The Australian Wine Research Institute

COMMERCIAL SERVICES

FERMENTATION AND SENSORY EVALUATION OF WINES RELATING TO THE APPLICATION OF *SCREEN* AND *SCREEN DUO* TO WINEGRAPES

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EXECUTIVE SUMMARY

This study has been conducted to investigate and evaluate the impact on fermentation and wine quality relating to the application of *Screen* and *Screen Duo* to winegrapes. Both *Screen* and *Screen Duo* use kaolin-based 'particle film technology' to protect crops from sunburn and heat stress with *Screen Duo* having the addition of a naturally occurring compound to stimulate the crop's ability to cope with these stresses.

The results outlined in this report were generated by the Commercial Services group of the Australian Wine Research Institute Ltd (AWRI). The Laboratory is NATA-accredited in the field of chemical testing and is GLP recognised for analytical testing and grape processing for residue studies. This study was not performed in accordance with the OECD GLP guidelines.

Samples of whole bunches of frozen grapes were received on behalf of Agricrop from David Bell on the 5 May 2010. Samples consisted of an untreated control and a sample treated with *Screen* and a sample treated with *Screen Duo*. The *Screen* treatment consisted of four (4) applications with the initial application at 2.5 kg / 100 L and subsequent applications at 1.25 kg / 100 L. Screen Duo consisted of four (4) applications with the initial application at 1.25 kg / 100 L and subsequent applications at 0.625 kg / 100 L.

All samples were placed in frozen storage on receipt and kept frozen prior to vinification. Samples were thawed then processed as per AWRI SOP7 - Sample preparation for the fermentation and juicing of fresh and frozen grapes.

As part of the fermentation and wine quality study, each grape sample was thawed, crushed and destemmed then separated into triplicate ferments. These ferments were inoculated and then fermentation was monitored by weight loss until 'dry' (< 1 g/L residual sugar). The wines were then cold-settled, racked from gross lees then left to clarify, before being racked again and presented to a sensory panel for difference testing.

This study concluded that the *Screen* and *Screen Duo* application had a minor effect on fermentation onset. Fermentation rates and completion were unaffected. No significant difference was noted in wine quality parameters or red wine colour. Aluminium levels, with both products, were significantly lower than the untreated control and posed no risk to export markets. Sensory difference testing showed a minor difference between the untreated control and the wines from Treatment 3 (*Screen Duo*) at the 95% confidence level. No significant difference was noted when comparing the untreated control with the wines from Treatment 2 (*Screen*). No major faults or taints were evident as a result of the treatment.

EXPERIMENTAL

Sample Handling and Preparation

Three frozen grape samples of approximately 20 kg each were received and the samples assigned AWRI Sample IDs AC60547 – AC60549 as per Table 1. All samples were placed in frozen storage on receipt and thawed for at least 24 hours prior to vinification.

Table 1. AWRI sample IDs and study sample descriptions

AWRI Sample ID	Variety	Treatment No.	Treatment Type
AC60547	Shiraz	1	Untreated control
AC60548	Shiraz	2	*Screen*
AC60549	Shiraz	3	*Screen Duo*

The fermentations were conducted and monitored in triplicate thus giving three replicates of each wine. The wine samples were assigned AWRI sample IDs AC60550 – AC60558 as outlined in Table 2.

Table 2 Cross-referenced grape and wine sample ID

AWRI Grape Sample ID	Treatment	AWRI Wine Sample ID
AC60547	Untreated control	AC60550
AC60547	Untreated control	AC60551
AC60547	Untreated control	AC60552
AC60548	*Screen*	AC60553
AC60548	*Screen*	AC60554
AC60548	*Screen*	AC60555
AC60549	*Screen Duo*	AC60556
AC60549	*Screen Duo*	AC60557
AC60549	*Screen Duo*	AC60558

Vinification Procedure

The samples were processed as per AWRI SOP7 – Sample preparation for the fermentation and juicing of fresh and frozen grapes, which is summarised below.

For each ferment, a 5 L glass fermentation vessel was thoroughly cleaned, rinsed with an ethanol solution then allowed to dry.

