Evaluation of Nitrogen Management Schemes in Cover Cropped Vineyards

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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
Horticulture

Master of Science in Life Sciences
In
Food Science and Technology

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2 May 2016
Blacksburg, VA

Keywords: Vitis vinifera, yeast assimilable nitrogen (YAN), chlorophyll content index (CCI), amino acids, ultra performance liquid chromatography (UPLC), foliar fertilization

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ABSTRACT

Vineyards in the Eastern United States are often prone to excessive vegetative growth. In order to suppress excessive vine vigor, many viticulturists have employed cover cropping strategies. Cover crops provide a myriad of agronomic benefits, however they are known to compete with the vine for water and nutrients. Due to the widespread use of cover crops in Eastern vineyards, many vineyards experience nitrogen (N) deficiencies in both the vegetative vine tissue and yeast assimilable nitrogen (YAN) in the juice. Soil applications of calcium nitrate and foliar applications of urea were assessed as a means of vineyard N amelioration at cover cropped sites comprised of Petit Manseng and Sauvignon blanc (*Vitis vinifera* L.). Perennial White and Crimson clover cover crops and foliar urea applications were also used in a Vidal blanc (*Vitis spp.*) vineyard. Treatments were imposed in the Sauvignon blanc vineyard for five years. The Petit Manseng and Vidal blanc vineyards were subjected to treatments for two years. Soil-applied N at bloom was most effective at increasing leaf petiole N at véraison, season-long chlorophyll content index (CCI), vine capacity and fruit yield. Fruit yield was increased due to more berries per cluster and greater berry weights. Increased rates of soil-applied N decreased the fruit weight:pruning weight ratio. Foliar-applied N after fruit set was most effective at increasing berry YAN. 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. Clover cover crops offered little to no benefit as a N source in the two-year period of evaluation. None of the N management schemes negatively impacted canopy density, fruit zone light interception, or botrytis bunch rot incidence. The combination of both a soil-applied and foliar-applied N fertilizer may be the most effective means to increase both vine capacity and YAN in vineyards where vineyard floor cover crops are compromising vine N status.

Acknowledgements

Without the guidance of Dr. Tony Wolf and Dr. Amanda Stewart, I don't know where I'd be. Their attention to detail and passion were essential in the completion of this project. Their friendship and guidance were paramount to my success and happiness.

I could not have done the amino acid analyses without the help of Sihui Ma. 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.

There is nothing as enjoyable and stimulating as working in vineyards and labs with your closest friends. I can't express enough gratitude to the Wolf Pack: Tremain "Tremainjamin" Hatch, Greg "The Badger" Klinger, Cain "Charlie" Hickey, Brycen "Bry-guy" Hill, Hannah "Hannanah" Kasabian, Rachael White, Dani Bunce and Dana Melby. The Wolf Pack was not only crucial in data collection, but they also held me together during the ups and downs of grad school and life. My love for them is unwavering.

I would also like to thank our collaborators at GMV and ISV for the donation of their land, fruit and assistance: Jeff White, Kelly White, Steve Brown, Nick Riffee.

I would like to thank those folks who have inspired me along my journey of grapes, wine and science. I would never have made it to Virginia Tech without these great mentors: Dr. Glen Creasy, Kirsten Creasy, Dr. Graeme Buchan, Timbo Deaker, Jase Thomson, Sue Blackmore, Dan Barwick, Claudia Weersing, Mike Weersing and Jim O'Donnell.

My family is my rock. They have been by my side through thick and thin. I cherish their love and guidance. I couldn't have asked for better kin.

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Ala – Alanine
AREC – Alson H. Smith Jr. AREC (Petit Manseng)
Arg – Arginine
Asn – Asparganine
Asp – Aspartic acid
CCI – Chlorophyll Content Index
CEFA – Cluster exposure Flux Availability
CEL – Cluster Exposure Layer
Cys – Cysteine
EPQA – Enhanced Point Quadrate Analysis
GDD – Growing Degree Days
Gln – Glutamine
Glu – Glutamic acid
Gly – Glycine
GMV – Glen Manor
His – Histidine
ILE – Isoleucine
ISV – Indian Springs Vineyard
LEFA – Leaf Exposure Flux Availability
LEL – Leaf Exposure Layer
Leu – Leucine
Lys – Lysine
Met – Methionine
OLN – Occlusion Layer Number
PDA – Photodiode array
Phe – Phenylalanine
% PPF – Photosynthetic photon flux
Pro – Proline
Ser – Serine
TA – Titratable acidity
Thr – Threonine
Tyr – Tyrosine
UPLC – Ultra Performance Liquid Chromatography

Introduction

Excessive vine vigor is a common characteristic of vineyards in the Eastern United States. This excessive vegetative growth can lead to deleterious effects summarized in table 1.

Table 1. Deleterious effects of excessive vegetative growth of grapevines

Impact upon fruit	Increase/decrease	Reference
fungal disease pressure	increase	(Austin et al. 2011; English et al. 1993)
phenolic concentration	decrease	(Diago et al. 2012; Verzera et al. 2016)
methoxypyrazines	increase	(Šuklje et al. 2014)
thiols	decrease	(Šuklje et al. 2014)
pH	increase	(Bledsoe et al. 1988)
potassium concentration	increase	(Bledsoe et al. 1988)
malic acid:tartaric acid ratio	increase	(Hunter et al. 2004)
monoterpenes	decrease	(Skinkis et al. 2010)

Excessive vegetative growth has led vintners to employ various methods to reduce the vigor within their farming systems. In recent years, many vintners have begun using cover crops to reduce vine vigor (Giese et al. 2014; Morlat and Jacquet 2003; Tesic et al. 2007; Wheeler et al. 2005). Cover cropping has been demonstrated to have many benefical effects upon the farming system, including acting as a nitrogen (N) and carbon (C) source (Ranells and Wagger 1996) and providing alternative food source for mealybugs (Clearwater 2000). More benefits of cover crops have been summarised in table 2.

Table 2. Benefits of cover crops in farming systems

Impact upon soil	Increase/decrease	Reference
soil erosion	decrease	(Gaffney et al. 1991)
soil compaction during wet periods	decrease	(Louw and Bennie 1991)
water infiltration	increase	(Celette et al. 2005)
weed suppression	increase	(Baumgartner et al. 2008)
microbial biodiversity within the soil	increase	(Ingels et al. 2005)

Not all aspects of cover cropping are benefical, however. The use of a cover crop can have significant drawbacks such as lower yields (Tesic et al. 2007), potential frost risk (Derr 2008), decreased

perennial N reserves (Celette et al. 2009), and depressed concentrations of Yeast Assimilable Nitrogen (YAN) (Gouthu et al. 2012).

Reductions in YAN concentrations represents a significant issue for vintners. Low YAN concentrations can lead to issues such as stuck and sluggish fermentations (Mendes-Ferreira et al. 2004), off odors (H_2S) (Jackson 2008), increased concentrations of higher alcohols (Webster et al. 1993) and lower concentrations of esters (Garde-Cerdán and Ancín-Azpilicueta 2008). A high YAN is not always beneficial either. If berry nitrogen is excessive it can lead to decreased anthocyanin concentrations (Keller and Hrazdina 1998), increased risk of botrytis infection (R'Houma et al. 1998), potential for atypical aging by indirectly increasing indole-3-acetic acid (Linsenmeier et al. 2004) and increased levels of ethyl carbamate (Bell and Henschke 2005). It is important for the vintner to achieve a YAN "balance" in the must in order to attain the highest wine quality.

Much of the previous viticulture research in relation to N has focused upon vine physiology as well as berry and must YAN; however, more research is needed to determine the impact of vine N supply upon secondary metabolites, such as flavor and aroma (Bell and Henschke 2005).

The seemingly ubiquitous use of competitive crops in the Eastern United States has presented challenges to vintners as far as supplying adequate N to the vine and resulting must. The viticultural aim of this study was to examine methods by which vintners might attain adequate vine and must N through vineyard management schemes, while still maintaining the benefits of a cover crop and gaining an understanding as to how these treatments impact wine flavor and aroma. The methods employed in the current study included the use of leguminous cover crops, foliar applications of urea and traditional soil applications of calcium nitrate.

Petit Manseng and Sauvignon blanc represent the third and fifth most planted white wine grapes in Virginia respectively. On average, Petit Manseng and Sauvignon blanc are farmed at higher tonnages per acre and sold for higher prices per ton than the state's most widely planted white variety,

Chardonnay (Virginia Wine Marketing Office 2014). Sauvignon blanc is also the third most planted white grape variety in the United States and is the fourth most popular white varietal wine in the country (California Department of Food and Agriculture 2014; Wine Institute 2014). The regional importance of Petit Manseng and the national and international economic significance of Sauvignon blanc warrant further investigation into how one might impact secondary metabolites within these varieties through N management schemes in the vineyard.

Varietal aromas (e.g. terpenes, norisoprenoids, pyrazines and thiols) have been demonstrated to be influenced by nitrogenous inputs to the musts and vines (Bell and Henschke 2005). Petit Manseng and Sauvignon blanc grapes and wines have been found to have significantly high concentrations of bound and free thiols (Darriet et al. 1995; Tominaga et al. 2000; Tominaga et al. 1996) These thiols contribute to the distinctive tropical fruit aromas of these wines and have been shown to be impacted by must nitrogen concentrations (Choné et al. 2006; Dufourcq et al. 2009; Lacroux et al. 2008; Peyrot des Gachons et al. 2005).

As was previously mentioned, the vegetative growth is relatively high in the Eastern United States. Higher leaf area and the fruit shading that results can increase concentrations of methoxypyrazines and depress the concentration and/or perception of the tropical fruit aromas of the volaitle thiols (Šuklje et al. 2014; Van Wyngaard 2013). Although a survey of thiol concentrations in Virginia wines has not been carried out, one has been conducted in New York and the researchers found that the Sauvignon blanc wines from New York had considerably lower concentrations of volatile thiols compared to wines from other regions, including New Zealand (Musumeci et al. 2015).
New Zealand Sauvignon blanc represents ~30% of the US Sauvignon blanc market, it sells for a premium price point and the market equity of these wines has been increasing dramatically in recent years (New Zealand Winegrowers 2013, 2014).

One of the distinguishing features of New Zealand Sauvignon blanc is its high concentration of thiols (Benkwitz et al. 2012b). There are many excellent Sauvignon blanc wines with low concentrations of thiols. If the concentration of these compounds is too high, it may be considered unpleasant to some consumers. However, it may be to the advantage of American vintners to devise strategies to increase the concentration of thiols in their Sauvignon blanc wines in order to remain qualitatively competitive with the wines from New Zealand.

Review of Literature

Varietal aroma

Wine flavor orginiates from many different sources. These can be considered "primary" or "varietal" aromas which arise as either free or bound compounds within the grape itself, "secondary" or fermentation aromas which develop during fermentation and "tertiary aromas" which develop as a wine ages in barrel and bottle. Secondary aromas were previously reviewed by the author and will not be covered here (Moss 2014a, 2014b, 2014c).

Varietal aroma includes classes of compounds such as terpenes, C13-norisoprenoids, methoxypyrazines and thiols. Grapes do not have exclusive aromatic compounds which are specific to the cultivar, rather each grape has a multitude of flavor and aroma compounds in a complex matrix which interact to deliver the unique aromatic character of the cultivar. Varietal aroma does not seem to be directly associated with sugar accumulation but is influenced by vintage weather and viticultural practices (González-Barreiro et al. 2015). Many of these aromatic compounds are glycosidically bound or form amino acid conjugates in the grape itself and are released during alcoholic fermentation (Park et al. 1991; Peyrot des Gachons et al. 2000).

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 3. 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 (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.

C_{13}-Norisoprenoids

Norisoprenoids are formed from the degradation of carotenoids, which are formed via the non-mevalonate pathway (MEP), and are responsible for a great diversity of aromas ranging from those perceived as flowery and fruity to petrol and kerosene (see table 4) (Robinson et al. 2014). Norisoprenoids are found in nearly all grape varieties and are known to be important contributors to the aromas of wines made from grapes including Riesling, Chardonnay, Merlot, Syrah, Cabernet Sauvignon, Sémillon and Sauvignon blanc (Arrhenius et al. 1996; Benkwitz et al. 2012a; Ferreira et al. 2000; Gürbüz et al. 2006; Mayr et al. 2014; Sefton et al. 1996; Simpson and Miller 1984).

β-Damascenone and β-ionone are largely considered to be the most important C_{13}-Norisoprenoids due to their low sensory thresholds and relatively high concentrations in wines (González-Barreiro et al. 2015).

Table 4. C13-norisoprenoids and their corresponding aromatic descriptors

Compound	**Aroma descriptors**
β-Ionone	Violet[a]
β-Damascenone	Bark, canned peach, baked apple, dry plum[b]
1,1,6-trimethyl-1,2-dihydronaphthalene (TDN)	Petrol[c], Kerosene[d]
(*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB)	Floral, geranium, tobacco, insecticide[e]

[a](Fretz et al., 2005) [b](Li et al., 2008) [c](Sacks et al., 2012) [d](Ross et al.,2014) [e](Janusz et al., 2003)

The norisoprenoids can be formed through several different mechanisms. β-ionone is known to arise from thermal degradation and photo-oxygenation of carotenoids (Isoe et al. 1969; Kanasawud and Crouzet 1990). A carotenoid cleavage dioxygenase, CCD1 has been found to cleave β-carotene at carbons 9,10 and 9',10' and form β-ionone in other crops (Ibdah et al. 2006; Lashbrooke et al. 2013; Simkin et al. 2004). However, some researchers found that CCD1 was not able to use β-carotene as a substrate in grapes to form to β-ionone (Gunata 2013; Mathieu et al. 2005). Another carotenoid cleavage dioxygenase, CCD4, has been found to cleave β-carotene and yield β-ionone in other plants (Huang et al. 2009; Rubio et al. 2008). More research into the functional characterization of CCDs may be necessary to attain a better understanding of their action and specificity.

