BLENHEIM, NEW ZEALAND
MAY 10, 2018

MICROBIOLOGICAL STRATEGIES TO OPTIMIZE WINE REGIONALITY AND PERSONALITY

THE *XXVIII^es ENTRETIENS SCIENTIFIQUES LALLEMAND*

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BLENHEIM, NEW ZEALAND, MAY 10, 2018

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PROCEEDINGS
OF

THE *XXVIIIes ENTRETIENS SCIENTIFIQUES LALLEMAND*

LALLEMAND®

ISBN 978-2-9818091-0-0 (printed version)
ISBN 978-2-9818091-1-7 (pdf version)

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Bibliothèque et Archives nationales du Québec 2019
Library and Archives Canada 2019

Lallemand Inc. Montréal, Canada H1W 2N8

Moduli Publicité . Communication Inc., Longueuil, Canada, J4G 2K1

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AROMA PROFILING OF SAUVIGNON BLANC: A GRAPE TO WINE ANALYSIS.

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Abstract

Grapevine berries display remarkably phenotypic plasticity, adapting to their immediate environments (microclimates), and modulating/adjusting their transcriptomes and metabolites to acclimate to perceived stress(es). Berry composition is considered a function of the cultivar, the environment and viticultural management practices. Understanding the links between the cultivar, the environment, and stress response molecules in grapevine therefore provides insight into a number of important concepts in wine sciences, such as cultivar typicity and terroir expression. Molecular and/or metabolite profiling techniques have proven to be powerful tools to understand how this plasticity is achieved, specifically when combined with environmental profiling to generate a clear understanding of the (stressful) factors influencing the berries. Leaf removal in the bunch zone is a common viticultural practice with several objectives, yet it has been difficult to conclusively link the physiological mechanism(s) and metabolic berry impact to this widely practiced treatment. We used a field-omics approach in a Sauvignon Blanc high altitude model vineyard, showing that early leaf removal in the bunch zone caused quantifiable and stable responses (over years) in the microclimate where the main perturbation was increased exposure. Both light quantity and quality (UVB attenuation) was modulated, leading to an interesting view of the impact of UVB on berry metabolism. The data also show that the berry developmental programme dictates the potential changes that can be made to metabolism at the specific developmental stage. We provide an explanation for how leaf removal leads to the shifts in grape metabolites typically linked to this treatment and how it follows through to wine quality impact factors. Our data will also be discussed from the perspective of metabolic potential of grape and wine matrices.

1. Introduction

1.1 "Cause and effect" is tricky in wine science

Wine production is a multi-step process that starts with an inherently variable raw material, the grapes, originating from a complex vineyard ecosystem where a multitude of factors can have an impact on the product quantity and perceived quality.

Grapevine is cultivated in 72 countries and considered to be the most economically important fruit crop worldwide. There is, however, an unpredictability associated with the cultivation of grapes in a field setting that is largely linked to the interaction between the specific grape genotype ("G") being cultivated, the environmental factors ("E") that the developing grapes are exposed to, and the specific viticultural (management) treatments implemented ("M"). Grapes and their composition (as a proxy to quality characteristics) are considered to be the result of the so-called GxExM interaction. "Terroir" is the term frequently used to indicate the impact of this interaction between the vines, their environment, and the effect of human intervention in a specific geographic location (reviewed in Fabres et al., 2017).

Producers and viticulturists are therefore challenged with a multitude of compounding factors to take into consideration when aiming to produce quality grapes. While some viticultural decisions are long term (for example, site selection, cultivar/clone per site, row orientation and spacing of vines and rows, and trellising system), others are seasonal, such as the choice to implement canopy manipulations (e.g., shoot thinning, shoot trimming, and leaf removal), bunch manipulations, chemical control and feeding regimes, or perhaps the addition of bio-stimulants (such as plant growth regulators or inactivated yeast products). Although viticultural manipulations such as leaf removal are widely used, it had not conclusively been linked to a physiological mechanism(s) and/or metabolic impacts in grapevine berries.

Grape research is currently benefitting from efforts to systematically describe and monitor the variable factors that can impact growth, development, and product quantity/quality. Interestingly, apart from affecting the whole plant's ability to adapt and cope with its environment, numerous stress-related pathways are directly related to the biosynthesis of quality-associated wine compounds. These include colour (e.g., anthocyanins, flavonols, and carotenoids), flavour and aroma (e.g., monoterpenes, sesquiterpenes, and norisoprenoids), mouthfeel (e.g., polyphenolics such as tannins), and antioxidants (e.g., terpenoids such as carotenoids and polyphenolics such as flavonoids) (refer to Figure 1).

1.2 The "field-omics" concept

The success of a specific experimental approach can be determined by accurately linking causality of a treatment to the results (cause and effect) and the repeatability thereof. The term "field-omics" was put forward in Alexandersson et al. (2014) to describe an experimental approach that could potentially be effective at mitigating the effects that highly variable field conditions may have on the outcome of crop studies. This approach relies on in-depth characterization of the environmental factors, as well as the progression of growth of the crop being studied to identify potential sources of unintended variability, as a context of data being generated and interpreted. This is particularly important when attempting vineyard experiments using omics techniques that provide snapshots in time of transcripts, proteins, and metabolites, all of which are strongly responsive to environmental influences. Not only have the grapevine profiling tools advanced, but the technologies available with which to characterize specific environmental conditions have improved dramatically as well. Sensors can be used to quantify the macro, meso, and microclimatic variables of the developing grapes in vineyards and studies. Several recent studies adopting this approach have contributed to the improvement of grapevine field experimental systems (for example Carbonell-Bejerano et al., 2016; Pinto et al., 2016; Reshef et al., 2017).

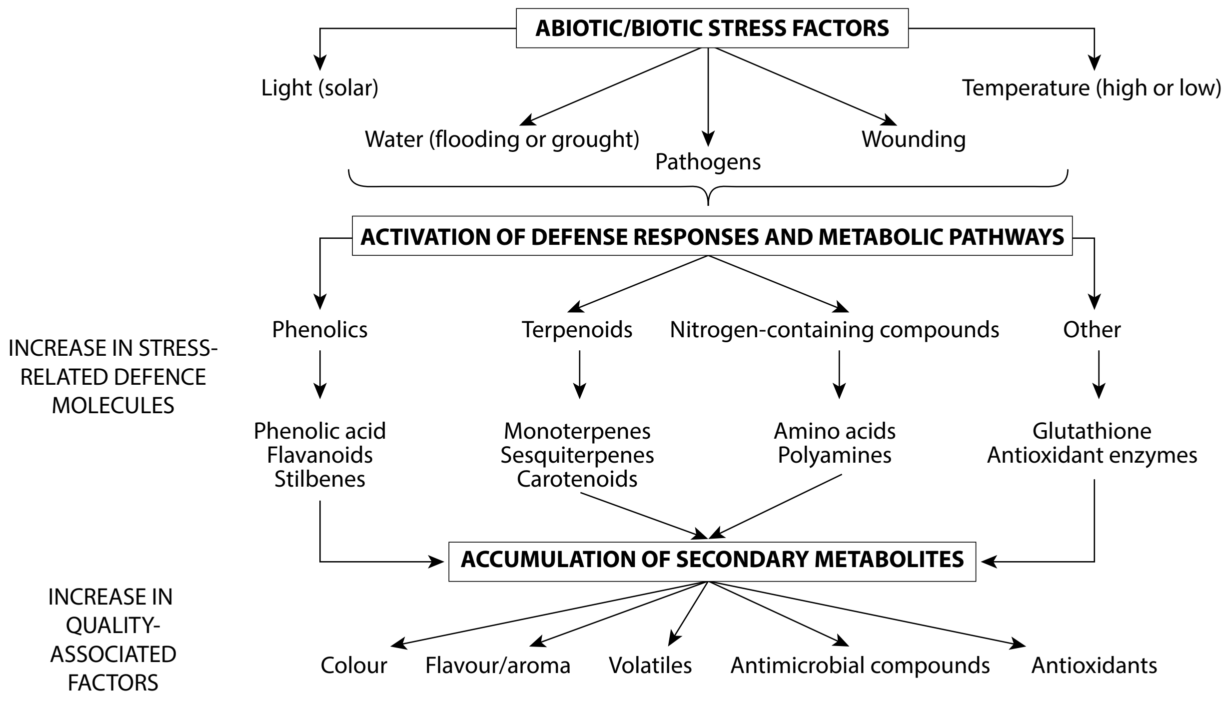

Figure 1. How stress impacts quality in grape berries. An overview of the climatic factors affected by leaf removal treatment and the consequent influence on quality-associated metabolites. The altered microclimate leads to stress responses, acclimation, and altered quality associated metabolites in grapevine berries (adapted from Gonzalez-Aguilar et al., 2010).

2. Research: Sauvignon Blanc field-omics studies where grape to wine analyses were implemented

Here we describe the outcomes of two experiments in a Sauvignon Blanc vineyard in the Southern hemisphere, using the field-omics approach:

Project A: A multi-season experiment to study the impact of light quantity and quality on berry growth, metabolism, and quality impact factors in the grapes and the resulting wines of Sauvignon Blanc

Light influences inflorescence formation and fruitfulness (Buttrose, 1969; Morgan et al., 1985; Srinivasan and Mullins, 1981), berry metabolic processes (Chorti et al., 2010; Downey et al., 2008; du Plessis et al., 2017; Martin et al., 2016; Reshef et al., 2017; Smart, 1987; Song et al., 2015; Suklje et al., 2014; Sun et al., 2017; Young et al., 2016), as well as berry characteristics. Of increased interest have been the impacts of ultraviolet B (UVB) radiation on grapevine, specifically in the Southern hemisphere which is known to receive higher levels of UVB. A number of approaches have been utilized to investigate UVB impacts in field trials, including the use of UVB screens installed over the bunch zone, encasement of the bunches in UV attenuating bags, and the complete covering of the grapevine in plastic films with different UV transmittance properties (De Oliveira et al., 2015; Gregan et al., 2012; Koyama et al., 2012; Liu et al., 2015). Evidence from these studies has revealed that grapevine is in fact well adapted to UVB exposure and typically shows acclimation responses that include morphological (Del-Castillo-Alonso et al., 2016; Doupis et al., 2016), metabolic (Del-Castillo-Alonso et al., 2016; Reshef et al., 2018; Song et al., 2015), and transcriptomic (Liu et al., 2015) changes, all of which contribute to the phenotypic plasticity of grapevine to the UVB stress signal. Despite these significant advancements, which coincided with the objectives of this study, the assessment of UVB impacts in white cultivar grape berries remained restricted.

Project B: Describing the impact of Lalvigne Aroma on Sauvignon Blanc wines and wine styles.

Inactivated yeast products have been widely used during wine-making, but the objective here was to describe the impact on the wines when the product (LalVigne Aroma) was sprayed in the vineyard at the onset of ripening as a biostimulant.

2.1 Experimental details:

The studies had the benefit of an existing highly characterized (model) vineyard where the field-omics principles were implemented in the experiments' design. The model vineyard was established in 2004 in Elgin in the Overberg region of the Western Cape coastal region of South Africa. At more than 250 m above sea level, Elgin is a high-altitude wine-grape growing region. The elevation and proximity to the Atlantic Ocean, and consequent exposure to the cooling sea breeze, make it the fourth coolest wine-grape-growing region in South Africa. This site was chosen as representative of a typical moderate climatic site for the production of a commercially desirable style of Sauvignon Blanc wine.

Sauvignon Blanc clone 316 was grafted on 101-14 Mgt and planted in a NW/SE row orientation with 2.5 m between-row and 1.8 m in-row spacing. The vines were trellised to a double cordon with a vertical shoot-positioning system and winter pruned to eight two-bud spurs per running meter of cordon. For study A, we used an early leaf removal treatment to study the impact of increased bunch exposure on grape composition throughout berry developmental stages (i.e., green pea size through till the ripe/harvest stage) (Young et al., 2016). The treatment involved leaf and lateral shoot removal in the bunch/fruiting zone (approximately 30-40 cm above the cordon) on the NE-facing side of the canopy (i.e., the facet of the vine that received morning sunlight exposure) at Eichorn-Lorenz stage 29 (~pea-size berries). UVB attenuation was achieved by installing UVB exclusion panels in the bunch zones. The canopies were manipulated to generate four distinct microclimates in the bunch zones: a high light (HL), low light (LL), as well as an HL and LL environment with attenuated UVB (HL-UVB and LL-UVB, respectively) (refer to Figure 2 on next page). Sensor measurements confirmed the light conditions for the four different microclimates as well as the other microclimatic factors in the canopy as well as in the bunch zones (described in Young et al, 2016 and Joubert et al, 2016).

Berry samples generated from this study were subjected to a range of metabolite profiling analyses as well as transcriptomic analysis (RNA sequencing) (Refer to Figure 3 on next page for a summary of the analyses performed).

For study B, the same vineyard was used, as was described in Suklje et al. 2016. The Lalvigne Aroma product was applied, as per the manufacturer's instructions and experimental wines were produced and chemically and sensorially analysed, generating treated and untreated samples according to a statistically sound experimental layout.

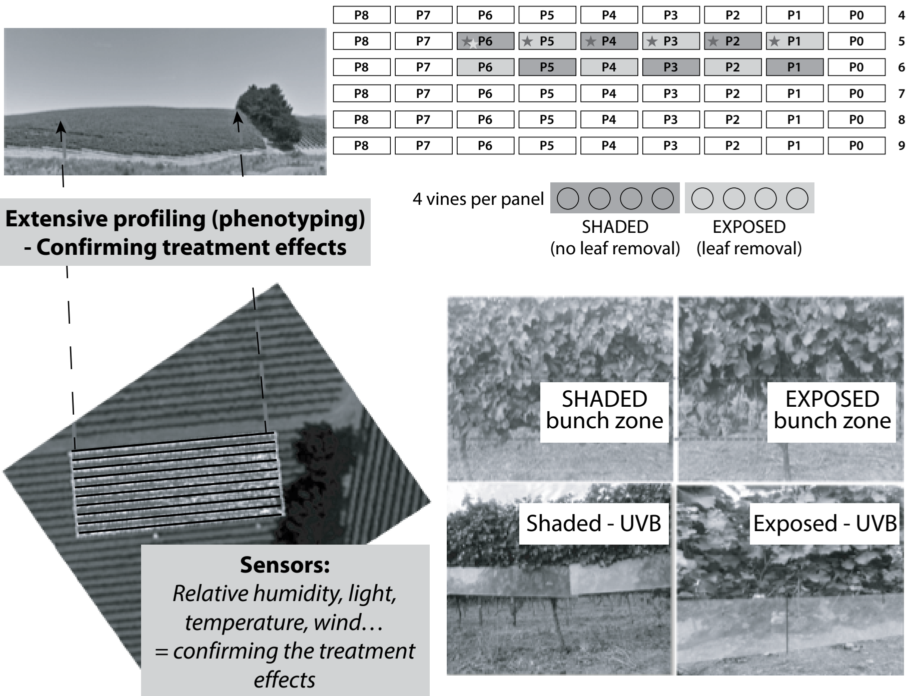

Figure 2. An overview of the experimental layout and microclimates created to study the impact of light exposure and light quality in a Sauvignon Blanc model vineyard in the Elgin region in South Africa.

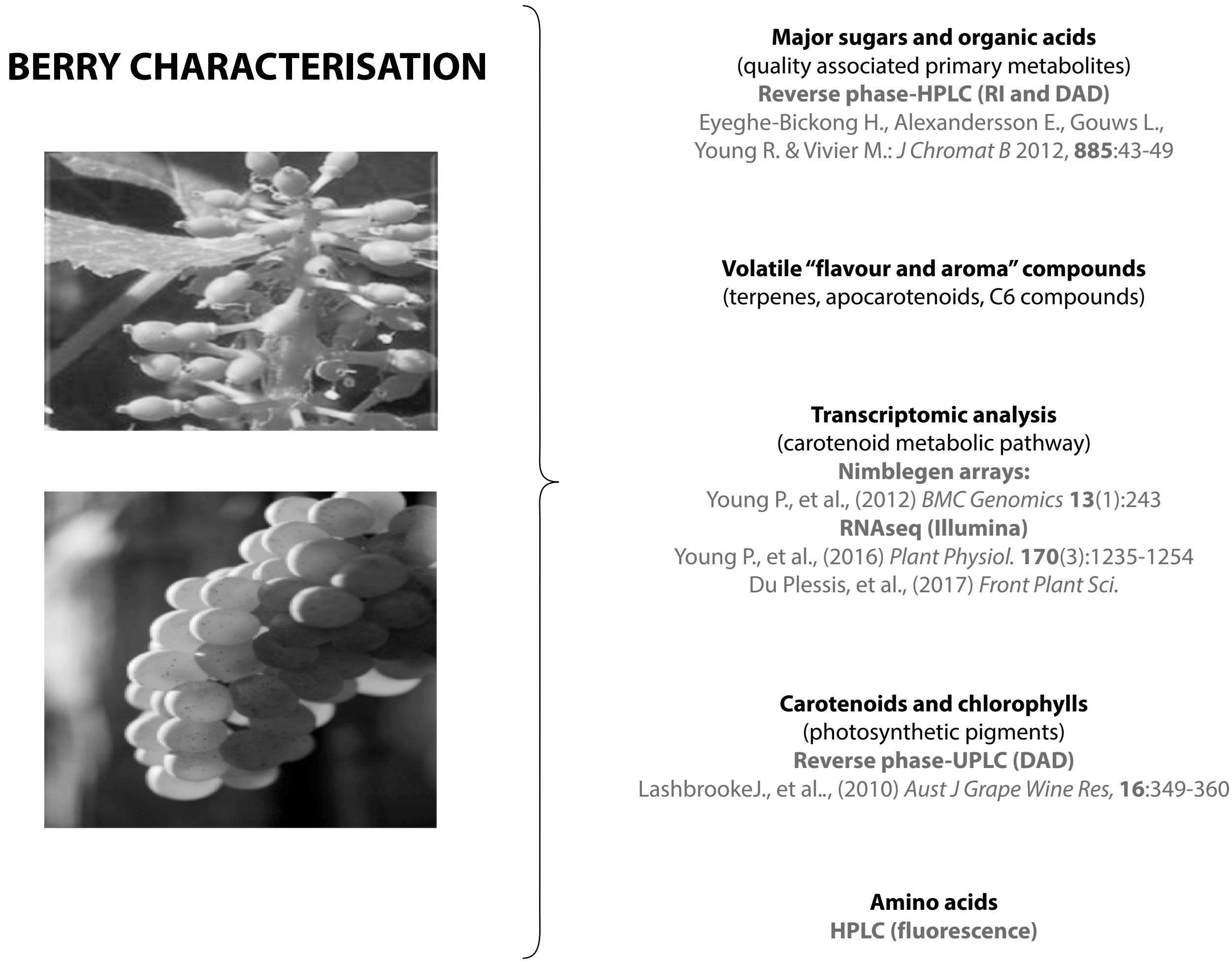

Figure 3. A summary of the transcriptomic and metabolite profiling conducted on Sauvignon Blanc berries at three berry developmental stages (green, veraison, and ripe).

2.2 Results and discussion:

The main outcomes of the experimental work for project A are contextualised in the sections below.

The increased exposure (in the high light microclimates) changed the metabolism of the berries

The grapevine's response to the increased exposure, and the subsequent effect on berry composition, is best described as acclimation: a plant's innate ability to respond and adapt to its environment. It was clear that different acclimation responses were used at the different berry stages (green versus ripe stages). Whole transcriptome analyses revealed that more than 90% of all the genes annotated in the grapevine genome remained unaffected by elevated light at every developmental stage evaluated. These unaffected genes were predominantly involved in primary metabolic processes, hereby confirming that not only were the berries largely unaffected on a metabolite level (Young et al., 2016), but that the underlying molecular mechanisms associated with primary metabolism remained constant as well (Du Pessis et al, 2017). The leaf removal treatment did not affect the typical ripening progression of the grapes (relative to the controls: HL vs. LL [control]; or HL [control] vs. HL-UVB, and LL [control] vs. LL-UVB). Physical berry parameters (e.g., weight and diameter) and the trends in primary metabolites (the major sugars and organic acids) were not significantly altered at

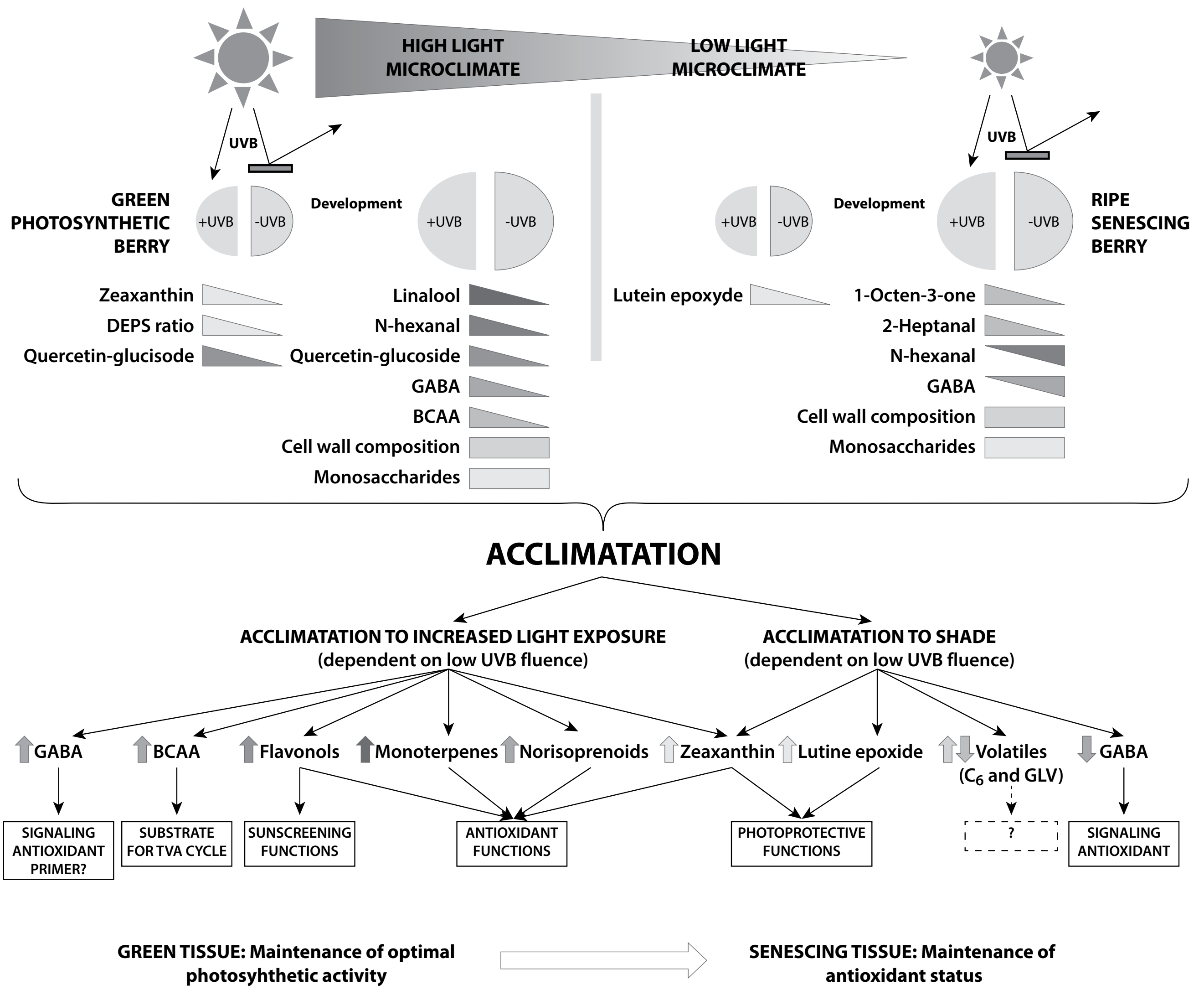

Figure 4. A comprehensive model summarising the early and late developmental stage metabolic responses in grape berries as a way to acclimate to variations in UVB light under HL and LL microclimates. In each light environment (HL and LL) both early and late developmental stages are represented as well as the attenuation of UVB. The coloured triangles indicate those compounds that reacted to UVB attenuation in each case, indicating the presence of an acclimation response in the berries. Each of the compound groups perform a specific function in the berry tissue and contribute to the acclimation of the berry via various physiological processes. These processes differ depending on the tissue type and are therefore associated with the developmental stage of the berry (Honeth, 2018).

any of the developmental stages by the leaf removal treatment. The berries, however, responded to the changes in the microclimate by altering the amino acid metabolism, and by utilising secondary metabolites prevalent at the specific developmental stage to counter potential stress. Figure 4 (on previous page) proposes an overview model of the respective UVB radiation elicited impacts under variable light exposure in the grape matrices, highlighting the most important metabolic responses. The field-omics approach employed in this study demonstrated considerable plasticity in grapevine responses to UVB attenuation, which were modulated by the level of light exposure and influenced by the developmental stage of the berries. The alterations in berry microclimate during development clearly influenced berry composition, thereby forming four individual berry matrices at the green stage and at maturity (Figure 4).

The results of this study demonstrated that specific metabolites responded to the modulation of UVB radiation in a metabolically plastic manner and these responses were dependent on light exposure and developmental stage. In the green developmental stage, when berries were still photosynthetically active, specific photoprotective carotenoids, namely the xanthophylls, reacted to the differences in UVB exposure. These xanthophylls are related to the violaxanthin and lutein epoxide cycles, both of which were demonstrated to be functional in the green berries under both the HL and LL microclimates. The lutein epoxide and violaxanthin cycles have been demonstrated to optimise the light harvesting process as well as facilitate energy dissipation, however, the work done on the responses induced by UVB light in the xanthophyll cycles is minimal. Here we showed that UVB radiation could be implicated in the formation of zeaxanthin under HL conditions, the directional change in the xanthophyll cycle associated with photoprotection through energy dissipation. Furthermore, under LL conditions, the variation in UVB was implicated in the metabolism of lutein epoxide, a molecule which serves a photoprotective role under conditions of sudden and localised light exposure under shaded conditions. These results demonstrated the grape berry's ability to shift its metabolism as a way to mitigate potential UVB damage and acclimate to the environment. The successful acclimation of green grape berries to UVB exposure was evidenced by the insignificant effect of UVB exposure on the chlorophylls and main carotenoids under both the HL and LL microclimates. These results indicated that photosynthetic processes were probably maintained in the green berries, implying that the perceived stress was mitigated through certain mechanisms such as this non-photochemical quenching via the xanthophyll cycles. Our results extended the current understanding of UVB impacts in grapevine fruits by showing that some of the specific carotenoids involved in photoprotection were responsive to levels of solar radiation (exposure), but that the UVB component in this light signal was required for the typical photoinhibition responses linked to the violaxanthin and lutein epoxide cycles. This provided novel insights into the underlying mechanisms employed by green developing grape berries to acclimate to UVB (Figure 4).

Ripe berries also responded metabolically to the variations in UVB under HL and LL microclimates, resulting mostly in the formation of compounds which have direct antioxidant and/or "sunscreening" abilities. The most typical UVB-induced response was the accumulation of polyphenolics, most notably with higher light exposure. The phenolics have received the most attention in UVB-related studies, and these results served to further corroborate the typical responses seen in grape berries while furthering our insights into white cultivar responses. Moreover, the variation in UVB induced several modulations in berry volatile compound composition. A noted increase in monoterpenes and norisoprenoids with ambient UVB assisted in the maintenance of the berry antioxidant status (Figure 4). The antioxidant capacity of isoprenes has been validated in plants, and it is possible that this is one of their biological functions in older (sink) tissues such as ripe berries. Similarly, the norisoprenoids may serve as sensing and signaling compounds when plants are subjected to stress as a way to mitigate potential damage by activating oxidative stress defense mechanisms (Ramel et al., 2012). These ripe berries also displayed interesting amino acid responses, which were modulated depending on the level of light exposure.

The variations in UVB elicited compositional changes to the wines

The juices yielded from these berries were distinct in their composition in each microclimate and displayed variable compositional changes during juice processing prior to fermentation. This in turn influenced the final wine composition, resulting in four wines unique to each microclimate. Furthermore, the manipulation of light and UVB exposure was shown to modulate metabolites mostly located in the skin tissue of the grape berry, including the monoterpenes, phenolics, and amino acids. These compounds contribute to the varietal characteristics of the wines, and the skin of the grape berry thus potentially represents an untapped pool of aromatic potential. Further experimentation to determine the effects of different extraction methods, such as extended skin contact or the usage of specific enzymes, could potentially benefit wine attributes.

The sensorial analysis of the final bottled wines following aging revealed perceptible differences associated with the four different treatments/controls. This further substantiates the fact that bunch microclimate modification will impact wine sensorial attributes. Here we highlighted the influence of UVB particularly on the wine characteristics, linking them to compositional changes in the berries, juice, and wine. The data revealed a strong association with tropical attributes to ambient UVB levels under HL conditions, implying that the UVB component of light is necessary for the formation of compounds linked to these aromas. Furthermore, modulation to berry microclimate may have influenced the oxidation potential of the different juice and wine matrices, resulting in the occurrence of specific aromatic descriptors. The potential impact of polyunsaturated fatty acids on wine aroma characteristics was also considered. These compounds were modulated by the variation in UVB radiation under HL and LL microclimates and may have contributed to the "green" characters associated with certain wines. Other studies conducted in the same vineyard revealed the impacts of UVB on other important Sauvignon Blanc aromatic compounds, including the thiols and methoxypyrazines (Suklje et al., 2014). The combination of these results and our profiling of the fermentation-derived compounds provides a chemical signature that links to the sensorial profiles of the four wines (refer to Figure 5).

Project B: *The wines made with Lalvigne Aroma were chemically and sensorially distinct from the untreated samples*

The results from project B confirmed that the addition of the inactivated yeast products did not significantly alter the industrial grape ripeness indicators at harvest (Suklje et al., 2016), but several impacts in terms of metabolites were observed in the juice and/or wines. Chemical analysis revealed that glutathione (GSH) levels were increased

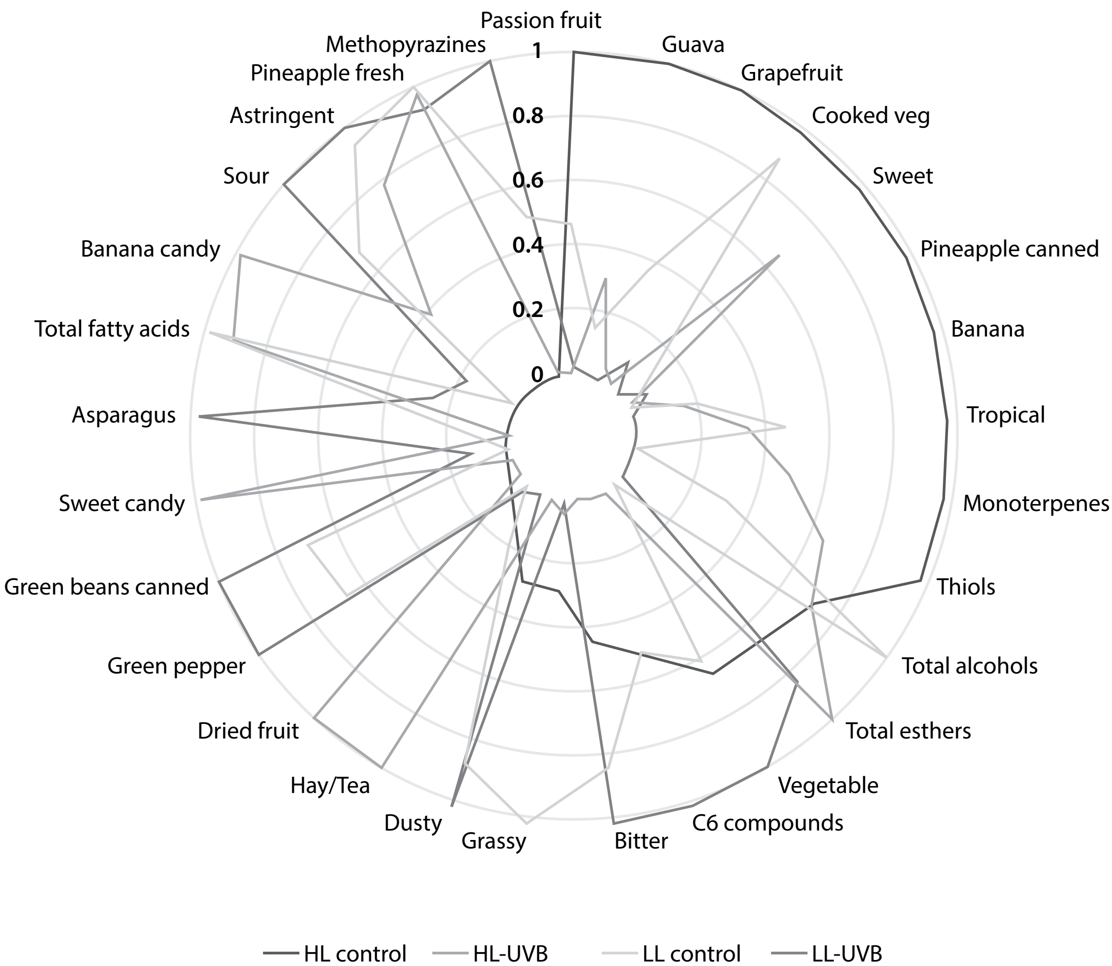

Figure 5. A representation of the main aromatic compound groups measured in the bottled wines as well as the sensorial descriptors associated with each wine. The graph was constructed from data attained in this study as well as the results presented in the study by Suklje et al. (2014). The data was normalised in order to better visualise the impacts of UVB in each wine (Honeth, 2018).

in all the matrices tested in the treated samples. GSH is a tripeptide that has several important functions in wine-making, most notably preventing must oxidations and playing and important role in protecting volatile thiols during bottle ageing. The amino acid levels were also increased in the treated samples after fermentation, as were the major volatiles in the wines, particularly the alcohol acetates. Interestingly, it was also observed that the rate of decay of the ethyl esters of straight chain fatty acids were two-fold lower in the treated samples. The sensorial analysis confirmed that the treated samples were perceived by a trained panel to be more tropical, with descriptors of peach, citrus, apple, and lemon dominating. The higher levels of certain volatiles and their longevity (i.e., their longer persistence), as well as the higher levels of GSH, an aroma protectant, contributed to a more aromatic wine, linked to the Lalvigne Aroma bio-stimulant in this experiment (Suklje et al., 2016).

