Enhanced Maturity and Botrytis Trial 2016

Isosceles Vineyard, Te Mata Estates
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Executive Summary

Enhanced maturity[1] as it pertains to wine grapes is a recent area of viticultural research and it builds on the background of scientific research undertaken on table grapes in the US and Israel.

This trial is the last in a series of trials beginning in the 2010/2011 season in a Hawke's Bay, New Zealand vineyard where Protector[hml] (a potassium soap complex) and potassium bicarbonate were combined and found to 'enhance the maturity' of wine grapes, as well as to provide substantial end of season botrytis resilience.

This report summarises three extensive hand sprayed trials (Chardonnay, Syrah and Merlot) in another Hawke's Bay vineyard (Te Mata Estate's Isosceles Vineyard) where the primary focus was to identify the most effective timing for enhanced maturity applications of HML32 with and without an additive of HML Silco (potassium silicate).

The best timings were taken through to wine and **improvements in wine quality** have been established both by chemical analysis and sensory analysis.

Considerable time and effort has been expended professionally reviewing historic phenological and climatic data related to the trial site with the aim of providing a formula to make reliable judgements regarding other varieties and other growing regions. Several concepts/timings have been examined to assist growers to ascertain the likely best spray time/timings, such as 5% flowering, establishing the lag phase, counting back from a theoretical harvest date, brix accumulation and veraison.

[1] 'Enhanced maturity' as it pertains to wine grapes can be defined as follows:
At any point, after the application to berries/bunches of a particular concentration of a particular potassium salt, when measured against an untreated control, there is

- *earlier maturity*
- *enhanced brix content*
- *enhanced colour*
- *thicker skins*
- *enhanced phenolics*
- *no obvious change in berry appearance*

The three features that are required to be present for 'enhanced maturity' to reliably occur are:-

- a potassium salt of the right type within a suitable concentration range
- application/s timed when the berry is most amenable to influence
- an adjuvant with the right properties to spread, penetrate, deposit and dry the potassium salt on the berry surface to facilitate interaction between the them.

The conclusion is that **the best plant growth stage to work from is 50% veraison** defined as 50% of berries being soft or 8.5 brix.

Hence the recommended spray timing would be:

Chardonnay: If sprayed twice – **the first application to be at 50% veraison followed by another application 10 days later.**
If only going to apply once – **apply 10 days after 50% veraison**.

Merlot and Syrah: If sprayed twice – **the first application to 7 days after 50% veraison followed by another application 10 days later.**
If only going to apply one – **apply 17 days after 50% veraison**.

This report also specifically notes a **significant improvement in resilience to botrytis** (both expressed as bunch botrytis or slip skin) achieved through applications at the same timing that produced 'enhanced maturity'.

This report confirms that

- Red grapes require different application timings to white grapes.
- 'Thicker skins' are generated.
- Other issues that influence maturity such as crop thinning/loading, water stress influence the level of enhancement achievable by these applications.
- Two applications (10 days apart) deliver a greater effect than single applications – but a single application in some instances might be all that is required in some circumstances.
- The use of potassium silicate as an additive to a single application did not improve a single application significantly – but may still have potential in machine sprayed applications.
- A reduction in yield is strongly indicated when the harvest brix is targeted at 22 to 23, if there was no rain event.

Summary of Trial Objectives

The objectives of the trial were to expand on previous research as follows:

1. To identify the best plant growth timing 'to link' applications of HML32 to achieve enhanced maturity.
2. To identify if different application timings are required for white or red varieties.
3. To establish/confirm that 2 applications are required, rather than one application at a more precise timing.
4. To document the relationship between brix accumulation and berry weight (yield analysis).
5. To explore through the use of a potassium silicate additive (HML Silco), the possibility of making one application instead of two.
6. To identify any changes in the berry itself in terms of firmness/thickness and whether that provides any disease resilience, especially against botrytis.
7. To confirm that there is little or no visual differences between treated and untreated berries in respect of shrivel etc. with the use of HML32.
8. To confirm that fermentation of like wines were normal and similar and through sensory evaluation that all wines contained no faults.
9. To report through sensory evaluation the underlying values of all wines

Objective 1: Timing of Applications

In this report, application timings have been defined as Days after Lag Phase as well as Days after 5% flowering. In earlier trials, days before a theoretical harvest date was used, as well as brix accumulation.

These timing constructs are problematic due to the variability from year to year. Dr Rob Agnew, Plant and Food Research, reviewed historic phenological and climatic data relating to the trial site with the aim of providing a formula to make reliable judgements regarding other varieties and other growing regions. Various plant stages were considered including the ones described above.

After all issues were considered, the stage that is most recommended is **50% veraison, defined as 50% softening of berries or 8.5 Brix.**

This growth stage has a number of advantages: it is close to when applications are required, there is a good history of the 50% veraison timepoint by region and by variety, and growers for the most part have access to it in real time. It is also something growers can individually make a judgement call in relation of their own crop and location in respect of data produced close by them.

For Chardonnay (best effects), the first application timing would be at 50% veraison followed by another application 10 days later. If only one application was to be made the target timing would be 10 days after 50% veraison.

For Merlot and Syrah (best effects), the first application would be 7 days after 50% veraison followed by another application 10 days later. If only one application was to be made then the target timing would be 17 days after 50% veraison.

Objective 2 : Identifying the application timing for red and white varieties
The trial data confirms that timing for reds and whites are different as described above.

Objective 3: **Number of Applications**
Two applications are clearly indicated to achieve better results than one application. The reasons for this are unknown but it is more likely to be around the ability of the berries to uptake, than simply timing. Data around timing/effects indicates a soft 'bell curve' in relation to timing/plant growth stage – meaning that timing can be less than optimal but still successful.

All offshore data to date also confirms 2 applications are better than 1.

Objective 4: Relationship between Brix and Yield
The Chardonnay trial confirms that from the time of application to harvest, loss of berry weight appears to be a feature of 'enhanced maturity' when there are no other external influences such as rain. Yield loss and brix accumulation are clearly linked.

Further study is however required as the Syrah trial gave confusing and unusual outcomes, especially when applications of HML32 included the additive (and particularly in the weeks immediately after application). Some results indicated heightened brix, but also an increase in yield.

Objective 5: Changes in berry firmness and resilience against disease

The trial confirmed that the applications of HML32 that enhanced maturity also resulted in firmer berries (thicker skins), which in turn provided significant resilience to end of season diseases – in this case botrytis (both as bunch botrytis and slip skin). This is perhaps the most significant finding of this report.

Objective 6: Reduced number of applications with an additive
The trial disclosed there was an improvement over the short term from a single application of HML32 with the additive (HML Silco) but at harvest there appeared to be no difference in outcome. This requires further study, particularly in respect of machine sprayed trials, where it is likely there will be step down in performance from hand spraying – but the addition of HML Silco might reduce the difference.

Objective 7: Visual effects on the Berries
The trial confirmed that HML32, when applied at the correct plant growth stage, under NZ conditions, does not cause any visual adverse effect on the berries or bunches, such as advanced raisoning (shrivel), for both red and white varieties. Additionally no phytotoxic effects were noted on foliage.

Objective 8: Fermentation and Wine Faults
In all cases, fermentation of the untreated control and the three treatment wines for each variety conformed to the same fermentation curve – indicating clearly that treatments did not affect the fermentation process.

All wines have no off flavours or faults.

Objective 9: Sensory evaluation and comment
An overview of sensory aspects is contained in the report by Ant Mackenzie, a senior consultant winemaker. The wines were tested in flights by wide audiences in Hawkes Bay, Gisborne and Marlborough with no negative comments received.

1.0 Introduction

This trial involved trials on three grape varieties (Chardonnay, Merlot and Syrah) in one vineyard. Each variety received applications of 3 different treatments applied at 5 day intervals from the lag phase to harvest. With the requirements of replication and the size of the plots, each trial measured approximately 0.5ha per variety at trial completion.

The data measured and collected consists of:

- A large folio of photographs taken of tagged bunches in the three varieties covering all treatments of one replicate. For the Chardonnay, one bunch per treatment/timing was taken. For the Syrah and Merlot, photographs of two representative bunches were taken as more variation through veraison was expected. Photographs were taken from the start of the trial, generally on the same day of application.
- Brix and yield data over a 7 week period. Four sets of brix and yield over all treatments of the Chardonnay (4 weeks before harvest). Three sets of brix and yield over all treatments of the Syrah (3 weeks before harvest).
- Comparative penetrometer readings at different time points.
- Videos of botrytis (slip skin) outcomes in the Merlot trial that could be recorded in no other way.
- In-house and independent botrytis disease assessments in the Syrah trial.
- Preliminary juice results, fermentation charts from trial wines (12) and AWRI 'Wine Cloud' tannin and phenolic comparisons for Merlot and Syrah.

Supporting information includes:

- Gubler grape powdery mildew prediction data and Bacchus botrytis prediction data
- Growing degree day accumulation from flowering and from the beginning of the trial
- Plant and soil background nutrient levels
- Water quality of spray make-up water.

It is accepted that the robustness of the trial is perhaps slightly weakened by the fact that the full suite of brix and yield data, penetrometer readings and botrytis assessments was not completed for all varieties and so the conclusions reached are specific to the variety. This occurred because of time constraints and resource requirements given the size of the trials, and because some of the outcomes were only seen at the point of harvest and could not be measured except in another variety. However it is the belief of the author that even allowing for the varieties being white or red, the outcomes if concentrated on one variety would have been the same.

1.1. History and Background

This is the 5th season in New Zealand that the phenomenon of enhanced maturity has been studied. It was in the 2010/2011 season that a combination of Protector^{hml} (Protector) and potassium bicarbonate (the genesis of HML32) markedly improved the maturity of Sauvignon Blanc as well as providing exceptional end of season rot resistance in challenging climatic conditions.

Unknown at the time, that trial aligned with scientific study already underway in California to enhance the maturity of table grapes. Potassium bicarbonate was included in the last season of that study and was the best performing potassium salt for the effects of increased brix, heightened colour and 'thicker' skins. This study was presented at the US Table Grape Commission conference in 2012 (Joseph Smilanick and others). It is accessible via the internet at -

http://www.henrymanufacturing.co.nz/products/hml-32/publications/potassium-effects-on-table-grapes.pdf.

Scientific study has continued in Israel where the 'mode of action' of this phenomenon was established in respect of specific potassium salts as mild desiccation (water loss) engendering the plant to replenish with a full nutrient stream – hence 'enhancement'. That study was reported by Amnon Lichter and others - Scientia Horticulturae 187 (2015) 58-64.

'Enhanced Maturity' and how it relates to wine grapes and their end of season diseases is a new area of viticultural research.

'Enhanced maturity' as it pertains to wine grapes can be defined as: - At any point, post application to berries of a particular potassium salt, when measured against an untreated control, there is

- earlier maturity
- enhanced brix content
- enhanced colour
- thicker skins
- enhanced phenolics

The three features that are required to be present for 'enhanced maturity' to reliably occur are:-

- a potassium salt of the right type within a concentration range
- application/s timed when the berry is amenable to influence
- an adjuvant with the right properties to spread, penetrate and dry the potassium salt on the berry surface at a rate to facilitate interaction.

Studies in New Zealand (2011/2012, 2012/2013, 2013/2014, 2014/2015) by the author and supported by many people, have revolved around specifying the rate of potassium bicarbonate in combination with Protector and the appropriate plant growth timing, with hand sprayed and machine sprayed trials on white and red wine grapes.

Successful treatments have been taken through to wine – the most comprehensive being described in the 2013/2014 presentation given to the Gimblett Gravels Technical Workshop. This presentation can be viewed on -
https://www.dropbox.com/s/u4ltx7am5mfbgl8/HML32%20Presentation%20%20Gimblett%20Gravel%20Growers%20Tech%20meeting%201%209%2014.pptx?dl=0.

The report for that trial is not on the Henry Manufacturing Limited website because of the failure of commercial trials in the season following (2014/2015) and the obvious need to improve the reliability of outcomes.

In 2014/15, 18 full size commercial machine sprayed trials were planned comparing wines made from adjacent treated and untreated blocks with conventional wine making methods. No phytoxicity issues occurred however, all but one vineyard failed to exhibit the enhancement expected (2 brix improvement from 2 applications).

The two areas identified as requiring further study were the plant growth timing and application. The potassium bicarbonate rate as included in HML32 was deemed to be satisfactory.

The intention of this report is to deepen understanding in respect of plant growth timing.

2.0 Trial Objectives

The objectives of the trial were as follows:

- To identify the critical plant growth timing for the application of HML32 to achieve enhanced maturity.
- To identify if different application timings are required for white or red varieties.
- To provide an adjustment formula, a 'best guess', to enable growers to make the necessary timing adjustments by variety and by their vineyard location using the trial data as a base.
- To document the relationship between brix accumulation and berry weight (yield analysis).
- To explore through the use of a potassium silicate additive (HML Silco), the possibility of making one application instead of two.
- To identify any changes in the berry itself in terms of firmness/thickness and whether that provides any disease resilience, especially against botrytis.
- To confirm that there is little or no visual differences between treated and untreated berries in respect of shrivel etc. with the use of HML32.
- To confirm that fermentation of like wines were normal and similar and through sensory evaluation that all wines contained no faults.
- To report through sensory evaluation the underlying values of all wines

3.0 Trial Site

The trial site is located on a vineyard owned by Te Mata Estate, Maraekakaho Rd, Bridge Pa, Hawkes Bay (see Figure 1). The trial was undertaken on three varieties - Chardonnay (planted 2000), Merlot (planted 2002) and Syrah (planted 2006).

The vines were 4 cane-pruned (Chardonnay and Merlot) and 2 cane- pruned (Syrah), VSP trellised and planted in a slightly north west by south west orientation. The rows were 2.5 metres apart, the bay length was eight metres with plants at approximately two metre spacing. All trials covered an area of approximate 0.5ha each when applications concluded, in an area that appeared to offer a consistent soil type.

The condition of the vineyard, the health of the vines, disease control (until close to harvest), and the consistency between vines is a credit to the management of the vineyard. All crops were drip line irrigated as needed after veraison with a minimum of water stress. Background nutrient levels of each variety and soil tests showed good levels, neither deficient nor excessive (see Appendix 1). A test of the irrigation water (and spray water) showed some levels of hardness (see Appendix 2).

The Growing Degree Days for the season followed a normal pattern as shown in Figure 2.

From a data point of view this meant there was a complete lack of background 'noise' in any of the trial sites.

The Chardonnay carried a crop of approximately 13 t/ha. The normal crop load is 8-10 t/ha for this area. It was therefore heavily cropped, particularly considering that the clone was Mendoza.

The Merlot was heavily cropped (to capacity). The crop load was not determined, but it was thought to be between 13t and 16t/ha.

The Syrah was crop managed to one bunch per shoot, and then after veraison the crop was then reduced again to 17 bunches per vine (premium quality grapes). Crop load was not determined or estimated.

Figure 1: Isosceles Vineyard Trial Site

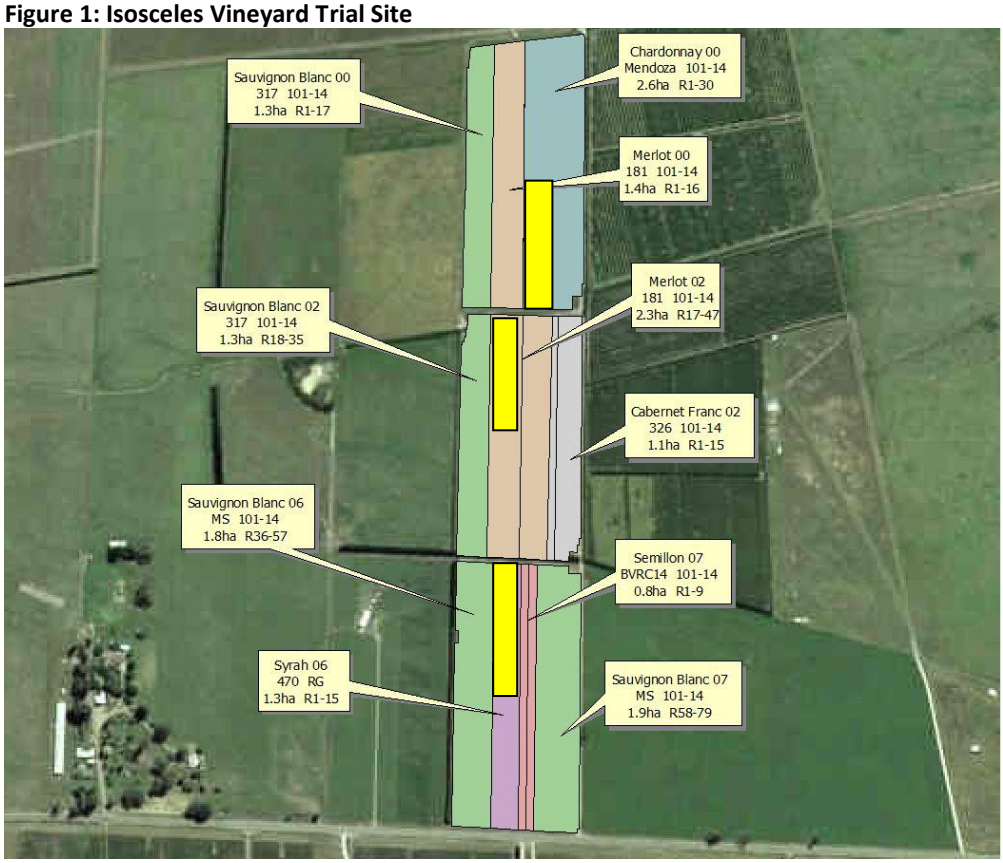
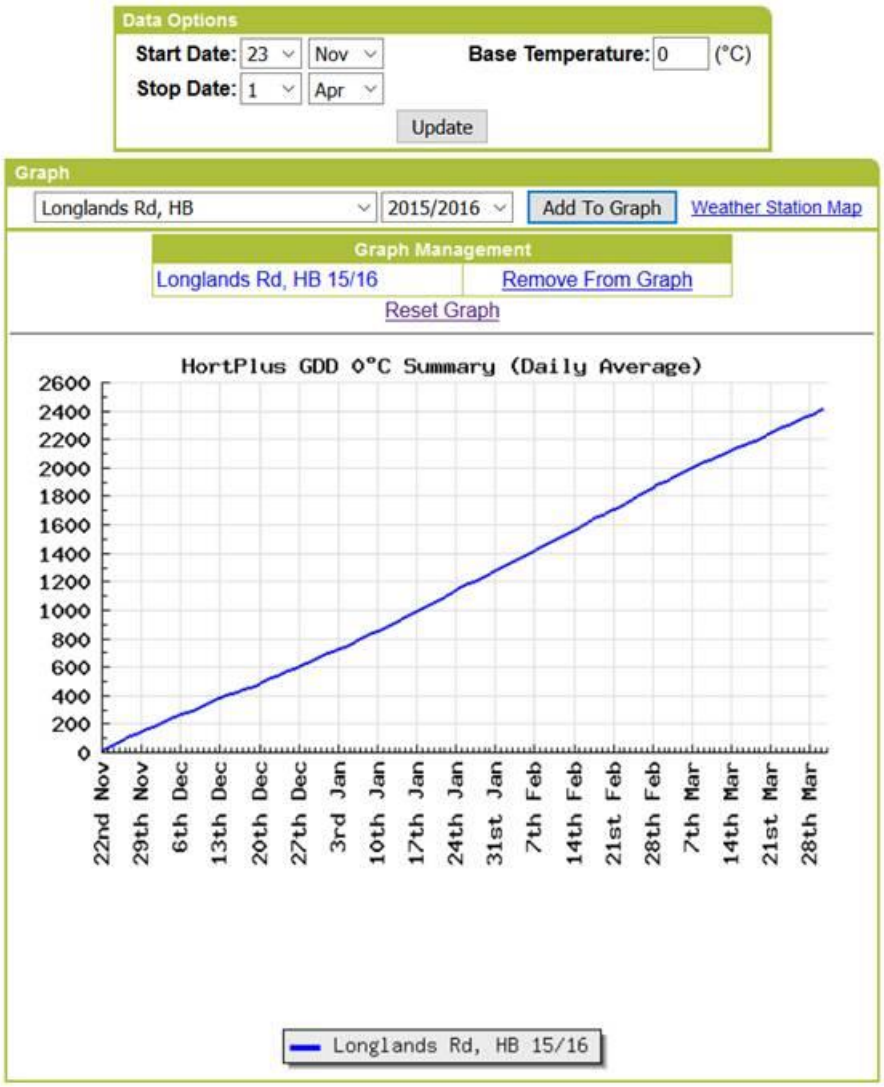


Figure 2: Growing Degree Days - Longlands Rd site



3.1. Trial Design

The trial plots were across 15 rows, the end selected as the one having the least soil variability. The first block of trial plots (to Treatment 26 (Day 40)) comprised 7 bays per row. Behind this block separated by a guard bay, the trial block was repeated for the treatments from Day 45 onwards.