The biosynthesis of β-damascenone is extraordinarily complex and varied. The synthesis, sensory imapct, occurrence and fate of β-damascenone has been reviewed by Sefton et al. (2011). β-damascenone has been found to be directly synthesized from neoxanthin via thermal oxidation (Bezman et al. 2005). The synthesis of β-damascenone is the result of bio-oxidative cleavage of a carotenoid substrate (neoxanthin) followed by a series of enzymatic transformations and finally an acid-catalyzed conversion to form β-damascenone (Sefton et al. 2011; Winterhalter and Gok 2013). 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) and (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) are also formed through a degradation of a parent carotenoid followed by further chemical conversion (Mendes-Pinto 2009; Winterhalter and Gok 2013).

It is interesting to note that although β-damascenone has an extremely low aroma threshold (0.05 μg/L), it may only play an indirect role in overall wine aroma even when its concentration is greater than the sensory threshold (Bindon et al. 2014; Guth 1997; Pineau et al. 2007). In a study upon interaction effects of fruity aromas in wine, it was found that norisoprenoids can enhance yeast derived esters at low concentrations and can provide an aromatic impact of raisin or dried plums at high concentrations (Escudero et al. 2007). Further study and clarification of the interactions between the wine matrix and aromatic compounds may allow for more reliable viticultural recommendations based upon aromatic optimization.

Vine nutrition and C_{13}-norisoprenoids

Little is known about the influence of vineyard N upon the formation of norisoprenoids (Burin et al. 2015; Linsenmeier and Lohnertz 2007). N fertilization has been demonstrated to increase carotenoids in grape leaves and this may also apply to fruit (Keller and Torres-Martinez 2002). It has been found that increased concentrations of leaf N correlate with an increased concentration of carotenoids in the leaf tissue (Chen and Cheng 2003a). Linsenmeier and Lohnertz (2007) evaluated norisoprenoid concentrations in wines made from Riesling vines which had been subjected to 0, 60 and 150 kg N/ha for

over a decade. Over the course of this study, increased N fertilization in the vineyard led to a decrease in TDN concentrations in the wine. Conversely, an increase of β-damascenone in response to increased N fertlization was reported by Linsenmeier and Lohnertz (2007). However, across vintages, only β-damascenone was found to be positively correlated with must N ($\alpha = 0.1\%$). β-damasacenone was also found to significantly increase with the foliar application of a commercial N fertilizer in two applications at véraison which resulted in a total N application of 900g/ha (Garde-Cerdán et al. 2015). Increasing YAN concentrations in must through the addition of di-ammonium phosphate has also been found to corrleate with higher concentrations of C_{13}-norisoprendoids in wine (Vilanova et al. 2012). The increase in must N status may increase the activity of yeast glycosidases which can result in a greater release of glycoconjugated norisoprenoids.

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 5. 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 compound which contain an –SH group. To date, research on thiols has focused primarily on their negative impact upon wine quality in the form of secondary odors formed by yeast (e.g. H_2S) and tertiary odors (e.g. mercaptans, dimethyldisulfide and thioacetic acid esters). These aromas are considered deleterious to wine quality and can impart aromas which have been described as rotten egg, onion, cabbage, burnt match and cabbage. However, not all thiols are associated with negative wine aromas.

The existence of positive volatile thiols in wine is a relatively new discovery, with the first being 4-mercapto-4-methylpentan-2-one (Darriet et al. 1995). Three positive volatile thiols have been identifed. These are 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and an acetate of 3MH, 3-mercaptohexyl acetate (3MHA) (Tominaga et al. 1996; Tominaga et al. 1998). The varietal thiols are important to the aromas of wines made from white varieties such as Sauvignon blanc, Petit Manseng, Gewürztraminer, Riesling, Colombard, Semillon, Koshu, Niagra and Cayuga White (Kobayashi et al. 2010; Musumeci et al. 2015; Tominaga et al. 2000) Although most of the research has focused upon thiols in Sauvignon blanc, due its significantly high concentrations of thiols, they are also present in red grapes above sensory thresholds. Negrette, Cabernet Sauvignon, Merlot, Syrah and Grenache are all red grapes in which the volatile thiols have been identified in concentrations greater

than the sensory threshold (Murat et al. 2001; Rigou et al. 2014; Rodriguez-Bencomo et al. 2009). The aromatic descriptors of the varietal thiols have been summarized in table 6.

Table 6. Aroma descriptors of key varietal thiols found in grapes and wine

Compound	Aroma description
4-mercapto-4-methylpentan-2-one (4MMP)	box tree[a], broom[a], passion fruit[b], black currant[b]
3-mercapto-hexan-1-ol (3MH)	passion fruit[c], gooseberry[b], grapefruit[c] and guava[b]
3-mercapto-hexylacetate (3MHA)	passion fruit[a], box tree[a]

[a](Dubourdieu et al. 2006) [b](Coetzee and du Toit 2012) [c](Tominaga et al. 1998)

3MH and 4MMP are found within the juice as non-volatile cysteinylated (Cys-3MH and Cys-4MMP) or glutathionylated conjugates (Glu-3MH and Glu-4MMP) (Darriet et al. 1995; Tominaga et al. 1998). 3MHA is a product of fermentation as a result of an enzymatic esterification of 3MH by acetyltransferase with acetic acid and is not found in the grape (Swiegers et al. 2007). The uptake of the conjugated thiols is thought to occur via amino acid uptake pathways in yeast (Subileau et al. 2008). Once inside the yeast cell, the amino-thiol junction is cleaved by the carbon-sulfur β-lyase enzyme that is present in some wine yeasts (Coetzee and du Toit 2012).

The exact mechanisms by which amino acid-thiol conjugates are formed is not fully understood. Capone et al. 2010 postulate that the formation of Glu-3MH is likely due to conjugation of glutathione (a derivative of glutamic acid, cysteine and glycine) and *(E)*-2-hexenal. It has also been postulated that the Glu-3MH is a precursor to Cys-3MH.

S-glutathione conjugates are known to be involved in the detoxification of exogenous compounds in plants, including herbicides such as 2,4-D and atrazine (Anders et al. 1988). In the plant, the toxic compound is bound to glutathione and then degraded by *γ*-glutamyltranspeptidase. Through the removal of glutamic acid, a carboxypeptidase and glycine, a cysteinylated thiol conjugate is formed (Peyrot des Gachons et al. 2002). The enzymes responsible for this transformation have been identified in grapes and the genes which regulate them are known to be up regulated by environmental stresses such as UV-C irradiation, water deficit, and cold/heat shock (Kobayashi et al. 2011).

The conversion of the glutothionylated and cysteinylated precursors of volatile 3MH by yeast has been evaluated by adding the conjugated form of each to a synthetic grape juice medium and fermenting it with a known thiol releasing yeast, *Saccharomyces Cerevisiae* VL 3 (Laffort) (Winter et al. 2011). The researchers then quantified the molar conversion of the cojugated form of 3MH into the volatile forms. They found that the molar conversion of cys-3MH was 2 times greater than that of Glu-3MH. However, it has been found that Glu-3MH can be present in musts in concentrations up to 37 times greater than Cys-3MH (Capone et al. 2010), attaining a more thorough understanding of the enzymatic conversion of Glu-3MH to Cys-3MH and how viticultural and enological factors influence this reaction might allow for managerial activities which could increase the concentration of the volatile thiol in the resulting wine.

Volatile thiols in wine are not just related to their conjugated precursors in the juice. A study was conducted in which the concentrations of Glu-3MH and Cys-3MH were determined and related to the final concentration of the corresponding volatile thiols in the finished wine (Pinu et al. 2012). Of the 55 wines made, the researchers found no correlation between the thiol conjugates found in the juice and the volatile thiols in the resulting wines. Capone et al. (2011) also found there to be no significant relationship between 3MH conjugated precursors and volatile 3MH. This suggests that there are other means by which volatile thiols are formed during alcoholic fermentation.

It has been demonstrated that volatile 3MH and 4MMP can be formed from other carbonyl compounds such as *E*-2-hexenal and mesityl oxide (Schneider et al. 2006). These researchers hypothesize that 3MH and 4MMP can be formed indirectly by the bonding of the carbonyl with a cysteine molecule followed by a conversion to the volatile thiol by yeast. They further theorized that thiols may be formed directly by a 1, 4 addition of H_2S to conjugated carbonyls followed by a reduction step to form the volatile thiol.

Viticultural practices are known to influence the concentrations of thiols. The conjugated thiols seem to increase from véraison until harvest in Sauvignon blanc (Peyrot des Gachons et al. 2005; Roland et al. 2010). It is not understood what impacts the rate of formation of thiol precursors during ripening, as research has demonstrated this to be highly variable. Roland et al. (2010) and Capone et al. (2011) both found dramatic increases in thiol precursor concentrations from 1 to 2 weeks before commercial harvest of Sauvignon blanc. However, Roland et al. (2010) found that while Cys-3MH increased dramatically 7 days before and after harvest for Sauvignon blanc, Glu-3MH concentration differed slightly. The researchers also demonstrated that one of the vineyard sites had its lowest concentration of thiols on the same day that another vineyard site had its highest concentration of thiols. Peyrot des Gachons et al. (2005) found that conjugated 3MH did not change its concentration very dramatically from véraison until harvest, but that conjugated 4MMP increased steadily from véraison onward and decreased prior to harvest. More research is needed to understand the mechanisms which impact the rate of thiol development, so as to provide vintners with the ability to make management decisions from véraison to harvest that will have a positive influence upon potential flavor and aroma.

Vine water status has also been shown to have an impact upon thiol precursors in the fruit (Peyrot des Gachons et al. 2005). Peyrot des Gachons et al. (2005) found limited thiol production under severe water deficits, but an increase in thiol conjugates within the fruit under moderate water stress.

One of the defining characteristic aromas of Sauternes (wines made from botrytized Semillon, Sauvignon blanc and Muscadelle) is grapefruit and tropical fruits. Thibon et al. (2009) found that there was up to a 275-fold increase in the concentration of Cys-3MH in Sauvignon blanc juice from botrytized fruit when compared to uninfected fruit. The authors suggested that *Botrytis cinerea* was not responsible for the production of Cys-3MH, but rather that it stimulated its formation via metabolic pathways in the grape itself. The 3-MH precursors in botrytised Sauterne juice was also correlated to significantly higher volatile 3MH in the resulting wine (Sarrazin et al. 2007). Sarrazin et al. (2007) found

a 12 – 60 fold increase of 3MH concentration in wines made from botrytised fruit. The amount of increase was dependent upon the stage of botrytis infection. The researchers suggested that the increase was due to the increased concentration of the thiol precursors in the juice of botrytised Sauvignon blanc and Semillon. The correlation between precursors and volatile thiols in the wine was in direct contradiction with other research on this subject and warrants further study.

Vine nutrition and thiols

Nitrogenous fertilization in the vineyard has been demonstrated to have a significant impact upon the resulting volatile thiol concentration in the wine and the thiol conjugates found in the fruit. Choné et al. (2006) applied 60 kg of N/ha to a Bordeaux vineyard that was historically low in N. The fruit of the control had a YAN concentration of 29 ppm at harvest, whereas the fruit from the treated vines had a YAN concentration of 174ppm. All of the cys- conjugate thiols increased, but not in the same fashion. Cys-4MMP increased by 76% and Cys-3MH increased by 341%. The researchers found a 30% reduction in phenols with a dramatic 670% increase in gluathione. Polyphenol quinones can react with thiols, resulting in their oxidation and loss of aromatic potential (Blanchard et al. 2004). Glutathione can prevent oxidiation of thiols by reacting with quinones to form a stable glutathionylated quinone adduct (Ugliano et al. 2011). Therefore, thiols are less likely to oxidize in wines made from juices of higher N concentrations.

Foliar applications of N in the form of urea have been found to be an effective means of increasing berry and must YAN concentrations when applied immediately around véraison (Dufourcq et al. 2009; Hannam et al. 2016; Hannam et al. 2014; Lacroux et al. 2008; Lasa et al. 2012). This methodology can increase berry YAN without the deleterious effects of increased vegetative growth. In their study of foliar nitrogen applied to Sauvignon blanc, Colombard, Gros Manseng and Négrette, Dufourcq et al. (2009) observed significant increases in berry YAN with applications of urea. However, the modulations were variable and in some cases modulation was not demonstrated, illustrating the

unpredictable variability of plant response to fertilization. Dufourcq et al. (2009) found that musts with higher YAN concentrations correlated with higher 3MH and 3MHA concentrations in the wine.

Dufourcq et al. (2009) also evaluated the impact of applying micronized sulphur (S) and urea together as foliar sprays. A synergistic effect between foliar N and S applications in wheat has been found (Tea et al. 2007). Tea et al. (2007) found that when foliar N and S were applied together, the uptake of both nutrients was better than when each was applied alone. Dufourcq et al (2009) did not find an increase in YAN concentrations with the N + S foliar spray treatment in relation to just an application of N. They found a 3 – 12 fold increase in total thiols in the plots sprayed with N and S compared to the control.

Lacroux et al (2008) applied the following treatments to a Sauvignon blanc vineyard in Bordeaux:

1. Control: no fertilization
2. 30 kg of N/ha applied to the soil after flowering
3. 10 kg of N/ha as a foliar urea spray in two applications before véraison
4. 10 kg of N/ha as a foliar urea spray + 5 kg S/ha as micronized sulphur in two applicatoins prior to véraison

Wines were made from these treatments and evaluated both chemically and sensorially.