3. General conclusions and future perspectives

One of the strengths of these studies lies in the use of a characterised vineyard and the benefits it brings to the experimental design. Utilising a field-omics approach, a holistic representation of grape berry responses to light exposure and UVB, as well as the addition of an inactivated yeast product, could be generated. Certain compounds were clearly translated directly from the berry to the juice and wine, including compounds such as the monoterpenes, norisoprenoids, and phenolic compounds. Other compounds such as the amino acids undergo various changes through the wine making process, and it is important to consider the physical location of the compounds in the berry tissues (compartmentalisation) and to follow these compounds through the different matrices to better understand the underlying mechanisms involved in the dynamic responses and extraction dynamics. It is indeed important to consider the deconstruction of the berry and the evolution of the grape and wine matrices to optimally link berry, juice, and wine metabolic potentials.

The two experiments confirmed that Sauvignon Blanc wine styles can be drastically impacted through vineyard manipulations – here the phenotypic plasticity of the cultivar was evident, yielding very different wines from the same geographical location through the addition of canopy manipulations to modulate light quantity and quality, or through the addition of a biostimulant. Apart from a deeper understanding of grapevine biology, this information also has the potential to inform and rationalise design changes to management practices to manipulate berry and juice composition and thereby influence wine style.

Acknowledgements

Financial contributions are gratefully acknowledged from Wine Industry Network for Expertise and Technology (Winetech), the National Research Foundation (NRF), and the Department of Science and Technology (DST), as well as Lallemand. Thank you to Zelmari Coetzee, Katja Suklje, Piet Loubser, Javier Quemada Tellez, and Carlos Suarez for their collaboration on the Lalvigne Aroma trial, as well as the invitation to compile a presentation for the Les Entretiens Scientifiques Lallemand held in Blenheim, New Zealand (May 5-12, 2018), and to Ann Dumont for arrangements during and after this event.

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CUSTOM NUTRITION FOR SPECIFIC WINE YEASTS TARGET: SAUVIGNON BLANC

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In an increasingly competitive market, optimizing the quality of wines, especially the organoleptic properties, is a major challenge for winemakers. Wine aroma is one of the principal attributes determining wine consumers' preferences.

The importance of nutrients such as nitrogen or lipids to the achievement of alcoholic fermentation is well known in the wine industry. Indeed, to assure a complete fermentation with a regular kinetic, in addition to looking at factors such as cellar hygiene and the choice of suitable yeast strains, winemakers have to ensure that musts have adequate nutritional, physical, and chemical conditions for optimum yeast development.

More recently, lipid content, temperature, as well as nitrogen and other micronutrients have been described to have a great impact on a large number of flavour compounds produced by biosynthesis by wine yeast during alcoholic fermentation.

The interactions between nitrogen and other key nutrients such as lipids and vitamins and their influences on yeast viability and yeast fermentative capacities will be developed in the first part of this article. The second part will aim at describing the importance of nutrients and micronutrients in yeast aroma metabolism as well as yeast's ability to synthesize aromas and especially to reveal varietal aromas in Sauvignon Blanc winemaking.

Nitrogen: Role and importance of nature (quality of nitrogen)

The nitrogen in musts is one of the essential elements that enable yeast to complete fermentation. This key compound involved in yeast growth and yeast metabolism enables biomass synthesis, but also influences the enzymes and membrane transporter activities necessary for yeast functioning. Nitrogen also affects many aspects of yeast metabolism, including the formation of volatile compounds that contribute significantly to the organoleptic qualities of wines. The composition—and quality—of the nitrogen (mineral or organic source) in the must significantly impacts the wine's final aroma profile.

Nitrogen is present in grape must in different forms: ammonium, amino acids, peptides, and proteins. The part of nitrogen that can be used by yeast during alcoholic fermentation is called "assimilable nitrogen."

YAN (for Yeast Assimilable Nitrogen) includes free α-amino acids (AA), ammonium, and some peptides. The ability of *Saccharomyces cerevisiae* to use small peptides has been recently well documented.

YAN concentrations in natural grape musts range from about 60 mg/L to 500 mg/L, depending on grape variety and vintage (usually, 1/3 of the nitrogen is found in ammonium form and 2/3 in amino acids) (Bely et al., 1990).

In nitrogen deficiency conditions (YAN < 150 mg/L for sugar concentration around 200 g/L - 220 g/L) yeast growth and fermentation speeds are limited. A low initial YAN concentration leads to slow fermentations, which is why adding nitrogen to the must has become a common and necessary practice during alcoholic fermentation.

Nitrogen from external sources added to the must to address a YAN deficiency comes in two forms: inorganic (ammonium salts [DAP, DAS]) and organic (proteins, peptides, tripeptides, and free amino-acids issued from yeast [inactivated yeast and yeast autolysate]).

A wide variety of studies have shown that the best time to add nitrogen is at 1/3 of fermentation (Bely M., Sablayrolles J, Barre P., 1990). This is when the yeast population has reached its maximum—i.e., when the must has reached full nitrogen depletion (all nitrogen in the must has been consumed by yeast for the multiplication phase and building biomass)—and is at its most beneficial for fermentation rate and kinetics. A single addition of nitrogen at the beginning of fermentation is not recommended as it leads to a very high yeast population, a sudden increase in fermentation speed accompanied by an exothermic reaction (heat production), and high nitrogen depletion. This quickly leaves the yeasts without any nitrogen left to convert sugar to ethanol. Sluggish or stuck fermentations can occur with a single addition of DAP (30 g/L, equivalent to 63 mg/L of YAN) at the onset of fermentation. To recover and increase a fermentation rate, it is essential to add nitrogen at 1/3 of fermentation (entry into the stationary phase). Otherwise, the fermentation rate will decrease drastically, fermentation will become slow, and, in the event of other deficiencies (e.g., sterols), the risk of stuck and sluggish fermentation is high (Sablayrolles, 1996).

When organic nutrition is used at the beginning of fermentation and at 1/3 of fermentation for better efficiency, the use of nitrogen is slower and more controlled. Consequently, fermentation is more regular—with no heat peaks and better temperature control—and the AF proceeds to completion as seen in Figure 1.

More and more studies describe the difference in efficiency between organic and inorganic nitrogen additions with respect to kinetics (INRA Pech-rouge trials, 2011, internal data).

Nitrogen and wine aroma profile

The metabolism of nitrogen, notably from amino acids, generates aroma compounds involved in the aroma matrix of wine: higher alcohols and their acetates. Yeast metabolism also influences the appearance or preservation of certain aroma precursors of an amino nature (cysteinylated precursors or glutathionylated precursors of varietal thiols). As a result, the nitrogen composition of the must can modulate the wine's aroma profile. The use of organic nutrients has also been shown to positively influence the formation of aroma compounds when used during alcoholic fermentation.

The metabolism of amino acids (anabolism and catabolism) by yeast leads to the formation of higher alcohols, esters acetate, and ethyl esters.

One study focused on modulating the profile of esters by adding nitrogen (AWRI-Lallemand project, 2012). It looked at DAP additions versus organic nutrient additions

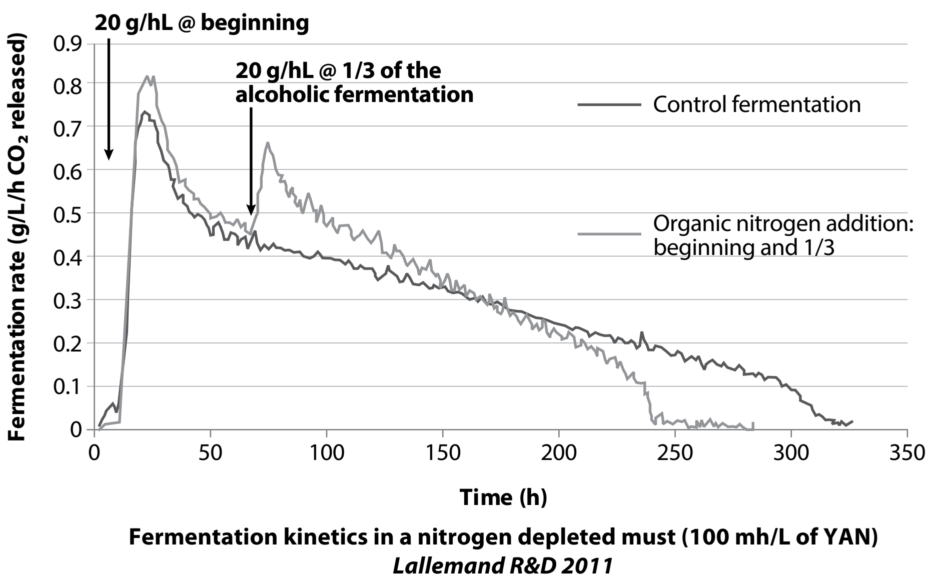

Figure 1. Impact of organic nutrition addition on alcoholic fermentation. 20 g/hL (8 mg/L of YAN) of organic nitrogen added at the beginning of fermentation, and another 20 g/hL (8 mg/L of YAN) at 1/3 of AF.

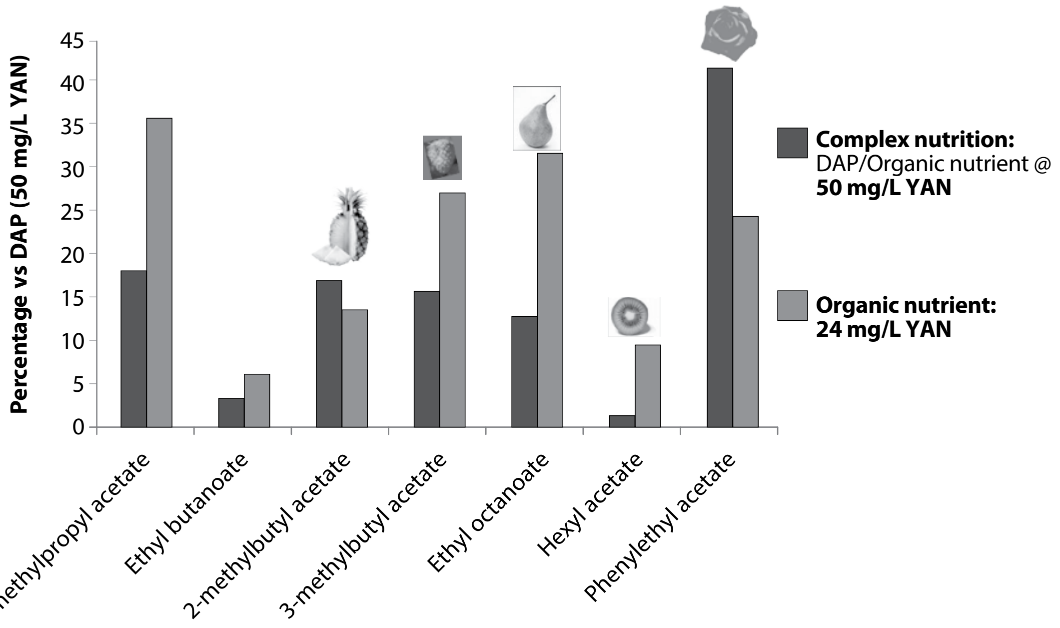

Figure 2. Chardonnay from the Yalumba winery fermented with two different sources of nitrogen: DAP at 50 mg/L of YAN (blue bars) and organic nutrient at 24 mg/L of YAN (green bars).

in Chardonnay grapes. Some of the results are reported below (Figure 2), comparing the synthesis of ester compounds with an addition of 50 mg/L of YAN under DAP form versus 24 mg/L of YAN under organic nutrient form. A significant increase in all aromatic compounds was observed with the organic nutrient, underscoring the greater efficiency of organic nitrogen vs. inorganic nitrogen on the formation of esters.

The organic nutrient's positive influence on the wine's sensory profile was also demonstrated in various other studies (data not shown).

Nitrogen-lipid interactions

Nitrogen management during alcoholic fermentation is very important but some recent studies have underlined the greater impact that nutrient interactions and nutritional imbalances have on the progress of fermentation and on yeast metabolism. Recently it has been demonstrated (Blondin and Tesnières., 2013, PlosOne: 8, e1645) that an imbalance between lipids and nitrogen leads to cell death during fermentation. This increased mortality could explain some occurences of sluggish or stuck fermentation mainly when nitrogen is added as a single nutrient in high amounts, and when there is a deficiency in another specific nutrient, such as lipids (Figure 3).

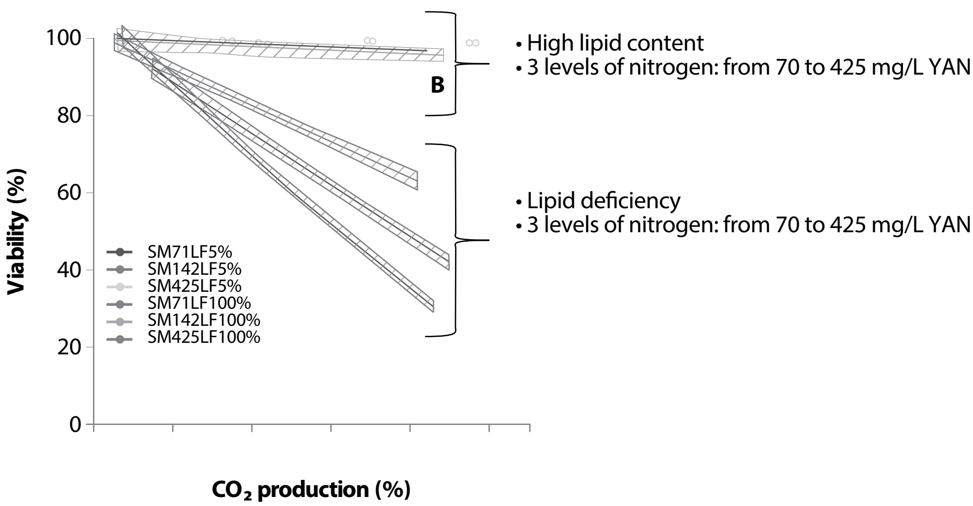

Figure 3. Yeast viability during alcoholic fermentation based on nitrogen and lipid levels in synthetic grape must.

In the event of a lipid deficiency, regardless of the initial assimilable nitrogen level, there is a quick decrease in viability during the stationary phase. This is not the case when the level of lipids is sufficient, even in cases where nitrogen is limited (YAN equivalent to 70 mg/L). The mortality rate is modulated by nitrogen concentration: the highest nitrogen with low lipids, the highest mortality. This was further investigated through a thesis written in partnership with the INRA. The objectives were to better understand the effect of nutritional disequilibrium on yeast cell death.

Nutritional imbalances

Nutrients available for yeasts in grape musts are known to have a strong impact on the kinetics of alcoholic fermentation. Depending on the availability of nutrients, yeast will be more or less active (with variable fermentation rates) and may or may not be able to withstand the stress of alcoholic fermentation (ethanol, low pH, etc.). A key feature of wine alcoholic fermentation is that yeasts face these stresses in non-growing, starvation conditions. Under various circumstances, nutrient disequilibrium can lead to significant yeast cell death, which can result in sluggish or stuck fermentations. It has been shown that cell death in wine alcoholic fermentation occurring in lipid-limited fermentations was modulated by the availability of nitrogen, and that nitrogen signaling is involved in the triggering of cell death. Under these conditions, the yeast reacts with a stress response system to preserve its viability throughout alcoholic fermentation. On the other hand, lipid deficiency does not cause yeast to respond adequately to stress, which leads to yeast cell death and thus the cessation of fermentation. This cell death is modulated by the level of nitrogen.

In cases of low lipid content and high nitrogen content, yeast mortality during alcoholic fermentation is increased and can occur in the first stage of fermentation (Tesnières et al., 2013). Studies were conducted to identify other nutritional imbalances leading to yeast cell death. In cases where there is a nutritional imbalance, such as a high nitrogen content and a deficiency of oleic acid, or pantothenate or nicotinic acid, a high rate of mortality has been observed (Figure 4). This new data demonstrates the importance of a good nutritional balance (minerals, vitamins, sterols, organic nitrogen) not only to preserve yeast viability and prevent the cessation of fermentation, but also to limit the risk of developing unpleasant organoleptic characteristics and deviations such as the production of H_2S. Several researchers have shown that an imbalance between a high level of assimilable nitrogen and a low pantothenic acid content in the must leads to a significant production of sulfur compounds such as H_2S (Wang 2003, Blondin 2016).

To conclude, yeast cell death in alcoholic fermentation is triggered by starvation for a set of micronutrients (including oleic acid, ergosterol, pantothenic acid, and nicotinic

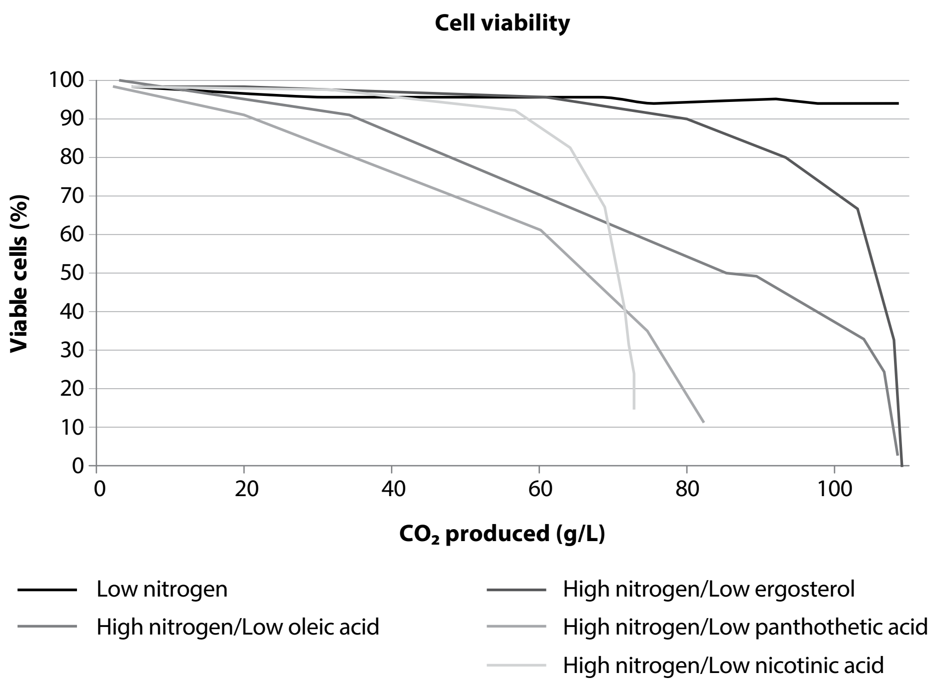

FIGURE 4. Nutritional imbalances leading to yeast cell death.

acid) whenever the nitrogen level is high, but not in low nitrogen conditions. Yeast mortality is controlled by the availability of residual nitrogen and involves the nitrogen signaling pathways that control the triggering of an appropriate stress response. Some of these micronutrient limitations can occur in winemaking situations depending on the practices used. For example, grape must clarification aimed at removing solid particles can deplete musts of lipids, especially sterol, and unsaturated fatty acids that are critical nutrients for yeast in alcoholic fermentation. The impact of the grape must's nitrogen content therefore needs to be considered for potential interaction with micronutrient limitations in managing wine alcoholic fermentation. Since nitrogen supplementation—which is usually performed with ammonium salt but can also involve more complex organic sources—is a common practice in enology, a better understanding of the effect of supplementation with different nitrogen sources and of the interactions with micronutrient limitations is required for enhanced nitrogen management.

Conclusion

Supplementation of grape musts with nitrogen sources is a common practice used to compensate for deficiencies in assimilable nitrogen. Today, the potential interactions between nitrogen and other micronutrients are not fully known. Here we examined the impact of nitrogen supplementation in light of previous results demonstrating that nitrogen sources could trigger yeast cell death when combined with some micronutrient limitations.

To conclude, yeast nutrition management is more complex than simply adding a single dose of inorganic nitrogen, which has been common practice for years. Many works have demonstrated the importance of an adapted, well-balanced approach to nutrition that takes into account the grapes' initial nutrient composition, the yeast strain selected, the temperature, and the desired wine aroma profile.

A reasoned and well-balanced nutritional approach that takes into account all nutrients and micronutrients is key to yeast activity and metabolism. What about the yeast's nutritional impact on wine aroma profile?

In the last 10 years, our knowledge and understanding of the role yeast nutrition plays on the production of volatile aromas has improved significantly. These volatile aromas have different primary origins:

1. Grape-derived (include volatile thiols, norisoprenoids, etc.)
2. Alcoholic and malolactic fermentation (include higher alcohols, esters, etc.)
3. Aging

Numerous studies have demonstrated the influence of conditions such as temperature. Nutrients (such as nitrogen) and lipid content of grape musts also have an influence on the yeast's ability to synthesize fermentative aromas (Rollero et al, 2016; Mouret et al.).

Further investigations addressed the impact of yeast nutrition on the revelation of varietal aromas in grapes varieties such as Sauvignon Blanc.

Sauvignon Blanc is grown all over the world, and its characteristic aroma typicity is becoming more and more popular, especially among the New Zealand wines.

Grapefruit, passion fruit, and boxwood are some of the most common and appreciated aroma descriptors for this type of wine, whereas the original must is practically neutral in these aromas. These volatile aromas are well known and correspond to 3 main volatile thiols: 3-mercaptohexanol, 3-mercaptohexylacetate, and 4-mercapto-4-methylpentan-2-one.

During the fermentation stage of winemaking, the yeast releases the varietal thiols from odourless precursors initially present in the must (Tominaga et al., 1998c; Tominaga & Dubourdieu, 2000). These precursors were described as S-cysteine conjugates: S-4-(4-methylpentan-2-one)-L-cysteine (Cys-4MMP), and S-3-(hexan-1-ol)-L-cysteine (Cys-3MH) (Tominaga et al., 1995). Therefore, the final thiol levels in wine depend on the pool of corresponding precursors available in the must, and on the fermentation process through which the transformation of the initial aromatic potential is more or less profitable. Within this process, yeast plays a key role on the release of volatile thiols from grape precursors. The mechanism involves 2 steps: First the uptake of the cysteinylated and glutathionylated precursors into the cell thanks to specific transporters (about 5 different ones have been recently described, with the most common being GAP1, OPT1, PTR2). Second, once the precursors are in the cell, the clivage reaction occurs thanks to the activity of the yeast C-S lyase, releasing the 4-MMP and 3-MH from their corresponding cysteine precursors, mediated by *IRC7gene*. (Roncoroni et al., 2011; Holt et al., 2011; Cordente et al., 2015).

The release of volatile thiols is dependent on yeast species and yeast strain (cf Figures 5 and 6). Belda et al, and Zott et al. showed how beta-lyase activity was widespread be-

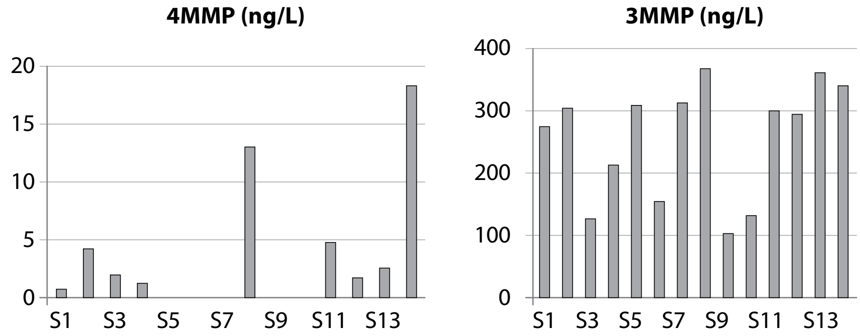

Figure 5 and 6. The release of volatile thiols is dependent on yeast species and yeast strain.

tween yeast species, with *Kluyveromyces* or *Torulaspora* having the highest beta-lyase activity and Metschnikowia the best ability to release 3-MH.

The release of volatile thiols is also influenced by environmental factors such as the nutrient and micronutrient content of grapes.

In a thesis submitted in 2012 by M. Subileau (France), research was undertaken to investigate parameters that influenced thiol release by *Saccharomyces cerevisiae*, from a controlled synthetic must to the complexities of Sauvignon Blanc juice. Figure 7 describes the quantification of the effect of 5 fermentation parameters (oxygen, sugar, ammonium, vitamins, and sterol) on 3MH production by a wine yeast at 22ºC, following a fractional factorial design. Addition of more ergosterols led to a 15% increase in 3MH, whereas excess ammonia resulted in a 68% decrease in 3MH. O2 sparging and high sugar concentration had a negative effect with a 30% reduction, and vitamins addition increased 3MH release by 30%.

Other studies looked at Cys-4MMP and Cys-3MH uptake into the cell and its influence on the possible limitation of thiol release (Subileau 2012), as well as the role of the general amino acid permease (Gap1p), which transports all amino acids and whose activity is repressed by nitrogen catabolite repression (NRC).

In winemaking, addition of DAP in the grape must and during alcoholic fermentation to limit the risk of stuck or sluggish fermentation is/was a common practice.

Depending on the amount added, this ammonium supplementation can extend the nitrogen catabolic repression (NRC) (Beltran et al., 2005). At the beginning of wine fermentation *GAP1* expression is repressed by the presence of ammonium ions in the medium.

Thus DAP addition can increase GAP1 down regulation and limit the uptake of cysteinylated precursor through GAP1p, leading to a reduced release of volatile thiols.

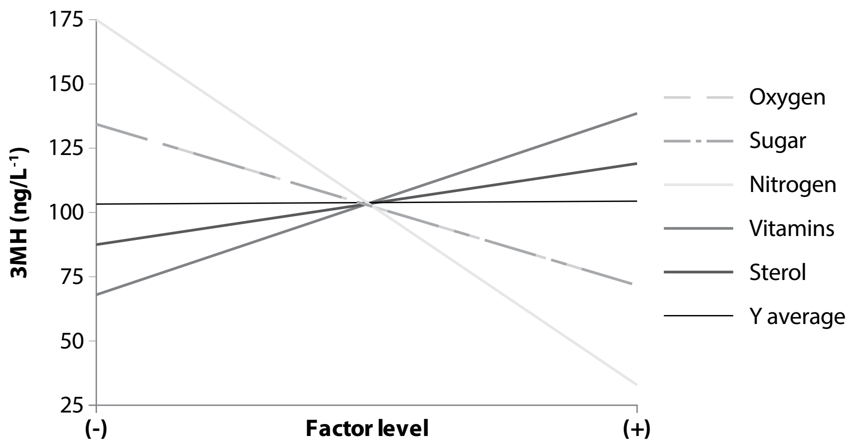

Figure 7. Nutritional imbalances leading to yeast cell death.

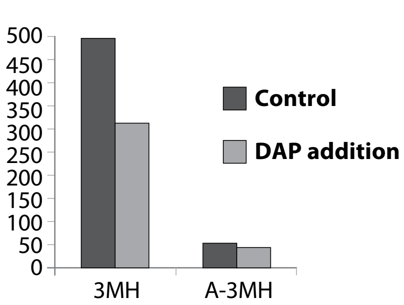

Figure 8. Sauvignon Blanc (Languedoc) Production of 3-MH (ng/L).

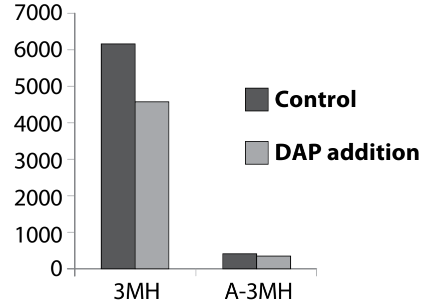

Figure 9. Sauvignon Blanc (Gers) Production of 3-MH (ng/L).

As shown in figures 8 and 9, addition of DAP decreased mainly 3-MH production in the final Sauvingnon Blanc wines from two different French wine areas.

During alcoholic fermentation, volatile thiols are produced during the yeast growth phase: cell multiplication reaches its maximum with a strong uptake of the nitrogen sources, including amino acids, allowing the highest uptake of cys-3MH and cys-4MMP. Meanwhile, yeasts exhibit a high enzymatic activity (beta-lyase activity).

As described in figures 10 and 11, cysteinylated precursor consumption occurred mainly during the exponential growth phase. 70% of Cys-4MMP was consumed within the first 48 hours. Cys-3MH was also rapidly consumed (within 2 days). At the same time, the bulk of 4MMP and 3MH was also produced early in the fermentation (within 2 days for 4MMP and 3 to 4 days for 3MH).

Our recent research took a more indepth look at the impact of yeast nutrition and micronutrition management on a wine's sensory profile. The target was to optimize and reveal the aromatic potential of the grapes through well-balanced nutrition based on the matrix, wine yeast, and timing of nutrient addition.

Previous research has demonstrated that the type of nutrition used impacts the formation of varietal thiols. Based on this knowledge, specific nutrients have been developed to enhance yeast capacity to uptake aroma precursors from grapes and to optimize their bioconversion in volatile varietal aromas. When added at the beginning of fermentation, these nutrients efficiently stimulate the enzymatic activity of the yeast, increasing the revelation of varietal aromas such as volatile thiols.

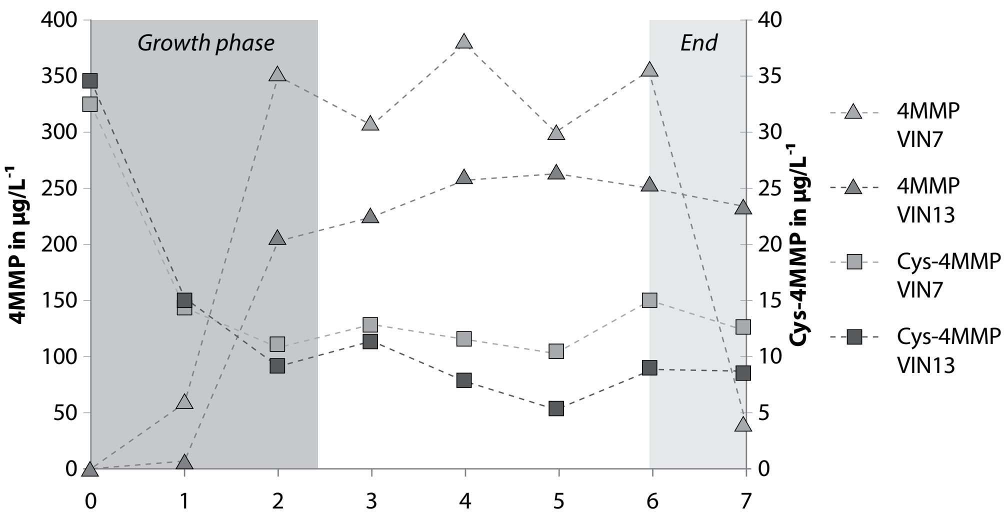

Figure10. Consumption of Cys-4MMP and production of 4MMP by strains VIN 7 and VIN 13.

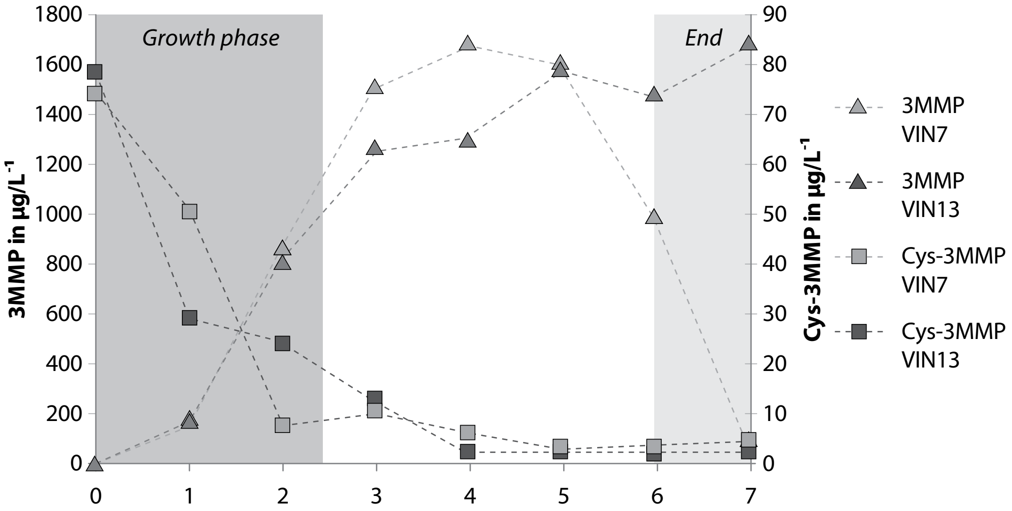

Figure 11. Consumption of Cys-3MMP and production of 3MMP by strains VIN 7 and VIN 13.