Each treatment has a replication of four, randomly laid out within. Each plot usually contained four plants (very few had three plants – none had two). The treatment plots are shown in Table 1: Trial layout and replication

(a)
o Day 65

6a	1a	17a	15a	25a	14a	7a	11a	12a	11a	21a	8a	21a	4a
5a	8a	24a	25a	9a	12a	8a	22a	10a	19a	23a	2a	13a	24a
20a	19a	21a	7a	13a	19a	17a	3a	25a	20a	7a	25a	11a	9a
16a	9a	22a	23a	18a	1a	26a	17a	22a	9a	1a	1a	12a	5a
26a	12a	2a	3a	6a	10a	24a	6a	14a	24a	4a	18a	7a	20a
13a	10a	11a	15a	23a	2a	5a	3a	18a	15a	2a	8a	19a	17a
4	14a	18a	4a	21a	16a	20a	13a	5a	16a	26a	22a	15a	26a
Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard
6	1	17	15	25	14	7	11	12	11	21	8	21	4
5	8	24	25	9	12	8	22	10	19	23	2	13	24
20	19	21	7	13	19	17	3	25	20	7	25	11	9
16	9	22	23	18	1	26	17	22	9	1	1	12	5
26	12	2	3	6	10	24	6	14	24	4	18	7	20
13	10	11	15	23	2	5	3	18	15	2	8	19	17
4	14	18	4	21	16	20	13	5	16	26	22	15	26
Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard
Headland													

o Day 40)

Table 2The treatments and the treatment descriptions are shown in Table 2.

There were three sets of treatments; HML32 plus a potassium silicate additive sprayed once at 5 day intervals, HML32 alone sprayed once at 5 day intervals and HML32 sprayed twice, 10 days apart. The three treatments sets are respectively referred to as HML32 Plus, HML32 Single and HML32 Twice in the results section. Each set of treatments were sprayed onto 4 different plots every five days. Each timing (or combination of timings) in each set represents an individual treatment.

Initially, the trial was designed to identify the critical timing from the Lag Phase (defined as being 55 days after flowering begins (5% flowering) through to Day 40 (post Lag Phase). However this was extended to end as close to harvest as possible in order to gain the most complete set of data possible.

The last treatment timing depended on harvest date. In the Chardonnay, treatments stopped at Day 55 (7 March 2016) as harvest was due in the week beginning 21 March 2016. In the Merlot and Syrah, treatments stopped at Day 65 (19 March 2016).

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Table 1: Trial layout and replication

2nd set (a)	6a	1a	17a	15a	25a	14a	7a	11a	12a	11a	21a	8a	21a	4a	3a
Day 45 to Day 65	5a	8a	24a	25a	9a	12a	8a	22a	10a	19a	23a	2a	13a	24a	14a
	20a	19a	21a	7a	13a	19a	17a	3a	25a	20a	7a	25a	11a	9a	16a
	16a	9a	22a	23a	18a	1a	26a	17a	22a	9a	1a	1a	12a	5a	6a
	26a	12a	2a	3a	6a	10a	24a	6a	14a	24a	4a	18a	7a	20a	23a
	13a	10a	11a	15a	23a	2a	5a	3a	18a	15a	2a	8a	19a	17a	10a
	4	14a	18a	4a	21a	16a	20a	13a	5a	16a	26a	22a	15a	26a	
	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard
1st set	6	1	17	15	25	14	7	11	12	11	21	8	21	4	3
(Day 0 to Day 40)	5	8	24	25	9	12	8	22	10	19	23	2	13	24	14
	20	19	21	7	13	19	17	3	25	20	7	25	11	9	16
	16	9	22	23	18	1	26	17	22	9	1	1	12	5	6
	26	12	2	3	6	10	24	6	14	24	4	18	7	20	23
	13	10	11	15	23	2	5	3	18	15	2	8	19	17	10
	4	14	18	4	21	16	20	13	5	16	26	22	15	26	
	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard	Guard
	Headland														

Table 2: Trial Treatments

	Colour of Timing	Blue	Red	White	Yellow	Lime green	Orange	Black-white	Yellow-Black	Pink-Black	Blue	Red	White	Yellow	Lime gree	Orange	Black-whit	Yellow-Bla	Pink-Black
Treatment	Colour of Treatment	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Day 35	Day 40	Day 45	Day 50	Day 55	Day 60	Day 65	Day 70	Day 75	Day 80	Day 85
1	Green - untreated																		
2	Blue/Orange-White	HML32/Si																	
3	Red/Orange-White		HML32/Si																
4	White/Orange-White			HML32/Si															
5	Yellow/Orange-White				HML32/Si														
6	Lime Green/Orange-White					HML32/Si													
7	Orange/Orange-White						HML32/Si												
8	Black-White/Orange-White							HML32/Si											
9	Yellow-Black/Orange-White								HML32/Si										
10	Pink-Black/Orange-White									HML32/Si									
20a	Blue/Orange-White										HML32/Si								
3a	Red/Orange-White											HML32/Si							
4a	White/Orange-White												HML32/Si						
5a	Yellow/Orange-White													HML32/Si					
6a	Lime Green/Orange-White														HML32/Si				
7a	Orange/Orange-White															HML32/Si			
8a	Black-White/Orange-White																HML32/Si		
9a	Yellow-Black/Orange-White																	HML32/Si	
11	Blue	HML32																	
12	Red		HML32																
13	White			HML32															
14	Yellow				HML32														
15	Lime green					HML32													
16	Orange						HML32												
17	Black-White							HML32											
18	Yellow-Black								HML32										
19	Pink-Black									HML32									
11a	Blue										HML32								
12a	Red											HML32							
13a	White												HML32						
14a	Yellow													HML32					
15a	Lime green														HML32				
16a	Orange															HML32			
17a	Black-White																HML32		
18a	Yellow-Black																	HML32	
20	Blue/White	HML32		HML32															
21	Red/Yellow		HML32		HML32														
22	White/Lime Green			HML32		HML32													
23	Yellow/Orange				HML32		HML32												
24	Lime Green/Black-white					HML32		HML32											
25	Orange/Yellow-Black						HML32		HML32										
26	Black-white/Pink-Black							HML32		HML32									
2a	Yellow-Black/Blue								HML32		HML32								
21a	Pink-Black/Red									HML32		HML32							
22a	Blue/White										HML32		HML32						
23a	Red/Yellow											HML32		HML32					
24a	White/Lime Green												HML32		HML32				
25a	Yellow/Orange													HML32		HML32			
26a	Lime Green/Black-white														HML32		HML32		
10a	Orange/Yellow-Black															HML32		HML32	
19a	Black-white/Pink-Black																HML32		HML32

3.2. Identification of Lag Phase

The Lag Phase is a recognised plant growth stage for grapes. It is a period where the berry stops growing for a short period immediately before veraison (ripening processes) begin.

The onset of the Lag Phase was identified using a method developed by Dr Steve Price, Cornell University. This involved slicing through a berry and seed with a razor blade and observing when seed resistance occurred. This assessment was undertaken on 11/12 January 2016.

Seed resistance was felt in all berries in the Chardonnay indicating that the lag phase had perhaps just passed. In the Merlot, resistance was felt in the big seed but less so in the smaller seed. In the Syrah there was even less resistance in the big seed and almost no resistance in the smaller seed. This variation is to be expected given the different flowering dates of each variety, shown in Table 3.

For the purpose of trial efficiency, each timing application was sprayed on the same day for all three grape varieties. Table 3 sets out the date of flowering and the date of the first timing application.

Table 3: Flowering Dates and Date of first application

	5% flowering	Day 0 (1st appl)	Days since 5% flowering (DaF)
Chardonnay	23 November 2015	13 January 2016	51 days
Merlot	29 November 2015	13 January 2016	45 days
Syrah	1 December 2015	13 January 2016	43 days

3.3. Application Dates and Days after flowering

While applications were scheduled to be undertaken every 5 days, unfavourable weather conditions meant that at times the application was made a day earlier or later. Table 4 shows the actual dates of application and the corresponding number of days after flowering. For the treatments having 2 applications (Treatments 20-26, 2a, 21a-24a), the second application was approximately 10 days after the first application.

Table 4: Applications Dates and Days after 5% Flowering (DaF)

			Chardonnay	Merlot	Syrah
Date of 1st application	**Treatment Numbers**	**Day from Lag Phase**	**Days after 5% flowering**	**Days after 5% flowering**	**Days after 5% flowering**
	1	Control			
13/01/2016	2,11,20	0	51	45	43
18/01/2016	3,12,21	5	56	50	48
23/01/2016	4,13,22	10	61	55	53
27/01/2016	5,14,23	15	65	59	57
1/02/2016	6,15,24	20	70	64	62
5/02/2016	7,16,25	25	74	68	66
11/02/2016	8,17,26	30	**80**	74	72
16/02/2016	9,18,2a	35	85	79	77
22/02/2016	10,19,21a	40	**91**	85	83
27/02/2016	20a,11a,22a	45	96	**90**	**88**
2/03/2016	3a,12a,23a	50	100	94	92
7/03/2016	4a,13a,24a	55	105 (last app)	**99**	**97**
12/3/2016	5a,14a,	60		104	102
19/3/2016	6a,15a	65		111	109

Note: the red figures indicate the treatments that were eventually harvested for microvins.

3.4. Application Rates and Method

HML32 was applied at a rate of 1.25L per 100 litres. HML Silco was applied at a rate of 425g per 100 litres.

All treatments were applied at high volume, to the bunch line only and on each side of the row. The application was to the point of run-off using one pass with an electric pump assisted hand gun. Spray applications were undertaken by Chris Henry. No attempt is made to provide a l/ha link between hand spraying and machine spraying as in the opinion of the author any figure supplied lacks credibility and is more misleading than helpful.

3.5. Assessment Protocols

Because the trial is within a commercial vineyard, the amount of crop loss needed to be minimised. The assessment protocols reflected that to some degree.

3.5.1. Brix Testing and Yield Assessment

The protocol for sampling berries for brix and yield assessment was to pluck 8 berries from the distal end of one representative, but random, bunch each side of the plant for all four plants in a bay, making a total of 64 berries. Bunches were selected from the lower cordon and always taken on the morning sun side of the row.

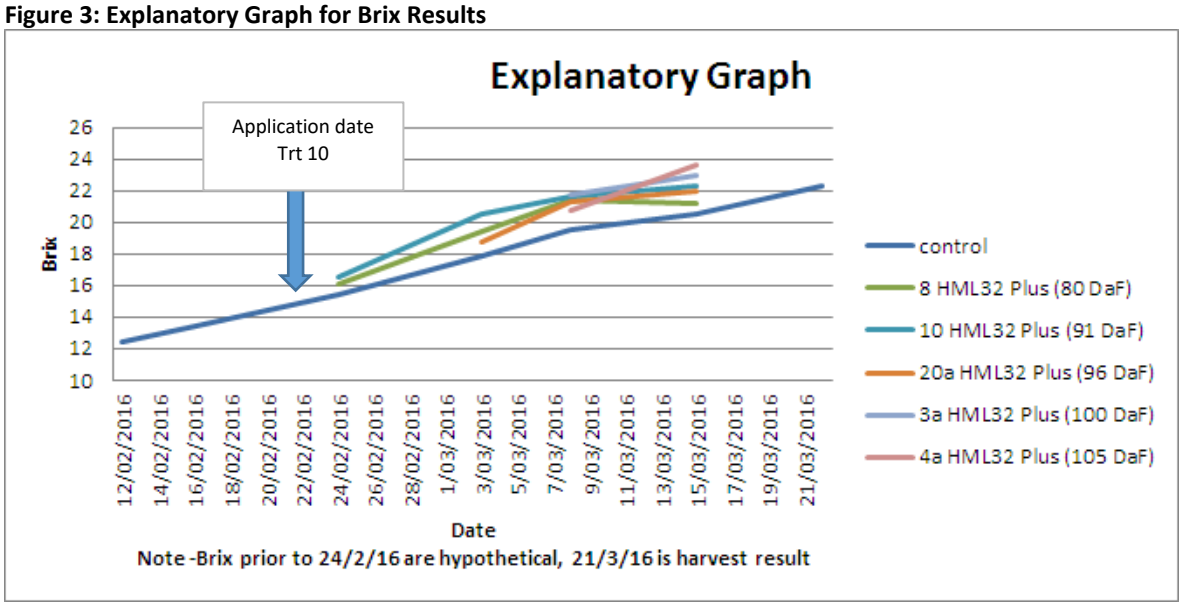
The samples were taken by Chris Henry and placed into pre-prepared numbered zip-top plastic bags. Susan Mains weighed and brix tested them in the field the same day (within an hour of sampling) using a balance scale and a portable digital brix meter.

Brix and weights were assessed on four occasions for the Chardonnay trial - 24 February 2016, 3 March 2016, 8 March 2016, 15 March 2016 and on three occasions for the Syrah - 22 March 2016, 28 March 2016 and 1 April 2016.

No brix/yield testing was undertaken on the Merlot. The Syrah data was used to determine the best treatment timings for testing from a maturity enhancement/disease perspective within the Merlot.

Figure 3 is an explanatory graph demonstrating the increase in Brix that was anticipated for the trial. The Brix results span a short period of time and it is necessary to consider the brix pattern prior to the first brix result to understand the context of that result. It is not simply a matter of comparing it with the control on the same day; it needs to be compared with the control leading up to that point and also in relation to when the treatment was applied. While the brix of elevated treatments may return to levels similar to control, it is the period of elevation and what that might mean in terms of enhanced wine quality (colour, phenolics etc) that is of interest.

Figure 3: Explanatory Graph for Brix Results



3.5.2. Penetrometer Testing

Penetrometer testing of the berries was used as a proxy for 'skin thickness'. Anecdotally most grape growers believe that increased skin thickness is related to a crop's ability to resist late season diseases such as botrytis.

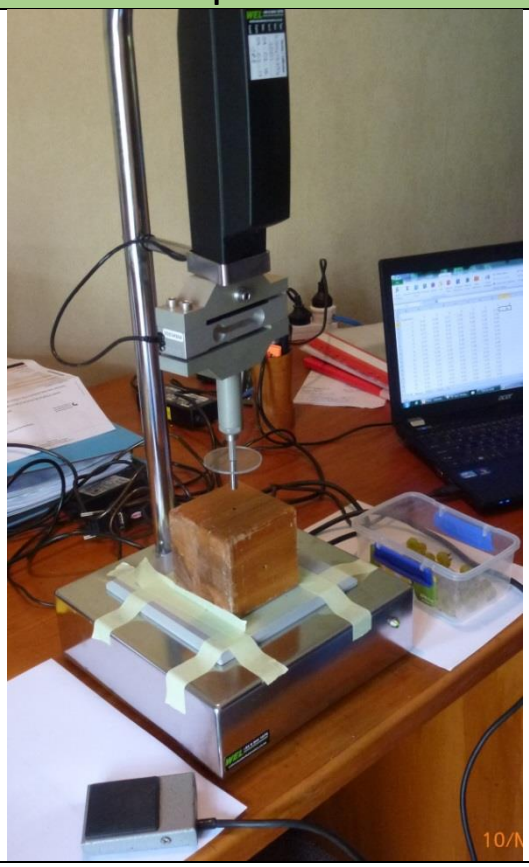
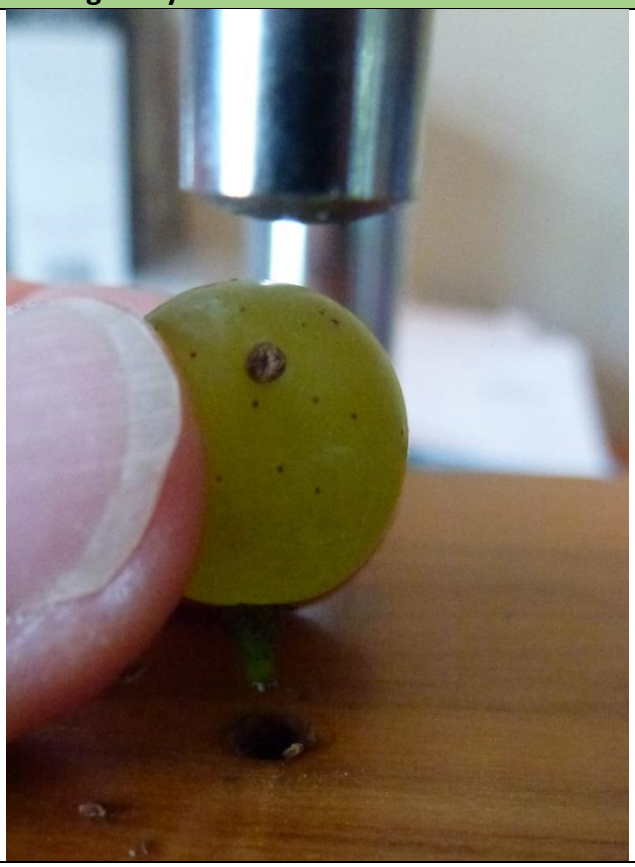
The original protocol for sampling berries for firmness was to take one bunch from each side of the plant for all four plants in a bay, making a total of 8 bunches. The sun facing side of the bunch was marked so that the berries to be removed for testing all came from the same side. Six to eight berries were cut from the bunch with the petiole in place.

The samples were taken by Chris Henry. Chris also cut the berries from each bunch to make the 50 berry sample. Berries were between 12 and 13 mm in diameter.

Helen Henry tested the firmness using an electric motorised penetrometer linked to software. It measured the force needed to depress the berry by 2 mm using an 8mm diameter flat probe and the result was automatically recorded in a spreadsheet.

The berry was located on a block of wood with a small hole into which the petiole was inserted. This ensured the berries were in the same place. The force was therefore applied to the end of the berry. This is shown in Figure 4. (Note: testing indicated that there was very little difference between the force required to depress the end of the berry by 2 mm or side of the berry by 2 mm.)

