Assessment of Vineyard Nitrogen Management upon Grape Chemistry

James Russell Moss

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degrees of

Master of Science in Life Sciences
In
Food Science and Technology

Amanda C. Stewart, Co-chair
Tony K. Wolf, Co-chair
Gregory M. Peck
Sean F. O'Keefe

29 July 2016
Blacksburg, VA

Key words: Vitis vinifera, foliar urea, calcium nitrate, YAN, UPLC

Assessment of Vineyard Nitrogen Management upon Grape Chemistry

James Russell Moss

ABSTRACT

To combat excessive vine vigor, many vintners have employed intensive cover cropping techniques. While cover crops provide a multitude of benefits to the farming system, they can compete for nutrients and water. The seemingly ubiquitous adoption of cover crops in the Eastern United States has led to vines and grape musts which are deficient in nitrogen (N). A must that is deficient in yeast assimilable nitrogen (YAN) can lead to the production of off aromas and stuck or sluggish fermentations. It has also been suggested that musts with limited amino nitrogen sources can result in wines with less fruity aromas than those with a higher starting amino acid content. Varying rates of calcium nitrate were applied to the soil at bloom and foliar urea was sprayed at a Sauvignon blanc and Petit Manseng (*Vitis vinifera* L.) vineyard. Perennial White and Crimson clover as well as foliar urea applications at véraison were utilized at a Vidal blanc (*Vitis spp.*) site. Foliar urea was effective at significantly increasing YANs in all experiments with some year to year variation in efficacy. Foliar urea applications slightly favored the production of ammonia over primary amino nitrogen. While most of the measured amino acids in fruit increased in concentration with the application of either soil or foliar N, foliar applications were more effective at increasing fruit amino acids. Of the amino acids measured, arginine and glutamine were the most increased by foliar urea applications, whereas proline was relatively unaffected. The use of clover as a perennial under-vine cover crop did not increase berry YAN. The application of foliar urea sprays may present an effective means by which vintners can easily increase must YANs and amino acid contents.

Acknowledgements

I am very fortunate to have had not one, but two great advisers over the course of this work; Dr. Tony Wolf and Dr. Amanda Stewart. I could not have asked for more passionate and knowledgeable folks. With their guidance, I was able to do more than I had expected and I gained invaluable experience during my tenure at Virginia Tech. I will always hold them in high esteem and count them among my closest friends and colleagues.

Sihui Ma was critical in conducting the amino acid analysis. Her drive will always be an inspiration to me. I will miss our late-night talks in the lab about China, wine and whatever else, as we derivatized and ran samples.

Tom "Tater Tom" Boudreau was critical in helping me quickly learn the NOPA and NH_4^+-N analyses. During our time together, Tom became one of my closest friends. I will miss using his desk for late-night lab dinners.

Our collaborators at GMV and ISV were crucial in the success of our research. This was industry focused research. I hope that other practitioners will find this work helpful in the management of their own vineyards and wineries.

Table of Contents

List of Tables

List of Abbreviations

Ala – Alanine
AREC – Alson H. Smith Jr. AREC (Petit Manseng)
Arg – Arginine
Asn – Asparganine
Asp – Aspartic acid
Cys – Cysteine
Gln – Glutamine
Glu – Glutamic acid
Gly – Glycine
GMV – Glen Manor
His – Histidine
ILE – Isoleucine
ISV – Indian Springs Vineyard
Leu – Leucine
Lys – Lysine
Met – Methionine
PDA – Photodiode array
Phe – Phenylalanine
Pro – Proline
Ser – Serine
TA – Titratable acidity
Thr – Threonine
Tyr – Tyrosine
UPLC – Ultra Performance Liquid Chromatography

Introduction

Due to the natural terroir of the Eastern United States, many vineyards experience exceedingly vigorous growth. Excessive vigor can lead to increased fungal disease pressure, due to a diminution of spray and light penetration (Austin et al. 2011). Increased vigor can also lead to higher juice pH due to increased potassium concentrations, as a result of leaf-on-leaf shading (Bledsoe et al. 1988; Dokoozlian and Kliewer 1995; dos Santos et al. 2007; Morrison and Noble 1990; Rojas-Lara and Morrison 1989; Smart et al. 1985). The increase in juice potassium brought on by increased leaf-on-leaf shading can also hinder the degradation of malic acid and lead to a higher malic to tartaric acid ratio, resulting in an unpleasantly tart acid profile in the wine (Hale 1977; Hunter et al. 2004; Lobit et al. 2006). Increased fruit shading can also lead to lower fruit temperatures. It is known that the concentration of methoxypyrazines is diminished via thermal degradation (Allen et al. 1991; Belancic and Agosin 2007; Scheiner et al. 2010). Also, it has been previously found that heat and sunlight can stimulate the formation of positive varietal aromas such as those arising from terpenes and thiols (Belancic et al. 1997; Skinkis et al. 2010; Song et al. 2015; Šuklje et al. 2014). It has also been found that higher heat (up to 35^{o}C) and sunlight can lead to increased phenolic concentrations, therefore high vine vigor can lead to wines with a lighter mouthfeel and less color due to less flavonoid development (Diago et al. 2012; Verzera et al. 2016).

To limit the deleterious effects of increased vine vigor, many viticulturists have begun using cover crops in their vineyards. Cover crops have previously been found to be an effective means of vigor suppression (Giese et al. 2014; Tesic et al. 2007). Cover crops are able to reduce vigor by competing with the vine for water and nutrients (Celette et al. 2009; Gouthu et al.

2012; Monteiro and Lopes 2007). Nitrate found dissolved in soil water is the primary form of N taken up by the vine roots (Keller 2015). Through competition for vine water, the cover crop is also able to effectively compete for N (Celette et al. 2009).

Competition for N brought on by the cover crop can lead to diminished perennial N reserves and less tissue N (Celette et al. 2009; Tesic et al. 2007). A decrease in N nutrition can lead to lower leaf chlorophyll content, which can lead to a lower photosynthetic rate (Chen and Cheng 2003). A lower CO_2 assimilation rate can lead to depressed berry growth and less ovule fertility and fruit set (Dokoozlian and Kliewer 1996; Ewart and Kliewer 1977; Ollat and Gaudillere 1998). Due to the aforementioned reasons, cover crops are often associated with lower fruit yields when compared to vineyards which do not use cover crops (Tesic et al. 2007).

Cover crop competition for N can also lead to reductions in Yeast Assimilable Nitrogen (YAN) (Gouthu et al. 2012; Pérez-Álvarez et al. 2015). A YAN of at least 140 mg/L is generally accepted as the lower limit for the successful completion of a dry wine fermentation (Butzke 1998). Yeast Assimilable Nitrogen concentrations lower than 140 mg/L can lead to stuck or sluggish fermentations (Mendes-Ferreira et al. 2004), less fruit fermentative aromas arising from esters (Garde-Cerdán and Ancín-Azpilicueta 2008), production of “eggy” smelling hydrogen sulfide (Bell and Henschke 2005), an increase in “solvent” aromas arising from a greater production of higher alcohols (D. R. Webster et al. 1993).

In recent years “YAN balance” has been a hot topic of conversation among winemakers. In general, many winemakers believe that organic N sources, coming from amino N, are more desirable than inorganic N, arising from ammonia. Higher alcohols are formed via the

transamination of amino acids, they can then undergo a further transformation during fermentation to form fruity smelling acetate esters (Boulton et al. 1996; Pretorious and Lambrechts 2000; Sumby et al. 2010). In a study evaluating the effect of different N sources upon wine sensory properties, it was found that musts supplemented solely with di-ammonium phosphate produced wines with higher concentrations of ethyl acetate and acetic acid, resulting in undesirable aromas related to volatile acidity (Torrea et al. 2011). However, ammonium (NH_4^+-N) is one of the most readily assimilable N sources for yeast (Jiranek et al. 1995a). The preferential utilization of ammonium can support a rapid growth in the initial yeast population, which can minimize the "lag phase" of fermentation. It is important to have a balanced must with both inorganic and organic YAN components in order to ensure fermentation with ideal kinetics and a resulting wine with a positive aromatic profile.

Berry N does not just impact fermentative aromas. Berry N can also have a dramatic impact upon varietal aromas. However, the research into the effect of vineyard N nutrition and varietal aromas is limited (Bell and Henschke 2005). Additional research into the relationship between vineyard N nutrition and the development of varietal aromas is needed.

There are four major classes of varietal aromas; terpenes, thiols, pyrazines and norisoprenoids. Two of the three varieties assessed in the current study, Petit Manseng and Sauvignon blanc, are known to produce an abundance of thiols (Darriet et al. 1995; Tominaga et al. 2000a). Thiols have been found to responsible for tropical fruit aromas akin to "passionfruit, box tree, gooseberry and guava" (Coetzee and du Toit 2012; Dubourdieu et al. 2006; Tominaga et al. 2000b).

New Zealand Sauvignon blanc has been gaining market share in the United States over the course of the last decade and currently represents nearly one third of all the Sauvignon blanc sold in the US (New Zealand Winegrowers 2013, 2014). The main distinguishing feature of NZ Sauvignon blanc is its remarkably high concentration of thiols (Benkwitz et al. 2012; Musumeci et al. 2015).

The purpose of the current study was to evaluate vineyard based N supplementation strategies and their implication for grape and wine quality, with particular interest paid to the development of varietal thiols in the resulting wines of Petit Manseng and Sauvignon blanc.

Review of Literature

Primary aroma

Primary aromas, also referred to as varietal aromas, are those aromas arising from the grape itself which persist and/or are heightened through the winemaking process. In concert with fermentative aromas (secondary aromas), they provide an aromatic fingerprint of a grape variety.

Varietal aromas consist of terpenes, norisoprenoids, methoxypyrazines and thiols. The biosynthesis of norisoprenoids and thiols and the effect of nitrogen upon their expression in grapes and wine has been previously discussed by the author and will not be mentioned in this document (Moss 2016).

Terpenes

Terpenes are responsible for imparting a myriad of different aromas. They are mostly associated with floral (e.g. geraniol, nerol, linalool) and citrus aromas (e.g. citronellol).

However, they can also convey aromas that are perceived as spicy or resinous (e.g. α-terpinene, p-cimene, β-mycrene, limonene) (King and Dickinson 2003). Five important terpenes and their corresponding aroma descriptors have been summarized in table 3.

Table 1. Important wine terpenes and their corresponding aroma descriptors

Compound	Aroma descriptors
Geraniol	floral, rose[a]
Linalool	coriander[a], flowery[b]
Citronellol	lemon[c], rose, sour[d]
Nerol	fruity and flowery[c]
α-terpineol	anise[c], spicy[e]

[a](Marais 1993)[b](Chisholm et al. 1994)[c](Lin and Rouseff 2001)[d](Hognadottir and Rouseff 2003) [e](Gürbüz et al. 2006)

A eucalyptus-like aroma arising from 1,8-cineole has been identified in wines. This compound can present itself in wine through its extraction from matter other than grapes (such as leaves) during fermentation of grapes grown near Eucalyptus trees (Capone et al. 2012; Capone et al. 2011b). 1,8-cineole has also been found to be produced within the berries of Tannat at concentrations higher than the sensory threshold (Farina et al. 2005).

Terpenes are thought to serve as a defensive mechanism to various stresses including herbivory (Kessler and Baldwin 2001; Loughrin et al. 1997), heat stress (Copolovici et al. 2005) and oxidative stress (Vickers et al. 2009). Terpenes may also play a role in signaling within and between plants, as their production when herbivory is induced can lead to their synthesis in nearby, vascularly isolated foliage, as found in *Vaccinium corymbosum* and a hybrid poplar (*Populus deltoides* × *nigra*) (Frost et al. 2007; Rodriguez-Saona et al. 2009).

The localization of freely volatile terpenes may differ by variety. Gomez et al. (1994), for example, found a significantly higher concentration of geraniol in the skin of Monastrell than in the juice or pulp; however, the highest concentration of geraniol was in the pulp of Tempranillo

(Gomez et al. 1994). It has been suggested that geraniol synthesis is restricted to the exocarp of the grape berry, whereas linalool is synthesized in both the meso- and exocarp (Luan and Wust 2002). In a study utilizing Muscat of Alexandria, Park et al. (1991) found that the highest concentration of free and bound monoterpenes (linalool, geraniol and nerol) was in the mesocarp when compared to the skins. However, over 46% of the monoterpenes measured in the study were found in the skins and 90% of the total terpenes occurred as glycosides, which can later be rendered volatile by yeast through glycosidase activity.

Monoterpenes can be found in many different grapes and wines. They are found at particularly high concentrations in Riesling, Gewürztraminer and Muscat varieties (González-Barreiro et al. 2015; Marais 1993). There have been over 40 terpene compounds identified in grapes (Marais 1993; Mateo and Jimenez 2000). Monoterpene diols have been the focus of much of the research, due to their low aroma thresholds and abundance in aromatic varieties such as Riesling and Muscat (Dimitriadis and Williams 1984; Gunata et al. 1985). Chief among the monoterpene alcohols are linalool, geraniol, α-terpineol, nerol and citronellol (Mateo and Jimenez 2000).

Terpenes exist in both a freely volatile (FVT) and a potentially volatile (PVT) form as glycosidically conjugated precursors (Dimitriadis and Williams 1984; Williams et al. 1981; Williams et al. 1982b). The glycosidically conjugated monoterpenes (PVT) are in a greater abundance than the freely volatile forms (Mateo and Jimenez 2000). PVT can be transformed into the volatile wine aroma through the hydrolysis of the C-OH bond between the carbohydrate and the terpene. This occurs through the action of terpene glycosidases which are present in yeast. Each yeast strain varies in its efficiency to carry out this hydrolysis and can

therefore have an impact upon the aromatic intensity and profile of varietal terpenes (Zoecklein et al. 1997). Over time, acid hydrolysis of terpenols can rearrange the ratios of each terpenol, thereby altering the aromatic profile of wine during the aging process (Simpson and Miller 1983; Williams et al. 1982a).

In grapes and wine, the monoterpenes have been studied to a far greater extent than the sesquiterpenes. However, sesquiterpenes have been detected in several German varieties including Riesling, Traminer and Müller-Thurgau (Schreier et al. 1976) as well as in the red Baga grape from Portugal (Coelho et al. 2006). Possibly the most significant sesquiterpene discovered to date has been rotundone which is responsible for a black pepper aroma. This compound has been found in Shiraz, Grüner Veltliner, Cabernet Sauvignon, Durif, Mourvedre, Schioppettino and Vespolina grape varieties (Mattivi et al. 2011; Wood et al. 2008). The aroma detection threshold for Rotundone in red wine was found to be very low at 16 ng/L (Wood et al. 2008). Due to the large diversity of sesquiterpenes found in grapes and wine and their seemingly low aromatic thresholds, it is likely that more of these compounds impart aromatic character to wine and have yet to be quantified.

Polyhydroxylated terpenes have also been found in grapes. These compounds do not make a direct contribution to wine aroma, but it is possible that they can be broken down into aromatic compounds. One study demonstrated that after heating muscat juice, dienediol (a hydroxylated linalool derivative) was broken down into nerol oxide and hotrienol, which can have a positive aromatic influence (Williams et al. 1980). These researchers did not evaluate if this rearrangement of the dienediols can result in a significant sensorial impact in grapes and wine. The breakdown of polyhydroxylated terpenes was hypothesized as a rationale behind the

presence of nerol oxide in aged Riesling wines (Simpson and Miller 1983). However, the highest concentration of nerol oxide found in the previous study was 70µg/L from a Riesling wine that was 12 years old, but the aroma threshold for nerol oxide is ~100µg/L (Marais 1993), therefore it is unlikely that nerol oxide contributed a considerable aromatic impact. The increase in nerol oxide over maturation has also been demonstrated in single variety Vinho Verde wines made from Loureiro and Alvarinho. However, the concentration of nerol oxide was also found to be well below the aromatic threshold (Oliveira et al. 2008). Further investigation into the importance of polyhydroxylated terpenes might be warranted, as to determine their potential contribution to the potential aromatic profile of aged terpene driven wines.