Lacroux et al. (2008) found that glutathione concentrations were significantly higher in foliar N and foliar N + S treatments than in the soil treatment or control. However, the foliar N + S treatment did not yield higher glutathione concentrations than foliar N alone. Nitrogen assimilation was not enhanced by N and S co-application. Volatile thiols in the wine did not increase from the soil N application when compared to the control. 4MMP increased in the foliar N treatment when compared to the control, but this was the only volatile thiol to increase in the foliar N treatment when compared to the control. However, 3MH, 3MHA and 4MMP were all at the highest concentrations in wines produced from the N +

S treatment. A tasting panel found that the N + S treatment was more aromatically intense when compared to the foliar N alone and the foliar N treatment was more intense than the soil N treatment and control. Soil N and control wines could not be differentiated by the tasting panel in that study, which was only conducted in one vineyard and with only one variety. A more robust study with more sites and varieties should be conducted to verify these results.

An increased tropical fruit aroma was described in wines made from Petit Manseng to which a foliar nitrogen and sulfur treatment was applied. However, in this study, volatile thiols were not measured and it could not be determined whether the increased tropical fruit aromas were coming from the volatile thiols and/or esters (Kelly 2013).

The mechanisms behind the apparent increase in volatile thiols associated with N+S foliar treatments has not been elucidated. It would seem that the conjugated thiols do not form a major source of the volatile thiols found in the resulting wine (Pinu et al. 2012). However, cysteine plays an important role in the formation of the volatile thiols during fermentation (Schneider et al. 2006). It may be possible that the foliar application of N and S through foliar fertilization increases the concentration of cysteine and/or other carbonyl compounds which are able to be transformed by yeast to form volatile thiols during fermentation. To date, the studies which have demonstrated increased thiol concentrations with N + S foliar applications have not provided an explanation as to the mechanism behind this result.

Enological factors which influence the concentrations of conjugated precurors and volatile thiols have been reviewed by the author in a previous article and will not be reviewed here (Moss 2015).

Materials and methods

Sites and treatments

Three vineyard sites with a history of low vine and juice nitrogen were chosen for this study. Two of the sites, Glen Manor Vineyards (GMV) and Indian Springs Vineyard (ISV), were commercially managed. The third site, The Alson H. Smith Jr. Agricultural Research and Extension Center (AREC), was managed as a commercial vineyard throughout the course of the study. AREC was the location of two separate experiments, AREC 1 and AREC 2.

GMV

GMV was located in Front Royal, VA (38°50'23.8"N 78°13'42.2"W). The site was planted in 1995 with Sauvignon blanc (*V. vinifera*) grafted to 3309C (*V. riparia* × *V. rupestris*) supported by an open Lyre trellis. The site was on a deep and well drained Myersville-Catoctin silt loam complex with a 15% South-West facing slope. The rows were in a North-South orientation with a spacing of 3.35 meters between the rows and 2.13 meters between plants within the row, resulting in a planting density of 1401 vines/ha. The intra-row and inter-row was cover-cropped with red fescue (*Festuca rubra)* and tall fescue (*Festuca arundinacea*), respectively.

Treatments have been annually imposed since the 2010 growing season. Three treatments and an unfertilized control were imposed upon six replicates of three vine plots with three-vine border plots separating treatments. Treatments included nitrogenous fertilizer applied to the soil in the form of calcium nitrate at rates of 30 kg N/ha (30 N soil) and 60 kg N/ha (60 N soil). Both soil N treatments were applied at bloom, however the 60 kg N/ha treatment was split equally and the second dose was applied at véraison to minimize N lost to leaching. The third treatment consisted of 30 kg N/ha applied as a foliar urea spray (30 N foliar). The foliar urea treatment was split into six equal applications beginning at bloom and implemented every 7-10 days. Each foliar treatment was applied with a backpack sprayer at the equivalent of 900 liters of water/ha.

AREC 1 and AREC 2

The AREC vineyard was located near Winchester, VA (39°06'43.4"N 78°17'04.9"W). AREC 1 and AREC 2 were planted with Petit Manseng (*V. vinifera)* on 420 A rootstock (*V. berlandieri* × *V. riparia*) in 2007 and 2009, respectively. Vines at AREC were supported by a Vertical Shoot Positioned (VSP) trellis. The soil was a deep and well drained Frederick-Poplimento sandy loam on a 2% easterly grade. Rows were in a northeast-southwest orientation. The vine rows were separated by 3 meters with 1.5 meters between plants within the row, resulting in a vine density of 2,222 vines/ha. The inter-rows were sown in tall fescue (*F. arundinacea*) and orchard grass (*Dactylis glomerata*), maintained by mowing throughout the growing season. The intra-row of AREC 1 was cover-cropped in creeping red fescue (*F. rubra*). The intra-row of AREC 2 was bare and maintained with glyphosate.

In both 2014 and 2015, four treatments and an unfertilized control were imposed at AREC 1 over five replicates in a completely randomized design. Experimental units in both AREC 1 and 2 consisted of five-vine plots. Treatments at AREC 1 were separated by five border vines. Treatments consisted of soil-applied nitrogen in the form of calcium nitrate at rates of 30 kg N/ha (30 N soil), 45 kg N/ha (45 N soil) and 60 kg N/ha (60 N soil). Each soil treatment was imposed at bloom. The 60 N soil treatment was split into equal applications, the first being applied at bloom and the second at véraison in order to avoid leaching. The fourth treatment consisted of 45 kg N/ha as calcium nitrate applied at bloom and a split application, separated by 7-10 days of 15 kg N/ha at véraison as a foliar urea spray at a rate of 900 liters of water/ha (45 N soil + 15 N foliar).

AREC 2 was established in 2014 with five replicates of two treatments and an unfertilized control in a randomized complete block design. One treatment consisted of 15 kg N/ha applied as foliar urea two weeks prior to véraison in two equivalent split applications separated by 7-10 days (15 N foliar). The second treatment was 15 kg N/ha applied as foliar urea, in conjunction with 5 kg S/ha as micronized sulfur applied two weeks prior to véraison in two equivalent split applications separated by

7-10 days (15 N foliar + 5 S foliar). Each foliar treatment was applied with a backpack sprayer at a water rate that was equivalent to 900 liters/ha.

ISV

ISV was located in Shenandoah County, VA (38°55'55.0"N 78°33'42.3"W) and was planted with own-rooted Vidal blanc (*Vitis ssp.*) with an inter-row spacing of 2.74 meters and an inter-plant spacing of 2.13 meters, resulting in a density of 1712 vines/hectare. The vines were trellised to VSP running in a slightly northwest-southeasterly direction on a southern facing slope with a 2-7% gradient. The soil at ISV was a well-drained Edom silty clay loam. The inter-rows were cover-cropped with a regularly mown voluntary sward. Historically, the vines have had an intra-row cover crop, but this was removed for this study and replaced with clover or maintained bare with herbicide. Due to the results from soil samples taken at the site, triple superphosphate (P_2O_5) was applied at a rate of 50 kg P/ha prior to the imposition of treatments in 2014.

Each experimental unit consisted of four vines replicated four times in a randomized complete block design. Experimental units were separated by four vine border plots. Four treatments and two controls were established at ISV. Crimson clover (*Trifolium incarnatum*) and Dutch White clover (*T. repens*), inoculated with NITRO-COAT® rhizobium (Outsidelands.com Inc., Independence, OR), were sown in all treatments at rates of 33.6 kg/ha and 15.7kg/ha, respectively. In 2014, due to project timing, seeds were sown in May. Due to difficulty in cover crop establishment in 2014, seeds were re-sown in 2015. In 2015, the cover crop was frost-seeded in March in order to assist with soil incorporation. Seeds for each treatment were broadcast by hand onto bare soil and incorporated down to a depth of approximately one centimeter using rakes.

The following treatments were applied at ISV: under-vine Crimson clover (Crimson), under-vine White clover (White), Crimson clover + 10 kg N/ha as foliar urea (Crimson + 10 N foliar), White clover + 10 kg N/ha as foliar urea (White + 10 N foliar).

Clover plots were maintained throughout the season as needed through spot applications of Fluazifop-P-butyl and glyphosate.

Two controls were established at ISV to evaluate the efficacy of the cover cropping strategies in relation to industrially standard practices. The two controls at ISV were as follows: bare intra-row and 15 kg N/ha applied to soil (15 N soil), bare intra-row and 15 kg N/ha applied to soil and 10 kg N/ha applied to the foliage (15 N soil + 10 N foliar).

The intra-rows of the 15 N soil and 15 N soil + 10 N foliar treatments were kept bare with the application of glyphosate throughout the season. All soil-applied nitrogen at ISV was in the form of calcium nitrate and was imposed at bloom. Foliar N treatments were applied with a backpack sprayer in the form of urea at the start of véraison in two equal split applications separated by 7-10 days at an equivalent water rate of 900 liters/ha.

Plant tissue analysis

Plant tissue analysis was conducted at each site in 2014 and 2015. Sixty petioles (thirty from each side of the canopy) attached to the first fully expanded leaf from the end of a count shoot were collected from each experimental unit at 90-100% véraison. The petioles were dried in an oven at 60^{o}C for 24 hr and sent to the Pennsylvania State University Agricultural Services Laboratory (University Park, PA) in 2014 and to Waypoint Analytical (Richmond, VA) in 2015 for an analysis of mineral nutrients.

Chlorophyll content index

A chlorophyll meter (CCM-200 plus, Apogee Instruments, Logan, UT) was used to measure the chlorophyll content index (CCI). CCI was calculated by measuring the ratio of radiation transmitted via a 0.71mm^2 aperture through the leaf at wavelengths of 653nm (red) and 931nm (near-infrared). The red wavelength was strongly absorbed by chlorophyll *a* and *b* and the near-infrared wavelength was used to compensate for physical differences in leaf tissue, such as tissue thickness.

CCI measurements were taken at GMV, ISV and AREC 1 in 2014 and 2015. CCI measurements were not taken at AREC 2, as foliar urea sprays were unlikely to significantly affect leaf chlorophyll content.

CCI measurements were taken three times through the growing season (pre-véraison, véraison and post-harvest) separated by approximately 30 days between measurements. Due to an equipment malfunction, no post-harvest measurement was made at GMV and ISV in 2014 and only a post-véraison measurement was made at ISV in 2014.

CCI readings were made on fully expanded leaves on count shoots, which were between the 5th and 8th nodal positions. Leaves were tagged upon first measurement and the same leaf was used in subsequent readings. Measurements were taken on the right side of the leaf in the third interveinal space from the leaf tip. These measurements were made once upon five random leaves throughout the experimental unit and readings were averaged.

Canopy Architecture

The Enhanced Point Quadrat Analysis (Meyers and Vanden Heuvel 2008) was performed on every site at 90-100% véraison in 2014 and 2015. Canopy insertions were made with a metal rod every 30 cm within each experimental unit. Data were recorded as “gaps”, “leaves” or “fruit” as the rod contacted those features. Photosynthetic photon flux density (PPFD) was measured on cloudless days at solar noon (± 1 hr) using an AccuPAR ceptometer (AccuPAR80, Decagon Devices, Inc., Pullman, WA). The ceptometer readings were taken by placing the instrument within the fruit zone parallel to, and just above, the cordon. Once in the canopy, a vertical, east and west recording of photon flux was made and averaged. PPFD was recorded on a per vine basis. Unobstructed ambient light readings were taken in the inter-row prior to taking each set of canopy PPFD readings. Data were analyzed using a specially designed EPQA software package (Meyers and Vanden Heuvel 2008).

Cover crop measurements

Cover crop performance was assessed qualitatively and quantitatively at ISV in 2015. Each cover cropped experimental plot was assessed. Therefore, the 15 N soil and 15 N soil + 10 N foliar treatments were not assessed, as they were kept bare through the application of herbicide.

Square quadrats with an internal area of 0.25m^2 were placed equidistant between every vine in each experimental unit. A turf-grass stand density scale was modified (Morris 2001) and used for the visual assessment of stand density by three people, as used elsewhere (Giese et al. 2014). Stand density was ranked on a scale from one to six with the numerical ranks as follows: 1 = 76-100% invasive species/bare ground; 2 = 51-75% invasive species/bare ground; 3 = 26-50% invasive species or bare ground; 4 = 10-25% invasive species/bare ground; 5 = <10% invasive species/bare ground; 6 = 100% ground cover by cover crop.

Above ground biomass was sampled from the same quadrats that were used for visual stand density estimation. Sampling took place by cutting the vegetation within the quadrat with sheers about 5 mm above the soil surface. The vegetation was then sorted into "weeds" and "cover crop" and dried in an oven for 48 hours at 60 ^{o}C. The dry weight of the biomass was then taken and averaged across each experimental unit, as used elsewhere (Giese et al. 2014).

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.

Components of yield

Crop yield data was collected at all sites in all years. All clusters were harvested, counted and weighed on a per vine basis. From this information, total vine yield and the number of clusters per vine was determined. Cluster weights were calculated by dividing the mass of the harvested clusters by the number of clusters per vine. Berry weights were determined by dividing the mass of the berries collected for primary fruit chemical analysis by the number of berries within the sample. The number of berries per cluster was calculated by dividing cluster weight by berry weight. All data collected on a per-vine basis was averaged across each experimental unit.

Pruning weights

Pruning weights were collected in the winters of 2015 and 2016 for all sites. Pruning weights were recorded on a per vine basis using a hand held scale and averaged across each experimental unit.