Figure 4: Penetrometer set-up

Penetrometer set-up	Placing berry on the block
	

The first set of testing for the Chardonnay was undertaken in accordance with this protocol but due to time constraints a full replicate was not tested. However sufficient testing was undertaken to enable the treatments exhibiting a difference to the control to be identified. Further penetrometer testing was limited to the control and those treatments that showed the highest brix and those that were taken for wine-making.

3.5.3. Disease assessment

A disease assessment was not part of the trial planning but botrytis was observed in all three grape varieties and provided an unexpected opportunity.

Outputs from the Bacchus Botrytis Risk Model for the flowering period and for the ripening period are shown in Figure 5 and Figure 6. Details of the specific botrytis infection periods are provided in Appendix 3.

Figure 5: Botrytis Infection Period - Flowering

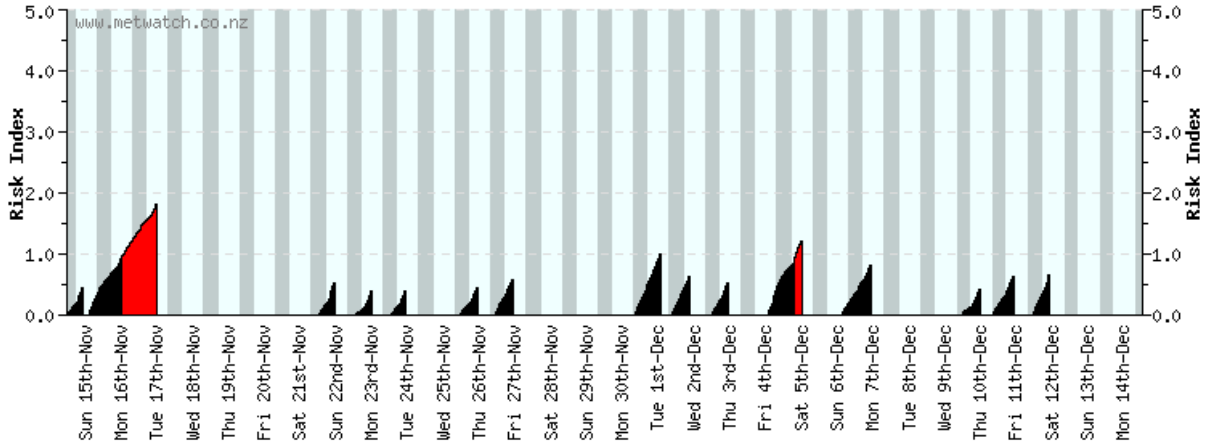
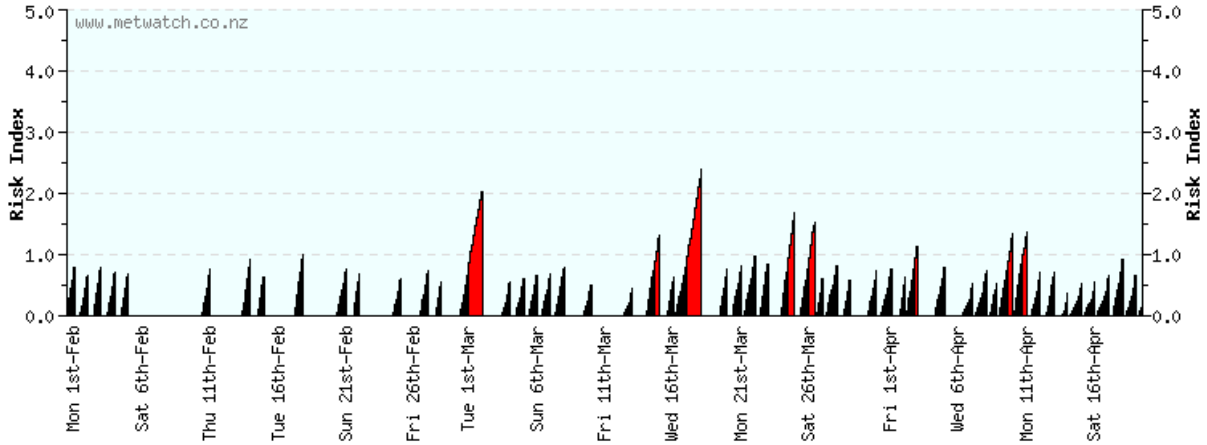


Figure 6:Botrytis Infection Period - Ripening



Botrytis (mostly as slip skin) was first observed in the Chardonnay when the main crop was being harvested. Up until this point the crop had been absolutely 'clean'. There was no time to undertake a botrytis assessment but a quick visual assessment indicated that the treatments that were picked for wine-making had less botrytis than the untreated control.

On 27 March 2016, five days later, the Merlot was heavily infected with botrytis, so much so that it was held to be beyond a meaningful assessment. The Syrah was also infected but not to the same extent.

Chris Henry undertook a botrytis assessment of Replicate 1 of the Syrah looking at the percentage of bunch infection for 25 bunches per treatment. This was a time consuming process as there were

so many treatments, however it highlighted the best performing treatments in terms of botrytis control.

Twenty five random but representative bunches were then picked from each plot of the best performing treatments, over the four replicates (100 bunches each treatment).

An independent qualified viticulturist then undertook an assessment from the picking bins within 1 hour of picking – assessing both incidence and severity of botrytis. The assessment was made blind.

Video footage and photographs were taken of Merlot and Syrah to demonstrate the amount of berry fall (slip skin) from the shaken treatments and the control.

3.5.4. Wine making and evaluation

For each variety, one treatment was selected from each treatment set, generally based on brix enhancement, and its fruit was harvested for wine-making. The control was also harvested. Therefore 4 wines were made for each variety.

For the Chardonnay, grapes were harvested using a target of 22.5 brix. The actual values were slightly different from that.

For the Merlot and Syrah, all selected treatments and the control were harvested at the same time due to the pressure of commercial harvesting requirements.

Forty kilograms of each treatment (all reps) were taken to the Eastern Institute of Technology in Hawke's Bay for microvin ferments.

The fermentation and winemaking process is described in Figure 7and Figure 8. Microvinification was undertaken by Karen Ball, Eastern Institute of Technology (EIT). This was overseen by Ant Mackenzie, a Hawke's Bay winemaker who had been involved in wine evaluations from an earlier trial conducted by Henry Manufacturing Ltd.

There were no acid or sugar adjustments and they were not fined in any way. No copper was added prior to bottling. This resulted in degrees of reductive notes but it was very minor and did not affect the subsequent qualitative evaluation of differences.

Wine tasting of the three wine flights were presented a three workshops - Gisborne, Hawke's Bay and Blenheim. An open invitation to winemakers and viticulturalists was made. Ant Mackenzie led the evaluation and discussion.

Winemakers had an opportunity to taste the wines and evaluate and discuss the differences then rank them in preference. At the Hawke's Bay workshop it was done blind; at the Gisborne and Blenheim workshops, participants knew which treatments they were tasting to assist in the evaluation.

Figure 7: Flow Diagram for the Microvinification of Red Wine Varieties at EIT Winery

2

Figure 1: Flow diagram for the Microvinification of Red Wine Varieties at EIT Winery

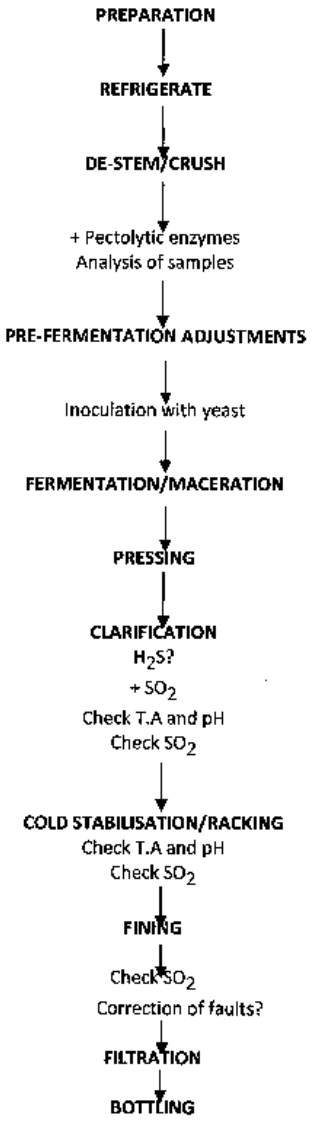
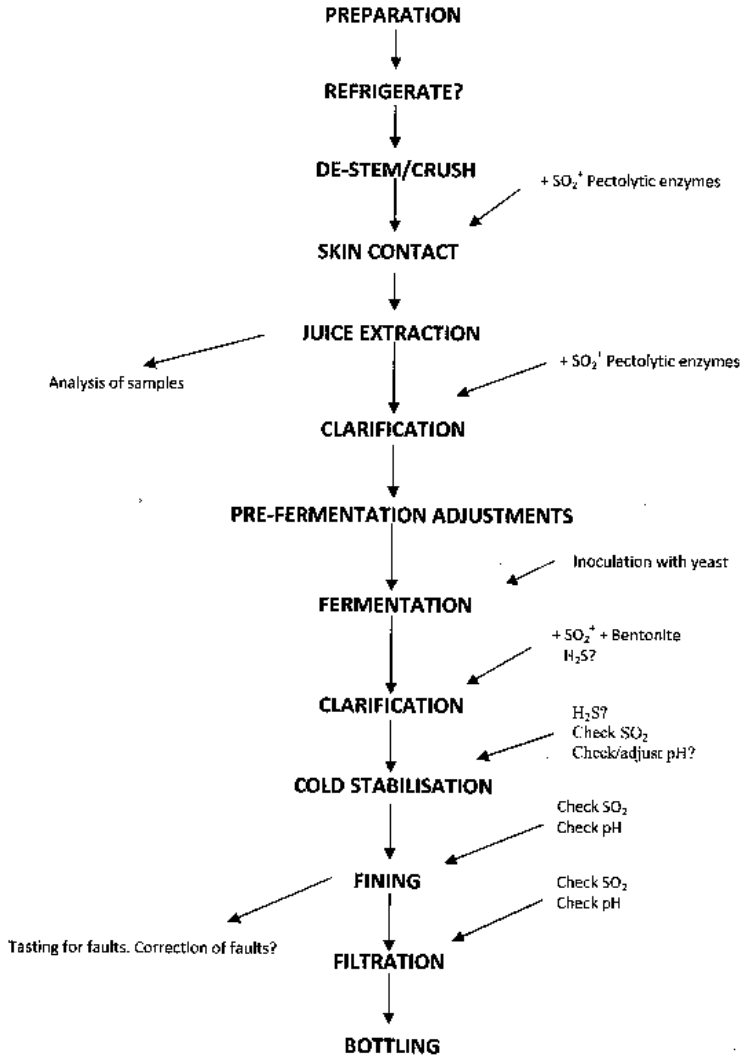


Figure 8: Flow Diagram for the Microvinification of White Wine Varieties at EIT Winery

3

Figure 2: Flow diagram for Microvinification of White Wine Varieties at EIT Winery



4.0 Results of Chardonnay Trial

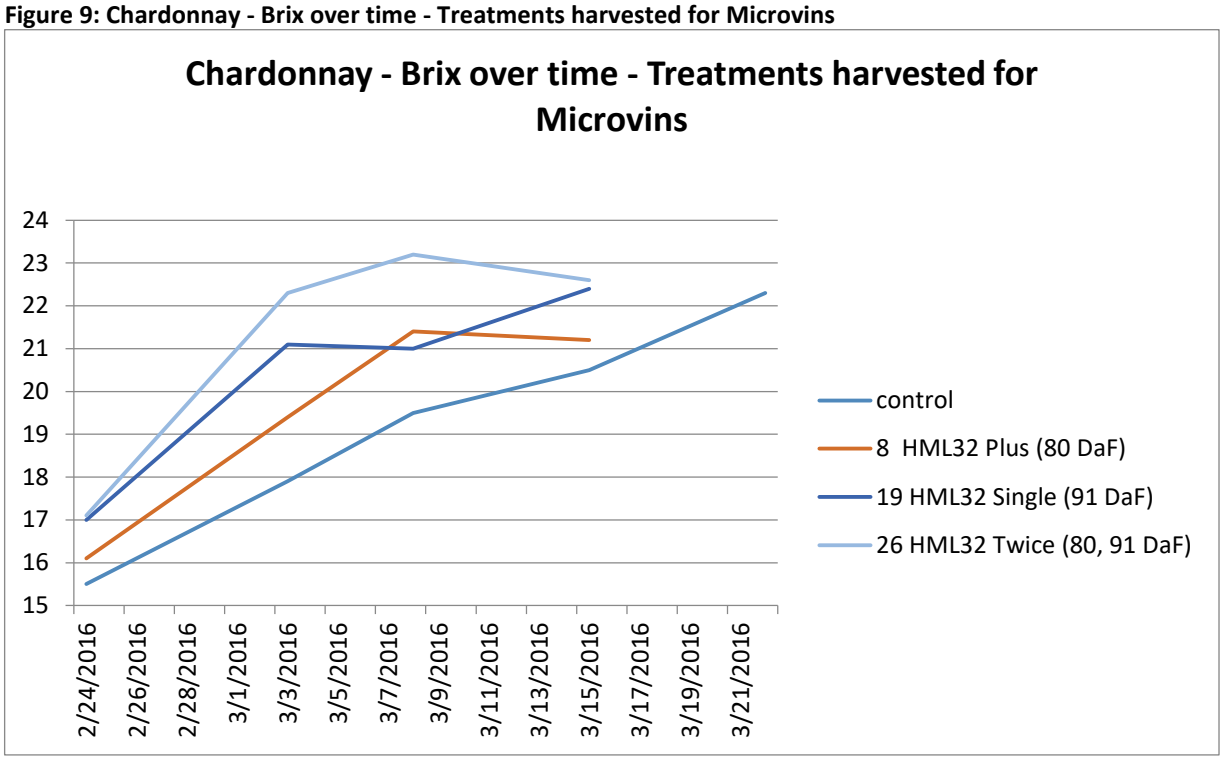
4.1. Brix

4.1.1. Brix over time

Brix tests were undertaken four times from 24 February to 15 March 2016 for each set of treatments. Appendices 4, 5 and 6 contain graphs of the results for HML32 Plus, HML32 Single and HML32 Twice respectively with the graphs separated into early treatments and later treatments. It is the later treatments which showed any increase in Brix levels.

Figure 9 shows the treatments which showed the best enhancement result for each treatment set. These are the treatments that were harvested for microvins.

Figure 9: Chardonnay - Brix over time - Treatments harvested for Microvins



4.1.2. Brix versus Weight

One of the known effects of increasing Brix is a decrease in weight, particularly if the primary mode of action is desiccation. Figure 10, Figure 11 and Figure 12 shows the Brix versus Weight relationship for each of the Treatment Sets. The green colour indicates the control and the red colour indicates the treatment that was harvested for microvins.

Figure 10: Chardonnay - Brix v Wgt (15 March 2016) - HML32 Plus

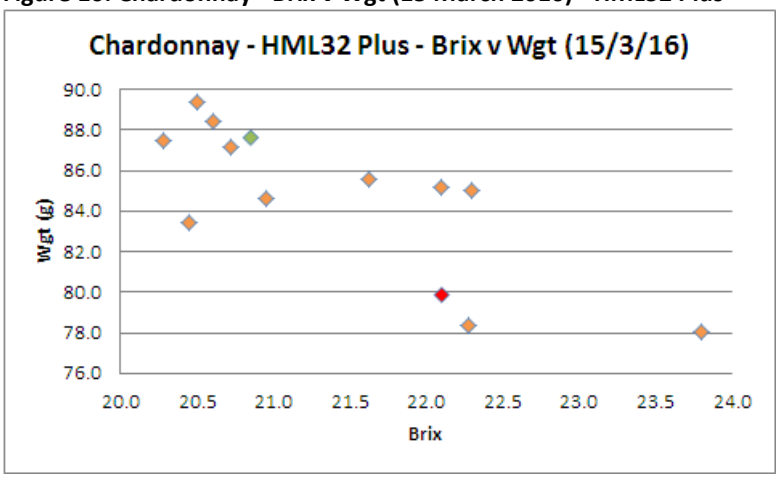


Figure 11: Chardonnay - Brix v Wgt (15 March 2016) - HML32 Single

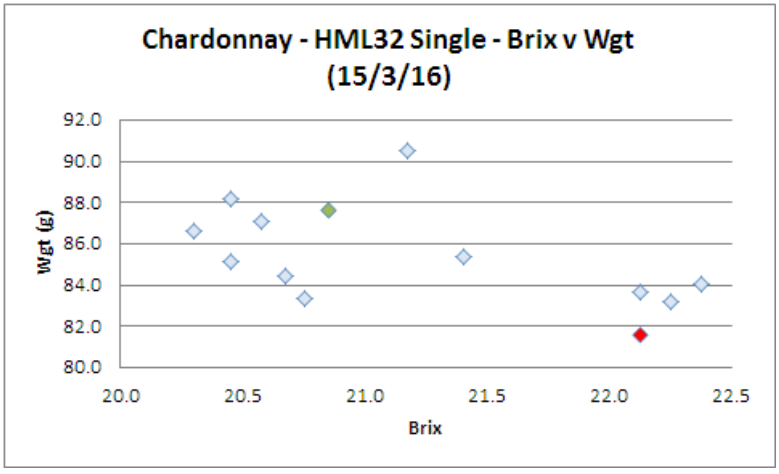
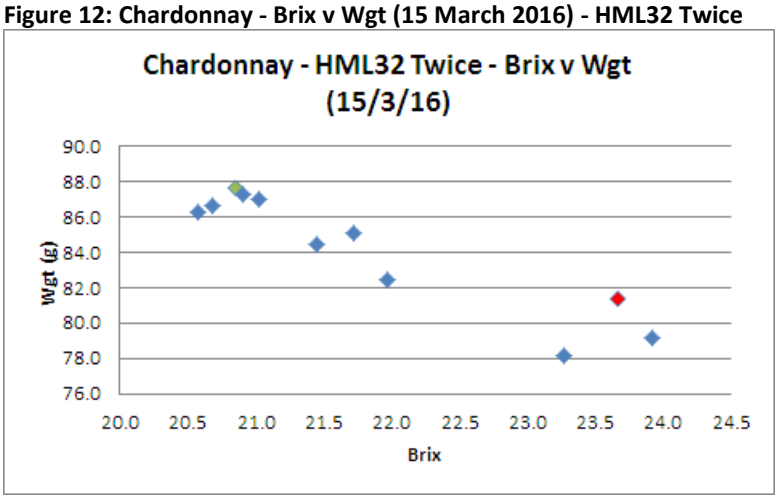


Figure 12: Chardonnay - Brix v Wgt (15 March 2016) - HML32 Twice



4.2. Penetrometer Results

Figure 13, Figure 14 and Figure 15 shows the results of the penetrometer testing undertaken on 9 March 2016. As discussed in Section 3.5.2, the testing was not undertaken on the full replicate. The green colour indicates the control and the red colour indicates the treatment that was harvested for microvins.