Vine nutrition and terpenes

It is difficult to separate the nutritive status of the vine from the production of volatile compounds. Increasing N nutrition to the vine can increase canopy density (Bell and Robson 1999). This increase in canopy density can then result in a decrease in solar radiation interception (Marais et al. 2001). Low sunlight exposure has been linked to a suppression of monoterpene biosynthesis (Belancic et al. 1997; Skinkis et al. 2010; Song et al. 2015; Zhang et al. 2014). To date, there has been only one study conducted which evaluated the relationship between monoterpene concentration and vineyard N management. That study found a variable effect of N fertilization upon monoterpene concentrations in 3 to 5 year old wines (D. Webster et al. 1993). In general, the total concentration of monoterpenes (geraniol, nerol and citronellol) in wine decreased with increasing N fertilization. Monoterpenes in the berry, or in young wine were not measured. Nitrogen fertilization and its effect upon monoterpenes could present an area for future research.

Monoterpenes can also be synthesized by *S. cerevisiae* during fermentation. Greater synthesis of linalool and citronellol has positively correlated with must YAN concentration (Carrau et al. 2005). Higher N concentrations in the must due to vineyard fertilization (or nitrogen addition in the winery) could lead to higher concentrations of monoterpenes in the resulting wine (Carrau et al. 2005). A link between monoterpene biosynthesis and phosphorus (P) nutrition has been found in other plants (Dragar and Menary 1995; Prasad et al. 2012). In grapes, P fertilization has been associated with an increase in freely volatile terpenes in musts and wine (Bravdo 2000). The relationship between P nutrition and monoterpene biosynthesis in the grape could present an area of future research.

Methoxypyrazines

3-Alkyl-2-methoxypyrazines (MPs) are a class of volatile compound that are largely responsible for the characteristic aromas of several vegetables including bell pepper, asparagus, peas and potatoes (Buttery et al. 1969; Buttery and Ling 1973; Luning et al. 1994; Murray et al. 1970). MPs are also found in processed food products such as cheddar cheese (Neta et al. 2008; Suriyaphan et al. 2001). 3-isobutyl-2-methoxypyrazine (IBMP) was the first MP to be identified in grapes of Cabernet Sauvignon (Bayonove et al. 1975). Besides IBMP, 3-isopropyl-2-methoxypyrazine (IPMP) and sec-butly-2-methoxypyrazine (SBMP) also make important contributions to juice and wine aroma. IBMP, IPMP and SBMP are considered the most important MPs found in grapes. Their aroma descriptors as determined through gas chromatography-olfactometry have been presented in table 5.

Table 2. Aroma descriptors for key methoxypyrazines found in wine

Compound	Aroma descriptors
3-isobutyl-2-methoxypyrazine (IBMP)	earthy[a], bell pepper[b]
3-isopropyl-2-methoxypyrazine (IPMP)	pepper, earthy[a]
sec-butyl-2-methoxypyrazine (SBMP)	bell pepper[c], earthy[a]

[a](Campo et al. 2005)[b](Culleré et al. 2004) [c](Neta et al. 2008)

MPs are not major odor active compounds in all grapes/wines, however it is well known that they play an integral role in the aromatic profile of wines made from Cabernet Sauvignon (Allen et al. 1994), Sauvignon blanc (Allen et al. 1991; Augustyn et al. 1982; Lacey et al. 1991), Carménère (Belancic and Agosin 2007; Dominguez and Agosin 2010), Cabernet Franc (Hashizume and Umeda 1996; Roujou de Boubée et al. 2000) and Merlot (Kotseridis et al. 1998; Sala et al. 2000).

The MPs contribute positive varietal aromas at low concentrations and have been found to have incredibly low thresholds of detection in the order of 1-2ng/L (Alberts et al. 2009; Allen et al. 1991; Parr et al. 2007). MPs are largely considered undesirable at higher concentrations. To date, no peer reviewed research exists which has attempted to understand the consumer rejection threshold of the methoxypyrazines, although levels of >10ng/L to 30ng/L have been posited (Candelon et al. 2010; Eebler 2014). Methoxypyrazines can not only contribute herbaceous aroma, but may also mask the positive fruity and floral aromas (Campo et al. 2005; King et al. 2011; van Wyngaard et al. 2014).

The methods of biosynthesis of MPs within the grape berry has not been fully elucidated. It has been proposed that the process may begin with the amidation of leucine, isoleucine and/or valine which then undergoes condensation with glyoxal to form a hydroxypyrazine (Eggers, 2006). The hydroxypyrazine is then ezymatically methylated to form

the final MP (Hashizume et al. 2001). Further research is needed in order to better understand the biosynthesis of MPs.

Vine nutrition and methoxypyrazines

The concentration of MPs reaches a peak around véraison (Harris et al. 2012). After véraison, MPs undergo rapid photodecomposition (Hashizume and Samuta 1999). Immature grapes and increased canopy density can result in higher concentrations of methoxypyrazines in the resulting product.

As methoxypyrazines are cyclic-nitrogenous compounds, derived from valine, leucine and isoleucine, N fertilization in the vineyard may directly influence their concentrations. Past research on N fertilization has mostly associated higher levels of MPs with increased fruit shading from increased vegetative growth, which in turn limits the photodecomposition of the MPs (Allen et al. 1991; Bell and Henschke 2005; Mendez-Costabel et al. 2014). More research is needed to elucidate the role which N fertilization might directly play in the biosynthesis of methoxypryazines.

Thiols

Thiols are any organic comound containing a sulfylhydryl (-SH) group. However, in the wine industry the term “thiol” is usually relegated to the positive varietal compounds known to impart tropical fruit aromas. The three main thiols found in wines are 3-mercaptohexan-1-ol (3MH), 3-mercaptohexyl acetate (3MHA) and 4-mercapto-4-methylpentan-2-one (4MMP). The bioysnthesis the aformentioned compounds and the impact of N fertilization upon these thiols has been previously discussed by the author and won’t be mentioned here (Moss 2016).

Instead, the remainder of this review will focus upon enological processes which can impact the concentration of these compounds in juice and wine.

Enological factors impacting thiols

New Zealand Sauvignon blanc is known to contain some of the highest concentrations of volatile thiols of any wine in the world (Benkwitz et al. 2012). Most of the Sauvignon blanc in Marlborough is harvested with machines. Machine harvesting is used out of necessity in Marlborough. Fortuitously, it has been demonstrated that machine harvesting of fruit results in higher concentrations of volatile thiols in wines compared to wines that originate from hand-harvested fruit (Allen et al. 2011; Olejar et al. 2015). Allen et al. (2011) hypothesized that an increase in enzymatic activity in "damaged" machine harvested fruit may play a role in the formation of thiol precursors. Thus explaining the increase in volatile thiols in the resulting wine.

The time of day at which the fruit is harvested may play a role in the concentration of thiols in the resulting wine. Oxidation will occur faster in warmer juice than cooler juice. This oxidation of the juice can then lead to loss of aromatic potential by the volatile thiols, as the oxidation can lead to formation of quinones, which will oxidize the volatile thiols after fermentation (Allen et al. 2011). Therefore, harvest should be conducted in the cooler periods of the day in order to minimize oxidation.

In smaller growing regions, as are present in the Eastern US, machine harvesting is not commonly practiced. Therefore, one can possibly mimic the effect of machine harvesting through the use of cold maceration. However, studies which have evaluated the impact of a traditional cold soak on thiol concentrations have simply measured the amino-acid conjugated

thiols in the must (Larcher et al. 2013; Maggu et al. 2007). The conjugated thiols tended to increase in must that has been cold-soaked. However, because the conjugated thiols in juice represent less than 10% of the final volatile thiol concentration in wine, the results from these studies can't be extrapolated to final wine.

A study has been conducted which used a novel cryogenic maceration technique. Sauvignon blanc was harvested and crushed and cold soak occurred at -4^{o}F (-20^{o}C) using dry ice. Upon reaching -4^{o}F, the must was allowed to warm over a 24-hour period to ambient temperature before it was pressed and vinified. In this study, concentrations of volatile thiols in wines made from hand-picked/cryogenically macerated fruit were greater than those measured in wines made from machine-harvested fruit. Through sensory analysis, the wines made from hand-harvested fruit were found to be less aromatic than the wines of machine-harvested fruit. However, no discernable sensorial difference was seen between the wines made from machine-harvested fruit and cold-soaked musts (Olejar et al. 2015). Further research is needed to determine the efficacy of cold soak as a means to increase the aromas coming from thiols.

Regardless of whether or not volatile thiols increase with skin contact, it may increase reduced glutathione (GSH) concentrations in the resulting wine. GSH is a naturally occurring antioxidant in grapes and is found in especially high concentrations in Sauvignon blanc. In wine, it can react with quinones which can oxidize the volatile thiols, thereby depressing their aromatic impact. GSH can also preserve esters, terpenes and wine color during aging (Roussis and Sergianitis 2008; Sonni et al. 2011).

A recent study on Sauvignon blanc found that skin contact of only 18 hours increased

the GSH concentration in the must by up to 55% greater than the initial concentration (Pons et al. 2015). Results were highly variable between batches, but always showed a higher glutathione concentration with skin contact. The same study found the GSH concentration to be highest in the cold-soaked must after only 30 minutes. After 30 minutes, the concentration of GSH decreased. This phenomenon occurs because most GSH is found in the skins of the grape. Therefore, even if cold soak doesn't increase the volatile thiol concentration in the wine, it may help to preserve their aroma through an increase in GSH. It is advisable to conduct a cold soak under CO_2 and with the addition of SO_2 in order to deactivate polyphenol oxidases and minimize oxidation, thereby allowing for the maximum retention of GSH (Du Toit et al. 2007)

The concentration of the conjugated thiols is known to increase during the oxidation of juice (Roland et al. 2010). However, the volatile thiols in wine are easily oxidized. Polyphenol oxidases may cause phenols such as caftaric acid and catechins to oxidize into quinones which can cause the rapid oxidation of thiols during fermentation. Therefore, wines made from musts which have been treated with an addition of 50ppm of SO_2 are likely to have higher concentrations of aromas arising from volatile thiols (Coetzee et al. 2013).

It's worth noting that the addition of ascorbic acid prior to fermentation may also provide additional protection against oxidation, as it is a more powerful reducer of quinones than SO_2 (Nikolantonaki et al. 2014) Ascorbic acid additions warrant further research with regard to its use in the production of high quality thiol driven wines, as the study by Nikolantonaki et al. (2014) was performed in a model wine. Also, ascorbic acid is unpredictable and can lead to the formation of pigments which SO_2 can't prevent and it has been implicated

in the formation of sotolon, a compound that produces a maple syrup like aroma in prematurely aged white wines (Barril et al. 2012; Pons et al. 2010).

Oxygen seems to be the archenemy of the volatile thiols. As mentioned previously, oxygen inclusion during the juice stage will lead to a depression in the final concentration of GSH. Less GSH in the wine may leave the thiols more vulnerable to oxidation by quinones. Therefore, pressing should be conducted under a reductive environment with carbon dioxide or nitrogen gas, in order to preserve volatile thiols.

With increasing pressure, one can extract more conjugated thiols in the press. However, with higher pressure, one will begin to extract more phenols, which can then oxidize to form quinones. The quinones will react with glutathione, thereby leaving the thiols more vulnerable to oxidation (Maggu et al. 2007). Therefore, it's worth adding SO_2 prior to pressing, as well as pressing in an anaerobic environment, in order to inhibit the polyphenol oxidases and maximize aromatic potential of the volatile thiols.

Various yeast strains have been demonstrated to produce wines with increased concentrations of volatile thiols. As the origin of the volatile thiols is not fully understood, it is not known entirely why some yeast strains result in wines with more volatile thiols than other strains. However, it is known that an enzyme (β-lyase) is responsible for the cleavage of the carbon-sulfur bond between the non-volatile amino acid/thiol conjugates. As the conjugated thiols can account for 10% of the volatile thiols, increased β-lyase activity can lead to higher thiol concentrations in wines. Yeast strains such as Levuline ALS, Zymaflore VL3, Anchor VIN 13 and Uvaferm SVG have demonstrated a remarkable ability to produce wines with high concentrations of volatile thiols (Dubourdieu et al. 2006; Kobayashi et al. 2010). Interestingly, a

co-fermentation of *Pichia kluyveri* and *Saccharomyces cerevisiae* has been demonstrated to significantly increase concentrations of 3MHA in the resulting wine (Anfang et al. 2009).

The amino-acid conjugated thiols in the juice are metabolized by yeast and the carbon-sulfur bond between the amino acid and the thiol is cleaved by an enzyme known as β-lyase. The addition of Diammonium phosphate (DAP) can interfere with the pathway that regulates amino acid transport and in turn, this can lead to wines with a lower volatile thiol concentration. This phenomenon is known as nitrogen catabolite repression (NCR) (Subileau et al. 2008). The use of Laffort's Dynastart®, a yeast rehydration that is made of yeast autolysates and inactivated yeast, has been shown to lead to wines with higher concentrations of volatile thiols than wines made from yeast rehydrated with DAP. However, both the organic and inorganic rehydration regimes had greater thiol concentrations than the control, which had no nutrient additions during fermentation (Winter et al. 2011).

Higher fermentative temperatures have been demonstrated to increase volatile thiols in wine (Masneuf-Pomarède et al. 2006). However, important aromas that are intrinsic to many white wine styles are lost at higher fermentation temperatures. Therefore, a fermentation temperature of 18-20^{o}C is recommended in order to obtain the greatest concentration of volatile thiols while still retaining some of the pleasant aromas produced during fermentation.

Thiols contain an –SH group. As such, when one adds copper to a final wine to remove sulfides, one will also be removing the volatile thiols. This loss occurs because the copper can bind with the sulfhydryl group of thiols and form copper sulfate, which will settle out. Copper can also directly cause thiols to oxidize into disulfides (Ugliano et al. 2011). Fungicides that contain copper can also be responsible for residual copper in the must. In order to remove any

residual copper from must, one can use an adsorbent copolymer known as polyvinyl imidazole-polyvinylpyrrolidone (PVI/PVP) (Mira et al. 2007).

As was previously mentioned, volatile thiols can rapidly oxidize and lead to dramatic aroma losses. Therefore, the permeability of wine packaging to oxygen is an important factor when one wants to conserve these aromas. It is well known that screwcap and technical corks are two of the most impermeable closures currently on the market, whereas synthetic corks are known to allow considerable oxygen ingress (Lopes et al. 2009). Screwcaps have been linked to reductive aromas, however one can minimize the risk of reductive aromas through encouraging a healthy fermentation and through a pre-bottle SO_2 addition that is not in excess of what is needed.

The Saranex liner, which consists of layers of low density polyethylene and Saran ®, has been found to minimize the reductive aromas and preserve wine aroma (Lopes et al. 2009). A crucial component of the Saranex liner is low density polyethylene which may play a direct role in minimizing the reductive aromas by scalping the unpleasant volatile sulfur compounds (Silva et al. 2012). The potential for reductive aromas to arise under screwcap can be minimized by encouraging a healthy fermentation, allowing greater ullage at bottling and by making efficient, rather than excessive, SO_2 additions (Dimkou et al. 2011).

Materials and methods

Sites and treatments

Three perennially nitrogen deficient vineyards were chosen for this experiment. All three vineyards were managed using commercially standard practices throughout the duration of these experiments. Brief site descriptions have been given below. More detailed site descriptions have been provided by the author in another text (Moss 2016).

GMV

Glen Manor Vineyards (GMV) was located near Front Royal, VA and was planted with Sauvignon blanc (*V. vinifera*). GMV was under vine cover cropped with red fescue (*Festuca rubra*). The alleyways were planted with tall fescue (*Festuca grundinacea*). Cover crops were maintained with mowing throughout the season.

A randomized complete block design consisting of 6 blocks, 3 treatments and a control was used at GMV. Three vines were used per experimental unit and each unit was separated by three vines. No exogenous N was applied to the control. Soil applied N treatments were applied as Calcium nitrate. A 30 kg N/ha soil (30 N soil) applied treatment was applied at bloom. A 60 kg N/ha soil (60 N soil) applied treatment was split into two equivalent applications, the first application was made at bloom and the second application was made one month later. 30 kg N/ha of urea (30 N foliar) was applied to the foliage starting at bloom and made in six 5 kg N/ha applications (0.56% concentration by weight) separated by 7-10 days.

Treatments were executed on an annual basis starting in 2010.