Yeast assimilable nitrogen (YAN)

Prior to YAN analyses, all samples were thawed and clarified through centrifugation at 3,500 RPM 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 3,500 RPM 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°C. After incubation at 55 °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°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 °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 °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°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 °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

Data were analyzed using JMP pro version 11 (SAS; Cary, NC). Two-way analysis of variance (ANOVA) was carried out on all data sets, where applicable. Model effects tested at AREC 2, GMV and ISV were treatment, block, year and the treatment*year interaction. Model effects tested at AREC 1 were treatment, year and treatment*year interaction.

One-way ANOVAs were carried out on all appropriate data sets in order to assess within year treatment differences. Model effects tested at AREC 2, GMV and ISV were treatment and block. The model effect tested at AREC 1 was treatment. A confidence level of 95% was used in all statistical analyses.

The means of all analyses containing three or more treatments were separated using Tukey's HSD ($\alpha = 0.05$). The means of all analyses containing two treatments were separated using Student's T-test ($\alpha = 0.05$).

Results

Temperature and rainfall

Total rainfall and heat summation for the 2015 growing season were slightly greater than in the 2014 season (Table 7). At the end of the growing season, the 2015 season had accumulated 156 more growing degree days than had accrued in 2014. The 2015 season also accrued 32mm more rain than the 2014 season (Table 7).

Table 7. Precipitation and heat accumulation at AREC vineyard site 2014-2015

Parameter/year	Apr	May	Jun	Jul	Aug	Sep	Oct	Total Apr-Oct
Rainfall (mm)								
2014	77	102	87	78	74	10	91	519
2015	87	92	122	54	31	96	68	551
GDD (<10°C)[a]								
2014	83	249	363	397	356	286	124	1858
2015	98	300	363	424	409	316	103	2014

[a] GDD (<10 °C) = growing degree days above 10 °C

Plant tissue analysis

GMV: Véraison petiolar N and Mg concentration at GMV were significantly increased by soil-applied N, with the highest N and Mg concentrations from the highest rate of fertilization (60 N soil) (Table 8 and 9). Phosphorus and K concentration in the petioles at véraison were slightly depressed by soil-applied N (Table 8 and 9). However, differences in petiolar K were only detected by the combined model (Table 8) and petiolar K was not significantly different between treatments within 2014 and 2015 (Table 9). Petiolar P concentration was only significantly depressed by soil-applied N (60 N soil) in 2014 ($P<0.05$). (Table 9). The higher soil treatment (60 N soil) depressed petiolar Mn and B concentrations (Table 10). However, within each year, N fertilization had no effect upon Mn concentrations and only significantly depressed B concentration in 2015 (Table 11). Year had a significant effect upon petiolar nutrient status for every nutrient except boron ($p<0.05$) (Table 8 and 10).

AREC 1: Soil-applied N increased petiolar N concentrations at véraison (Table 12 and 13). The highest petiolar N concentration was found in the 45 N soil treatment in both 2014 and 2015 (Table 13). N applications depressed petiolar P at véraison (Table 12), but this reduction in P was only significant in 2015 ($p<0.05$) (Table 13). The treatment-year interaction for P at AREC 1 was significant ($p<0.05$). The 45 N soil + 15 N foliar treatment increased petiolar Ca concentration (Table 12). However, a significant increase in Ca concentration was not detected within each year (Table 13). Treatment did not have a significant effect upon micronutrients at AREC 1 (Table 14 and 15). Year significantly affected all petiolar nutrients, except N ($p<0.05$) (Table 12 and 14).

AREC 2: Petiolar nutrient status was not significantly affected by treatment (Table 16-19). Year had a significant effect upon petiolar nutrient concentrations of K, Mn, Fe, Cu, B, Zn and Na ($p<0.05$).

ISV: Treatments at ISV did not have a significant effect upon petiolar N at véraison (Table 20 and 21). Foliar N and clover cover crops significantly increased petiolar P concentrations at véraison ($p<0.05$) (Table 20). However, significance between treatments with regards to petiolar P was not detected within each year (Table 21). The Crimson + 10 N foliar treatment significantly increased petiolar K concentrations relative to the 15 N soil + 10 N foliar and White + 10 N foliar treatments (Table 20). However, this result was only significant in 2014 (Table 21). Treatment did not have a significant effect upon micronutrients (Table 22 and 23). Year had a significant effect upon every nutrient, aside from Mg ($p<0.05$)(Table 20 and 22).

Table 8. GMV: Macronutrient composition of petioles at véraison in response to soil and foliar N fertilization (2014 – 2015)

Treatment[ab]	N%	P%	K%	Ca%	Mg %
Control	0.64 bc	0.38 a	4.81 a	1.21	0.52 bc
30 N soil	0.66 ab	0.23 bc	4.46 ab	1.26	0.59 b
60 N soil	0.67 a	0.18 c	4.03 b	1.21	0.67 a
30 N foliar	0.63 c	0.32 ab	4.58 a	1.19	0.49 c
Trt[c]	0.0457	0.0001	0.0017	ns[d]	<0.0001
Yr	<0.0001	0.0240	<0.0001	<0.0001	<0.0001
Trt × Yr	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] 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

[d]ns = not significant

Table 9. GMV: Macronutrient composition of petioles at véraison in response to soil and foliar N fertilization by year (2014 and 2015)

	N%		P%		K%		Ca%		Mg %	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	0.72 ab	0.56 ab	0.33 a	0.43	5.48	4.14	1.08	1.35	0.39 c	0.65 ab
30 N soil	0.75 ab	0.58 ab	0.20 bc	0.27	4.91	4.01	1.11	1.40	0.48 b	0.69 ab
60 N soil	0.76 a	0.59 a	0.16 c	0.20	4.48	3.59	1.07	1.35	0.58 a	0.77 a
30 N foliar	0.71 b	0.55 b	0.29 ab	0.35	5.07	4.10	1.05	1.34	0.40 c	0.57 b
Trt[c]	0.0254	0.0113	0.0007	ns[d]	ns	ns	Ns	ns	<0.0001	0.0152

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

[d]ns = not significant

Table 10. GMV: Micronutrient composition of petioles at véraison in response to soil and foliar N fertilization (2014 – 2015)

Treatment[ab]	**Mn ppm**	**Fe ppm**	**Cu ppm**	**B ppm**	**Al ppm**	**Zn ppm**	**Na ppm**
Control	297 ab	23	18	31 a	6	55	47
30 N soil	300 ab	23	17	30 ab	4	54	46
60 N soil	258 b	23	16	28 b	5	55	47
30 N foliar	338 a	24	17	29 ab	5	65	46
Trt[c]	0.0115	ns[d]	ns	0.0021	ns	Ns	ns
Yr	<0.0001	<0.0001	<0.0001	ns	0.0015	0.0003	<0.0001
Trt × Yr	ns	ns	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] 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

[d]ns = not significant

Table 11. GMV: Micronutrient composition of petioles at véraison in response to soil and foliar N fertilization by year (2014 and 2015)

	Mn ppm		Fe ppm		Cu ppm		B ppm		Al ppm		Zn ppm		Na ppm	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	192	401	10	36	26	9	30	31 a	4	9	43	67	59	35
30 N soil	204	396	10	35	24	9	30	30 ab	4	4	47	61	56	35
60 N soil	171	345	10	37	23	9	29	27 b	4	6	43	67	57	37
30 N foliar	213	464	12	37	25	10	29	29 ab	4	6	45	84	59	33
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	0.0193	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 12. AREC 1: Macronutrient composition of petioles at véraison in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	**N%**	**P%**	**K%**	**Ca%**	**Mg %**
Control	0.59 c	0.15 a	6.06	1.97 b	0.32
30 N soil	0.67 b	0.11 b	5.61	2.10 ab	0.33
45 N soil	0.74 a	0.09 b	5.72	1.96 b	0.39
60 N soil	0.67 b	0.10 b	5.47	1.98 b	0.35
45 N soil + 15 N foliar	0.69 ab	0.10 b	5.37	2.32 a	0.38
Trt[c]	<0.0001	<0.0001	ns	0.0055	ns
Yr	ns[d]	<0.0001	<0.0001	<0.0001	0.0004
Trt × Yr	ns	0.0070	ns	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] 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

[d]ns = not significant

Table 13. AREC 1: Macronutrient composition of petioles at véraison in response to soil and foliar N fertilization by year (2014 and 2015)

	N%		P%		K%		Ca%		Mg %	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	0.60 b	0.59 b	0.10	0.20 a	6.49	5.64	1.75	2.20	0.29	0.35
30 N soil	0.65 ab	0.68 ab	0.09	0.15 ab	6.05	5.18	1.89	2.30	0.30	0.37
45 N soil	0.73 a	0.75 a	0.08	0.10 b	6.20	5.24	1.75	2.17	0.35	0.43
60 N soil	0.66 ab	0.68 ab	0.09	0.12 b	5.88	5.05	1.76	2.21	0.31	0.39
45 N soil + 15 N foliar	0.68 a	0.69 ab	0.08	0.12 b	5.90	4.85	2.08	2.56	0.33	0.44
Trt[c]	0.0025	0.0032	ns[d]	0.0010	ns	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; 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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 14. AREC 1: Micronutrient composition of petiole at véraison in response to soil and foliar N fertilization (2014 – 2015)

Treatment[ab]	**Mn ppm**	**Fe ppm**	**Cu ppm**	**B ppm**	**Al ppm**	**Zn ppm**	**Na ppm**
Control	177	24	7	26	7	59	105
30 N soil	177	19	7	27	5	56	104
45 N soil	189	20	7	27	6	57	111
60 N soil	187	19	7	25	4	56	101
45 N soil + 15 N foliar	206	18	7	26	6	59	110
Trt[c]	ns[d]	ns	ns	ns	Ns	ns	ns
Yr	<0.0001	<0.0001	<0.0001	<0.0001	0.0462	<0.0001	<0.0001
Trt × Yr	ns	ns	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; 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] 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

[d]ns = not significant

Table 15. AREC 1: Micronutrient composition of petiole at véraison in response to soil and foliar N fertilization by year (2014 and 2015)

	Mn ppm		Fe ppm		Cu ppm		B ppm		Al ppm		Zn ppm		Na ppm	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	124	231	14	34	3	12	21	30	7	7	47	72	151	60
30 N soil	131	223	8	31	4	11	24	31	6	4	48	64	152	56
45 N soil	137	241	8	33	4	11	23	31	6	6	47	67	160	62
60 N soil	145	228	10	28	3	11	22	29	6	3	43	70	149	54
45 N soil + 15 N foliar	163	250	7	29	3	11	22	30	6	5	47	70	159	62
Trt[c]	ns[d]	ns	ns	ns	ns	ns	Ns	ns	ns	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; 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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 16. AREC 2: Macronutrient composition of petioles at véraison in response to foliar N fertilization with and without micronized sulfur (2014-2015)

Treatment[ab]	**N%**	**P%**	**K%**	**Ca%**	**Mg %**
Control	0.57	0.20	5.94	1.90	0.15
15 N foliar	0.58	0.23	5.93	1.93	0.15
15 N foliar + 5 S foliar	0.58	0.20	5.75	1.83	0.14
Trt[c]	ns[d]	ns	ns	ns	ns
Yr	ns	ns	<0.0001	ns	ns
Trt × Yr	ns	ns	ns	ns	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] 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

[d]ns = not significant

Table 17. AREC 2: Macronutrient composition of petioles at véraison in response to foliar N fertilization without and without micronized sulfur by year (2014 and 2015)

	N%		P%		K%		Ca%		Mg %	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	0.58	0.56	0.19	0.22	6.85	5.02	1.87	1.94	0.14	0.15
15 N foliar	0.59	0.58	0.21	0.26	6.85	5.02	1.86	1.99	0.15	0.15
15 N foliar + 5 S foliar	0.59	0.58	0.21	0.20	6.60	4.90	1.76	1.90	0.14	0.15
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 18. AREC 2: Micronutrient composition of petioles at véraison in response to foliar N fertilization with and without micronized sulfur (2014 – 2015)

Treatment[ab]	**Mn ppm**	**Fe ppm**	**Cu ppm**	**B ppm**	**Al ppm**	**Zn ppm**	**Na ppm**
Control	197	19	6	38	6	44	117
15 N foliar	172	24	7	37	6	47	113
15 N foliar + 5 S foliar	198	21	7	36	6	48	118
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns
Yr	<0.0001	<0.0001	<0.0001	0.0227	ns	0.0004	<0.0001
Trt × Yr	ns	ns	ns	ns	ns	ns	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] 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

[d]ns = not significant

Table 19. AREC 2: Micronutrient composition of petioles at véraison in response to foliar N fertilization with and without micronized sulfur by year (2014 and 2015)

	Mn ppm		Fe ppm		Cu ppm		B ppm		Al ppm		Zn ppm		Na ppm	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	134	259	7	32	2	10	37	39	6	6	37	50	182	52
15 N foliar	114	231	7	41	3	11	35	38	5	7	42	51	174	52
15 N foliar + 5 S foliar	145	252	9	33	3	11	35	38	6	7	38	58	183	52
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 20. ISV: Macronutrient composition of Vidal blanc petioles at véraison in response to treatments (2014-2015)

Treatment[ab]	**N%**	**P%**	**K%**	**Ca%**	**Mg %**
15 N soil	0.66	0.30 b	1.81 abc	2.88	0.87
15 N soil + 10 N foliar	0.69	0.34 ab	1.54 bc	2.91	0.93
Crimson	0.65	0.42 a	1.69 abc	2.78	0.78
Crimson + 10 N foliar	0.64	0.41 a	2.11 a	2.97	0.80
White	0.65	0.37 ab	2.00 ab	2.82	0.88
White + 10 N foliar	0.67	0.40 a	1.28 c	2.88	0.90
Trt[c]	ns[d]	0.0056	0.0007	ns	ns
Yr	0.0001	0.0058	0.0060	<0.0001	ns
Trt × Yr	ns	ns	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] 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