The grapes were machine crushed and destemmed and approximately 50 mg/L of free sulfur dioxide added to the must using a potassium metabisulfite solution. Approximately 4-5 kg (where possible) of the must was transferred to each replicate 5L sterilised fermentation vessel and a subsample taken for pH, titratable acidity (TA) and Brix analysis (see Results section). Based on these results, the pH of each ferment was adjusted to approximately 3.5 with an addition of a tartaric acid solution. Diammonium phosphate (200 mg/L) was also added to each ferment. A stock solution of EC1118 rehydrated active dried wine yeast was prepared as per manufacturers recommendations and the must inoculated with the rehydrated yeast at 250 mg/L then gently mixed. Inoculation time (t = 0 hrs) and the total mass of each vessel was recorded and the vessels placed in a 25°C constant temperature room.

The fermentation vessels were checked, weighed and shaken, twice daily until pressing then daily, until no significant loss of mass was observed. If hydrogen sulfide formation was detected in the ferments during fermentation then a further 50 mg/L of diammonium phosphate was added to all ferments.

After seven (7) days, each ferment was pressed twice using a stainless steel basket press with mixing of the pomace between pressings. The pomace was weighed and charted then the ferment returned to the original vessel and allowed to complete fermentation with daily monitoring. Ferments were considered to be 'dry' when measured at <1 g/L residual sugar using the *Clinitest* method. The wines were racked from gross lees to a sterilised 2L vessel, approximately 100 mg/L of free sulfur dioxide was added using a potassium metabisulfite solution following which the wines were left to cold settle at approximately 4°C. During cold-settling, glass marbles were used in the containers to reduce ullage and prevent oxidation.

After a further 30 days the wines were racked from lees again prior to being presented for sensory assessment. An aliquot of each wine was taken for routine wine analyses by the Analytical Laboratory using NATA-accredited methods, AWRI LM28 – Prediction of alcohol, pH. TA, VA, SG, acetic acid and residual sugar (glucose + fructose) in wine using FOSS WineScan and LM29 – Determination of free and total sulfur dioxide in wine by flow injection analysis (FIA). Results of the routine wine analyses are outlined in Table 4 in the Results section of this report.

Due to concerns regarding regulatory limits for aluminium in Germany, the replicate finished wines were analysed for residual aluminium content. This analysis was subcontracted to a third-party laboratory to be performed using inductively-coupled plasma with mass spectrometry (ICP-MS). Results of this analysis are outlined in Table 5 of the Results section.

All replicate wines were also analysed for red wine colour due to potential effects on wine colour. This analysis was performed using AWRI GM107 – Determination of colour and tannin in grapes, juice and wine using Modified Somers. Results of this analysis are outlined in Table 5.

Any problems occurring during the winemaking process were treated after consultation with the Winemaking and Extension Services group at the AWRI. All winemaking was carried out in accordance with sound winemaking practices and applied to all wines in the study.

Sensory Analysis

The objective of the sensory assessment was to establish whether the wine made from treated grapes was different to the wine made from the untreated control grapes using a balanced-reference triangle difference test carried out in accordance with the Australian Standard AS 2542.2.2. Testing was performed under amber lighting to avoid bias due to any colour differences.

The individual wines were informally assessed by the Sensory Manager, Project Manager and sensory staff to ensure that they showed no obvious faults or taints and were suitable for assessment. The replicate wines were examined individually to ensure there were no large differences between the replicates and that each treatment could be pooled prior to sensory assessment to accrue sufficient volume.

RESULTS

Fermentation Study

A representative subsample of must from each of the six samples was taken during crushing for Brix, pH and titratable acidity (TA) analyses. Results of these analyses are outlined in Table 3 below. The pH of each must was adjusted to approximately 3.5 prior to fermentation using a tartaric acid solution.

Table 3. Results of routine grape analysis

Sample ID	pH	TA (g/L)	% sugar (°Brix)
AC60547	4.14	3.0	24.5
AC60548	4.10	2.9	24.6
AC60549	4.04	3.0	23.0

The individual fermentation data (Appendix1, Tables 7-9) has had the actual mass data adjusted for the loss and removal of the marc, due to pressing, so as to maintain the continuity of the fermentation profile. A small disturbance in the fermentation curves at the time of pressing is due to small losses of liquid and marc that occur during this process. The data was then adjusted to show the rate of fermentation as a percentage of the total weight lost over the period of fermentation with the initial weight expressed as 100%. This was done to allow easier comparison of the treatments and to assist in interpretation. Average fermentation curves for each treatment are shown in Appendix 2, Figure 1. All ferments went to completion as confirmed by the *Clinitest* method.