AREC 1 and AREC 2

The Alson H. Smith Jr. Agricultural Research and Extension Center (AREC) located near Winchester, VA was planted in Petit Manseng (*V. vinifera*). Two experiments were established at this site.

AREC 1 was established in a completely randomized design. The alleyways were cover cropped in tall fescue (*F. arundinacea*) and orchard grass (*Dactylis glomerata*) and mown as needed. Four treatments and an unfertilized control were imposed in 2014 and 2015. All soil fertilization was applied as Calcium nitrate. Soil N was applied at three rates: 30 kg N/ha (30 N soil), 45 kg N/ha (45 N soil) and 60 kg N/ha (60 N soil). Soil N fertilization was executed at bloom. The 60 N soil treatment was applied in split applications with the first being at bloom and the second being applied one month later. Another treatment included the application of 45 kg N/ha to the soil at bloom and 15 kg N/ha applied to the foliage (45 N Soil + 15 N foliar) as urea in two equivalently split applications at 100% véraison. The foliar urea was applied at a concentration of 0.8% by weight and applications were separated by 7-10 days.

AREC 2 was established in a randomized complete block design with 5 blocks, two treatments and an unfertilized control. Both treatments included 15 kg N/ha applied as urea in equivalent split applications separated by 7-10 days at a concentration of 0.8% by weight at 100% véraison (15 N foliar and 15 N foliar + 5 S foliar). 5 Kg S/ha of micronized sulfur was included in the 15 N foliar + 5 S foliar treatment, with 2.5 kg S/ha being applied per spray.

Experimental units at AREC 1 and 2 consisted of five vines each.

ISV

Indian Springs Vineyard (ISV) was located near Woodstock, VA and was planted in Vidal blanc (*Vitis ssp.*). The alleyways at ISV were cover cropped in native vegetation and mown throughout the season to maintain ease of access. Where not sown with a clover cover crop, the under-vine area was maintained bare with herbicide at a width of approximately 1 meter.

A randomized complete block design consisting of four blocks, four treatments and two controls with four vine experimental units each separated by four vine border plots. Crimson clover (*Trifolium incarnatum*) and Dutch white clover (*T. repens*) were sown at rates of 33.6 kg/ha and 15.7 kg/ha respectively. Treatments utilizing clover as an under-vine cover crop included: Crimson clover alone (Crimson), White clover alone (White), Crimson clover and a 10 kg N/ha application of foliar urea (Crimson + 10 N foliar), White clover and a 10 kg N/ha application of foliar urea (White + 10 N foliar).

Two controls meant to mimic industrially standard practices were imposed at ISV. One control consisted of 15 kg N/ha applied to the soil as Calcium nitrate at bloom (15 N soil). The other control included 15 kg N/ha applied to the soil as Calcium nitrate at bloom and 10 kg N/ha of urea applied to the foliage (15 N soil + 10 N foliar).

All foliar urea at ISV was applied at the start of véraison in two equivalent split applications at a concentration of 0.56% by weight.

Primary chemistry

Berry samples for primary chemistry, YAN and amino acid analyses were taken at commercial harvest on all sites. At GMV and ISV, 60 berries per experimental unit were

randomly selected. Due to the lyre trellis at GMV, the two canopies were sampled individually. At AREC 1 and 2, 100 berries were randomly selected per experimental unit.

Berry samples were crushed by hand and the juice was collected for analysis. Soluble solids (^{o}Brix) was measured immediately after crushing with a digital refractometer (Pocket PAL-1, Atago USA Inc., Bellevue, WA). The juice was held in 50 ml centrifuge tubes at 10^{o}C for no more than 48 hours prior to measuring pH and titratable acidity (TA). pH measurements were made from 5ml of juice pipetted into 40ml of distilled water which was stirred throughout the measurement period. TA was then taken from the same sample used for pH, using an automatic titrator (848 Titrino Plus, Metrohm, Herisau, Switzerland). Juice was titrated with 0.1 N NaOH to a pH endpoint of 8.2 and TA was recorded as g/L as tartaric acid equivalents. Remaining juice was then stored at -80^{o}C and used at a later date to measure YAN and amino acid profiles.

Yeast assimilable nitrogen (YAN)

Prior to YAN analyses, all samples were thawed and clarified through centrifugation at 2301 ×g for 10 min.

Ammonia (NH_4^+-N) was measured using an enzymatic kit (K-AMIAR kit, Megazyme, Bray, Ireland). Primary amino nitrogen (PAN) was determined by the *o*-phthaldialdehyde analysis (NOPA) (Dukes and Butzke 1998). A UV/vis spectrophotometer (Gensys 10S, Thermo Fisher Scientific, Waltham, MA, USA) was used to determine both NH_4^+-N and PAN concentrations.

Each NH_4^+-N and PAN analysis was conducted in triplicate with external standards being measured after every 24 samples. Analytical replicates were then averaged for each sample.

Amino acids

Amino acid profiles of the juices from 2014 and 2015 in all experiments were analyzed the same way. Prior to derivatization and analysis, juice samples were thawed and centrifuged at 2301 ×g for 10 min, then filtered through a PTFE 0.22 µm membrane filter (MicroSolv, Eatontown, NJ). AccQ·Tag Ultra kits (Waters Corporation, Milford, MA) were used to derivatize and analyze 18 different amino acids through the use of UPLC/PDA (Acquity H-class UPLC, Waters Corporation, Milford, MA). Each sample was spiked with 2.5 mM concentrations of Norvaline (NVA) as an internal standard.

The Waters Amino Acid Hyrdrolysate Standard was used as the calibration standard. The standard contained 2.5 mM concentrations of the following amino acids dissolved in 0.1N HCl: Histidine (His), Asparagine (Asn), Serine (Ser), Arginine (Arg), Glycine (Gly), Glutamic acid (Glu), Threonine (Thr), Alanine (Ala), ɤ-aminobutryic acid (GABA), Proline (Pro), Lysine (Lys), Tyrosine (Tyr), Valine (Val), Isoleucine (Ile), Leucine (Leu) and Phenylalanine (Phe). Cysteine (Cys) was at a concentration of 1.25 mM in the standard solution. ɤ-aminobutryic acid (GABA), Aspartic acid (ASP), Glutamine (Gln) and Norvaline (NVA) dissolved in 0.1N HCl were added at a concentration of 2.5 mM to the existing standard prior to analysis.

To derivatize each sample, 70 µL of AccQ·Tag Ultra Borate buffer was first added to a clean recovery vial. Then 10 µL of the calibration sample to the vials and were vortexed for approximately 10 sec. Then 20 µL of reconstituted AccQ·Tag Ultra reagent was added to the vial and immediately vortexed for approximately 10 sec. The solution was then allowed to sit for about one minute before being loaded into a heating block set to 55^{o}C. After incubation at 55 ^{o}C for 10 min, the samples were removed from the heating block and analyzed using a

Waters Acquity H-Class UPLC system with PDA detector (Waters Corporation, Milford, MA). 1 µL of each sample was injected onto a Waters AccQ·Tag Ultra Column 2.1×100mm, 1.7 µm at a temperature of 43^{o}C with a flow rate of 0.7 mL/min. The Empower™ Software package (Waters Corporation, Milford, MA) was used for system control and data collection.

If individual amino acid concentration exceeded the calibration range (>50 mM/L), the samples were diluted with UPLC grade deionized water and re-run. Samples were run at dilution factors of 2:1, 5:1, 10:1 and 100:1.

Winemaking

Wines were made from Petit Manseng and Sauvignon blanc at AREC 2 and GMV, respectively. Fermentations at AREC 2 ceased prior to reaching dryness in both 2014 and 2015. AREC 2 fermentations will not be discussed. At GMV, wines were made from the control and foliar treatments in 2014 and 2015. The fruit was harvested and held at 10 ^{o}C for 48 hours prior to destemming with a mechanical de-stemmer. The de-stemmed fruit was collected in a sanitized plastic bucket preloaded with dry ice in order to minimize oxidation of the juice through displacement of oxygen by sublimated CO_2. The fruit was then promptly pressed using a vertical water press (Hydro 40, Zambelli, Camisano Vicentino, Italy). The juice was dispensed directly into carboys and continuously gassed with CO_2. Sulfur dioxide in the form of potassium metabisulfite was added to a concentration of 30 ppm in 2014 and 50 ppm in 2015. A commercial pectinase was also added after pressing at a concentration of 1.32 ml/hl (Pec 5L, Scott Laboratories, Petaluma, CA). The juice was settled for 48 hours at 3 ^{o}C, racked, and inoculated with VIN 7 in 2014 and VIN 13 in 2015 (Anchor Wine Yeast, Johannesburg, SA). The yeast was rehydrated according to the manufacturer's specifications and dosed at a rate of 30

g/hl. In both years, fermentation took place in a walk-in refrigeration unit that was set to 18^{O}C. In 2014, fermentations were carried out in duplicate in 3.79 liter carboys topped with a rubber bung and airlock. Fermentation was monitored daily with a hydrometer. Once fermentation reached 0 ^{O}Brix on the hydrometer, fermentation was monitored until dryness (<10 g/L of residual sugar) with Clinitest (Bayer, Leverkusen, Germany). However, after the first year this method of fermentation monitoring was deemed to be too oxidative for the purposes of this experiment and the fermentation methods were improved for the 2015 harvest following a microscale fermentation protocol previously reported by others (Allen et al. 2011). These bottles were equipped with rubber bungs and airlocks and fermentations were carried out in triplicate. The bottles were weighed daily throughout the duration of fermentation. When bottle weights remained unchanged for more than 2 days, the residual sugar concentration was measured through the use of Clinitest reducing sugar assay (Bayer, Leverkusen, Germany). Upon completion of fermentation in 2014 and 2015, 100 ppm of SO_2 was added to the wines and they were then syphoned under inert N gas into clear, 118 ml glass bottles with foil lined plastic screwcaps (Wheaton, Millville, NJ). The bottles were stored in darkness at 4 ^{O}C until being shipped to Hill Laboratories for thiol analysis (Hamilton, NZ).

Data analysis

All data was analyzed with JMP pro 11 (SAS; Cary, NC). Two-way analysis of variance was conducted on all data from ISV, AREC 2 and GMV with the model effects tested being treatment, block, year and the treatment-year interaction. Two-way ANOVA was also conducted upon the data from AREC 1 with the model effects being treatment, year and the treatment-year interaction.

All data sets were also analyzed using one-way ANOVA, as to evaluate treatment effects within individual years. Treatment and block were used as model effects at AREC 2, GMV and ISV. Treatment was the model effect analyzed at AREC 1.

Means were separated using Tukey's Honestly Significant difference or Student's T-test when appropriate. A confidence level of 95% was used in all statistical analyses.

Results

Primary chemistry and yeast assimilable nitrogen

GMV: The 60 N soil and 30 N foliar treatments significantly increased juice pH relative to the control ($p<0.05$) (Table 3). However, this treatment effect was minimal and insignificant within 2014 and 2015 (Table 4). Also, the treatment-year interaction was significant with regards to juice pH ($p<0.05$). Foliar N significantly increased YAN concentrations relative to all other treatments and the control (Table 3 and 4). The 30 N foliar treatment increased juice YAN, relative to the control by 107% and 131% in 2014 and 2015, respectively. N fertilization increased both ammonia-N (NH_4^+-N) and primary amino nitrogen (PAN) (Table 3 and 4). However, PAN was not significantly affected by treatment in 2014 (Table 4). N fertilization only had an effect upon the inorganic to organic assimilable N (NH_4^+-N: PAN) ratio in 2015. N fertilization increased the inorganic to organic N ratio with increasing levels of soil-applied N, but was most dramatic with the foliar-applied urea, in which the 30 N foliar treatment resulted in a 160% increase in the NH_4^+-N to PAN ratio, relative to the control. Year had a significant effect upon soluble solids, pH, titratable acidity (TA), ammonia and the inorganic to organic N ratio (NH_4^+-N: PAN) ($p<0.05$) (Table 3).

AREC 1: Soluble solids, pH and TA were all highest within the 45 N soil + 15 N foliar treatment (Table 5). However, soluble solids were not different between treatments in 2014 and 2015 (Table 6) and TA was only significantly affected by treatment in 2015 ($p<0.05$) (Table 6). Also, there was a significant treatment-year interaction with pH ($p<0.05$). YAN was significantly increased by the foliar application of urea ($p<0.05$) (45 N soil + 15 N foliar) (Table 5 and 6). Relative to the control, the combined soil and foliar application of N increased the YAN concentration by 92% and 197% in 2014 and 2015, respectively (Table 6). Relative to the 45 N soil treatment, the addition of 15 N foliar improved YAN concentrations by 40% and 149% in 2014 and 2015 (Table 6). Applications of foliar urea significantly increased the concentration of NH_4^+-N and PAN (Table 5 and 6). The application of N fertilizer increased the inorganic to organic N ratio, but this response was not significant between treatments within each year (Table 6). Year had a significant effect upon pH, TA, NH_4^+-N, PAN, YAN and the inorganic to organic N ratio ($p<0.05$) (Table 5).

AREC 2: The application of foliar urea at véraison significantly increased juice pH ($p<0.05$) (Table 7). However, the pH response was only significant in 2015 ($p<0.05$) (Table 8). Foliar urea increased YAN concentrations significantly ($p<0.05$) (Table 7); however, the response was due principally to the significant response in 2015 (Table 8). There was no statistically significant difference between the YAN of juices coming from the 15 N foliar and 15 N foliar + 5 S foliar treatments (Table 8). In 2015, foliar urea treatments were effective at improving both NH_4^+-N and PAN concentrations (Table 8). The application of foliar urea significantly increased the NH_4^+-N to PAN ratio in 2015, but not 2014 (Table 8). Year had a significant effect upon soluble

solids, pH, TA, PAN and the inorganic to organic N ratio ($p<0.05$) (Table 7). A significant treatment by year interaction was found for the PAN and YAN concentrations ($p<0.05$) (Table 7).

ISV: Treatment had no significant effect upon soluble solids, pH or TA (Table 9 and 10). YAN concentrations of the 15 N soil + 10 N foliar and White + 10 N foliar treatments were significantly greater than those from other treatments ($p<0.05$) (Table 9). Foliar treatments increased both the ammonia and amino nitrogen components of YAN, without significantly affecting the inorganic to organic N ratio (Table 9). The 15 N soil + 10 N foliar significantly increased the inorganic to organic N ratio in 2015, relative to the Crimson, Crimson + 10 N foliar and White treatments ($p<0.05$) (Table 10). Year had a significant effect upon soluble solids, pH, NH_4^+-N, PAN, YAN and the inorganic to organic N ratio ($p<0.05$) (Table 9). There was a significant treatment-year interaction with NH_4^+-N concentration ($p<0.05$) (Table 9).

