

Nitrogen and Sulphur Foliar Fertilisation

F.A. Bruwer, W. du Toit, A. Buica*

Department of Viticulture and Oenology, P/Bag X1 Matieland, Stellenbosch 7600, South Africa

Submitted for publication: October 2018

Accepted for publication: February 2019

Key words: Foliar fertilisation, nitrogen, sulphur, Chenin blanc, Sauvignon blanc, yeast assimilable nitrogen (YAN), glutathione, thiols

The effects of fertilisation can reverberate from grapes through to wines. In wine, non-volatile compounds mainly influence the taste and flavour of wines, while volatile compounds play an important role in the aromatic expression. This review includes information on the presence of non-volatile and volatile compounds reportedly affected by nitrogen and sulphur foliar fertilisation, (bio)synthesis, and evolution throughout winemaking, their chemical properties, and their implications. The second part presents the status of the research on elucidating the influence and contribution of foliar fertilisation practices on the chemical compounds throughout winemaking, from the grape to the wine.

INTRODUCTION

A wine's aroma, taste and flavour can contribute to the wine's overall quality and determine if winemakers and consumers find it appealing (Marais, 1994). The aroma of a wine is the result of various interactions between different chemical compounds found in the wine. These compounds are generated at different stages and through various processes; some compounds originate from the grapes, while others are generated during fermentation or wine ageing. The composition of a grape berry depends on various factors, such as grape variety, environmental conditions, viticultural practices, and terroir. Monoterpene and methoxypyrazines are derived from the grape, while volatile thiols, esters, higher alcohols and fatty acids are released by yeast from their precursors during alcoholic fermentation (Fischer, 2007).

The nutrient requirements of a grapevine depend on its age, cultivar variety, yield, soil type and properties (Holzapfel & Treeby, 2007). A vine nutrition deficiency occurs often and due to various reasons, and negatively affects the aroma profile of a wine due to sluggish or stuck fermentation (Monteiro & Bisson, 1991). Nutrition deficiencies can be corrected by carrying out fertilisation applications. Foliar nitrogen and sulphur fertilisation applications can positively influence the levels of nitrogen and sulphur compounds in the must, the success rate of alcoholic fermentation, and the resulting wine's composition and aroma (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Lasa *et al.*, 2012; Hannam *et al.*, 2014; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b; Helwi *et al.*, 2017; Kelly *et al.*, 2017). Results obtained through aroma and chemistry research in foliar fertilisation studies can contribute to the knowledge base of aromas and chemical compounds of

specific cultivar varieties.

The first part of this literature review will focus on various wine compounds and classes of compounds present in grapes and wines that were shown to be affected by foliar fertilisation. The second part will present the status of the research on elucidating the influence and contribution of different fertilisation practices in relation to the chemical compounds of the grapes, juice and wine, but also on the resulting wine's aromatic expression.

CLASSES OF CHEMICAL COMPOUNDS PRESENT IN THE GRAPE, MUST, JUICE AND WINE THAT ARE AFFECTED BY FOLIAR FERTILISATION

Standard oenological parameters (pH, total acidity, Balling and percentage ethanol)

In the past, the determination of harvest date did not include parameters such as pH and TA, and nowadays relying solely on sugar level is not considered a very accurate index to follow (Du Plessis & Van Rooyen, 1982). Optimal grape ripeness can be determined by including the levels of °B, pH, TA and colour of the grape skin and seeds (Deloire, 2012). Van Schalkwyk and Archer (2000) reported various optimal grape ripeness ranges for different classes of South African wines. The sugar content of white wine grapes at harvest ranges from 19.5 to 23°B, while grapes for sparkling wines are harvested at lower levels (18 to 20°B). Red wines nowadays fall within the same range as white wine, or can be slightly higher, and sweet and dessert wine grapes are harvested at higher sugar levels (22 to 26°B) (Van Schalkwyk & Archer, 2000). The pH ranges for white, red and sweet wine grapes are similar (3.2 to 3.4), while sparkling wines

*Corresponding author: E-mail address: abuica@sun.ac.za

Acknowledgements: The authors would like to thank Winetech for financial support

have lower levels (2.8 to 3.2), and dessert wines have higher levels (3.3 to 3.7) (Van Schalkwyk & Archer, 2000). The TA levels of red (6.5 to 7.5 g/L), sweet (6.5 to 8 g/L), and dessert wine grapes (6.5 to 8 g/L) are lower compared to those of sparkling wines, at 7 to 9 g/L, and white wines, which range from 7 to 8 g/L (Van Schalkwyk & Archer, 2000).

During winemaking, methods such as skin contact, maceration and pressing can influence the pH and TA levels in the must and juice. Free-run juice results in higher sugar and lower acid levels compared to pressed juice (Amerine *et al.*, 1982). The method of adding tartaric acid to wines to lower their pH levels is effective and should be performed before the start of alcoholic fermentation (Pambianchi, 2001). During alcoholic fermentation, the yeast converts the sugar into alcohol and carbon dioxide (CO₂). If the acidity is below 3 g/L, the alcoholic fermentation is somewhat reduced; on the other hand, low pH levels help to inhibit the growth of undesirable bacteria (Amerine *et al.*, 1982). During alcoholic fermentation, the alcohol content increases and the solubility of the acids decreases; therefore TA levels decrease and cause the pH to increase (Pambianchi, 2001; Robinson & Harding, 2014). Cold and protein stabilisation of white wine is done generally at -4°C for a minimum of two weeks. During this procedure, tartaric acid precipitates as potassium bitartrate salt, and the TA of the resulting wine decreases and the pH increases further (Pambianchi, 2001; Robinson & Harding, 2014).

The initial sugar level of the harvested grapes and alcohol levels of the resulting wine are correlated. In South Africa, the residual sugar levels differ for different classes of wines: sparkling wines are from < 3 to > 50 g/L, dry still wines are < 5 g/L), semi-dry wines are > 5 to ≤ 12 g/L), semi-sweet wines vary from > 12 to < 30 g/L), late harvest wines are ≥ 20 g/L, natural sweet wine is > 20 g/L), and noble late harvest wines are > 50 g/L (WOSA, 2017). During wine maturation in barrels, the wine diffuses through the small oak pores, and alcohol diffuses slower than water. Thus, in a dry cellar, the ethanol strength can increase, while in a humid cellar, the ethanol strength will decrease. It is therefore important to top up the barrels regularly during maturation (Robinson & Harding, 2014).

Non-volatile compounds

Various non-volatile compounds are constituents of grapes, juices and wines. These compounds are not aromatic, but some can be considered precursors to aromatic compounds, while others can influence the perception of aroma compounds through interactions. The compounds reviewed in this section include normal oenological parameters, ammonia and amino acids, and glutathione (GSH). Standard oenological parameters, including pH, total acidity (TA), sugar content (Balling) and ethanol content, are commonly measured during the winemaking process. Knowing the nitrogen levels of the must is crucial for successful alcoholic fermentation, and therefore the yeast assimilable nitrogen (YAN) levels are measured. GSH includes the reduced and oxidised forms and can be considered an indicator of the oxidation status.

Yeast assimilable nitrogen

Grapes contain various nitrogen compounds that are grouped into two forms: mineral (NH₄⁺, NO₃⁻ and NO₂⁻) and organic (free amino acids, nucleic acids, proteins, ethyl carbamate and urea) (Kuhn *et al.*, 2014). Grape juice and must contain various nutrients and it is important to know which nitrogen compounds are abundant and are required by the yeast for metabolism (Henschke & Jiranek, 1993). The major sources of nitrogen utilised by yeast are known as YAN, and the constituents thereof are free amino nitrogen (FAN) and ammonia nitrogen (NH₃). Bell (1994) reported that, during grape ripening, there is a gradual increase in total nitrogen and amino acid nitrogen levels in Cabernet Sauvignon grapes, while ammonium levels decrease. Similar increases and decreases during ripening have been reported by other researchers (Kliewer, 1968; Löhner & Schaller, 1992; Hilbert *et al.*, 2003). Henschke and Jiranek (1993) reported that the nitrogen content of grape juice ranges from 60 to 2 400 mg N/L. The yeast assimilable amino acid nitrogen is distributed in different parts in the berry: 10 to 15% in the seed, 19 to 29% in the skin, and 61 to 65% in the pulp (Stines *et al.*, 2000). The assimilable nitrogen content of must provides a good estimation of the vine nitrogen status (Van Leeuwen *et al.*, 2000).

YAN measurements should ideally be performed directly on the must or juice just before alcoholic fermentation to get the most representative results (Bell & Henschke, 2005). Juice samples can underestimate the total berry YAN because the majority of amino acids are contained in the skins of the grape (Stines *et al.*, 2000). Grapes from vineyards with a history of low YAN levels could be analysed for YAN before harvest to get an indication of the levels. In such cases, nitrogen supplementation with di-ammonium phosphate (DAP) at the start of alcoholic fermentation is suggested. Research studies recommend that the minimum YAN level required by the yeast before alcoholic fermentation is between 140 and 150 mg N/L (Henschke & Jiranek, 1993; Spayd *et al.*, 1993; Bell & Henschke, 2005). Lower YAN levels increase the risk of having slow, lagging or stuck alcoholic fermentations, and also the risk of producing hydrogen sulphide (Henschke & Jiranek, 1991). Even though 140 to 150 mg N/L is seen as the critical value for YAN, nitrogen levels practically should be increased to at least 200 mg N/L for a successful fermentation (Leonardelli, 2013). It has been suggested that the nitrogen requirement of yeast differs according to the sugar content of the juice: < 21°B (200 to 250 mg N/L), 21 to 23°B (250 to 300 mg N/L), 23 to 25°B (300 to 350 mg N/L), and > 25°B (350 to 400 mg N/L) (Wilton, 2015).

Ammonia nitrogen

Ammonia nitrogen (NH₃, or NH₄⁺ for the ionic form, ammonium) is an important component of YAN. As mentioned above, the ammonia nitrogen concentration declines over time as the grape ripens (Bell, 1994). Various researchers have reported the percentage of ammonia nitrogen of the total YAN content found in berries and juices from different cultivars (Bell, 1994; Spayd *et al.*, 1994; Conradie, 2001; Ribéreau-Gayon *et al.*, 2006; Petrovic *et al.*, 2019). Of the total YAN, ammonia nitrogen levels varied from 32

to 80% in the berries and from 9 to 40% in the juice (Huang & Ough, 1989). Ammonia nitrogen is readily assimilated by the yeast, and is the most preferred nitrogen source during alcoholic fermentation (Monteiro & Bisson, 1991; Henschke & Jiranek, 1993). At the end of alcoholic fermentation, the ammonia nitrogen levels are usually depleted and it is therefore important to know the levels in the grape must before alcoholic fermentation (Ribéreau-Gayon *et al.*, 2006).

The major source of ammonia nitrogen is the berry itself, but additions of DAP to deficient musts can influence the levels thereof and also the total YAN concentration (Henschke & Jiranek, 1991, 1993; Monteiro & Bisson, 1991). Henschke and Jiranek (1993) reported a range of 5 to 325 mg N/L ammonia nitrogen in grapes, while a recent study on South African grape musts has shown that ammonia nitrogen levels ranged from 0 to 165 mg N/L over three seasons (Petrovic *et al.*, 2019).