Each yeast is different in its capacity to convert glutathionylated and cysteinylated precursors into thiols, and each wine yeast has different nutritional demands for its metabolism. Based on this, our studies on the impact of nutritional stimulation on thiol bioconversion resulted in the development of an optimized nutrient: Stimula Sauvignon blanc™. It stimulates and enhances the bioconversion of grape precursors into varietal thiols. The ideal timing for the addition was determined based on yeast metabolism: when the yeast actively consumes nitrogen, multiplies, and reaches its peak enzymatic activity (during the growth phase), bioconversion of thiol precursors into varietal thiols is at its maximum. This precise timing corresponds to the beginning of fermentation.

Figure 12 examines two different wine strains and decribes the impact on the 3-MH level at the end of the growth phase when Stimula SVG B is added at the early stage of fermentation. The uptake of 3-MH precursors is not impacted by the stimula supplementation; however, the conversion into volatile thiols is significantly improved compared to the control fermentation where nothing was added.

Several trials were conducted to evaluate the impact of Stimula SVGB on the production of 3 volatile thiols for

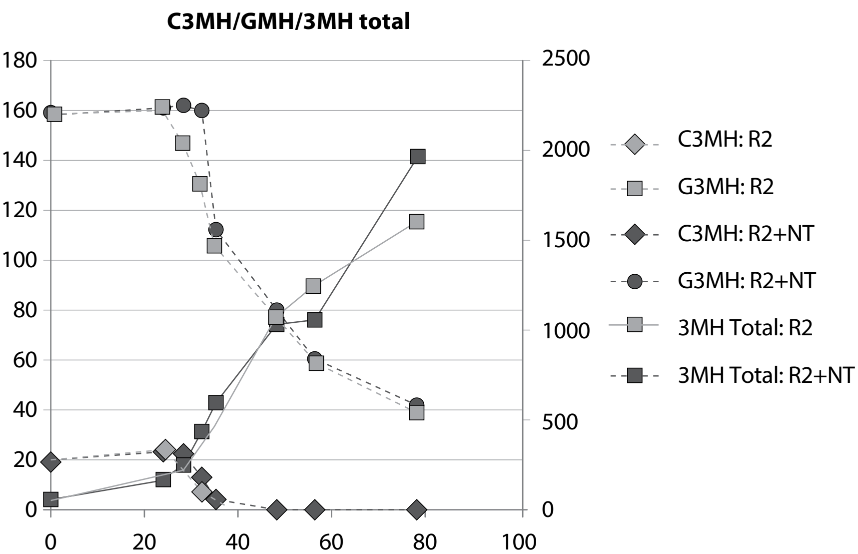

Figure 12. Impact on the 3-MH level at the end of the growth phase when Stimula SVG B is added at the early stage of fermentation in two wine strains.

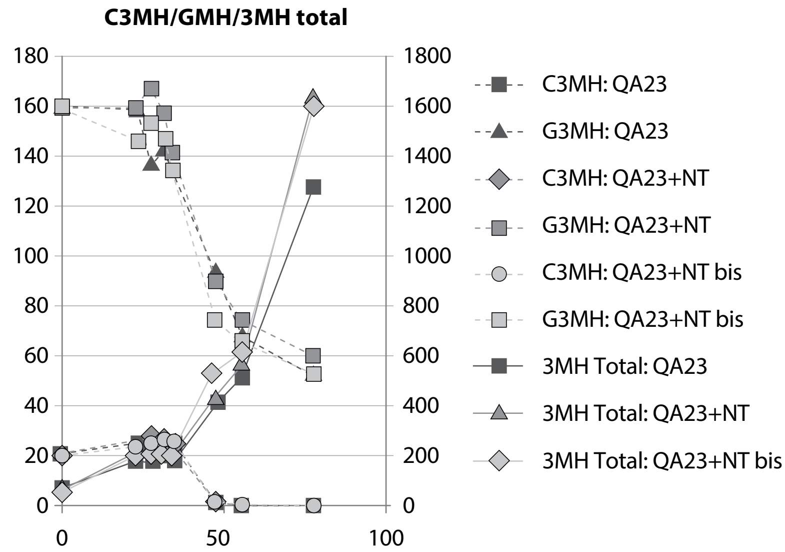

Figure 13. Impact of Stimula SVGB on the production of three volatile thiols for different wine yeasts.

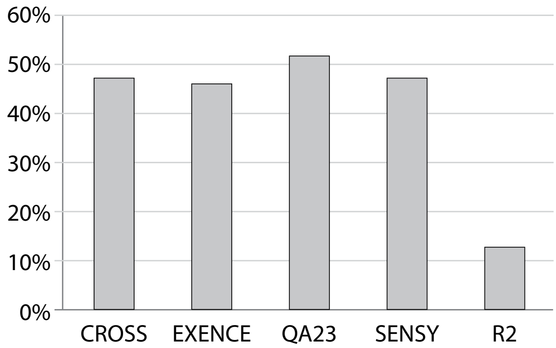

Figure 14. 4MMP increase with Stimula SVG addition.

different wine yeasts. For example, in the 4MMP synthesis evaluation in Figure 14, we can observe a strong increase of 4MMP production with the addition of Stimula SVG, reaching 50% for some yeast strains.

During the 2017 southern hemisphere harvest, trials in New Zealand wineries were carried out to compare Stimula SVG addition with the winery protocol for thiol production (control fermentation).

In both trials, analytical results exhibited a tremendous increase in 4MMP with the Stimula SVG, as shown in Figure 15.

At another winery, the use of Stimula at the beginning of fermentation increased 3-MH by 35%. (Table 1)

Yeast and yeast nutrition management play an essential role in the release of varietal thiols.

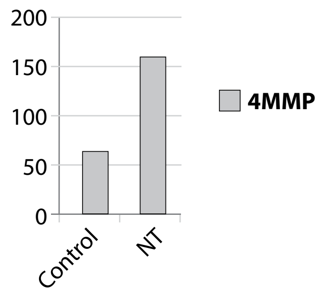

Figure 15. New Zealand wineries trials comparing Stimula SVG to control fermentation.

Understanding of the nutritional mechanisms that influence yeast cell viability, vitality, metabolism, and aroma biosynthesis has increased dramatically in the past 5 years, as has fundamental knowledge of the chemistry and biochemistry of varietal thiols and wine aromas in general. These studies have made it possible to develop new microbiotools for winemakers with the same goal always in mind: to reveal the aromatic potential of the grapes and achieve a successful fermentation resulting in a unique aromatic wine profile.

Table 1. 35% increase of 3-MH with the use of Stimula SVG at the beginning of fermentation in New Zealand winery.

	Control	NT
3-MH (ng/L)	8742	11836
3-MHA (ng/L)	1539	1671
3-MMP (ng/L)	ND	ND

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MARLBOROUGH SAUVIGNON BLANC – DIVERSITY THROUGH TERROIR – VILLA MARIA'S EXPERIENCES WITH SAUVIGNON BLANC SUBREGIONALITY

Kathrin Jankowiec

Villa Maria Estate, Marlborough, New Zealand

Marlborough – no other wine region made such an entry in to the wine world when it first appeared on the wine map. New Zealand, and especially Marlborough, shot to fame basically overnight by producing outstanding Sauvignon Blanc. The unique and fruit-forward style had many critics in awe, yet at the same time the distinctive style, with its passionfruit and herbal aromas, has drawn criticism for being a "one-trick pony."

Showcasing the different terroirs – or the sense of place – has always been important to Villa Maria. We have long been producing Single Vineyard wines to display the region's diversity to change and challenge the perception of what a Marlborough Sauvignon should taste like.

Marlborough Sauvignon is a very recent phenomenon; it was only about 40 years ago that the rapid rise of Sauvignon Blanc started with the first plantings. When the first vines were established, no one could have predicted the varietal's success and the astonishing pace at which the industry would develop. Between 2000 and 2008 the industry grew by over 500%, with new areas and vineyards developing rapidly as demand exceeded supply. Whilst new sites developed, Marlborough's winemakers and grape growers quickly learned with each vintage about the impact of terroir, climate, new vineyard techniques, and styles.

But what makes Marlborough so exceptional for growing grapes? The answer lies in an amalgam of factors.

Marlborough sits on the northeastern coast of the South Island of New Zealand, surrounded by the inland Kaikoura Ranges to the south and the Richmond Ranges to the north. These rugged mountains, reaching 3,000 m in elevation are responsible for New Zealand's driest and sunniest climate. Blenheim boasts about 2,400 sunshine hours annually with relatively high levels of UV light. A marked diurnal temperature variation of at least 10 degree Celsius is a crucial climatic influence that ensures the retention of acid and flavour in the grapes.

With its latitude of 41.3°, Marlborough is aligned with Europe's wine growing regions, but its climate differs from those regions as its proximity to the ocean and prevailing winds create a temperate maritime climate with cool summers and mild winters.

In geological terms, New Zealand is a very young country as it was the last landmass to appear from the sea. Marlborough was shaped and eroded by glaciers and rivers as recently as 14,000 years ago, and a major fault line moves right through the region and divides its geological features. This results in a patchwork of soil types, mostly dominated by free-draining alluvial soils.

The discovery of certain distinct differences in soil composition, climate, geography, and rainfall led us to define three major subregions: the Wairau Valley, the Southern Valleys, and the Awatere Valley.

Each area has emerged with its own individual style of wine, shaped and influenced by the varied growing conditions.

Wairau Valley

The Wairau Valley was the first region and the main focus of the first plantings in the 1970s.

The valley stretches from the ocean at Cloudy Bay just south of the Richmond Ranges along the Wairau River inland to the west.

Within Marlborough we consider the Wairau Valley the warmest and wettest of the three subregions.

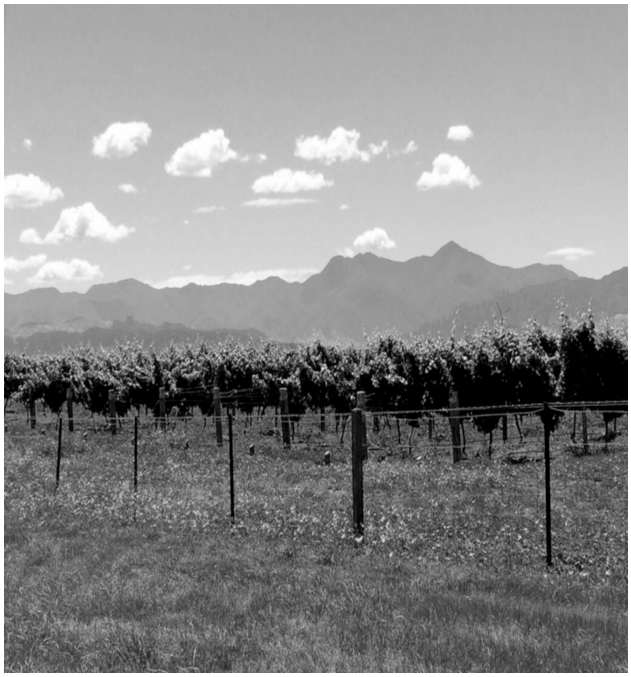
The soils and mesoclimate of the Wairau Valley are diverse. Old, gravelly riverbed soils dominate, with alluvial deposits and big river stones. Generally the soils are free-draining and infertile. The area covers a range of cool, dry inland sites and coastal regions moderated by sea breezes. The style of Sauvignon from the Wairau is ripe and tropical, with a pungent style of passionfruit and guava notes that leap out of the glass. Villa Maria identified many remarkable sites within the valley that led us at the winery to divide the region further into smaller subregions when assessing our wines.

One example is the area around Dillons Point where a very distinctive style of Sauvignon is grown. Villa Maria has sourced some fruit in the past from this region, which has contributed to a portion of the Wairau Reserve Sauvignon.

Dillons Point in the lower Wairau Valley is closely located to the ocean and hence influenced by cooling sea breezes. The area boasts a particularly unique soil as a former swamp land that has been drained. Soils are made up of peat, peat gravels, and silt. The land is rich in nutrients as it contains a high amount of organic matter. The water table is elevated here, and grapevines have ample access to water and nutrients. This synergy leads to vigorous growth in the vineyard. The big, lush canopies and the high fruitfulness of the vines have to be balanced in order to achieve a parallel development of physiological ripeness and flavour precursors. If these challenges are well mitigated, this subregion rewards with a distinct and pungent flavour profile. The wines display high concentrations of thiols; tropical notes of passionfruit and grapefruit are accompanied by a flinty mineral aroma that seduces the senses.

Moving inland towards the mid Wairau Valley is where most of the early plantings originated. The Rapaura region or so called "Golden Mile" is a very diverse area with a huge variability in soil types. Largely made up of alluvial deposits and stony riverbeds, the free-draining soils are much less fertile compared to the lower Wairau. Many vineyards here sit on buried or visible river terraces and river stones can be found under the vines to reflect heat back into the fruit zone. The climate is also warmer as the cooling influence from the ocean recedes.

Flavours of citrus and grapefruit attribute to the style of Sauvignon found here.



The Northbank subregion is situated just south of the Richmond ranges, which protect the valley from weather events. Villa Maria has seen some great success with Sauvignon Blanc from this region and has started to invest further with significant plantings in the last couple of years. The Richmond ranges influence the climate considerably; cloud cover is more common and consequently the region experiences colder, wetter weather. The tight soils are dominated by loam, clay, and alluvial deposits. Due to the climatic influences the growing season is more compressed and ripening is slightly delayed compared to that of the mid Wairau. Sauvignon from here exhibits very attractive boxwood thiols and greener herbaceous notes.

Southern Valleys

South of the Wairau Valley tucked in the foothills of the Wither Hills Range are the Southern Valleys, the second subregion within Marlborough. The side valleys of Ben Morven, Brancott, Omaka, Fairhall, and Waihopai spread like fingers from the hills, and plantings expand from the valley floor off the fringes of the Wairau Valley up into the rolling hillsides. The valleys provide scope to position vineyards on perfectly aligned slopes with northerly aspects. Cold air flow from the downward stream off the Wither hills creates a colder, much drier climate than that found in the middle of the Wairau Valley.

The valleys have been influenced by glacial outwash, but the river system has less of an influence here. Heavy soils predominate including silts, peat, clay, permeable enriched clay or compact clay subsoils, providing a premium growing area not only for Sauvignon Blanc but also for Pinot Noir and Chardonnay. The soil's high clay content supplies plenty of nutrients and water to the vine and, as a result, produces bigger berries and bunches with a high concentration of flavour. The dry and cold climate presents some challenges for grape growing; drought and frost danger has to be mitigated through careful irrigation and frost protection programs. Villa Maria has successfully produced a Single Vineyard Sauvignon Blanc from this area since 2007. The tight soils and microclimate of the Ben Morven Valley deliver Sauvignon Blanc with pungent aromatics of gooseberry, cut grass, and tropical stone fruits, as well as a very distinct texture. The mouthfeel is generous, weighty, and voluptuous.

The Waihopai Valley is located on the western end and differs from the rest of the Southern Valleys in climate and elevation. The Waihopai is similar to the Southern Valleys in terms of soil type, yet more gravel appears along the Waihopai River. As the land steadily gains altitude towards the hills to the south, the Waihopai climate gets colder and drier. As a result, we see a compressed growing season and later ripening but also thicker skins and great flavour accumulation. Wines from here are weighty with a more pronounced herbaceous note.

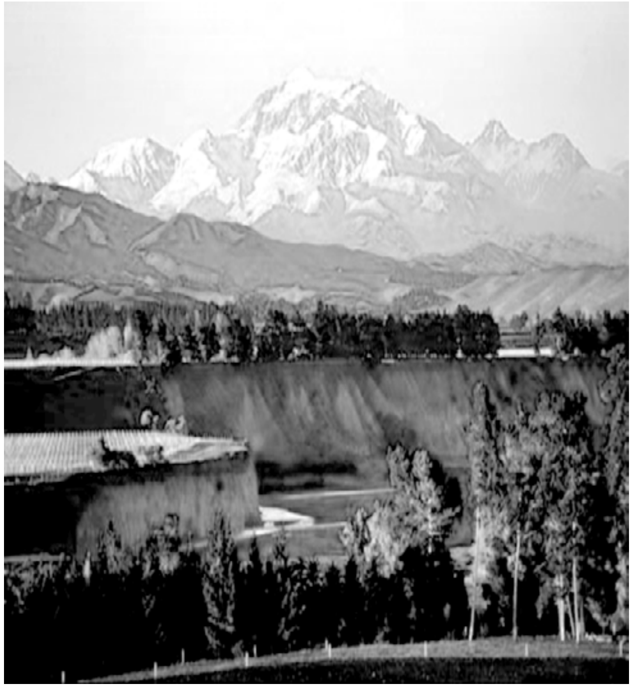
Awatere Valley

About a 30-minute drive south of Blenheim Township is the third major subregion of Marlborough. The Awatere Valley was the latest subregion to be developed and extensive plantings have taken place in recent years. Villa Maria had the foresight to recognise its potential early and invest in the subregion, which now contributes to about 50% of our Sauvignon Blanc plantings across Marlborough.

The Awatere Valley is the coldest and driest of the three viticulture areas and also the most geographically varied. Lying south of the Wairau Valley, the vast subregion stretches inland from high country sites to exposed vineyards close to the sea. Villa Maria vineyards range from sites at sea level to vineyards at 340 m in elevation, and we showcase several Single Vineyard and Reserve wines from these distinct areas. Awatere soils range from alluvial gravel and silt loams to wind-blown loess with a diverse composition of stones and a wider range of parent material, including volcanic rock. Over the years, the Awatere River has carved cliffs into the riverbank, exposing defined soil horizons and providing sheltered terraces with a unique microclimate. Cold prevailing easterly sea breezes as well as cold air flow from snow-covered mountains influence the climate here. Crop tends to ripen later in this harsh environment, creating a longer and slower growing and ripening season.

The coastal Awatere is mostly influenced by ocean breezes, and soils here are predominantly alluvial but the subsoil is made up of marine sediment. The impervious papa subsoil restricts topsoil depth and fertility so berries tend to be small and retain crisp acidity. Vineyard irrigation is fed from the Awatere River, which carries papa outwash with it. The water in this river is slightly alkaline. Coastal Awatere Sauvignon Blanc is in the pungent green spectrum. Crisp, focussed, and mineral wines with herbaceous flavours and a lip smacking salinity define this area.

Moving inland to the central and upper Awatere, the region gets more diverse in soil type. The higher altitude and colder climate along with poorer stony soils restrict yield levels naturally. Berries and vines are small; the yield per hectare is significantly lower compared to the fertile ground in the Wairau. Ripening is delayed by about two to three weeks, and thick skins provide ample flavour. Sauvignon Blanc from this area is herbaceous in character, with tomato leaf, nettle, lime, and lemongrass flavour with tangy acidity and a distinct minerality.



Marlborough's rich and diverse natural environment is reflected in the wide ranging styles of Sauvignon Blanc shaped by soil and climate. Through the introduction of single site Sauvignon Blanc, consumers are invited to experience the region's terroir and its multifaceted nuances. Marlborough is a well known and recognized "trademark" that is now synonymous with NZ Sauvignon Blanc, yet the journey through Marlborough offers up a cornucopia of Sauvignon Blanc expressions. Sauvignon Blanc has a bright future as we continue to explore, nurture, and experiment.

SHAPING SAUVIGNON BLANC – FROM A VINEYARD SITE SELECTION AND WINEMAKING PERSPECTIVE

Heather Stewart

Saint Clair Family Estate, Marlborough, New Zealand

The project related to consumer preference of Sauvignon Blanc with different winemaking protocols to optimize specific sites was of interest to Saint Clair as the UK is one of the most important markets. And although as wine producers and winemakers we need to be true to ourselves, our preferences, and our own signature style, at the end of the day we are making wine for the market, so it is important to know what the people want in order to be successful and profitable.

Saint Clair's signature style with our Sauvignon Blanc is a very expressive, thiol-driven, rich tropical salty wine, and in our winemaking this is what we endeavour to bring out in almost all our parcels of Sauvignon Blanc. And this may be to the detriment of vineyards with really green or mineral or citrus notes, which are really important blending components to add complexity, but don't usually end up in our top wines. This project allowed us to investigate how to enhance the inherent characters of these greener or more mineral blocks to increase their expressiveness, quality, and performance.

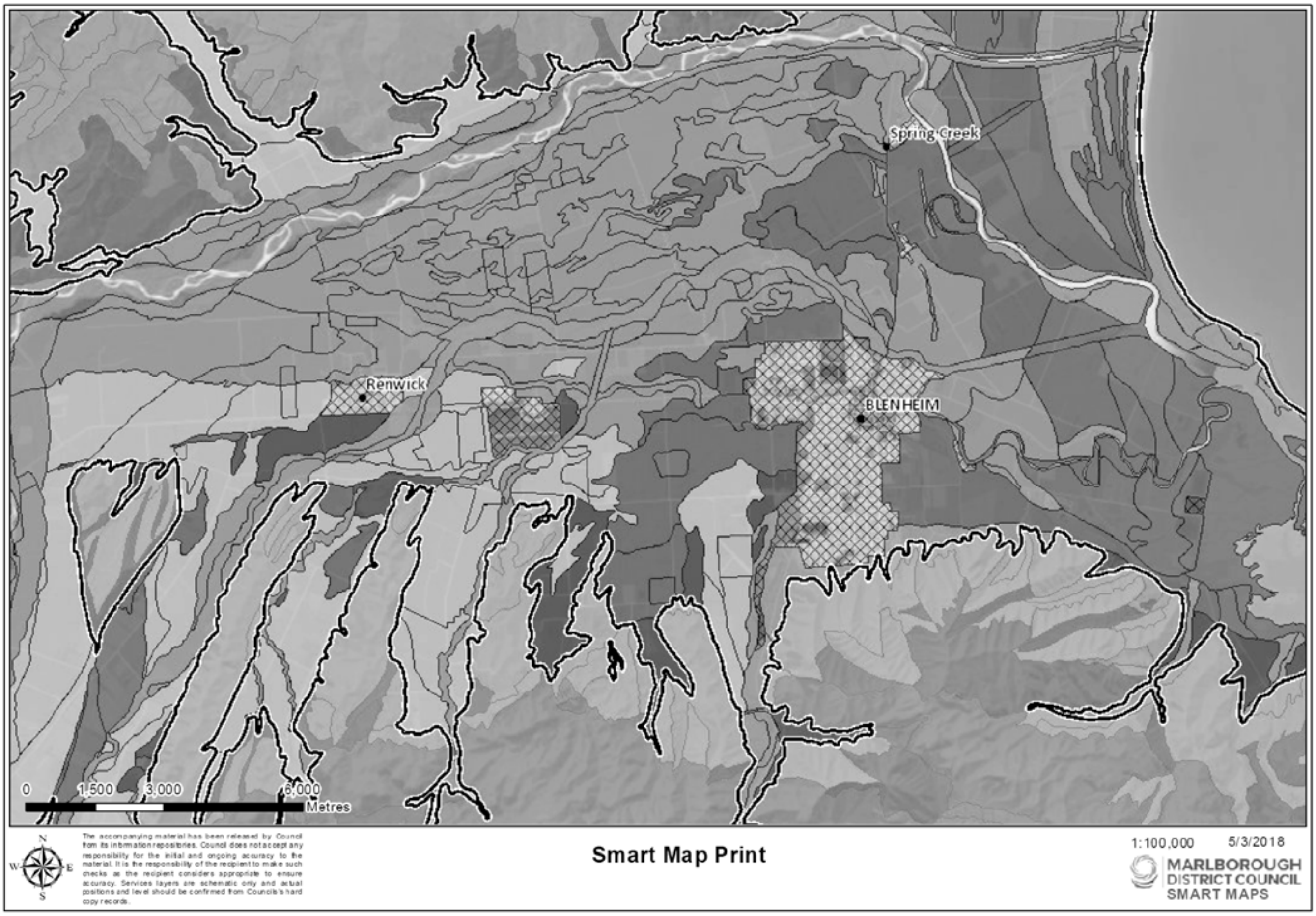
Our aim was to develop four specific styles of Sauvignon Blanc using site selection and winemaking techniques, with a particular focus on the use of biotechnology.

Marlborough Soil Types

Our Sauvignon Blanc is planted all over the region. The different subregions of Marlborough have quite different soil types and microclimates, so our vineyards produce very different flavour profiles. Our preferred subregion for Sauvignon Blanc, where most of the grapes for our top wines come from, is Dillons Point in the Lower Wairau. This region has very fertile flood silt soils, rich in nutrients and trace minerals. These soils produce really high quality Sauvignon Blanc and this is where three of the four wines for this project were grown. Even though the vineyards are from the same subregion and in very close proximity to each other, they produce quite different flavours. This is in part because of the braided river system which has changed path over time, depositing stony, sandy loam over the gravels, so that even within one row in a vineyard the soil can vary. The 'mineral/citrus' wine was grown much further up the Wairau Valley on the Waihopi River on much less fertile, stony soils.

Sauvignon Blanc Production at Saint Clair Family Estate

- Machine harvest to increase thiol precursors
- Ascorbic & PMS to grapes in gondola
- Press grapes and cold settle juice with bentonite and enzyme
- Warm juice to 18°C & inoculate with selected yeast & nutrient
- Ferment at 12-14°C with DAP add as necessary
- Chaptilisation where necessary
- Chill to stop fermentation with 3 g/L RS, then settle yeast
- Rack, sulphur, grade, blend



- Balance acid & RS as required, fine if necessary
- Stabilise, filter, prepare for bottling late in year

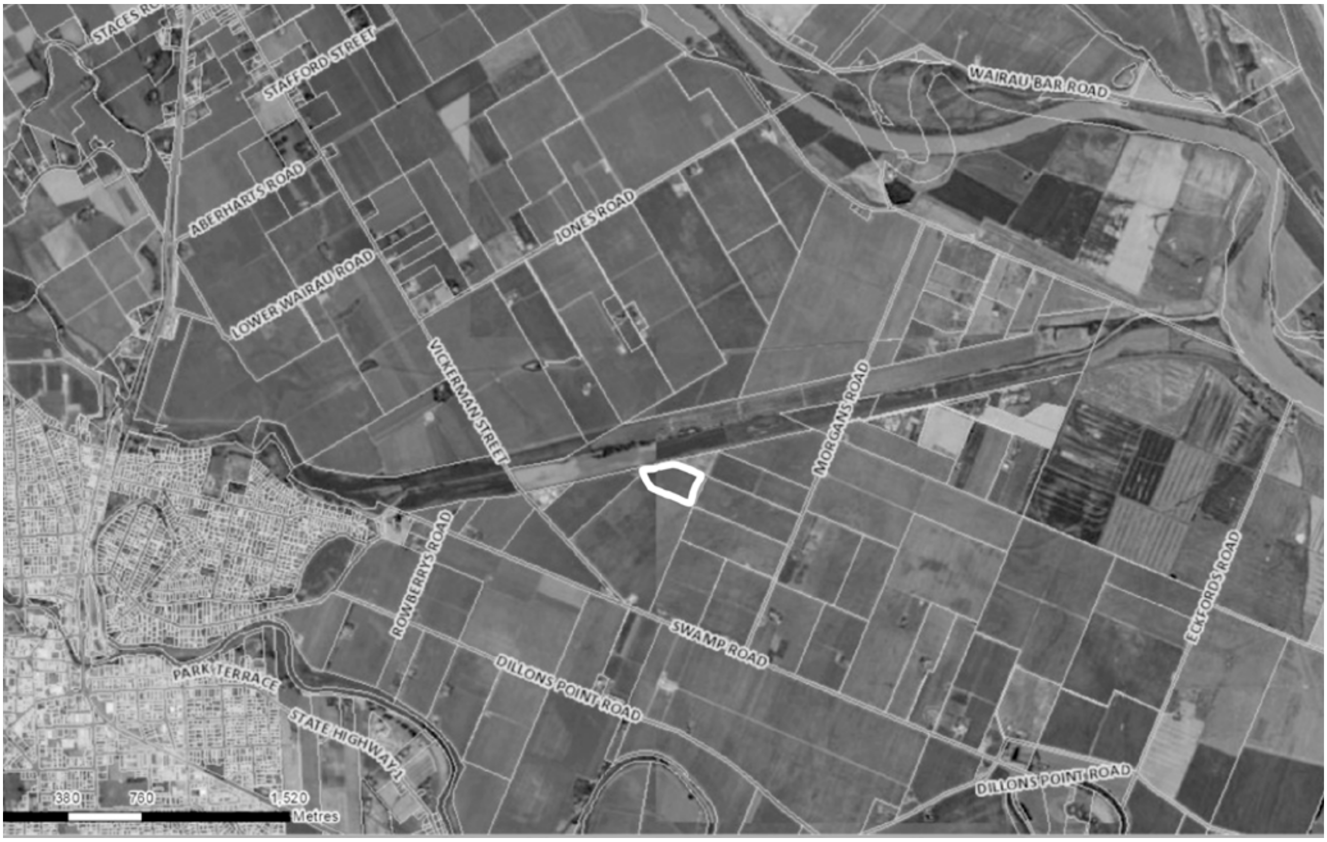
Other than for our barrel-fermented Sauvignons, which are hand harvested, we machine harvest our Sauvignon Blanc, partly for practical reasons but also because research has shown that machine harvesting and de-stemming results in much higher levels of thiols than hand-harvested whole bunch pressed fruit. We add ascorbic acid and PMS (for SO_2) to the grapes in the gondola at harvest to prevent oxidation of flavour compounds. We press immediately, cold settle with the assistance of some enzyme and bentonite, rack clean, then warm and inoculate with selected yeasts. We ferment our Sauvignon Blanc at around 12 and 14 degrees. In 2017 we had a very challenging, wet season, with very low Brix levels, so almost every must needed a sugar add to increase potential alcohol. We usually arrest the fermentation early by chilling to keep a few grams of RS in the wine for balance and palate weight, but in 2017 we tended to let the wine run dry to maximise the alcohol, then added back around 3 g/L RS using grape juice concentrate. Post-fermentation we let the yeast settle out for a couple of weeks with the wine set down cold, before racking off lees and sulphuring. We usually balance, and we also fine as needed. The thiol, green, and mineral wines for this MW project are all made similarly according to this regimen, but using different Lallemand biologicals.

Thiol-Driven Tropical Sauvignon Blanc
Morgans Rd, Dillons Point, Lower Wairau

This area is very low lying, just above sea level. Very close to the ocean (~3-4 km), so it has a strong maritime climate with cooling sea breezes and a large diurnal temperature change. Ripening is very slow, and this subregion is our latest to be picked. The slow physiological ripening and long hang time allow more time for the thiol precursors to form, as these are formed in the last stages of ripening.

Thiol-Driven Tropical Sauvignon Blanc Viticulture

- Dillons Point, Lower Wairau
- *Microclimate*- Sea level, close to the ocean, slow ripening and long hang time, allowing thiol precursors to form
- *Soils*- Fertile nutrient-rich flood silts. Saline loamy alluvium



Dillons Point, Lower Wairau.

- *Vines*- 4 cane VSP
- *Harvest*-

The fertile flood silt deposits in this area create more vigour and cooler soils, and this also contributes to slow ripening, allowing thiol precursor formation. There is some saline loamy alluvium in this vineyard, and we do get quite a lot of salt character in the wine from this site. Fertile soils in this area allow a bigger crop load, and this also helps slow ripening and increase hang time, so the vines are pruned to 4 canes, with VSP trellising.

Thiol-Driven Tropical Sauvignon Blanc Winemaking

- IOC BE Thiols™ yeast 200ppm – promotes 3MH production
- GoFerm Protect Evolution ™rehydration nutrient 250ppm – aromatic precursor assimilation
- Optimum White™ 200ppm – antioxidant
- Stimula Sauvignon Blanc™ 400ppm – amino nitrogen nutrient optimising volatile thiols
- Fermaid O™ 200ppm – nitrogen-rich inactivated yeast
- Pure-lees Longevity™ – inactivated yeast with high dissolved oxygen uptake

Thiols are formed by yeasts during fermentation from precursors present in the must. So for this thiol-driven wine the choice of yeast is really important. In 2017 we trialled 6 different yeasts in a controlled trial and found that the yeast strain could affect thiol production by up to 28% (for 3MHA).

Table 1. Thiol-Driven Tropical Sauvignon Blanc Viticulture.

Hectares	Planted	Row spacing	Plant spacing	Clone	Rootstock	T/ha	Harvest date
4.28	2003	2.8 m	2.0 m	MS	101-14	18.3	2 Apr.

Harvest

Harvest method	Brix	FSO_2	TSO_2	pH	TA	YAN	Settling
Machine	18.9	8ppm	23ppm	3.15	10.7 g/L	251ppm	40

We chose IOC BE Thiols™ yeast, which promotes 3MH production, bringing out fruity thiol characters without excessive plant-based notes.

We rehydrated with GoFerm Protect Evolution™, for all wines, which provides the yeast with a high amount of sterols, vitamins, and minerals, allowing for better aromatic precursor assimilation, which obviously is very important for Sauvignon Blanc.

Optimum White™ was also added to the juice post racking at 200ppm for all four wines. This is a natural yeast derivative rich in glutathione and polysaccharides that protects against oxidation, preserving the thiols and esters. It also enhances wine complexity and has a smoothing effect to bring more roundness to the wine.