Table 3. GMV: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	°Brix	pH	TA (g/L)	NH_4^+-N[c] (mg N/L)	PAN[c] (mg N/L)	YAN[c] (mg N/L)	NH_4^+-N:PAN
Control	21.86	3.28 b	8.16	21.85 b	46.40 b	68.25 c	0.62
30 N Soil	21.53	3.30 ab	8.26	26.82 b	58.69 b	85.50 bc	0.53
60 N Soil	21.12	3.32 a	7.99	34.80 b	66.54 ab	101.34 b	0.54
30 N foliar	21.59	3.31 a	8.03	60.37 a	88.53 a	148.89 a	0.78
Trt[-d]	ns[e]	0.0080	ns	<0.0001	0.0001	<0.0001	ns
Yr	<0.0001	<0.0001	<0.0001	0.0007	ns	ns	0.0029
Trt × Yr	ns	0.0096	ns	ns	ns	ns	ns

[a]Control = no N fertilization; 30 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom; 60 N soil = application of 30 kg N/ha as calcium nitrate at bloom and véraison; 30 N foliar = six applications of 5 kg N/ha as urea starting at bloom and separated by 7-10 days
[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)
[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)
[d]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction
[e]ns = not significant

Table 4. GMV: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to soil and foliar N fertilization by year (2014 and 2015)

	°Brix		pH		TA (g/L)		NH_4^+-N[c] (mg N/L)		PAN[c] (mg N/L)		YAN[c] (mg N/L)		NH_4^+-N:PAN	
Treatment[ab]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	20.26	23.47	3.2	3.35	9.14	7.17	32.93 b	10.77 c	40.13	52.67 c	73.07 b	63.42 c	1.04	0.20 c
30 N soil	19.79	23.28	3.23	3.37	9.00	7.53	32.85 b	20.78 b	53.92	63.45 b	86.73 b	84.23 b	0.73	0.33 b
60 N soil	19.96	22.28	3.24	3.4	8.77	7.22	44.33 ab	25.28 b	64.17	68.92 b	108.48 ab	94.18 b	0.72	0.37 b
30 N foliar	20.00	23.13	3.27	3.35	8.44	7.73	70.97 a	49.77 a	80.62	96.43 a	151.57 a	146.22 a	1.03	0.52 a
Trt[d]	ns[e]	ns	ns	ns	ns	ns	0.0341	<0.0001	ns	<0.0001	0.0095	<0.0001	ns	<0.0001

[a]Control = no N fertilization; 30 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom; 60 N soil = application of 30 kg N/ha as calcium nitrate at bloom and véraison; 30 N foliar = six applications of 5 kg N/ha as urea starting at bloom and separated by 7-10 days
[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)
[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)
[d] Significance of effects using one-way ANOVA. Trt = significance of treatment effects
[e]ns = not significant

Table 5. AREC 1: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	°Brix	pH	TA (g/L)	NH_4^+-N[c] (mg N/L)	PAN[c] (mg N/L)	YAN[c] (mg N/L)	NH_4^+-N:PAN
Control	27.28 ab	3.18 ab	9.61 ab	31.92 c	57.29 b	89.23 b	0.54 b
30 N soil	27.31 ab	3.17 b	9.90 ab	36.98 bc	60.28 b	97.26 b	0.60 ab
45 N soil	27.59 ab	3.17 b	9.94 ab	47.99 b	67.89 b	115.88 b	0.69 a
60 N soil	26.73 b	3.19 ab	8.92 b	48.83 b	70.87 b	119.70 b	0.67 ab
45 N soil + 15 N foliar	27.79 a	3.23 a	10.28 a	83.56 a	125.86 a	209.41 a	0.68 ab
Trt[d]	0.0182	0.0114	0.0456	<0.0001	<0.0001	0.0001	0.02
Yr	ns[e]	<0.0001	<0.0001	<0.0001	0.04560	<0.0001	<0.0001
Trt × Yr	ns	0.0029	ns	ns	0.00830	ns	ns

[a]Control = no N fertilization; 30 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom; 45 N soil = 45 kg N/ha applied to soil as calcium nitrate at bloom; 60 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom and véraison; 45 N soil + 15 N foliar = 45 kg N/ha applied to soil as calcium nitrate at bloom and two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[e]ns = not significant

Table 6. AREC 1: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to soil and foliar N fertilization by year (2014 and 2015)

	°Brix		pH		TA (g/L)		NH_4^+-N[c] (mg N/L)		PAN[c] (mg N/L)		YAN[c] (mg N/L)		NH_4^+-N:PAN	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	27.22	27.34	3.15 ab	3.22 b	10.4	8.81 ab	40.89 c	22.96 b	64.72 b	49.84 b	105.61 c	72.82 b	0.62	0.45
30 N soil	27.60	27.02	3.12 ab	3.21 b	10.78	9.03 ab	45.58 bc	28.38 b	67.61 b	52.94 b	113.18 bc	81.32 b	0.67	0.53
45 N soil	27.78	27.40	3.11 b	3.24 ab	10.94	8.95 ab	65.23 b	30.74 b	79.92 b	55.86 b	145.15 b	86.62 b	0.83	0.55
60 N soil	26.46	27.00	3.19 a	3.19 b	9.86	7.98 b	64.85 b	32.82 b	82.65 b	58.78 b	147.80 b	91.60 b	0.78	0.55
45 N soil + 15 N foliar	27.90	27.68	3.15 ab	3.30 a	11.29	9.27 a	91.19 a	75.94 a	111.56 a	140.16 a	202.75 a	216.08 a	0.83	0.54
Trt[d]	ns[e]	ns	0.0477	0.0027	ns	0.0467	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	ns	ns

[a]Control = no N fertilization; 30 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom; 45 N soil = 45 kg N/ha applied to soil as calcium nitrate at bloom; 60 N soil = 30 kg N/ha applied to soil as calcium nitrate at bloom and véraison; 45 N soil + 15 N foliar = 45 kg N/ha applied to soil as calcium nitrate at bloom and two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d]Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[e]ns = not significant

Table 7. AREC 2: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to foliar N fertilization (2014-2015)

Treatment[ab]	**°Brix**	**pH**	**TA (g/L)**	**NH_4^+-N[c] (mg N/L)**	**PAN[c] (mg N/L)**	**YAN[c] (mg N/L)**	**NH_4^+-N:PAN**
Control	24.78	3.08 b	12.87	43.63	52.18 b	95.80 b	1.38
15 N foliar	25.17	3.13 a	12.94	55.95	86.80 a	142.75 a	0.69
15 N foliar + 5 S foliar	25.27	3.11 ab	13.16	58.06	80.06 a	138.12 ab	0.78
Trt[d]	ns[e]	0.0382	ns	ns	0.0004	0.04	ns
Yr	<0.0001	<0.0001	<0.0001	ns	<0.0001	ns	0.0449
Trt × Yr	ns	ns	ns	0.0454	0.016	0.0118	ns

[a]Control = no N fertilization; 15 N foliar = two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison; 15 N foliar + 5 S foliar = two 7.5 kg N/ha applications of urea and 2.5 kg S/ha as micronized sulfur separated by 7-10 days at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[e]ns = not significant

Table 8. AREC 2: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to foliar N fertilization by year (2014 and 2015)

	°Brix		pH		TA (g/L)		NH_4^+-N[c] (mg N/L)		PAN[c] (mg N/L)		YAN[c] (mg N/L)		NH_4^+-N:PAN	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	22.34	27.22	2.95	3.22 b	15.79	9.96	61.92	17.80 b	49.16	55.2 b	118.82	72.78 b	2.44	0.31 b
15 N foliar	22.76	27.58	3.00	3.25 ab	15.70	10.19	54.06	57.80 a	68.80	104.8 a	122.84	162.66 a	0.82	0.55 a
15 N foliar + 5 S foliar	23.28	27.26	2.97	3.25 ab	16.49	9.83	47.90	68.20 a	53.12	107 a	101.02	175.22 a	0.93	0.63 a
Trt[d]	ns[e]	ns	ns	0.0187	ns	ns	ns	0.0002	ns	<0.0001	ns	<0.0001	ns	0.0009

[a]Control = no N fertilization; 15 N foliar = two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison; 15 N foliar + 5 S foliar = two 7.5 kg N/ha applications of urea and 2.5 kg S/ha as micronized sulfur separated by 7-10 days at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[e]ns = not significant

Table 9. ISV: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to treatments (2014-2015)

Treatment[ab]	°Brix	pH	TA (g/L)	NH_4^+-N[c] (mg N/L)	PAN[c] (mg N/L)	YAN[c] (mg N/L)	NH_4^+-N:PAN
15 N soil	23.04	3.39	6.98	26.49 c	75.58 bcd	102. 09 bc	0.34
15 N soil + 10 N foliar	22.04	3.43	6.91	43.92 a	95.5 ab	139.43 a	0.45
Crimson	22.68	3.42	6.57	24.86 c	68.05 cd	92.90 bc	0.34
Crimson + 10 N foliar	23.01	3.44	6.50	31.93 bc	88.99 abc	120.09 bc	0.36
White	22.34	3.43	6.84	25.82 c	65.09 d	90.90 c	0.37
White + 10 N foliar	21.75	3.44	6.60	42.85 ab	97.87 a	140.72 a	0.41
Trt[d]	ns[e]	ns	ns	<0.0001	<0.0001	<0.0001	ns
Yr	<0.0001	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001
Trt × Yr	ns	ns	ns	0.0258	Ns	ns	ns

[a]15 N soil = 15 kg N/ha applied to soil as calcium nitrate at bloom; 15 N soil + 10 N foliar = 15 kg N/ha applied to soil as calcium nitrate at bloom and two applications of 5 kg N/ha applied to foliage as urea at véraison; Crimson = under vine Crimson clover cover crop; Crimson + 10 N foliar = under vine Crimson clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison; White = under vine White clover cover crop; White + 10 N foliar = under vine White clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[e]ns = not significant

Table 10. ISV: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to treatments by year (2014 and 2015)

Treatment[ab]	Brix		pH		TA (g/L)		NH_4^+-N[c] (mg N/L)		PAN[c] (mg N/L)		YAN[c] (mg N/L)		NH_4^+-N:PAN	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
15 N soil	22.53	23.73	3.35	3.45	7.09	6.84	39.66 b	13.65 bc	88.50 ab	64.16 ab	128.15 abc	77.86 bc	0.45	0.21 ab
15 N soil + 10 N foliar	20.83	23.25	3.39	3.48	6.72	7.1	66.18 a	21.65 a	103.27 ab	87.40 a	169.79 ab	109.05 a	0.65	0.25 a
Crimson	22.63	23.53	3.37	3.53	6.42	6.62	39.81 b	9.93 c	77.80 ab	58.30 b	117.61 bc	68.20 c	0.52	0.17 b
Crimson + 10 N foliar	22.03	23.33	3.35	3.49	6.84	6.29	49.78 ab	13.43 bc	102.27 ab	76.97 ab	152.05 abc	90.39 abc	0.51	0.17 b
White	21.05	22.45	3.38	3.5	6.65	6.55	41.90 b	9.73 c	72.53 b	57.65 b	114.43 c	67.38 c	0.57	0.17 b
White + 10 N foliar	21.5	23.18	3.38	3.47	6.91	6.77	67.11 a	18.6 ab	112.52 a	83.22 a	179.64 a	101.80 ab	0.60	0.22 ab
Trt[d]	ns[e]	ns	ns	ns	ns	ns	0.0006	0.0002	0.0241	0.0006	0.0034	0.0004	ns	0.0035

[a]15 N soil = 15 kg N/ha applied to soil as calcium nitrate at bloom; 15 N soil + 10 N foliar = 15 kg N/ha applied to soil as calcium nitrate at bloom and two applications of 5 kg N/ha applied to foliage as urea at véraison; Crimson = under vine Crimson clover cover crop; Crimson + 10 N foliar = under vine Crimson clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison; White = under vine White clover cover crop; White + 10 N foliar = under vine White clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison

[b]Within columns, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]NH_4^+-N = ammonia nitrogen; PAN = primary amino nitrogen; YAN = yeast assimilable nitrogen (YAN = NH_4^+-N + PAN)

[d]Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[e]ns = not significant

Amino acids

GMV: Relative to the control, the 30 N foliar treatment significantly increased the concentration of Ser, Gln, Arg, Gly, Asp, Thr, Ala, GABA, Pro, Val, Ile and Leu ($p<0.05$) (Table 11). More than 70% of amino acid concentration was comprised of Pro, Arg, GABA, Glu, Ala and Gln (Table 12). As a proportion of the free amino acids measured, Gln, Arg, Ala and GABA were the only amino acids which increased their relative contribution to the overall pool of amino acids measured ($p<0.05$) (Table 55). Relative to the control, the 60 N soil treatment increased the total concentration of free amino acids by 53% (Table 11). The 30 N foliar treatment increased the total concentration of free amino acids by 106% (Table 11). The Pro to Arg ratio decreased significantly with both soil and foliar N fertilization ($p<0.05$) (Table 12). Year had a significant effect upon the absolute concentration of every acid measured, except His, Gln and Phe ($p<0.05$) (Table 11). There was a significant treatment-year interaction with His, Gly, Ile, Leu ($p<0.05$) (Table 11). Year had a significant effect upon the proportional contribution of each amino acid, except Arg, GABA, Val, Ile and Phe (Table 12).

AREC 1: The 45 N soil + 15 N foliar treatment significantly increased ($p<0.05$) the concentration of every amino acid measured, except for Lys (Table 13). Pro, Thr, Arg and Ser represented more than 75% of the amino acids measured (Table 14). The 45 N soil + 15 N foliar treatment increased the concentration of free amino acids by 99% (Table 13). His, Ser, Gln, Arg, Thr, Ala, Thr, Met, Val, Leu and Phe significantly increased in their relative proportional contribution to the overall concentration of free amino acids ($p<0.05$) (Table 14). The 45 N soil + 15 N foliar treatment significantly decreased the Pro to Arg ratio by over 250% (Table 14). Year had a

significant effect upon the concentration of every amino acid measured, except Gln, Glu, Thr and Lys (Table 13). Year had a significant effect ($p<0.05$) upon the proportional contribution of each amino acid measured, except Gly, Lys, Val and Ile (Table 14). Treatment-year interactions were significant ($p<0.05$) for the concentration of each amino acid, but Gln, Asp, Ala and Lys (Table 13). Treatment-year interactions were significant for the proportion of the following amino acids relative to the total concentration of amino acids measured: His, Ser, Gly, Asp, Glu, Ala, Tyr, Met, Val and Phe ($p<0.05$) (Table 14).

AREC 2: Relative to the control, the 15 N foliar and 15 N foliar + 5 S foliar treatments significantly increased the concentration of Ser, Gln, Arg, Thr, Ala and Pro ($p<0.05$) (Table 15). Both foliar treatments significantly increased the concentration of free amino acids by over 90% ($p<0.05$) (Table 15). Proline contributed over 60% of the total free amino acids measured (Table 59). Ser, Arg, Thr and Pro represented over 85% of the amino acids in the control, 15 N foliar and 15 N foliar + 5 S N foliar samples (Table 16). Both the 15 N foliar and 15 N foliar + 5 S foliar treatments significantly lowered the Pro to Arg ratio ($p<0.05$) (Table 16). Year significantly affected the concentration of every amino acid, except Asn, Ala and Lys ($p<0.05$) (Table 15). Year had a significant effect upon the relative proportion of Gln, Gly, Asp, Glu, Ala, Lys, Tyr and Leu ($p<0.05$) (Table 16). Treatment-year interactions were significant for the concentration of Asn, Ala and Pro ($p<0.05$) (Table 15). Treatment-year interactions were significant in regard to the relative concentration of Leu ($p<0.05$) (Table 16).

ISV: The only amino acid significantly increased by the White + 10 N foliar treatment was Arg, which increased by 25% relative to the 15 N soil treatment ($p<0.05$) (Table 17). Ser, Gln, Arg, Thr and Pro represented more than 70% of the free amino acids measured (Table 18). The proportion of Gln and Arg to the pool of free amino acids was significantly increased by the White + 10 N foliar treatment ($p<0.05$) (Table 18). The White + 10 N foliar treatment did not have a significant effect upon the total concentration of amino acids measured, but it did significantly decrease the Pro to Arg ratio ($p<0.05$) (Table 18). Year had a significant effect upon the concentration of Arg, Gly, Glu, Thr, Pro, Tyr, Val and Phe ($p<0.05$) (Table 17). Year had a significant effect upon the relative concentration of Ser and Phe ($p<0.05$) (Table 18).