Free amino nitrogen

FAN includes free or primary amino acids, while secondary amino acids do not fall under this group. All amino acids contain the carboxyl (-COOH) and amino (-NH₂ or -NH-) functional groups (Ribéreau-Gayon *et al.*, 2006). From a structural point of view, the difference between primary and secondary amino acids is due to the level of substitution of the N in the amino group; in this case, -NH₂ is for primary and -NH- for secondary amino structures (Ribéreau-Gayon *et al.*, 2006).

Amino acids are the most prevalent form of total nitrogen in grape juice and wine. Amino nitrogen distribution in Riesling and Cabernet Sauvignon berries is 10 to 15% in the seeds, 19 to 29% in the skin, and 61 to 65% in the pulp (Stines *et al.*, 2000). The total FAN levels vary in grapes and grape juice depending on the year, and amino acids usually represent 30 to 40% of the total nitrogen in ripe grapes (Ribéreau-Gayon *et al.*, 2006) and 51 to 92% of juice YAN at harvest (Bell, 1994; Spayd *et al.*, 1994; Conradie, 2001).

Several factors can influence the amino acid composition and concentration levels in grapes, including the grape cultivar, rootstock, site, seasonal conditions, and viticultural management (Kliewer, 1968; Etievant *et al.*, 1988). Bell and Henschke (2005) compiled a list of all the amino acids found in whole grapes and/or juice at harvest. During the growth phase of alcoholic fermentation, yeast metabolises grape amino acids, while others are produced by the enzymatic degradation of proteins and some are excreted by live yeasts at the end of fermentation (Lehtonen, 1996). Under anaerobic conditions, amino acids are not metabolised by the yeast during alcoholic fermentation (Duteutre *et al.*, 1971; Ingledew *et al.*, 1987; Long *et al.*, 2012).

Nitrogen compounds, including amino acids, contribute to the formation of compounds like esters, higher alcohols, hydrogen sulphide (H₂S), monoterpenes and volatile thiols during the winemaking process (Henschke & Jiranek, 1993; Bell & Henschke, 2005). Various volatile compounds are formed from amino acids during alcoholic fermentation, therefore amino acids can be considered precursors of certain aroma compounds (Henschke & Jiranek, 1993; Bell & Henschke, 2005).

Amino acids can be divided into different groups based on their role or structure: yeast-preferred, branched, sulphur-containing, and other amino acids (Godard *et al.*, 2007; Ljungdahl & Daignan-Fornier, 2012). Yeast-preferred amino acids (aspartic acid (ASP), glutamic acid (GLU), asparagine (ASN), serine (SER), arginine (ARG), alanine (ALA) and glutamine (GLN)) are considered the most important because yeast metabolises them first (Monteiro & Bisson, 1991; Godard *et al.*, 2007). ARG and proline (PRO) are usually the most abundant amino acids in grapes, and ARG is preferred by the yeast as a nitrogen source (Garde-Cerdán & Ancín-Azpilicueta, 2008; Holzapfel *et al.*, 2015). The addition of DAP to juice inhibits the ARG utilisation, and ARG will only be used after ammonia nitrogen has been metabolised by the yeast. Keto acids, such as pyruvic and α -ketoglutaric acid, bind to sulphur dioxide (SO₂), react to phenols during winemaking and are formed during the Ehrlich pathway by the amino acids ALA and GLU (Ough *et al.*, 1990).

Branched amino acids (valine (VAL), leucine (LEU), phenylalanine (PHE) and isoleucine (ILE)) are precursors of volatile esters (Antalick *et al.*, 2014). Higher alcohols are formed during the Ehrlich pathway from these amino acids, but a greater proportion are synthesised from sugars (Bell & Henschke, 2005). The relationship between higher alcohols and amino acid assimilation during the fermentation cycle is not clear (Bell & Henschke, 2005). The acids, along with these alcohols, can form esters such as isoamyl acetate and phenylethyl acetate. These amino acids are accumulated in the early stages of alcoholic fermentation and do not support and contribute to the high growth rates during fermentation. The majority of esters are enzymatically synthesised by the yeast from alcohols and medium- and long-chain fatty acids through esterification reactions (Lambrechts & Pretorius, 2000).

Sulphur-containing amino acids are methionine (MET), cysteine (CYS) and cystine (Cys-Cys). They are involved in yeast metabolism under certain conditions and can result in H₂S production (Henschke & Jiranek, 1991; Giudici & Kunkee, 1994). When MET becomes depleted in the early stages of alcoholic fermentation, the sulphate reduction sequence (SRS) pathway is activated to reduce sulphate to H₂S and to release a surplus thereof alongside mercaptans from the cell (Bell & Henschke, 2005). DAP additions before or during alcoholic fermentation can inhibit the production of H₂S (Bell & Henschke, 2005). GSH is formed during alcoholic fermentation from glutamate, GLY and CYS (Castellarin *et al.*, 2012). Research done by Elskens *et al.* (1991) and Hallinan *et al.* (1999) suggests that GSH can be degraded to CYS and finally to H₂S under nitrogen-deficient conditions. Volatile thiols are found in small amounts in juice must, and their precursors, such as non-volatile, non-glycosylated, odourless S-CYS conjugates, have been identified (Tominaga *et al.*, 1998a, 1998b, 1998c). During alcoholic fermentation, the yeasts degrade the S-CYS thiol precursors to release the volatile thiols (Tominaga *et al.*, 1998a, 1998b, 1998c; Murat *et al.*, 2001a).

All other amino acids (aminobutyric acid (GABA), lysine (LYS), threonine (THR), glycine (GLY), tyrosine (TYR), tryptophan (TRP), histidine (HIS) and ornithine

(ORN); hydroproline (HYP) and PRO are secondary amino acids and are not included in the FAN value) are used by the yeast only in the case that other nitrogen sources are depleted first (Duteutre *et al.*, 1971). PRO is among the two dominant amino acids that make up the bulk of the total amino acids (Kliewer, 1968). PRO cannot be assimilated by yeast in the absence of oxygen; therefore, at the end of alcoholic fermentation, the PRO levels have not changed or decreased and high levels are still found in the resulting wine (Ribéreau-Gayon *et al.*, 2006).

Glutathione

GSH was first discovered by Cheynier *et al.* (1989) in red and white French grapes. It is a sulphur-containing tripeptide (γ - glutamyl- L- cysteinyl- GLY) and occurs as a natural antioxidant in grapes and must (Anderson, 1998). GSH is formed from the amino acids GLU, GLY and CYS, and contains a nucleophilic -SH centre (Anderson, 1998; Castellarin *et al.*, 2012). GSH is synthesised enzymatically in the grape berry, and the reduced GSH (GSH-R or simply GSH) form is the most abundant thiol-containing compound present at harvest. Šuklje *et al.* (2012) reported that, of the total GSH content in grapes, more than 90% is present in its reduced form.

During crushing, GSH needs to be present in high levels in the must to protect it fully (Singleton *et al.*, 1985). Du Toit *et al.* (2007) and Kritzinger (2012) report that GSH can be used as a marker of oxidation in winemaking due to its sensitivity to oxidation. GSH reacts with polyphenol *o*-quinones in juice and wine and consequently limits the effect of oxidation (Cheynier *et al.*, 1989; Makhokina *et al.*, 2014). Being reactive to quinones, GSH plays an important role in juice by protecting various volatile thiols from oxidation (Chone *et al.*, 2006), and also stopping the formation of unstable aromas in wine (Papadopoulou & Roussis, 2001).

The electrophilic -SH group of GSH-R is reactive, leading to the formation of 2- S- glutathionyl- caftaric acid or grape reaction product (GRP1) (Singleton *et al.*, 1985). The *o*- quinone of caffeoic acid reacts most readily with GSH-R, and phenols can form derivatives of GSH. The GRP is colourless and traps the *o*-quinone, preventing the must from browning and other reactions from taking place (Kritzinger *et al.*, 2013). The GRP can further be oxidised by laccase and yields an *o*-quinone, which can brown the polymers and also produce 2,5- di- sulphur- glutathionylcaftaric acid (GRP2) by the addition of another GSH (Singleton *et al.*, 1985; Cheynier *et al.*, 1989). GSH can also react with oxygen and undergo oxidation, resulting in the oxidised GSH form (GSH-O or GSSG).

The GSH concentration increases during grape ripening, and the highest levels occur at the start of véraison (Adams & Liyanage, 1993). Due to enzymatic and redox reactions, GSH levels decrease rapidly during crushing (Adams & Liyanage, 1993). Free-run juices during pressing are reported to have higher GSH concentrations compared to other higher press fractions (Patel *et al.*, 2010). Levels of GSH-R during alcoholic fermentation usually decrease, and this can be due to the metabolism of the yeast (Du Toit *et al.*, 2007). During wine ageing, GSH (unspecified GSH-R or GSH-O)

concentrations decrease (Lavigne *et al.*, 2007; Ugliano *et al.*, 2011). In South Africa, GSH-R concentrations are present in ranges from 1 to 71 mg/L in juice and up to 35 mg/L in wine (Du Toit *et al.*, 2007; Janeš *et al.*, 2010; Fracassetti *et al.*, 2011). Reported GHS-O levels found in South African Sauvignon blanc juices ranged from 0.46 to 2.93 mg/L (Du Toit *et al.*, 2007).

Volatile compounds

In addition to the grape-derived aroma compounds, the metabolism of sugar by the yeast during alcoholic fermentation leads to the formation of volatile compounds, such as esters, fatty acids and higher alcohols (Francis & Newton, 2005). These compounds arise as primary metabolites of yeast and sugar and the metabolism of amino acids (Henschke & Jiranek, 1993; Swiegers *et al.*, 2005). The nitrogen status of the must also contributes to the formation of these compounds (Henschke & Jiranek, 1993), but a too-high nitrogen content can reduce the production thereof. The odour thresholds for major volatiles are measured in mg/L, while methoxypyrazines and volatile thiols are much lower and measured in ng/L in juice and wine.

Major volatile compounds

Esters

Esters are an important group of volatile compounds and contribute to the pleasant 'fruity' and 'floral' aromas in wines (Swiegers *et al.*, 2005). Esters can be grouped into two groups, namely acetate esters and ethyl esters. The most significant esters are ethyl ethanoate (ethyl acetate), 3-methylbutyl acetate (isoamyl acetate), 2-methylpropyl ethanoate (isobutyl acetate), ethyl hexanoate (ethyl caproate) and 2-phenylethyl acetate (phenethyl acetate) (Thurston *et al.*, 1981). Ethyl esters contribute 'apple' aromas, while acetate esters are associated with 'fruity' aromas (Saerens *et al.*, 2008). Yeast plays a crucial role in the formation of esters. Esters are produced by the metabolism of yeast through lipid and acetyl-CoA metabolism, through the action of alcohol acetyl transferase, an alcohol and a coenzyme A-activated acid condensate (Lambrechts & Pretorius, 2000).

Various factors, such as yeast strain, clarification, and temperature during the winemaking process can influence the levels of esters present in wines. During ageing, ester concentrations can decrease due to hydrolysis or oxidation, and the resulting wines can experience a loss of 'fruity' aromas (Marais, 1978). During bottle maturation of white wine, loss of 'fruitiness' can be linked to the loss of acetate esters, and they tend to diminish more rapidly than ethyl esters (Ramey & Ough, 1980). Ethyl acetate concentrations are much higher compared to those of other esters found in wine.