After inoculation we added Stimula Sauvignon Blanc™, which is an amino nitrogen nutrient that optimizes the uptake of 4-MMP and 3-MH precursors and their bioconversion to volatile thiols. We added 400ppm the day after inoculation, as the uptake of thiol precursors occurs in the very early stage of fermentation. We trialled this product pre-release.

For nutrients during all four fermentations we added Fermaid O™, a blend of inactivated yeast fractions rich in organic nitrogen and that doesn't contain added ammonia salts (DAP). We added 200ppm about a quarter of the way through fermentation.

Thiol-Driven Tropical Sauvignon Blanc Fermentation Kinetics

Fermentation was completed to dryness in 10 days. It was really clean throughout, and had really nice struck match characters.

We did a "control" to this wine for our own interest, using our usual yeast, Yeast X. Fermentation was 4 days shorter with Lallemand's products and acidity was lower, but most significantly, the 3MHA and 3MH in particular were much higher on the Lallemand wine.

Thiol-Driven Tropical Sauvignon Blanc Control Wine

***Control* - Yeast X - Nutrient X**
***Lallemand* – IOC BE Thiol™ & GoFerm Protect™ & Stimula Sauvignon Blanc™**

The trial doesn't control for each additive, but from the 6-way yeast trial we did on that same vintage, we know that yeast X and IOC BE Thiols™ produce almost exactly the same level of thiols, so we can assume the increase in

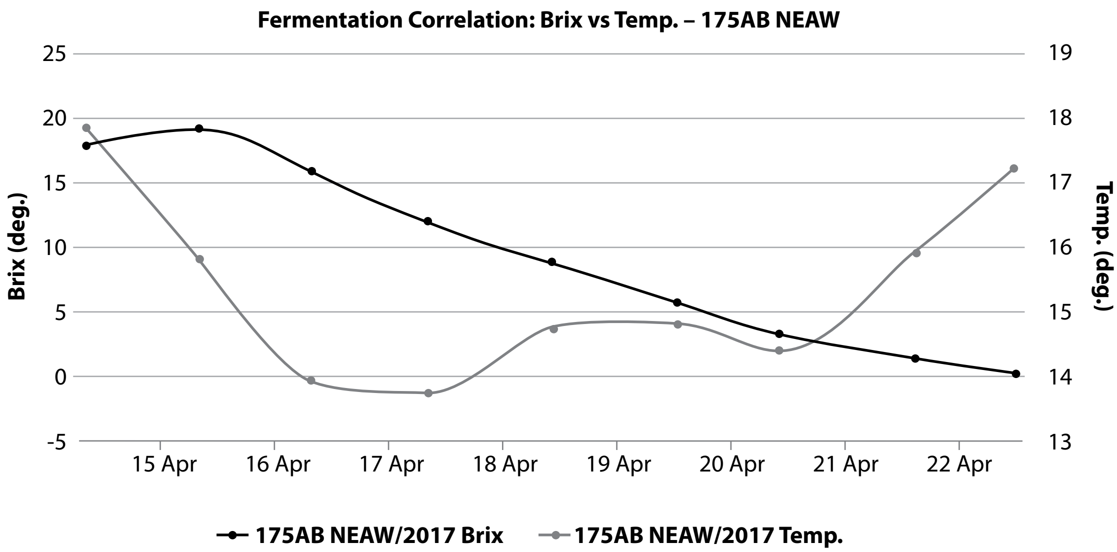


Figure 1. Thiol-Driven Tropical Sauvignon Blanc Fermentation Kinetics.

Table 2. Thiol-Driven Tropical Sauvignon Blanc Control Wine.

	IOC BE Thiol & Stimula	Control Yeast X
3MH Passionfruit grapefruit	12,613 ng/L	9,748 ng/L
3MHA Grapefruit zest, passionfruit, boxwood, herbaceous, sweet sweaty	4,669 ng/L	3,125 ng/L
4MMP Boxtree, broom, blackcurrant bud, cat's pee	17 ng/L	32 ng/L
Methoxypyrazine Green capsicum, grassy, tomato leaf, herbaceous	6.9 ng/L	7.1 ng/L

Table 3. Thiol-Driven Tropical Sauvignon Blanc Finished Wine.

	FSO_2	TSO_2	pH	TA	RS	Alcohol	Score
Post-racking & sulphuring	36ppm	118ppm	3.17	8.6 g/L	0.6 g/L	12.85%	15.5
Pre-bottle	31ppm	121ppm	3.23	7.1 g/L	3.4 g/L	12.85%	

thiols is due to the Stimula Sauvignon blanc. The control wine had more purity but the Lallemand wine had more complexity and was more expressive. Struck match and smoky passionfruit, more tropical aromas, and a wider flavour spectrum. Really good result for a new product, and we've used it again this vintage.

Thiol-Driven Tropical Sauvignon Blanc Finished Wine

- Smoky passion fruit, struck match, tropical, expressive, complex

This shows analyses on the finished wine both at the end of fermentation and at bottling once we had balanced and stabilised the wine.

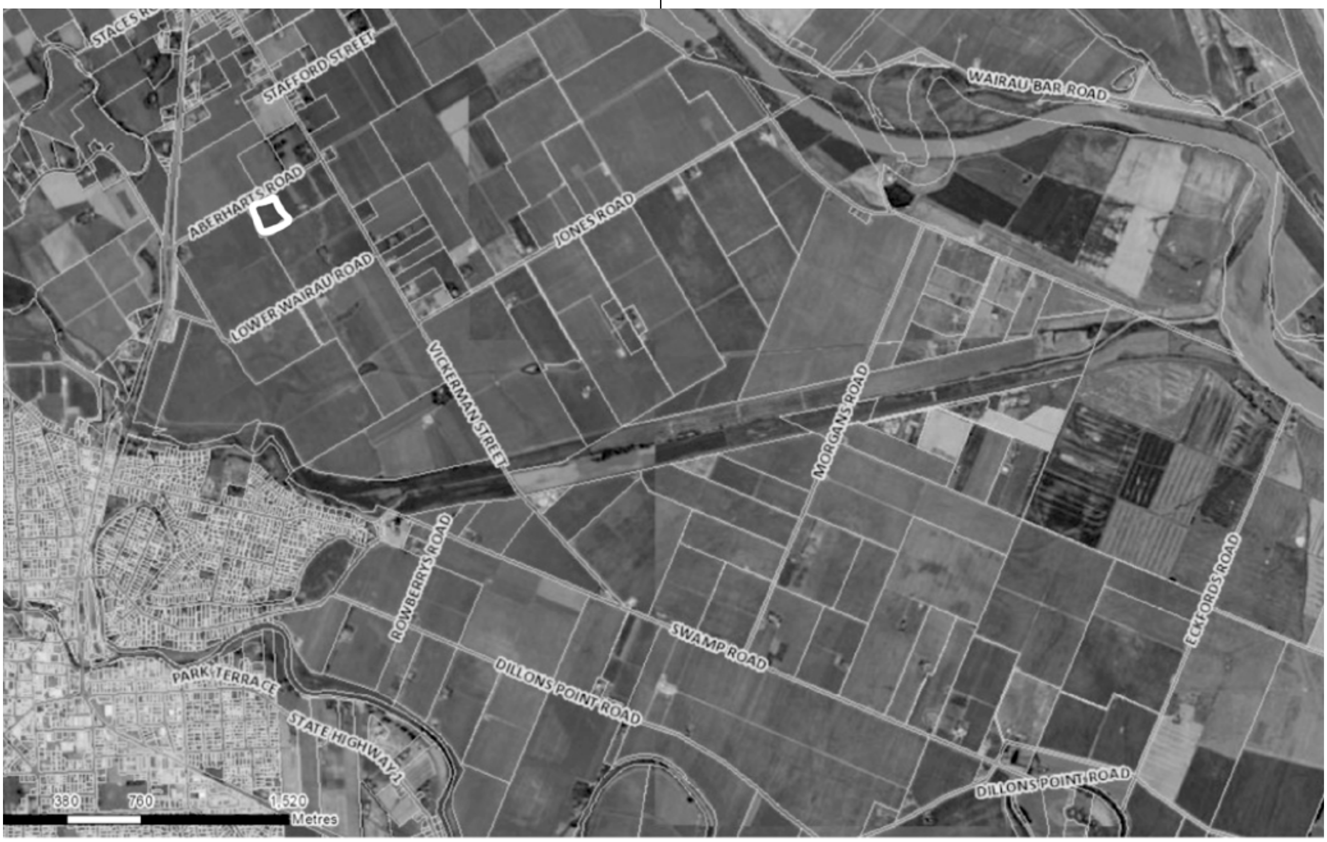
We also added Pure-lees Longevity™ to all wines at racking, except the barrel-fermented Sauvignon Blanc. This is a specific inactivated yeast that has high dissolved oxygen uptake and prevents against oxidation, which would cause us to lose all the aromas we had taken so much care to get into the wine. Ideally we would have used this on each racking or transfer.

Green Herbaceous Sauvignon Blanc
Aberharts Rd, Dillons Point, Lower Wairau

Green Herbaceous Sauvignon Blanc Viticulture

- Dillons Point, Lower Wairau
- *Microclimate*- Sea level, close to the ocean, slow ripening and long hang time.
- *Soils*- Fertile nutrient-rich flood silts. Imperfectly drained loamy alluvium.
- *Vines*- 4 cane VSP. Wide row spacing, tall vigorous canopy leaving more methoxypyrazines in grapes.

Soils- The soils are the same as the previous vineyard, and this vineyard is just as expressive, but has a totally differ-



Dillons Point, Lower Wairau. Very close proximity to the previous vineyard, about 3 km to the NW.

Table 4. Green Herbaceous Sauvignon Blanc Viticulture.

Hectares	Planted	Row spacing	Plant spacing	Clone	T/ha	Harvest date
2.66	2004	3.0 m	2.0 m	MS	13.9	9 Apr.

Harvest

Harvest method	Brix	FSO_2	TSO_2	pH	TA	YAN	Settling
Machine	20.4	6ppm	14ppm	3.17	10.7 g/L	300ppm	30

ent spectrum. It consistently produces green Sauvignon Blanc, almost to the point of gherkin or jalapeno pepper, and is a really good blender but usually too green to be a stand-alone wine.

The microclimate and soils don't really explain this greenness, to us anyway, so all I can put it down to is the vines. This vineyard has wider row spacing and a very tall canopy, over 2 m high, which creates more shading. It is exceptionally vigorous, with more canopy through veraison, which protects the grapes from UV, and may create more methoxypyrazines in the grape, which give those green, grassy, herbaceous characters.

This vineyard also produces thiols, but methoxypyrazines are definitely the dominant flavour compounds. Methoxypyrazines, by the way, come from the grapes, unlike thiols, which are formed from precursors during fermentation.

You can see this had almost 2 Brix higher than the previous vineyard, so the greenness is not simply due to ripeness.

Green Herbaceous Sauvignon Blanc Winemaking

- Lalvin R2™ yeast 200ppm – *Saccharomyces cerevisiae bayanus* promotes terpenes, higher alcohols and esters
- GoFerm Protect Evolution™ rehydration nutrient 250ppm
- Optimum White™ 200ppm
- Fermaid O™ 200ppm
- Pure-lees Longevity™ 200ppm
- Green capsicum, cucumber, citrus, tomato leaf, herbal notes

We chose Lalvin R2™ yeast, which is a *Saccharomyces cerevisiae bayanus* hybrid. It was recommended for the herbaceous wine as it is widely and successfully used in France for this style of Sauvignon. Past research projects had also shown it to be good at enhancing other aroma and flavour compounds, including thiols, so I thought it could be good to broaden the spectrum of the wine a bit.

Green Herbaceous Sauvignon Blanc Fermentation Kinetics

R2 can have quite high H_2S production with low nutrient, and this ferment did get a bit of sulphide at around 12 Brix despite a decent YAN, so we added some DAP to finish it off. Fermentation took 9 days.

The finished wine scored 16 in our grading tasting, and our tasting notes included green capsicum, cucumber, citrus, tomato leaf, and herbal notes.

You may note the SO_2 analysis pre-bottle, which is one of the real-world challenges in a commercial winery, where a harvest intern adds 10x the prescribed amount of PMS. And it's always on a trial block or Reserve wine. It did decrease from 90ppm down to 60 by the time it bottled, and none of us could detect the FSO_2.

Table 5. Green Herbaceous Sauvignon Blanc Winemaking.

	FSO_2	TSO_2	pH	TA	RS	Alcohol	Score
Post-racking & sulphuring	38ppm	91ppm	3.25	8.9 g/L	0.6 g/L	13.2%	16
Pre-bottle	59ppm	157ppm	3.42	7.4 g/L	4 g/L	13.2%	

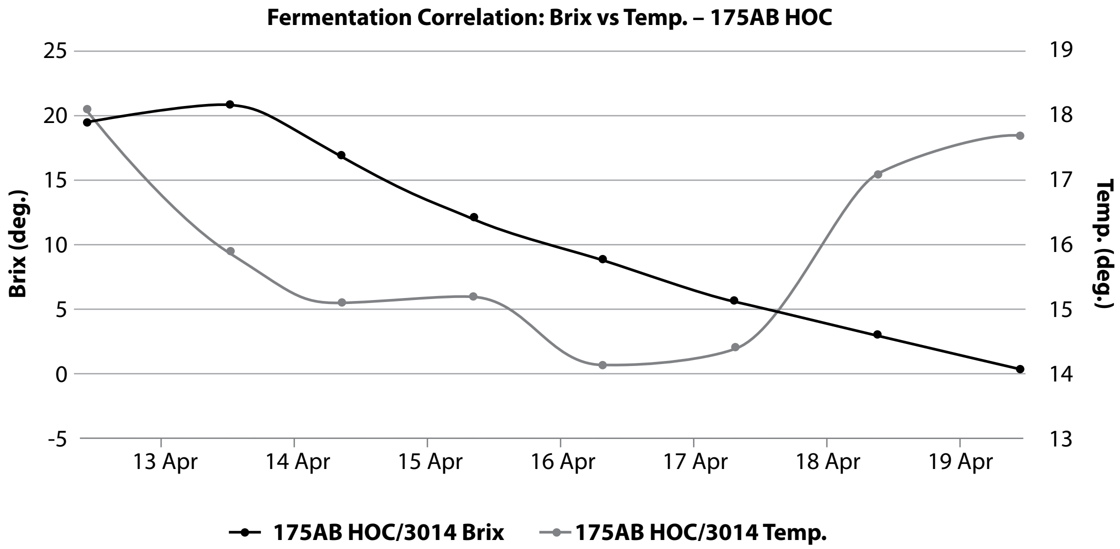


Figure 2. Green Herbaceous Sauvignon Blanc Fermentation Kinetic.

Table 6. Green Herbaceous Sauvignon Blanc Fermentation Kinetic.

Free SO_2	Total SO_2	pH	Titratableacidity	Residual sugar	Alcohol	Score
38ppm	91ppm	3.25	8.9 g/L	0.6 g/L	13.2%	16
59ppm	157ppm	3.42	7.4 g/L	4 g/L	13.2%	

Classic Mineral Citrus Sauvignon Blanc
Guernsey Road, Waihopi Valley

Vineyard - Guernsey Road, at the beginning of the Waihopi Valley, on the first of the river terraces right next to the Waihopi River. A good depiction in this map of the fertility created from the historical path of the braided rivers, which can be seen all over Marlborough.

Table 7. Classic Mineral Citrus Sauvignon Blanc Viticulture.

Hectares	Planted	Row spacing	Plant spacing	Clone	Rootstock	T/ha	Harvest date
8.41	2004	2.5 m	1.8 m	MS	S04 & 3309	16.1	10 Apr.

Harvest

Harvest method	Brix	Free SO_2	Total SO_2	pH	Titratable acidity	YAN	Post settling NTU
Machine	19.5	8ppm	19ppm	3.05	9.8 g/L	134 mg/L	45

Classic Mineral Citrus Sauvignon Blanc Viticulture

Waihopi Valley, on river terrace

- *Microclimate*- Sunny, protected from NW winds, cool nights.
- *Soils*- Very free-draining stony, loamy, sandy alluvium with river stones. Low vigour.
- *Vines*- 3 cane VSP.

Soils – The soils are very free-draining stony, loamy, and sandy alluvium. There is some topsoil with river stones very close to the surface. I don't know if minerality comes directly from the stones but this vineyard often produces wines with river stone characters. The soils don't have the fertility that the other vineyards in the Lower Wairau have, and the flavours are therefore more restrained with less thiols and more citrus. You can see that the YAN result is lower than for the other blocks.

Classic Mineral Citrus Sauvignon Blanc Winemaking

- Cross Evolution™ yeast 200ppm – *Saccharomyces cerevisiae* var. *cerevisiae* for mouthfeel, aromatic intensity, fresh fruit and floral notes
- GoFerm Protect Evolution™ rehydration nutrient 250ppm
- Optimum White™ 200ppm
- Fermaid O™ 200ppm
- Pure-lees Longevity™ 200ppm
- Gravel & river stone notes with citrus & orange pith chalky phenolics

For this wine we used Cross Evolution™, a *Saccharomyces cerevisiae* natural cross hybrid. It increases mouthfeel,

Table 8. Classic Mineral Citrus Sauvignon Blanc Winemaking.

	FSO_2	TSO_2	pH	TA	RS	Alcohol	Score
Post-racking & sulphuring	30ppm	88ppm	3.00	8.4 g/L	1.4 g/L	13.1%	14
Pre-bottle	26ppm	74ppm	3.13	7.1 g/L	3.2 g/L	13.1%	

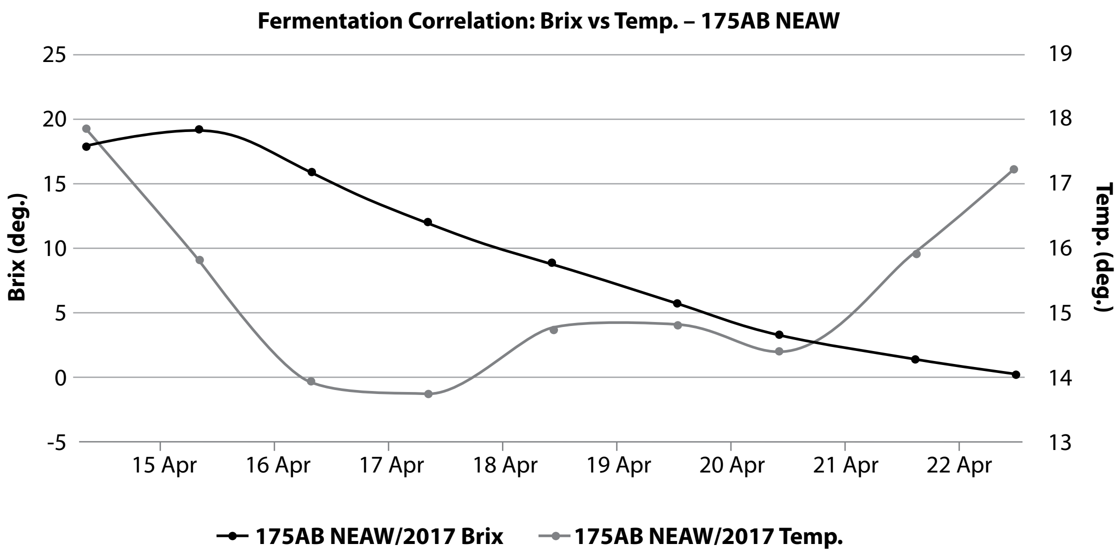


Figure 3. Classic Mineral Citrus Sauvignon Blanc Fermentation Kinetics.

has high aromatic intensity, and enhances fresh fruit and floral characters. Recommended for Viognier, Chenin Blanc, Gewurz, Pinot Gris, etc.

Classic Mineral Citrus Sauvignon Blanc Fermentation Kinetics

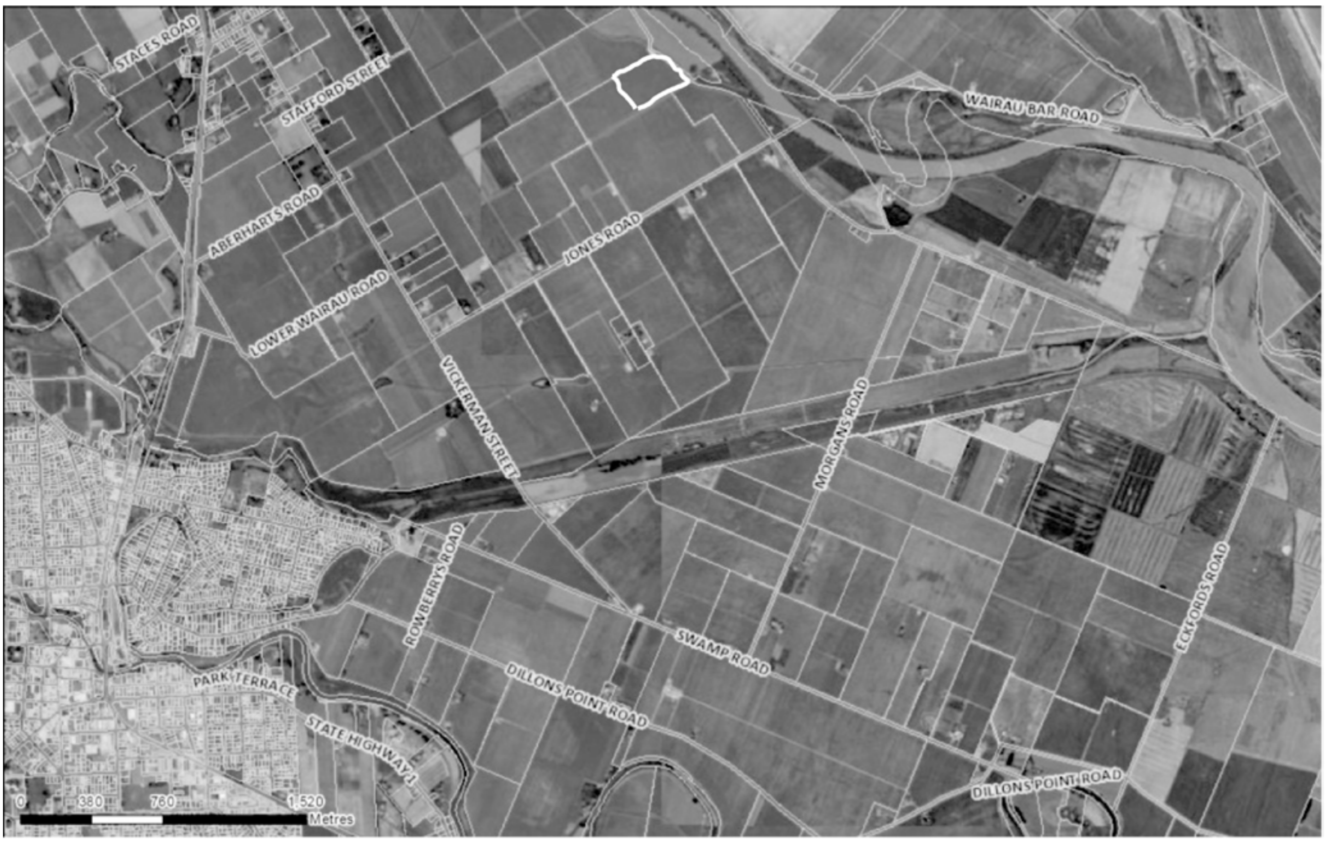
Cross Evolution™ can have a longer lag phase, and we did see a slightly longer lag phase with this ferment, so it took 11 days to ferment to dryness.

Although I love a slightly restrained, elegant, minerally Sauvignon Blanc, at Saint Clair we tend to favour more expressive Sauvignons. It was this wine in particular that I was excited about unleashing the potential of. I don't know that the wine looked better than it had in past years; however, it definitely wasn't the best Sauvignon Blanc season, so that might have worked against us. There were some gravel and river stone notes, with citrus and orange pith and some powdery phenolics/chalky texture. I would really like to do more trials with this yeast and others in a better vintage to realise the full potential of this vineyard.

Barrel-Fermented Sauvignon Blanc
Jones Roadd, Dillons Point, Lower Wairau

We have been making a barrel-fermented Sauvignon Blanc, named Barrique, since 2013 at Saint Clair. It began as a covert little interest project, and it turned out really well, so we bottled and released it, and in subsequent years we have increased the volume and done a lot of trials to learn more about the process and tweak the winemaking and viticulture. We've found that the fruit needs to be expressive and robust with intensity of flavour; however, we don't really like it to be too green or too tropical. A balanced block with thiol, a nice mineral note, laced with a bit of herbaceousness, seems to achieve what we are looking for.

The vineyard that we used for this barrel-fermented Sauvignon Blanc is a site again in the Dillons Point subregion, so it has good expression and intensity, with lots of thiol, but also a nice mineral thread, which I think lends well to this style. This vineyard is right next to the Wairau River, and this may possibly be responsible for some of the mineral notes.



Jones Roadd, Dillons Point, Lower Wairau.

Table 9. Barrel-Fermented Sauvignon Blanc Viticulture.

Hectares	Planted	Row spacing	Plant spacing	Clone	Rootstock	Harvest date
6.52	2006	2.7 m	2.0 m	MS	101-14	20 April

Harvest

Harvest method	Brix	FSO_2	TSO_2	pH	TA	YAN	Settling NTU
Hand pick	19.7	13ppm	23ppm	3.11	10.7 g/L	326ppm	Varied 250

Barrel-Fermented Sauvignon Blanc Viticulture

- Dillons Point, Lower Wairau
- *Microclimate*- Sea level, close to the ocean, slow ripening and long hang time.
- *Soils*- On Wairau River, changing from poorly drained loamy alluvium to well drained loamy & sandy alluvium. High water table.
- *Vines*- 4 cane VSP. Rows are not north-south, with more greenness on south side

Soils - The vineyard has a high water table, which keeps the soil cooler and slows ripening, for good flavour development, but also keeps more green characters.

Vineyard - The rows in this vineyard do not run north-south, but almost at a 45-degree angle, so the south side of the vines gets less sun exposure, which also keeps more herbaceousness in the grapes.

We hung the rows for the barrel-fermented Sauvignon Blanc a week or so longer than the rest of the vineyard to get extra ripeness, and it was our very last fruit to come in for the 2017 vintage. However, due to the nature of the season, the Brix was still just under 20. As we didn't want to overdo the thiols and because we wanted to whole bunch press this fruit, we hand harvested it. In the troublesome 2017 vintage this also allowed us to pick around any disease.

Barrel-Fermented Sauvignon Blanc Winemaking

- Whole bunch press, with press cut at 600 L/T
- PMS & light settling
- Rack, warm, inoculate, chaptilise
- Transfer to 2x seasoned French puncheons, 5x seasoned French barriques
- Lalvin CY3079™ yeast 200ppm – barrel ferment & sur lie, with early yeast autolysis

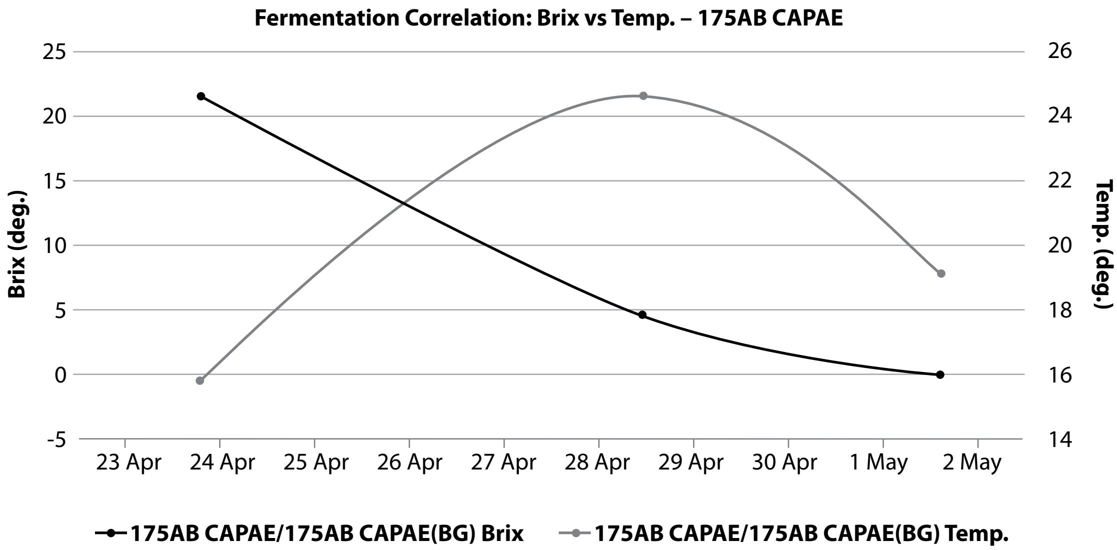


Figure 4. Barrel-Fermented Sauvignon Blanc Fermentation Kinetics.

Table 10. Barrel-Fermented Sauvignon Blanc Fermentation Kinetics.

	FSO2	TSO2	pH	TA	RS	Alcohol
Post-racking & sulphuring	11ppm	89ppm	3.16	9.3 g/L	0.6 g/L	12.8%
Pre-bottle	29ppm	99ppm	3.53	6.2 g/L	1.6 g/L	13.1%

- GoFerm Protect Evolution™ rehydration nutrient 250ppm
- Optimum White™ 200ppm
- Fermaid O™ 200ppm

We press cut at about 600 L/T, and used only the free run juice. We added a bit of PMS to the juice and lightly settled it for about 12 hours before racking, warming, inoculating, and chaptilising in tank, then filled 2 seasoned French oak puncheons and 5 seasoned French oak barriques.

One thing we haven't looked at in our Barrique trials is yeast or other biological additives, other than wild fermentation. The yeast recommended was Lalvin CY3079™, which was developed for white Burgundy and is good for barrel ferment and sur lie aging. It provides rich, full, round mouthfeel and some nice secondary characters due to early autolysis. The early autolysis of CY3079™ slows the end of fermentation, which you can see on the fermentation graph.

Barrel-Fermented Sauvignon Blanc Fermentation Kinetics

We once again added Optimum White™ and Go Ferm Protect™, and a third of the way through fermentation we made a Fermaid O™ add. We didn't really control the fermentation temperature, and it reached almost 25 degrees. The fermentation took 10 days to dryness, which I was happy with. I probably should have put the barrels through MLF, as the acid was quite high, but I wanted to preserve the elegance of the flavours.

The wine stayed in oak until January this year. We lightly fined it with skim milk, which we haven't usually had to do and balanced it, which again we haven't usually had to do. As I just picked the three best barrels it was logistically too hard to cold stabilise. I forgot to add Longevity to this wine.

- Oxidative winemaking led to less classic Marlborough
- Sauvignon Blanc characters.
- Stone fruit, tamarillo, guava, flint, less acid, more palate weight & roundness.
- Lees stirring & oak provides savoury & spice notes.

Where the other three wines were made reductively, this wine is made oxidatively, which gives less overt fruit characters, and less of the classic Sauvignon Blanc flavours, but more stone fruit, tamarillo, guava, flinty notes, less acid, and more palate weight and roundness. The lees stirring gives it nice savoury notes and the oak provides a hint of spice. When we came to put this into oak we were down to our very last barrels, and unfortunately we only had 2016/one-year-old barrels left, so there is more oak influence in this wine than I would have liked. This became especially evident once we had taken the wine out of barrel. Ideally we would have used older oak with no oak character.

The wines we produced are all really expressive and distinct from each other. Stylistic diversity in Sauvignon Blanc is really important and something we should be focussing more on in the future.

CREATING MARLBOROUGH SAUVIGNON BLANC IN DIFFERENT STYLES, AND ASSESSING UK CONSUMER AND JOURNALIST PREFERENCES FOR THESE WINES

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Abstract

The Marlborough wine region is home to a distinct style of Sauvignon Blanc. Within this large region, different vineyard sites make wines with distinctive characteristics. We worked with the Saint Clair winery to create four distinctive styles of Marlborough Sauvignon: tropical, citrus, barrel and herbaceous, by means of different vineyard sources and winemaking protocols, including the use of specific yeasts and microbial-derived products. We then subjected these wines to aroma analysis and sensory analysis to establish their differences. The four wines were used in a consumer preference and description study, where we examined the responses of a set of UK consumers to these wines, and compared them with a group of wine journalists. The consumers and journalists both preferred the aromas of the citrus wine. With all factors considered, the citrus wine was preferred by journalists, while the consumers liked the tropical wine equally. The journalists' least favourite wine was tropical, while the consumers' was the barrel. The consumer group could also be segmented by age, gender and average spend. Aroma analysis showed that it is not just one aroma compound that is responsible for preference. These results suggest that segmenting the market by preference groups and then targeting them with wines made in specific styles could be an effective strategy for wineries.

INTRODUCTION

With each vintage, winemakers face choices on how to handle the grapes coming in. Their goal is to make wine that maximizes the fruit's potential to meet stylistic and quality goals. They want to make wines that fit in with the signature style of the winery, and which also manage to bring out the good personality traits of the vineyards they are working in. Ultimately, they need to make wines that people will want to buy, so consumer perception of these wines is critical. The tools at their disposal include the timing of the harvest, how the fruit is processed, the use of winemaking technology and, perhaps most critically, the microbiological toolkit that they have available.