Table 11. GMV: Juice amino acid concentrations (mg/L) at harvest in response to soil and foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	60 N soil	30 N foliar	Trt[e]	Yr	Trt × Yr
His	23.06 a	11.34 b	14.52 b	<0.0001	ns[f]	0.0147
Asn	nd[d]	nd	nd	-	-	-
Ser	23.26 b	34.47 ab	42.13 a	0.0016	0.0427	ns
Gln	27.19 c	58.46 b	86.03 a	<0.0001	ns	ns
Arg	78.15 c	161.50 b	239.84 a	<0.0001	<0.0001	ns
Gly	12.13	11.67	11.45	ns	<0.0001	0.0252
Asp	12.59 b	16.36 ab	21.03 a	0.0053	0.0307	ns
Glu	52.51	64.06	68.02	ns	<0.0001	ns
Thr	20.56 b	45.6 a	59.95 a	<0.0001	0.0007	ns
Ala	47.53 b	84.83 a	112.43 a	<0.0001	0.0285	ns
GABA	71.41 b	82.96 ab	100.60 a	0.0129	<0.0001	ns
Pro	98.12 b	147.55 b	220.58 a	<0.0001	0.0093	ns
Lys	nd	nd	nd	-	-	-
Tyr	nd	nd	nd	-	-	-
Cys	nd	nd	nd	-	-	-
Met	nd	nd	nd	-	-	-
Val	14.57 b	19.53 ab	24.04 a	0.0093	0.0049	ns
Ile	7.32 b	8.78 b	11.70 a	0.0018	0.0099	0.0054
Leu	10.03 b	10.95 b	13.85 a	0.0110	0.0292	0.0060
Phe	14.02	12.26	17.98	ns	ns	ns
Total	502.37 c	768.76 b	1033.12 a	<0.0001	0.0003	ns

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]Control = no N fertilization; 60 N soil = application of 30 kg N/ha as calcium nitrate at bloom and véraison; 30 N foliar = six applications of 5 kg N/ha as urea starting at bloom and separated by 7-10 days

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 12. GMV: Juice amino acid concentrations as a percentage of total amino acid concentration in response to soil and foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	60 N soil	30 N foliar	Trt[e]	Yr	Trt × Yr
His	4.70% a	1.8% b	1.47% b	<0.0001	0.0293	ns[f]
Asn	nd[d]	nd	nd	-	-	-
Ser	4.57%	4.49%	4.08%	ns	0.0083	ns
Gln	5.27% b	7.72% a	8.51% a	<0.0001	<0.0001	ns
Arg	15.62% b	21.01% a	22.76% a	<0.0001	ns	0.0004
Gly	2.28% a	1.47% b	1.08% c	<0.0001	<0.0001	0.0006
Asp	2.97% a	2.30% b	2.13%b	0.0002	<0.0001	ns
Glu	9.68% a	8.10% b	6.35% c	<0.0001	<0.0001	ns
Thr	3.80% b	5.69% a	5.66% a	<0.0001	0.0018	0.0429
Ala	9.31% b	11.18% a	10.88% a	0.0039	ns	ns
GABA	14.06% a	10.82% b	9.58% b	<0.0001	0.0003	ns
Pro	20.87%	19.21%	21.56%	ns	ns	ns
Lys	nd	nd	nd	-	-	-
Tyr	nd	nd	nd	-	-	-
Cys	nd	nd	nd	-	-	-
Met	nd	nd	nd	-	-	-
Val	2.77% a	2.49% ab	2.30% b	0.0283	ns	0.0055
Ile	1.34%	1.05%	1.00%	ns	ns	0.0413
Leu	3.11% a	1.22% b	1.71% ab	0.0275	0.0370	ns
Phe	3.45%	1.56%	2.20%	ns	ns	ns
Pro:Arg	1.34 a	0.95 b	0.96 b	0.0059	ns	ns

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɣ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]Control = no N fertilization; 60 N soil = application of 30 kg N/ha as calcium nitrate at bloom and véraison; 30 N foliar = six applications of 5 kg N/ha as urea starting at bloom and separated by 7-10 days

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 13. AREC 1: Juice amino acid concentrations (mg/L) at harvest in response to soil and foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	45 N soil + 15 N foliar	Trt[e]	Yr	Trt × Yr
His	10.80	52.23	0.0002	<0.0001	0.0006
Asn	4.00	8.55	<0.0001	0.0012	0.0007
Ser	33.43	122.00	<0.0001	<0.0001	<0.0001
Gln	18.30	167.49	<0.0001	ns[f]	ns
Arg	89.48	374.32	<0.0001	0.0188	0.003
Gly	4.44	6.28	0.0103	<0.0001	0.0013
Asp	8.01	15.74	<0.0001	0.0006	ns
Glu	12.37	34.40	<0.0001	ns	0.0004
Thr	30.70	97.40	<0.0001	ns	0.0074
Ala	22.78	99.07	<0.0001	0.0003	ns
GABA	23.38	32.83	<0.0001	<0.0001	0.0031
Pro	1028.46	1482.66	0.0002	<0.0001	0.0066
Lys	2.94	2.15	ns	ns	ns
Tyr	6.21	29.29	<0.0001	<0.0001	<0.0001
Cys	nd[d]	nd	-	-	-
Met	1.00	6.52	<0.0001	0.0167	0.0046
Val	16.07	40.33	<0.0001	<0.0001	<0.0001
Ile	7.61	14.89	<0.0001	<0.0001	<0.0001
Leu	12.54	30.95	<0.0001	<0.0001	<0.0001
Phe	9.41	37.75	<0.0001	<0.0001	<0.0001
Total	**1337.07**	**2653.10**	**<0.0001**	**<0.0001**	**0.0009**

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Student's T-test (α=0.05)

[c] Control = no N fertilization; 45 N soil + 15 N foliar = 45 kg N/ha applied to soil as calcium nitrate at bloom and two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 14. AREC 1: Juice amino acid concentrations as a percentage of total amino acid concentration in response to soil and foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	45 N soil + 15 N foliar	Trt[e]	Yr	Trt × Yr
His	0.84%	1.62%	0.0139	0.0099	0.0056
Asn	0.40%	0.36%	ns[f]	0.0003	ns
Ser	3.04% b	4.71% a	<0.0001	<0.0001	<0.0001
Gln	1.73% b	7.25% a	<0.0001	0.0006	ns
Arg	8.18% b	15.79% a	<0.0001	<0.0001	ns
Gly	0.36% a	0.22% b	0.0021	ns	0.0109
Asp	0.84%	0.76%	ns	<0.0001	0.0158
Glu	1.22%	1.46%	ns	<0.0001	0.0139
Thr	2.90% b	4.14% a	0.0004	<0.0001	ns
Ala	2.37% b	4.95% a	<0.0001	<0.0001	0.0036
GABA	1.94% a	1.32% b	0.0003	0.0003	ns
Pro	72.30% a	51.68% b	<0.0001	<0.0001	ns
Lys	0.17%	0.07%	ns	ns	ns
Tyr	0.44% b	0.99% a	<0.0001	0.0111	0.0002
Cys	nd[d]	nd	-	-	-
Met	0.08% b	0.25% a	0.0004	0.0150	0.0475
Val	1.26% b	1.50% a	0.0065	ns	0.0073
Ile	0.58%	0.55%	ns	ns	ns
Leu	0.91% b	1.12% a	0.0291	0.0291	ns
Phe	0.71% b	1.24% a	0.0009	0.0138	0.0003
Pro:Arg	12.97	3.69	<0.0001	<0.0001	0.0002

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Student's T-test (α=0.05)

[c]Control = no N fertilization; 45 N soil + 15 N foliar = 45 kg N/ha applied to soil as calcium nitrate at bloom and two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 15. AREC 2: Juice amino acid concentrations (mg/L) at harvest in response foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	15 N foliar	15 N foliar + 5 S foliar	Trt[e]	Yr	Trt × Yr
His	13.20 b	22.90 ab	28.23 a	0.0048	0.0023	ns[f]
Asn	6.29	4.70	5.55	ns	ns	0.0208
Ser	28.46 b	56.19 a	64.72 a	0.0008	0.0227	ns
Gln	19.84 b	54.30 a	62.33 a	0.0005	0.0345	ns
Arg	82.75 b	270.41 a	296.13 a	<0.0001	0.0008	ns
Gly	6.346	6.833	6.548	ns	0.0296	ns
Asp	10.205	9.561	10.978	ns	0.0009	ns
Glu	14.01 b	18.43 ab	20.48 a	0.0161	0.0002	ns
Thr	22.95 b	58.25 a	64.65 a	0.0001	0.0036	ns
Ala	11.26 b	25.19 a	29.66 a	0.0001	ns	0.0469
GABA	18.71 b	23.38 ab	30.20 a	0.0272	0.0004	ns
Pro	1041.58 b	1914.19 a	1955.63 a	<0.0001	<0.0001	<0.0001
Lys	2.96	3.90	4.70	ns	ns	ns
Tyr	8.67 b	17.86 ab	21.39 a	0.0110	0.0004	ns
Cys	nd[d]	nd	nd	-	-	-
Met	nd	nd	nd	-	-	-
Val	20.80 b	28.03 ab	33.84 a	0.0138	0.0024	ns
Ile	13.62	14.38	16.97	ns	0.0047	ns
Leu	19.78	23.88	29.18	ns	0.0036	ns
Phe	16.50	21.01	25.47	ns	0.0049	ns
Total	**1350.73 b**	**2570.85 a**	**2704.59 a**	**<0.0001**	**<0.0001**	**<0.0001**

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]Control = no N fertilization; 15 N foliar = two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison; 15 N foliar + 5 S foliar = two 7.5 kg N/ha applications of urea and 2.5 kg S/ha as micronized sulfur separated by 7-10 days at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 16. AREC 2: Juice amino acid concentrations as a percentage of total amino acid concentration in response to foliar N fertilization (2014-2015)

Amino acid[ab]	Control[c]	15 N foliar	15 N foliar + 5 S foliar	Trt[e]	Yr	Trt × Yr
His	1.02%	0.88%	1.04%	ns[f]	ns	ns
Asn	0.5% a	0.18% b	0.22% b	0.0066	ns	ns
Ser	2.15%	2.21%	2.56%	ns	ns	ns
Gln	1.38% b	2.34% ab	2.97% a	0.0049	<0.0001	ns
Arg	6.1% b	10.41% a	12.31% a	0.0030	ns	ns
Gly	0.48%	0.29%	0.30%	ns	0.0003	ns
Asp	0.74%	0.41%	0.55%	ns	<0.0001	ns
Glu	1.04%	0.78%	1.01%	ns	<0.0001	ns
Thr	1.69% b	2.26% ab	2.56% a	0.0256	ns	ns
Ala	0.84%	1.00%	1.21%	ns	0.0091	ns
GABA	1.51% a	0.89% b	1.17% ab	0.0374	ns	ns
Pro	76.80%	74.16%	69.05%	ns	ns	ns
Lys	0.20%	0.16%	0.22%	ns	0.0154	ns
Tyr	0.71%	0.66%	0.75%	ns	0.0207	ns
Cys	nd[d]	nd	nd	-	-	-
Met	nd	nd	nd	-	-	-
Val	1.59% a	1.10% b	1.34% ab	0.0305	ns	ns
Ile	1.02% a	0.56% b	0.67% b	0.0055	ns	ns
Leu	1.49% a	0.94% b	1.16% ab	0.0190	ns	0.0293
Phe	1.20%	0.82%	0.95%	ns	ns	ns
Pro:Arg	15.15 a	7.89 b	6.48 b	0.0137	ns	ns

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɣ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Tukey's HSD (α=0.05)

[c]Control = no N fertilization; 15 N foliar = two 7.5 kg N/ha applications of urea separated by 7-10 days at véraison; 15 N foliar + 5 S foliar = two 7.5 kg N/ha applications of urea and 2.5 kg S/ha as micronized sulfur separated by 7-10 days at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 17. ISV: Juice amino acid concentrations (mg/L) at harvest in response White clover and foliar N fertilization (2014-2015)

Amino acid[ab]	15 N soil[c]	White + 10 N foliar	Trt[e]	Yr	Trt × Yr
His	19.12	17.35	ns[f]	ns	ns
Asn	3.80	9.94	ns	ns	ns
Ser	31.28	33.00	ns	ns	ns
Gln	79.48	118.28	ns	ns	ns
Arg	209.85 b	262.70 a	0.0498	0.0002	ns
Gly	8.71	5.86	ns	0.0234	ns
Asp	43.62	30.05	ns	ns	ns
Glu	57.04	53.13	ns	0.0147	ns
Thr	26.47	29.37	ns	0.0149	ns
Ala	80.06	80.70	ns	ns	ns
GABA	73.86	61.61	ns	ns	ns
Pro	699.67	644.14	ns	<0.0001	ns
Lys	nd[d]	nd	-	-	-
Tyr	12.44	12.97	ns	0.0447	ns
Cys	nd	nd	-	-	-
Met	nd	nd	-	-	-
Val	16.33	17.75	ns	0.0270	ns
Ile	3.73	6.05	ns	ns	ns
Leu	8.07	11.85	ns	ns	ns
Phe	23.13	18.25	ns	0.0022	ns
Total	**1384.73**	**1408.25**	**ns**	**<0.0001**	**ns**

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Student's T-test (α=0.05)

[c]15 N soil = 15 kg N/ha applied to soil as calcium nitrate at bloom; White + 10 N foliar = under vine White clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Table 18. ISV: Juice amino acid concentrations as a percentage of total amino acid concentration in response to white clover and foliar N fertilization (2014-2015)

Amino acid[ab]	15 N soil[c]	White + 10 N foliar	Trt[e]	Yr	Trt × Yr
His	1.32%	1.15%	ns[f]	ns	ns
Asn	0.38%	0.72%	ns	ns	ns
Ser	2.34%	2.52%	ns	0.0019	ns
Gln	5.58% b	9.00% a	0.0351	ns	ns
Arg	14.90% b	18.38% a	0.0065	ns	ns
Gly	0.59%	0.39%	ns	ns	ns
Asp	3.05%	2.15%	ns	ns	ns
Glu	3.98%	3.77%	ns	ns	ns
Thr	1.91%	2.10%	ns	ns	ns
Ala	5.87%	5.92%	ns	ns	ns
GABA	5.33%	4.57%	ns	ns	ns
Pro	51.01%	44.90%	ns	ns	ns
Lys	nd[d]	nd	-	-	-
Tyr	0.90%	0.91%	ns	ns	ns
Cys	nd	nd	-	-	-
Met	nd	nd	-	-	-
Val	1.20%	1.31%	ns	ns	ns
Ile	0.37%	0.46%	ns	ns	ns
Leu	0.63%	0.84%	ns	ns	ns
Phe	1.56%	1.18%	ns	0.0202	ns
Pro:Arg	3.53	2.45	0.0174	ns	ns

[a]His = histidine; Asn = asparagine; Ser = serine; Gln = glutamine; Arg = arginine; Gly = glycine; Asp = aspartic acid; Glu = glutamic acid; Thr = threonine; Ala = alanine; GABA = ɤ-aminobutryic acid; Pro = proline, Lys = lysine; Tyr = tyrosine; Cys = cysteine; Met = methionine; Val = valine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine

[b]Within row, means with different letters indicate differences of means using Student's T-test (α=0.05)

[c]15 N soil = 15 kg N/ha applied to soil as calcium nitrate at bloom; White + 10 N foliar = under vine White clover cover crop and two applications of 5 kg N/ha applied to foliage as urea at véraison

[d]nd = not detectable

[e]Significance of effects using two-way ANOVA. Trt = significance of treatment effects; Yr = significance of year effects; Trt × Yr = significance of treatment by year interaction

[f]ns = not significant

Discussion

Juice pH increased with soil and foliar-applied N fertilization at all sites, except ISV. However, the result was inconsistent from year to year at GMV and AREC 1 due to the significant treatment-year interaction. At AREC 2, pH was consistently higher among foliar N treatments, however this difference was only statistically significant in the second year of the study. Others have found juice pH to increase in response to foliar N treatments (Hannam et al. 2014; Lasa et al. 2012). Whereas others have found foliar urea treatments to have no impact upon juice pH (Garde-Cerdán et al. 2014; Hannam et al. 2016). Hannam et al. (2014) found that pH increased slightly with foliar N applications in Merlot in two years of a three-year experiment. However, Hannam et al. (2014) did not observe a significant impact of foliar urea upon juice pH on any of the other three varieties evaluated in that study. The changes in pH found in the current study were small and unlikely to dramatically alter wine quality.

Juice TA was significantly different between the 60 N soil and the 45 N soil + foliar treatment at AREC 1, with the 60 N soil treatment having a lower TA. Some have found higher rates of soil supplied N to depress titratable acidity (Ewart and Kliewer 1977). However, as Bell and Henschke (2005) point out, the response of TA to N fertilization is often variable. Juice TA significantly increased with a foliar application of urea at AREC. The relationship between TA and foliar urea treatments may depend upon grape cultivar. For example, Lasa et al. (2012) found that TA increased with post-véraison foliar urea treatments in Sauvignon blanc, whereas Hannam et al. (2014) found that foliar urea applied at véraison decreased TA in Pinot gris.

Soluble solids were not impacted by any treatment except for at AREC 1, where the SSC of the 60 N treatment was significantly less than that of the 45 N soil + 15 N foliar treatment.

It's worth noting that this result was only seen in the combined years model, and not within either year.