Fatty acids

Fatty acids contribute to the wine aroma and have an important impact on wine quality (Bell & Henschke, 2005). The most abundant fatty acids in wine include acetic, decanoic, hexanoic, octanoic and decanoic acid. Volatile fatty acid composition ranges from 500 to 1 000 mg/L, and acetic acid, ranging from 0.2 to 2 g/L, accounts for more than 90% of the fatty acids. Generally, red wines have higher

concentrations of these acids than white wine (Lambrechts & Pretorius, 2000). Low concentrations can contribute positively to the complexity and aroma of wines (Coetze, 2011), while unwanted flavours, such as 'cheesy', 'vinegar' and 'rancid', are due to too high concentrations of fatty acids (Lambrechts & Pretorius, 2000). Bacterial spoilage can be linked to elevated levels of acetic acid (Ribéreau-Gayon *et al.*, 2006).

During the early stages of alcoholic fermentation and the biosynthesis of long-chain fatty acids, medium-chain fatty acids like hexanoic, octanoic and decanoic acid are produced as intermediates. Acetic acid is formed as a metabolic intermediate in the synthesis of acetyl-CoA from pyruvic acid, or is formed directly from acetaldehyde by aldehyde dehydrogenases (Bell & Henschke, 2005). Long-chain unsaturated fatty acids, such as oleic and linoleic acid, can enhance alcoholic fermentation, but are not yeast-derived products and originate from the waxy cuticle of grape skins (Lambrechts & Pretorius, 2000). Among the many factors that can influence the fatty acid levels are yeast strain, sugar concentration, inoculation rate, juice clarification, fermentation temperature, nitrogen, oxygen exposure and SO_2 additions (Henschke & Jiranek, 1993; Garde-Cerdán *et al.*, 2009; Coetze, 2011). The nitrogen concentration levels of the must play a crucial role in the volatile acidity in wine.

Contradictory results, related to increases, decreases or stability of certain fatty acids, have been reported during ageing (Roussis *et al.*, 2005; Blake *et al.*, 2009; Lee & Steenwerth, 2011).

Higher alcohols

Higher alcohols, also known as fusel alcohols, are secondary yeast metabolites and can positively or negatively influence the aroma of the wine (Bell & Henschke, 2005). Higher alcohols have more than two carbon atoms in their structure and can be grouped into two categories: aliphatic and aromatic alcohols (Lambrechts & Pretorius, 2000). Aliphatic alcohols include pentan-1-ol (amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), 2-methylpropan-1-ol (isobutanol) and propan-1-ol (propanol), while aromatic alcohols include 2-phenylethanol (phenylethyl alcohol) and 4-(2-hydroxyethyl) phenol (tyrosol).

Higher alcohols are formed in two ways during alcoholic fermentation. Firstly, by being synthesised anabolically from intermediates of the sugar metabolism (e.g. glucose), and secondly by being synthesised catabolically from branched amino acids, such as LEU, ILE, THR and VAL (Boulton *et al.*, 1996; Dickinson *et al.*, 2003). Alcohols are formed via the Ehrlich pathway, and the branched amino acids are deaminated and α -keto-acids are decarboxylated and reduced to the corresponding alcohol (Bell *et al.*, 1979).

Must containing high levels of amino acids will produce higher levels of higher alcohols (Swiegers *et al.*, 2005). Concentrations below 300 mg/L add positively to the complexity of wines, whereas more than 400 mg/L can have a detrimental effect and display unpleasant 'fusel' and 'solvent-like' aromas, with the exception of 2-phenyl ethanol ('rose' and 'floral' aromas) (Lambrechts & Pretorius, 2000). Isoamyl alcohol is usually found in wines, with the

highest levels ranging from 45 to 490 mg/L at the end of alcoholic fermentation (Lambrechts & Pretorius, 2000). During ageing, alcohols can be oxidised to form aldehyde, causing the concentration levels to decrease (Marais & Pool, 1980).

Methoxypyrazines

Methoxypyrazines are nitrogen-containing compounds that are derived from grapes and are situated in the skin and exocarp of grape berries (Marais, 1994). Methoxypyrazines are formed by the catabolism of secondary amino acids, such as VAL, GLY and MET present in the grape (Cheng *et al.*, 1991). Three methoxypyrazine compounds have been identified in Sauvignon blanc, namely 2-isobutyl-3-methoxypyrazine (IBMP), 2-sec-butyl-3-methoxypyrazine (SBMP), and 2-isopropyl-3-methoxypyrazine (IPMP) (Lacey *et al.*, 1991; Marais, 1998).

The accumulation of methoxypyrazines in the berries can be influenced by various factors, such as environmental parameters, clone, canopy management, soil and terroir (Swiegers *et al.*, 2006). During berry ripening, methoxypyrazine levels increase and the highest levels are obtained at véraison, but the levels also start to decrease during véraison (Lacey *et al.*, 1991; Swiegers *et al.*, 2006). Lacey *et al.* (1988) reported that higher climatic temperatures will lead to lower methoxypyrazine concentrations due to their degradation during ripening and their sensitivity to sunlight, while higher concentrations are obtained in cooler climates. Various research studies link vines with excessive growth to the production of grapes with higher methoxypyrazine concentrations before harvest, irrespective of cluster light exposure. The majority (95.5%) of methoxypyrazines are located in the skin of grapes at harvest time (Roujou de Boubee *et al.*, 2000). The highest levels of IBMP are found in the free-run juice, and increased levels are also found after skin contact (Marais, 1998; Marais *et al.*, 1999; Roujou de Boubee *et al.*, 2000). Methoxypyrazine concentrations can decrease due to clarification processes, while the levels are reported to be stable during alcoholic fermentation (Sala *et al.*, 2004; Kotserides *et al.*, 2008). It can be concluded that viticultural practices can influence the methoxypyrazine concentrations much more than winemaking practices (Roujou de Boubee *et al.*, 2000).

Methoxypyrazine sensory detection thresholds in water are very low: at 2 ng/L for IBMP, 1 ng/L for SBMP and 2 ng/L for IPMP (Lacey *et al.*, 1991; Alberts *et al.*, 2009). Methoxypyrazines are known to contribute to the 'green' aromas present in Sauvignon blanc wines. IBMP contributes to the 'herbaceous' and 'green pepper' aromas, while SBMP contributes to the 'asparagus' and 'green beans' aromas, and IPMP is associated with 'pea' and 'bell pepper' aromas (Ebeler & Thorngate, 2009). Wines with high methoxypyrazine levels can be perceived negatively if the aroma notes are not in balance with other compounds. IBMP is the methoxypyrazine that is found with the highest concentration in Sauvignon blanc musts and wines, while IPMP and SBMP are found at much lower concentrations (Lacey *et al.*, 1991). Van Wyngaard (2013) performed a survey of South African Sauvignon blanc wines and found that the levels of IBMP ranged from 0.4 to 44 ng/L.

Volatile thiols

Volatile thiols are sulphur-containing compounds that are present in wine at very low concentrations and the aromas they produce are powerful. Volatile thiols are generally known to contribute to the positive tropical aroma characteristics of Sauvignon blanc wines, such as 'citrus', 'gooseberry', 'grapefruit', and 'passion fruit' (Tominaga *et al.*, 1998c, 2000). These compounds are sulphur-containing substances with additional functional groups, such as alcohol, ester or ketone. Darriet *et al.* (1995) identified the first volatile thiol, 4-mercaptop-4-methyl-pentan-2-one (4MMP) in Sauvignon blanc wines in 1995. Since then, various researchers have focused on these compounds and Tominaga *et al.* (1996, 1998a) identified four other volatile thiols in Sauvignon blanc. 3MH and 3MHA were only recently identified in South African Chenin blanc wines (Wilson, 2017; Coetzee *et al.*, 2018). The odour thresholds of these volatile thiols in model wine solution are 0.8 ng/L for 4MMP, 60 ng/L for 3MH and 4 ng/L for 3MHA (Tominaga *et al.*, 1998b).

Three pathways that can lead to the formation of 4MMP and 3MH have been proposed (Roland *et al.*, 2011). One pathway leading to the formation of 3MH includes the *trans*-2-hexenal and *trans*-2-hexenol alongside H₂S, which acts as a sulphur donor. 4MMP and 3MH share the other two pathways and include cysteinylated and glutathionylated precursors. 4MMP and 3MH are synthesised in the berry and their precursors are in a cysteinylated bound form.

Tominaga *et al.* (1998c) identified the precursors of volatile thiols as odourless, non-volatile, non-glycosylated sulphur-CYS conjugates. These conjugates are cleaved by the yeast via its beta-lyase activity during alcoholic fermentation, and the non-volatile thiols S-3-(hexan-1-ol)-cysteine (CYS-3MH) and S-3-(4-mercaptop-4-methylpentan-2-one)-cysteine (CYS-4MMP) become active compounds (Roland *et al.*, 2010). The last pathway includes the glutathionylated precursors, such as S-3-(hexan-1-ol)-glutathione (GSH-3MH), which are released during alcoholic fermentation. 3MH precursors are located mainly in the skins, while 4MMP precursors are mostly found in the skin and pulp (Roland *et al.*, 2011).

Only trace amounts of 4MMP and 3MH are found in grapes and musts (Dubourdieu *et al.*, 2006). During alcoholic fermentation, these volatile thiols are released from their odourless non-volatile precursors. 3MHA is produced from the acetylation of 3MH during alcoholic fermentation by the yeast ester and forms alcohol acetyltransferase (Roland *et al.*, 2011). Various factors, such as terroir and winemaking processes prior to alcoholic fermentation, can influence the levels of precursors present in the grapes (Murat *et al.*, 2001b; Dubourdieu *et al.*, 2006). Volatile thiols are susceptible to oxidation during ageing, and a decrease in levels can be expected (Coetzee, 2014). During wine ageing, 3MHA is usually converted to 3MH via acid hydrolysis (Nikolantonaki *et al.*, 2010; Herbst-Johnstone *et al.*, 2011) or by the breakdown of 3MH disulphide present in the wines (Capone *et al.*, 2010; Sarrazin *et al.*, 2010).

FERTILISATION EFFECTS: FROM THE VINEYARD TO THE FINISHED WINE

Grapevines have the potential to grow successfully

and produce quality grapes when grown in favourable environmental conditions. The nutrition levels of a grapevine can influence the grape's composition and eventually the wine's composition and quality (Bell & Henschke, 2005). A grapevine's nutrition can be affected by various factors, such as the canopy shading (Perez-Harvey & Witting, 2001), canopy temperature (Ewart & Kliewer, 1977), cultivar (Christensen, 1984; Huang & Ough, 1989), rootstock (Christensen, 1984; Huang & Ough, 1989), season (Bell & Robson, 1999), site (Huang & Ough, 1989), soil management (Bell *et al.*, 1979), training system (Kliewer *et al.*, 1991), and the timing, rate and form of application of fertiliser (Bell *et al.*, 1979; Peacock *et al.*, 1991; Spayd *et al.*, 1994; Christensen & Peacock, 2000; Conradie, 2001; Dufourcq *et al.*, 2007; Lacoux *et al.*, 2008; Jreij *et al.*, 2009).

The nutrient requirements of a grapevine depend on its age, cultivar, yield, soil type and soil properties (Holzapfel & Treeby, 2007). Nutrition deficiencies often occur for various reasons and can be rectified by carrying out fertiliser applications.