The first goal of this study was to look at how winemakers can influence the final flavour profile of Marlborough Sauvignon Blanc. It is widely believed that different subregions in Marlborough produce Sauvignon Blanc with differing characteristics. We were interested in seeing how winemaking – and specifically the use of microbial tools – can help reveal these differences in the wine. Working in conjunction with Saint Clair Family Estate, a medium-sized winery in Marlborough with access to a number of vineyard sites, we created four different wines from the 2017 vintage. These were made in commercial quantities to avoid some of the problems of microvinifications. The winemaking protocols were based on Lallemand's knowledge of what their microorganisms or derivatives can accomplish under specific conditions, as well as with the experience of the Saint Clair white winemaker, Heather Stewart, as to the characteristics of wines from the various vineyard sites. The wines were all produced with a different end style in mind: tropical, herbaceous, citrus and barrel fermented.

In the second part of study we used these wines in an attempt to understand the preferences of different UK consumer groups for Marlborough Sauvignon Blanc styles, and compared their preferences to those of a group of wine journalists.

One of the motivations for this work was the declining consumption of alcoholic beverages in the UK. Over the course of the last 12 months total beer, wine and spirits sales have fallen 1.6% (Nielsen). 85% of the spend is from those over the age of 45, and 20% of consumers under 25 identify as teetotal (Kantar Worldpanel). Wine as a total category continues to lose spend to other sectors such as craft beer and gin. If we are able to understand more about consumer preference and how this relates to winemaking protocols, we stand a chance of making wines tailored to these preferences. This can help maximize market share and potentially stem the decline in wine consumption.

Winemaking protocols

The fruit from different vineyard sites was treated differently to produce four distinct styles of Sauvignon Blanc. One of the challenges was the fact that 2017 was a difficult vintage in Marlborough.

Tropical-style Sauvignon Blanc

78.53 tonnes of Sauvignon Blanc were machine harvested on April 2, 2017, from Dillons Point vineyard in the Lower Wairau. Juice yield after pressing was 787 litres per tonne. This is a low-lying area just 3–4 km from the sea, so the climate has a maritime influence, with cooling sea breezes and a large diurnal temperature range. Ripening is slow, and this subregion is the latest to be picked. This is a subregion known for making high-thiol Sauvignons. The fertile soils result in large canopies, and with the slow physiological ripening and long hang time this is thought to result in higher levels of thiol precursors. The fertile flood silt deposits in this area create more vigour and cooler soils. There is some saline loamy alluvium in this vineyard, and wines from this site can have a slight salty character. The high fertility allows for larger crops, which also delays ripening. Vines are pruned to four canes.

Winemaking Protocol:

- Juice was chaptalized at 2% 12 days after harvest.
- IOC BE Thiols™ yeast added at 200 ppm. This helps promote the polyfunctional thiols 3-mercaptohexanol (3MH), which, along with 3-mercaptohexanol acetate (3MHA), contribute passionfruit and grapefruit aromas to the wine.
- GoFerm Protect Evolution™rehydration protector added at 250ppm. This is in order to encourage aromatic precursor assimilation.
- Optimum White™ at 200ppm. This acts as an antioxidant to protect aromatic compounds.
- Stimula Sauvignon Blanc™ at 400ppm. This is an amino-acid-containing nitrogen nutrient optimising the production of volatile thiols.
- Fermaid O™ at 400 mg/L initially and then a third of the way through fermentation at 200 mg/L. This is a nitrogen-rich nutrient.
- Pure-lees Longevity™ was also added at 200 ppm. This is an inactivated yeast with high dissolved oxygen uptake.

Follows the winemakers' description notes:

- Struck match
- Cleaner
- Smoky passionfruit
- Wider flavour spectrum
- More expression on palate
- More expressive on nose
- More tropical
- Clove
- Shorter
- Stone fruit

Table 1. Juice analysis (free run) for the tropical-style Sauvignon Blanc.

Parameters	
Brix	18.9
pH	3.15
TA (tartaric acid equivalent)	10.7 g/L
YAN	251 mg/L

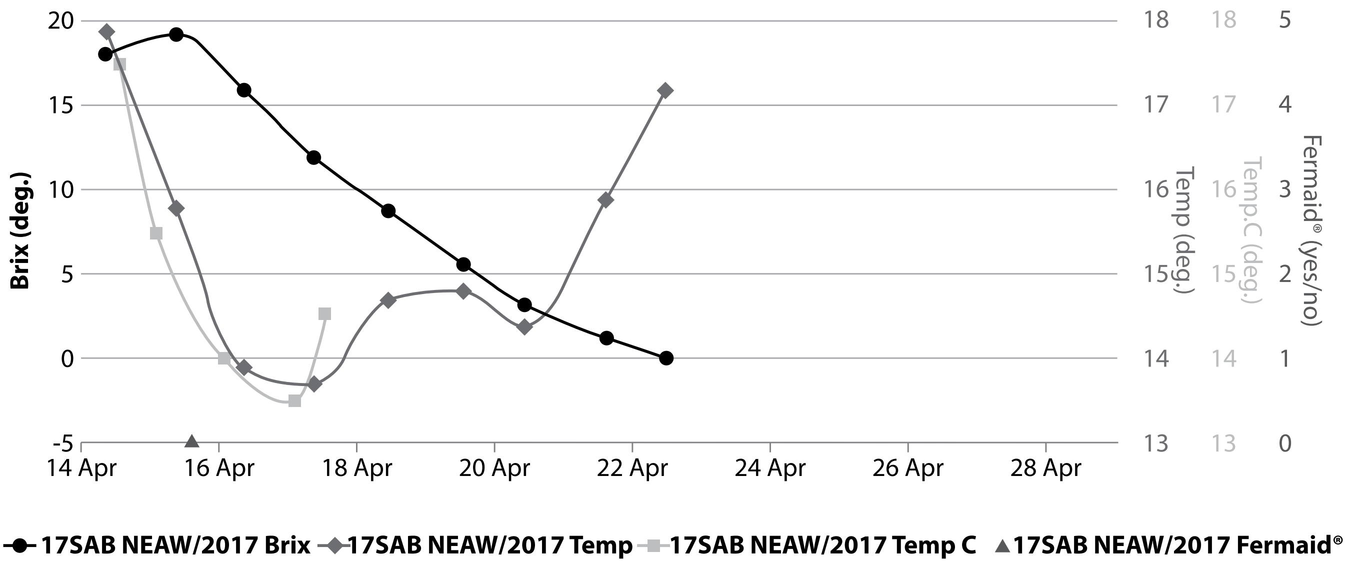

Figure 1. Brix and temperature of the tropical-style Sauvignon Blanc during fermentation.

Table 2. Analysis of the tropical-style Sauvignon Blanc wine.

Parameters	Post-ferment - set zero degrees	Post racked & SO_2
Alcohol %	12.77	12.85
Residual sugar (g/L)	0.7	0.6
pH		3.17
TA (g/L)		8.6
Free SO_2 (mg/L)		36
Total SO_2 (mg/L)		118

Herbaceous-style Sauvignon Blanc

36.85 tonnes of Sauvignon Blanc were harvested on April 9, 2017, from a vineyard in Dillons Point, in the Lower Wairau, with a juice yield of 781 litres per tonne. The vineyard is 3 km northwest of the previous one, so it shares many of the same physical attributes.

The soils are the same as the previous vineyard, but the wines that come from it are usually quite different. It consistently produces a very green expression of Sauvignon Blanc, with characteristics of green pepper and cucumber. It is a really good blending component, but is usually too green to be a stand-alone wine. The microclimate and soils don't really explain this greenness, so it is likely due to viticulture. This vineyard has wider row spacing and a very tall canopy, over 2 metres high, which creates some shading of neighbouring rows. It is an exceptionally vigorous site, with more canopy through veraison, which protects the grapes from light: this may lead to higher levels of methoxypyrazines, which contribute green, grassy, herbaceous characters to the wine.

This vineyard also produces high levels of thiols, but the green-tasting methoxypyrazines are definitely the dominant flavour compounds. It also had higher brix levels at picking so the green flavours aren't simply due to less ripeness.

Winemaking protocol:

- Juice was chaptalized by 3.2% on April 13
- Lalvin R2™ yeast at 200ppm. *Saccharomyces cerevisiae bayanus* promotes terpenes, higher alcohols and esters
- GoFerm Protect Evolution™ at 250 ppm. This is a rehydration protector.
- Optimum White™ at 200ppm
- Fermaid O™ at 200ppm
- Pure-lees Longevity™ at 200ppm

Table 3. Juice analysis for the herbaceous-style Sauvignon Blanc (free run).

Parameters	
Brix	20.4
pH	3.17
TA	10.7 g/L
YAN	300 mg/L

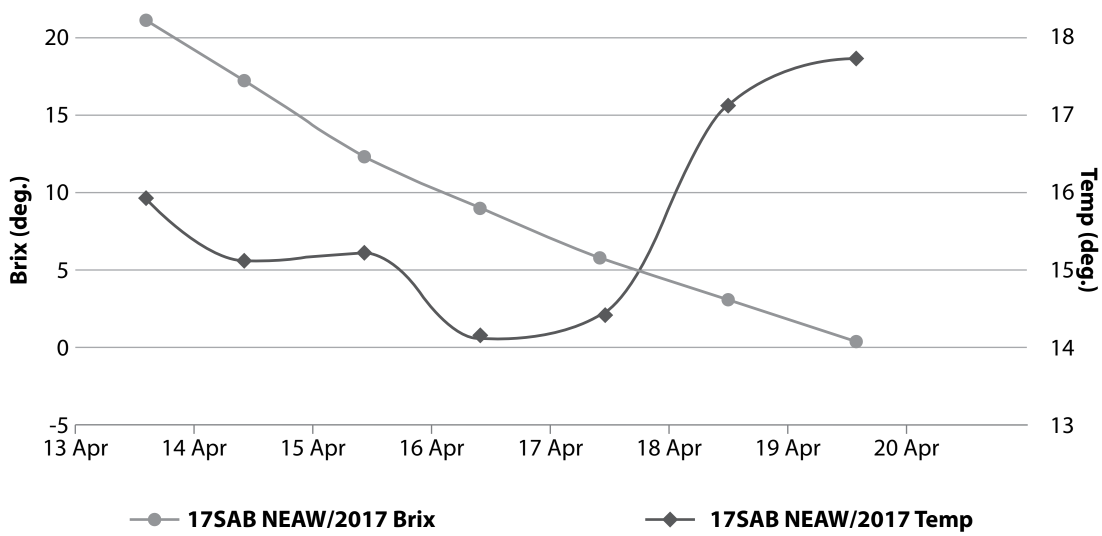

Figure 2. Brix and temperature of the herbaceous-style Sauvignon Blanc during fermentation.

During fermentation, Fermaid O™ at 200 ppm was added at the beginning of fermentation, followed by DAP addition during fermentation at 12.2° Brix. After racking and SO2 addition, Pure-Lees Longevity™ was added at 200 ppm.

Follows the winemakers' description notes:

- Green capsicum
- Cucumber
- Citrus/lime thread
- Herbal note on nose
- Tomato leaf

Table 4. Analysis of the herbaceous-style Sauvignon Blanc wine.

Parameters	Post-ferment - set zero degrees	Post racked & SO_2	Current analysis post $KHCO_3$ & GJC addition
Alcohol %	13.15	13.42	13.24
Residual sugar (g/L)	2.1	0.6	3.1
pH		3.25	3.36
TA (g/L)		8.9	8.6
Free SO_2 (mg/L)		38	90
Total SO_2 (mg/L)		91	157
VA (g/L)			0.38

Table 5. Juice analysis (free run) for the citrus-style Sauvignon Blanc.

Parameters	
Brix	19.5
pH	3.05
TA	9.8 g/L
YAN	134 mg/L

Citrus-style Sauvignon Blanc

135.97 tons of Sauvignon Blanc were harvested on April 10, 2017, from the Guernsey Road Vineyard with a juice extraction rate of 780 tons/litre. This vineyard is at the beginning of the Waihopai Valley, on the first of the river terraces right next to the Waihopai River. It's on a river terrace with free-draining stony, loamy, sandy alluvium with some river stones. The site is sunny and protected from northwest winds, and nights are cool. Three cane pruning, vertical shoot positioning. This site often produces wines that have a 'river stone' character, although this can't be directly from the river stones themselves. It is a low-fertility site and produces wines with lower thiol levels. YANs are relatively low.

Winemaking protocol:

- Chaptalized at 2% on April 14
- Cross Evolution™ yeast at 200 ppm. *Saccharomyces cerevisiae* var. *cerevisiae* for mouthfeel, aromatic intensity, fresh fruit and floral notes
- GoFerm Protect Evolution™ rehydration protector at 250 ppm
- Optimum White™ at 200 ppm
- Fermaid O™ at 200 ppm
- Pure-lees Longevity™ at 200 ppm

The juice was inoculated with Cross Evolution™. The juice was chaptalized at 2% 12 days after harvest on April 14. Fermaid O™ was added at 400 mg/L initially and then a third of the way through fermentation at 200 mg/L.

Follows the winemakers' description notes:

- Gravel
- Dry
- River stone
- Crisp
- Citrus
- Powdery phenolics
- Hint of green
- Orange pith

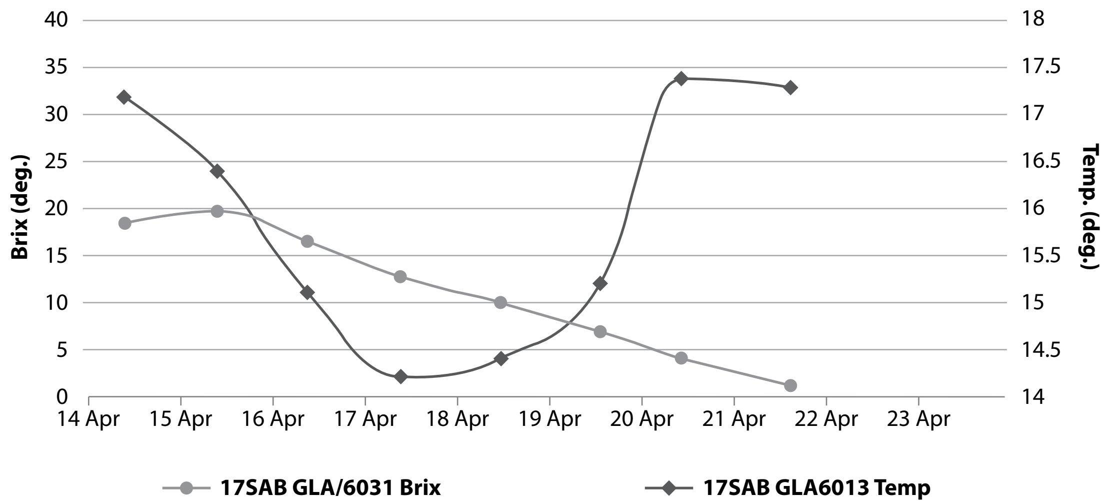

Figure 3. Brix and temperature of the citrus-style Sauvignon Blanc during fermentation.

Table 6. Analysis of the citrus-style Sauvignon Blanc wine.

Parameters	Post-ferment - set zero degrees	Post racked & SO_2	Current analysis post $KHCO_3$ & GJC addition
Alcohol %	12.77	13.17	13.06
Residual sugar (g/L)	6.4	1.4	2
pH		3.0	3.16
TA (g/L)		8.4	7.8
Free SO_2 (mg/L)		30	37
Total SO_2 (mg/L)		88	95
VA (g/L)			0.24

Barrel-fermented Sauvignon Blanc

Saint Clair has made a barrel-fermented Sauvignon Blanc (named Barrique) since 2013. The winery's experience is that ideally the fruit needs to be expressive and robust with intensity of flavour, but it can't be too green or too tropical. A balanced block that produces fruit with some thiol character, mineral notes laced with a hint of herbaceousness that is able to contribute the characteristics necessary to achieve the required style.

The vineyard used is in the Dillons Point subregion, so it has good expression and intensity, with lots of thiol, but also a nice mineral thread, which lends well to this style. This vineyard is right next to the Wairau River, and this may be responsible for some of the mineral notes.

The vineyard has a high water table, which keeps the soil cooler and slows ripening, for good flavour development, but also keeps more green characters. The rows are oriented at a 45-degree angle to the north-south axis, so the south side of the vines gets less sun exposure, which

Table 7. Juice analysis (free run) for the barrel-fermented Sauvignon Blanc.

Parameters	
Brix	19.7
pH	3.11
TA	10.7 g/L
YAN	326 mg/L

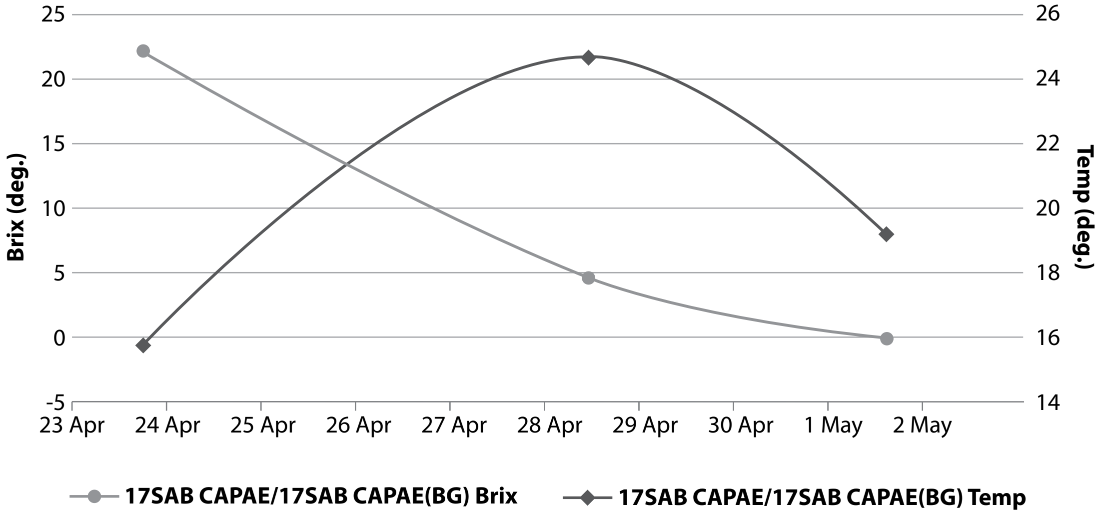

Figure 4. Brix and temperature of the barrel-fermented Sauvignon Blanc during fermentation.

Table 8. Analysis of the barrel-fermented Sauvignon Blanc wine.

Metric	Post-ferment	Current analysis post SO_2
Alcohol %	12.76	12.79
Residual sugar (g/L)	0.6	2.4
pH	3.05	3.16
TA (g/L)	9.2	9.3
Free SO_2 (mg/L)		11
Total SO_2 (mg/L)		121

also keeps more herbaceous character in the grapes. The grapes for this wine were hung a week or so longer than the rest of the vineyard to get extra ripeness, and it was the very last fruit to come in for the 2017 vintage. However, due to the nature of the season, the Brix was still just under 20. The goal was not to overdo the thiols and because we decided to whole bunch press this fruit, the grapes were hand harvested (which also contributes to lower thiol levels). In the troublesome 2017 vintage this also allowed us to pick around any disease.

Winemaking protocol:

- Whole bunch press, with press cut at 600 litres/tonne
- Added some SO_2 and light settling
- Racked, warmed, inoculated, chaptalized
- Transfer to two seasoned French puncheons and five seasoned French barriques
- Lalvin CY3079™ yeast at 200ppm. Barrel ferment and leave on lees, with early yeast autolysis
- GoFerm Protect Evolution™ rehydration protector at 250ppm
- Optimum White™ at 200ppm
- Fermaid O™ at 200ppm

The juice was inoculated with the yeast Lalvin CY3079™ first rehydrated in Go-Ferm Protect™ and Optimum White™ was added to the juice. Everything was added based on the manufacturer's instructions. After inoculation and chaptalization (37.7 g/L) in tank, the juice was transferred to oak for fermentation. During fermentation, Fermaid O™ at 200 ppm was added at the beginning of fermentation.

Follows the winemakers' description notes:

- Ripe tamarillo palate
- Savoury notes
- Struck match/hint of smoke
- Nectarine
- Hint of spice
- Nice phenolic structure

Aroma compound analysis

Aroma compounds analysis of the wines was carried out by NYSEOS (Montpellier, France) to determine the concentrations of major aroma compound classes: thiols, ethyl acetates, esters, pyrazines and terpenols. This analysis was carried out using the same finished bottle samples that were also used for the consumer research. The following figures show the key results.

Levels of three different polyfunctional thiols were analysed. The first was 4-mercaptopentan-2-one (4MMP), which has boxwood aromas and a detection threshold of 1ng/L (Figure 5). Tropical had the lowest levels, while citrus had the highest. The other two wines were both well above threshold for this aroma.

Herbaceous and tropical had similarly elevated levels of 3MH (3-mercaptohexan-1-ol, 60 ng/L sensory perception threshold), and also similar levels of 3MHA (3-mercaptohexyl-acetate, perception threshold at 1 ng/L), both of which were well over the perception threshold. 3MHA hydrolyses to become 3MH, so its levels are normally lower. Barrel-fermented is the lowest on both these compounds. 3MH has been described as citrus (grapefruit) and passion fruit, whereas 3MHA has been described as passion fruit, boxwood and grapefruit rind (Figure 6).

Esters are important aroma compounds in wine that contribute fruity notes. Isoamyl acetate (Figure 7) is quite high for the tropical wine, enhancing the fruity notes. Other esters are present at similar levels in all the wines, with the exception of the barrel-fermented Sauvignon.

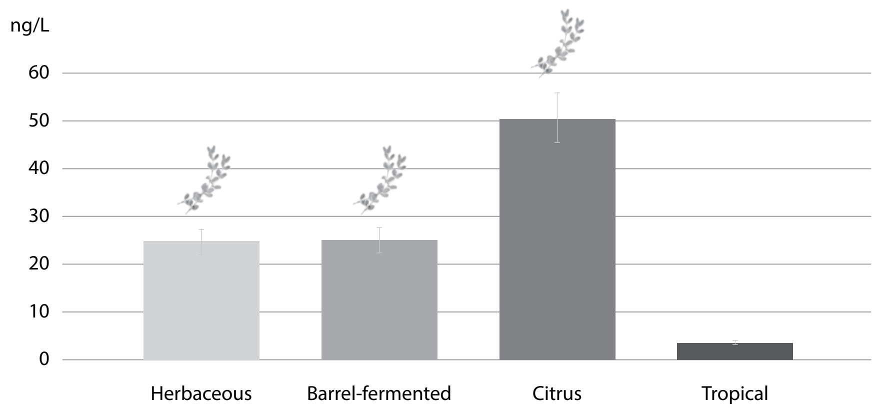

Figure 5. 4MMP analysis in the 4 different Sauvignon Blanc wines.

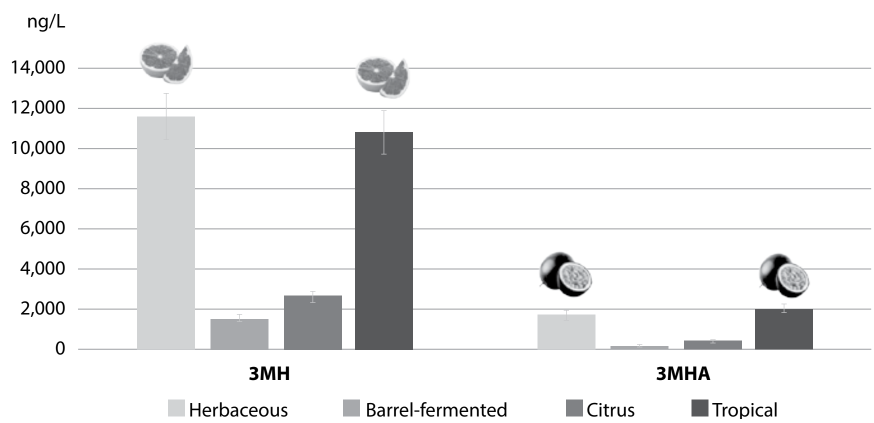

Figure 6. 3MH and 3MHA (Fruity thiols) analysis in the 4 different Sauvignon Blanc wines.

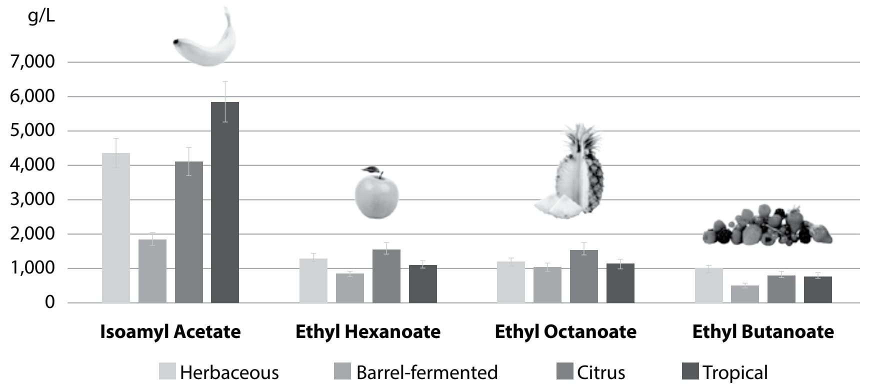

Figure 7. Major esters in the four different Sauvignon Blanc wines.

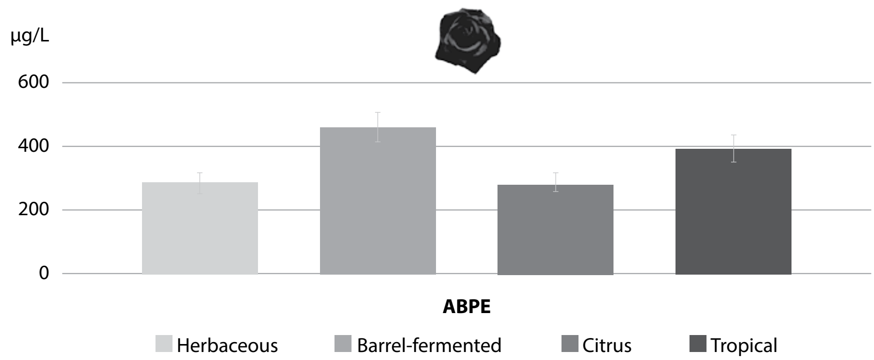

Figure 8. Phenyl ethyl acetate in the four different Sauvignon Blanc wines.

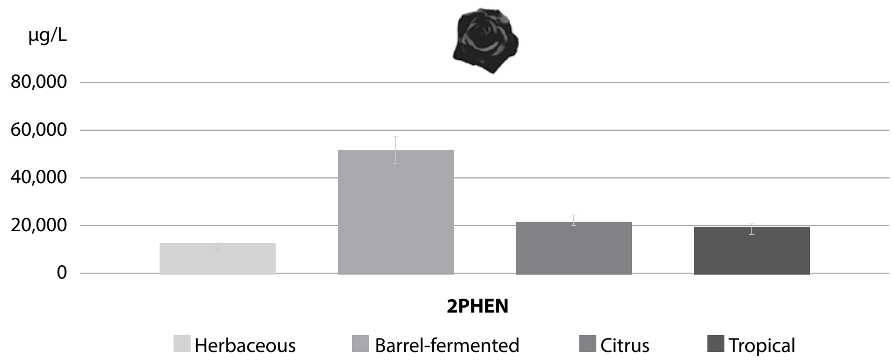

Figure 9. 2-phenyl ethanol in the four different Sauvignon Blanc wines.

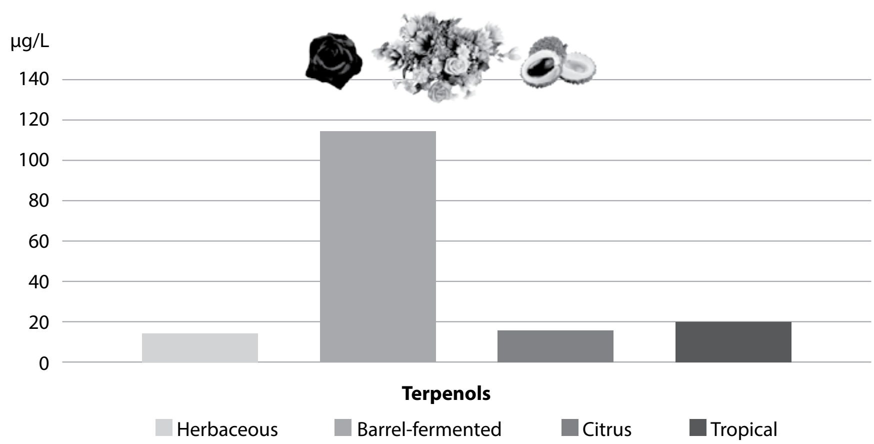

Figure 10. Terpenols in the four different Sauvignon Blanc wines.

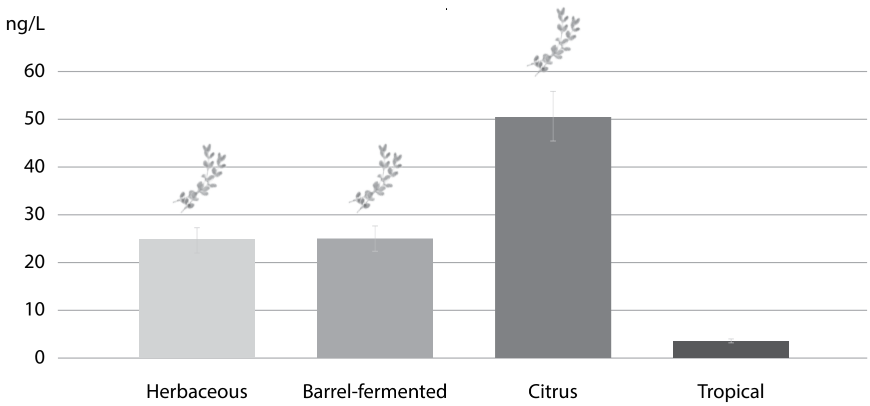

Figure 11. Methoxypyrazines in the four different Sauvignon Blanc wines.

Barrel-fermented is the highest in 2-phenylethanol, phenylethyl acetate and terpenols. It's clearly the highest on the floral notes (Figs 9-10)

The level of methoxypyrazines (Fig. 11) was below sensory detection threshold (0.02μg/L) in two wines, but above threshold in the herbaceous and tropical wine styles.

As the aromatic analysis demonstrates, the wines reflected their designated styles effectively, for example with the higher levels of green thiol and pyrazine character in the herbaceous wine, and high isoamyl acetate and 3MHA in the tropical wine.

Consumer preferences

We examined consumer preference using a survey. Each participant provided their age, gender, average spend on Sauvignon Blanc as well as their preferences for each wine. We broke down this preference into aroma and flavour profiles. We also asked participants to describe the predominant aroma and flavour descriptors they detected in each wine, and asked them to rate acidity, mouthfeel and body. Drawing on experience of preference testing used by the Australian Wine Research Institute (AWRI)'s tasting panels, and from Lezaeta et al's trialling of two different preference methods, we used a 9-point hedonic scale to record consumer preference. This was used to record aroma, flavour and mouthfeel preference.

How much do you like the aroma of the wine?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

How much do you like the flavour of the wine?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

How much do you like the mouthfeel of the wine?

- Dislike extremely
- Dislike very much
- Dislike moderately
- Dislike slightly
- Neither like nor dislike
- Like slightly
- Like moderately
- Like very much
- Like extremely

To assess each consumer's evaluation of aroma and flavour, we used a Check All That Apply (CATA) approach. In order to ensure consumers from all three groups could relate to the CATA options, the vocabulary chosen for the descriptors was selected as the most commonly used adjectives for flavour and aroma when describing Sauvignon Blanc wines based on a review of 40 widely available Sauvignon Blanc wines and taking into account key descriptors from the winemaker notes. The same descriptors were provided for both the aroma and flavour descriptor questions:

Passionfruit Pineapple Melon Mango Peach
Pear Apricot Lemon Lime Grapefruit
Gooseberry Nettle Cut Grass Herbal
Chalky Stony Vanilla Coconut

Please circle the descriptors that best describe the aroma of the wine.

Check all that apply.

Please circle the descriptors that best describe the flavour of the wine.

Check all that apply.

To avoid being too prescriptive and restrictive, the consumers were also provided with a prompt for any further descriptors of flavour and aroma which enabled a free-form answer.

Are there any other descriptors you would use to describe the aroma of the wine?

Each consumer was also asked to identify which aroma and flavour was most dominant to them.

Of the descriptors you selected in Q2 which one is the most dominant/strongest?

Which one of the flavours you selected is the strongest, most dominant?

Analysis of the results enabled correlations to be drawn between the extent to which a consumer liked or disliked a wine and the aromas and flavours they claimed to identify as dominant.

To avoid potentially negative connotations of 'acidity' amongst consumers, the word refreshing was used to gauge their preference of this attribute.

How refreshing do you find the wine?

- Not at all refreshing
- Not refreshing
- Neither refreshing nor not refreshing
- Refreshing
- Extremely refreshing

Finally, the standard descriptors used for body were used.

How would you describe the body of the wine? (Please circle)

Light bodied Medium bodied Full bodied

The same questions were asked in the same sequence for each wine. Each tasting was conducted with the wines poured in a randomized order, blind, to each consumer. Wines were served lightly chilled to each consumer. As much as possible this was at 10˚C although the range of locations and tasting times of each participant meant there was some variance.

The Participants

The study collated responses from 271 participants. These included 247 consumers and 24 journalists. As broad a geographical spread as possible was incorporated, with participants from Edinburgh, Bristol, Manchester, Leeds, York, Birmingham and London.