[d]ns = not significant

Table 21. ISV: Macronutrient composition of Vidal blanc petioles at véraison in response to treatments by year (2014 and 2015)

	N%		P%		K%		Ca%		Mg %	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
15 N soil	0.68	0.65	0.34	0.26	1.70 ab	1.93	2.64	3.12	0.78 ab	0.96
15 N soil + 10 N foliar	0.71	0.68	0.37	0.31	1.41 ab	1.67	2.76	3.07	0.85 ab	1.01
Crimson	0.67	0.63	0.45	0.39	1.65 ab	1.74	2.6	2.96	0.84 ab	0.71
Crimson + 10 N foliar	0.68	0.61	0.41	0.40	1.92 a	2.31	2.8	3.14	0.73 b	0.86
White	0.69	0.61	0.4	0.33	1.84 ab	2.17	2.66	2.97	0.78 ab	0.98
White + 10 N foliar	0.68	0.65	0.43	0.38	1.01 b	1.56	2.67	3.10	0.98 a	0.83
Trt[c]	ns[d]	ns	ns	ns	0.0474	ns	ns	ns	0.0303	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 22. ISV: Micronutrient composition of Vidal blanc petioles at véraison in response to treatments (2014-2015)

Treatment[ab]	Mn ppm	Fe ppm	Cu ppm	B ppm	Al ppm	Zn ppm	Na ppm
15 N soil	67.68	20.59	6.11	35.68	5.53	65.91	68.14
15 N soil + 10 N foliar	65.59	22.69	6.74	38.96	6.73	70.68	67.48
Crimson	70.79	22.48	6.21	37.18	5.52	75.63	68.19
Crimson + 10 N foliar	70.21	22.71	6.77	37.99	5.87	75.44	64.00
White	74.82	20.31	5.93	37.74	3.86	73.90	68.45
White + 10 N foliar	81.54	23.49	6.57	39.88	4.95	75.09	70.02
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns
Yr	<0.0001	<0.0001	<0.0001	<0.0001	0.0202	<0.0001	0.0090
Trt × Yr	ns	ns	ns	ns	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] 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

[d]ns = not significant

Table 23. ISV: Micronutrient composition of Vidal blanc petioles at véraison in response to treatments by year (2014 and 2015)

	Mn ppm		Fe ppm		Cu ppm		B ppm		Al ppm		Zn ppm		Na ppm	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
15 N soil	37	98	9	32	4	8	39	32	5	7	56	76	69	68
15 N soil + 10 N foliar	43	89	11	35	4	9	44	34	4	9	61	81	72	63
Crimson	40	101	9	36	4	9	41	34	4	7	62	89	69	68
Crimson + 10 N foliar	40	101	10	36	4	10	41	35	4	7	64	87	68	60
White	48	102	10	31	4	8	41	34	4	3	62	86	74	63
White + 10 N foliar	51	112	9	38	4	10	45	35	4	6	66	84	78	63
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Chlorophyll content index

GMV: Soil-applied N at GMV significantly increased the season long CCI (Table 24). This result was significant in both 2014 and 2015 ($p<0.05$) (Table 25). The highest rate of soil-applied N (60 N soil) resulted in the highest CCI values. Year had a significant effect upon CCI ($p<0.05$) (Table 24).

AREC 1: The 45 N soil and 45 N soil + 15 N foliar treatments had significantly higher season-long CCIs than the control ($p<0.05$) (Table 26). However, the season-long CCI was only statistically significant in the first year of the study (Table 27). In general, soil-applied N increased the season-long CCI values. Year had a significant effect upon CCI ($p<0.05$) (Table 26).

ISV: Treatments had no significant effect upon the season-long CCI at ISV in 2015 (Table 28).

Table 24. GMV: Season long chlorophyll content index in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	Season long CCI
Control	14.13 c
30 N soil	16.09 b
60 N soil	18.35 a
30 N foliar	15.85 b
Trt[c]	<0.0001
Yr	0.0062
Trt × Yr	ns[d]

[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] 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

[d]ns = not significant

Table 25. GMV: Season long chlorophyll content index in response to soil and foliar N fertilization by year (2014 and 2015)

	Season long CCI	
Treatment[ab]	**2014**	**2015**
Control	13.85 c	14.4 c
30 N soil	15.83 b	16.33 b
60 N soil	17.58 a	19.12 a
30 N foliar	15.37 bc	16.35 b
Trt[c]	0.0001	<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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 26. AREC 1: Season long chlorophyll content index in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	**Season long CCI**
Control	19.01 b
30 N soil	20.91 ab
45 N soil	23.02 a
60 N soil	20.45 ab
45 N soil + 15 N foliar	22.03 a
Trt[c]	0.0021
Yr	0.0008
Trt × Yr	ns[d]

[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] 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

[d]ns = not significant

Table 27. AREC 1: Season long chlorophyll content index in response to soil and foliar N fertilization by year (2014 and 2015)

	Season long CCI	
Treatment[ab]	**2014**	**2015**
Control	18.14 b	19.89
30 N soil	19.55 ab	22.28
45 N soil	22.07 a	23.97
60 N soil	19.32 ab	21.58
45 N soil + 15 N foliar	20.82 ab	23.25
Trt[c]	0.0269	ns[d]

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

[d]ns = not significant

Table 28. ISV: Season long chlorophyll content index in response to treatments (2015)

Treatment[ab]	**Season long CCI**
15 N soil	20.20
15 N soil + 10 N foliar	20.53
Crimson	18.45
Crimson + 10 N foliar	18.32
White	19.60
White + 10 N foliar	19.60
Trt[c]	ns[d]

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

[d]ns = not significant

Linear regression analysis

No correlation between petiolar N and season-long CCI was detected at GMV (Figure 1). There was a significant positive relationship between véraison petiolar N and season-long CCI at AREC 1 and ISV ($p<0.05$) (Figure 2 and 3).

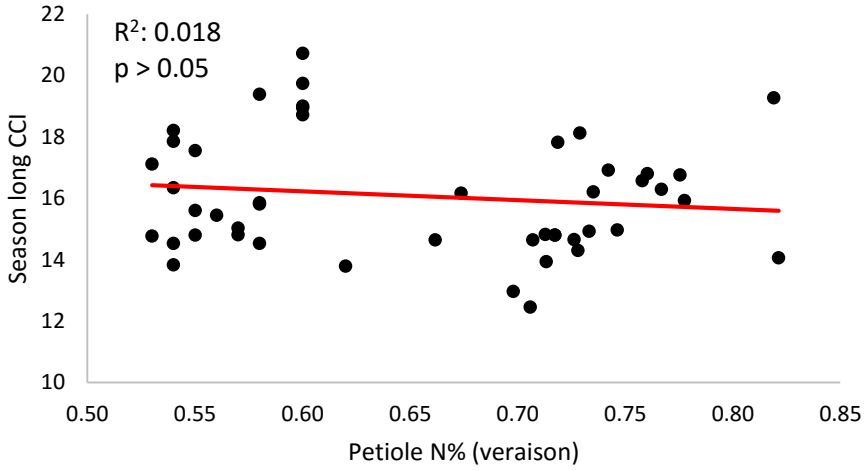

Figure 1. GMV: Regression analysis of season-long CCI and Petiole N% at véraison (2014-2015)

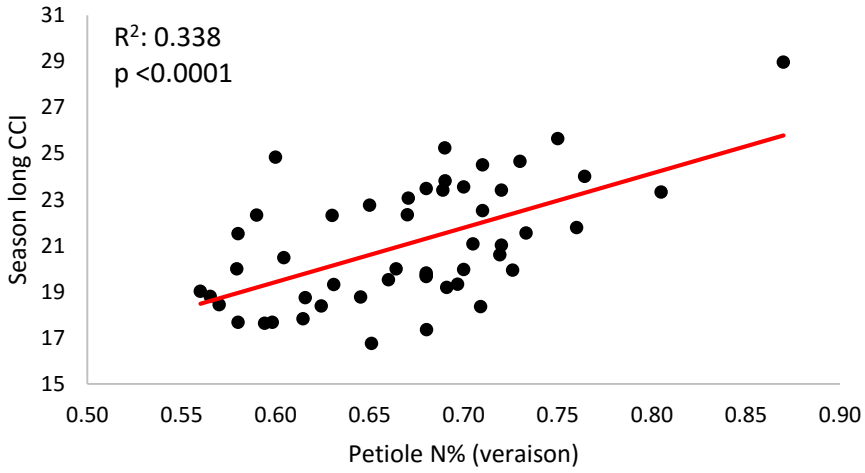

Figure 2. AREC 1: Regression analysis of season-long CCI and petiole N% at véraison (2014-2015)

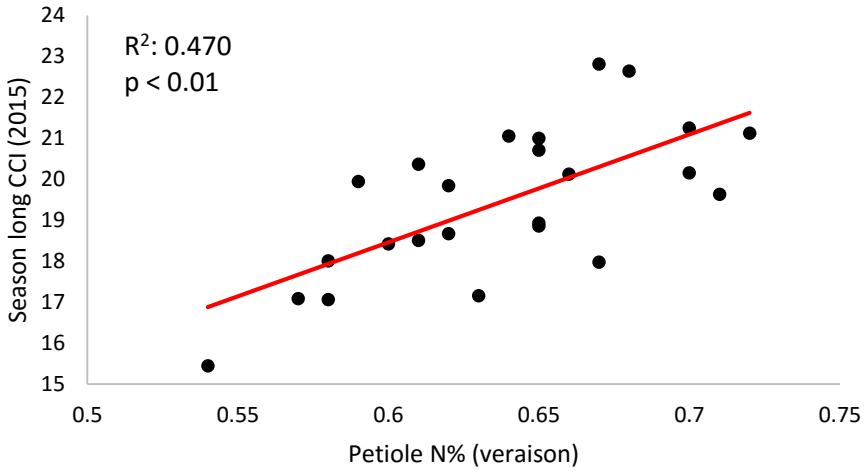

Figure 3. ISV: Regression analysis of Season long CCI and petiole N% at véraison (2015)

Components of yield and pruning weights

GMV: Relative to the control, soil and foliar-applied N significantly increased total vine yield, cluster weight, berry weight, berries per cluster and the yield to pruning weight ratio ($p<0.05$) (Table 29). The most dramatic increase in total yield, cluster weight, berry weight and pruning weights came from the 60 N soil treatment (Table 29). The 60 N soil treatment also significantly reduced the yield to pruning weight ratio relative to the control and 30 N foliar treatment ($p<0.05$) (Table 29). Total vine yield, cluster weight, berry weight, berries per cluster and pruning weights were significantly affected by treatment each year ($p<0.05$) (Table 30). The 60 N soil treatment significantly lowered ($p<0.05$) the yield to pruning weight ratio relative to the control and 30 N foliar treatment in 2014, but not 2015 (Table 30). Year had a significant effect upon total vine yield, clusters per vine and pruning weights ($p<0.05$) (Table 29).

AREC 1: The highest berry weight came from the 60 N soil treatment (Table 31 and 32). However, this result was only significant in 2015 and there was a significant treatment-year interaction ($p<0.05$) (Table 31 and 32). No other component of yield was affected by treatment. Pruning weights and the yield to pruning weight ratio was unaffected by treatment. Year had a significant effect upon total vine yield, clusters per vine, berry weight, berries per cluster, pruning weights and the yield to pruning weight ratio (Table 31).

AREC 2: Treatments did not significantly affect any component of yield, pruning weights or the yield to pruning weight ratio (Table 33 and 34). Year had a significant effect upon clusters per vine, pruning weights and the yield to pruning weight ratio ($p<0.05$) (Table 33).

ISV: Treatments had no significant effect upon any component of yield, pruning weights or the yield to pruning weight ratio (Table 35 and 36). Treatment had no effect upon pruning weights or the yield to pruning weight ratio (Table 37). Year had a significant effect upon total vine yield, clusters per vine, cluster weight, berry weight and berries per cluster ($p<0.05$) (Table 35).