Nitrogen supplementation is the most commonly performed soil fertilisation, as it is a primary constituent and is required by the grapevine for growth and reproduction. How much nitrogen to apply depends on the production and quality of the crops, but also on numerous research trials and years of grower experience (Christensen & Peacock, 2000). Drip irrigation is the most common way of nitrogen application, and the use of liquid materials such as aqua ammonia, urea and ammonium nitrate solution has increased, while the use of anhydrous ammonia has declined due to the high cost (Christensen & Peacock, 2000). The application rates of drip irrigation depend on vineyard conditions and this is usually applied in the spring. Research studies have reported the following foliar rates to be applied to vines: 11 to 28 kg/ha N applied to vines of medium vigour and 33 to 44 kg/ha N to below-average vines (Christensen & Peacock, 2000). Organic sources of nitrogen include farm manure, grape pomace and compost, while cover crops can also be used to add nitrogen to the soil (Christensen & Peacock, 2000).

Over the past few years, foliar fertilisation has been used widely where deficiencies or imbalances cannot be rectified by soil fertiliser applications. Foliar fertilisation is also applied as a method to improve a crop's quality and yield (Christensen & Peacock, 2000). Foliar fertiliser applications show advantages such as low cost, lack of soil fixation, being independent of root uptake, using small quantities of fertiliser, giving rise to increased quality and yield of crops, and to quick plant uptake, response and assimilation (Oosterhuis, 2009; Lasa *et al.*, 2012). Foliar applications are preferred over soil applications when the topsoil is dry, the soil has low available nutrients, or with decreased root activity. Foliar applications are applied mostly in the case when small fertilisation corrections are to be made. The limitations of foliar fertilisation applications include leaf burn, leaf necrosis, low penetration rate, solubility problems, being washed off by rain, limited amounts can be applied at a time, and correct weather conditions are required (Watson *et al.*, 2000). Many foliar fertilisers are soluble in water and can be applied directly to the leaves of a grapevine.

Foliar fertilisation chemicals can traverse from the leaf to the stomata via two pathways, namely an aqueous pathway and a lipoidal route (Oosterhuis, 2009). The uptake of the nutrients depends on the element's inorganic form, whether it is combined in an organic form, its ionic concentration, or the environmental conditions, which influence the time the nutrients remain in solution on the leaf (Oosterhuis, 2009). Water deficit increases the wax of the cuticle and reduces the absorption of the foliar-applied nutrient (Oosterhuis, 2009), and foliar fertilisation should therefore not be applied to grapevines experiencing a drought.

Nitrogen foliar fertilisation can be applied using urea (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Wolf, 2012; Garde-Cerdán *et al.*, 2015; Juhasz, 2015; Verdenal *et al.*, 2015a; Garde-Cerdán *et al.*, 2016; Geffroy *et al.*, 2016a; Hannam *et al.*, 2016; Verdenal *et al.*, 2016; Dienes-Nagy *et al.*, 2017) or amino acids (Garde-Cerdan *et al.*, 2015). Application levels for these studies ranged from 2 to 36 kg N/ha. Sulphur foliar applications (5 and 10 kg S/ha) used elemental soluble sulphur (Dufourcq *et al.*, 2009; Juhasz, 2015; Geffroy *et al.*, 2016a) or micronised sulphur (Lacroux *et al.*, 2008). Elemental sulphur is a slow-release substrate and is widely and frequently applied in the industry at various stages during the growing season of vines. Sulphur has a direct fungicidal effect and fungistatic activity on plants by increasing the plant's resistance to powdery mildew (*Uncinula* spp.), downy mildew and botrytis (*Botrytis cinerea*) (Omnia, 2015). Sulphur can also be sprayed to control mites, which can have a detrimental effect on the growth of buds and leaves during the growing season. In South Africa, the minimum sulphur content or requirement for a plant is 900 g S/kg elemental sulphur per year (Department of Agriculture, Forestry and Fisheries, 2018). A 30-day withholding period of sulphur applications is required, with 10-day intervals between applications, otherwise residual sulphur can influence the chemical composition of the grapes and can give rise to negative associated aromas from compounds such as H₂S. Studies with foliar applications have not focused on applying sulphur separately to vines (Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009). A synergy between nitrogen and sulphur results in interactions between the pathways (Téa *et al.*, 2003; Lacroux *et al.*, 2008). The timing of foliar sprays is critical, and nitrogen and sulphur can be applied at different stages, such as during véraison (Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Garde-Cerdán *et al.*, 2015; Juhasz, 2015; Verdenal *et al.*, 2015a; Garde-Cerdán *et al.*, 2016; Geffroy *et al.*, 2016a; Hannam *et al.*, 2016; Verdenal *et al.*, 2016; Dienes-Nagy *et al.*, 2017), before véraison (Lacroux *et al.*, 2008) or at the flowering stage (Wolf, 2012; Verdenal *et al.*, 2015a).

Foliar fertilisation studies on nitrogen and sulphur applications cover a wide range of red and white cultivars and were performed in various places, such as Canada, Chile, France, Spain, South Africa, Switzerland and Virginia, USA. White cultivars included Chardonnay (Dienes-Nagy *et al.*, 2017), Chasselas (Verdenal *et al.*, 2015a, 2015b, 2016; Dienes-Nagy *et al.*, 2017; Koestel *et al.*, 2017), Colombard (Dufourcq *et al.*, 2005, 2009; Geffroy *et al.*, 2016a, 2016b), Gewürztraminer (Dienes-Nagy *et al.*, 2017), Gros Manseng (Dufourcq *et al.*, 2007, 2009; Geffroy *et al.*, 2016a, 2016b),

Melon (Dufourcq *et al.*; Geffroy *et al.*, 2016a, 2016b), Négrette (Dufourcq *et al.*, 2005, 2009; Geffroy *et al.*, 2016a, 2016b), Sauvignon blanc (Dufourcq *et al.*, 2011; Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Jreij *et al.*, 2009; Geffroy *et al.*, 2010; Lasa *et al.*, 2012; Wolf, 2012; Helwi *et al.*, 2014; Juhasz, 2015; Geffroy *et al.*, 2016a, 2016b; Dienes-Nagy *et al.*, 2017; Helwi *et al.*, 2017) and Viognier (Hannam *et al.*, 2014). Red cultivars such as Cabernet Sauvignon (Hannam *et al.*, 2014; Gutiérrez-Gamboa *et al.*, 2017a), Carignan (Geffroy *et al.*, 2012, 2016a, 2016b), Duras (Dufourcq *et al.*, 2005), Fer Servadou (Geffroy *et al.*, 2012, 2016a, 2016b), Malbec (Dufourcq *et al.*, 2005), Merlot (Lasa *et al.*, 2012; Wolf, 2012; Hannam *et al.*, 2014, 2016), Petit Manseng (Wolf, 2012; Kelly, 2013; Kelly *et al.*, 2017), Pinot Gris (Hannam *et al.*, 2014, 2016), Pinot Noir (Hannam *et al.*, 2014) and Tempranillo (Garde-Cerdán *et al.*, 2015) were included. Only soil nitrogen fertilisation studies have been performed on Chenin blanc (Conradie, 1981; Conradie & Saayman, 1989). Foliar nitrogen fertilisation applications do not only influence the concentrations and composition of nitrogen present in the grape berry, but also indirectly influence the nitrogen levels in the must, alcoholic fermentation, and the resulting wine's composition and aroma (Petering *et al.*, 1991; Bell & Henschke, 2005).

Vineyard

A grapevine's response to fertiliser applications depends on various factors, such as the genetic makeup of the cultivar, canopy management and the terroir. The chemical analysis of vine leaves, sap and soil can indicate the nitrogen content of a grapevine and can affect how the vine reacts to an application of foliar nitrogen (Bell *et al.*, 1979; Kliewer *et al.*, 1991; Spayd *et al.*, 1994; Bell & Robson, 1999; Conradie, 2001; Lacroux *et al.*, 2008; Kelly, 2013; Hannam *et al.*, 2014; Kelly *et al.*, 2017). The nitrogen requirement for every wine cultivar is different; for instance, the shoots, leaves and fruit of Chenin blanc accumulated up to 27 kg N/ha after soil application, and up to 60% of nitrogen reserves originate from nitrogen absorbed post-harvest (Conradie, 1986). Nitrogen-deficient grapevines (low YAN) that received nitrogen resulted in increased vine nitrogen status, which led to nitrogen metabolism and carbohydrate accumulation (Bell & Henschke, 2005).

Increased levels of nitrogen in the roots, canes and trunk increase the grapevine's ability to store nitrogen and carbohydrates (Kliewer *et al.*, 1991; Bell & Robson, 1999). Therefore, with increased growth of the roots, the uptake of nutrients, water and nitrogen is increased (Kliewer & Cook, 1971). By applying small additions of nitrogen to a vineyard with adequate nitrogen levels, no increases in the growth and yield will happen (Kliewer *et al.*, 1991). High vine nitrogen status may interrupt the grapevines' natural balance, causing them to become excessively vegetative and maintain vegetative growth at the expense of yield (Kliewer & Cook, 1971). It is very important that the source and sink ratio are balanced in vines, otherwise competition can occur between the sinks for carbohydrates, and grapes can have reduced composition and quality (Ewart & Kliewer, 1977). When a canopy changes due to increased growth, the microclimate in the canopy, including factors such as light, temperature,

radiation, wind and humidity, are altered.

Only a few foliar fertilisation studies have focused on studying the effect of the applications on the grapevine. Most research studies on fertilisation have made use of urea, an inorganic form of nitrogen, because of the easy control of application. Factors such as the canopy nitrogen status, interior leaves, clusters, leaf layers, leaf area, pruning mass, bunch weight, yield and bunches per vine in the vineyard during ripening were studied. Kelly *et al.* (2017) reported that the petioles had relatively low nitrogen levels prior to fertilisation and found no differences in the number of interior leaves or clusters among fertilised treatments (15 kg/ha urea and 15 kg/ha urea with 5 kg/ha micronised sulphur (Microthiol Disperss), applied twice before véraison), but the control had deficient YAN levels and had a greater percentage of leaf layers compared to the vines with fertiliser applications.

Another study, in which 15 kg/ha urea were applied thrice to Petit Manseng, showed no impact on the primary or secondary leaf area; however, the pruning mass per vine increased significantly compared to the control (Helwi *et al.*, 2014). An alternative study, using foliar urea applications at 1% and 2% w/v rates applied on Merlot two weeks before véraison, at véraison and two weeks after véraison, resulted in no difference in the grapevine nitrogen status, but an increase in canopy density occurred in the second year of the experiment (Hannam *et al.*, 2014). Lacroux *et al.* (2008) reported that the plots in their study were nitrogen deficient (low initial N-tester values) before performing nitrogen and nitrogen with sulphur foliar applications twice before véraison on Sauvignon blanc vines. Higher levels compared to the initial nitrogen levels were observed with the N-tester (leaf blade nitrogen content) analysis for both foliar applications, as well as a 60% increase in YAN levels (Lacroux *et al.*, 2008).