The Consumers

The Consumers were carefully selected to provide as broad a group as possible, consisting of recruited Coop shoppers, engaged wine consumers using the Local Wine School Network and non-wine affiliated consumers from other areas. The gender breakdown was 53% female, 39% male and 8% unspecified gender.

The consumers covered a broad range of ages:

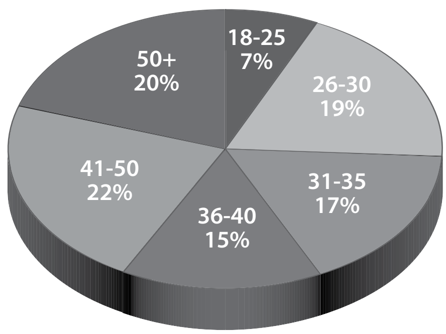

Figure 12

As shown in the chart below, the consumers also had a broad average typical spend on a bottle of Sauvignon Blanc:

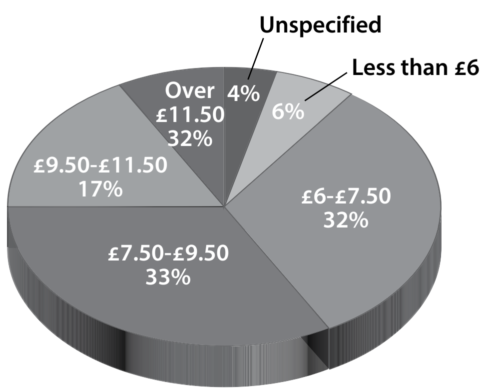

Figure 13

The Journalists

Journalists were selected as wine-specific journalists from newspapers, specialty magazines and blogs. The gender breakdown was 50% female, 36% male, and 14% unspecified gender.

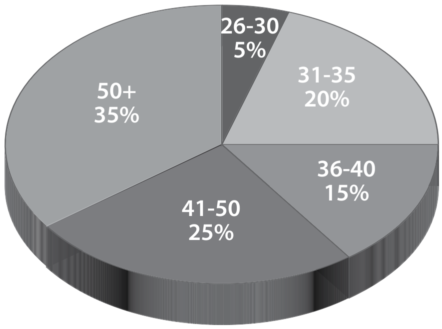

FIGURE 14

Journalists showed a typical spend spread more closely related to that of the consumers:

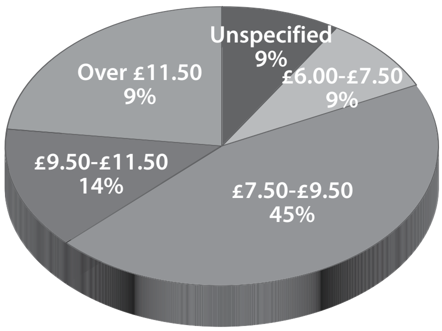

FIGURE 15

Results

The following mean scores show the outcome of the responses to the question, 'To what extent do you like the aroma of the wine?'.

The aromatic preference for both consumers and journalists was for the citrus wine. The journalists ranked the barrel-fermented style higher than the consumer group. Analyzing the aromatic preferences further showed that male consumers had a greater preference for the barrel-fermented style than women did. The citrus style was the preferred style aromatically for consumers typically spending below £6, between £6 and £7.50, and between £9.50 and £11.50 but those who typically spent £9.50-£11.50 preferred the tropical style and those spending over £11.50 the barrel-fermented style.

The barrel-fermented style was more favoured by those between the ages of 31 and 40 and over the age of 50. The variation of preferences by age, gender and typical spend highlights the importance of knowing the target demographic of a wine if winemaking style is going to factor in the consumer preferences of that particular group.

Aromas (CATA responses) Citrus Wine

The table below shows the three most dominant flavours detected by each respondent group. The most dominant aromas for the consumers were grapefruit and gooseberry, followed by pineapple and pear. Gooseberry was the most selected option as 'most dominant aroma' for the journalist group. It is clear that the consumers are detecting the same aromas as the professionals:

Referring back to the analysis of the wine, the citrus wine showed relatively low levels of 3MH and 3MHA. Although the 3MH level in citrus is above the detection threshold, this wine shows much lower levels than the herbaceous and tropical styles that are ranked less favourably with the consumer group. This suggests that high-thiol Sauvignon might not be the preferred style by groups of consumers such as this, and that while they are identifying grapefruit and gooseberry characters in the wine – and liking them –

TABLE 9 AND 10. Mean scores of the responses to the question, 'To what extent do you like thearoma of the wine?' by each respondent group.

Tropical	Mean score
Consumer	6.62
Journalist	5.9

Citrus	Mean score
Consumer	6.67
Journalist	6.77

Barrel	Mean score
Consumer	5.66
Journalist	6.16

Herbaceous	Mean score
Consumer	6.39
Journalist	5.95

TABLE 11. Three most dominant flavours detected by each respondent group for the citrus wine.

Citrus Consumer	Citrus Journalist
Grapefruit = Gooseberry	Gooseberry
Pineapple	Pineapple
Pear	Passionfruit = Grapefruit

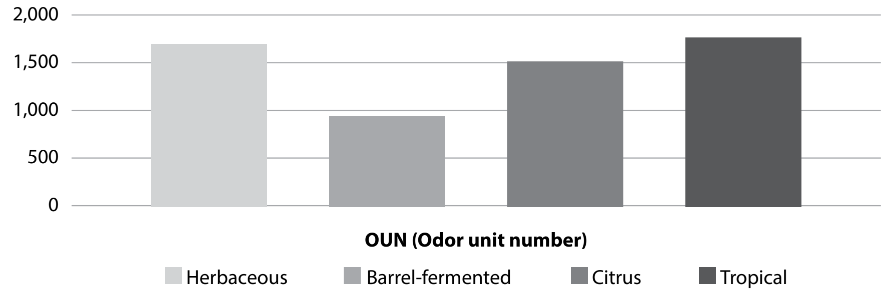

Figure 16. Aroma intensity of each wine (Odor Unit Number).

these might not be due to thiols, but rather other aroma compounds (alone or in combination). We should be cautious about thinking that thiols are the route to consumer preference in Sauvignon Blanc. Neither the herbaceous nor tropical wines with higher levels of 3MH and 3MHA scored as well with the consumer group, suggesting a move in consumer preference for a more restrained style. Considering that New Zealand Sauvignon is known for its more overt, pungent style, it is interesting to note that it is not the most aromatically intense wine here that is favoured by the tasters. We might have expected the tropical wine to be the most popular.

Furthermore, it is the interplay of other aromas that contribute to the overall perception of the wine. The citrus wine had the highest level of 4MMP (50 ng/L), C13-norisoprenoid (2.05 μg/L), ethyl octanoate (1506 μg/L; reflected in the 'pineapple' picked out by the consumers and journalists) and ethyl hexanoate of the four wines. It showed the most complex range of esters with good concentrations of the main esters giving complexity which could have contributed to its rating. More research is needed on the interplay between these compounds to understand their impact on the sensory perception of Sauvignon Blanc.

Aromas Freeform Responses Citrus Wine

Gathering freeform responses demonstrated that whilst consumers may use more simplistic language, they still detected some of the same attributes as the professional tasters, with 'body odour' being detected by both groups, and apple by the consumers. This suggests that consumers are no less able than their professional counterparts to freely assess the aromas in wine.

Figure 17. Aromas freeform responses by each respondent group for citrus wine.

The body odour character was much more frequently cited for the tropical wine (with higher levels of 3MH: 10 820 ng/L) and the consumers did reflect the high levels of iso-amyl acetate (5861µg/L) with their 'banana' and 'banana sweets' descriptors. All groups detected green characters in the herbaceous wine (frequently verbalised as 'green' and 'fresh' in the consumer group, 'asparagus' 'peas' and more 'vegetal' attributes in the journalist group).

The frequency with which they use adjectives as aromatic attributes (fruity, subtle, sweet, fresh) rather than noun, perhaps supports a different descriptive approach which could be useful for producers planning their labelling and marketing.

Aromas (CATA responses) Tropical Wine

Consistency of aroma ranking is shown again in the tropical and herbaceous wines. Whilst grapefruit and gooseberry aromas dominate in the responses for the tropical wine, passionfruit was also picked out consistently by each group of tasters. (Table 12)

The tropical wine did have the highest level of 3MHA of all four wines, as well as the highest levels of Isoamyl acetate.

Aromas (CATA responses) Herbaceous Wine

The herbaceous-style wine reflected the high levels of 3MHA and 3MH: (Table 13)

The barrel-fermented wine was consistently categorized as coconut and vanilla across all groups. Interestingly, the barrel-fermented wine was the highest in 2-phenylethanol, phenylethyl acetate and terpenols, yet only the consumers referenced detection of a floral character in their freeform answers for the aroma descriptors.

Flavour preference and flavour dominance

The tropical wine was the consumer favourite on flavour, although they scored closely with a mean of 6.42 for the tropical and 6.35 for the citrus. The journalists scored citrus top with a mean of 6.35 and the tropical with 6.27. The barrel-fermented wine was the least favourite of both groups.

Table 14 ranks the dominant flavours picked out by the consumers. As discussed, tropical and herbaceous both have higher 3MH so the dominance of this character does not correlate with the overall ranking of the wine.

Aroma vs. Flavour Preference

Sensostat analysed the survey results using an Analysis of Variance (ANOVA) and a Least Significant Difference (LSD) test. Results with the same letter cannot be considered as having significantly different mean and, as shown below, this would suggest that there is much less variance of preference for flavour.

This suggests that a greater value focus is placed on aromatic preference than on flavour when a taster is evaluating a wine. (Figure 18)

In contrast there is clearly more variability in preference on aroma with differentiation between the mean (Figure 19).

Other Sensory Attributes

Both groups ranked the citrus wine top for 'refreshing' (Table 15).

The ranking for 'mouthfeel' was less consistent, suggesting that a clearer definition with set parameters is needed to enable tasters to more ably define their preferences (Table 16).

Table 12. Aromas (CATA responses) Tropical Wine.

Tropical Consumer	Tropical Journalist
Grapefruit	Gooseberry
Gooseberry	Pineapple
Passionfruit	Passionfruit

Table 13. Aromas (CATA responses) Herbaceous Wine.

Herbaceous Consumer	Herbaceous Journalist
Grapefruit	Gooseberry
Gooseberry	Pineapple
Passionfruit	Passionfruit

Table 14. Dominant flavours picked out by the consumers and journalists.

Tropical Consumer	Tropical Journalist
Grapefruit	Grapefruit
Gooseberry	Gooseberry
Peach = Passionfruit	Mango

Citrus Consumer	Citrus Journalist
Grapefruit	Grapefruit
Gooseberry	Peach
Pear = Lime	Melon

Herbaceous Consumer	Herbaceous Journalist
Gooseberry	Gooseberry
Passionfruit	Lime
Grapefruit	Grapefruit

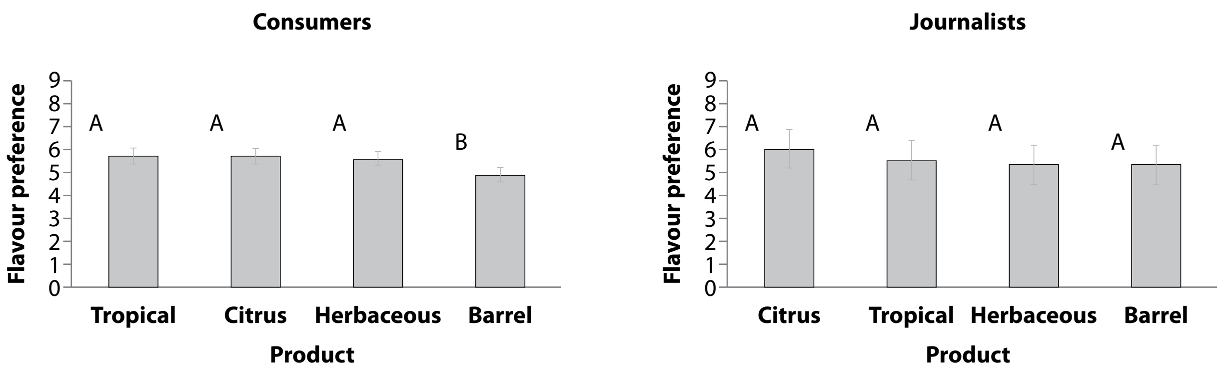

Figure 18. Mean of flavour preference by each respondent group.

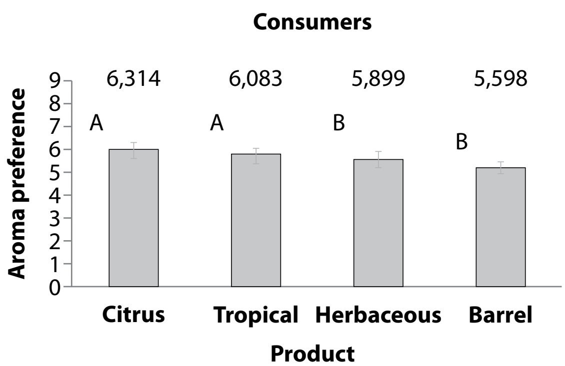

Figure 19. Mean of aroma preference by each respondent group.

Table 15. Other Sensory Attributes – "Refreshing".

	Consumer Preference	Journalist Preference
Most refreshing	Citrus	Citrus
	Tropical	Herbaceous
	Herbaceaous	Tropical
Least refreshing	Barrel	Barrel

Table 16. Other Sensory Attributes – Mouthfeel

	Consumer Preference	Journalist Preference
Favourite	Herbaceous	Citrus
	Tropical	Barrel/Herbaceous
	Citrus	Barrel/Herbaceous
Least favorite	Barrel	Tropical

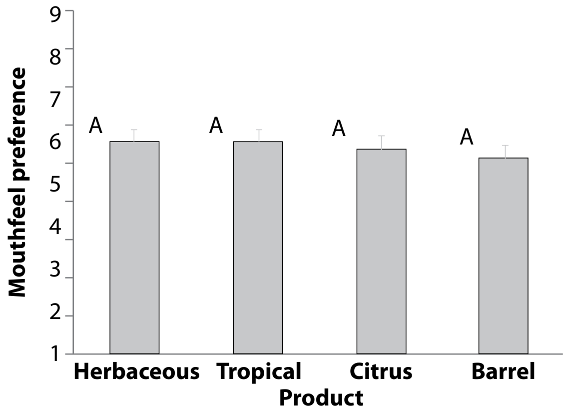

Figure 19. Mean of mouthfeel preference.

Again using the analysis of variance (ANOVA) and a Least Significant Difference (LSD) test no significant difference in preference was apparent for mouthfeel (Figure 20).

Overall preferences

Overall preferences were calculated by scoring each wine based on its ranking for mean preference scores on aroma, flavour, refreshment and mouthfeel. Evaluation of 'body' could not be ranked as the question required tasters to rank it, but not qualify whether they 'liked' the body in the same way as they ranked their preferences for each other element hedonically. This is something that could be amended in future studies. While the citrus wine was the favourite aromatically for the consumers, with mouthfeel and refreshment factored in, it tied with the tropical wine, both scoring well ahead of the herbaceous wine and the barrel-fermented wine, the least popular style. Journalists also preferred the citrus wine overall, followed by the herbaceous, barrel-fermented and tropical.

Conclusion

This study demonstrates that there is a range of preferences for wine style. Winemakers have the tools to maximize site potential with specific winemaking protocols. It is possible to make wines that better reflect their sense of place, but also wines that specific consumer groups prefer. In a crowded market, having knowledge of consumer preference and the right toolkit to be able to make wines that fit in with these preferences can present a competitive edge. Interestingly, this study highlighted that while consumers and journalists agreed on some points, they also had differing preferences. It would be interesting to extend this study to see whether there are significant differences in preference between the wine trade and the customers that the trade is trying to serve. For this sort of research to be useful, wineries and retailers need to have a means of marketing to the specific target preference segments, once these have been identified.

The aroma compound analysis here showed that making wines to suit consumer preferences isn't as simple as focusing on one aroma compound and then maximizing its level. For Sauvignon Blanc, there has been a lot of work on the impact of polyfunctional thiols. These are found at elevated levels in Marlborough Sauvignon Blanc, and there has been an effort by many wineries to devise protocols that result in wines with higher levels of 3MHA and 3MH in particular. This study indicates that elevated levels of these thiols isn't a reliable indicator of consumer preference. Increasing aromatic intensity or sensory perception levels of a given compound does not correlate with a wine being more popular. The overall preference of the citrus wine suggests a preference for a wine with a greater interplay of a wider range of aromatics and more moderate levels of certain compounds such as 3MH in comparison with the other wines. Ultimately, winemakers know their sites and the winemaking potential of their sites, and with a greater understanding of consumer preference they can use an integrated winemaking approach to optimize the potential of their vineyards in line with favourable consumer preferences.

NEW ZEALAND PINOT NOIR ON THE GLOBAL STAGE: STYLES, CHALLENGES, OPPORTUNITIES

Jamie Goode

www.wineanorak.com

Pinot Noir is one of the most widely loved and sought after of all red grape varieties. Over the last few decades, the notion that Pinot only performs well in Burgundy and is a poor traveler has been disproved with increasingly successful wines being produced in Australia, California, Oregon, South Africa, Chile, and, of course, New Zealand. Pinot's talent is its ability to produce elegant, floral reds that are lighter in body and which are particularly good at expressing their vineyard origins. This article presents an overview of how Pinot is doing around the world, and puts New Zealand Pinot Noir into this perspective. It also includes a discussion of how New Zealand Pinot might best be placed for future development, arguing that a focus on making high-end wines from privileged sites is the best way forward.

The goal of this paper is to look at Pinot Noir around the world, and answer the question: where does New Zealand fit in? But before I set out, there are two concepts I want to explore that are relevant to this discussion, and which would be worth bearing in mind. First, we need to segment the market. There is no such person as "the consumer," and when it comes to selling wine, the rules are different in different market segments. Customers are different and behave differently, and the routes to market are different. For the purposes of this discussion, I would like to think in terms of three segments. The first is the bottom end, where wine is pretty much a commodity. People shopping here have a tight budget and just want something that tastes OK, with no nasty surprises. Then at the other end of the spectrum we have the fine wine dimension, which operates under completely different rules. Here we encounter the complex concept of terroir: the idea that there are specific characteristics of the site that can then shape the characteristics of the wine in such a way as to impart a local taste. Here, price is less of an issue: there are people who will pay a lot for what they regard to be the very best wines. And quality is not just about the liquid in a bottle: the reputation and heritage of fine wines is also important. In between these two segments lies the middle ground.

Where do you want to play in this market? And, perhaps more importantly, where can you play? If you have a lot of wine to sell you might find yourself forced to play at the bottom end, selling large volumes of wines very cheaply at low (or no) margin. The fine wine end is where everyone would like to play, but in order to play there you need to make wines that are recognized to be of very high quality, and you need to have a good reputation, and the right routes to market. It's a profitable segment, but volumes here are limited. And then there's the middle ground, where many wines end up fighting it out for relatively few slots: it's a segment that has been struggling for a while now. But if you can sort distribution then it's a nice place to be.

The second concept is that of wines of style versus wines of place. Expressing place in a wine is quite a challenge, and terroir can easily be lost. If you pick too late and use lots of new oak, you end up with a wine of style. If you pick too early, you can also end up with a wine of style. If you have *Brettanomyces*, you can end up with a wine of style. Too much or clumsy intervention can result in a wine that says more of the process than the place. There

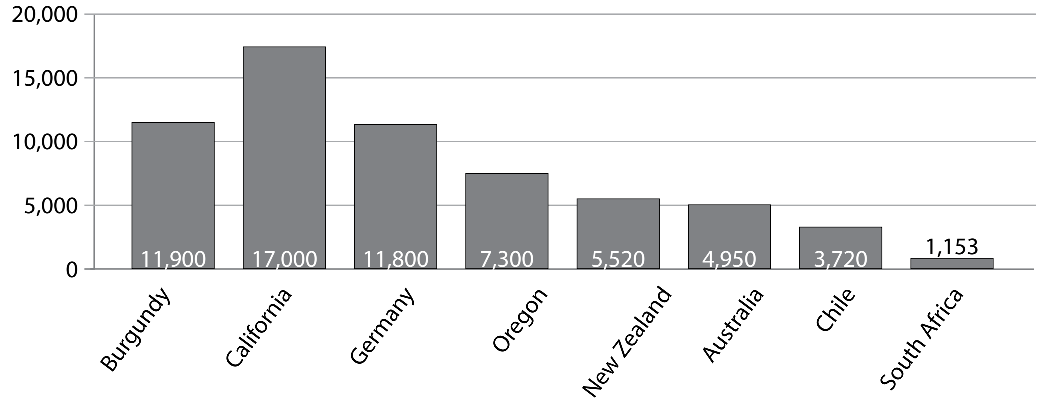

Figure 1. Major Pinot Noir producers by hectares (including Pinot Noir for sparkling).

are lots of wines of style: we need more wines of place. But, of course, you need an interesting place to make an interesting wine of place. Not all wines need to be wines of style, but at the high end the market wants wines of place, and wines of place can't be copied.

So let's go on a tour of the world's Pinot Noir hotspots. Bear in mind, as we discuss vineyard area, that Pinot Noir is also used for sparkling wine, and these figures include both still and sparkling.

Burgundy

We need to start here, the home of Pinot. It has 11,900 hectares of Pinot Noir. This is a region that's all about parcellation. The vineyards have been delineated into 1,247 climats (with each being a recognized vineyard block with specific characters), and overall the region has some 84 AOPs. There is also a quality hierarchy: the Burgundians have long recognized that not all vineyard sites are created equal. The most expensive Burgundies are among the most expensive wines of all, and price is tightly connected to the notion of terroir. This is interesting, because if terroir is in fact a bit of a myth, as some have suggested (most notably Professor Mark Matthews in his book *Terroir and Other Wine Growing Myths*), then by lavishing care and attention on a vineyard lower down in the Burgundy hierarchy, you might expect to be able to fashion an astonishing wine. The robustness of Burgundy's terroir-based vineyard hierarchy, even where there are now strong economic motives to invest in producing the very best wines, suggests that there is something to this notion of terroir. Red Burgundy is seen very much as the benchmark for great Pinot Noir. Not all Burgundy is good; not all expensive Burgundy is good. But the best wines are thrilling, and are increasingly sought after. Even if you are wealthy, you can't just go and buy them. Top producers allocate their wines carefully, so you'll have to stand in line and wait your turn if you want some, even if you can afford the top bottles.

California

California has a lot of Pinot Noir. There are some 17,000 hectares of it planted, and there's a wide range of quality levels and style choices. Serious Pinot began here in the early 1970s in Santa Barbara County, with vineyards such as Sanford and Benedict in the Santa Rita Hills (first planted 1971) and Bien Nacido. There's a lot of good Pinot here these days, and also in Sonoma, especially in the Sonoma Coast appellation. Top Californian Pinots are highly prized and fetch good money. While there are some exports, a lot is destined for the domestic market, which has plenty of consumers with high disposable income and a penchant for local wine. One of the strengths of California's high-end Pinot scene is that there exist several famous vineyards which then sell their grapes to a range of different producers. This is building up equity in places and not just producers.

Oregon

The strength of Oregon is that it is so strongly focused on Pinot. Things began here in the mid-1960s when David Lett planted Pinot Noir in the Willamette Valley. There are now 7,300 hectares of Pinot in Oregon, and most of the wines are made on a boutique scale, although Rex Hill/A to Z Wineworks is a producer who makes a significant quantity of affordable Pinot Noir. Oregon's Willamette Valley is now subdivided into different AVAs, each with their own characteristics. This is the beginning of a parcellation of the vineyard area by physical characteristics of the vineyard sites, which is an interesting direction to take as it adds value to the wines. Top Oregon wines are very highly sought after, and this is an exciting place for Pinot.

Australia

Pinot Noir arrived in Australia with the James Busby vine imports in the 1830s. Currently there are some 4,950 hectares of Pinot Noir in Australia, and regions such as the Mornington Peninsular, Adelaide Hills, Tasmania, and the Yarra Valley all have an excellent reputation and a bevy of top quality producers specializing in Pinot Noir. The exploration of terroir continues in each of these regions, and the scene is a very healthy one indeed.

Germany

With 11,800 hectares of Spätburgunder (the German name for Pinot), Germany should be better known internationally for its Pinot Noir than it is. The reason for it flying under the radar a bit is because most is for local consumption. The key regions for Pinot Noir are the Ahr Valley (a small region specializing in the variety, with schist soils) and Baden, but there is also some excellent Pinot in Franconia. The best German Pinots are truly world class, but as with many countries, quality is variable.

Chile

Pinot plantings have increased quite rapidly in Chile, and now there are some 3,720 hectares of the variety. Much of the wine produced is inexpensive, and lacks the elegance and texture that Pinot is capable of, but these can serve as useful affordable introductions to the grape. Of late, though, some of the cooler areas such as Aconcagua Costa, Limari, San Antonio, and Bio Bio have been turning out some convincing wines. A work in progress.

South Africa

South Africa's winelands are mostly a bit too warm for Pinot Noir to show its best, but there are 1,150 hectares of Pinot planted, much of which is destined for sparkling wine. But there are two cooler regions that have done some nice things with Pinot: Hemel-en-Aarde and Elgin. Both regions are now making some very good wines, albeit in quite small quantities.

And New Zealand?

New Zealand Pinot Noir has a relatively short history, and we can trace the current industry back to the mid-1980s. Three regions emerged with a talent for Pinot Noir. The first New Zealand Gold Medal Pinot was the 1981 Babich Pinot Noir, which achieved this distinction at the Royal Easter Show. The show success of Danny Schuster's 1982 St Helena Pinot Noir established the North Canterbury region as having potential for this variety, based on fruit from an acre of Pinot planted by Graeme Steans in the Kaitura Valley. Then there was Martinborough, with Ata Rangi and Martinborough Vineyard leading the way, developing this small region as a Pinot specialist. Larry McKenna made the first Martinborough Vineyard Pinot in 1984, and was to achieve gold medal success with the 1986. The first Ata Rangi Pinot Noir was the 1985, made by Clive Paton from the Abel clone, named after the customs officer who recognized the significance of some vines smuggled in from Burgundy. And perhaps most significantly of all, the emergence of Central Otago as a region with a particular tal-

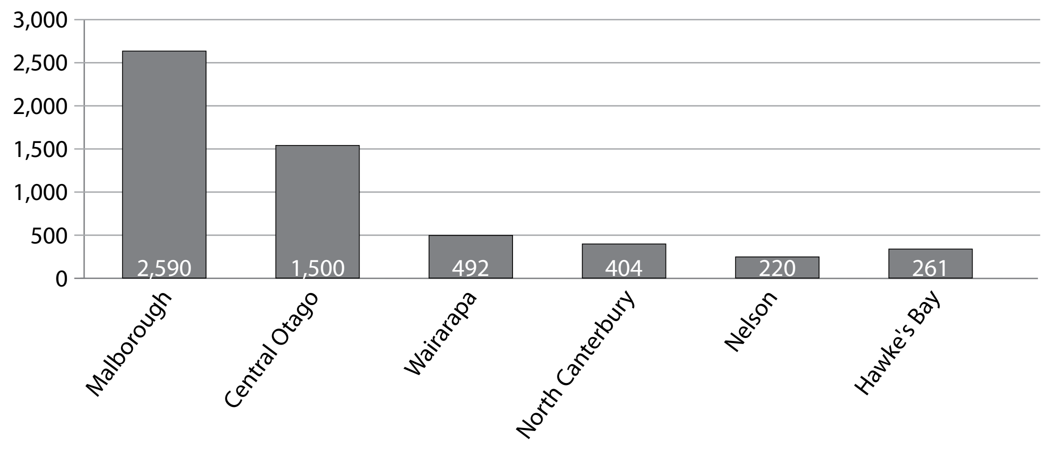

Figure 2. Pinot Noir plantings by region.

ent for Pinot. A group of pioneers all began making wine at the same time in the mid-1980s, including Alan Brady (Gibbston Valley), Rolfe and Lois Mills (Rippon), Verdun and Sue Edwards (Black Ridge), Bill Grant (William Hill), and Ann Pinckney (Taramea). These three regions are still out in front, but there's also some good Pinot being made in Nelson, and most significantly, Marlborough has emerged as a serious player. There were 141 hectares of Pinot Noir in New Zealand in 1989, 1,100 hectares in 2000, and 4,777 hectares in 2009. Currently (2018) there are 5,698 hectares in production.

If we look at Pinot Noir plantings, Marlborough is out in front with 2,590 hectares. Central Otago follows with 1,500, and then there's quite a jump down to Wairarapa (492 ha) and North Canterbury (404 ha). Marlborough's Pinot Noir has improved considerably with a concentration of planting in sites that suit this variety best. Here, this means the clay-influenced soils of the southern valleys of the Wairau, and also the Awatere. It is generally less successful when planted on the braided, heterogeneous old riverbed soils that are characteristic of much of the Wairau: these are ideal for Sauvignon Blanc, which copes well with variations in ripeness across the same vineyard block.

Progress report

New Zealand Pinot Noir is particularly strong in the mid-price segment (retail NZ$18-35). Pinot is quite tricky to grow and is never going to be really cheap, but with other world regions focusing on a more premium, boutique offering, New Zealand can deliver very good quality at relatively affordable prices – something that California and Oregon struggle to do. New Zealand also has a position at the bottom end of the market (retail NZ$12-15), and some of these wines are OK. But the question needs to be asked: should New Zealand be playing there? Does cheap Pinot Noir damage the brand proposition, and position New Zealand as a country that makes good commercial wine? In some ways, the market is able to deal with this. For example, the market has no problems with California wine, which can be incredibly cheap and incredibly expensive. The various segments of the market are quite separate there. But perhaps in New Zealand the segments aren't far enough distanced from each other to avoid this sort of contagion. Sparkling wine might be a better destination for Pinot Noir grapes from terroirs less well suited to fine wine production.

What of the New Zealand fine wine dimension? It has some strengths, but needs work. Most Pinot Noir producers tend to cluster in their pricing. There need to be more absolutely world class New Zealand Pinot Noirs. Currently, there are a handful of producers who have the track record and global recognition for their Pinot Noir. These would include Ata Rangi, Felton Road, Burn Cottage, Bell Hill, and Pyramid Valley, and this is reflected in their pricing. This band needs to grow.

Currently, the market isn't taking Marlborough Pinot Noir seriously enough. Part of the reason for this is that the region established its identity for Sauvignon Blanc. 80% of the region's 27,000 hectares of vineyard is planted to this variety, and it enjoys a global reputation for its distinctive, pungent, aromatic style. While Marlborough Sauvignon is a success story and helped put New Zealand wine on the map, it has made it difficult to get the message of the region's Pinot Noir out to the world. But when Pinot Noir is grown on the right sites, it can excel, and there are now some very serious Pinots being made in Marlborough. The hot spots are the series of valleys in the south of the Wairau Valley, heading into the Wither Hills, and also in the Awatere Valley, on the other side of the Wither Hills. It is the identification of privileged sites for Pinot in Marlborough that has led to a rise in quality. The top 20 Marlborough Pinots would be a strong match for the top 20 from Central Otago, Wairarapa, or North Canterbury. These are now well established as the four regions doing best work with this variety.

How do we decide what makes a Pinot Noir great?

It's time for a slight diversion, to consider the question of how we decide what makes a Pinot Noir great. It's not just simply a question of hedonics: which one tastes nicest. Indeed, greatness cannot be conferred by one person's opinion, and if a wine is only great to me yet uninteresting to others: it's simply autobiography. We need to move beyond hedonics. Greatness in wine is conferred by the community of judgement. We recognize that within the wine trade we have individuals who by virtue of their training and experience and aesthetic sensibility are in possession of worthwhile opinions, and it is when these opinions are pooled – as we sit together and taste and share our verdicts – that we can identify wines of particular merit. We decide together what is greatness when it comes to Pinot Noir. Such verdicts aren't, of course, universally held, but on the whole, we tend to agree which the best wines are.

Style choices

So let's consider the way forward for New Zealand Pinot Noir, and begin with the issue of style. Some markets demand colour in their Pinots, but I think it would be a mistake for New Zealand producers to focus on making darker Pinots. At the high end, the market is now accepting of lighter coloured Pinots. Instead of focusing on color, producers should focus on what Pinot can do so well: it's a variety that produces wines with a hallmark of finesse, elegance, perfume, and pleasure. These should be the primary goals, and if there's colour with this, then great. More serious examples of Pinot Noir need layers of flavour, and some non-fruit complexity. New Zealand usually has no problems making fruity Pinots. The challenge is to make Pinots that maintain the fruit presence but which supplement this with something extra. In contrast, many Burgundies have lots of non-fruity components but could do with some lush, pure fruit of the sort that New Zealand delivers so effortlessly.

But there is no place in Pinot Noir for *Brettanomyces*, which even at low levels strips away fruit and texture. It can handle a little bit of reduction, though, which can frame the palate quite nicely, but this is quite a tightrope to walk if it is a deliberate style choice.

It's about the interpretation of place: making a wine that in some way expresses its terroir involves human activity. The winegrower acts as an interpreter, and there is no single correct interpretation of a site. However, there are intelligent and less intelligent interpretations of site, and it is possible to make a wine in such a way that it could have come from just about anywhere. At a low price point it's entirely appropriate to make a Pinot Noir that tastes simply of the variety: a wine of style. But for the fine wine dimension, it's important to capture the place, even if to some this seems like a vague, fanciful, or ill-defined notion. People who buy wines of style typically buy the cheapest version of the wine that meets their style criteria. Why would they pay more? But once you have a wine of place, no one from outside that place can copy it. It is in the interest of New Zealand's Pinot producers to make wines that capture their places.