Figure 13: Chardonnay Penetrometer Results - KgF, n=50 - HML32 Plus

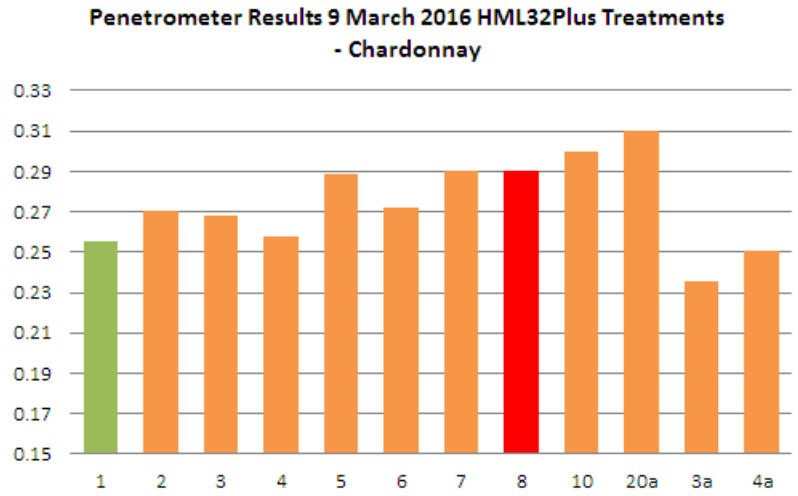


Figure 14: Chardonnay Penetrometer Results - KgF, n=50 - HML32 Single

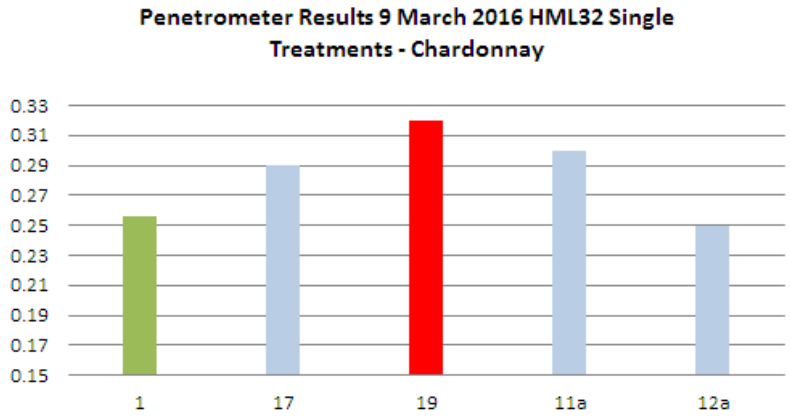
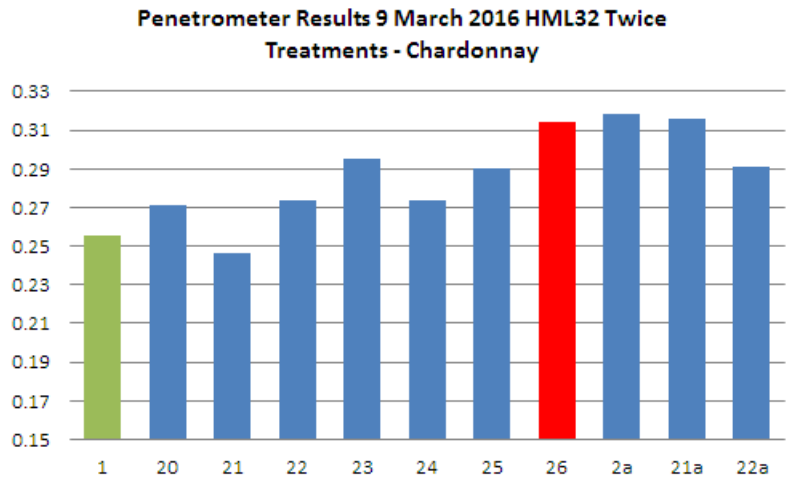


Figure 15: Chardonnay Penetrometer Results - KgF, n=50 - HML32 Twice



The results appear to indicate that there was increased firmness of the berry for the later treatments and that also corresponds to increased firmness of the berry where the brix has been enhanced.

Table 5 shows the penetrometer readings for the Control and the Treatments that were harvested for microvins along with the Brix results from the field. It indicates that the firmness of the berries drop over time which is to be expected as berries ripen but that the treatments with enhanced maturity maintained a comparatively higher level of firmness.

Table 5: Chardonnay Penetrometer Readings - changes over time

	Date of harvest	Brix at Harvest	Penetrometer reading (kgF) at 9 March 2016 (n=50)	Penetrometer reading (kgF) at 22 March 2016 (n-50)
Control	22 March 2016	22.3	0.26	0.23
Treatment 8	15 March 2016	23.0	0.29	0.25
Treatment 19	15 March 2016	22.5	0.32	0.25
Treatment 26	8 March 2016	23.3	0.31	0.28

4.3. Disease Assessment

During the Brix sampling of the Chardonnay on 15 March 2016, a low level botrytis infection was observed mostly as single berry infections.

Seven days later, when harvesting the Control replicates just ahead of harvest on 22 March 2016, the amount of botrytis observed had increased considerably.

There was very little time to make any meaningful assessment of botrytis. Little crop remained in the treatments which had presented the best brix enhancement, as the majority of it had been harvested for microvins. These remaining bunches were picked into harvest bins so a visual assessment could be made with the untreated control.

Only 1-3 bunches of grapes in each of the 'enhanced' treatments had botrytis (out of 8kg to 10kg). There was a much higher incidence (but unmeasured) level of botrytis in the untreated control.

Photographs were taken but they did not clearly disclose the difference.

4.4. Microvins

For the Chardonnay, grapes were harvested when the brix reached a target of 22.5 or as close as possible to it. The first treatment to reach 22.5 or above was Treatment 26 (HML32 sprayed twice - Day 30 and Day 40), 2 weeks ahead of the Control. This was followed by Treatments 8 (HML32 plus

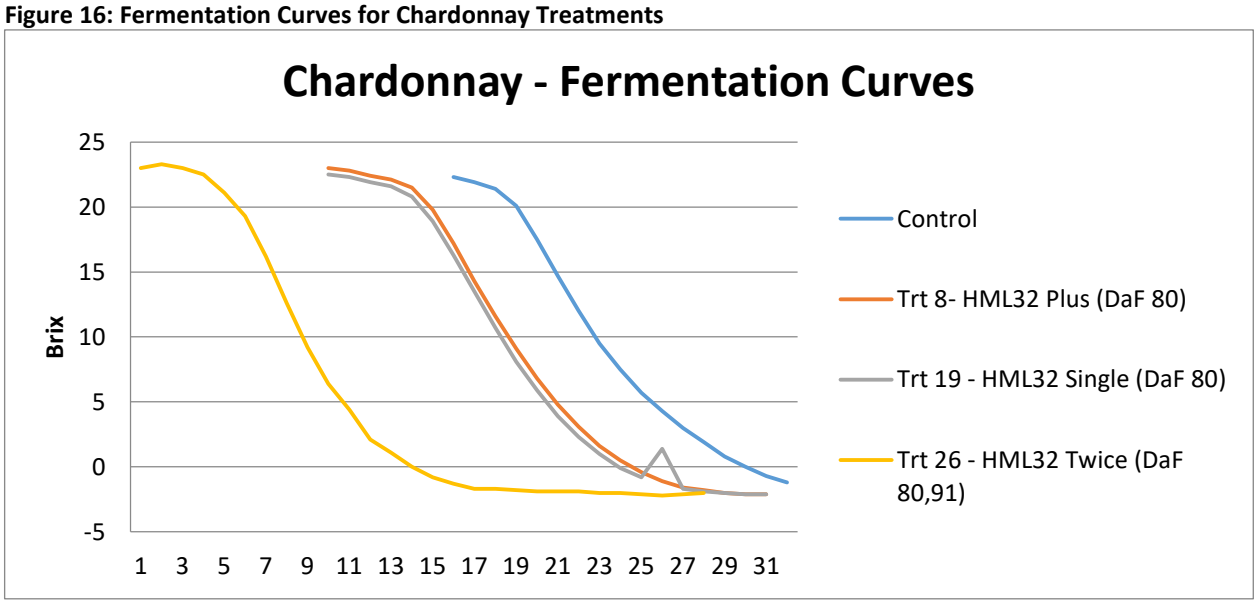
Silco sprayed once) and 19 (HML32 sprayed once) one week later, and finally the control a week after that. The pH, TA and Brix of the juice as measured at EIT are shown in **Error! Reference source not found.**.

Table 6: Juice - pH, TA, Brix Chardonnay Treatments and Control

	Date of harvest	pH	TA	Brix
Control	22 March 2016	3.27	7.94	22.3
Treatment 8 (HML32Plus)	15 March 2016	3.33	7.80	23.0
Treatment 19 (HML32Single)	15 March 2016	3.26	7.76	22.5
Treatment 26 (HML32Twice)	8 March 2016	3.22	9.57	23.3

Fermentation proceeded evenly as shown by the curves for each of the four wines in Figure 16. These indicate that the treatments did not affect the fermentation process. The wines were placed in the chiller after ferment and then racked off and bottled.

Figure 16: Fermentation Curves for Chardonnay Treatments



Note: DaF means days after 5% flowering

4.4.1. Comparative Sensory Evaluation

While it was an informal evaluation, most participants detected favourable differences in the treated Chardonnay wines when compared to the control, as well as between the treatments. When ranking their preference, any one of the treatment wines was generally the first preference compared to the control wine. One Marlborough winemaker thought the control presented classic Chardonnay elements and that the other wines would have needed some acid adjustment. However, all wines were of commercial wine quality indicating that the treatments did not have any downside to final wine quality; rather they enhanced wine quality in a number of different ways.

Ant Mackenzie's evaluation of the Chardonnay wines is summarised as:

At the Hawke's Bay workshop I did not detect a big difference between the wines but now knowing what they are, I do detect some differences. The last wine (Treatment 26 HML32 sprayed

twice) was my preference but my 2nd preference was the control. The last wine was the richest, softest and the roundest yet it was the highest TA. Treatment 26 was harvested two weeks ahead of the control; if it had been left to hang on the vine for as long as possible, I would expect even more enhanced characteristics.

His tasting notes are provided in Appendix 10.

5.0 Results of Merlot Trial

5.1. Brix over time

No samples for Brix testing were taken of the Merlot treatments due to the need to prioritise resources.

5.2. Disease assessment

On 27 March 2016, a day after 3 days of rain totalling 14 mm, and the 3 days preceding that having heavy dews, the Merlot became heavily infected with botrytis, so much so that it was beyond formal assessment. Two days later, on 29 March 2016, the Merlot was due to be harvested. That morning Chris Henry walked through the Merlot trial and shook the cordon wire of the untreated bays and observed significant berry fall, indicating slipskin. He then did the same to the treatments that had shown enhanced brix activity in the Syrah (Treatments 4a, 13a and 22a) and found the amount of berry fall to be significantly less than the untreated control. The treatments applied 5 days before and and 5 days after those treatments also disclosed a profound improvement over the untreated control.

Equipment was quickly collected to undertake a number of 'shake tests' on the control and on what were considered to be the best treatments in the Merlot before then harvesting for microvins. The commercial harvest was also getting under way. A video record was made in Replicate 3 of the control and Treatment 4a. In Replicate 4, a video record was made of the control (note the release of botrytis spores) and Treatments 4a, 13a and 22a. The video can be seen on https://www.dropbox.com/s/ktxrd7qzbv2eebo/STU0220%20Botrytis-Trial%202.0.mp4?dl=0

Figure 17 shows the amount of berry fall for each treatment.

Figure 17: Berry Fall from 'Shake Tests' Merlot Rep 4

Treatment 22a	Treatment 13a	Treatment 4a	Control
			

All treatments relate to the same timing application being Day 55 after lag phase , with treatment 22a being Day 45 and Day 55 timings.

5.3. Penetrometer Results

No penetrometer readings were taken on the Merlot Trial.

5.4. Microvins

The four replicates of Merlot Treatments 4a, 13a and 22a and the Control were harvested on 29 March 2016 (just ahead of the commercial harvest) for microvins. The pH, TA and Brix of the juice as measured the next day is shown in Table 7.

As mentioned, microvinification was undertaken by Karen Ball, Eastern Institute of Technology (EIT) and overseen by Ant Mackenzie, a Hawke's Bay winemaker who had been involved in wine evaluation from earlier trial conducted by Henry Manufacturing Ltd.

There were no acid or sugar adjustments and they were not fined in any way. No copper was added prior to bottling. This resulted in degrees of reductive notes but it was very minor and did not affect the subsequent qualitative evaluation of differences.

The unfiltered fermented wine (pre cold stabilisation) was analysed by AWRI wine cloud and the tannins and phenolics are shown in Table 8. The treatments show an increase in tannins and phenolics over the control.

Table 7: Juice - pH, TA and Brix of Merlot Treatments and Control - 30 March 2016

Juice Values	pH	TA	Brix
Control	3.37	6.10	22.1
Treatment 4a (HML32Plus)	3.41	5.70	23.6
Treatment 13a (HML32Single)	3.36	6.10	22.6
Treatment 22a (HML32Twice)	3.38	5.95	24.0

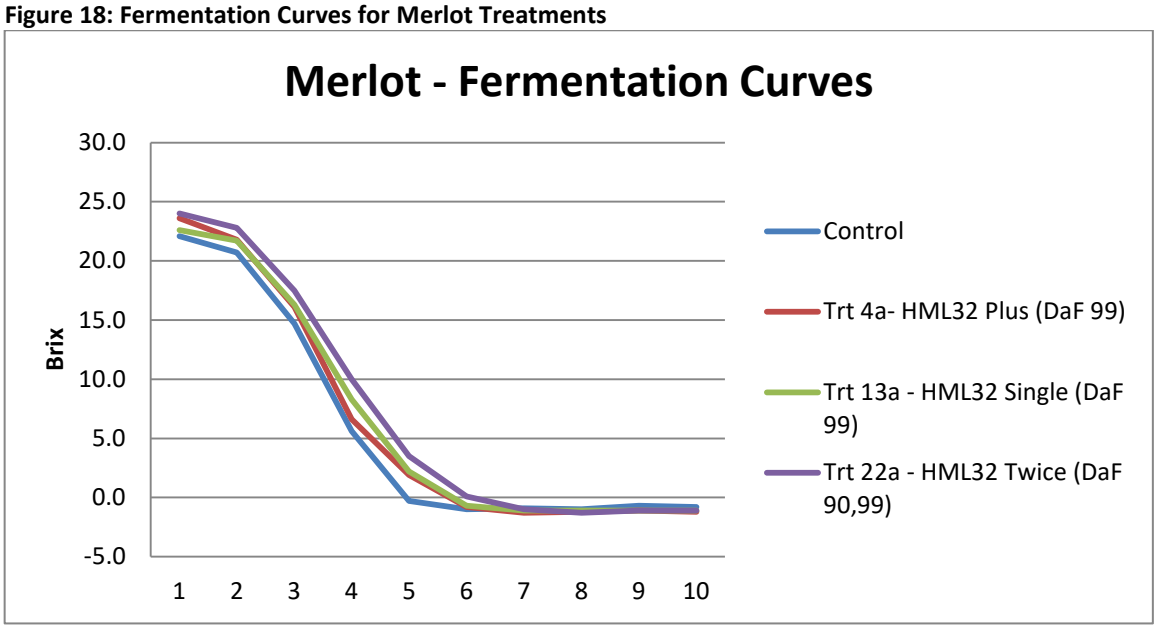
Table 8: Unfiltered Merlot wine after fermentation, pre-cold stability Tested AWRI 11 May 2016

Wine Cloud	Vintage	Total Tannins	Total Pigments	Total Phenolics	Pigmented Tannins	Free Anthocyanins
Control	2016	0.82	14.89	39.98	0.86	13.46
Treatment 4a (HML32Plus)	2016	1.16	26.32	51.36	1.00	24.65
Treatment 13a (HML32Single)	2016	1.38	33.41	58.55	1.19	31.42
Treatment 22a (HML32 Twice)	2016	1.56	36.62	62.73	1.36	34.35

Note 1:Tannins recorded in g/L epicatechin equivalents
Note 2: Other results recorded in Absorbance Units and are therefore comparative results not quantitative.

Fermentation on skins proceeded evenly as shown by the curves for each of the four wines made from the Merlot in Figure 18. These indicate that the treatments did not affect the fermentation process.

Figure 18: Fermentation Curves for Merlot Treatments



Note: DaF means days after 5% flowering

5.4.1. Comparative Sensory Evaluation

For the Merlot wines, all participants detected favourable differences in the wines when compared to the control and between the treatments. The treatment wines were plusher and more vibrant. There was a clear enhancement of colour in the treatments as reflected in the wine cloud analysis compared to the control. Again, all wines were of commercial wine quality.

Ant Mackenzie's evaluation of the Merlot wines is summarised as:

All three treated wines were better than the control. The treatment of HML32 sprayed twice had a broader flavour spectrum with tannins that coats the whole mouth and my second preference was HML32 and Silco (Treatment 4a) which was full, rich, plums and spice with front phenolics. The control was slighter greener, tarry with an edgier palate.

His tasting notes are provided in Appendix 10.

6.0 Results of Syrah Trial

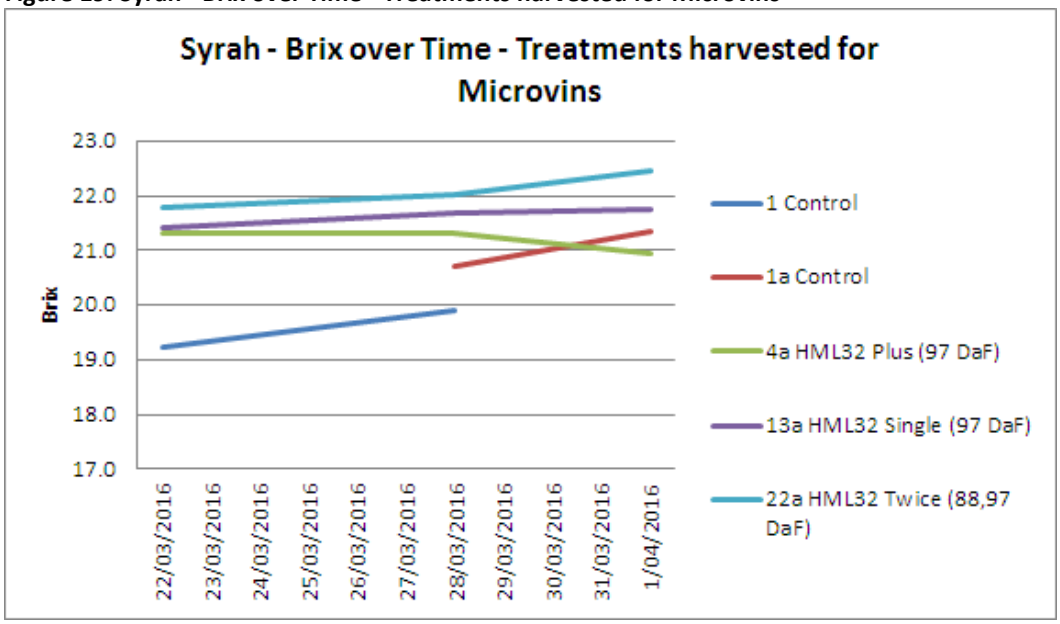
6.1. Syrah Brix over time

Brix tests were undertaken 3 times from 22 March 2016 to 1 April 2016 for each set of treatments. Appendices 7, 8 and 9 contain graphs of the results for HML32Plus, HML32Single and HML32Twice respectively with the graphs separated into early treatments and later treatments. It is only the later treatments which showed any increase in Brix levels, **but the outcomes are confused, and do not reflect the substantial enhanced maturity result as was obtained on the Chardonnay**.