The individual fermentation data and the average fermentation curves for each treatment are illustrated in Figure 1. Comparison of the treatments and untreated control, using the means and standard errors of the data from the fermentation replicates, demonstrated no significant difference in fermentation rate between the untreated control (Treatment 1) and Treatment 3. A minor difference occurred between the fermentation rates of Treatment 1 and both treatments from approximately 48 to 160 hours post inoculation, with the Untreated Control fermenting at a faster rate. This difference is relatively consistent throughout the quoted time period and could be due to a small difference in the fermentation onset between the untreated control and the treated samples. There was no significant difference between the fermentation rates of the treatments. There was no significant difference between the treatment replicates with respect to the ability to complete fermentation.

Overall, the treatments were not considered to have had any adverse effect on the fermentation process.

Results of routine wine analyses performed on all wines and replicates, using AWRI NATA accredited methods LM28 and LM29, are outlined in Table 4.

The analytical results showed a high degree of consistency between the fermentation replicates for all analyses. One replicate from each of Treatment 2 (AC60555) and Treatment 3 (AC60557) had a total sulphur dioxide concentration that was significantly different from the other treatment replicates. Some experimental differences in sulphur dioxide levels are quite typical.

Table 4. Results of routine wine analyses

	Sample ID	pH	Alc. % (v/v)	VA g/L	TA g/L	G + F g/L	SG	FSO_2 mg/L	TSO_2 mg/L
Control	AC60550	3.68	13.8	0.37	5.7	0.4	0.9933	35	80
	AC60551	3.68	13.9	0.38	5.8	0.5	0.9932	40	83
	AC60552	3.69	14.0	0.38	5.8	0.6	0.9932	38	78
Screen	AC60553	3.67	13.9	0.37	5.7	0.7	0.9931	36	77
	AC60554	3.70	14.1	0.37	5.5	1.0	0.9930	38	76
	AC60555	3.68	13.7	0.40	5.8	0.5	0.9934	40	98
Screen Duo	AC60556	3.70	13.8	0.41	5.7	0.6	0.9936	38	75
	AC60557	3.73	13.7	0.45	5.8	0.6	0.9937	39	96
	AC60558	3.74	13.8	0.47	5.8	0.4	0.9936	35	75

VA – volatile acidity; TA – titratable acidity (pH 8.2); G+F – glucose + fructose (residual sugar); SG – specific gravity; FSO_2 – free sulfur dioxide; TSO_2 – total sulfur dioxide

Based on the average replicate result and the measurement of uncertainty for the analytical methods, there were no significant differences between the untreated control wines and the wines from either treatment for all analyses.

Aluminium concentrations and red wine colour results in the replicate finished wines are outlined in Table 5.

Table 5. Results of aluminium concentration and red wine colour

	Sample ID	Aluminium mg/L	Anthocyanin mg/L	Hue	Colour Density a.u.	Total Pigment a.u.
Control	AC60550	0.47	40	0.55	10.3	4.54
	AC60551	0.31	40	0.55	9.8	4.43
	AC60552	0.32	43	0.55	9.6	4.54
Screen	AC60553	0.22	47	0.55	10.2	4.80
	AC60554	0.2	44	0.55	10.4	4.80
	AC60555	0.19	51	0.54	10.4	5.07
Screen Duo	AC60556	0.097	46	0.55	10.5	4.82
	AC60557	0.13	45	0.55	10.3	4.86
	AC60558	0.12	45	0.55	10.8	5.01

a.u. – absorption units

Based on the average replicate result and the measurement of uncertainty for the analytical methods, the wines from both Treatments 2 and 3 both had significantly lower levels of aluminium than the untreated control wines. Aluminium has a regulatory limit in wine for the German market of 8 mg/L. Concentrations of aluminium in the wines from both treatments are considerably lower than this limit and hence, do not pose an issue to export markets.

For the red wine colour results, there were no significant differences between the untreated control wines and the wines from either treatment for all red wine colour results. Hence, the treatment is not considered to have any impact on wine colour.