Bell and Henschke (2005) posited that the most dependable outcome of vineyard N fertilization was an increase in nitrogenous compounds within the grape berry. The results of the current study support this assertion. N fertilization increased berry YAN in both years, at each site. Although in many cases soil-applied N increased berry YAN, foliar urea consistently resulted in the most dramatic increases in YAN. Foliar urea did not significantly increase berry YAN at AREC 2 in 2014. This was the only year/site at which berry YAN was not significantly increased by foliar urea treatments. This inter-annual variation in foliar urea efficacy at increasing berry YAN was also found by Hannam et al. (2014). Hannam et al. (2014) suggested that the differential responses from year to year were likely the result of climatic and/or managerial practices, rather than varietal or site specific differences.

Foliar urea has been shown to increase berry YAN in numerous studies (Ancín-Azpilicueta et al. 2013; Garde-Cerdán et al. 2015; Hannam et al. 2014; Tozzini et al. 2013; Verdenal et al. 2015). Studies comparing both soil and foliar-applied N found that the most dramatic impact upon berry YAN came from the application of urea to the foliage around véraison, which is in agreement with the current study (Hannam et al. 2016; Lacroux et al. 2008). In a study of ^{15}N labelled urea applied to the foliage of Chasselas (*V. vinifera*), it was found that bunches were the strongest sink for foliar N when applied at both flowering and véraison (Verdenal et al. 2015). The highest berry YAN at GMV came from the 30 N foliar treatment which was applied from flowering over the course of 6 sprays separated by 7-10 days and finished prior to véraison. This result indicated that although véraison may be the most

efficient period to apply foliar urea in order to increase berry YAN (Lasa et al. 2012), application of foliar urea from flowering onward may also increase berry YAN status. Schreiber et al. (2002) found that about 30% of the N that was applied to the foliage was assimilated by the grape berries, whereas only 2% of the N applied to the soil was partitioned into the fruit. This difference in N partitioning with regards to method of application is likely why foliar-applied urea resulted in a greater improvement of YAN status which, in most cases, was more significant than the impact of soil-applied N treatments.

The co-application of urea and micronized sulfur (S) to the foliage of wheat has been previously found to assist in the assimilation of both N and S when compared to the sole application of either nutrient (Tea et al. 2007). However, the mechanism behind the apparent synergistic interaction between foliar urea and micronized sulfur has not been elucidated (Tea et al. 2007). In one year of a two-year grape study, Kelly et al. (2013) found that the co-application of urea and micronized S improved berry YAN significantly more than when urea was applied alone. However, in both years at AREC 2, the combined foliar application of urea and micronized sulfur was unable to significantly improve the berry YAN status more than just the application of urea alone. Lacroux et al. (2008) also did not find a significant increase in YAN with a co-application of urea and micronized S. The co-application of N and S warrants further investigation. I would propose establishing a dosing experiment in which the total amounts of N and S applied are varied. It may be that the amounts of N and S applied in the current study were insufficient to produce a measurable response.

The lowest YAN concentrations at ISV came from the clover cover cropped treatments. Non-leguminous cover crops can compete with the vine for N, depressing berry YAN (Pérez-

Álvarez et al. 2015; Sweet and Schreiner 2010). The majority of the species within the cover crop stands at ISV were non-leguminous weeds which may have resulted in N competition and lead to a deleterious impact upon berry YAN. Moss (2016) presented data which quantified cover crop biomass and stand density at ISV.

Foliar urea treatments have been previously found to increase the concentration of both NH_4^+-N and PAN in the juice (Hannam et al. 2016). Foliar urea applications were also found to increase both the inorganic and organic constituents of YAN in the current study. When foliar urea had a significant impact upon the NH_4^+-N to PAN ratio, it tended to increase the concentration of inorganic N to PAN. However, this result was not consistent across all years and varieties. Treatment-year interactions were significant for PAN at AREC 1 and 2 and for ammonia at ISV. Therefore, the ratio of inorganic to organic YAN sources may be more dependent upon seasonal variables than nitrogenous fertilization. The apparent lack of inorganic N:organic N response to N fertilization has positive enological consequences, as amino-nitrogen is often a preferred nitrogen source by winemakers as high ammonium concentrations can lead to greater acetic acid production (Torrea et al. 2011) and even increased H_2S production if ammonium is fully utilized prior to the completion for fermentation (Jiranek et al. 1995b). The significant increase in PAN concentrations has important implications for wine quality, as amino acids are precursors to volatile compounds which are produced during alcoholic and malolactic fermentation, such as esters, varietal thiols, volatile fatty acids, higher alcohols and carbonyls (Äyrapaa 1971; Duhamel et al. 2015; Garde-Cerdán and Ancín-Azpilicueta 2008; Schneider et al. 2006). However, these aromatic compounds were not measured in the current study and could be the focus of future research.

A YAN concentration of 140 mg N/L is generally accepted as being the minimum concentration needed to successfully bring a fermentation of a must destined for a normal table wine to dryness (Butzke 1998). Foliar urea treatments were able to attain the YAN minimum in most years across all sites in the current study. The 140 ppm YAN minimum was only not reached at AREC 2 and ISV in 2014 and 2015 respectively.

Most amino acids measured increased in concentration in response to nitrogen fertilization in the form of soil-applied calcium nitrate for foliar urea at all sites, but ISV. Arginine (Arg) was the only amino acid positively influenced by the White + 10 N foliar treatment at ISV. The apparent lack of amino acid response to the White + 10 N foliar treatment is not surprising, as the PAN concentrations between the 15 N soil and White + 10 N foliar treatments were not dramatically different in 2014 and 2015. However, the PAN concentrations coming from the White + 10 N foliar treatments were consistently higher, which may mostly be due to the positive effect this treatment had upon Arg.

While the 60 N soil treatment did result in an increase of most amino acids at GMV, the 30 N foliar treatment was most effective at increasing amino acid concentrations. Hannam et al. (2016) also found that while soil-applied N increased some amino acids, foliar applications of urea were more effective in this regard. The most responsive amino acids to foliar urea across GMV, AREC 1 and AREC 2 were Arginine (Arg), Glutamine (Gln), Tyrosine (Tyr), Alanine (Ala) and Threonine (Thr). D'Atillio (2013) also found Arg, Ala, Thr and Gln to be among the most responsive amino acids to foliar urea applications. The least affected were Lysine (Lys), Glycine (Gly), ɣ-aminobutryic acid (GABA), Asparagine (Asn) and Aspartic acid (Asp). This is partially consistent with Hannam et al. (2016) who found that Arg, Gln, Val, Ala and Ile increased the

most in response to foliar urea applications, whereas Pro, GABA, Glu, Asp and Phe were the least responsive.

Interestingly, Histidine (His) increased in response to foliar applications of urea in all vineyards, except GMV. This may have occurred due to varietal differences.

The significant increase in threonine has important implications for wine quality. Hernandez-Orte (2002) found that threonine has the most appreciable effect upon wine aroma. Esters confer a general fruity character to a wine and are produced during fermentation at concentrations well above their odor threshold (Pretorious and Lambrechts 2000). Higher alcohols are synthesized through the transamination of amino acids, which can then form the alcohol group of the acetate esters (Boulton et al. 1996; Sumby et al. 2010). The supplementation of musts with amino acids has been demonstrated to increase the concentration of esters and the production of a wine that was perceived as being fruitier than those wines which had not been supplied additional amino acids (Torrea et al. 2011). A study conducted upon Tempranillo found that wines made from grapes sprayed with urea had increased concentrations of esters and scored higher in aromatic intensity and fruitiness than wines made from grapes which had not received a foliar urea treatment (Ancín-Azpilicueta et al. 2013). Therefore, due to the apparent ability for foliar urea applications to significantly increase many of the amino acids, this practice can have a positive impact upon wine quality, depending upon stylistic goals and winemaking ethos.

Juice Arg and Gln concentrations more than doubled, relative to the control, each year in response to foliar urea treatments at each site, but ISV. This has positive winemaking implications, as Arg and Gln are two of the most readily assimilated amino-N sources by

Saccharomyces cerevisiae (Bell and Henschke 2005; Garde-Cerdán et al. 2007; Jiranek et al. 1995a).

Foliar and soil N application significantly depressed the Pro to Arg ratio. Lasa et al. (2012) reported a decrease in the Pro to Arg ratio in response to foliar urea treatments. Others have also reported a significant decrease in the Pro to Arg ratio in relation to soil-applied N (Conradie 2001; Rodriguez-lovelle and Gaudillere 2002).

Foliar urea treatments had a widely variable response upon the amino acid profile of the Petit Manseng at AREC 1, as demonstrated by the significant treatment-year interaction in most of the amino acids measured. Amino acid profiles can be affected by various environmental stresses and by the degree of fruit maturity (Cramer et al. 2007; Kliewer 1968; Matthews and Anderson 1988). Other studies have found that foliar urea treatments often have extremely variable effects upon individual juice amino acids (Garde-Cerdán et al. 2014; Hannam et al. 2016; Lasa et al. 2012).

The tendency for amino acid concentrations to increase with increasing levels of soil and foliar N fertilization has important wine quality implications.

Wines were made from the Sauvignon blanc at GMV and the Petit Manseng in 2014 and 2015. The Petit Manseng fermentations ceased prior to completion in both years and were therefore not utilized for thiol analysis. The 2014 and 2015 Sauvignon blanc from GMV was bottled and sent to a contract laboratory for the analysis of volatile thiols (Hill laboratories; Hamilton, New Zealand). However, many of the thiols were “not detectable”. Thiols are known to oxidize readily (Allen et al. 2011; Nikolantonaki et al. 2010). In this study, small quantities of wine were produced. Therefore, wines were bottled in non-traditional, small format vessels.

Screwcap bottles with foil liners were used as this type of closure has previously been found to be highly reductive (Lopes et al. 2009). However, Lopes et al. (2009) conducted their study on traditional wine bottles. No oxygen permeability data for the vessels used in this study existed prior to bottling. Therefore, it is possible that oxidation of the wines occurred within the packaging material. Also, it should be noted that commercial harvest at GMV occurred when the fruit was at 20-22^{o}Brix. It is known that thiol aromatic potential of a must increases with ripening (Capone et al. 2011a; des Gachons et al. 2005). Also, in a recent survey of wines from New York state, it was found that the concentration of thiols in the Sauvignon blanc wines were lower than those reported from other regions of the world (Musumeci et al. 2015). Therefore, the thiol concentrations of the Sauvignon blanc may have been limited from the outset.

To improve results of similar studies, the thiol concentrations of the commercial wines made from the property being used in the study should be evaluated in order to assess whether or not they can be detected prior to making wines from these sites. Also, small-lot wine making is inherently oxidative, so if wine lots can be scaled up to larger quantities, this may alleviate some of the oxidative risk. Wines being made for thiol research should also be bottled in traditional 750 ml bottle-cork or bottle-screwcap combinations in order to avoid potential oxidation within the packaging, and to best approximate the extent of the impact these treatments could be expected to have on commercial wines.

Conclusion

Foliar applications of urea were more effective at increasing YAN and amino acid concentrations than were soil applications. The ammonia to primary amino nitrogen ratio was slightly increased by foliar urea applications in some years. However, the relative concentration of the inorganic and organic N sources tended to remain similar to the controls when foliar urea was applied. Increased YAN and amino acid concentrations associated with the foliar urea treatments could have positive consequences for wine aroma, although this could not be demonstrated with the wine-making techniques used in this study. Foliar urea tended to have the most dramatic increase upon concentrations of arginine and glutamine, which are two of the most readily assimilated amino nitrogen sources. Soluble solids and titratable acidity was largely unaffected by any of the treatments in this study. However, juice pH was slightly increased by N application, but the increase was not of great enological consequence. Further research is needed with regards to N fertilization and primary aroma production, specifically in relation to foliar applied urea treatments.

References

Alberts, P., M.A. Stander, S.O. Paul, and A. de Villiers. 2009. Survey of 3-Alkyl-2-methoxypyrazine content of South African Sauvignon blanc wines using a novel LC– APCI-MS/MS method. Journal of agricultural and food chemistry 57:9347-9355.

Allen, M., M. Lacey, R. Harris, and W. Brown. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. American Journal of Enology and Viticulture 42:109-112.

Allen, M.S., M.J. Lacey, and S. Boyd. 1994. Determination of methoxypyrazines in red wines by stable isotope dilution gas chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry 42:1734-1738.

Allen, T., M. Herbst-Johnstone, M. Girault, P. Butler, G. Logan, S. Jouanneau, L. Nicolau, and P. Kilmartin. 2011. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon blanc wines. Journal of Agricultural and Food Chemistry 59:10641-10650.

Ancín-Azpilicueta, C., R. Nieto-Rojo, and J. Gómez-Cordón. 2013. Effect of foliar urea fertilisation on volatile compounds in Tempranillo wine. Journal of the Science of Food and Agriculture 93:1481-1485.

Anfang, N., M. Brajkovich, and M.R. Goddard. 2009. Co-fermentation with Pichia kluyveri increases varietal thiol concentrations in Sauvignon Blanc. Australian Journal of Grape and Wine Research 15:1-8.

Augustyn, O., A. Rapp, and C. Van Wyk. 1982. Some volatile aroma components of Vitis vinifera L. cv. Sauvignon blanc. South African Journal for Enology and Viticulture 3:53-60.

Austin, C., G. Grove, J. Meyers, and W. Wilcox. 2011. Powdery Mildew Severity as a Function of Canopy Density: Associated Impacts on Sunlight Penetration and Spray Coverage. American Journal of Enology and Viticulture 23-31.

Äyrapaa, T. 1971. Biosynthetic formation of higher alcohols by yeast. Dependence on the nitrogenous nutrient level of the medium. Journal of the Institute of Brewing 77:266-276.

Barril, C., A.C. Clark, and G.R. Scollary. 2012. Chemistry of ascorbic acid and sulfur dioxide as an antioxidant system relevant to white wine. ANALYTICA CHIMICA ACTA 732:186-193.

Bayonove, C., R. Cordonnier, and P. Dubois. 1975. Etude d'une fraction caracteristique de l'arome du raisin de la variete Cabernet-Sauvignon: mise en evidence de la 2-methoxy-3-isobutylpyrazine. Comptes Rendus de l'Academie des Sciences 281:75-78.

Belancic, A., and E. Agosin. 2007. Methoxypyrazines in grapes and wines of Vitis vinifera cv. Carmenere. American Journal of Enology and Viticulture 58:462-469.

Belancic, A., E. Agosin, A. Ibacache, E. Bordeu, R. Baumes, A. Razungles, and C. Bayonove. 1997. Influence of sun exposure onthe aromatic composition of chilean muscat grape cultivars moscatel de alejandria and moscatel rosada. American Journal of Enology and Viticulture 48:181-186.

Bell, S., and P. Henschke. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research 11:242-295.

Bell, S., and A. Robson. 1999. Effect of nitrogen fetilization on growth, canopy density and yield of Vitis vinifera L. cv. Cabernet Sauvignon. American Journal of Enology and Viticulture 50:351-358.

Benkwitz, F., T. Tominaga, P. Kilmartin, C. Lund, M. Wohlers, and L. Nicolau. 2012. Indentifying the chemical composition related to the distinct aroma characteristic of new zealand sauvignon blanc wines. American Journal of Enology and Viticulture 63:62-72.

Bledsoe, A., W. Kliewer, and J. Marois. 1988. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. American journal of enology and viticulture 39:49-54.

Boulton, R., V. Singleton, L. Bisson, and R. Kunkee. 1996. *Principles and Practices of Winemaking*. Springer Science and Business Media inc, New York City.

Bravdo, B. 2000. Effect of mineral nutrition and salinity on grape production and wine quality. Acta Horticulturae 512:23-30.

Buttery, R., R. Seifert, D. Guadagni, and L. Ling. 1969. Characterization of some volatile constituents of bell peppers. Journal of Agricultural and Food Chemistry 17:1322-1327.

Buttery, R.G., and L.C. Ling. 1973. Earthy aroma of potatoes. Journal of Agricultural and Food Chemistry 21:745-746.

Butzke, C.E. 1998. Survey of yeast assimilable nitrogen status in musts from California, Oregon, and Washington. American Journal of Enology and Viticulture 49:220-224.

Campo, E., V. Ferreira, A. Escudero, and J. Cacho. 2005. Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data. Journal of Agricultural and Food Chemistry 53:5682-5690.