The reasons for this can be attributed to rain and heavy dews from the 21st March to the 26th March, which re-hydrated berries and set off the botrytis infection. It may also be attributable to the Syrah having been crop thinned to a low level (post veraison).

Figure 19 shows the treatments which showed the best enhancement result for each treatment set. These are the treatments that were harvested for microvins.

Figure 19: Syrah - Brix over Time - Treatments harvested for Microvins



6.2. Syrah Disease Assessment

While Henry Manufacturing Limited always endeavours to use independent parties to undertake trial assessment, the unexpected discovery of the severe botrytis in the Merlot and Syrah occurred over the Easter Holiday period.

Chris Henry undertook a botrytis assessment of **one replicate** (Rep 1) of the Syrah trial (incidence and severity of 25 random representative bunches) in order to ascertain the botrytis control efficacy over the 41 different treatments. These results are shown in Figure 21,

Figure 22 and Figure 23 for each treatment set being HML32Plus, HML32Single and HML32Twice respectively – % Incidence is shown in **Dark Blue** and Severity is shown in **Orange**. For treatment descriptions, refer back to Table 2.

An independent and blind assessment of Incidence and Severity was undertaken by Bridget Wilton over all **four reps** of the best performing treatments and these are shown in the same graphs in **Light Blue** and **Light Orange**.

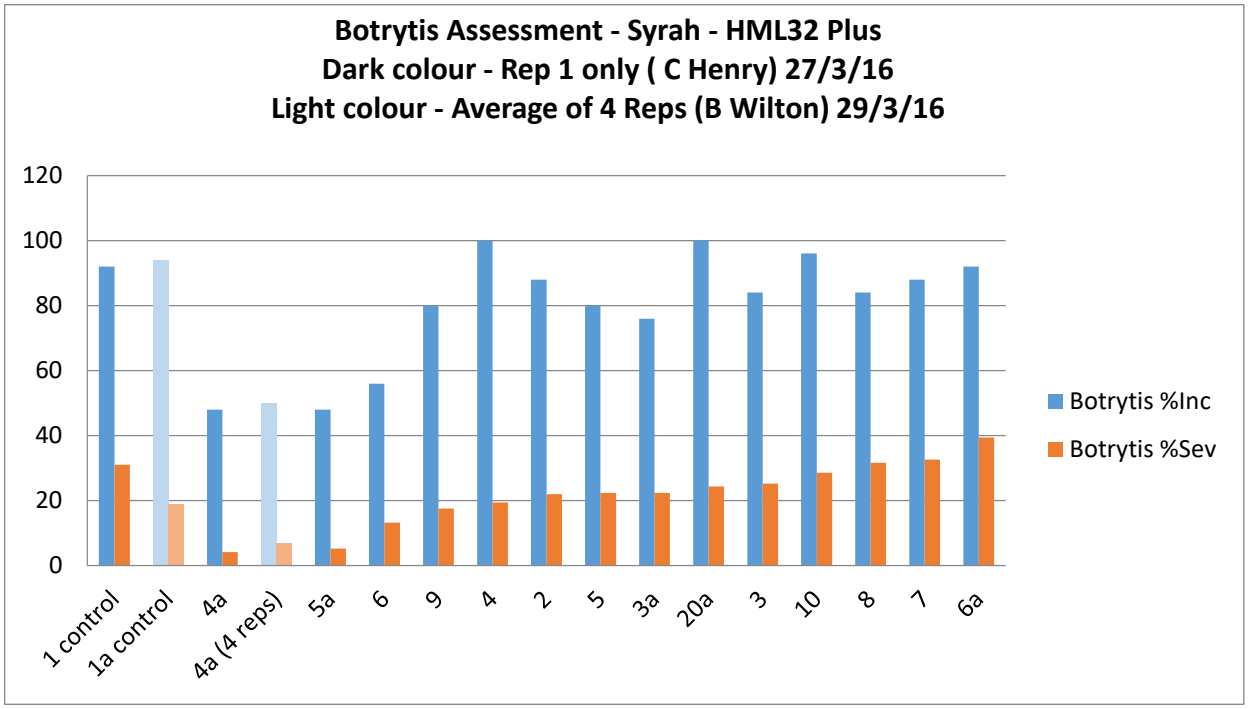
There appeared to be some variation within the trial plots with the untreated plots in the extended trial area appearing to be not as severely infected as the first trial area. Given that the treatments showing the best efficacy were all in the extended trial area, 4 untreated bays within the second trial plot was assessed. These are shown as Treatment 1a extended plots.

A 'Shake Test' was done on all reps of the treatments that had been independently assessed. These were videoed and a photograph of the berry fall was taken. Figure 20 shows the results from Rep 1. It is noted that the incidence and severity of botrytis appeared to increase across the trial plot.

Figure 20: Berry Fall from 'Shake Tests' Syrah Rep 1

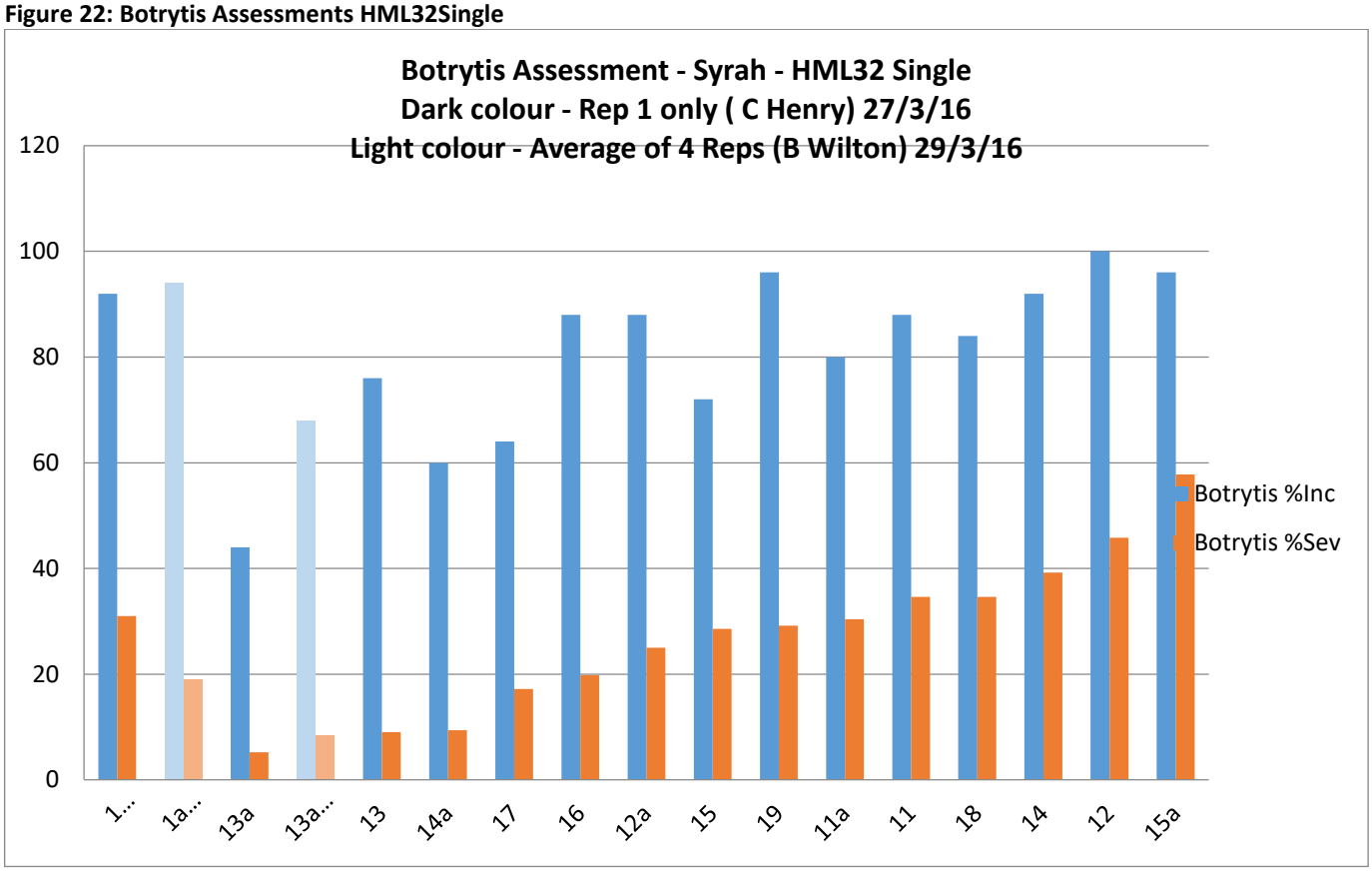
Treatment 22a	Treatment 13a	Treatment 4a	Control
			

Figure 21: Botrytis Assessment - HML32Plus Treatments



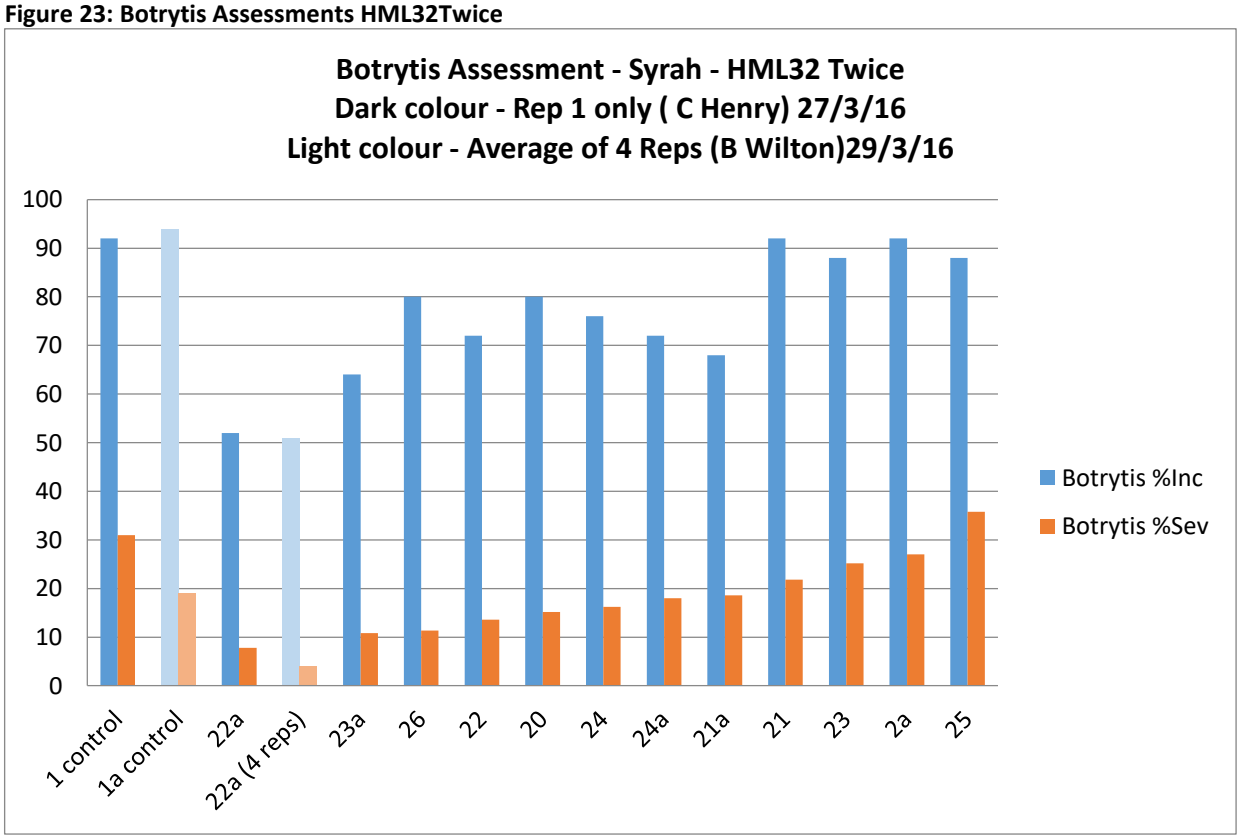
Note: refer to Table 4 for Treatment Timings

Figure 22: Botrytis Assessments HML32Single



Note: refer to Table 4 for Treatment Timings

Figure 23: Botrytis Assessments HML32Twice



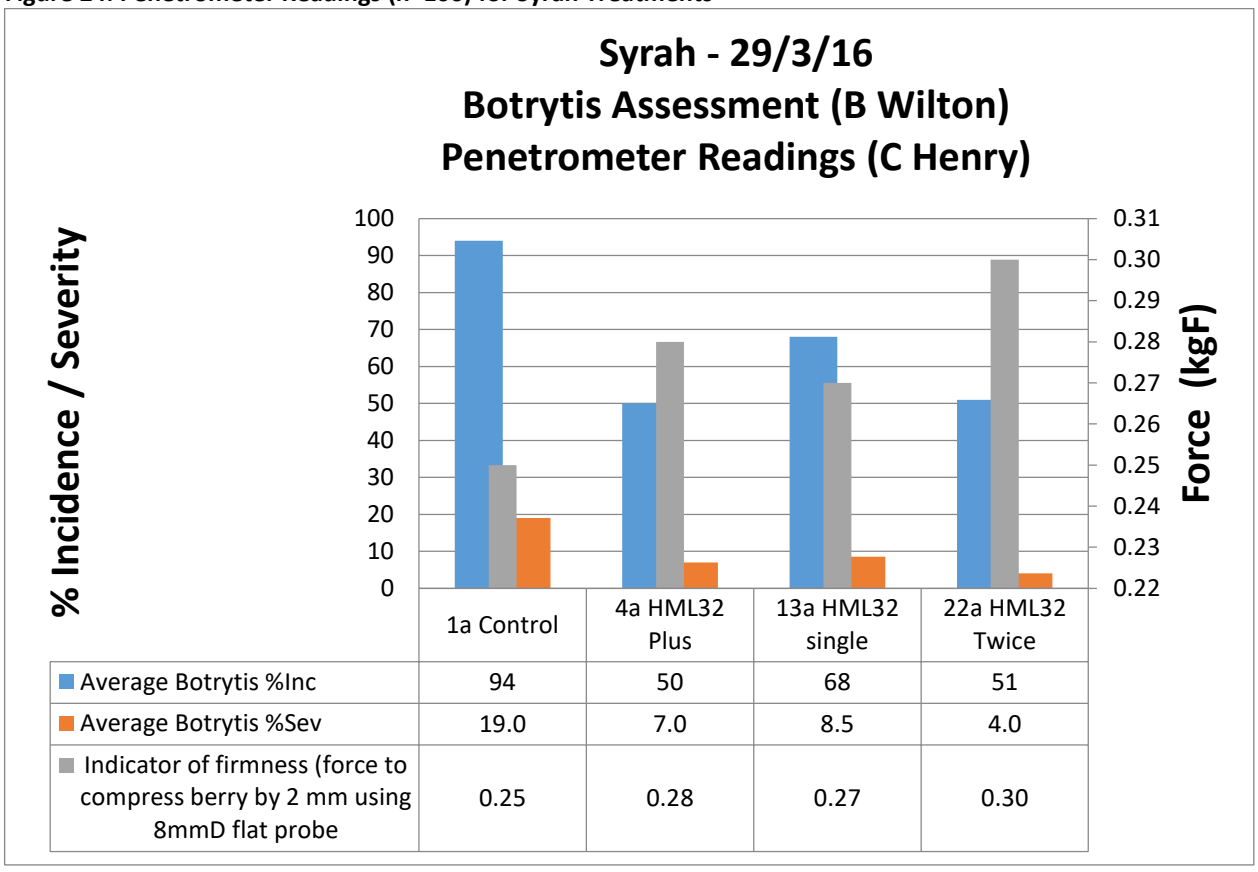
Note: refer to Table 4 for Treatment Timings

The treatments 4a, 13a and 22a with the greatest disease efficacy (and the highest brix elevation, with the exception of 4a) were all associated with an application on Day 55 after lag phase or some 97 days after flowering. Treatment 22a received its first application at 88 days.

6.3. Penetrometer Readings

Bunches of grapes were taken from each of the best performing treatments and one hundred (100) berries selected for penetrometer testing. The numerical results shown in Figure 24 indicate that Treatment 22a (2 applications) was firmer that Treatments 4a and 13a (single applications) and all treatments were firmer than the Untreated Control.

Figure 24: Penetrometer Readings (n=100) for Syrah Treatments



	1a Control	4a HML32 Plus	13a HML32 single	22a HML32 Twice
Average Botrytis %Inc	94	50	68	51
Average Botrytis %Sev	19.0	7.0	8.5	4.0
Indicator of firmness (force to compress berry by 2 mm using 8mmD flat probe	0.25	0.28	0.27	0.30

6.4. Microvins

The four replicates of Syrah Treatments 4a, 13a and 22a and the Control were harvested on 29 March 2016 for microvins. It was understood that commercial harvest was imminent but it was another week before it was harvested. The pH, TA and Brix of the juice as measured the next day is shown in Table 9.

Table 9: Juice - pH, TA and Brix of Syrah Treatments and Control - 30 March 2016

	pH	TA	Brix
Control	3.16	8.20	21.8
Treatment 4a (HML32Plus)	3.17	8.20	22.2
Treatment 13a (HML32Single)	3.20	7.95	22.4
Treatment 22a (HML32Twice)	3.18	7.9	22.5

As mentioned, microvinification was undertaken by Karen Ball, Eastern Institute of Technology (EIT) and overseen by Ant Mackenzie, a Hawke's Bay winemaker who had been involved in wine evaluation from earlier trial conducted by Henry Manufacturing Ltd.

There were no acid or sugar adjustments and they were not fined in any way. No copper was added prior to bottling. This resulted in degrees of reductive notes but it was very minor and did not affect the subsequent qualitative evaluation of differences.

The unfiltered fermented wine (pre cold stabilisation) was analysed by AWRI wine cloud and the tannins and phenolics are shown in Table 10. The treatments show an increase in tannins and phenolics over the control.