The total yield has been found to increase upon the application of nitrogen to low nitrogen-containing vines (Bell *et al.*, 1979; Kliewer *et al.*, 1991; Spayd *et al.*, 1994; Bell & Robson, 1999; Conradie, 2001; Hannam *et al.*, 2014), while other studies have found no increases in the yield (Lacroux *et al.*, 2008; Kelly, 2013; Kelly *et al.*, 2017). This can be due to the initial nitrogen levels present in the vines prior to foliar fertiliser applications. Lacroux *et al.* (2008) also found no significant differences in bunch weight, yield, bunches per vine and pruning weight, and this can be due to the late application of nitrogen in the season. A study using a 2% w/v (28 to 36 kg N/ha/year) urea application found an increased yield of Pinot Gris, an increased number of clusters/vine for Merlot (plot 2) and Viognier, while another Merlot plot and more Pinot Gris vineyards had an increased number of clusters/vine in the following season (Hannam *et al.*, 2014). The variable yields obtained can be due to the optimal or suboptimal timing of applications, fertiliser materials, dosage, and climatic and environmental conditions (Oosterhuis, 2009).

Grapes

During the ripening period of the grape berry, the composition, colour, flavour, size, texture and susceptibility to pathogens change (Gomes *et al.*, 2013). Tartaric acid

concentrations increase during the first stages of ripening and usually remain constant after véraison. Compounds such as amino acids, micronutrients and volatile aroma compounds accumulate during the first phase of berry growth, affecting the composition of the grape berry and eventually the composition of the resulting wine (Gomes *et al.*, 2013). During véraison, the grape berry undergoes dramatic changes and transforms from small, acidic and hard berries with very little sugar to softer, sweeter, larger, less acidic, and strongly flavoured and coloured berries. Aromatic compounds such as methoxypyrazines, which are produced during the first stage, decline during the ripening of the grape berries (Gomes *et al.*, 2013). This can be due to the increase in sunlight and temperature levels in the cluster, and leaf-removal practices (Šuklje *et al.*, 2016).

During ripening, the grape berries rely on carbohydrates produced from photosynthesis for their development and growth. Most of the accumulation of fructose and glucose occurs after véraison. The physiological ripeness of grape berries is reached when the grapes achieve sufficient sugar levels without losing too much acidity. The phenolic and aromatic content of the berries should also be taken into consideration (Gomes *et al.*, 2013). For a winemaker, optimal grape maturity is essential for successful alcoholic fermentation and for producing quality wines. Generally, canopies with low light produce grapes with reduced concentrations of soluble solids, total phenols and anthocyanins, while the levels of pH, TA, methoxypyrazines and potassium in the grapes increases (Kliewer, 1968; Ewart & Kliewer, 1977; Šuklje *et al.*, 2016).

Foliar fertilisation research studies have analysed °B, TA, pH, berry composition, total nitrogen content, berry volume, YAN, amino acids, ammonium, IBMP, and susceptibility to pathogens in the grapes during grape berry ripening and at harvest (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Mundy *et al.*, 2009; Hannam *et al.*, 2014; Dienes-Nagy *et al.*, 2017; Helwi *et al.*, 2017; Kelly *et al.*, 2017). No foliar fertilisation studies focused on all the methoxypyrazine compounds, except on IBMP, or on the composition of separate amino acids during ripening or at harvest.

Both 1% (14 to 18 kg N/ha/year) and 2% w/v (28 to 36 kg N/ha/year) foliar urea applications increased the soluble solids at harvest for Merlot and Pinot Gris in 2010, but the 2% urea treatment resulted in a reduction in soluble solids in Merlot in the two following seasons, *viz.* 2011 and 2012 (Hannam *et al.*, 2014). The 2% urea treatment caused increases in the pH levels in Merlot and Pinot Gris in 2010, but only in Pinot Gris in 2012. For all three vintages, TA levels were reduced in the grape juice of Pinot Gris with the 2% urea foliar application (Hannam *et al.*, 2014). Similarly, Kelly *et al.* (2017) also found increased pH levels with foliar urea (15 kg N/ha twice before véraison) and urea with micronised sulphur (15 kg N/ha with 5 kg S/ha twice before véraison) treatments in 2011 compared to the control, while no difference was observed in the °B and TA levels (Kelly *et al.*, 2017). Lacroux *et al.* (2008) found no differences in the grape composition at the harvest date for the oenological parameters of Sauvignon blanc.

During ripening, the total nitrogen content of the grapes increases, but the levels can plateau after an initial increase

and, in some cases, even decline towards the end of ripening (Kliewer & Cook, 1971; Bell, 1994). Jreij *et al.* (2009) showed that a soil application of 60 kg N/ha ammonium nitrate (one week after bud break) with a 5 kg N/ha foliar application (at véraison) resulted in an 11% increase in berry volume compared to the control and the 30/2.6 kg N/ha treatment. The 60/5 kg N/ha treatment also showed an 11% increase in total berry nitrogen at the maturity stage compared to the control and other treatment (Jreij *et al.*, 2009).

Hannam *et al.* (2014) reported that foliar applications of 2% urea solution (28 to 36 kg N/ha/year) increased the YAN levels of grape juice at harvest in almost all the vineyards in all three vintages of their study. These YAN levels were more than double compared to the control (Hannam *et al.*, 2014). YAN levels were increased by 60% with applications of foliar nitrogen (10 kg N/ha urea, two applications prior to véraison) and foliar nitrogen (10 kg N/ha urea twice before véraison) with sulphur (5 kg S/ha micronised sulphur twice before véraison) compared to the control (Lacroux *et al.*, 2008). Dienes-Nagy *et al.* (2017) reported that YAN levels increased proportionally with foliar urea applications compared to the control over three vintages. This shows that vines are able to redistribute the nitrogen that is absorbed from the leaves to the grape berry.

The total amino acid concentration has been found to increase from véraison to harvest (Kliewer, 1968; Bell, 1994), while in other cases the concentration stabilised and declined after véraison (Kliewer, 1968). During grape berry ripening, the ammonium concentration of the grape berries declines (Bell, 1994). Foliar urea applied to white cultivars resulted in an increase in primary and secondary amino acids, but a decrease in ammonium nitrogen levels was observed during grape berry ripening compared to the control (Dienes-Nagy *et al.*, 2017). Bell (1994) reported that PRO accumulation increases during grape berry ripening, while some researchers have reported PRO levels to stabilise and slowly decline until harvest (Bell & Henschke, 2005). PRO generally accumulates due to abiotic stresses such as salt stress, cold stress, water stress and heat stress (Kavi Kishor & Sreenivasulu, 2014). ARG increased from véraison and reached a maximum at and started to decline during ripening (Kliewer, 1968; Bell, 1994), while other studies found that ARG continued to increase until harvest.

The susceptibility of grapes to the fungal pathogen *Botrytis cinerea* can be enhanced by a high content of foliar urea applications, although Lacroux *et al.* (2008) found contradictory results that showed no increase in the incidence of *Botrytis*. A strong positive correlation between sugar concentration and susceptibility to *Botrytis cinerea* in intact grape berries of Sauvignon blanc has been found, and the wounding of unripe berries with low sugar can also increase the susceptibility to the fungal pathogen (Mundy *et al.*, 2009).

Grape must to wine

After grapes are harvested, some grape compounds can be subject to degradation due to biological, chemical or physical processes. These processes can influence the composition and quality of the grapes and also of the resulting wine. The nitrogen composition of grape berries at harvest, including

the total nitrogen content, primary and secondary amino acids, ammonium and thiol precursors, can be influenced positively by nitrogen fertilisation (Conradie, 2001; Chone *et al.*, 2006). Mechanical machine harvesting can activate a variety of processes, such as oxidation, thereby releasing the precursors of volatile thiols or microbial metabolism. Harvested white grapes usually undergo crushing and destemming, and SO₂ is added to prevent oxidation. Thereafter, the grape undergoes a period of skin contact, after which it is pressed. During alcoholic fermentation, the grape must is transformed into wine by the yeast. In other words, the high sugar concentration of glucose and fructose is converted by various metabolic steps, leading to the production of CO₂ and ethanol. The yeast growth depends on the available nutrients, such as nitrogen provided by the grapes (Henschke & Jiranek, 1993). When the must is deficient in nitrogen content (< 140 mg N/L, low YAN), the winemaker can adjust the nitrogen levels by adding a source of nitrogen, usually DAP. During alcoholic fermentation, a large number of aroma compounds such as volatile thiols and major volatiles are released from their precursors (Lambrechts & Pretorius, 2000; Swiegers *et al.*, 2005) and contribute to the 'fermentation bouquet' (Bell & Henschke, 2005). The maturation of wine in the bottle or in an oak barrel can influence the chemical compound makeup and aromatic expression (Botha, 2015).

Research studies on foliar and soil fertilisation have focused on measuring °B, alcohol, CYS-3MH, FAN, GSH, GSH-3MH, malic acid, pH, TA, tartaric acid, YAN, amino acids, esters, methoxypyrazines, higher alcohols and volatile thiols in the must, juice, fermentation juice and/or resulting wines (Conradie, 2001; Chone *et al.*, 2006; Lacroux *et al.*, 2008; Dufourcq *et al.*, 2009; Lasa *et al.*, 2012; Helwi *et al.*, 2014; Geffroy *et al.*, 2016a; Dienes-Nagy *et al.*, 2017; Gutiérrez-Gamboa *et al.*, 2017b).

Oenological parameters, such as °B, alcohol, pH, TA, tartaric acid and malic acid, have been measured and no differences were found in the composition of the grape juice and must between the treatments (Lacroux *et al.*, 2008). Lacroux *et al.* (2008) reported that, with Sauvignon blanc, the soil ammonium nitrate application (30 kg N/ha) resulted in higher alcohol levels in the wines compared to the control and foliar fertilisation applications. Foliar applications did not significantly affect the oenological parameters of Tempranillo and Monastrell grape juice (Garde-Cerdan *et al.*, 2015), but late foliar applications of urea (15 days after véraison) increased the acidity of wines made with Sauvignon blanc and Merlot (Lasa *et al.*, 2012).

GSH in musts and wine plays a crucial role by protecting varietal volatile thiols from oxidation, and therefore protects the aroma production of Sauvignon blanc wines (Lavigne *et al.*, 2007). The GSH levels in wines were higher for applications of foliar urea (10 kg N/ha twice before véraison), and foliar urea (10 kg N/ha twice before véraison) with sulphur (5 kg S/ha twice before véraison), compared to the control (nitrogen deficient) and soil fertilisation (Lacroux *et al.*, 2008). The urea with sulphur application did not increase the GSH levels compared to the urea treatment (Lacroux *et al.*, 2008). Foliar urea and foliar urea with sulphur applied at véraison and two weeks later did not show

significant effects on the GSH levels in the musts compared to the control (Gutiérrez-Gamboa *et al.*, 2017a). Soil nitrogen fertilisation of 60 kg N/ha at berry set to Sauvignon blanc showed a 670% increase in GSH compared to the control (Chone *et al.*, 2006).

Helwi *et al.* (2014) applied soil fertilisation (50 kg N/ha, two applications), soil (100 kg N/ha, two applications) and foliar fertilisation of urea (15 kg N/ha, three applications) to Sauvignon blanc in two locations. The glutathionylated precursor of 3MH (PGSH-3MH) increased during ripening for all the treatments, and with soil and foliar treatments the levels were higher compared to the control. The results were highly correlated with the nitrogen status of the grapes. The cysteinylated precursor of 3MH (PCYS-3MH) was stable in the berries from one of the Sauvignon blanc plots during berry development, but increased with nitrogen foliar application at mid-maturity and decreased thereafter (during maturity) for all the treatments. Conversely, on the other Sauvignon blanc plot, the PCYS-3MH concentration increased during ripening and was higher in the soil and foliar nitrogen applications compared to the control (Helwi *et al.*, 2014).