Identifying talented sites

It's for this reason that I think the future for New Zealand Pinot Noir lies in identifying great sites. The place is what matters and winegrowers collectively should look to making the place famous and not the producer. This work is already well underway, and I'd like to illustrate it with some examples from Marlborough, where I have spent quite a bit of time of late.

The first talented site to be identified was Clayvin. Mike Eaton deserves a lot of credit for this, the first hillside, close-planted vineyard in the region, which he began planting back in 1991 on a shoestring. At the top of the Brancott Valley, it is organically farmed and is now owned by Giesen. As well as Giesen, Fromm and Te Whare Ra are also making Pinot Noir from this site, and it is interesting to taste the wine side by side, to see the family resemblances and also the producer's style footprint.

The Cowley family own the Auntsfield vineyard, which is tucked into the Wither Hills at the end of the Ben Morven valley. As well as Auntsfield, Corofin are also making Pinot Noir from this vineyard.

Sam Weaver planted the Churton vineyard, which sits on a ridge between the Omaka and Waihopai valleys. Churton, Fromm, and Novum make single-vineyard wines from this vineyard.

The Settlement Vineyard, Omaka, and the Yarrum Vineyard on the boundary of the Omaka and Brancott valleys are both owned by Ivan Sutherland/Dog Point. They sell Pinot Noir to a range of growers and are exciting sites for this variety.

Medway Vineyard: Deep into the Awatere Valley, this is owned by Yealands. It made a stunning Pinot Noir in 2017.

Ballochdale: at the far end of the Awatere, this has made some stunning Pinots in the past. 2016 was the last year that Villa Maria (Esk) sourced from here. It is a north-facing glacial terrace at 300 m, with the cold air draining into deep gorges, thus minimizing frost risk.

The future

These are just a few examples. The future for New Zealand's Pinot Noir fine wine dimension is to build on the largely producer-driven foundations that have already been laid, and begin exploring parcellation and segmentation. Not all sites are created equal, and it is the identification of New Zealand's Grand Crus, and the emergence of famous places alongside famous producers, that will drive things forward.

In the obsession with vineyard sites, we mustn't forget the art of winemaking. Great sites need interpreting, and wines don't make themselves. The mastery of fermentation, and then of elevage, are vital skills in fashioning fine wines. The vineyard provides the ceiling for quality, but it is the winemaker that then determines whether this ceiling is reached. Any discussion of great terroir must also involve a discussion of interpretative winemaking, and there are now many tools of elevage available.

New Zealand has been making great Pinot Noir for a while now. But in wine terms, it is still early days. The world will be watching with interest as this exciting category continues to develop.

UNIQUE SPECIFIC AUTOLYSATE TO HELP WITH PINOT NOIR COLOUR AND TEXTURE MANAGEMENT

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Introduction

Consumer demand for fruity red wines with intense colour and good mouthfeel continues to grow. Meeting this demand in Pinot Noir winemaking is challenging, especially in terms of colour and texture management. Pinot Noir grapes exhibit a peculiar polyphenolic composition: low total anthocyanins, no acylated or coumarylated anthocyanins, and a high tannin content that comes mostly from seeds (Mazza et al., 1999). Winemaking practices such as cold maceration and aging on lees are well-established methods for dealing with the characteristics of this varietal. Indeed yeast and Pinot Noir wines have been closely linked for decades for better (colour stabilization, texture/balance improvement…) and for worse (*Brettanomyces* and other undesirable microorganisms, sulfur off-flavours).

With respect to colour and texture, research on the impact of different yeast strains has illustrated how yeast impact on tannin content and colour intensity is strain-dependent (Carew et al., 2013). As such, yeast-derived winemaking and aging tools offer an opportunity for colour and texture improvement.

Aging on lees is a widespread traditional winemaking technique aimed in part at reducing astringency and bitterness while increasing body and aromatic length and complexity. Aging on lees can also help stabilize the colour of red wines. During this step, winemakers reap the many well-known benefits—including the release of mannoproteins—provided by adding dead or dying autolyzed yeast (Rodriguez et al., 2005). To avoid the inconvenience of traditional aging on lees, a practice has developed over the past 15 years where specific inactivated yeasts are added to promote the release of polysaccharides (Guadalupe et al., 2007, and Rodriguez-Bencomo et al., 2010). The concept that certain polysaccharides can bind with tannins and thereby reduce the astringency of wines has been around for a number of years.

In the past two decades, specific inactivated yeast (SIY) products have been developed in order to provide tools to modulate wine astringency and improve wine texture. More recently it has been evidenced that, depending on the process applied to yeast biomass, yeast-derivate fractions can differ in terms of composition and solubility, thus impacting wine quality differently (Mekoue et al., 2015). Polyphenols can interact in different ways with these fractions. Phenolic oligomers and polymeric tannins are the major polyphenols involved in interactions with SIYs. The initial finding that polyphenols are absorbed in the yeast cell wall gave way to the more recent discovery of their massive trapping in the yeast's internal space, followed by the precipitation demonstrated by Mekoue et al in 2015. Intracellular compounds can lead to the formation of either large aggregates with a following precipitation, or the formation of soluble complexes that remain in solution.

A recent study focused on the interactions between mannoproteins and grape or wine polyphenols was conducted at the INRA Montpellier (Science Pour l'Oenologie research unit) (Mekoue et al., 2016). Interactions in solution between grape skin tannins with an average degree of polymerization of 27 and yeast parietal mannoproteins led to the formation of finite-size submicronic aggregates that were stable over time and remained in suspension. These findings support the hypothesis that mannoproteins released by specific inactivated yeasts can help improve the taste of red wine by binding with tannins. It is likely that using this type of product (high in mannoproteins) at the beginning of the winemaking process will limit aggregation of tannins and anthocyanins early on, thus improving the colour and mouthfeel of red wine. Recent scientific advances have provided more precise tools for characterizing wine yeasts and their products, leading to the development of a new yeast autolysate (MEX-WY1) with unique mannoprotein properties based on an innovative combination of a special strain of *Saccharomyces cerevisiae* (WY1) and a specific inactivation process (MEX).

Development of the specific yeast autolysate

Physico-chemical characterization of the specific yeast autolysate (MEX-WY1)

Specific yeast strain with special parietal mannoprotein properties evidenced by atomic force microscopy

In recent research conducted in partnership with INSA Toulouse, atomic force microscopy (AFM) was used to characterize properties of wine yeast cell walls (Schiavone et al., 2014). Wine yeasts that displayed strong mannoprotein-producing capacity were selected and AFM used to explore the unique properties of the WY1 strain of *Saccharomyces cerevisiae*. Figure 1 shows AFM topographical images of two cells of the WY1 and WY2 strains (Fig. 1A and 1B) and corresponding images of their adhesion (Fig. 1C and 1D). WY1 was particularly adhesive, and due to its high mannoprotein content and the length (average

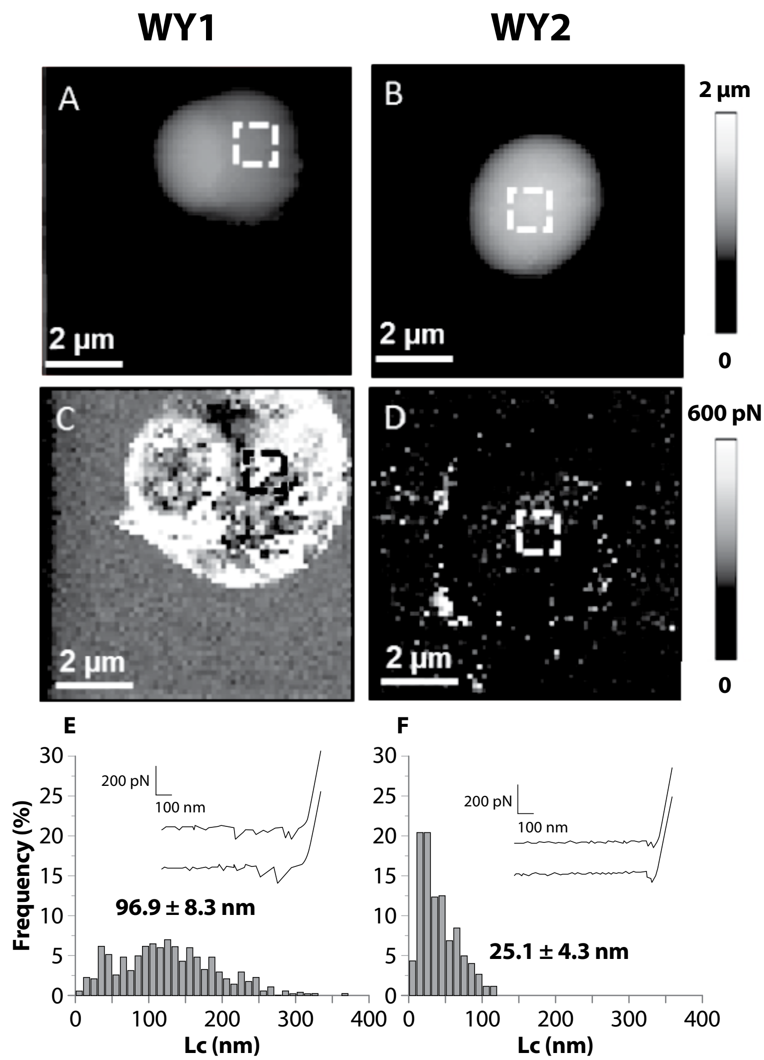

Figure 1. AFM images of the height (A, B) and adhesion (C, D) of strains WY1 and WY2. Distribution and average total length (Lc) of mannoproteins fully stretched on yeast cell walls.

length: 96.9 nm) of its mannoprotein chains stretched on the cell wall (Fig. 1E and 1F), it interacted strongly with the lectin Concanavalin A.

An innovative inactivation process combined with a unique yeast strain leading to an original autolysate with specific properties

Various autolysis conditions and thermal or physicochemical inactivation procedures were applied to the WY1 yeast to release its high content and long chain mannoproteins. Following several screening and optimizations in the lab, a specific physicochemical treatment was selected (MEX process) for its ability to disrupt yeast and release high molecular weight parietal mannoproteins. Figure 1 shows transmission electron microscopy (TEM) images from autolysates obtained through a classic thermal process (Fig. 2.A = SWYT-WY1) in comparison to the MEX treatment (Fig. 2.B = MEX-WY1). The autolysates obtained through thermal and physicochemical treatments had very different appearances. Although thermally inactivated WY1 yeasts maintained a certain cellular integrity and were more than 60% insoluble, physicochemical inactivated yeasts using the MEX process released more components that were 80% soluble.

Size exclusion chromatography (SEC) confirmed that the MEX soluble fraction contained a high level of high molecular weight polysaccharides compared to the classical thermal process (Figure 3).

Exploring into the action mechanism

Further experiments were undertaken at lab-scale in order to determine the mechanism of action of MEX-WY1 autolysate interactions with polyphenols extracted from Merlot grape skin. Interaction experiments were performed in a synthetic must with added Merlot grape skin polyphenols and the soluble fraction of the yeast autolysate (MEX-WY1-S) at a dose rate equivalent to the application of 30 g/hL of the total MEX-WY1. After 24 h contact (stirred at ambient temperature), samples were centrifuged and

2.A: SWYT-WY1 **2.B: MEX-WY1**

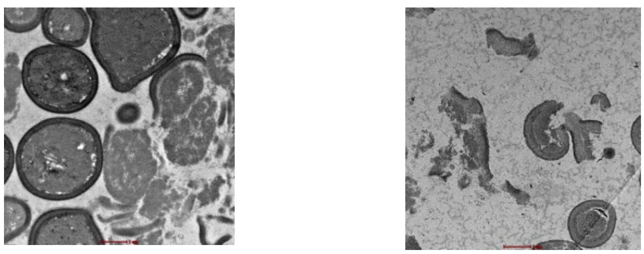

Figure 2. Microscopic (TEM) images of yeast derivatives produced either with a classical thermal process (A, SWYT-WY1) or a specific inactivation process (B, MEX-WY1).

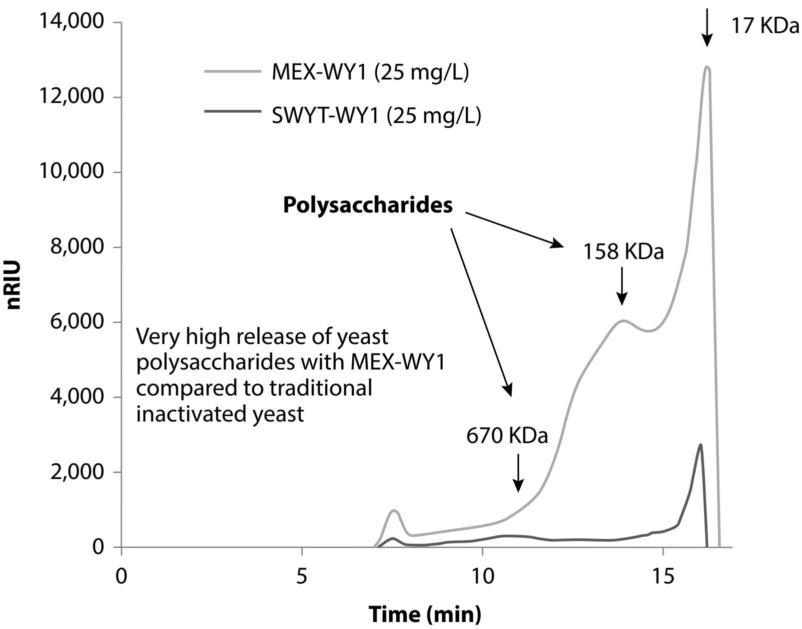

Figure 3. Size exclusion chromatography of SWYT-WY1 and MEX-WY1 soluble fractions

the supernatants were analyzed. Total Polyphenols (TP) and Total Red Pigments (TRP) were determined using UV-visible spectrophotometry, and BSA precipitable tannins and polymeric pigments were determined according to the procedure described by Boulet et al. (2016). Absorbency differences at 280 (ΔA 280) between the untreated and BSA-treated wines indicate the amount of tannins and pigments the protein (BSA) precipitated.

Interactions between polyphenols and MEX-WY1 soluble components did not lead to visible aggregation and precipitation. Only a small measurable decrease of the TP and TRP indexes was observed (around 5% of TP and 6% of TRP) between the control (synthetic must + polyphenols alone) and the samples after interactions.

BSA precipitation determination showed a lower precipitation of tannins with the addition of the whole MEX-WY1 soluble fraction (Fig. 4 A) compared to the control. This would suggest a reduction of astringency with the addition of the specific autolysate. The very low PT and TRP decrease indicated the formation of stable complexes with high molecular weight tannins and pigments. This stabilization of polyphenols in solution by MEX-WY1-S could enable colour stabilization during fermentation and a reduction in astringency, as their complexation with autolysate's soluble components would make tannins unavailable to interact with salivary proteins that are involved in astringency perception.

To identify the specific soluble component involved in these interactions, MEX-WY1-S was fractionated into low (< 10 kDa) and high (> 10 kDa) molecular weight fractions and interactions with polyphenols were performed. The MEX-WY1-S autolysate was able to reduce tannin precipitation after BSA addition. This would indicate a lower precipitation with salivary proteins, thus a lower astringency. When fractionated, the high molecular weight components were more effective regarding the reduction of tannin precipitation. (Fig. 4 B).

Thus, these studies have demonstrated the role of macromolecules in MEX-WY1 autolysate in wine quality improvement, specifically colour stability and astringency. These macromolecules are mainly composed of mannoproteins with unique properties, obtained through the combination of a special yeast strain and a specific inactivation process.

Beyond the science, proof of impact in winemaking conditions

The final step in this study was to evaluate the performance of the MEX-WY1 specific autolysate under red winemaking conditions.

To study the effect of adding the specific autolysate MEX-WY1 under large-scale production conditions, numerous trials were conducted at pilot scale (1 hL) and production (50-200 hL) scale on various grape varieties in different grape growing areas in both hemispheres. For each trial, the objective was to compare standard red wine production (control) with MEX-WY1 autolysate (addition rate of 30 g/hL at the beginning of alcoholic fermentation) under the same winemaking process. Fermentation kinetics were monitored and the resulting wines were analyzed at different stages (post-alcoholic fermentation, post-malolactic fermentation, and post-stabilization). Batch homo-

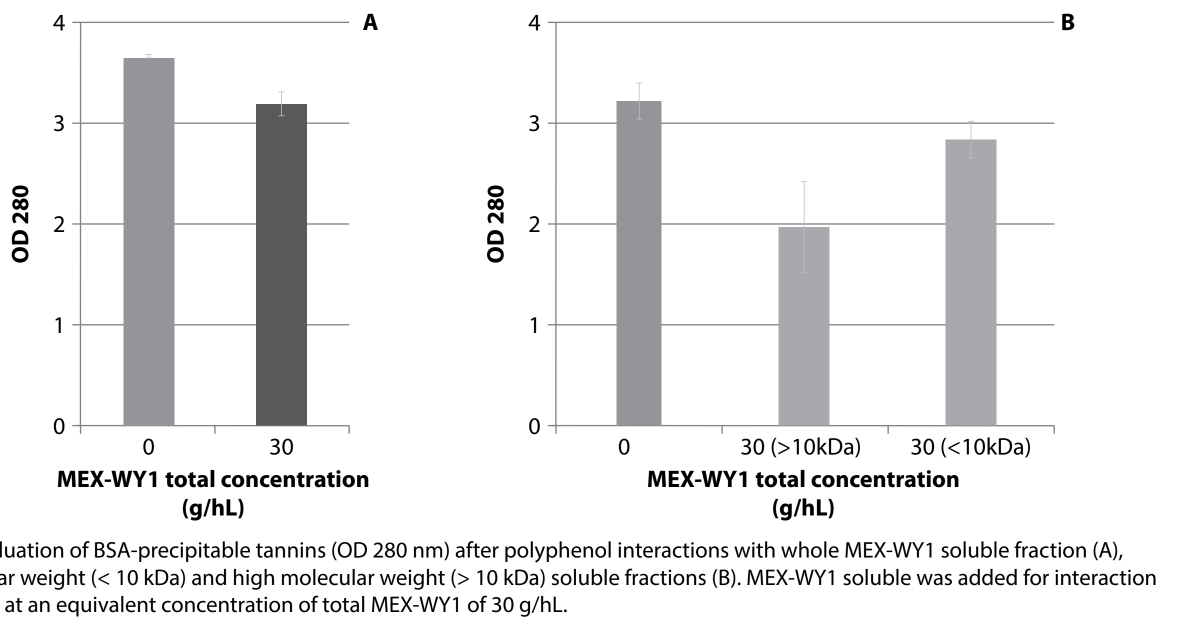

Figure 4. Evaluation of BSA-precipitable tannins (OD 280 nm) after polyphenol interactions with whole MEX-WY1 soluble fraction (A), low molecular weight (< 10 kDa) and high molecular weight (> 10 kDa) soluble fractions (B). MEX-WY1 soluble was added for interaction experiments at an equivalent concentration of total MEX-WY1 of 30 g/hL.

geneity was checked by measuring classic physicochemical parameters. The colour of the wines was evaluated through spectrophotometry and by measuring tristimulus values (CieLab). The wines were subjected to a post-stabilization sensory analysis and the saliva precipitation index (SPI) assay.

Fermentation kinetics in the numerous trials were not affected by the addition of MEX-WY1. The effect of MEX-WY1 on colour stability and wine sensory qualities are described below.

Effect on the colour of red wine

In numerous trials, the addition of the specific autolysate MEX-WY1 at the beginning of fermentation was observed to have a positive effect on wine colour. An example is shown in Figure 5, which shows the colour (parameters L, a, b) measured in Pinot Noir wines from trials conducted in New Zealand (Marlborough, 2016). The wine from the fermentation using MEX-WY1 had a darker, redder colour. The ΔE calculated based on the three parameters was 4.7. It is widely recognized that a trained professional is able to detect an average ΔE of 3 in red wine.

Another example is shown in Figure 6., which highlights the impact the addition of MEX-WY1 has on wine colour parameters after alcoholic fermentation (Fig. 6. A.) and on the corresponding wines after bottling (Fig. 6.B.). Colour intensity was higher after alcoholic fermentation when compared to the control and this improvement of colour was confirmed even after malo-lactic fermentation and after bottling, with a ΔE of 2.5.

Effect on the sensory qualities of red wine (fruitiness, mouthfeel, overall quality)

Trials using the specific autolysate MEX-WY1 demonstrated that several sensory characteristics of red wine can be improved: reduced astringency, better overall mouthfeel, and riper, fruitier aromas.

- Significant reduction in astringency:

The Saliva Precipitation Index (SPI) measures the reactivity of salivary proteins to polyphenols in wine and it is a good estimate of wine astringency (Rinaldi et al., 2012). Figure 7 shows SPI of Grenache wine made with the Thermo Flash process, which is known to promote significant

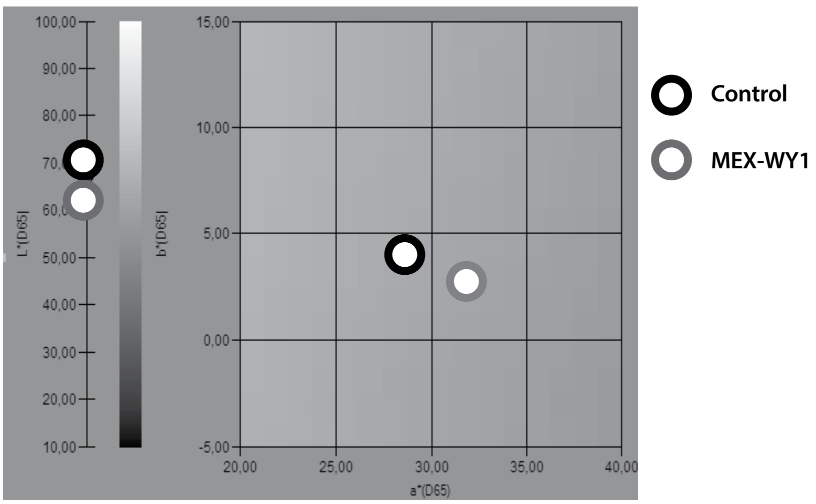

Figure 5. Wine colour as determined by CieLab measurements (L, a, b parameters) in Pinot Noir wines (Marlborough, New Zealand, 2016) from MEX-WY1 (MEX-WY1 added at the beginning of fermentation) and Control fermentations.

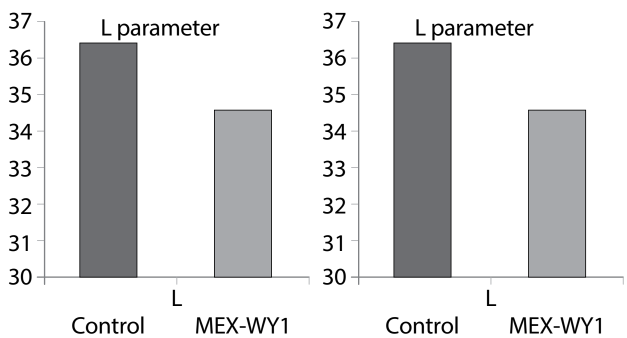

Figure 6. Pinot Noir, Burgundy, 2017, comparative trial: L Analysis (L, a, b) after alcoholic fermentation (6. A.) and after bottling (6. B.).

phenolic extraction and can lead to pronounced astringency. We can see that wine fermented with MEX-WY1 has significantly lower SPI compared with the control (38 versus 52). This difference directly correlates with reduced astringency in the MEX-WY1 wine.

- Overall improvement in the mouthfeel and structure of red wine:

Apart from the reduced astringency observed, most of the trials demonstrated an overall improvement in the perceived wine structure and mouthfeel.

Figure 8 illustrates the impact the early addition of the specific autolysate has on the sensory attributes of a Tempranillo treated wine compared to the control: higher tannic structure and fuller body.

Thus, the mechanisms and interactions observed in the model studies above have an impact not only on wine astringency, but also on other taste characteristics related to the wine's mouthfeel and structure.

- Enhanced fruit maturity:

In a number of the winery trials, some unexpected differences in aroma were noted, including fruit maturity and vegetal and grass characteristics. For example, Cabernet Sauvignon (Bordeaux, France, 2016) wine made from grapes harvested and fermented under the same condition, either with or without the addition of the specific autolysate MEX-WY1 at a rate of 30 g/hL at the beginning of fermentation, showed a different aroma sensory profile (Figure 9). The MEX-WY1 treatment produced a significant difference (10% confidence level) in "fruit maturity," i.e., more mature fruit notes, compared to the control. The control wine was considered to be slightly more vegetal and the MEX-WY1 wine to have more red/black fruit notes.

Summary

Recent research has given us a much better understanding of how yeast and phenolic compounds interact in red wine, enabling us to better characterize the biochemical and biophysical properties of yeast with unique wine-relevant characteristics. We have described the development of a specific yeast autolysate with unique wine sensory

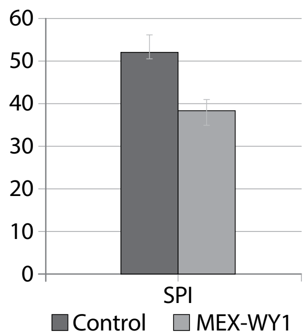

FIGURE 7. Saliva Precipitation Index (SPI) measured in Grenache wine (France, Côtes du Rhône, 2016). The only variable was the addition of the specific autolysate at 30g/hL at the beginning of fermentation in the MEX-WY1 treatment compared to the Control without MEX-WY1.

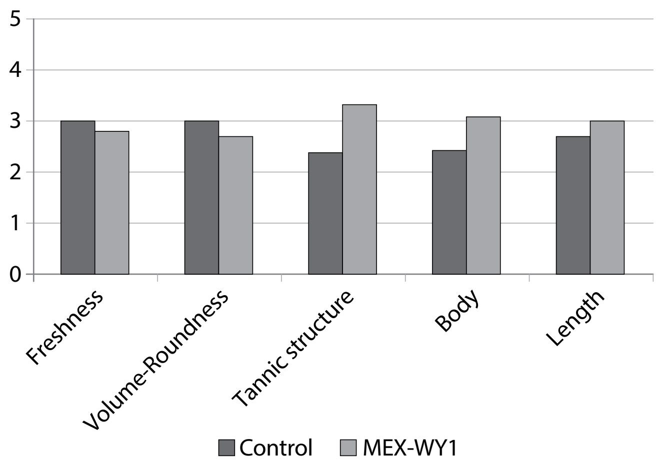

FIGURE 8. Winery-trial, Tempranillo wines made with the specific autolysate MEX-WY1 added (30g/hL) at the beginning of fermentation (MEX-WY1 treatment) or without (Control treatment), 2016. Sensory analysis by a panel of professionals.

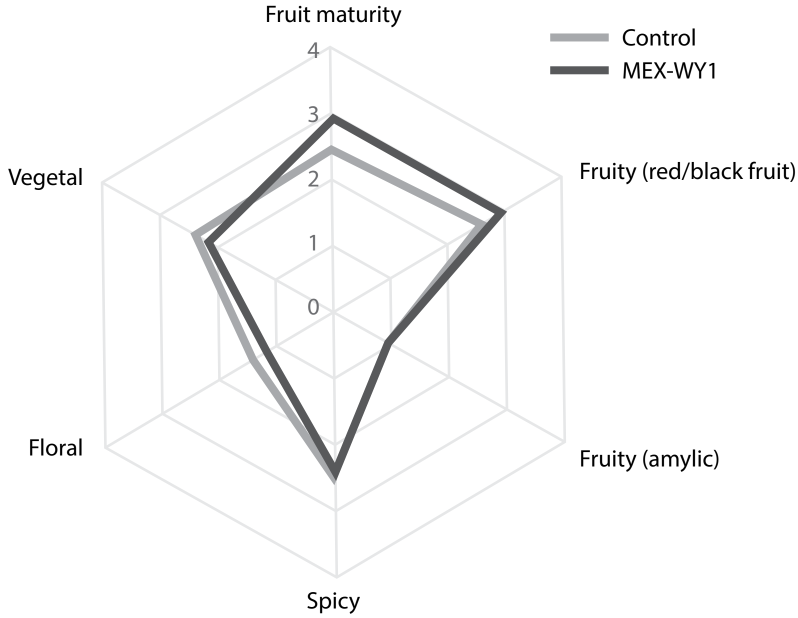

Figure 9. Aroma analysis by a panel of second-year student enologists (Toulouse, France, March 2017) of a Cabernet Sauvignon (Bordeaux, France, 2016) made with the specific autolysate MEX-WY1 added (30g/hL) at the beginning of fermentation (MEX-WY1 treatment) or without (Control treatment)

impacting properties. A yeast autolysate (MEX-WY1) was prepared from a wine yeast with distinctive characteristics. Studies using model grape must revealed the involvement of mannoproteins in the soluble fraction of the autolysate in the formation of stable complexes that contribute to colour stabilization and reduction in wine astringency.

Winery trials demonstrated that adding the specific autolysate MEX-WY1 at the beginning of fermentation had a positive effect on wine sensory characteristics such as colour, mouthfeel, and fruitiness in red wine, especially Pinot Noir wines. Thus, the new specific autolysate constitutes a unique tool to improve colour and texture management in Pinot Noir.

MEX-WY1 has been released as commercial product, OPTI-MUM RED™.

The research work described in this article is from a collaboration between INRA/Montpellier Supagro, LISBP/INSA Toulouse, and Lallemand.

Special acknowledgement to Master's student Elissa Abi Habib.

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CO-INOCULATION STRATEGIES IN PINOT NOIR

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Abstract

Malolactic fermentation (MLF) is an integral step in red winemaking that goes beyond wine de-acidification, as MLF will also influence the composition of volatile fermentation-derived compounds with concomitant effects on wine sensory properties and the wine colour profile. Long-established winemaking protocols for MLF induction generally involve inoculation of bacteria starter cultures post-alcoholic fermentation; however, more recently there has been a trend to introduce bacteria earlier in the fermentation process. Co-inoculation greatly reduces the overall fermentation time, and the rate of alcoholic fermentation is generally not affected by the presence of bacteria. In addition, the fermentation-derived wine volatiles profile is distinct from wines produced where bacteria were inoculated at a later stage of alcoholic fermentation. In most red wine studies an overall slight decrease in wine colour density is observed following MLF, but this is not influenced by the MLF inoculation regime. However, there can be differences in anthocyanin and pigmented polymer composition, with co-inoculation exhibiting the most distinct profile. Studies in Pinot Noir have shown some important loss in colour following MLF. The rapid onset and speed of completion of MLF can influence "clarity" of Pinot Noir wine. In this study, a wine with a higher clarity value (CieLab, L value) is less coloured than a wine with a lower clarity value. Another study showed that a delay in MLF did not affect loss of colour, but a longer delay in MLF reduced the loss of polymeric pigment, and a 200-day delay to initiate MLF resulted in no losses compared to the control. Much higher colour density and polymeric pigment content have been observed in Pinot Noir wines inoculated with *Lactobacillus plantarum* compared with *O. oeni*.

Introduction

Red wine colour depends on anthocyanin extraction from grape skin and its stabilization in wine in a coloured form. The extraction and stabilization of phenolics can be a particular challenge for Pinot Noir winemakers. Compared with other red wine grape varieties, Pinot Noir grapes have low anthocyanin content, and what anthocyanin is present is of the less-stable non-acetylated variety.

Stabilization of anthocyanins occurs through reactions between anthocyanins and tannins to form pigmented tannins and through co-pigmentation of anthocyanins. In addition to being low in anthocyanin concentration, Pinot Noir grapes have a low skin-to-seed tannin ratio compared with many other red wine grape varieties.

Winemaking practices may influence red wine colour, but most studies have focused on physical and chemical parameters, including temperature and length of maceration, rather than the role of wine microorganisms such as wine yeast and malolactic bacteria *Oenococcus oeni* (*O. oeni*). Malolactic fermentation (MLF) occurs in wine as the result of the metabolic activity of wine lactic acid bacteria (LAB). MLF reduces wine acidity and modifies wine flavour, both of which are considered to be beneficial to wine quality. Additionally, the use of selected strains of wine LAB allows for better control of the time frame of L-malic acid degradation. Sensory studies show that flavour compounds produced by wine LAB impart recognizable changes to the flavour characteristics of wine (Laurent et al. 1994, Costello et al. 2012 a,b, and Knoll et al. 2011). The timing of the bacterial addition also influences the sensory profile of Shiraz wine, including wine colour and phenolics, and volatile fermentation-derived compounds (Abrahamse and Bartowsky 2012a). Burgundy winemakers often delay MLF because the resulting wines have anecdotally been reported to have superior colour. Gerbaux and Briffox (2003) showed the influence of the rapid onset and speed of completion of MLF on the "clarity" of Pinot Noir wine. In this study, a wine with a higher CieLab 'L' clarity number is less coloured than a wine with a lower clarity value. Recent studies reported that MLF can influence red wine colour independent of pH (Burns and Osborne 2013) and that the loss of colour over time may be associated with a reduction in polymeric pigment content. Polymeric pigments play a role in a wine's long-term colour stability because they are resistant to SO_2 bleaching and oxidation.