Table 10: Unfiltered Syrah wine after fermentation, pre cold stability Tested AWRI 11 March 2016

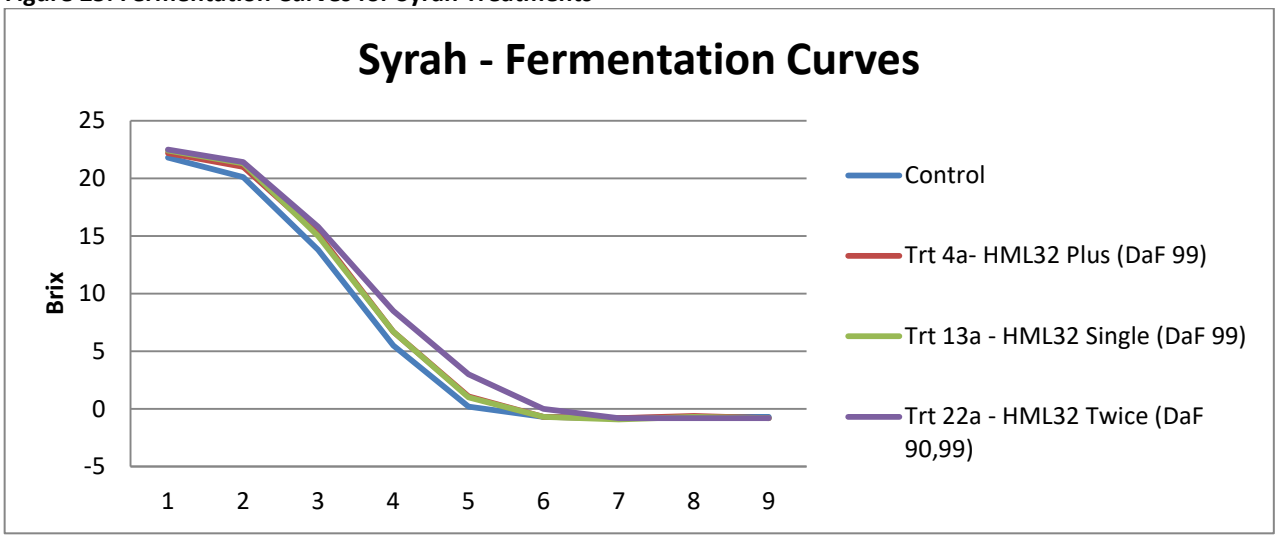
Wine Cloud	Vintage	Total Tannins	Total Pigments	Total Phenolics	Pigmented Tannins	Free Anthocyanins
Control	2016	0.68	20.81	40.85	0.84	19.41
Treatment 4a (HML32Plus)	2016	0.78	24.68	44.57	0.88	23.22
Treatment 13a (HML32Single)	2016	0.77	25.96	45.24	0.86	24.53
Treatment 22a (HML32 Twice)	2016	1.12	32.08	54.72	0.91	30.56

Note 1:Tannin recorded in g/L epicatechin equivalents

Note 2: Other results recorded in Absorbance Units and are therefore comparative results not quantitative.

Fermentation on skins proceeded evenly as shown by the curves for each of the four wines made from the Syrah in Figure 25**Error! Reference source not found.**. These indicate that the treatments did not affect the fermentation process.

Figure 25: Fermentation Curves for Syrah Treatments



Note: DaF means days after 5% flowering

6.4.1. Comparative Sensory Evaluation

For the Syrah wines, all participants detected favourable differences in the wines when compared to the control and between the treatments. Descriptions ranged from peppery spice to fruity spice and preferences were varied across the treatments. Treatment wines were found to have more mid palate weight and concentrate than the control which was a bit thinner. Discussion in Hawke's Bay in particular was around how each wine might be used. Again, all wines were of commercial wine quality.

Ant Mackenzie's evaluation of the Syrah wines is summarised as:

There is a bigger range of differences in these wines which all had the same residual sugar. I found the treatment of HML32 sprayed twice sweeter, richer and more supple with tannins at the front of the palate and more fruit and spice notes. Treatment 4a (HML32 with Silco sprayed once) was also spicy but with gamey notes. It was vibrant and bright. The control I found to have slighter greener tannins, good richness and back palate.

His tasting notes are provided in Appendix 10.

7.0 Discussion and Conclusions

7.1. Enhanced Maturity

As with many trials of this nature, the objectives of a trial are simply defined at the beginning but as data is collected and other issues are observed, the trial becomes meaningful in areas outside the original objectives.

It must be remembered that 'Enhanced maturity' (as produced by applications of HML32) does not occur in isolation from other aspects that influence maturity.

In past research on table grapes, Smilanick (pers.comm.) found that 'enhancement effects' could be muted by such things as high potassium soil fertilising, crop load reduction to bring on earlier maturity or high temperatures (>38°C). In the experience of the author, some degree of water stress after veraison exaggerates 'enhanced effects', likewise exposure of bunches to the sun, the counter being that significant rain suppresses brix effects in the short term, but comparative phenolic improvements appear to remain.

The difference in outcomes for each of the three varieties is to a certain extent supported by those factors described in the previous paragraph. The Chardonnay and the Merlot were largely cropped to capacity. The Syrah was in contrast crop thinned to a low level (post veraison), hence the brix difference between treatments was small. But this was not reflected in 'Wine Cloud' results which showed substantial improvement. All of this confused by end of season rains, which impacted the Merlot and Syrah to a larger degree than the Chardonnay, which was harvested much earlier.

7.2. Botrytis Efficacy

Anecdotally growers associate 'thick skins' with heightened resilience of grape berries against end of season rots. Growers apply calcium sprays with the objective of thickening the skin. Thicker skins are a phenomenon that anecdotally occurs between conventional grown grapes and organically grown grapes.

Thickened skin as a result of 'enhanced maturity' applications has been clearly demonstrated in Smilanick's work on table grapes by comparative penetrometer testing and dissection with the use of an electron microscope.

Thickened skin has been noted within all previous trials of HML32, but this trial is the first time we have measured effects – also with a penetrometer. Penetrometer testing shows that skins are thickened by applications at the same timing as 'enhanced maturity' also that 2 applications produce better effects.

Botrytis efficacy data was collected from this trial in the field from the Syrah alone. An assessment was made by the author over all treatments (41) of one replicate to determine any differences in botrytis outcomes. This information was used to identify and collect samples from each replicate (4 x 25 bunches) of the treatments that disclosed efficacy as well as the untreated control and these treatments were assessed blind by an expert.

Substantial botrytis efficacy was achieved by applications of HML32 made at the same timing that achieve 'enhanced maturity'.

Botrytis efficacy was also captured by video of vigorously shaking those same treatments in the Merlot which was heavily infected with botrytis bunch rot, and botrytis as slip skin. The difference in efficacy is clearly demonstrated on that video (including the cloud of botrytis spores from the untreated). The fruit drop was visually recorded in bins (but not weighed).

Anecdotally successful treatments were also disclosed by the harvest machine operator who told the vineyard manager he had to slow his machine on those successful treatments to harvest the grapes.

7.3. Timing of application(s)

The main focus of this trial was to ascertain spray timing - when the berry is most amenable to influence. This was measured as days after onset of flowering as well as from the 'lag phase'. To a large extent it has been successful in producing that outcome, with peak efficacy appearing to occur at different points for Reds and Whites.

- Whites, 80 days to 91 days after onset of flowering.
- Reds, 88 days to 99 days after onset of flowering.

Figure 26, Figure 27 and Figure 28 shows the growth stage when the best treatments of the Chardonnay, Merlot and Syrah were sprayed. For the Merlot and the Syrah bunches, the untreated photographs were used as no photographic record of the later treatments (Post Day 40) was kept. The untreated plots reflect the growth stage at the timing of the treatment application.

Figure 26: Chardonnay growth stage when best treatments applied

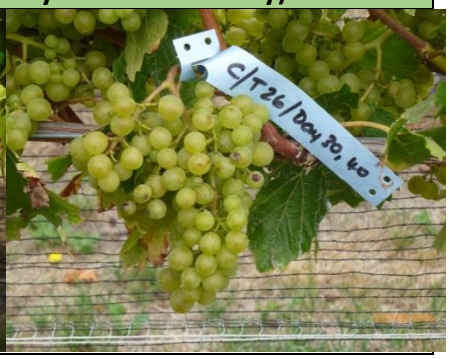
Treatment 8 HML32Plus - Day 30 (photo taken on 9 February)	Treatment 19 HML32Single - Day 40 (photo taken on 22 February)	Treatment 26 HML32Twice Day 30/40 (photo taken on day 40 - 22 February)
		

Figure 27: Merlot growth stage when best treatments applied

Merlot 29 February 2016 - Timing for Treatment 22a HML32Twice - first spray of two applications	Merlot - 7 March 2016 - Timing for Treatments 4a, 13a HML32Plus and HML32Single sprayed once and second spray for Treatment 22a HML32Twice
	

Figure 28: Syrah growth stage when best treatments applied

Syrah - 27 February 2016 - 2 days prior to Timing for Treatment 22a HML32Twice - first spray of two applications	Syrah - 7 March 2016 - Timing for Treatments 4a, 13a HML32Plus and HML32Single - sprayed once and second spray for Treatment 22a HML32 sprayed twice
	

The data also indicates that **applications at different times** (outside the most amenable period) also appear to influence 'enhanced maturity' **outcomes in different ways**.

- If made earlier than the amenable period, no enhancement was observed.
- Applications made early within the amenable period tended to accumulate sugars slower and retain their increase.
- Applications made later within the amenable period undergo a more rapid accumulation of brix.

In this trial, application timings have been defined as Days after Lag Phase as well as Days after 5% flowering. In previous trials, other timing constructs were used such as days before a theoretical harvest date, and brix accumulation.

These timing constructs are problematic due to the variability from year to year. Dr Rob Agnew, Plant and Food Research, reviewed historic phenological and climatic data relating to this trial site with the aim of providing a formula to make reliable judgements regarding other varieties and other growing regions. Various plant stages /timing constructs were considered including the ones described above.

After all issues were considered, the plant growth stage that is recommended is **50% veraison, defined as 50% softening of berries or 8.5 Brix.**

This growth stage has a number of advantages:

- it is close to when applications are required,
- there is a good history of the 50% veraison timepoint by region and by variety; and
- growers for the most part have access to it in real time.

It is also something growers can individually make a judgement call in relation of their own crop and location in respect of data produced close by them.

For Chardonnay (for best effect), the first application timing would be at 50% veraison followed by another application 10 days later. If only one application was to be made the target timing would be 10 days after 50% veraison.

For Merlot and Syrah (for best effect), the first application would be 7 days after 50% veraison followed by another application 10 days later. If only one application was to be made then the target timing would be 17 days after 50% veraison.

7.4. Berry firmness and resilience

Another focus of the trial was to ascertain whether any change occurs in the berry itself in terms of firmness/'skin thickness' and if it did, whether any disease resilience , especially against botrytis was associated with it.

This trial **confirms an increase in berry resilience** (as indicated by skin firmness) occurs at the same time as other aspects of enhancement, and that an **improved botrytis outcome (including slip skin)** follows as a consequence.

7.5. Enhanced maturity and yield

Another focus of this trial was to ascertain the relationship between enhanced maturity and yield.

The Chardonnay trial confirms that from the time of application to harvest, loss of berry weight appears to be a feature of 'enhanced maturity' when there are no other external influences such as rain. The relationship appears to be direct but the percentage increase in brix does not equate to the same percentage loss of berry weight – perhaps indicating other more complex mechanisms.

The Syrah trial gave confusing and unusual outcomes, especially when **applications of HML32 included the additive (**particularly in the weeks immediately after application). Some results indicated heightened brix, but also an increase in yield. This requires **further study.**

7.6. Effect of an 'additive'

A focus of this trial was to investigate whether the addition of a potassium silicate 'additive' to HML32 would improve the outcomes of one application of HML32 as an alternative to making 2 applications. The trial disclosed there was improvement over the short term but over the long term no difference in outcome. **This requires further study, particularly in respect of machine sprayed trials.**

7.7. Visual appearance of berries

A focus of the trial was to confirm that in both red and white grapes, application/s of HML32 make little visual difference to berries, such as advanced raisoning. The trial confirmed that under New Zealand conditions, **visually there was no difference**.

7.8. Wine Fermentations

Fermentation Curves for each of the four wines made from the Chardonnay, Merlot and Syrah plots indicate that the treatments did not affect the fermentation process.

7.9. Wine Quality

Participants in the three workshops held in Gisborne, Hawke's Bay and Blenheim provided an informal qualitative evaluation of the microvin wines made from the best timing of each treatment set (ie HML32 and potassium silicate sprayed once (HML32 Plus), HML32 sprayed once (HML32 Single) and HML32 sprayed twice (HML32 Twice)) and the control.

All wines for each variety were found to be of commercial quality and all treatment wines in all three varieties were found to have enhanced elements over the respective control wines.

For the Chardonnay, the achievement of hitting the target brix two weeks ahead of the control can have benefits in terms of avoiding late season diseases but if conditions allowed it to be left hanging on the vine for those two weeks, further enhancement of the juice quality could be expected.

For the Syrah, all treatment wines provided a greater richness and more tannins over the control, even though the difference in brix was only about half a brix. This provides opportunities to the wine maker for blending as well as the potential to make a higher quality single vineyard wine.

For the Merlot, all treatment wines provided a greater richness over the control with an increase in brix from 0.5 to nearly 2 brix and a significant increase in tannins and phenolics. Heavily cropped Merlot can be difficult to ripen and these treatments were able to ripen and enhance the juice quality.

The ability to enhance ripening and juice quality in the vineyard is an additional tool for winemakers to produce high quality, high value wine.

8.0 Acknowledgements

Chris Henry would like to acknowledge his thanks and appreciation to Larry Morgan and Dakin Andersen of Te Mata Estates for allowing this trial to be undertaken; Susan Mains for her determination of Brix and Berry weights in the field, Karen Ball of EIT for making the 12 microvin wines, and to Ant Mackenzie for overseeing the winemaking and presenting the findings at the Enhanced Maturity Workshops in Hawke's Bay, Gisborne and Blenheim and to the participants at those workshops who contributed their expertise to the evaluation of the wines.

Rob Agnew of Plant and Food Research (Marlborough) deserves special mention for his advice and input into growth stages and climate data, which will give growers the best chance of applying the data produced in this trial and others.

Appendix 1: Grape and Soil Nutrient Analysis

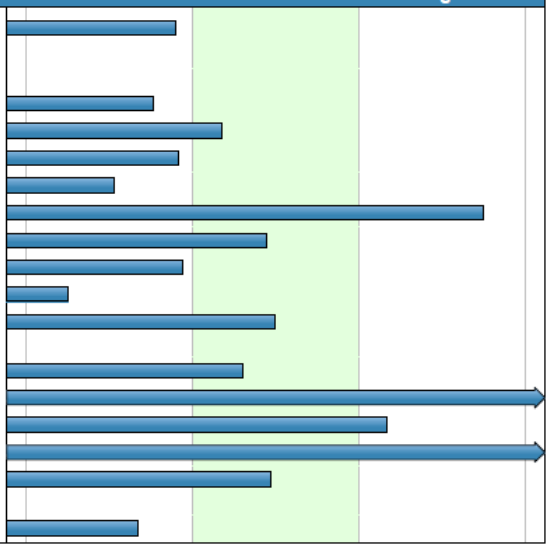
Example of Chardonnay Grape Nutrient Levels

ANALYSIS REPORT Page 1 of 7

Client:	Te Mata Estate	**Lab No:**	1510506	shMEBSpv1
Address:	C/- Fruitfed Supplies	**Date Registered:**	04-Dec-2015	
	PO Box 322	**Date Reported:**	08-Dec-2015	
	HASTINGS 4156	**Quote No:**		
		Order No:	59046013	
		Client Reference:	Isosceles	
		Submitted By:	C Pinker	

Sample Name: ISOS - Chardonnay 00 **Lab Number:** 1510506.1
Sample Type: Grape Dissected, Flowering, Chardonnay (D103)

Analysis		Level Found	Medium Range
Nitrogen*	%	2.5	2.6 - 3.3
Nitrate-N (Petiole)	mg/kg	< 100	300 - 1000
Phosphorus	%	0.21	0.25 - 0.40
Phosphorus (Petiole)	%	0.23	0.18 - 0.45
Potassium	%	1.0	1.1 - 1.5
Potassium (Petiole)	%	1.5	2.0 - 3.5
Sulphur	%	0.65	0.30 - 0.50
Calcium	%	1.56	1.20 - 2.00
Magnesium	%	0.19	0.20 - 0.40
Magnesium (Petiole)	%	0.20	0.25 - 0.45
Sodium	%	0.050	0.00 - 0.100
Iron	mg/kg	73	40 - 150
Manganese	mg/kg	530	40 - 200
Zinc	mg/kg	100	30 - 80
Copper	mg/kg	196	6 - 12
Boron	mg/kg	42	30 - 55
Molybdenum	mg/kg	0.11	0.15 - 1.0



Example of Soil Nutrient Levels

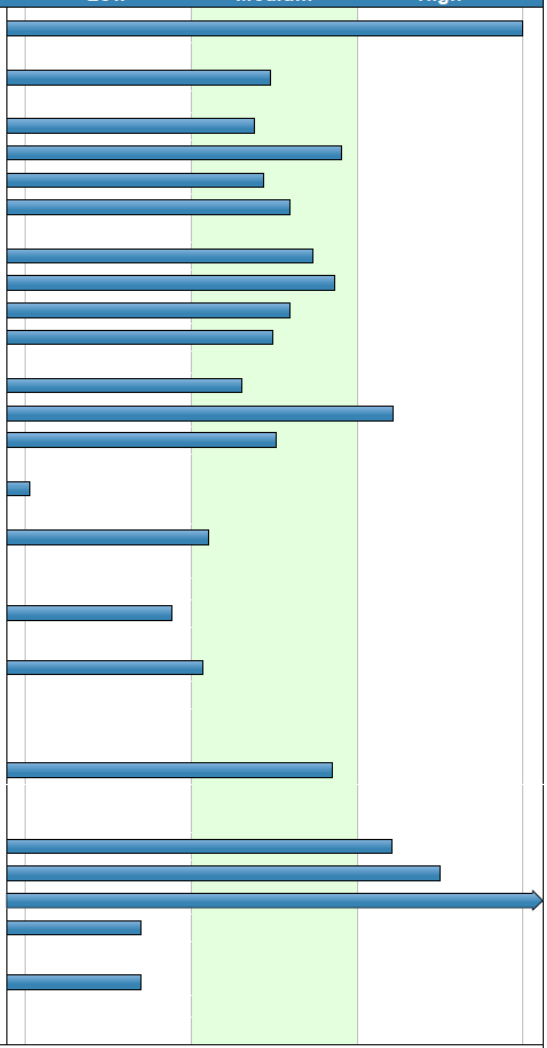
ANALYSIS REPORT

Page 1 of 26

Client:	Te Mata Estate	**Lab No:**	1165801	shMEBSpv1
Address:	C/- Fruitfed Supplies	**Date Registered:**	12-Aug-2013	
	PO Box 322	**Date Reported:**	20-Aug-2013	
	HASTINGS 4156	**Quote No:**		
		Order No:	59035301	
		Client Reference:		
		Submitted By:	C Pinker	

Sample Name: 1892 Chard West **Lab Number:** 1165801.1
Sample Type: SOIL Grape, Vineyard (S49)

Analysis		Level Found	Medium Range
pH	pH Units	7.0	5.8 - 6.8
Olsen Phosphorus	mg/L	27	15 - 40
Potassium	me/100g	0.55	0.40 - 0.80
Calcium	me/100g	11.4	6.0 - 12.0
Magnesium	me/100g	1.88	1.00 - 3.00
Sodium	me/100g	0.24	0.00 - 0.40
Potassium	%BS	3.5	2.0 - 4.0
Calcium	%BS	72	50 - 75
Magnesium	%BS	11.8	7.0 - 15.0
Sodium	%BS	1.5	1.0 - 2.0
CEC	me/100g	16	12 - 25
Total Base Saturation	%	88	60 - 85
Volume Weight	g/mL	0.80	0.60 - 1.00
Sulphate Sulphur	mg/kg	4	20 - 50
Available Nitrogen (15cm Depth)*	kg/ha	83	75 - 150
Anaerobically Mineralisable N*	µg/g	69	
Organic Matter*	%	6.1	7.0 - 17.0
Total Carbon*	%	3.6	
Total Nitrogen*	%	0.32	0.30 - 0.60
C/N Ratio*		11.1	
Anaerobically Mineralisable N/Total N Ratio*	%	2.1	
Phosphorus (Mehlich 3)*	mg/L	81	30 - 90
Iron (Mehlich 3)*	mg/L	190	
Manganese (Mehlich 3)*	mg/L	86.6	8.0 - 65.0
Zinc (Mehlich 3)*	mg/L	7.03	0.80 - 4.00
Copper (Mehlich 3)*	mg/L	33.6	0.4 - 2.0
Boron (Mehlich 3)*	mg/L	0.48	0.60 - 1.20
Cobalt (Mehlich 3)*	mg/L	0.5	
Aluminium (Mehlich 3)*	mg/L	624	900 - 1300
Soil Sample Depth*	mm	0-150	
MAF Units		K 9 Ca 11 Mg 34 Na 9	



Appendix 2: Water Quality of Spray Make-up Water

Analytical Research Laboratories

ARL analytical research laboratories

890 Waitangi Road,
Awatoto,
PO Box 989
Napier 4140

Phone: 0800 100 668
Fax: (06) 835 9223
Email: arl@arllab.co.nz
Website: www.arllab.co.nz

Customer:	HENRY MANUFACTURING LTD			**Customer No:**	60856023
	PO BOX 12015			**Samples Received:**	26/01/2016 14:19
	AHURIRI			**Report Issued:**	28/01/2016
				Total samples:	1
	NAPIER 4144	**Service Person:**	Customer Centre	**Order Number:**	W 26-1-16
		Name:			
		Email:	chrishenry@actrix.co.nz		

60856023-W 26-1-16

WATER ANALYSIS REPORT

Sample Name: Pernod - Omaranui Rd	**Lab Number: 1334161**	**Temp on receipt oC:**	27.7
Date & Time Sampled:	26/01/2016 14:19	**Order Number:**	W 26-1-16

Nutrient	Result	Uncertainty of measurement +/-
pH	7.8	0.15
Calcium mg/L	54.0	-
Magnesium mg/L	5.6	-
Potassium mg/L	2.3	-
Sodium mg/L	23.0	1.4
Copper mg/L	0.01	-
Zinc mg/L	0.14	-
Manganese mg/L	<0.1	0.015
Iron mg/L	<0.1	-
Conductivity mS/m at 25oC	40.26	-
Total Dissolved Solids mg/L **	270	-
Total Alkalinity (as CaCO3) mg/L	58	-
Chloride mg/L	32	-
Hardness (as CaCO3) mg/L	158	-
Bicarbonate me/L (as CaCO3)	1.2	-
Free Carbon Dioxide mg/L	<5	-
Boron mg/L	<0.1	-
Sodium Absorption Ratio	0.56	-

Analysis comment:

M. A. E. Smith **Mae Smith, NZCS, for ARL**

IANZ ACCREDITED LABORATORY

Tests indicated as not accredited are outside the scope of the laboratory's accreditation.