Candelon, N., S. Shinkaruk, B. Bennetau, C. Bennetau-Pelissero, M.-L. Dumartin, M. Degueil, and P. Babin. 2010. New approach to asymmetrically substituted methoxypyrazines, derivatives of wine flavors. Tetrahedron 66:2463-2469.

Capone, D., D. Jeffery, and M. Sefton. 2012. Vineyard and fermentation studies to elucidate the origin of 1,8-cineole in Australian red wine. Journal of Agricultural and Food Chemistry 60:2281-2287.

Capone, D., M. Sefton, and D. Jeffery. 2011a. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. Journal of Agricultural and Food Chemistry 59:4649-4658.

Capone, D., C. Van Leeuwen, D. Taylor, D. Jeffery, K. Pardon, G. Elsey, and M. Sefton. 2011b. Evolution and occurence of 1,8-cineole (eucalyptol) in Australian wine. Journal of Agricultural and Food Chemistry 59:953-959.

Carrau, F., K. Medina, E. Boido, L. Farina, C. Gaggero, E. Dellacassa, and P. Henschke. 2005. De novo synthesis of monoterpenes by Saccharomyces cerevisiae wine yeasts. FEMS microbiology letters 243:107-115.

Celette, F., A. Findeling, and C. Gary. 2009. Competition for nitrogen in an unfertilized intercropping system: the case of an association of grapevine and grass cover in a Mediterranean climate. European Journal of Agronomy 30:41-51.

Chen, L., and L. Cheng. 2003. Carbon assimilation and carbohydrate metabolism of concord grape (Vitis labrusca L.) leaves in response to nitrogen supply. Journal of the American Society for Horticultural Science 128:754-760.

Chisholm, M., L. Guiher, T. Vonah, and J. Beaumont. 1994. Comparison of some french-american hybrid wines with white riesling using gas chromatography-olfactometry. American Journal of Enology and Viticulture 45:201-212.

Coelho, E., S. Rocha, I. Delgadillo, and M. Coimbra. 2006. Headspace-SPME applied to varietal volatile components evolution during Vitis vinifera L. cv. Baga ripening. Analytica Chimica Acta 563:204-214.

Coetzee, C., and W.J. du Toit. 2012. A comprehensive review on Sauvignon blanc aroma with a focus on certain positive volatile thiols. Food Research International 45:287-298.

Coetzee, C., K. Lisjak, L. Nicolau, P. Kilmartin, and W.J. Toit. 2013. Oxygen and sulfur dioxide additions to Sauvignon blanc must: effect on must and wine composition. Flavour and Fragrance Journal 28:155-167.

Conradie, W. 2001. Timing of nitrogen fertilisation and the effect of poultry manure on the performance of grapevines on sandy soil. II. Leaf analysis, juice analysis and wine quality. South African Journal for Enology and Viticulture 22:60-68.

Copolovici, L., I. Filella, J. Llusia, U. Niinemets, and J. Penuelas. 2005. The capacity for thermal protection of photosynthetic electron transport varies for different monoterpenes in quercus ilex. Plant Physiology 139:485-496.

Cramer, G.R., A. Ergül, J. Grimplet, R.L. Tillett, E.A. Tattersall, M.C. Bohlman, D. Vincent, J. Sonderegger, J. Evans, and C. Osborne. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Functional & integrative genomics 7:111-134.

Culleré, L., A. Escudero, J. Cacho, and V. Ferreira. 2004. Gas chromatography−olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. Journal of Agricultural and Food Chemistry 52:1653-1660.

Darriet, P., T. Tominaga, V. Lavigne, J.-N. Boidron, and D. Dubourdieu. 1995. Identification of a powerful aromatic component of Vitis vinifera L. var. sauvignon wines: 4-mercapto-4-methylpentan-2-one. Flavour and Fragrance Journal 10:385-392.

des Gachons, C.P., C.V. Leeuwen, T. Tominaga, J.P. Soyer, J.P. Gaudillère, and D. Dubourdieu. 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of Vitis vinifera L cv Sauvignon blanc in field conditions. Journal of the Science of Food and Agriculture 85:73-85.

Diago, M.P., B. Ayestarán, Z. Guadalupe, S. Poni, and J. Tardáguila. 2012. Impact of Prebloom and Fruit-Set Basal Leaf Removal on the Flavonol and Anthocyanin Composition of Tempranillo Grapes. American journal of enology and viticulture:ajev. 2012.11116.

Dimitriadis, E., and P. Williams. 1984. The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. American Journal of Enology and Viticulture 35:66-71.

Dimkou, E., M. Ugliano, J.B. Dieval, S. Vidal, O. Aagaard, D. Rauhut, and R. Jung. 2011. Impact of Headspace Oxygen and Closure on Sulfur Dioxide, Color, and Hydrogen Sulfide Levels in a Riesling Wine. American Journal of Enology and Viticulture 62:261-269.

Dokoozlian, N., and W. Kliewer. 1995. The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. American Journal of Enology and Viticulture 46:219-226.

———. 1996. Influence of light on grape berry growth and composition varies during fruit development. Journal of the American Society for Horticultural Science 121:869-874.

Dominguez, A., and E. Agosin. 2010. Gas chromatography coupled with mass spectrometry detection for the volatile profiling of Vitis vinifera CV. carmenere wines. Journal of the Chilean Chemical Society 55:385-391.

dos Santos, T.P., C.M. Lopes, M.L. Rodrigues, C.R. de Souza, J.M. Ricardo-da-Silva, J.P. Maroco, J.S. Pereira, and M.M. Chaves. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. Scientia Horticulturae 112:321-330.

Dragar, V., and R. Menary. 1995. Mineral nutrition of Olearia phlogopappa: effect on growth, essential oil yield, and composition. Communications in Soil Science & Plant Analysis 26:1299-1313.

Du Toit, W.J., K. Lisjak, M. Stander, and D. Prevoo. 2007. Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 55:2765-2769.

Dubourdieu, D., T. Tominaga, I. Masneuf, C. Peyrot des Gachons, and M. Murat. 2006. The role of yeasts in grape flavour development during fermentation: the example of Sauvignon blanc. American Journal of Enology and Viticulture 57:81-88.

Duhamel, N., F. Piano, S.J. Davidson, R. Larcher, B. Fedrizzi, and D. Barker. 2015. Synthesis of alkyl sulfonic acid aldehydes and alcohols, putative precursors to important wine aroma thiols. Tetrahedron Letters 56:1728-1731.

Dukes, B.C., and C.E. Butzke. 1998. Rapid determination of primary amino acids in grape juice using an o-phthaldialdehyde/N-acetyl-L-cysteine spectrophotometric assay. American Journal of Enology and Viticulture 49:125-134.

Eebler, S.H., A. 2014. A comparison of extraction techniques for GC-MS/MS analysis of Oodor-active methoxypyrazines in wines. *In* 42nd Annual Winter Colloquium. UC Davis.

Ewart, A., and W.M. Kliewer. 1977. Effects of controlled day and night temperatures and nitrogen on fruit-set, ovule fertility, and fruit composition of several wine grape cultivars. American Journal of Enology and Viticulture 28:88-95.

Farina, L., E. Boido, F. Carrau, G. Versini, and E. Dellacassa. 2005. Terpene compounds as possible precursors of 1,8-cineole in red grapes and wines. Journal of Agricultural and Food Chemistry 53:1633-1636.

Frost, C., H. Appel, J. Carlson, C. De Moraes, M. Mescher, and J. Schultz. 2007. Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. Ecology Letters 10:490-498.

Garde-Cerdán, T., and C. Ancín-Azpilicueta. 2008. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. LWT-Food Science and Technology 41:501-510.

Garde-Cerdán, T., R. López, J. Portu, L. González-Arenzana, I. López-Alfaro, and P. Santamaría. 2014. Study of the effects of proline, phenylalanine, and urea foliar application to Tempranillo vineyards on grape amino acid content. Comparison with commercial nitrogen fertilisers. Food chemistry 163:136-141.

Garde-Cerdán, T., A.R. Marsellés-Fontanet, M. Arias-Gil, O. Martín-Belloso, and C. Ancín-Azpilicueta. 2007. Influence of SO2 on the consumption of nitrogen compounds through alcoholic fermentation of must sterilized by pulsed electric fields. Food chemistry 103:771-777.

Garde-Cerdán, T., P. Santamaría, P. Rubio-Bretón, L. González-Arenzana, I. López-Alfaro, and R. López. 2015. Foliar application of proline, phenylalanine, and urea to Tempranillo vines: Effect on grape volatile composition and comparison with the use of commercial nitrogen fertilizers. LWT-Food Science and Technology 60:684-689.

Giese, G., C. Velasco-Cruz, L. Roberts, J. Heitman, and T.K. Wolf. 2014. Complete vineyard floor cover crops favorably limit grapevine vegetative growth. Scientia Horticulturae 170:256-266.

Gomez, E., A. Martinez, and J. Laencina. 1994. Localization of free and bound aromatic compounds among skin, juice and pulp fractions of some grape varieties. Vitis 33:1-4.

González-Barreiro, C., R. Rial-Otero, B. Cancho-Grande, and J. Simal-Gándara. 2015. Wine aroma compounds in grapes: a critical review. Critical reviews in food science and nutrition 55:202-218.

Gouthu, S., P.A. Skinkis, J. Morre, C.S. Maier, and L.G. Deluc. 2012. Berry nitrogen status altered by cover cropping: Effects on berry hormone dynamics, growth and amino acid composition of Pinot Noir. Food chemistry 135:1-8.

Gunata, Y., C. Bayonove, R. Baumes, and R. Cordonnier. 1985. I. Extraction and determination of free and glycosidcally bound fractions of some grape aroma components. Journal of Chromatography 331:83-90.

Gürbüz, O., J. Rouseff, and R. Rouseff. 2006. Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography−olfactometry and gas chromatography−mass spectrometry. Journal of Agricultural and Food Chemistry 54:3990-3996.

Hale, C. 1977. Relation between potassium and the malate and tartrate contents of grape berries. Vitis-Berichte ueber Rebenforschung mit Dokumentation der Weinbauforschung (Germany, FR).

Hannam, K., G. Neilsen, D. Neilsen, A. Midwood, P. Millard, Z. Zhang, B. Thornton, and D. Steinke. 2016. Amino acid composition of grape (Vitis vinifera L.) juice in response to applications of urea to the soil or foliage. American Journal of Enology and Viticulture:ajev. 2015.15015.

Hannam, K., G. Neilsen, D. Neilsen, W. Rabie, A. Midwood, and P. Milard. 2014. Late-season foliar urea applications can increase berry yeast-assimilable nitrogen in winegrapes (Vitis vinifera L.). American Journal of Enology and Viticulture 65:89-95.

Harris, S., I. Ryona, and G. Sacks. 2012. Behavior of 3-Isobutyl-2-hydroxypyrazine (IBHP), a key intermediate in 3-Isobutyl-2-methoxypyrazine (IBMP) metabolism, in ripening wine grapes. Journal of Agricultural and Food Chemistry 60:11901-11908.

Hashizume, K., and T. Samuta. 1999. Grape maturity and light exposure affect berry methoxypyrazine concentration. American Journal of Enology and Viticulture 50:194-198.

Hashizume, K., K. Tozawa, M. Endo, and I. Aramaki. 2001. S-Adenosyl-L-methionine-dependent O-methylation of 2-hydroxy-3-alkylpyrazine in wine grapes: a putative final step of methoxypyrazine biosynthesis. Bioscience, biotechnology, and biochemistry 65:795-801.

Hashizume, K., and N. Umeda. 1996. Methoxypyrazine content of Japanese red wines. Bioscience, biotechnology, and biochemistry 60:802-805.

Hernandez-Orte, P., J.F. Cacho, and V. Ferreira. 2002. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. Journal of Agricultural and Food Chemistry 50:2891-2899.

Hognadottir, A., and R. Rouseff. 2003. Identification of aroma active compounds in orange essense oil using gas chromatography-olfactometry and gas chromatography-mass spectometry. Journal of Chromatography A 998:201-211.

Hunter, J., C. Volschenk, J. Marais, and G. Fouche. 2004. Composition of Sauvignon blanc grapes as affected by pre-veraison canopy manipulation and ripeness level. South African Journal of Enology and Viticulture 25:13-18.

Jiranek, V., P. Langridge, and P. Henschke. 1995a. Amino acid and ammonium utilization by Saccharomyces cerevisiae wine yeasts from a chemically defined medium. American Journal of Enology and Viticulture 46:75-83.

———. 1995b. Regulation of hydrogen sulfide liberation in wine-producing Saccharomyces cerevisiae strains by assimilable nitrogen. Applied and Environmental Microbiology 61:461-467.

Keller, M. 2015. *The science of grapevines: anatomy and physiology*. Academic Press.

Kessler, A., and I. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141-2144.

King, A., and J. Dickinson. 2003. Biotransformation of hop aroma terpenoids by ale and lager yeasts. FEMS Yeast Research 3:53-62.

King, E., P. Osidacz, C. Curtin, S. Bastian, and I. Francis. 2011. Assessing desirable levels of sensory properties in Sauvignon Blanc wines–consumer preferences and contribution of key aroma compounds. Australian Journal of Grape and Wine Research 17:169-180.

Kliewer, W. 1968. Changes in the concentration of free amino acids in grape berries during maturation. American Journal of Enology and Viticulture 19:166-174.

Kobayashi, H., H. Takase, K. Kaneko, F. Tanzawa, R. Takata, S. Suzuki, and T. Konno. 2010. Analysis of S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine in Vitis vinifera L. cv. Koshu for aromatic wines. American Journal of Enology and Viticulture 61:176-185.

Kotseridis, Y., A.A. Beloqui, A. Bertrand, and J. Doazan. 1998. An analytical method for studying the volatile compounds of Merlot noir clone wines. American journal of enology and viticulture 49:44-48.

Lacey, M.J., M.S. Allen, R.L. Harris, and W.V. Brown. 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. American Journal of Enology and Viticulture 42:103-108.

Lacroux, F., O. Tregoat, C. Van Leeuwen, A. Pons, T. Tominaga, V. Lavigne-Cruege, and D. Dubourdieu. 2008. Effect of foliar nitrogen and sulphur application on aromatic expression of vitis vinifera L. cv. Sauvignon blanc. Journal International des Sciences de la Vigne et du Vin 42:1-8.

Larcher, R., G. Nicolini, L. Tonidandel, T. Román Villegas, M. Malacarne, and B. Fedrizzi. 2013. Influence of oxygen availability during skin-contact maceration on the formation of precursors of 3-mercaptohexan-1-ol in Müller-Thurgau and Sauvignon Blanc grapes. Australian Journal of Grape and Wine Research 19:342-348.

Lasa, B., S. Menendez, K. Sagastizabal, M. Cervantes, I. Irigoyen, J. Muro, P. Aparicio-Tejo, and I. Ariz. 2012. Foliar application of urea to Sauvignon blanc and Merlot vines: doses and time of application. Plant Growth Regulation 67:73-81.

Lin, J., and R. Rouseff. 2001. Characterization of aroma-impact compounds in cold-pressed grapefruit oil using time-intensity GC-olfactometry and GC-MS. Flavour and Fragrance Journal 16:457-463.

Lobit, P., M. Genard, P. Soing, and R. Habib. 2006. Modelling malic acid accumulation in fruits: relationships with organic acids, potassium, and temperature. Journal of Experimental Botany 57:1471-1483.

Lopes, P., M.A. Silva, A. Pons, T. Tominaga, V. Lavigne, C. Saucier, P. Darriet, P.-L. Teissedre, and D. Dubourdieu. 2009. Impact of oxygen dissolved at bottling and transmitted through closures on the composition and sensory properties of a Sauvignon Blanc wine during bottle storage. Journal of agricultural and food chemistry 57:10261-10270.

Loughrin, J., D. Potter, T. Hamilton-Kemp, and M. Byers. 1997. Diurnal emission of volatile compounds by Japanese beetle-damaged grape leaves. Phytochemistry 45:919-923.

Luan, F., and M. Wust. 2002. Differential incorporation of 1-deoxy-d-xylulose into (3 S)-linalool and geraniol in grape berry exocarp and mesocarp. Phytochemistry 60:451-459.