The nitrogen content of the must was increased by 50% and 100% respectively with 10 and 20 kg N/ha foliar applications to white cultivars (Sauvignon blanc) (Geffroy *et al.*, 2016a). Dufourcq *et al.* (2009) reported significant increases in berry YAN with urea foliar fertilisation at véraison. Nitrogen soil applications resulted in a 10% decrease in amino acids in relation to YAN (Spayd *et al.*, 1994). Foliar urea treatments on white cultivars, such as Chasselas, Sauvignon blanc and Chardonnay, showed a correlation between YAN and PRO levels in the wines (Dienes-Nagy *et al.*, 2017). The amino acid content of a vine can be influenced and is dependent on various factors, such as the cultivar and rootstock genetics, climatic conditions, terroir, nitrogen content, soil, and canopy management practices (Conradie, 2001; Bell & Henschke, 2005; Lee & Schreiner, 2010). Increases of 44% and 75% in ARG and ALA respectively were obtained from urea with sulphur applications at véraison and two weeks later (Gutiérrez-Gamboa *et al.*, 2017a). This study also showed that, by comparing applications of foliar urea to foliar urea with sulphur, the latter application resulted in an improved amino acid content (Gutiérrez-Gamboa *et al.*, 2017a). Lasa *et al.* (2012) showed that, with foliar applications of urea, the accumulation and synthesis of amino acids such as ARG, GLN, THR and ALA were significantly increased. This gives rise to better alcoholic fermentation kinetics and the higher production of ethyl esters, which are essential for quality wines (Lasa *et al.*, 2012).

Methoxypyrazine levels in the must can determine the levels in the resulting wine. Helwi *et al.* (2017) reported that nitrogen soil fertilisation did not significantly affect the IBMP levels in Sauvignon blanc berries, wine or must compared to the control (deficient YAN). IBMP levels were slightly higher in the resulting wine than in the must (3 to 5 ng/L vs 0.5 to 1 ng/L) (Helwi *et al.*, 2017). Another study reported that a high level of irrigation with additional nitrogen (60 kg/ha at fruit set) promoted canopy growth, with higher levels of IBMP during fruit maturation, in Merlot vines (Mendez-

Costabel *et al.*, 2014). No foliar fertilisation research studies have focused on the impact of foliar nitrogen fertilisation on the methoxypyrazine content, such as the IBMP, IPMP and SBMP levels. Researchers speculate that foliar fertilisation with urea can increase these levels in the grapes and consequently in the resulting wine.

Various wines produced for nitrogen fertilisation studies have resulted in lower concentrations of higher alcohols. Higher alcohols are directly related to amino acid metabolism and are directly influenced by the YAN levels. Higher alcohols reach a peak between 200 and 300 mg N/L YAN, and degrade if the YAN levels increase (Ugliano *et al.*, 2007). Increased levels of esters are found mostly with nitrogen fertilisation applications (Lasa *et al.*, 2012). In this case, wines are described as being 'fruitier' compared to the control. Fatty acids, ethyl esters and acetates contribute to the fruity aroma of white wines, and their levels increase with DAP additions and higher YAN levels in the must (Ugliano *et al.*, 2007).

In vineyards containing low (< 140 mg N/L) and moderate nitrogen (140 to 300 mg N/L) (Gardner, 2014) to which nitrogen fertiliser applications were applied, there was an increase in YAN, 3MH, 3MHA and 4MMP levels, which were much higher compared to the control (Peyrot des Gachons *et al.*, 2005; Chone *et al.*, 2006). 4MMP was at higher levels after the foliar urea application (10 kg N/ha twice before véraison) compared to the control for Sauvignon blanc, while 3MHA and 3MH levels were not higher (Lacroux *et al.*, 2008). The application of foliar urea (10 kg N/ha twice before véraison) with sulphur (5 kg S/ha twice before véraison) resulted in higher concentrations of 3MH, 3MHA and 4MMP compared to the control (Lacroux *et al.*, 2008). Geffroy *et al.* (2016a) also reported that 10 and 20 kg N/ha foliar applications with 5 and 10 kg S/ha respectively resulted in musts with a three- to four-fold gain in varietal thiols in the case of white cultivars, including Sauvignon blanc. Dufourcq *et al.* (2009) reported significant increases in berry YAN with urea foliar fertilisation at véraison, and these musts correlated with higher 3MH and 3MHA concentrations in the resulting wine. Helwi *et al.* (2014) applied soil fertilisation (50 kg N/ha – two applications and 100 kg N/ha – two applications) and foliar fertilisation of urea (15 kg/ha – three applications) to Sauvignon blanc and reported that all the wines produced from vines with higher nitrogen status contained more 3MH.

Wines produced from grapevines that received applications of soil ammonium nitrate (30 kg/ha), foliar urea (10 kg N/ha, twice before véraison), and foliar urea (10 kg N/ha, twice before véraison) with sulphur (5 kg S/ha, twice before véraison), underwent sensory evaluation (Lacroux *et al.*, 2008; Geffroy *et al.*, 2016a). The wines that received foliar urea with sulphur applications had significantly higher aroma intensities, while wines that received foliar urea had decreased intensities (Lacroux *et al.*, 2008). Geffroy *et al.* (2016a) also reported that the foliar applications of 10 and 20 kg/ha N and 5 and 10 kg/ha S resulted in wines with more intense and increased notes of 'grapefruit' and 'tropical fruit', while no undesirable sulphur-related notes were perceived.

CONCLUSIONS

A wine's flavour and aroma expression are influenced by various reactions and interactions of chemical compounds (Fischer, 2007). These non-volatile and volatile compounds can be derived from the grape, produced from the yeast metabolism during alcoholic or malolactic fermentation, or produced during barrel or bottle ageing (Fischer, 2007).

Various foliar fertilisation studies show that nitrogen and nitrogen with sulphur applications can positively affect various volatile and non-volatile compounds in grapes, musts and the resulting wines. Foliar fertiliser applications of nitrogen and nitrogen with sulphur applied at véraison have shown a positive impact on berry chemical content, yeast growth and metabolism, and produce more aromatic wines. By undertaking nitrogen and sulphur foliar applications at véraison, the concentrations of sulphur- and nitrogen-containing compounds can be increased. Methoxypyrazines and major volatiles are important volatile compounds and have not been analysed in foliar fertilisation research studies. Only a few research studies have determined vine nitrogen status before and after foliar applications (Lacroux *et al.*, 2008; Jreij *et al.*, 2009; Helwi *et al.*, 2014). Only two studies have undertaken sensory analysis of the finished wines, but these do not mention the age of the wines (Lacroux *et al.*, 2008; Geffroy *et al.*, 2016a). Another void in research studies on foliar fertilisation is that the chemical and sensory evolution of wines during bottle maturation has not been considered. The information gained by performing sensory analysis during different stages of bottle maturation can add to the knowledge base of the aroma of these wines.

Most foliar studies have mentioned and proven that they have found positive correlations where fertiliser applications were done prior to and at véraison, due to the vine's nutrient uptake patterns and requirements. Many research studies have been done on the biochemical and chemical origins of non-volatile and aroma compounds, and the effects of the environment, and viticultural and winemaking practices on the concentrations on these compounds. By performing chemical and sensory analysis to understand the influence of the effect of foliar fertilisation with nitrogen and nitrogen with sulphur on the final wines, the wine industry can make decisions at the viticultural and winemaking level to produce wines with more desirable sensory attributes.

LITERATURE CITED

Adams, D.O. & Liyanage, C., 1993. Glutathione increases in grape berries at the onset of ripening. *Am. J. Enol. Vitic.* 44(3), 333-338.

Alberts, P., Stander, M.A., Paul, S.O. & De Villiers, A., 2009. Survey of 3-alkyl-2-methoxypyrazine content of South African Sauvignon blanc wines using a novel LC-APCI-MS/MS method. *J. Agr. Food Chem.* 57(20), 9347-9355.

Amerine, M., Berg, H., Kunkee, R., Ough, C., Singleton, V. & Webb, A., 1982. Technology of winemaking. Fourth. Westport, Connecticut, United States of America: The AVI Publishing Company, INC.

Anderson, M.E., 1998. Glutathione: An overview of biosynthesis and modulation. *Chem.-Biol. Interact.* 111-112, 1-14.

Antalick, G., Perello, M.C. & De Revel, G., 2014. Esters in wines: New insight through the establishment of a database of french wines. *Am. J. Enol. Vitic.* 65(3), 293-304.

Bell, A.A., Ough, C.S. & Kliewer, W.M., 1979. Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis Vinifera* var. Thompson seedless grapevines. *Am. J. Enol. Vitic.* 30(2), 124-129.

Bell, S.J., 1994. The effect of nitrogen fertilisation on the growth, yield and juice composition of *Vitis vinifera* cv. Cabernet Sauvignon grapevines. Thesis, University of Western Australia, Perth, Australia.

Bell, S.J. & Henschke, P.A., 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* 11(3), 242-295.

Bell, S.J. & Robson, A., 1999. Effect of nitrogen fertilisation on growth, canopy density and yield of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* 50, 351-358.

Blake, A., Kotseridis, Y., Brindle, I., Inglis, D., Sears, M. & Pickering, G., 2009. Effect of closure and packaging type on 3- alkyl- 2- methoxypyrazines and other impact odorants of Riesling and Cabernet franc wines. *J. Agr. Food Chem.* 57, 4680-4690.

Botha, A., 2015. The use of different oak products during the fermentation and ageing of Chenin blanc: Sensory properties, perceived quality, and consumer preference. (December). MSc Agric, Stellenbosch University

Boulton, R., Singleton, V., Bisson, L. & Kunkee, R., 1996. Principles and practices of winemaking. Chapman & Hall, New York, USA.

Capone, D., Sefton, M., Hayasaka, Y. & Jeffery, D., 2010. Analysis of precursors to wine odorant 3-mercaptophexan-1-ol using HPCL-MS/MS: Resolution and quantitation of diastereomers of 3-S-cysteinylhexan-1-ol and 3-S-glutathionylhexan-1-ol. *J. Agric. Food Chem.* 58, 1390-1395.

Castellarin, S.D., Bavaresco, L., Falginella, L., Gonçalves, M.I.V.Z. & Di Gaspero, G., 2012. Phenolics in grape berry and key antioxidants. In: Hernâni, G., Chaves, M. & Delrot, S. (eds). The biochemistry of the grape berry. Bentham Science Publishers, Sharjah, United Arab Emirates pp. 89 – 110.

Cheng, T., Reineccius, G., Bjorklund, J. & Leete, E., 1991. Biosynthesis of 2-methoxy-3-isopropylpyrazine in *Pseudomonas perolens*. *J. Agric. Food Chem.* 39, 1009-1012.

Cheynier, V., Souquet, J.M. & Moutounet, M., 1989. Glutathione content and glutathione to hydroxycinnamic acid ratio in *Vitis vinifera* grapes and musts. *Am. J. Enol. Vitic.* 40(4), 320-324.