Table 29. GMV: Components of yield and pruning weights in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	Yield (kg/vine)	Clusters per vine	Cluster weight (g)	Berry weight (g)	Berries per cluster	Pruning weights (kg/vine)	Y:P[e]
Control	11.19 c	61.19	183.39 c	1.56 c	117.36 b	0.98 c	11.58 a
30 N Soil	13.76 b	64.44	214.43 b	1.65 b	130.14 a	1.34 b	10.53 ab
60 N soil	14.96 a	64.46	233.25 a	1.71 a	136.29 a	1.75 a	8.77 b
30 N foliar	12.80 b	62.97	206.44 b	1.62 b	127.26 a	1.23 bc	10.98 a
Trt[c]	<0.0001	ns	<0.0001	<0.0001	<0.0001	<0.0001	0.0034
Yr	<0.0001	<0.0001	ns	ns	ns	0.0047	ns
Trt × Yr	ns[d]	ns	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] 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

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 30. GMV: Components of yield and pruning weights in response to soil and foliar N fertilization by year (2014 and 2015)

	Yield (kg/vine)		Clusters per vine		Cluster weight (g)		Berry weight (g)		Berries per cluster		Pruning weights (kg/vine)		Y:P[e]	
Treatment[ab]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	10.33 b	12.06 c	55.5	66.89	186.43 b	180.35 c	1.55 b	1.58 c	120.41 b	114.31 b	0.90 c	1.06 b	12.71 a	11.54
30 N soil	12.69 a	14.84 ab	57.39	71.5	221.27 a	207.60 b	1.65 a	1.64 b	133.87 a	126.41 ab	1.26 ab	1.49 ab	10.35 ab	10.16
60 N soil	13.34 a	16.58 a	57.97	70.95	230.51 a	235.99 a	1.69 a	1.73 a	136.18 a	136.41 a	1.55 a	1.95 a	8.99 b	8.75
30 N foliar	11.61 ab	14.09 b	55.07	69.56	211.47 ab	202.30 bc	1.66 a	1.59 c	127.69 ab	127.65 ab	1.06 bc	1.37 ab	13.07 a	10.81
Trt[c]	0.0007	<0.0001	ns[d]	ns	0.0004	0.0001	0.0017	<0.0001	0.0167	0.0050	0.0004	0.0053	0.0258	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 31. AREC 1: Components of yield and pruning weights in response to soil and foliar N fertilization (2014 -2015)

Treatment[ab]	Yield (kg/vine)	Clusters per vine	Cluster weight (g)	Berry weight (g)	Berries per cluster	Pruning weights (kg/vine)	Y:P[e]
Control	3.42	31.53	106.68	1.17 a	93.04	1.09	3.29
30 N soil	3.72	31.51	117.27	1.15 ab	102.20	1.17	3.17
45 N soil	3.78	33.29	113.10	1.13 ab	100.57	1.11	3.63
60 N soil	3.24	30.90	104.34	1.18 a	90.24	1.09	3.11
45 N soil + 15 N foliar	3.59	33.44	108.00	1.08 b	100.63	1.09	3.49
Trt[c]	ns[d]	ns	ns	0.0084	ns	ns	ns
Yr	0.0041	0.0019	ns	<0.0001	<0.0001	0.0062	<0.0001
Trt × Yr	ns	ns	ns	0.0257	ns	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] 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

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 32. AREC 1: Components of yield and pruning weights in response to soil and foliar N fertilization by year (2014 and 2015)

	Yield (kg/vine)		Clusters per vine		Cluster weight (g)		Berry weight (g)		Berries per cluster		Pruning weights (kg/vine)		Y:P[e]	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	4.00	2.85	34.65	28.40	114.06	99.30	1.05	1.28 ab	108.33	77.76 b	1.07	1.12	3.82	2.76
30 N soil	3.95	3.49	32.58	30.43	118.69	115.85	1.08	1.22 ab	109.51	94.89 a	1.11	1.23	3.46	2.87
45 N soil	4.10	3.45	35.78	30.78	113.85	112.35	1.05	1.22 ab	108.75	92.40 ab	1.00	1.20	4.26	3.01
60 N soil	3.31	3.19	31.28	30.52	104.35	104.32	1.04	1.33 a	101.34	79.15 b	1.02	1.15	3.25	2.98
45 N soil + 15 N foliar	3.68	3.50	34.52	32.36	107.33	108.67	1.02	1.14 b	105.34	95.93 a	1.01	1.16	3.89	3.09
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	0.0136	ns	0.0016	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 33. AREC 2: Components of yield and pruning weights in response to foliar N fertilization (2014 -2015)

Treatment[ab]	Yield (kg/vine)	Clusters per vine	Cluster weight (g)	Berry weight (g)	Berries per cluster	Pruning weights (kg/vine)	Y:P[e]
Control	2.55	34.35	74.60	1.25	59.78	0.68	3.90
15 N foliar	2.56	33.66	77.19	1.24	62.53	0.75	3.53
15 N foliar + 5 S foliar	2.48	35.02	70.32	1.23	57.09	0.68	3.85
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns
Yr	ns	0.0070	ns	ns	ns	<0.0001	<0.0001
Trt × Yr	ns	ns	ns	ns	ns	ns	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] 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

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 34. AREC 2: Components of yield and pruning weights in response to foliar N fertilization by year (2014 and 2015)

	Yield (kg/vine)		Clusters per vine		Cluster weight (g)		Berry weight (g)		Berries per cluster		Pruning weights (kg/vine)		Y:P[e]	
Treatment[ab]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	2.78	2.31	37.1	31.6	76.09	73.11	1.23	1.27	61.81	57.75	0.63	0.73	4.53	3.26
15 N foliar	2.72	2.41	35.84	31.48	77.02	77.35	1.23	1.24	62.74	62.33	0.68	0.82	4.08	2.99
15 N foliar + 5 S foliar	2.5	2.45	37.28	32.76	66.28	74.37	1.2	1.26	55.03	59.15	0.61	0.75	4.28	3.41
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Table 35. ISV: Components of yield in response to treatments (2014 -2015)

Treatment[ab]	Yield (kg/vine)	clusters per vine	cluster weight (g)	berry weight (g)	berries per cluster
15 N soil	10.55	52.17	207.40	1.96	106.53
15 N soil + 10 N foliar	10.65	53.09	203.49	1.96	103.79
Crimson	10.81	56.88	196.56	1.93	102.24
Crimson + 10 N foliar	10.42	51.35	207.91	1.95	106.93
White	10.90	57.34	195.74	1.94	100.92
White + 10 N foliar	11.67	57.66	211.45	1.98	107.65
Trt[c]	ns[d]	ns	ns	ns	ns
Yr	0.0016	<0.0001	0.0093	<0.0001	<0.0001
Trt × Yr	ns	ns	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] 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

[d]ns = not significant

Table 36. ISV: Components of yield in response to treatments by year (2014 and 2015)

	Yield (kg/vine)		Clusters per vine		Cluster weight (g)		Berry weight (g)		Berries per cluster	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
15 N soil	11.08	9.86	57.81	44.64	195.11	223.78	2.04	1.86	95.86	120.75
15 N soil + 10 N foliar	12.20	9.10	60.19	46.00	208.18	198.80	2.05	1.86	101.48	106.10
Crimson	11.51	10.11	63.00	50.75	187.49	205.63	2.05	1.82	91.63	112.86
Crimson + 10 N foliar	11.07	9.54	57.81	42.72	195.83	224.03	2.01	1.88	97.57	119.40
White	11.70	10.11	63.88	50.81	190.55	200.93	1.98	1.91	96.36	105.49
White + 10 N foliar	12.69	10.66	68.38	46.94	192.03	230.88	2.06	1.89	93.22	122.07
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 37. ISV: Pruning weights in response to treatments (2015)

Treatment[ab]	Pruning weights (kg/vine)	Y:P[e]
15 N soil	1.08	10.73
15 N soil + 10 N foliar	1.09	8.78
Crimson	0.97	11.53
Crimson + 10 N foliar	0.94	10.92
White	0.90	11.71
White + 10 N foliar	1.00	11.47
Trt[c]	ns[d]	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

[e]Y:P = Yield to pruning weight ratio

Canopy architecture

Treatment had no effect upon any metric of canopy architecture at any site, except ISV (Table 38 – 45). At ISV, significantly more ambient light was able to reach the fruit zone (%PPF) in the Crimson + 10 N foliar treatment than the 15 N soil + 10 N foliar treatment, ($p<0.05$) (Table 44). However, the significance of the relationship between treatment and %PPF was not found within each year (Table 45). The Crimson + 10 N foliar treatment had a significantly higher leaf exposure layer number (LEL) in 2015 in relation to the 15 N soil treatment. Year had a significant effect upon cluster exposure layer (CEL) at GMV and AREC 1 ($p<0.05$) (Table 38 and 40). Year had a significant effect upon occlusion layer number (OLN) at GMV and AREC 2 ($p<0.05$) (Table 38 and 42). Cluster exposure flux layer (CEFA) and leaf exposure flux availability (LEFA) were significantly affected by year at GMV, AREC 1 and AREC 2 ($p<0.05$) (Table 38, 40 and 42). Year had a significant effect upon photosynthetic photon flux at all four experimental sites ($p<0.05$) (Table 38, 40, 42 and 44).

Table 38. GMV: Select EPQA metrics in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	Occlusion layer number (OLN)	Cluster exposure layer (CEL)	Leaf exposure layer (LEL)	Cluster exposure flux availability (CEFA)	Leaf exposure flux availability (LEFA)	Photosynthetic photon flux (%PPF)
Control	1.71	0.21	0.11	0.55	0.55	4.58%
30 N soil	1.88	0.26	0.14	0.49	0.51	3.74%
60 N soil	1.81	0.30	0.14	0.47	0.53	3.74%
30 N foliar	1.83	0.27	0.12	0.52	0.54	4.38%
Trt[c]	ns[d]	ns	ns	ns	ns	ns
Yr	0.0002	0.0048	ns	0.0005	0.0103	<0.0001
Trt × Yr	ns	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] 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

[d]ns = not significant

Table 39. GMV: Select EPQA metrics in response to soil and foliar N fertilization by year (2014 and 2015)

	Occlusion layer number (OLN)		Cluster exposure layer (CEL)		Leaf exposure layer (LEL)		Cluster exposure flux availability (CEFA)		Leaf exposure flux availability (LEFA)		Photosynthetic photon flux (%PPF)	
Treatment[ab]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	1.62	1.77	0.21	0.23	0.11	0.12	0.61	0.48	0.57	0.53	6.04%	3.05%
30 N soil	1.72	2.03	0.20	0.29	0.13	0.15	0.56	0.44	0.52	0.50	4.89%	2.73%
60 N soil	1.60	2.01	0.16	0.44	0.13	0.16	0.61	0.38	0.56	0.50	4.87%	2.53%
30 N foliar	1.74	1.93	0.23	0.33	0.13	0.11	0.55	0.43	0.56	0.52	5.90%	2.88%
Trt[c]	ns[d]	ns	ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 40. AREC 1: Select EPQA metrics in response to soil and foliar N fertilization (2014-2015)

Treatment[ab]	Occlusion layer number (OLN)	Cluster exposure layer (CEL)	Leaf exposure layer (LEL)	Cluster exposure flux availability (CEFA)	Leaf exposure flux availability (LEFA)	Photosynthetic photon flux (%PPF)
Control	2.43	0.50	0.18	0.37	0.49	15.64%
30 N soil	2.38	0.43	0.17	0.39	0.50	14.28%
45 N soil	2.30	0.41	0.17	0.38	0.49	12.33%
60 N soil	2.33	0.45	0.17	0.40	0.51	14.76%
45 N soil + 15 foliar	2.25	0.42	0.17	0.41	0.50	13.61%
Trt[c]	ns[d]	ns	ns	ns	ns	ns
Yr	ns	0.0004	ns	0.0065	<0.0001	<0.0001
Trt × Yr	ns	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; 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] 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

[d]ns = not significant

Table 41. AREC 1: Select EPQA metrics in response to soil and foliar N fertilization by year (2014 and 2015)

	Occlusion layer number (OLN)		Cluster exposure layer (CEL)		Leaf exposure layer (LEL)		Cluster exposure flux availability (CEFA)		Leaf exposure flux availability (LEFA)		Photosynthetic photon flux (%PPF)	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Control	2.46	2.40	0.50	0.50 a	0.19	0.17	0.43	0.31	0.53	0.45	26.52%	2.05%
30 N soil	2.48	2.28	0.57	0.29 b	0.16	0.18	0.38	0.39	0.54	0.46	24.13%	1.96%
45 N soil	2.47	2.12	0.47	0.34 b	0.21	0.13	0.40	0.36	0.49	0.48	18.58%	1.91%
60 N soil	2.37	2.30	0.50	0.40 ab	0.18	0.16	0.45	0.34	0.55	0.47	27.26%	2.25%
45 N soil + 15 N foliar	2.28	2.22	0.49	0.34 b	0.16	0.18	0.43	0.40	0.55	0.45	25.18%	2.04%
Trt[c+]	ns[d]	ns	ns	0.0060	ns	ns	Ns	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; 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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 42. AREC 2: Select EPQA metrics in response to foliar N fertilization (2014-2015)

Treatment[ab]	Occlusion layer number (OLN)	Cluster exposure layer (CEL)	Leaf exposure layer (LEL)	Cluster exposure flux availability (CEFA)	Leaf exposure flux availability (LEFA)	Photosynthetic photon flux (%PPF)
Control	2.5	0.4	0.22	0.38	0.45	10.77%
15 N foliar	2.5	0.5	0.21	0.31	0.44	7.93%
15 N foliar + 5 S foliar	2.68	0.56	0.23	0.3	0.45	10.19%
Trt[c]	ns[d]	ns	ns	Ns	ns	ns
Yr	0.0192	ns	ns	0.0038	0.0006	<0.0001
Trt × Yr	ns	ns	0.0412	Ns	ns	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] 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

[d]ns = not significant

Table 43. AREC 2: Select EPQA metrics in response to foliar N fertilization by year (2014 and 2015)

	Occlusion layer number (OLN)		Cluster exposure layer (CEL)		Leaf exposure layer (LEL)		Cluster exposure flux availability (CEFA)		Leaf exposure flux availability (LEFA)		Photosynthetic photon flux (%PPF)	
Treatment[ab]	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	2.46	2.56	0.40	0.40	0.24	0.20	0.44	0.32	0.48	0.44	18.21%	3.32%
15 N foliar	2.44	2.57	0.50	0.51	0.24	0.20	0.34	0.29	0.46	0.44	13.02%	2.84%
15 N foliar + 5 S foliar	2.42	2.95	0.53	0.60	0.19	0.28	0.36	0.25	0.50	0.40	17.32%	3.06%
Trt[c]	ns[d]	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

Table 44. ISV: Select EPQA metrics in response to treatments (2014-2015)