Luning, P.A., T. de Rijk, H.J. Wichers, and J.P. Roozen. 1994. Gas chromatography, mass spectrometry, and sniffing port analyses of volatile compounds of fresh bell peppers (Capsicum annuum) at different ripening stages. Journal of agricultural and food chemistry 42:977-983.

Maggu, M., R. Winz, P. Kilmartin, M. Trought, and L. Nicolau. 2007. Effect of skin contact and pressure on the composition of Sauvignon blanc must. Journal of Agricultural and Food Chemistry 55:10281-10288.

Marais, J. 1993. Terpenes in the aroma of grapes and wine: A review. South African Journal of Enology and Viticulture 4:49-58.

Marais, J., F. Calitz, and Haasbroek. 2001. Relationship between microclimatic data, aroma component concentrations and wine quality parameters in the prediction of Sauvignon blanc wine quality. South African Journal of Enology and Viticulture 22:22-26.

Masneuf-Pomarède, I., C. Mansour, M.-L. Murat, T. Tominaga, and D. Dubourdieu. 2006. Influence of fermentation temperature on volatile thiols concentrations in Sauvignon blanc wines. International Journal of Food Microbiology 108:385-390.

Mateo, J., and M. Jimenez. 2000. Monoterpenes in grape juice and wines. Journal of Chromatography A 881:557-567.

Matthews, M.A., and M.M. Anderson. 1988. Fruit ripening in Vitis vinifera L.: responses to seasonal water deficits. American Journal of enology and Viticulture 39:313-320.

Mattivi, F., L. Caputi, S. Carlin, T. Lanza, M. Minozzi, D. Nanni, L. Valenti, and U. Vrhovsek. 2011. Effective analysis of rotundone at below-threshold levels in red and white wines using solid-phase microextraction gas chromatography/tandem mass spectometry. Rapid Communications in Mass Spectrometry 25:483-488.

Mendes-Ferreira, A., A. Mendes-Faia, and C. Leao. 2004. Growth and fermentation patterns of Saccharomyces cerevisiae under different ammonium concentrations and its implications in winemaking industry. Journal of Applied Microbiology 97:540-545.

Mendez-Costabel, M., K. Wilkinson, S. Bastian, C. Jordans, M. McCarthy, C. Ford, and N. Dokoozlian. 2014. Effect of increased irrigation and additional nitrogen fertilisation on the concentration of

green aroma compounds in Vitis vinifera L. Merlot fruit and wine. Australian journal of grape and wine research 20:80-90.
Mira, H., P. Leite, S. Catarino, J.M. Ricardo-da-Silva, and A.S. Curvelo-Garcia. 2007. Metal reduction in wine using PVI-PVP copolymer and its effects on chemical and sensory characters. VITIS 46:138-147.
Monteiro, A., and C.M. Lopes. 2007. Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal. Agriculture, ecosystems & environment 121:336-342.
Morrison, J., and A. Noble. 1990. The effects of leaf and cluster shading on the composition of cabernet sauvignon grapes and on fruit and wine sensory properties. American Journal of Enology and Viticulture 41:193-200.
Moss, R. 2016. Evaluation of nitrogen management schemes upon vine performance in cover cropped vineyards. Masters thesis, Virginia Polytechnic and State University.
Murray, K., J. Shipton, and F. Whitfield. 1970. 2-methoxypyrazines and the flavour of green peas (Pisum sativum). Chemistry and Industry:897-898.
Musumeci, L.E., I. Ryona, B.S. Pan, N. Loscos, H. Feng, M.T. Cleary, and G.L. Sacks. 2015. Quantification of Polyfunctional Thiols in Wine by HS-SPME-GC-MS Following Extractive Alkylation. Molecules 20:12280-12299.
Neta, E., R. Miracle, T. Sanders, and M. Drake. 2008. Characterization of alkylmethoxypyrazines contributing to earthy/bell pepper flavor in farmstead cheddar cheese. Journal of food science 73:C632-C638.
New Zealand Winegrowers. 2013. Annual report 2013. *In*. New Zealand Winegrowers, Auckland.
———. 2014. Celebrate the fifth annual international sauvignon blanc day friday 16 may. *In*. New Zealand Winegrowers, Auckland.
Nikolantonaki, M., I. Chichuc, P. Teissedre, and P. Darriet. 2010. Reactivity of volatile thiols with polyphenols in a wine-model medium" impact of oxygen, iron and sulfur dioxide. Analytica Chimica Acta:102-109.
Nikolantonaki, M., P. Magiatis, and A.L. Waterhouse. 2014. Measuring protection of aromatic wine thiols from oxidation by competitive reactions vs wine preservatives with ortho-quinones. Food Chemistry 163:61-67.
Olejar, K.J., B. Fedrizzi, and P.A. Kilmartin. 2015. Influence of harvesting technique and maceration process on aroma and phenolic attributes of Sauvignon blanc wine. Food chemistry 183:181-189.
Oliveira, J., P. Oliveira, R. Baumes, and O. Maia. 2008. Changes in aromatic characteristics of Loureiro and Alvarinho wines during maturation. Journal of Food Composition and Analysis 21:695-707.
Ollat, N., and J. Gaudillere. 1998. The effect of limiting leaf area during stage I of berry growth on development and composition of berries of Vitis vinifera L. cv. Cabernet Sauvignon. American journal of enology and viticulture 49:251-258.
Parr, W.V., J.A. Green, K.G. White, and R.R. Sherlock. 2007. The distinctive flavour of New Zealand Sauvignon blanc: Sensory characterisation by wine professionals. Food Quality and Preference 18:849-861.
Pérez-Álvarez, E.P., T. Garde-Cerdán, P. Santamaría, E. García-Escudero, and F. Peregrina. 2015. Influence of two different cover crops on soil N availability, N nutritional status, and grape yeast-assimilable N (YAN) in a cv. Tempranillo vineyard. Plant and Soil 390:143-156.
Pons, A., V. Lavigne, P. Darriet, and D. Dubourdieu. 2015. Glutathione Preservation during Winemaking with Vitis Vinifera White Varieties: Example of Sauvignon blanc Grapes. AMERICAN JOURNAL OF ENOLOGY AND VITICULTURE 66:187-194.
Pons, A., V. Lavigne, Y. Landais, P. Darriet, and D. Dubourdieu. 2010. Identification of a Sotolon Pathway in Dry White Wines. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 58:7273-7279.

Prasad, A., S. Kumar, A. Pandey, and S. Chand. 2012. Microbial and chemical sources of phosphorous supply modulate the yield and chemical composition of essential oil of rose-scented geranium (Pelargonium species) in sodic soils. Biology and Fertility of Soils 48:117-122.

Pretorious, I., and M. Lambrechts. 2000. Yeast and its importance to wine aroma: a review. South African Journal of Enology and Viticulture 21 (special issue):97-129.

Rodriguez-lovelle, B., and J. Gaudillere. 2002. Carbon and nitrogen partitioning in either fruiting or non-fruiting grapevines: effects of nitrogen limitation before and after veraison. Australian Journal of Grape and Wine Research 8:86-94.

Rodriguez-Saona, C., L. Rodriguez-Saona, and C. Frost. 2009. Herbivore-induced volatiles in the perennial shrub, Vaccinium corymbosum, and their role in inter-branch signaling. Journal of Chemical Ecology 35:163-175.

Rojas-Lara, B., and J. Morrison. 1989. Differential effects of shading fruit or foliage on the development and composition of grape berries. Vitis 28:27-63.

Roland, A., J. Vialaret, A. Razungles, P. Rigou, and R. Schneider. 2010. Evolution of S-cysteinylated and S-gutathionylated thiol precursors during oxidation of Melon b. and Sauvignon blanc musts. Journal of Agricultural and Food Chemistry 58:4406-4413.

Roujou de Boubée, D., C. Van Leeuwen, and D. Dubourdieu. 2000. Organoleptic impact of 2-Methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. Journal of Agricultural and Food Chemistry 48:4830-4834.

Roussis, I.G., and S. Sergianitis. 2008. Protection of some aroma volatiles in a model wine medium by sulphur dioxide and mixtures of glutathione with caffeic acid or gallic acid. FLAVOUR AND FRAGRANCE JOURNAL 23:35-39.

Sala, C., M. Mestres, M. Martı, O. Busto, and J. Guasch. 2000. Headspace solid-phase microextraction method for determining 3-alkyl-2-methoxypyrazines in musts by means of polydimethylsiloxane–divinylbenzene fibres. Journal of Chromatography A 880:93-99.

Scheiner, J.J., G.L. Sacks, B. Pan, S. Ennahli, L. Tarlton, A. Wise, S.D. Lerch, and J.E.V. Heuvel. 2010. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. American journal of enology and viticulture 61:358-364.

Schneider, R., F. Charrier, A. Razungles, and R. Baumes. 2006. Evidence for an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. Analytica Chimica Acta 563:58-64.

Schreier, P., F. Drawert, and A. Junker. 1976. Identification of volatile constituents from grapes. Journal of Agricultural and Food Chemistry 24:331-336.

Silva, M., M. Jourdes, P. Darriet, and P. Teissedre. 2012. Scalping of light volatiles sulfur compounds by wine closures. Journal of Agricultural and Food Chemistry 60:10952−10956.

Simpson, R., and G. Miller. 1983. Aroma composition of aged Riesling wines. Vitis 22:51-63.

Skinkis, P., B. Bordelon, and E. Butz. 2010. Effects of sunlight on berry and wine monoterpenes and sensory characteristics of traminette. American Journal of Enology and Viticulture 61:147-156.

Smart, R., J. Robinson, G. Due, and C. Brien. 1985. Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. Vitis 24:119-128.

Song, J., R. Smart, H. Wang, B. Dambergs, A. Sparrow, and M. Qian. 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of Vitis vinifera L. cv. Pinot noir wine. Food Chemistry 173:424-431.

Sonni, F., E.G. Moore, A.C. Clark, F. Chinnici, C. Riponi, and G.R. Scollary. 2011. Impact of Glutathione on the Formation of Methylmethine- and Carboxymethine-Bridged (+)-Catechin Dimers in a Model Wine System. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 59:7410-7418.

Subileau, M., R. Schneider, J. Salmon, and E. Degryse. 2008. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon blanc at the level of precursor transport. FEMS Yeast Research 8:771-780.

Šuklje, K., G. Antalick, Z. Coetzee, L.M. Schmidtke, H. Baša Česnik, J. Brandt, W.J. Toit, K. Lisjak, and A. Deloire. 2014. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis vinifera L. cv. Sauvignon Blanc wine. Australian Journal of Grape and Wine Research 20:223-233.

Sumby, K.M., P.R. Grbin, and V. Jiranek. 2010. Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food Chemistry 121:1-16.

Suriyaphan, O., M. Drake, X. Chen, and K.R. Cadwallader. 2001. Characteristic aroma components of British Farmhouse Cheddar cheese. Journal of agricultural and food chemistry 49:1382-1387.

Sweet, R.M., and R.P. Schreiner. 2010. Alleyway cover crops have little influence on Pinot noir grapevines (Vitis vinifera L.) in two western Oregon vineyards. American journal of enology and viticulture 61:240-252.

Tea, I., T. Genter, N. Naulet, M. Lummerzheim, and D. Keiber. 2007. Interaction between nitrogen and sulfur by foliar application and its effects on flour bread-making quality. Journal of the Science of Food and Agriculture 87:2853-2859.

Tesic, D., M. Keller, and R. Hutton. 2007. Influence of vineyard floor management practices on grapevine vegetative growth, yield, and fruit composition. American Journal of Enology and Viticulture 58:1-11.

Tominaga, T., R. Baltenweck-Guyot, G. Catherine Peyrot Des, and D. Dubourdieu. 2000a. Contribution of Volatile Thiols to the Aromas of White Wines Made From Several Vitis vinifera Grape Varieties. American Journal of Enology and Viticulture 51:178-181.

Tominaga, T., R. Baltenweck-Guyot, C.P. Des Gachons, and D. Dubourdieu. 2000b. Contribution of volatile thiols to the aromas of white wines made from several Vitis vinifera grape varieties. American Journal of Enology and Viticulture 51:178-181.

Torrea, D., C. Varela, M. Ugliano, C. Ancin-Azpilicueta, I.L. Francis, and P.A. Henschke. 2011. Comparison of inorganic and organic nitrogen supplementation of grape juice–Effect on volatile composition and aroma profile of a Chardonnay wine fermented with Saccharomyces cerevisiae yeast. Food Chemistry 127:1072-1083.

Tozzini, L., P. Sabbatini, and G.S. Howell. 2013. Increasing nitrogen availability at veraison through foliar applications: Implications for leaf assimilation and fruit ripening under source limitation in 'Chardonnay'(Vitis vinifera L.) grapevines. HortScience 48:608-613.

Ugliano, M., M. Kwiatkowski, S. Vidal, D. Capone, T. Siebert, J. Dieval, O. Aagaard, and E. Waters. 2011. Evolution of 3-Mercaptohexanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure and closure-derived oxygen. Journal of Agricultural and Food Chemistry 59:2564-2572.

van Wyngaard, E., J. Brand, D. Jacobson, and W.J. du Toit. 2014. Sensory interaction between 3-mercaptohexan-1-ol and 2-isobutyl-3-methoxypyrazine in dearomatised Sauvignon Blanc wine. Australian Journal of Grape and Wine Research 20:178-185.

Verdenal, T., J. Spangenberg, V. Zufferey, F. Lorenzini, J.L. Spring, and O. Viret. 2015. Effect of fertilisation timing on the partitioning of foliar-applied nitrogen in Vitis vinifera cv. Chasselas: a 15N labelling approach. Australian Journal of Grape and Wine Research 21:110-117.

Verzera, A., G. Tripodi, G. Dima, C. Condurso, A. Scacco, F. Cincotta, D.M.L. Giglio, T. Santangelo, and A. Sparacio. 2016. Leaf removal and wine composition of Vitis vinifera L. cv. Nero d'Avola: the volatile aroma constituents. Journal of the Science of Food and Agriculture 96:150-159.

Vickers, C., J. Gershenzon, M. Lerdau, and F. Loreto. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nature Chemical Biology 5:283-291.

Webster, D., C. Edwards, S. Spayd, J. Peterson, and B. Seymour. 1993. Influence of vineyard nitrogen fertilization on the concentrations of monoterpenes, higher alcohols, and esters in aged riesling wines. American Journal of Enology and Viticulture 44:275-284.

Webster, D.R., C.G. Edwards, S.E. Spayd, J.C. Peterson, and B.J. Seymour. 1993. Influence of Vineyard Nitrogen Fertilization on the Concentrations of Monoterpenes, Higher Alcohols, and Esters in Aged Riesling Wines. American Journal of Enology and Viticulture 44:275-284.

Williams, P., C. Strauss, and B. Wilson. 1980. Hydroxylated linalool derivitatives as precursors of volatile monoterpenes of muscat grapes. Journal of Agricultural and Food Chemistry 28:766-771.

———. 1981. Classification of the monoterpenoid composition of muscat grapes. American Journal of Enology and Viticulture 32:230-235.

Williams, P., C. Strauss, and B. Wilson. 1982a. Use of C18 reversed-phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape juice and wine. Journal of Chromatography 235:471-480.

Williams, P., B. Wilson, and R. Massy-Westropp. 1982b. Novel monoterpene disaccharide glycosides of vitis vinifera grapes and wines. Phytochemistry 21:2013-2020.

Winter, G., T. Westhuizen, V. Higgins, V. Curtin, and M. Ugliano. 2011. Contribution of cysteine and gluathione conjugates to the formation of the volatile thiols 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) during fermentation by Saccharomyces cerevisiae. Australian Journal of Grape and Wine Research 17:285-290.

Wood, C., et al. 2008. From wine to pepper: Rotundone, an obscure sesquiterpene, is a potent and spicy aroma compound. Journal of Agricultural and Food Chemistry 56:3738-3744.

Zhang, H., P. Fan, C. Liu, B. Wu, S. Li, and Z. Liang. 2014. Sunlight exclusion from Muscat grape alters volatile profiles during berry development. Food Chemistry 164:242-250.

Zoecklein, B., J. Marcy, J. Williams, and Y. Jasinski. 1997. Effect of native yeasts and selected strains of saccharomyces cerevisiae on glycosyl glucose, potential volatile terpenes, and selected agylcones of white riesling (vitis vinifera L.) wines. Journal of Food Composition and Analysis 10:55-65.