Overall, the treatment was not considered to have had any significant impact on routine wine quality parameters or red wine colour. Levels of aluminium are significantly lower in the treated wine samples compared to the untreated control wines. The levels of aluminium are well below the regulatory limit for wine exported to Germany.

Sensory Analysis

The replicate wines were examined individually and samples AC60551 (untreated control) and AC60554, AC60555 (Treatment 2) showed some very slight 'reductive' characters and were removed from the pooling of wines to avoid any bias from the panel. No gross winemaking faults or taints were present, all other treatment replicates were pooled prior to sensory assessment to accrue sufficient volume.

Table 6 below provides the results of the triangle test.

Table 6. Results of triangle test, n=30 responses

Test no.	Comparison	No. of correct responses	Significance (p)
1	Untreated Control vs *Screen*	13	n.s.
2	Untreated Control vs *Screen Duo*	15	0.0435

n.s. – not significant

The results of the difference testing, based on a 95% confidence level, were as follows:

The difference test comparing the Untreated Control with the wine from Treatment 2 (*Screen*) gave thirteen (13) correct responses from the total of thirty (30), indicating the panel saw no significant difference between the wines. No major faults or taints that could be related to the treatment were detected by the sensory panel.

The difference test comparing the Untreated Control with the wine from Treatment 3 (*Screen Duo*) gave thirteen (15) correct responses from the total of thirty (30), indicating the panel saw a significant difference between the wines with a 4.4% probability that the difference is false. Inconsistent panel descriptions of the differences meant they could not be used to elucidate the cause. No major faults or taints that could be related to the treatment were detected by the sensory panel.

OVERALL CONCLUSION

The fermentation data and plots comparing the Untreated Control and the samples treated with both *Screen* and *Screen Duo* indicated that the treatments had no adverse effect on fermentation rate and completion. A slight delay in fermentation onset was noted but fermentation rates and completion were unaffected. No consistent differences in routine wine quality parameters were noted in association with the treatments.

Aluminium levels were significantly lower in the treated sample wines and were well below any regulatory limits for any export markets at this time. Red wine colour levels were not significantly affected by either treatment.

Sensory analysis using balanced difference testing showed a significant difference between the Untreated Control wine and the wine from Treatment 3 with a 4.4% probability of a false prediction. The difference noted was at the threshold for the 95% confidence interval. No significant difference was noted when comparing the untreated control and the wine from Treatment 2.

Overall, the application of *Screen* is considered to have a very minor impact on fermentation onset but fermentation rates and completion were unaffected. No detrimental impact on red wine colour and aluminium levels was shown by the treatment. Sensory analysis demonstrated no significant impact on wine quality.

The application of *Screen Duo* is considered to have a very minor impact on fermentation onset but fermentation rates and completion were unaffected. No detrimental impact on red wine colour and aluminium levels was shown by the treatment. Sensory analysis demonstrated no obvious faults or taints but a minor difference was noted when comparing the untreated control and the wines from Treatment 3.

APPENDIX 1

Table 7. Individual fermentation data for untreated control (Treatment 1) samples

Sample reference		AC60550		AC60551		AC60552			
Treatment		Untreated control		Untreated control		Untreated control		Average	2 x std error
Date	Time (hrs)	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left		
Initial Mass	0.00	6.1042	100.0	6.0664	100.0	6.0803	100.0	100.0	0.0
8-Jun-10	18.00	6.0873	95.8	6.0517	96.4	6.0634	95.8	96.0	0.4
8-Jun-10	24.00	6.0723	92.0	6.0394	93.3	6.0480	92.0	92.5	0.9
9-Jun-10	42.00	6.0153	77.7	5.9815	79.0	5.9887	77.4	78.0	1.0
9-Jun-10	48.00	5.9937	72.3	5.9592	73.5	5.9659	71.7	72.5	1.1
10-Jun-10	66.00	5.9453	60.2	5.9101	61.4	5.9185	60.0	60.5	0.9
10-Jun-10	72.00	5.9270	55.6	5.8910	56.7	5.8999	55.4	55.9	0.8
11-Jun-10	90.00	5.8869	45.6	5.8504	46.7	5.8612	45.8	46.0	0.7
12-Jun-10	110.00	5.8210	29.0	5.7847	30.5	5.7962	29.8	29.8	0.8
13-Jun-10	134.00	5.7672	15.6	5.7310	17.2	5.7417	16.3	16.4	1.0
15-Jun-10	158.00	5.7105	1.4	5.6676	1.6	5.6821	1.6	1.5	0.2
17-Jun-10	206.00	5.7051	0.0	5.6612	0.0	5.6757	0.0	0.0	0.0
Mass of Marc (kg)		0.3463		0.3635		0.4110			
Total mass lost (kg)		**0.3991**		**0.4052**		**0.4046**			