Results are based on established methods of analysis, available on request. Results apply to the sample(s) as received
* Test was subcontracted to an outside facility. Bacteriological test(s) subcontracted to Hills Laboratory (IANZ accredited).
** Calculated from conductivity test result. Metals (total) determined directly by EPA 200.2 digestion and ICP-MS.

Tests not Accredited

Tests subcontracted

...

Appendix 3: Details of Specific Botrytis Events over the Period of Maturity

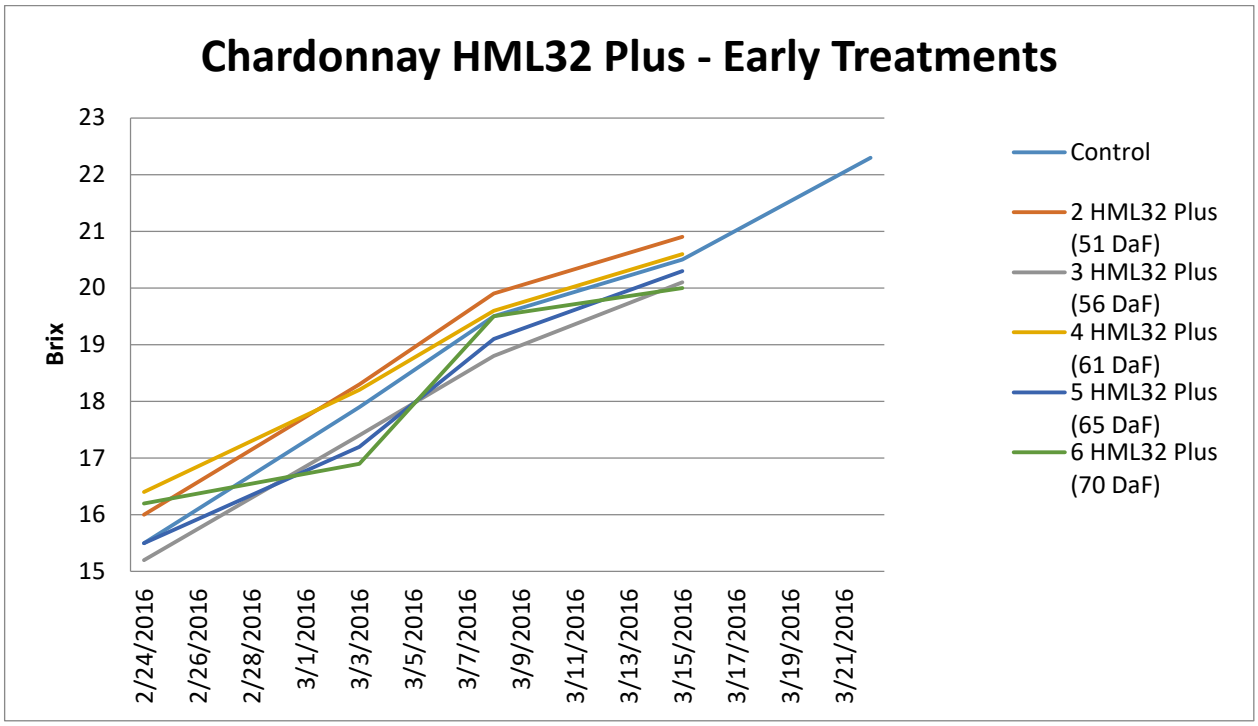
Period of Maturity

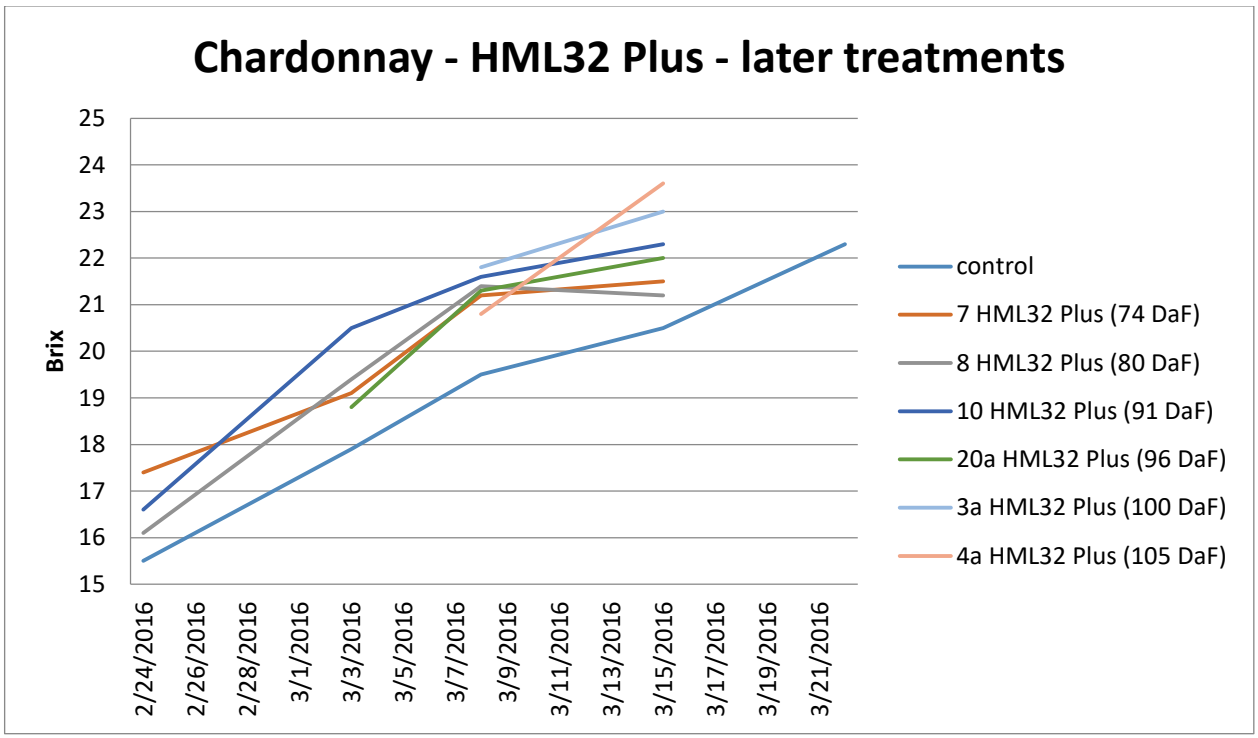
Grape Botrytis (Bacchus) event summary for Longlands Rd, HB

Start Time	Stop Time	Wetness Period (Hrs)	Total Rainfall (mm)	Average Temp. (°C)	Broken Wetness	Event Risk
Mon 29th Feb 10pm	Wed 2nd Mar 12pm	38	7	14.4	No	Severe
Mon 14th Mar 3pm	Tue 15th Mar 1pm	22	0.5	16.6	No	Severe
Wed 16th Mar 6pm	Fri 18th Mar 3pm	45	7.9	14.4	No	Severe
Thu 24th Mar 12pm	Fri 25th Mar 12pm	24	32	18.7	No	Severe
Fri 25th Mar 10pm	Sat 26th Mar 11pm	25	5.5	15.8	No	Severe
Sat 2nd Apr 6pm	Sun 3rd Apr 12pm	18	0.2	17.6	No	Severe
Sat 9th Apr 1pm	Sun 10th Apr 12pm	23	5.3	15.4	No	Severe
Sun 10th Apr 3pm	Mon 11th Apr 1pm	22	0.5	16.7	No	Severe

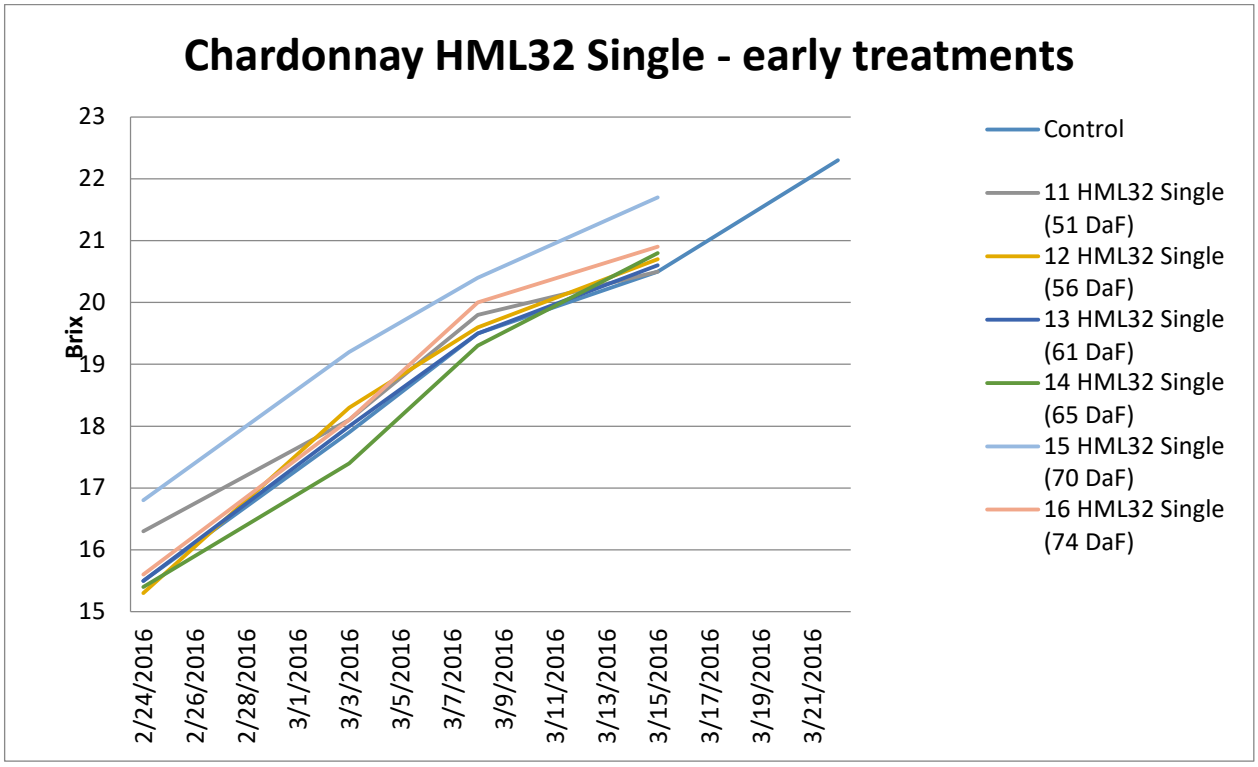
For a Botrytis infection period to be recorded by the model takes 13.5 hours of wetness at 20°C
About 33 hours at 10°C

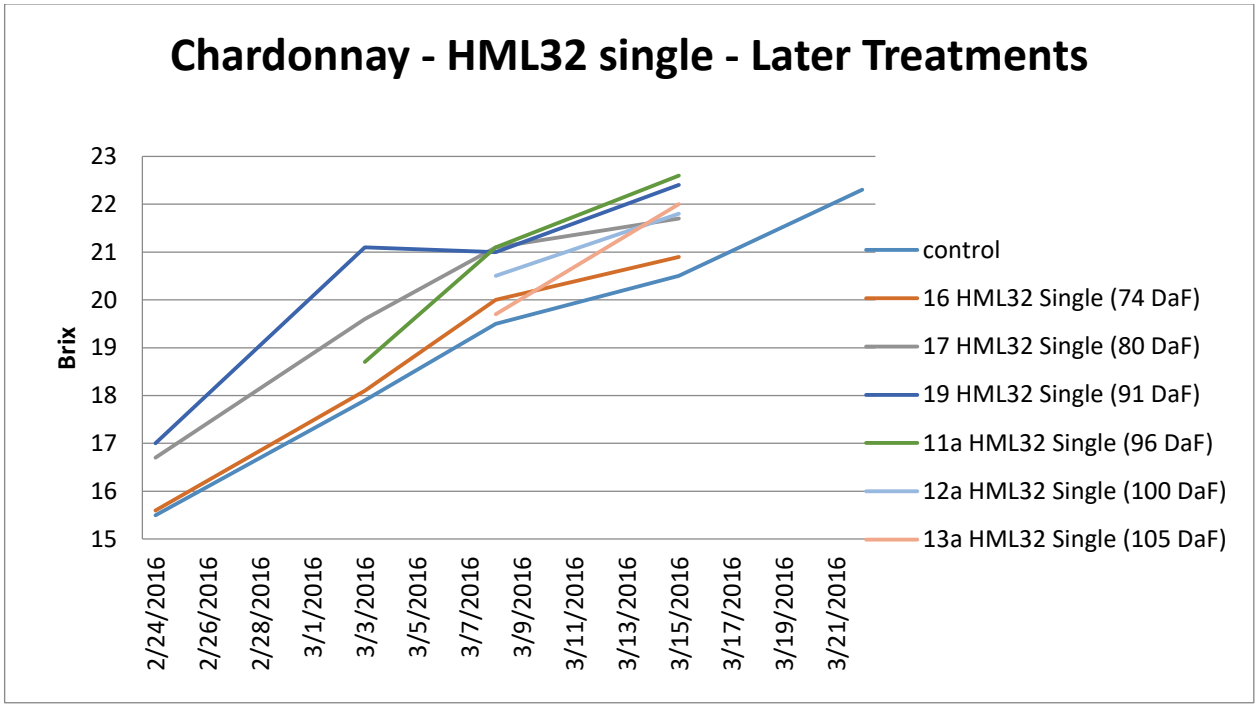
Appendix 4: Chardonnay Brix Results - HML32 Plus Treatments