Red Wine Colour And Pinot Noir Challenges

Wine colour is of course a very important attribute of red wine, and it is the first thing a consumer will notice in the glass. The formation of wine colour is influenced by numerous chemical and microbial parameters which are all interlinked (Figure 1). Colour is primarily affected by anthocyanins present in grapes and their extraction during winemaking (Fulcrand et al 2006). Stable polymeric pigments can be derived through reactions between anthocyanins and the yeast metabolites pyruvic acid or acetaldehyde, which are resistant to SO_2 bleaching. Moreover, they play a role in long term wine colour stability.

Pinot Noir grapes present several major winemaking challenges as they have tightly packed bunches which are susceptible to disease, low skin-to-seed tannin ratio, and low anthocyanin content predominated by the less stable acetylated form, which can hinder stable wine colour formation (Figure 2).

The role of bacteria and MLF in red wine colour is not well understood, however it is beginning to unravel. Wine LAB can metabolise acetaldehyde, including the SO_2-bound form, which then will impact wine colour (Asenstorfer et al 2003, Osborne et al 2000, Wells and Osborne 2012).

Timing Of Selected Wine Lactic Acid Bacteria Inoculation

Although not recommended, MLF can be conducted by indigenous wine LAB present in the winery infrastructure, which may occur during alcoholic fermentation (AF) or immediately after its completion. Traditionally, when selected cultures of known wine LAB are used, inoculation is performed at the completion of AF. Before 2003, researchers at the Université de Bordeaux recommended making the wine LAB addition only after completion of AF. They felt this timing would avoid the production of acetic acid and D-lactic acid, compounds derived from the heterofermentative carbohydrate metabolism of LAB (Ribéreau-Gayon et al. 1975). They proposed that wine LAB added at earlier points during AF may result in slow or stuck yeast fermentation, or result in MLF inhibition due to yeast antagonism. To date, none of these concerns have been observed when both AF and MLF have been properly managed.

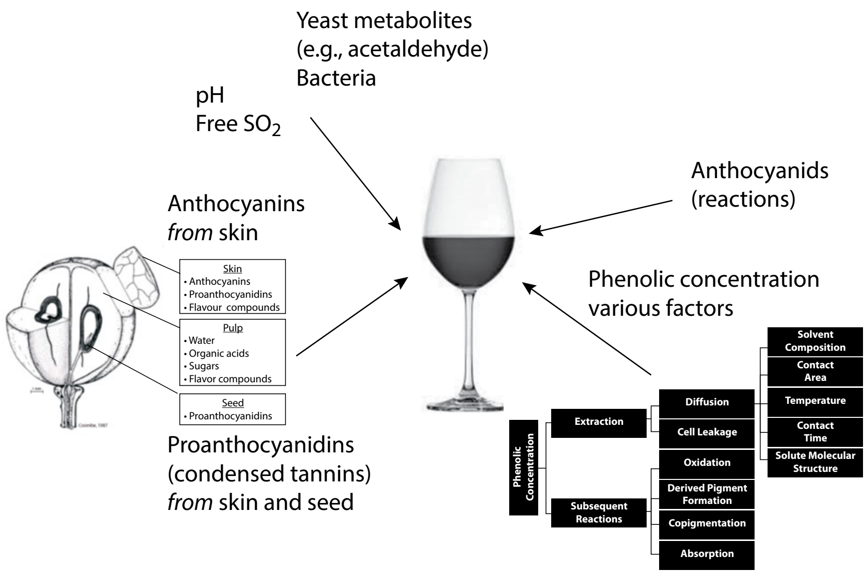

Figure 1. Red wine colour is influenced my numerous chemical and microbial parameters (adapted from Setford et al 2017).

The inoculation of selected wine LAB into juice along with yeast was proposed because it was thought that nutrient availability would be heightened, and the absence of alcohol would allow wine LAB to better acclimatize to environmental conditions and grow more vigorously. Beelman and Kunkee (1985) showed that MLF in the presence of fermentable sugars does not necessarily lead to the production of excessive amounts of acetic acid, as long as yeast fermentation starts promptly and goes to completion (Krieger 2002, and Sieczkowski 2004). There are several time points during wine production when selected wine LAB can be added (Figure 3).

- Co-inoculation with yeast and selected wine LAB; Selected wine LAB added 24 to 48 hours after yeast addition (48 to 72 hours if 80 to 100 ppm of SO_2 is added at crushing)
- Early inoculation; Selected wine LAB added during active AF or at an approximate density of 1040/1030 (8°/10°Brix)
- Post-alcoholic fermentation; At the end of, or just after, completion of AF
- Delayed inoculation; 2 to 6 months after completion of AF

Co-Inoculation

There are definite advantages for using co-inoculation in the production of wines destined for early consumption. This process enables wine LAB to acquire ethanol tolerance, allowing MLF to occur during the last third of AF (Vuchot 2004) and finish quickly. Co-inoculation can ensure the early implantation and dominance of selected wine LAB. Use of this method promotes the early stabilization of the wines, renders them marketable at an earlier date, and minimizes the possibility of developing spoilage organisms, such as acetic acid bacteria and *Brettanomyces* yeast (Pillet et al 2007). This technique, combined with the use of reliable yeast strains with good fermentation characteristics that will support MLF and satisfactory yeast nutrition, will ensure healthy yeast and malolactic fermentations with good kinetics.

Laboratory studies by Azzolini et al. (2010) using co-inoculation in a variety of red table wines with pH levels greater than 3.5 found that acetic acid levels were within acceptable limits (Figure 4.)

The trend towards harvesting higher maturity grapes has resulted in the processing of higher pH musts and the production of wines containing increased levels of alcohol.

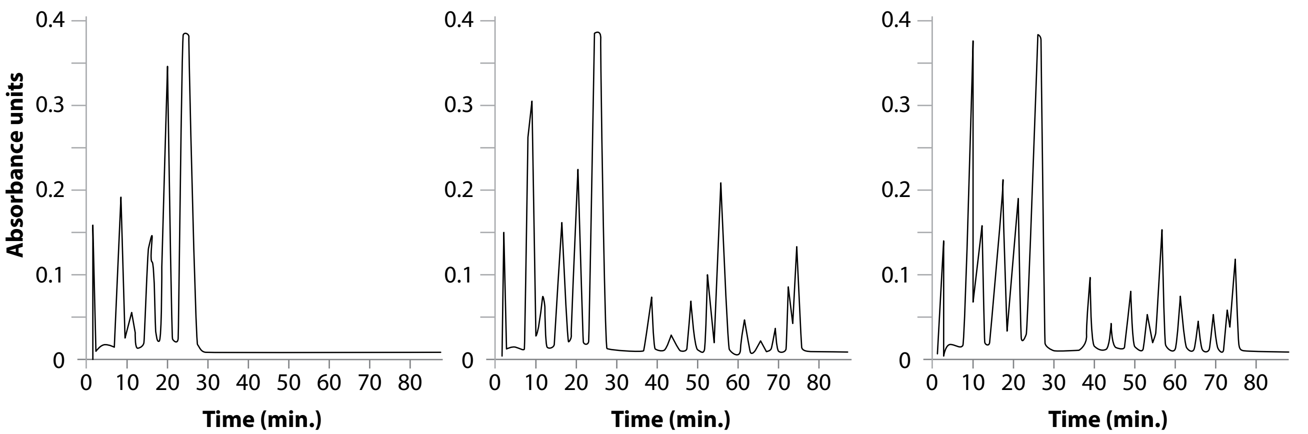

Figure 2. Pinot Noir has a different anthocyanin composition than most red grape varieties (Mazza et al 1999).

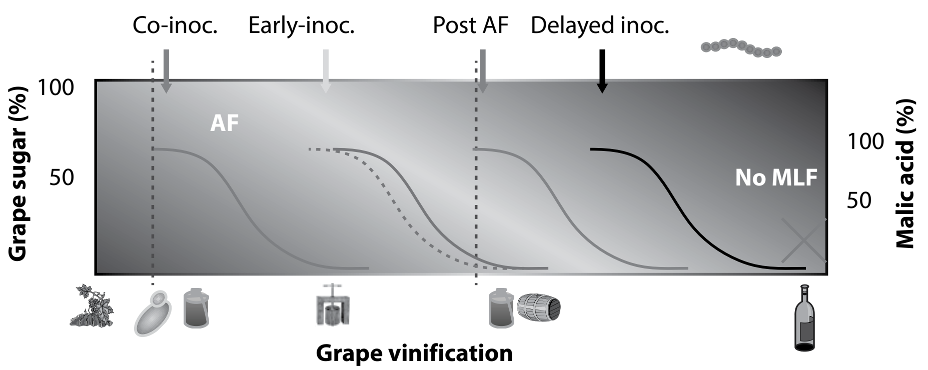

Figure 3. Inoculation regimes for selected wine lactic acid bacteria (Adapted from Bartowsky, AWRI, 2010).

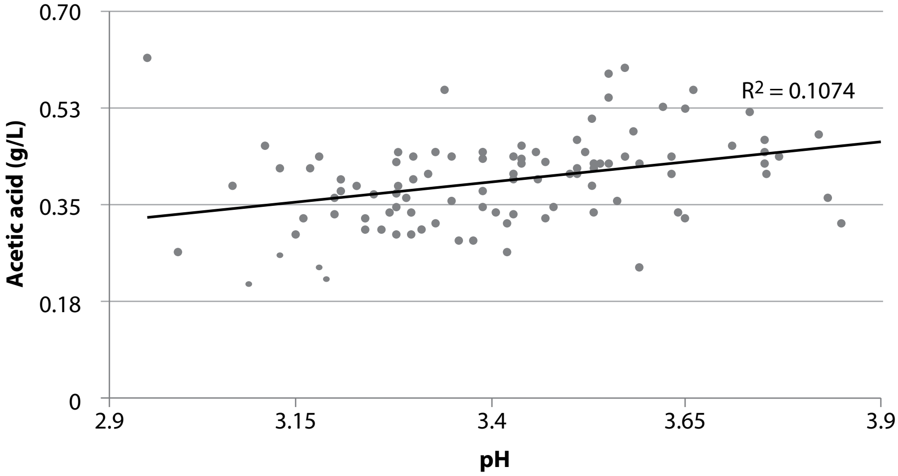

Figure 6. Degradation of malic acid (top graph) and acetaldehyde (bottom graph) by 12 strains of commercial wine lactic acid bacteria inoculated in wine post-AF (Jackowetz and Mira de Orduna 2012).

These conditions favour the growth of indigenous bacteria and often *O. oeni* does not prevail at the end of AF. The use of indigenous wine LAB is problematic and not recommended, so under the conditions described above, direct inoculation of *Lactobacillus plantarum* in the freeze-dried form may be a good option (du Toit et al. 2011). The use of this new generation of selected wine LAB starter culture offers several advantages. The *L. plantarum* MLF inoculum provides early implantation and dominance, as well as predictable and complete MLF. As it degrades hexose sugars by the homofermentative pathway, which poses no risk of acetic acid production from the residual sugars that may be present in high pH wines, it is an interesting alternative to the customary *O. oeni* starter culture (du Toit et al. 2011).

Abrahamse and Bartowsky (2012a) showed that co-inoculation greatly reduced the overall fermentation time in a Shiraz wine by up to 6 weeks, that the rate of alcoholic fermentation was not affected by the presence of bacteria, and that the fermentation-derived wine volatiles profile was distinct from wines produced where bacteria were inoculated late or post-alcoholic fermentation. An overall slight decrease in wine colour density observed following MLF was not influenced by the MLF inoculation regime. However, they found differences in anthocyanin and pigmented polymer composition, with co-inoculation exhibiting the most distinct profile (Table 1).

Sequential inoculation – Post-alcoholic fermentation

Traditional inoculation at the end of AF does not pose the risk of bacterial metabolism of sugars and the resultant increase in VA, nor does excessive lactic acid production, known as *piqûre lactique*, occur. Inoculation at this time point avoids much of the toxicity attributed to some carboxylic acids, such as fumaric acid, as their concentration declines after AF (Lafon-Lafourcade 1983). The merit of inoculation at the end of AF can also be related to the availability of nutrients, nitrogen-containing bases, peptides, amino acids, and vitamins that result from yeast death and subsequent bacterial autolysis (Kunkee 1967).

Another advantage is simply logistical. When using sequential inoculation, wines that should undergo MLF can be separated from wines whose acidity needs to be conserved. The vinification process can be conducted so that only one type of fermentation at a time is monitored.

Table 1. Spectrophotometric analysis of Shiraz wines following alcoholic and malolactic fermentation using different *O. oeni* inoculation timing (Abrahamse & Bartowsky 2012 a,b)

Inoculation treatment	Colour density (Au)	Hue	Total anthocyanins (mg/L)	Total phenolics (au)	SO_2-resistant pigments
Co-inoculation	12.0 ± 0.7	0.5 ± 0.0	475 ± 45	44 ± 4	2.2 ± 0.1
Mid alcoholic	12.7 ± 0.8	0.7 ± 0.1	284 ± 105	38 ± 2	3.2 ± 0.2
Pressing	12.0 ± 0.9	0.6 ± 0.0	390 ± 52	42 ± 3	3.0 ± 0.2
Post-alcoholic	11.4 ± 0.3	0.6 ± 0.0	380 ± 43	41 ± 2	2.9 ± 0.2
No MLF	15.5 ± 1.8	0.6 ± 0.0	430 ± 52	44 ± 2	3.6 ± 0.8
Values are mean ± SD Au absorbance units					

Often, this is perceived as a lower risk of cross-contamination.

However, exposure to the high levels of ethanol present may result in delayed MLF, especially in wines produced in hot climates. If wine conditions are not limiting, selected wine LAB added after the AF can achieve cell concentrations comparable to those inoculated into must. In cases of nutrient limitation or adverse wine chemical parameters, the addition of a bacterial nutrient will support MLF. In instances where alcohol levels exceed 14.5% v/v, selected wine LAB strains tolerant to alcohol should be used or they should be acclimatized before inoculating into wine. The additive inhibiting the effect of ethanol, pH, and SO_2 must be considered, and the strain best adapted to the conditions should be chosen.

Delayed inoculation

Over the past 15 years, the quality of wine LAB starter cultures has substantially improved. The starter cultures available for direct inoculation into wine are easy to manage and allow for better control over MLF. Using this new generation of wine LAB starter cultures permits the early onset and rapid completion of MLF. In the Burgundy region of France, as well as other wine regions that produce mainly Pinot Noir wines, the rapid development of MLF is contrary to their traditional winemaking techniques, which have relied on spontaneous MLF. The increasing use of active, direct inoculation wine LAB starter cultures has led to more rapid MLF. Gerbaux and Briffox (2003) showed the influence of the rapid onset and speed of completion of MLF on the 'clarity' of Pinot Noir wine. Results are shown in Figure 5.

Increased colour stabilization was achieved under the following conditions: (i) Increased time between completion of AF and onset of MLF; (ii) Decreased speed of MLF; (iii) SO_2 addition delayed until after completion of MLF (Gerbaux and Briffox 2003).

Conditions such as high SO_2, temperatures below 10°C, or the addition of Lysozyme will inhibit or delay MLF, help stabilize colour, and avoid colour loss in lightly pigmented wines. Burn and Osborne (2013) state, "Delaying MLF did not impact loss of colour at 520 nm but delaying MLF for increasing time periods reduced the loss of polymeric pigment to the point that after 200 days no loss was noted compared to the control." The Mira de Orduña team have shown wine LAB play an essential role in post-AF acetaldehyde removal (Jackowetz et al. 2012). Their study with 12 commercially available strains of *O. oeni* on the kinetics of major SO_2 binding compounds during MLF in wine showed a delay of five to seven days for the degradation of acetaldehyde compared to malic acid metabolism (Figure 6). They propose to wait for completion of acetaldehyde degradation if bound SO_2 levels are sought to be reduced.

In a study by Burns and Osborn (2015) investigating potential causes for the loss of Pinot Noir wine colour and polymeric pigment after MLF, they suggested the role of acetaldehyde and/or pyruvic acid degradation by *O. oeni* (Table 2) during MLF as a cause for reduced polymeric pigment formation independent of the pH change (Figure 7). *Oenococcus oeni* strains were able to metabolise both acetaldehyde and pyruvic acid to varying degrees, which in turn impacted wine colour.

Pinot Noir wines that had undergone MLF were supplemented with acetaldehyde and/or pyruvic acid to equivalent concentrations in control wines that did not undergo MLF. These wines were stored for 90 days and wine colour measured again. Wines with added acetaldehyde had

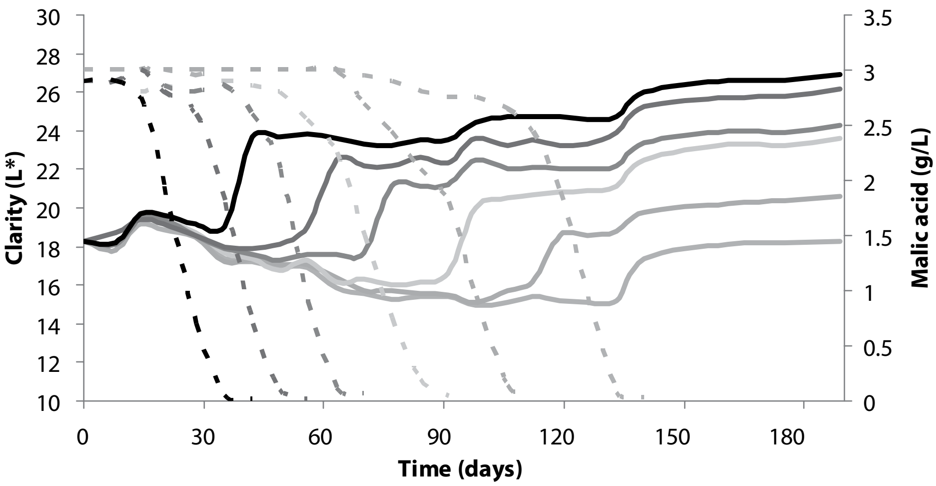

FIGURE 5. Influence of malolactic fermentation kinetics on the clarity of Pinot Noir wine (Gerbaux and Briffox, 2003).

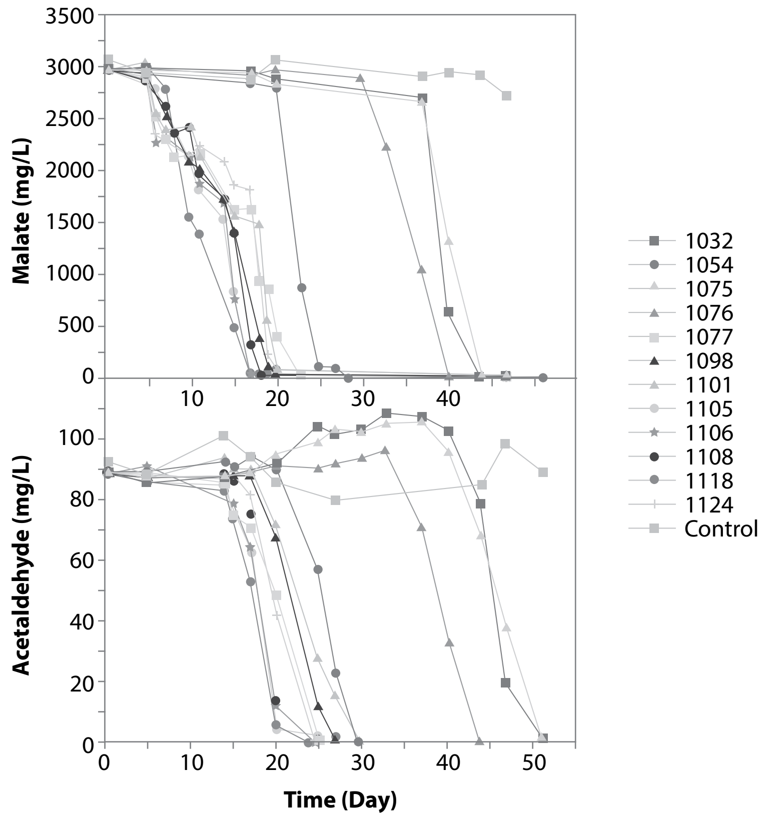

Figure 6. Degradation of malic acid (top graph) and acetaldehyde (bottom graph) by 12 strains of commercial wine lactic acid bacteria inoculated in wine post-AF (Jackowetz and Mira de Orduna 2012).

Table 2. Pinot Noir – Degradation of acetaldehyde and pyruvic acid by O. oeni following simultaneous (co-inoculation together with the yeast) and sequential inoculation (VP41, Alpha, MLB). Acetaldehyde and pyruvic acid concentrations were determined at end of AF (pre-MLF) and MLF (post-MLF).

Treatment	Acetaldehyde pre-MLF (mg/L)	Acetaldehyde post-MLF (mg/L)	Pyruvic acid pre-MLF (mg/L)	Pyruvic acid post-MLF (mg/L)
Control	18.26	19	29.66	29.52
Simultaneous	7.21	8.83	11.25	10.51
VP41™	19.02	10.34	30.66	18.79
Alpha™	18.66	6.86	32.04	17.78
MLB	20.55	4.95	28.95	8.42

more colour and polymeric pigment than MLF wines with no additions, and those wines with added pyruvic acid had no change in colour or concentration of polymeric pigment (Figure 8).

Additional experiments were undertaken to better understand why MLF wines contained lower polymeric pigments. Since increased polymeric pigment formation in wines where MLF was delayed was probably due to the presence of acetaldehyde in the wine, acetaldehyde and pyruvic acid were added back to the MLF wines to the concentrations present in the control wine that had not undergone MLF (Figure 8). Addition of pyruvic acid did not reduce colour or the loss of polymeric pigment caused by MLF. By contrast, compared to the wines that underwent MLF only, the addition of acetaldehyde increased colour intensity and polymeric pigment content.

More recent studies investigated different LAB strains with different acetaldehyde degradation capabilities to better understand how to minimize colour loss caused by MLF; *O. oeni* O-MEGA™ with a slower acetaldehyde degradation, *O. oeni* ALPHA™ and *L. plantarum* ML-PRIME™ which metabolizes acetaldehyde slowly on a delayed basis (Table 3).

Acetaldehyde degradation in the wine fermented with *L. plantarum* Prime (ML Prime™) was much higher than in the wines fermented with the two *O. oeni* strains (Omega™ and Alpha™) and close to the concentrations in the Control wine without MLF. Colour and polymeric pigment

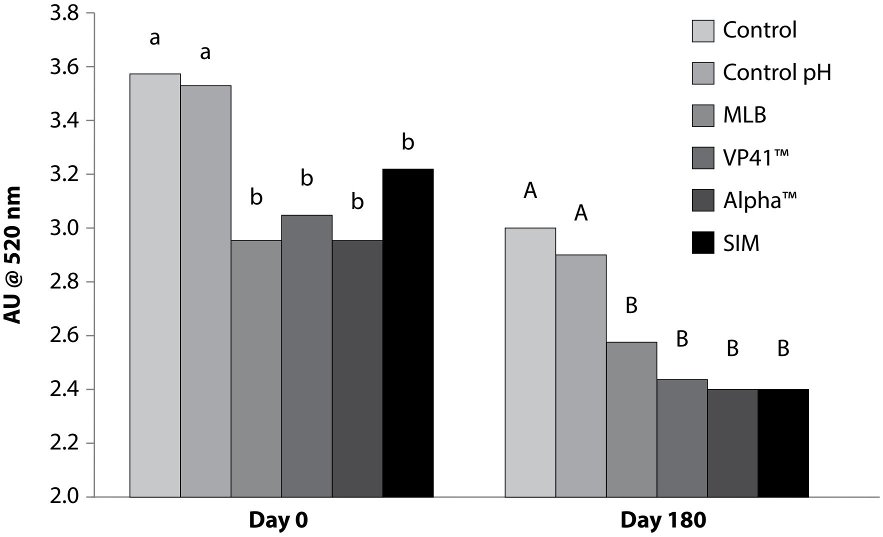

Figure 7. Pinot Noir colour was measured directly after malolactic fermentation (day 0) and 180 days after MLF with different wine LAB strains – simultaneous (SIM) and sequential inoculation (MLB, VP41, Alpha) and pH adjustment back to the pH of the control wine.

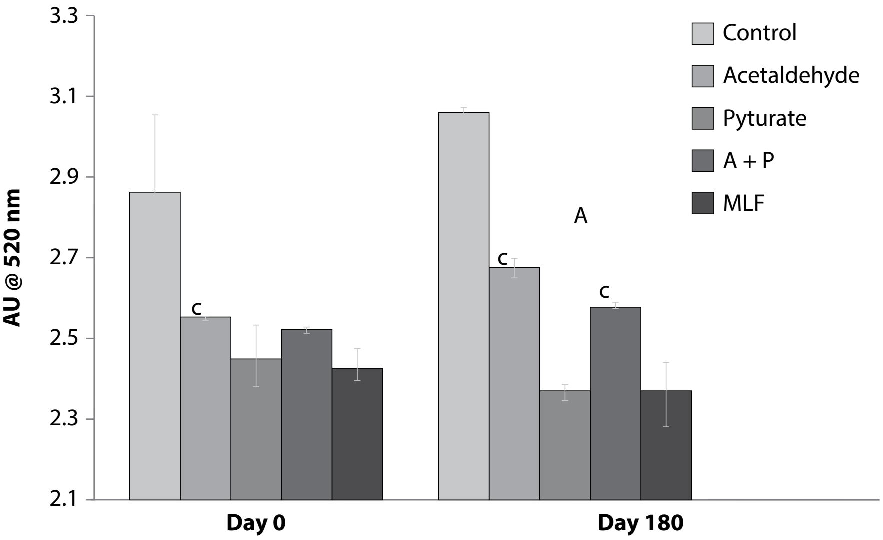

Figure 8. Pinot Noir colour – small improvements in colour at 520 nm with acetaldehyde (A) and pyruvate (P) additions back to the MLF wines (Burns and Osborne, 2015).

Table 3. Acetaldehyde concentration (mg/L) in a Pinot Noir wine pre- and post-MLF and a control wine without MLF.

	Pre-MLF	Post-MLF
Control	8.37	9.2
ML-Prime™	8.37	7.67
O-MEGA™	8.37	2.33
Enoferm ALPHA™	8.37	1.7

values were measured in the wines post-MLF (Day 0) and again after 30 and 90 days storage at cellar temperatures (Figure 9). While a reduction in colour was observed in wines that underwent MLF with Alpha or Omega, no loss of colour or polymeric pigment was noted in wines that underwent MLF with Prime.

Although results from these studies suggest that winemakers may be able to improve the polymeric pigment content of Pinot Noir wine by delaying MLF and storing the wine at cool cellar temperatures, Burns and Osborne also highlighted that delaying MLF by more than 100 days may increase the risk of microbial spoilage especially if *Brettanomyces* is part of the winery's microflora. Gerbaux

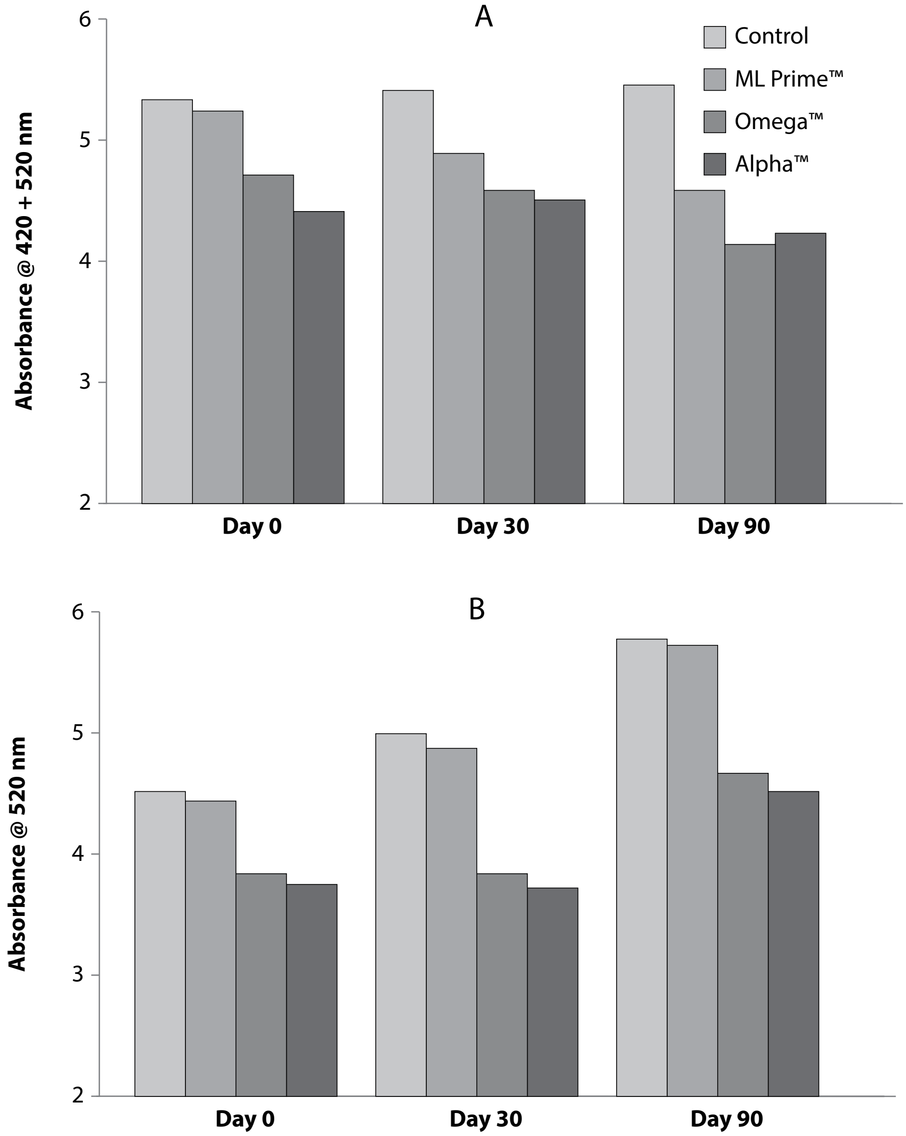

Figure 9. Wine colour @ 420 + 520 nm (A) and polymeric pigment content (B) @ 520 nm of Pinot Noir wines that did not undergo MLF (Control) or underwent MLF with different malolactic bacteria strains. Wines were assessed after 0, 30, and 90 days storage at 13°C.

and Briffox (unpublished 2017) have shown the presence of *O. oeni* clearly suppressing growth of *Brettanomyces* (Figure 10). A Pinot Noir wine was contaminated prior to inoculation with *O. oeni* strains IFV 04A1 + 11B3, and IFV 27.1 with a *Brettanomyces* at 100 cfu/ml. In the presence of *O. oeni* IFV 27.1 growth of *Brettanomyces* was limited and they finally died off. The volatile phenol concentrations remained low at 110 μg/ml due to the biocontrol with *O. oeni*.

CONCLUSIONS

It is known that following MLF there is a slight decrease in red wine colour; however, this is not dependent upon the timing of bacteria inoculation. Pinot Noir wine colour presents its own unique challenges, particularly because of its low tannin and anthocyanin content, with a bias towards a less stable acetylated form. Recent studies have shown that MLF in Pinot Noir can lead to colour loss that is independent of the pH change brought about by MLF. Some of this could be due to the *O. oeni's* ability to metabolise acetaldehyde, an integral component of stable wine colour. *Lactobacillus plantarum* has been shown to better preserve wine colour than *O. oeni*. Recent studies have indicated that delaying the onset of MLF (> 90 days) can mitigate the loss of wine colour. However, this approach to use a delayed MLF for more stable colour must be carefully weighed against potential microbial spoilage, including *Brettanomyces* and biogenic amine formation (indigenous LAB).

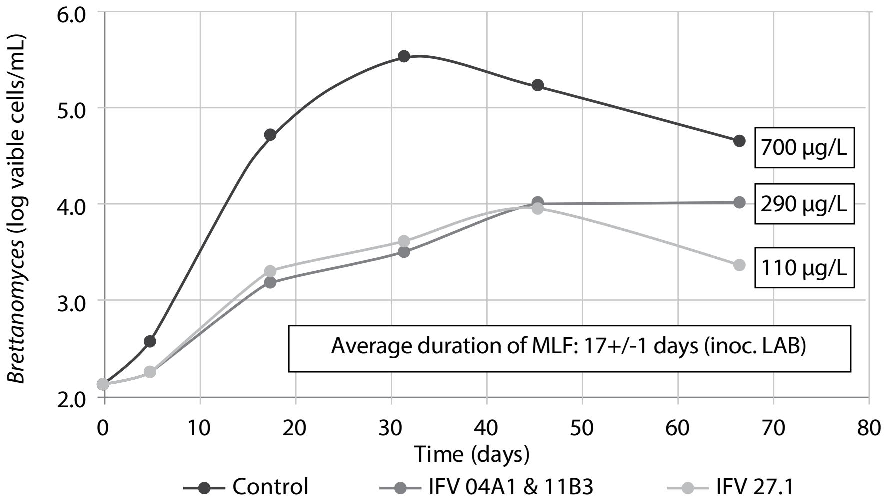

Figure 10. Influence of selected wine bacteria strains on growth of Brettanomyces and production of volatile phenols by Brettanomyces in Pinot Noir wine; 13% v/v, pH 3.4, 16°C (Control)

Acknowledgements

The authors thank their research partners Vincent Gerbaux and Caroline Briffox (IFV Beaune, France), Ramón Mira de Orduña (Cornell University USA, EIC Changins CH), and James Osborne (Oregon State University USA) for contributing their research and unpublished results to this paper.

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