Treatment[ab]	Occlusion layer number (OLN)	Cluster exposure layer (CEL)	Leaf exposure layer (LEL)	Cluster exposure flux availability (CEFA)	Leaf exposure flux availability (LEFA)	Photosynthetic photon flux (%PPF)
15 N soil	2.27	0.45	0.16	0.35	0.48	2.40% ab
15 N soil + 10 N foliar	2.32	0.51	0.16	0.31	0.47	1.90% b
Crimson	2.61	0.49	0.25	0.31	0.41	2.18% ab
Crimson + 10 N foliar	2.27	0.53	0.24	0.28	0.43	2.65% a
White	2.34	0.42	0.18	0.34	0.47	2.51% ab
White + 10 N foliar	2.41	0.45	0.20	0.33	0.44	2.20% ab
Trt[c]	ns[d]	ns	ns	Ns	ns	0.0486
Yr	ns	ns	ns	Ns	ns	<0.0001
Trt × Yr	ns	ns	ns	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] 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

[d]ns = not significant

Table 45. ISV: Select EPQA metrics in response to treatments by year (2014 and 2015)

	Occlusion layer number (OLN)		Cluster exposure layer (CEL)		Leaf exposure layer (LEL)		Cluster exposure flux availability (CEFA)		Leaf exposure flux availability (LEFA)		Photosynthetic photon flux (%PPF)	
Treatment[ab]	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
15 N soil	2.36	2.19	0.43	0.47	0.18	0.15 b	0.34	0.37	0.46	0.51	2.74%	2.06%
15 N soil + 10 N foliar	2.36	2.29	0.51	0.51	0.18	0.15 ab	0.32	0.30	0.46	0.48	2.14%	1.67%
Crimson	2.51	2.72	0.44	0.54	0.25	0.25 ab	0.36	0.27	0.42	0.42	2.50%	1.86%
Crimson + 10 N foliar	2.20	2.36	0.56	0.51	0.18	0.30 a	0.28	0.29	0.48	0.40	3.32%	1.97%
White	2.15	2.53	0.42	0.42	0.13	0.25 ab	0.35	0.35	0.51	0.43	2.75%	2.27%
White + 10 N foliar	2.37	2.45	0.46	0.45	0.19	0.21 ab	0.33	0.34	0.45	0.44	2.56%	1.84%
Trt[c]	ns[d]	ns	ns	ns	ns	0.0307	Ns	ns	ns	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] Significance of effects using one-way ANOVA. Trt = significance of treatment effects

[d]ns = not significant

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 46). However, this treatment effect was minimal and insignificant within 2014 and 2015 (Table 47). 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 46 and 47). 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 46 and 47). However, PAN was not significantly affected by treatment in 2014 (Table 47). 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 46).

AREC 1: Soluble solids, pH and TA were all highest within the 45 N soil + 15 N foliar treatment (Table 48). However, soluble solids were not different between treatments in 2014 and 2015 (Table 49) and TA was only significantly affected by treatment in 2015 ($p<0.05$) (Table 49). 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 48 and 49). 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 49). 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 49). Applications of foliar urea significantly increased the concentration of NH_4^+-N and PAN (Table 48 and 49). The application of N fertilizer increased the

inorganic to organic N ratio, but this response was not significant between treatments within each year (Table 49). Year had a significant effect upon pH, TA, NH_4^+-N, PAN, YAN and the inorganic to organic N ratio ($p<0.05$) (Table 48).

AREC 2: The application of foliar urea at véraison significantly increased juice pH ($p<0.05$) (Table 50). However, the pH response was only significant in 2015 ($p<0.05$) (Table 51). Foliar urea increased YAN concentrations significantly ($p<0.05$) (Table 50); however, the response was due principally to the significant response in 2015 (Table 51). 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 51). In 2015, foliar urea treatments were effective at improving both NH_4^+-N and PAN concentrations (Table 51). The application of foliar urea significantly increased the NH_4^+-N to PAN ratio in 2015, but not 2014 (Table 51). Year had a significant effect upon soluble solids, pH, TA, PAN and the inorganic to organic N ratio ($p<0.05$) (Table 50). A significant treatment by year interaction was found for the PAN and YAN concentrations ($p<0.05$) (Table 50).

ISV: Treatment had no significant effect upon soluble solids, pH or TA (Table 52 and 53). 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 52). Foliar treatments increased both the ammonia and amino nitrogen components of YAN, without significantly affecting the inorganic to organic N ratio (Table 52). 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 53). 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 52). There was a significant treatment-year interaction with NH_4^+-N concentration ($p<0.05$) (Table 52).

Table 46. 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 47. 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 48. 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 49. 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 50. 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 51. 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 52. 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 53. ISV: Primary fruit chemistry and yeast assimilable nitrogen (YAN) in response to treatments 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**
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 54). More than 70% of amino acid concentration was comprised of Pro, Arg, GABA, Glu, Ala and Gln (Table 55). 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 54). The 30 N foliar treatment increased the total concentration of free amino acids by 106% (Table 54). The Pro to Arg ratio decreased significantly with both soil and foliar N fertilization ($p<0.05$) (Table 55). Year had a significant effect upon the absolute concentration of every acid measured, except His, Gln and Phe ($p<0.05$) (Table 54). There was a significant treatment-year interaction with His, Gly, Ile, Leu ($p<0.05$) (Table 54). Year had a significant effect upon the proportional contribution of each amino acid, except Arg, GABA, Val, Ile and Phe (Table 55).

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 56). Pro, Thr, Arg and Ser represented more than 75% of the amino acids measured (Table 57). The 45 N soil + 15 N foliar treatment increased the concentration of free amino acids by 99% (Table 56). 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 57). The 45 N soil + 15 N foliar treatment significantly decreased the Pro to Arg ratio by over 250% (Table 57). Year had a significant effect upon the concentration of every amino acid measured, except Gln, Glu, Thr and Lys (Table 56). Year had a significant effect ($p<0.05$) upon the proportional contribution of each amino acid measured, except Gly, Lys, Val and Ile (Table 57). Treatment-year interactions were significant ($p<0.05$) for the concentration of each amino acid, but Gln,

Asp, Ala and Lys (Table 56). 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 57).

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 58). Both foliar treatments significantly increased the concentration of free amino acids by over 90% ($p<0.05$) (Table 58). 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 59). Both the 15 N foliar and 15 N foliar + 5 S foliar treatments significantly lowered the Pro to Arg ratio ($p<0.05$) (Table 59). Year significantly affected the concentration of every amino acid, except Asn, Ala and Lys ($p<0.05$) (Table 58). Year had a significant effect upon the relative proportion of Gln, Gly, Asp, Glu, Ala, Lys, Tyr and Leu ($p<0.05$) (Table 59). Treatment-year interactions were significant for the concentration of Asn, Ala and Pro ($p<0.05$) (Table 58). Treatment-year interactions were significant in regard to the relative concentration of Leu ($p<0.05$) (Table 59).

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 60). Ser, Gln, Arg, Thr and Pro represented more than 70% of the free amino acids measured (Table 61). 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 61). 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 61). Year had a significant effect upon the concentration of Arg, Gly, Glu, Thr, Pro, Tyr, Val and

Phe ($p<0.05$) (Table 60). Year had a significant effect upon the relative concentration of Asn and Phe ($p<0.05$) (Table 61).

Table 54. 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 = 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 ($\alpha=0.05$)

[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 55. 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 56. 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 = 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 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 57. 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 58. 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 59. 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 60. 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 61. 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

Cover crop stand density and biomass

White clover produced significantly more above ground biomass and a denser stand than Crimson clover in 2015 ($p<0.05$) (Table 62). However, in both the White clover and Crimson clover plots at ISV, the majority of the biomass and plant species within the stand came from non-leguminous weeds.

Table 62. ISV: Average cover crop stand density rating and proportion of aboveground biomass from Crimson and White clover cover crops in 2015

Cover crop[ab]	Cover crop biomass (% of total)	Stand density rating[c]
White clover	40.38% a	3.08 a
Crimson clover	17.88% b	1.50 b
Trt[d]	0.0298	<0.0001

[a]White clover and Crimson clover cover crops (both alone and with foliar N)
[b]Within column, means with different letters indicate differences in means using Student's T-test (α=0.05)
[c]Stand density rating 1 = 76-100% invasive species/bare ground; 2 = 51-75% invasive species/bare ground; 3 = 26-50% invasive species or bare ground; 4 = 10-25% invasive species/bare ground; 5 = <10% invasive species/bare ground; 6 = 100% ground cover by cover crop
[d]Significance of treatment effect using one-way ANOVA. Trt = Significance of treatment effect

Discussion

Excessive vegetative growth is a common characteristic of vineyards in the Eastern United States. Therefore, many viticulturists have opted to employ intensive cover cropping strategies in their vineyards, as cover crops have been demonstrated to effectively reduce vine vigor (Giese et al. 2014; Morlat and Jacquet 2003; Tesic et al. 2007; Wheeler et al. 2005). The vigor suppression of a cover crop is due to competition with the vine for both water and nutrients (Monteiro and Lopes 2007; Sweet and Schreiner 2010). The predominant soil N source for grapevines is in the form of nitrate (NO_3^-)(Keller et al. 2001b). Nitrates are dissolved in soil solution and reach the root via mass flow (Barber et al. 1963). Competition between grapevines and companion cover crops for nitrogen has been observed by others (Celette et al. 2009; Giese et al. 2014; Pérez-Álvarez et al. 2015; Sweet and Schreiner 2010; Tesic et al. 2007; Wheeler et al. 2005). Under perennial and complete floor cover cropping scenarios, vineyards can be subject to vine tissue and juice N deficiencies. The current study sought to evaluate several nitrogen management schemes with the aim of increasing vine tissue and juice N, while maintaining the agronomic benefits of the cover crop.

An increase in vine tissue N status in response to soil-applied N has been previously demonstrated (Bell and Robson 1999; Bell and Francis 2013; Conradie and Saayman 1989b; Ekbic et al. 2010; Keller et al. 2001b; Linsenmeier et al. 2008; Peuke 2009). However, the response of petiole N concentration to soil-applied N can be unreliable, resulting in only a marginal increase in tissue N concentration or an insignificant effect (Conradie and Saayman 1989b; Ekbic et al. 2010) While both soil- and foliar-applied N were evaluated in the current study, soil-applied nitrogen was more successful at raising petiole N status at véraison. In general, increasing the rate of N fertilization increased petiolar N concentration. However, the 45 N soil treatment at AREC 1 resulted in the highest petiole N concentration, not the 60 N soil treatment. The 60 N soil treatment was split into two equal applications of 30 kg N/ha, with the first being applied at bloom and the second application just prior to

véraison, whereas the 45 N soil treatment at AREC 1 was completely imposed at bloom. As N uptake is greatest at bloom, the 45 N soil treatment may have been more efficiently assimilated than the 60 N soil treatment, as more N was applied to the soil at the period of maximum N uptake (Schreiner et al. 2006). Petiole N concentration was not significantly impacted by the treatments at ISV. This strongly suggests that the application of 15 kg N/ha to the soil was insufficient for increasing vine N status, even in plots with a bare soil surface directly underneath the trellis.

A véraison petiolar N concentration of between 0.8-1.2% has been recommended as the optimum range for grapevines in the Eastern United States (Wolf 2008). No treatment in the current study was able to reach this "optimal" concentration. Perhaps this warrants further investigation as to the validity such a high "optimal" véraison petiolar N range. It should be noted that due to the great temporal variability of petiolar N status, leaf blade or whole leaf samples may be the most effective means of chemically assessing vine tissue N status (Davenport and Horneck 2011; Dominguez et al. 2015; Romero et al. 2014).

The clover cover crops at ISV did not affect vine N status. However, petiolar N status was maintained relative to the 15 N soil treatment. A similar maintenance of vine N status has been found by Pérez-Álvarez et al. (2015). The maintenance of vine N may have been due to rhizodeposition of nitrogen by the clover cover crops (Fustec et al. 2011). The potential N maintainenance could be explored in the future by comparisons of white clover with non-leguminous cover crops in the same experiment.

The application of foliar urea alone was ineffective at significantly increasing petiole N concentrations, as has been reported by others (Hannam et al. 2014). Between 17 and 80% of N applied in the form of foliar urea can be assimilated by the berries (Lasa et al. 2012; Schreiber et al. 2002). Also, the foliar urea treatments at ISV, AREC 1 and 2 were imposed around véraison, which corresponds to the phenological stage at which point the grape cluster becomes the strongest N sink and is highly

competitive with the other organs of the grapevine for N nutrition (Conradie 1991). Therefore, while foliar urea applications may effectively elevate berry YAN, it should not be relied upon to improve vine tissue N and vine capacity when the vineyard is in a state of N deficiency.

As reported by others we too found a significant reduction in vine P concentration in response to N fertilization (Bell and Robson 1999; Bell and Francis 2013; Conradie and Saayman 1989b; D'Attilio 2014; Linsenmeier et al. 2008; Peuke 2009; Spayd et al. 1993). Soil-applied N has been shown to increase petiolar mass (Bell and Robson 1999). The depression of petiolar P concentration in the current study in response to soil-applied N may be due to a dilution caused by greater petiole dry weights in response to N fertilization. Others have suggested that the decreased P concentration in response to soil N fertilization is in response to soil acidification caused by the application of nitrogenous fertilizer (Bell and Robson 1999; Conradie and Saayman 1989b); however, N fertilizer was applied as calcium nitrate in the current study, which is known to increase soil pH over time(Pierre 1928; Riley and Barber 1971; Sarkar and Wynjones 1982), although soil pH was not monitored in the current study.