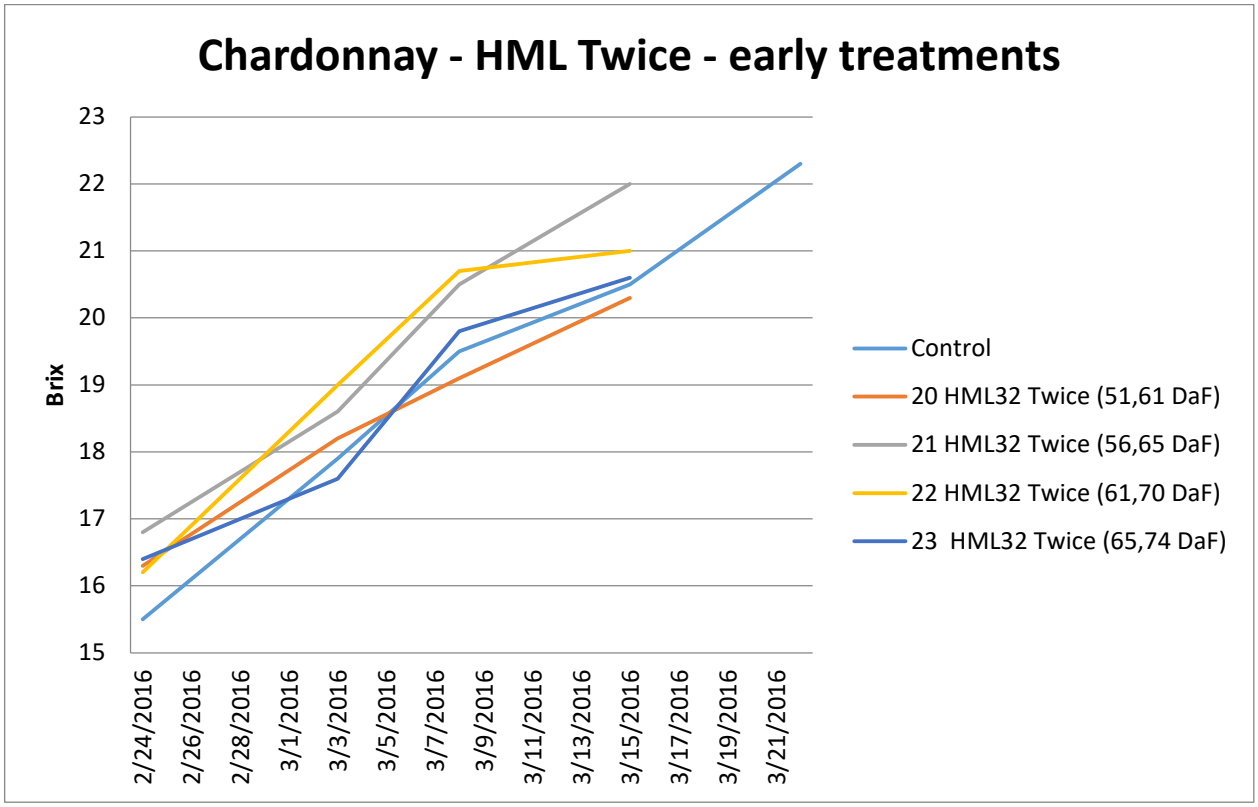


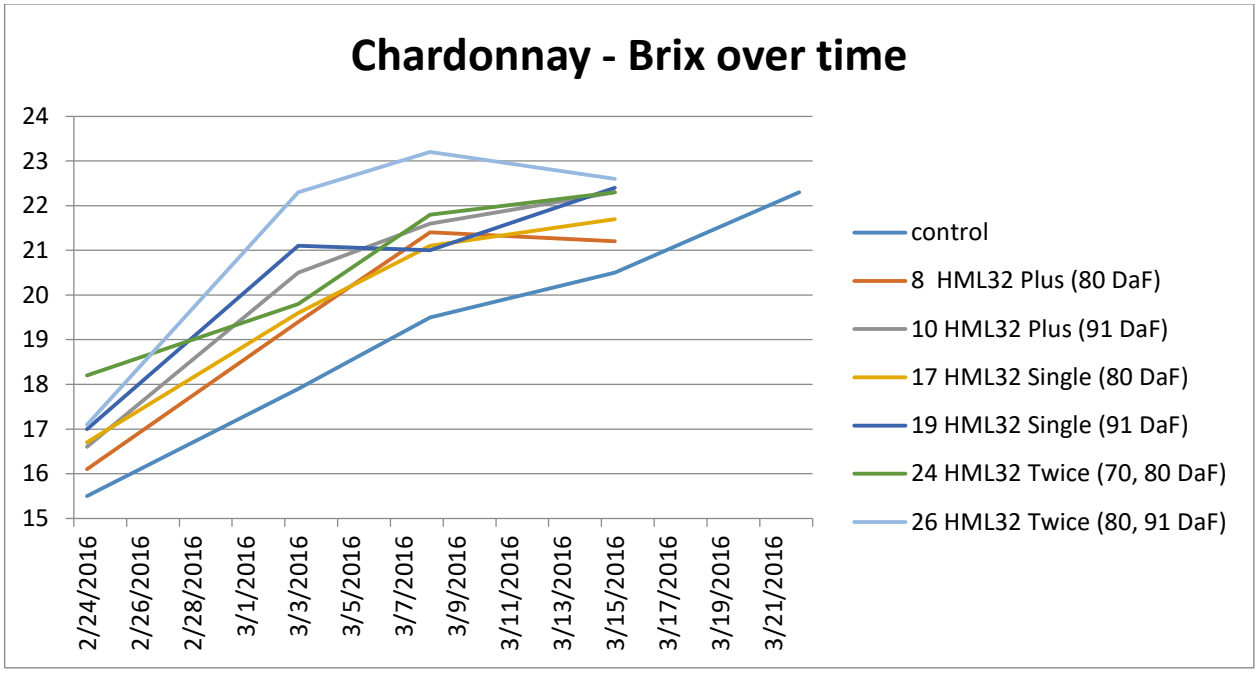
Appendix 5: Chardonnay Brix Results - HML32 Single Treatments



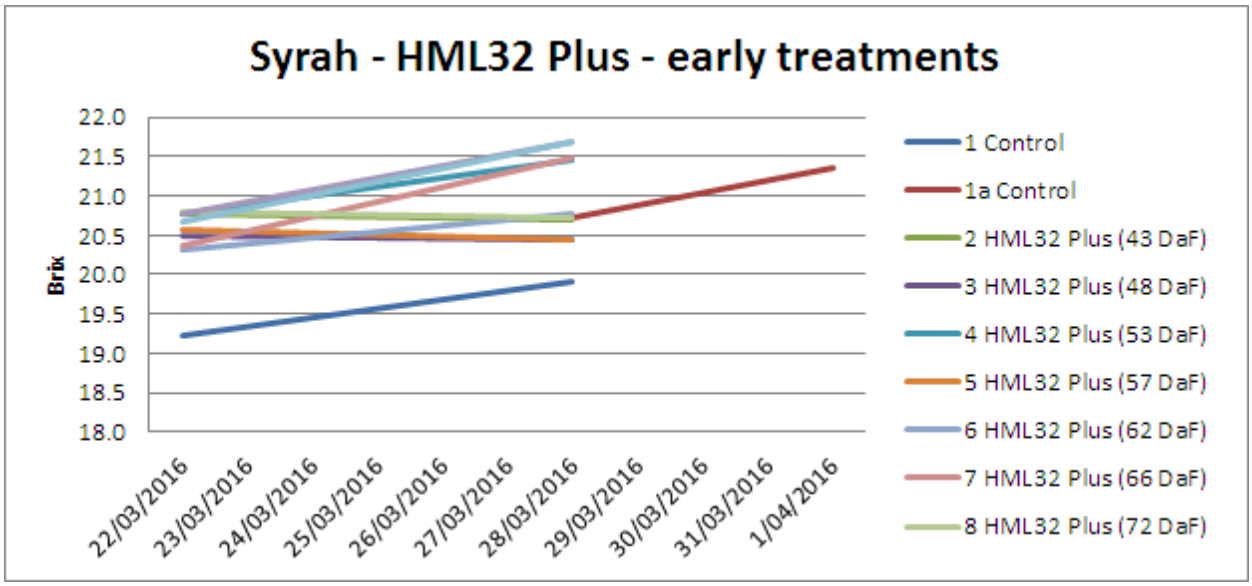


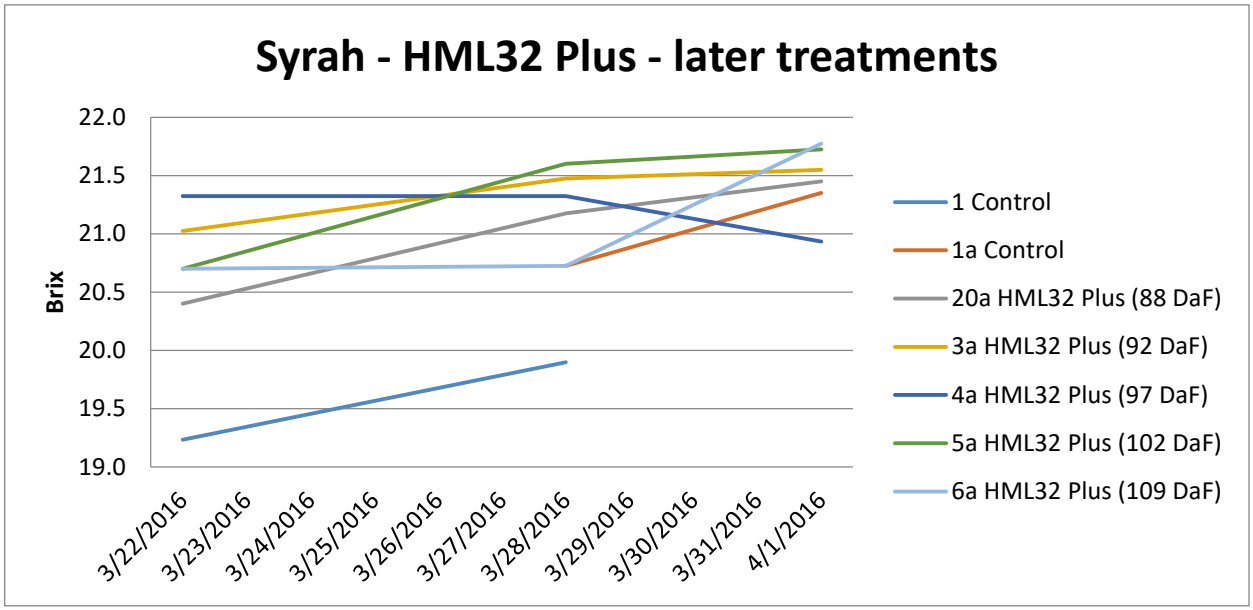
Appendix 6: Chardonnay Brix Results - HML32 Twice Treatments



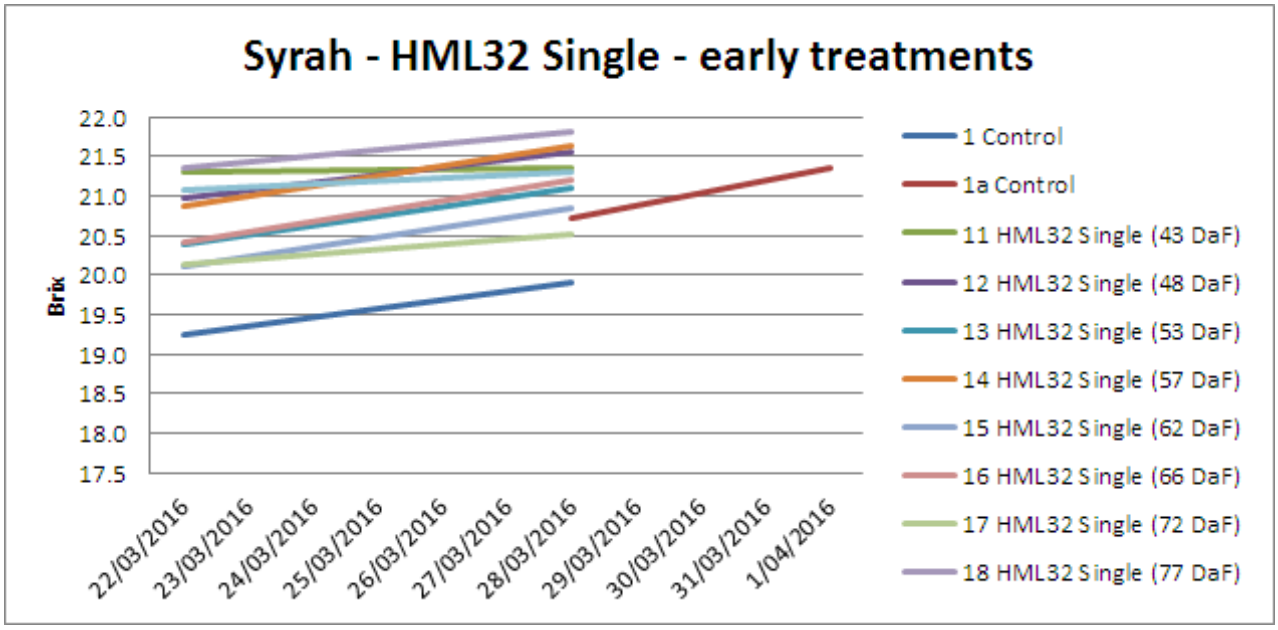


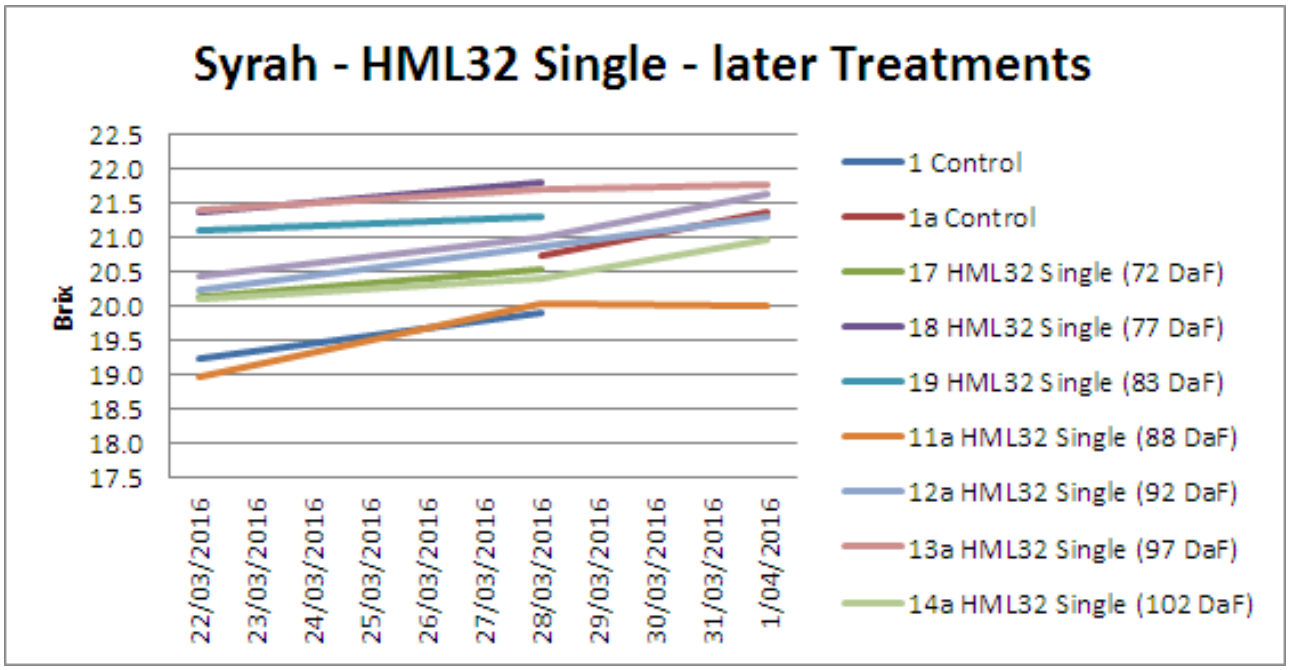
Appendix 7: Syrah Brix Results - HML32 Plus Treatments



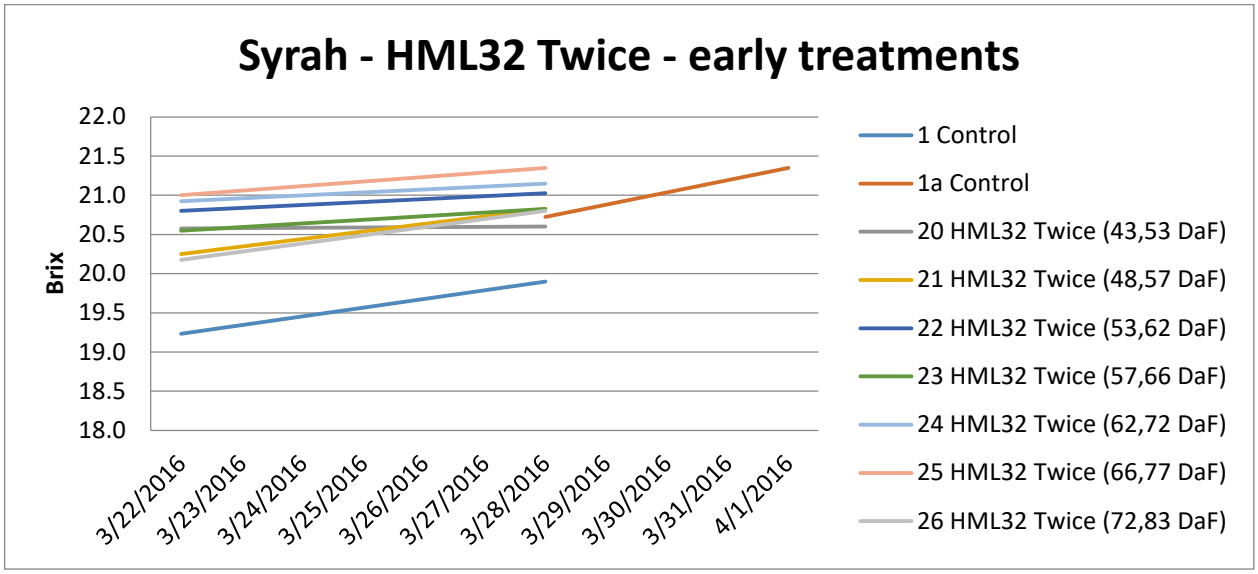


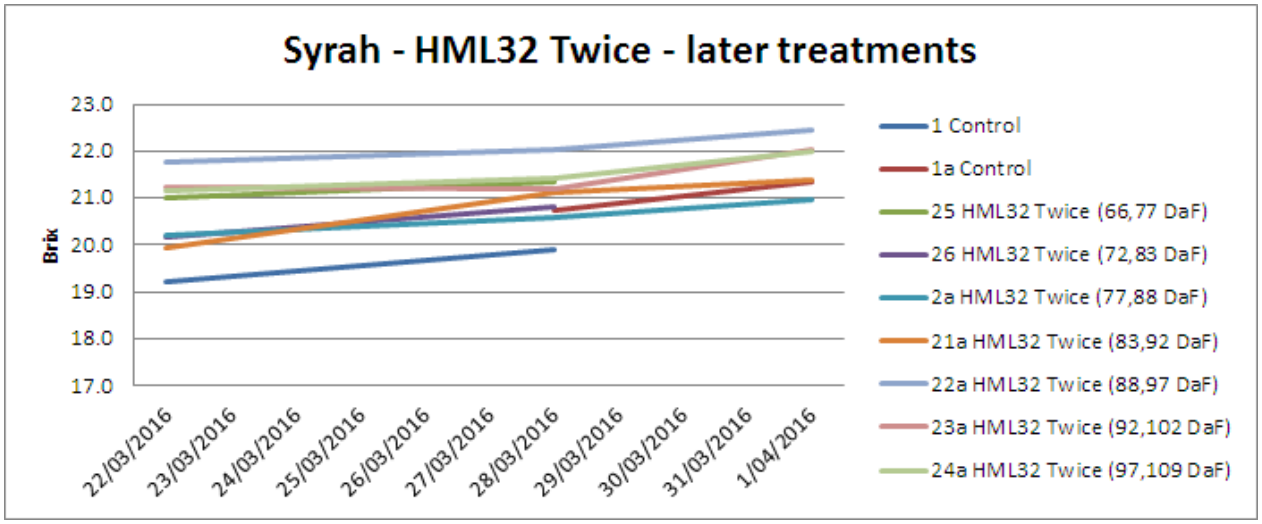
Appendix 8: Syrah Brix Results - HML32 Single Treatments





Appendix 9: Syrah Brix Results - HML32 Twice Treatments





Appendix 10: Ant Mackenzie's Tasting notes

Chardonnay	Aroma	Taste	Preference (1 being 1st)
Control	Lean, citrus, apple	Rich, round, pure, long	3
Treatment 8 (HML32 plus Silco sprayed once)	Lean, citrus, apple	Vibrant palate, full rich long	2
Treatment 19 (HML32 sprayed once)	Leaner, slightly grubby	Bright vibrant palate	4
Treatment 26 (HML32 sprayed twice)	Mineral, citrus, pure	Richer palate but vibrant, slightly salty	1

Syrah	Colour/Aroma	Taste	Preference (1 being 1st)
Control	Pepper, spice, fruit	Coarse, edgier palate, some tannin	4
Treatment 4a (HML32 plus Silco sprayed once)	Spice, meaty, gamey	Finer, front palate tannins, vibrant, bright	3
Treatment 13a (HML32 sprayed once)	Plush florals, spice, pretty fruit notes	Finer, front palate tannins, rich round	2
Treatment 22a (HML32 sprayed twice)	More dark, fruit, plum, spice notes	Rich, pure, sweeter, supple, front palate, riper notes	1

Merlot	Colour/Aroma	Taste	Preference (1 being 1st)
Control	Meaty	Slightly greener, coarser palate	4
Treatment 4a (HML32 plus Silco sprayed once)	Finer florals, plums, cassis	Sweeter, richer, front phenolics	2
Treatment 13a (HML32 sprayed once)	Meaty, dense, a greener edge	Graphite, broader tannins	3
Treatment 22a (HML32 sprayed twice)	Fine pure rose, aromatic	Sweet, rich, mouth coating tannins	1