Chhone, X., Lavigne-Cruege, V., Tominaga, T., Van Leeuwen, C., Castagnede, C., Saucier, C. & Dubourdieu, D., 2006. Effect of wine nitrogen status on grape aromatic potential: Flavor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. cv. Sauvignon blanc. *J. Int. Sci. Vigne Vin.* 1-6.

Christensen, P., 1984. Nutrient level comparisons of leaf petioles and blades in twenty-six grape cultivars over three years (1979–1981). *Am. J. Enol. Vitic.* 35, 124-133.

Christensen, P. & Peacock, W., 2000. Mineral nutrition and fertilization. In: Christensen, L.P. (ed.). Raisin production manual. University of California, Agriculture & Natural Resources, Communication Services, Oakland, CA. pp. 102 – 114.

Coetzee, C., 2011. Oxygen and sulfur dioxide additions to Sauvignon blanc must: Effect on must and wine composition. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Coetzee, C., 2014. Oxidation treatments affecting Sauvignon blanc wine sensory and chemical composition. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Coetzee, C., Schulze, A., Mokwena, L., Du Toit, W.J. & Buica, A., 2018. Investigation of thiol levels in young commercial South African Sauvignon blanc and Chenin blanc wines using propiolate derivatization and GC-MS/MS. *S. Afr. J. Enol. Vitic.* 39(2), 180-184.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. & Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. *Food* 1, 1-22.

Conradie, W.J., 1981. Nutrient consumption by Chenin blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. *S. Afr. J. Enol. Vitic.* 2(1), 15-18.

Conradie, W., 1986. Utilisation of nitrogen by grape-vine as affected by time of application and soil type. *S. Afr. J. Enol. Vitic.* 7(2), 76-83.

Conradie, W.J., 2001. Timing of nitrogen fertilisation and the effect of poultry manure on the performance of grapevines on sandy soil. II. Leaf analysis, juice analysis and wine quality. *S. Afr. J. Enol. Vitic.* 22(2), 53-59.

Conradie, W.J. & Saayman, D., 1989. Effects of long-term nitrogen, phosphorus, and potassium fertilization on Chenin blanc vines. II. Leaf analyses and grape composition. *Am. J. Enol. Vitic.* 40(2), 91-98.

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

Deloire, A., 2012. New method to determine optimal ripeness for different white wine styles. *Practical Winery & Vineyard.* , 2-5.

Department of Agriculture, Forestry and Fisheries, 2018. Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, No. 36 of 1947. Online: <http://www.fertas.co.za/wp-content/uploads/2017/01/Fertilizer-regulation-Page-1-of-3.pdf> [accessed March 2018].

Dickinson, J., Salgado, L. & Hewlins, J., 2003. The catabolism of amino acids to long chain and complex alcohols in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 278, 8028-8034.

Dienes-Nagy, Á., Vuichard, F., Spring, J.-L. & Lorenzini, F., 2017. Evolution of major N containing substances in response to the foliar urea treatment (Poster). *Int. Conf. WAC* Switzerland. March

Dubourdieu, D., Tominaga, T., Masneuf, I., Peyrot des Gachons, C. & Murat, M.L., 2006a. The role of yeasts in grape flavor development during fermentation: The example of Sauvignon blanc. *Am. J. Enol. Vitic.* 57(1), 81-88.

Dufourcq, T., Davaux, F., Charrier, F., Pou-Pault, P. & Schneider, R., 2011. La fertilisation foliaire en azote de la vigne et ses conséquences sur la composition des mouts et des vins. French Institute of Vine and Wine. France. 2016

Dufourcq, T., Serrano, E., Gontier, L. & Ollat, N., 2005. Rapport surface foliaire et poids de récolte: incidences sur l'alimentation hydrique de la vigne, la qualité du moût, la qualité du vin de quatre cépages de Midi-Pyrénées. Extrait du GESCO. Actes de colloque

Dufourcq, T., Nardi, S., Geffroy, O., Serrano, E., Lopez, R. & Cacho, J., 2007. Influence de l'itinéraire de vinification sur le potentiel aromatique des vins de Gros Manseng des vins. French Institute of Vine and Wine, Villenave-d'Ornon, France.

Dufourcq, T., Charrier, F., Poupault, P., Schneider, R., Gontier, L. & Serrano, E., 2009. Foliar spraying of nitrogen and sulfur at veraison: A viticultural technique to improve aromatic composition of white and rosés wines. 16th International GiESCO Symposium, Viticulture & Enology, UC Davis, Davis, CA, USA. pp. 379 – 383.

Du Plessis, C.S. & Van Rooyen, P.C., 1982. Grape Maturity and Wine Quality. *South African Journal for Enology and Viticulture.* 3(2), 41-5.

Duteutre, B., Bourgeois, C. & Chollot, B., 1971. Study of the assimilation of proline by brewing yeasts. *J. I. Brewing* 77(1), 28-35.

Du Toit, W.J., Lisjak, K., Stander, M. & Prevoo, D., 2007. Using LC-MSMS to assess glutathione levels in South African white grape juices and wines made with different levels of oxygen. *J. Agr. Food Chem.* 55(8), 2765-2769.

Ebeler, S.E. & Thorngate, J., 2009. Wine chemistry and flavor: Looking into the crystal glass. *J. Agric. Food Chem.* 57(18), 8098-8108.

Elskens, M., Jasper, C. & Penninckx, M., 1991. Glutathione as an endogenous sulfur source in the yeast *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* 137, 637-644.

Etievant, P., Schlich, P., Bouvier, J.-C., Symonds, P. & Bertrand, A., 1988. Varietal and geographic classification of French red wines in terms of elements, amino acids and aromatic alcohols. *J. Sci. Food Chem.* 45, 24-41.

Ewart, A. & Kliewer, W., 1977. Effects of controlled day and night temperatures and nitrogen on fruit-set, ovule fertility, and fruit composition of several wine grape cultivars. *Am. J. Enol. Vitic.* 28, 88-95.

Fischer, U., 2007. Flavours and fragrances. In: Berger, R.G. (ed). *Flavours and fragrances: Chemistry, bioprocessing and sustainability*. Springer, Berlin, Heidelberg, New York. pp. 241 – 267.

Fracassetti, D., Lawrence, N., Tredoux, A.G.J., Tirelli, A., Nieuwoudt, H.H. & Du Toit, W.J., 2011. Quantification of glutathione, catechin and caffeic acid in grape juice and wine by a novel ultra-performance liquid chromatography method. *Food Chem.* 128(4), 1136-1142.

Francis, I.L. & Newton, J.L., 2005. Determining wine aroma from compositional data. *Aust. J. Grape Wine R.* 11(2), 114-126.

Garde-Cerdán, T. & Ancín-Azpilicueta, C., 2008. Effect of the addition of different quantities of amino acids to nitrogen-deficient must on the formation of esters, alcohols, and acids during wine alcoholic fermentation. *LWT-Food Sci. Technol.* 41(3), 501-510.

Garde-Cerdán, T., Gutierrez-Gamboa, G., Gonzalo-Diago, A., Moreno-Simunovic, Y. & Martinez-Gil, A., 2016. Effect of different foliar nitrogen applications on the must amino acids and glutathione composition in Cabernet Sauvignon vineyard. *MacroWine*, June 2016, Changins, Switzerland.

Garde-Cerdán, T., Lorenzo, C., Lara, J.F., Pardo, F., Ancin-Azpilicueta, C. & Salinas, M.R., 2009. Study of the evolution of nitrogen compounds during grape ripening. Application to differentiate grape varieties and cultivated systems. *J. Agr. Food Chem.* 57, 2410-2419.

Garde-Cerdán, T., Portu, J., Lopez, R. & Santamaría, P., 2015. Effect of foliar applications of proline, phenylalanine, urea, and commercial nitrogen fertilizers on stilbene concentrations in Tempranillo musts and wines. *Am. J. Enol. Vitic.* 66(4), 542-547.

Gardner, D., 2014. High-YAN fermentations. *Wines & Vines* December. Online: www.winesandvines.com/features/article/142517/High-YAN-Fermentations [accessed March 2017].

Geffroy, O., Charrier, F., Poupault, P., Schneider, R., Lopez, R., Gontier, L. & Dufourcq, T., 2016a. Foliar nitrogen and sulfur spraying allow to produce wines with enhanced concentration in varietal thiols. *MacroWine* June 2016, Changins, Switzerland

Geffroy, O., Dufourcq, T., Lopez, R., Serrano, E., Gracia-Moreno, E., Cacho, J. & Ferreira, V., 2012. Impact de pulvérisations foliaires azotées réalisées à la véraison sur les caractéristiques aromatiques des vins rouges. . *Vigne Vin Sudouest*. Online: <https://www.vignevin-sudouest.com/publications/itv-colloque/vinaromas-aromes-vin/documents/feilhes-francais.pdf> [accessed March 2017]

Geffroy, O. & Dufourcq, T. 2010. Evaluation de 3 préparations biologiques appliquées par voie foliaire sur vigne pour corriger le statut azoté des mouts. *Vigne Vin Occitanie*. Online: <http://www.vignevin-occitanie.com/wp-content/uploads/2018/10/4-preparation-foliaire-biologique-foliaire.pdf> [accessed March 2017]

Geffroy, O., Lopez, R., Geffroy, O., Charrier, F., Poupault, P., Schneider, R., Lopez, R., Gontier, L. & Dufourcq, T., 2016b. Boosting varietal thiols in white and rosé wines through foliar nitrogen and sulfur spraying. *Proc. 16th Aust. Wine Ind. Tech. Conf.*, pp. 102 – 105. July, Adelaide, Australia

Giudici, P. & Kunkee, R.E., 1994. The effect of nitrogen deficiency and sulfur-containing amino acids on the reduction of sulfate to hydrogen sulfide by wine yeasts. *Am. J. Enol. Vitic.* 45(1), 107-112.

Godard, P., Urrestarazu, A., Vissers, S., Kontos, K., Bontempi, G., Van Helden, J. & André, B., 2007. Effect of 21 different nitrogen sources on global gene expression in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 27(8), 3065-3086.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Gonzalo-Diago, A., Moreno-Simunovic, Y. & Martínez-Gil, A., 2017a. Effect of different foliar nitrogen applications on the must amino acids and glutathione composition in Cabernet Sauvignon vineyard. *LWT-Food Sc. Technol.* 75, 147-154.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Portu, J., Moreno-Simunovic, Y. & Martínez-Gil, A.M., 2017b. Foliar nitrogen application in Cabernet Sauvignon vines: Effects on wine flavonoid and amino acid content. *Food Res. Int.* 96, 46-53.

Hallinan, P., Saul, D. & Jiranek, V., 1999. Differential utilisation of sulfur compounds for H2S liberation by nitrogen-starved wine yeasts. *Aust. J. Grape Wine Res.* 5, 82-90.

Hannam, K.D., Neilsen, G.H., Neilsen, D., Midwood, A.J., Millard, P., Zhang, Z., Thornton, B. & Steinke, D., 2016. Amino acid composition of grape (*Vitis vinifera* L.) juice in response to applications of urea to the soil or foliage. *Am. J. Enol. Vitic.* 67(1), 47-55.

Hannam, K.D., Neilsen, G.H., Neilsen, D., Rabie, W.S., Midwood, A.J. & Millard, P., 2014. Late-season foliar urea applications can increase berry yeast-assimilable nitrogen in winegrapes (*Vitis vinifera* L.). *Am. J. Enol. Vitic.* 65(1), 89-95.