Although not always statistically significant, petiolar potassium (K) concentrations were diminished in response to soil-applied N at GMV. Diminished K nutrition due to soil-applied N has been found by others (Hilbert et al. 2003; Linsenmeier et al. 2008; Peuke 2009). However, in their long-term study, Conradie and Saayman (1989) found that nitrogenous fertilization did not impact tissue K concentrations. The concentration of K in the petioles at ISV was significantly lower in the White + 10 N foliar treatment in relation to the Crimson + 10 N foliar treatment. However, significance was only found in the first year of the study. Although stand density and above ground biomass was only measured in 2015, it was anecdotally observed that the cover crops behaved similarly in both years. The White clover established a much denser stand with a greater biomass than that of the Crimson clover treatments. Sweet and Schreiner (2010) found that clover/grass mixes can effectively compete with the vine for K. As there was quite a lot of bare ground within the plots sown with Crimson clover, which was

associated with their poor stand rating, the White clover plots may have been more competitive for K. Tesic et al. (2007) found that cover crops were able to effectively compete with the vine for K; however, this response was inconsistent from year to year, as we also observed at ISV.

Keller et al. (2001) found that N fertilization increased the translocation of Mg in the xylem. Others have also noticed an increase in tissue Mg in response to N fertilization (Bell and Francis 2013; Hilbert et al. 2003; Linsenmeier et al. 2008). These results are in agreement with the current study, in which increased Mg concentrations were found in the véraison petiole samples at GMV and AREC 1.

Calcium was mostly unaffected by fertilization treatments. However, the application of foliar urea resulted in a statistically significant increase in petiolar Ca concentration between the 45 N soil and 45 N soil + 15 N foliar treatments at AREC 1. However, when each year was evaluated individually, it was apparent that these results were not significant between treatments, but the similar trend did exist, with the highest Ca concentration coming from the 45 N soil + 15 N foliar treatments. This result is of little or no consequence, as foliar N had no effect upon petiole Ca in the same variety at AREC 2, nor with Sauvignon blanc at GMV.

The only apparent treatment effects on micronutrients were a slight depression of Mn and B in the case of soil-applied N. Upon evaluating each year individually, it seems that the Mn was not significantly affected by treatment within each year. However, boron was depressed by soil N in 2015. This result is in agreement with Peuke (2009), who found that boron concentration in leaves of vines fertilized with N decreased with increasing levels of N fertilization. Nitrate is taken up by roots through active proton cotransport via a membrane bound proton pump, H^+-ATPase (Glass et al. 1992; Siddiqi et al. 1990). Boron is essential to the activity of the H^+-ATPase pump (Camacho-Cristóbal and González-Fontes 2007; Ferrol et al. 1993). With increasing levels of NO_3^- fertilization and the subsequent increase in H+-ATPase activity, it may deplete plant boron concentrations. Like petiolar P, the response of manganese and boron to soil N application may also be a function of dilution due to increased dry

matter accumulation. Also, the depression of boron concentration in petioles at véraison in response to soil-applied N is not viticulturally significant in that B tissue values were still well within an acceptable range (Wolf, 2008).

Soil-applied N significantly increased season-long CCI values at GMV and AREC 1, similar to petiolar N, CCI readings were highest in the 60 N soil treatment at GMV and the 45 N soil treatment at AREC 1. Leaf chlorophyll content and CCI values have been found to be highly correlated in species as diverse as grapevines, sugar maple, birch and Asian pear (Cate and Perkins 2003; Filimon et al. 2014; Ghasemi et al. 2011; Richardson et al. 2002; Taskos et al. 2015). The relationship between N fertilization, petiolar N and CCI is not surprising, as leaf chlorophyll content has been found to increase with increasing concentrations of N (Bell and Francis 2013; Ghasemi et al. 2011; Keller 2005; Keller and Koblet 1995; Keller et al. 2001b; Taskos et al. 2015). A significant positive relationship existed between season-long CCI and petiole N% at véraison at AREC 1 and ISV. However, this significant relationship was not apparent at GMV. This may be due to the methodology by which leaf N was measured. In previous studies which evaluated the efficacy of leaf N prediction with the CCM-200, total leaf N% was measured, rather than just petiolar N (Ghasemi et al. 2011; Taskos et al. 2015). This difference in methodology may impact the strength of this correlation. The lack of a positive relationship between leaf petiolar N and CCI at GMV was also observed by D'Attilio (2014). Petiolar N is more variable than leaf blade N (Dominguez et al. 2015). Also, leaf blades have been found to contain nearly triple the concentration of N as petioles, which may improve the sensitivity of the analysis for N (Romero et al. 2010). However, Dominguez et al. (2015) also found that the stability period for N was longer during veraison within the petioles and Romero et al. (2010) found that the position of the leaf did not affect petiolar N concentrations as much as it did the leaf blade. Therefore, either methodology (leaf blade or petiole sampling) has sources of error which cannot be overcome. The use of whole leaf sampling, as a means of reducing these sources of error and variability, warrants further investigation. Treatment did not

have a significant effect upon season-long CCI at ISV in 2015 (table 22). This is not surprising, as treatments also did not have a significant effect upon petiole N at ISV (table 15).

Yield and pruning weights followed a similar trend to CCI and petiolar N% at GMV and AREC 1, with higher yields and pruning weights associated with increasing levels of soil and foliar-applied N. It's interesting to note that although the soil-applied N had the greatest effect upon yield, the 30 N foliar treatment at GMV also significantly increased yield. Hannam et al. (2016) also found foliar urea increased vine yields in their study on Pinot gris and Merlot. In the current study, GMV was in the fourth and fifth year of treatments and a statistically significant yield differential was not found in previous years (D'Attilio 2014). In the previous study conducted by D'Atilio (2014), yield increased with soil-applied N treatments, but these yield increases were not statistically significant. A number of studies have also found an increase in fruit yield in response to soil-applied N (Conradie and Saayman 1989a; Keller et al. 1998; Keller et al. 2001a; Linsenmeier et al. 2008; Spayd et al. 1993). Conradie and Saayman (1989b) also found a delayed yield response with N fertilization treatments, with a N fertilization treatment increasing yield only after three years. The component of yield most affected by N fertilization at GMV was cluster weight. The increase in cluster weight with N fertilization was due to both more berries per cluster as well as greater individual berry weights. An increase of berry weight and the number of berries per cluster in response to increased N supply has also been demonstrated by others (Amiri and Fallahi 2007; Cheng et al. 2004; Duchene et al. 2001; Keller et al. 1998; Keller et al. 2001a; Spayd et al. 1993). Increased leaf chlorophyll and N content has been correlated with greater CO_2 assimilation (Chen and Cheng 2003b; Keller 2005; Keller et al. 2001b). Lower assimilation rates have been associated with limited berry growth (Dokoozlian and Kliewer 1996; Finger et al. 2002; Ollat and Gaudillere 1998). As N fertilization increased leaf chlorophyll content in N fertilized treatments, CO_2 assimilation may have also increased, thereby increasing berry size. The increase in fruit weight in response to N fertilization was likely due to increased cellular division and/or enlargement in the

pericarp brought on by increased assimilate supply, as has been found in cucumber, tomato and apple (Bohner and Bangerth 1988; Marcelis 1993; Xia et al. 2009).

It is commonly understood that limited N in the previous year can affect the vegetative and reproductive growth of the subsequent year, including bloom and fruit set (Cheng et al. 2004; Duchene et al. 2001; Guilpart et al. 2014; Zapata et al. 2004). Cheng and Xia (2004) found that berry size and the number of berries per cluster were affected by both reserve and current season N supply. As the Sauvignon blanc at GMV was in a state of N deficiency at the beginning of this long-term experiment in 2011, N reserves were likely increased over time. This may explain why neither berry number per cluster nor berry weight were significantly impacted until year 2014 and 2015. Berry number per cluster may have increased due to improved fruit set/ovule fertility brought on by improved N nutritional status (Ewart and Kliewer 1977). It is also possible that the improved N status of the grapevine increased the branching of the anlage in the previous season, which resulted in more flowers per inflorescence, as was speculated by others (Guilpart et al. 2014). Keller et al. (2001a) found that N fertilization did not increase the number of flowers per inflorescence, however the researchers only evaluated the effect of N fertilization in one year. Carbohydrate (CHO) reserves of the grapevine also play a crucial role in both inflorescence number per shoot and flower number per inflorescence (Bennett et al. 2005). Bennett et al. (2005) found a significant correlation between dormant CHO status of the grapevine and current season pruning weights. Therefore, pruning weights may be a good proxy for vine CHO status. Pruning weights significantly increased with both rates of soil-applied N at GMV. Therefore, it is likely that vine CHO at GMV was increased through N application and the increase in berries per cluster observed with increasing levels of soil N may have been due to improved fruit set and/or more flowers per inflorescence.

Soil-applied N increased vine vegetative growth at a faster rate relative to crop yield, as indicated by the significantly lowered yield to pruning weight ratio at GMV. Spayd et al. (1993) also

reported an increase in the crop weight to pruning weight ratio in response to N fertilization. A crop weight to pruning weight ratio of 5-10 has been proposed as being indicative of vines which are considered balanced (Kliewer and Dokoozlian 2005; Smart and Robinson 1991). If this ideal crop to pruning weight ratio is to be applied, the soil-applied N treatments at GMV brought the vines into balance. Kliewer and Dokoozlian (2005) found a significant negative correlation between the leaf area to crop weight to pruning weight ratio, it stands to reason that soil-applied N increased leaf area. Bell and Robson (1999) found that total vine leaf area, leaf area per leaf and the number of leaves per vine increased with increasing levels of nitrogen fertilization.

Canopy density was not affected by treatment at GMV, AREC 1 or at AREC 2. EPQA measurements were taken at véraison. It should be noted that these vineyards were commercially managed and that standard canopy management practices were undertaken prior to véraison (i.e. leaf removal, shoot positioning and hedging). These practices would have mitigated any potential increase in vine vigor caused by N fertilization. Others have found an increase in N supply can increase canopy density (Bell and Robson 1999; Bell and Francis 2013). Increasing N supply has been found to increase total vine leaf area, area per leaf and the number of leaves per vine (Bell and Robson 1999; Cheng et al. 2004; Keller et al. 1998). Others have found that the effect of N fertilization upon canopy density was inconsistent from year to year (Hannam et al. 2013). Bell and Francis (2013) found that although leaf layer number increased with N fertilization, the fruit zone light environment was unaltered.

The only significant treatment effect upon the canopy architecture metrics measured in the current study was at ISV. Significantly less light reached the fruit zone of the 15 N soil + 10 N foliar treatment than the Crimson + 10 N foliar treatment. However, this metric was not significant between treatments within each year of the study. Although the combined year model found statistical significance, it is unlikely that the treatment differences were biologically significant.

Infections of the grape cluster by the fungal pathogen *Botrytis cinerea* can lead to devastating crop losses and severe consequences for fruit and wine quality. Botrytis can cause juice/wine browning through the rapid enzymatic oxidation of phenolic compounds, diminish the aromatic impact of monoterpenes, and directly impart a mushroom-like aroma (Boidron 1978; La Guerche et al. 2006; Macheix et al. 1991; Miklosy et al. 2004). Others have found that increased N supply led to a greater incidence of *B. cinerea* in grapes (Keller et al. 2001a; R'Houma et al. 1998). In the current study, incidence of *B. cinerea* infection was recorded at each site in 2015 (data not presented) to investigate a potential disease response to N fertilization. Of the 4 sites, only two clusters were harvested with evidence of *B. cinerea* (both at GMV in a 30 N soil and 60 N soil experimental unit). Lacroux et al. (2008) also found that soil and foliar N applications did not significantly affect incidence of *B. cinerea*. Canopy density, weather, inoculum levels and physical damage to the grape berry are all known to affect the severity of *B. cinerea* infections (Broome et al. 1995; English et al. 1993). Keller et al. (2001a) hypothesized that the increased incidence of *B. cinerea* associated with N fertilization was due to increased cluster compactness caused by increased fruit set. Others have also found that more compact clusters are more susceptible to *B. cinerea* infection (Hed et al. 2009; Vail and Marois 1991). Cluster compactness was not measured in the current study; however, N fertilization was found to significantly increase berry weights and the number of berries per cluster at GMV and AREC 1. The increase in berry and berries per cluster may have resulted in an increase in cluster compactness. Therefore, although a significant relationship between N fertilization and *B. cinerea* incidence was not observed in the current study, it is still recommended that growers be aware of the potential risk and manage their vineyard accordingly, should they opt to utilize an N fertilization scheme.

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 veraison 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 (table 40-43, 46 and 47).

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.

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 (table 45 and 47).

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 (table 50 and 51). 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 (Table 41). 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.

Conclusions

The application of nitrogenous fertilizers to the soil increased vine nitrogen status and leaf chlorophyll content. After four years of fertilization in a N deficient vineyard, soil-applied N resulted in increased yields by increasing vine size and capacity, without negatively impacting canopy architecture. Soil-applied N was more effective than foliar-applied N at increasing vine tissue N concentrations than at increasing YAN or juice amino acid concentrations. Conversely, foliar applications of urea were more effective at increasing YAN and amino acid concentrations than were soil applications. 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 herein. The application of nitrogenous fertilizers to the soil at bloom (~30-60 Kg N/ha) and a small application of urea to the foliage around véraison (~10-15 kg N/ha) has the possibility to both increase vine N status, capacity and juice N while maintaining the agronomic benefits of the cover crop. The use of white clover as a perennial cover crop under the vine may assist in the maintenance

vine N status, but this hypothesis was not confirmed over the course of this study and warrants further investigation. Future studies could include an evaluation of vineyard N fertilization schemes upon varietal aromas and their precursors. Foliar-applied N provides the researcher with an opportunity to decouple the relationship between methoxypyrazines, canopy density and N fertilization, as foliar-applied N appeared to have no impact upon canopy density.

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