Table 8. Individual fermentation data for Treatment 2 (*Screen*) samples

Sample reference		AC60553		AC60554		AC60555			
Treatment		Treatment 2		Treatment 2		Treatment 2		Average	2 x std error
Date	Time (hrs)	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left		
Initial Mass	0.00	6.0831	100.0	6.0847	100.0	6.0375	100.0	100.0	0.0
8-Jun-10	18.00	6.0720	97.2	6.0716	96.8	6.0286	97.7	97.2	0.5
8-Jun-10	24.00	6.0595	94.1	6.0578	93.4	6.0196	95.3	94.3	1.1
9-Jun-10	42.00	6.0135	82.7	6.0116	82.1	5.9753	83.7	82.8	1.0
9-Jun-10	48.00	5.9979	78.8	5.9957	78.2	5.9574	79.0	78.7	0.5
10-Jun-10	66.00	5.9598	69.4	5.9579	68.9	5.9181	68.8	69.0	0.4
10-Jun-10	72.00	5.9445	65.6	5.9432	65.3	5.9026	64.7	65.2	0.5
11-Jun-10	90.00	5.9112	57.3	5.9101	57.1	5.8674	55.5	56.7	1.1
12-Jun-10	110.00	5.8514	42.4	5.8514	42.7	5.8041	38.9	41.4	2.4
13-Jun-10	134.00	5.7937	28.1	5.7925	28.3	5.7447	23.4	26.6	3.2
15-Jun-10	158.00	5.7026	5.5	5.7022	6.1	5.6740	4.9	5.5	0.7
17-Jun-10	206.00	5.6805	0.0	5.6773	0.0	5.6552	0.0	0.0	0.0
Mass of Marc (kg)		0.5451		0.4703		0.5415			
Total mass lost (kg)		**0.4026**		**0.4074**		**0.3823**			

Table 9. Individual fermentation data for Treatment 3 (*Screen Duo*) samples

Sample reference		AC60556		AC60557		AC60558			
Treatment		Treatment 3		Treatment 3		Treatment 3		Average	2 x std error
Date	Time (hrs)	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left	Mass (kg)	% Wt. Left		
Initial Mass	0.00	6.0268	100.0	6.0685	100.0	5.9340	100.0	100.0	0.0
8-Jun-10	18.00	6.0172	97.4	6.0568	97.0	5.9340	100.0	98.2	1.9
8-Jun-10	24.00	6.0068	94.7	6.0450	94.0	5.9216	96.6	95.1	1.5
9-Jun-10	42.00	5.9607	82.4	6.0015	82.9	5.8724	83.0	82.8	0.4
9-Jun-10	48.00	5.9447	78.1	5.9854	78.8	5.8553	78.2	78.4	0.4
10-Jun-10	66.00	5.9053	67.6	5.9476	69.2	5.8172	67.7	68.2	1.0
10-Jun-10	72.00	5.8890	63.3	5.9319	65.2	5.8008	63.2	63.9	1.3
11-Jun-10	90.00	5.8561	54.5	5.8968	56.2	5.7669	53.8	54.8	1.4
12-Jun-10	110.00	5.7947	38.2	5.8329	39.9	5.7051	36.7	38.2	1.9
13-Jun-10	134.00	5.7385	23.2	5.7743	24.9	5.6483	21.0	23.0	2.3
15-Jun-10	158.00	5.6739	6.0	5.6984	5.6	5.5843	3.3	4.9	1.7
17-Jun-10	206.00	5.6515	0.0	5.6765	0.0	5.5725	0.0	0.0	0.0
Mass of Marc (kg)		0.4420		0.4329		0.4343			
Total mass lost (kg)		**0.3753**		**0.3920**		**0.3615**			

APPENDIX 2

Figure 1. Comparison of fermentation profiles for control and treated samples