%	2.01

honeydew, sooty mould, or other microorganisms were observed during harvest. However, approximately four weeks earlier, fresh honeydew was present in the vineyard, and the sooty mould appeared to be actively growing. At that stage, other moulds, including low levels of *Botrytis* and what was believed to be *Penicillium*, were also observed. Therefore, while the condition of and careful selection of the fruit used for this trial might be considered ideal for the examination of the effects of sooty mould alone, it might represent an atypical situation in a commercial setting. In addition, it is possible that any small differences between the three wines presented here, will become more evident with time.

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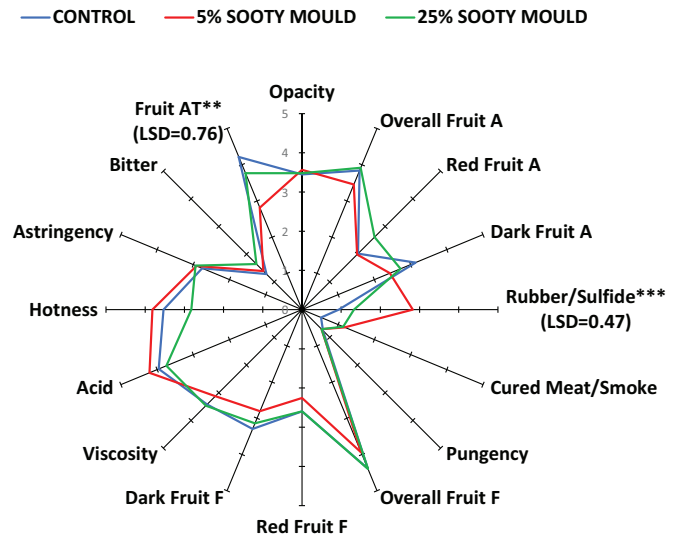


Figure 1. Spider plot of mean attribute scores for the control, 5% and 25% sooty mould treatments. Significant differences (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$) were only found for two attributes: 'rubber/sulfide' and 'fruit aftertaste'. Post-hoc Fisher's least significant difference (5%) values are included for significant attributes ($P \leq 0.05$). A: Aroma, F: Flavour

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