Helwi, P., Guillaumie, S., Darriet, P., Van Leeuwen, C. & Thibon, C., 2014. The impact of vine nitrogen status on aroma potential expression in *Vitis vinifera* L. cv. Sauvignon blanc. Online: <http://congresdesterroirs.org/articles/lire/248> [accessed March 2017].

Helwi, P., Habran, A., Guillaumie, S., Thibon, C., Hilbert, G., Renault, C., Gomes, E. & Darriet, P., 2017. Can wine aromas be manipulated by nitrogen applications in the vineyard? International Conference WAC. Proceedings of the International Conference WAC March 2017, Beaune, France.

Henschke, P.A. & Jiranek, V., 1991. Hydrogen sulfide formation during fermentation: Effect of nitrogen composition in model grape must. International Symposium on Nitrogen in Grape and Wine. Jube 1991, Seattle, Washington, USA

Henschke, P. & Jiranek, V., 1993. Yeasts – metabolism of nitrogen compounds. In: Fleet, G.H. (ed). *Wine microbiology and biotechnology*. Harwood Academic Publishers, Chur, Switzerland. pp. 77 – 164.

Herbst-Johnstone, M., Nicolau, L. & Kilmartin, P.A., 2011. Stability of varietal thiols in commercial Sauvignon blanc wines. *Am. J. Enol. Vitic.* 62(4), 495-502.

Hilbert, G., Soyer, J.-P., Molot, C., Giraudon, J., Milin, S. & Gaudillère, J.-P., 2003. Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* 42(2), 69-76.

Holzapfel, B.P. & Treeby, M.T., 2007. Effects of timing and rate of N supply on leaf nitrogen status, grape yield and juice composition from Shiraz grapevines grafted to one of three different rootstocks. *Aust. J. Grape Wine Res.* 13(1), 14-22.

Holzapfel, B.P., Watt, J., Smith, J.P., Šuklje, K. & Rogiers, S.Y., 2015. Effects of timing of N application and water constraints on N accumulation and juice amino N concentration in “Chardonnay” grapevines. *Vitis* 54(4), 203-211.

Huang, Z. & Ough, C., 1989. Effect of vineyard locations, varieties and rootstocks on the juice amino acid composition of several cultivars. *Am. J. Enol. Vitic.* 40, 135-139.

Ingledew, W., Magnus, C. & Sosulski, F., 1987. Influence of oxygen on proline utilisation during the wine fermentation. *Am. J. Enol. Vitic.* 38(3), 246-248.

Janeš, L., Lisjak, K. & Vanzo, A., 2010. Determination of glutathione content in grape juice and wine by high-performance liquid chromatography with fluorescence detection. *Anal. Chim. Acta* 674(2), 239-242.

Jreij, R., Kelly, M.T., Deloire, A., Brenon, E. & Blaise, A., 2009. Combined effects of soil-applied and foliar-applied nitrogen on the N composition and distribution in water stressed *Vitis vinifera* L. cv. Sauv. blanc grapes. *J. Int. Sci. Vigne Vin* 43(4), 179-187.

Juhasz, A., 2015. Effect of foliar fertilization on wine aroma for white wine cv. Sauvignon blanc (*Vitis vinifera* L.). Thesis, Hochschule Geisenheim University, Germany. MSc Thesis

Kavi Kishor, P.B. & Sreenivasulu, N., 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* 37(2), 300-311.

Kelly, M.K., 2013. Effect of foliar nitrogen and sulfur applications on aroma profile of *Vitis vinifera* L. cv. Petit Manseng using modified quantitative descriptive analysis, SPME GC-MS and electronic nose technology. Thesis, Virginia Polytechnic Institute, Virginia State University, USA.

Kelly, M., Gill Giese, W., Velasco-Cruz, C., Lawson, L., Ma, S., Wright, M. & Zoecklein, B., 2017. Effect of foliar nitrogen and sulfur on petit manseng (*Vitis vinifera* L.) grape composition. *J. Wine Res.* 28(3), 165-180.

Kliewer, W.M., 1968. Changes in the concentration of free amino acids in grape berries during maturation. Annual Meeting of the American Society of Enologists, June 1968, Del Coronado, CA, USA.

Kliewer, W.M. & Cook, J.A., 1971. Arginine and total free amino acids as indicators of the nitrogen status of grapevines. *J. Am. Soc. Hortic. Sci.* 96, 581-587.

Kliewer, W.M., Bogdanoff, C. & Benz, M., 1991. Responses of Thompson seedless grapevines trained to single and divided canopy trellis systems to nitrogen fertilisation. *Internat. Symp. Nitrogen Grapes Wine*, Seattle, USA. pp. 282 – 289.

Koestel, C., Dienes-Nagy, Á., Fuchsmann, P., Nardone, D., Zufferey, V., Spring, J.-L., Lorenzini, F. & Rösti, J., 2017. Impact of different nitrogen levels in the vineyard and wine-making processes on the chemical profile of Chasselas wines. Proceedings of the International Conference WAC 2017, Beaune, France. March

Kotserides, Y., Spink, M., Brindle, I.D., Blake, A., Sears, M., Chen, X., Soleas, G., Inglis, D. & Pickering, G.J., 2008. Quantitative analyses of 3-alkyl-2-methoxypyrazines in juice and wine using stable isotope labelled internal standard assay. *J. Chromatogr. A* 1190, 294-301.

Kritzinger, E.C., 2012. Winemaking practices affecting glutathione concentrations in white wine. Thesis, Stellenbosch University, Private Bag X1, 7602, Matieland (Stellenbosch), South Africa.

Kritzinger, E.C., Stander, M.A. & Du Toit, W.J., 2013. Assessment of glutathione levels in model solution and grape ferments supplemented with glutathione-enriched inactive dry yeast preparations using a novel UPLC-MS/MS method. *Food Addit. Contam. Part A, Chem. Anal. Control Expo. Risk Assess.* 30(1), 80-92.

Kuhn, N., Guan, L., Dai, Z.W., Wu, B.H., Lauvergeat, V., Gomès, E., Li, S.H., Godoy, F., Arce-Johnson, P. & Delrot, S., 2014. Berry ripening: Recently heard through the grapevine. *J. Exp. Bot.* 65(16), 4543-4559.

Lacey, M.J., Allen, M.S., Harris, R.L.N. & Brown, W.V., 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. *Am. J. Enol. Vitic.* 42(2), 103-138.

Lacey, M.J., Brown, W. V., Allen, M.S. & Harris, R.L.N., 1988. Alkyl methoxypyrazines and Sauvignon blanc character. Cool Climate Vitic. Enol, Symp, 11-15 Jan, Auckland, New Zealand

Lacroux, F., Tregot, O., Van Leeuwen, C., Pons, A., Tominaga, T., Lavigne-Cruège, V. & Dubourdieu, D., 2008. Effect of foliar nitrogen and sulphur application on aromatic expression. *J. Int. Sci. Vigne Vin* 3(42), 1-8.

Lambrechts, M.G. & Pretorius, I.S., 2000. Yeast and its importance to wine aroma - A review. *S. Afr. J. Enol. Vitic.* 21(Special Issue), 97-129.

Lasa, B., Menendez, S., Sagastizabal, K., Cervantes, M.E.C., Irigoyen, I., Muro, J., Aparicio-Tejo, P.M. & Ariz, I., 2012. Foliar application of urea to "Sauvignon blanc" and "Merlot" vines: Doses and time of application. *Plant Growth Regul.* 67(1), 73-81.

Lavigne, V., Pons, A. & Dubourdieu, D., 2007. Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection. Changes in concentration in dry white wines during alcoholic fermentation and aging. *J. Chromatogr. A* 1139(1), 130-135.

Lee, J. & Schreiner, R.P., 2010. Free amino acid profiles from "Pinot noir" grapes are influenced by vine N-status and sample preparation method. *Food Chem.* 119(2), 484-489.

Lee, J. & Steenwerth, K.L., 2011. Rootstock and vineyard floor management influence on "Cabernet Sauvignon" grape yeast assimilable nitrogen (YAN). *Food Chem.* 127(3), 926-933.

Lehtonen, P., 1996. Determination of amines and amino acids in wine – A review. *Am J. Enol. Vitic.* 47(2), 127-133.

Leonardelli, M., 2013. Why, when, and how to measure YAN. The Grape and Wine Institute, University of Missouri, Columbia, USA.

Ljungdahl, P.O. & Daignan-Fornier, B., 2012. Regulation of amino acid, nucleotide, and phosphate metabolism in *Saccharomyces cerevisiae*. *Genetics* 190(3), 885-929.

Löhnertz, O. & Schaller, K., 1992. Uptake of nitrogen by the grape berries of different cultivars during grape and development. *Wein-Wissenschaft* 47, 135-139.

Long, D., Wilkinson, K.L., Poole, K., Taylor, D.K., Warren, T., Astorga, A.M. & Jiranek, V., 2012. Rapid method for proline determination in grape juice and wine. *J. Agric. Food Chem.* 60(17), 4259-4264.

Makhotkina, O., Araujo, L.D., Olejar, K., Herbst-Johnstone, M., Fedrizzi, B. & Kilmartin, P.A., 2014. Aroma impact of ascorbic acid and glutathione additions to Sauvignon blanc at harvest to supplement sulfur dioxide. *Am. J. Enol. Vitic.* 65(3), 388-393.

Marais, J., 1978. The effect of pH on esters and quality of Colombar wine during maturation. *Vitis* 19, 151-154.

Marais, J., 1994. Sauvignon blanc cultivar aroma: A review. *S. Afr. J. Enol. Vitic.* 15(2), 41-45.

Marais, J., 1998. Effect of grape temperature, oxidation and skin contact on Sauvignon blanc juice and wine composition and wine quality. *S. Afr. J. Enol. Vitic.* 19(1), 10-16.

Marais, J. & Pool, H., 1980. Effect of storage time and temperature on the volatile composition and quality of dry white table wines. *Vitis* 19, 151-14.

Marais, J., Hunter, J.J. & Haasbroek, P.D., 1999. Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. *S. Afr. J. Enol. Vitic.* 20(1), 1-30.

Mendez-Costabel, M.P., Wilkinson, K.L., Bastian, S.E.P., Jordans, C., McCarthy, M., Ford, C.M. & Dokoozlian, N., 2014. Effect of winter rainfall on yield components and fruit green aromas of *Vitis vinifera* L. cv. Merlot in California. *Australian Journal of Grape and Wine Research.* 20(1), 100-10.

Monteiro, F.F. & Bisson, L.F., 1991. Biological assay of nitrogen content of grape juice and prediction of sluggish fermentations. *Am. J. Enol. Vitic.* 42(1), 47-57.

Mundy, D.C., Hill, G.N. & Beresford, R.M., 2009. Factors affecting susceptibility of Sauvignon blanc grape berries to *Botrytis cinerea* infection. Romeo Bragato Conference. 20-22 Aug, Hawke's Bay, New Zealand

Murat, M.L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T. & Dubourdieu, D., 2001a. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *Am. J. Enol. Vitic.* 52(2), 136-139.

Murat, M.L., Tominaga, T. & Dubourdieu, D., 2001b. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rose wine by assaying the cysteinylated precursor of 3-mercaptopropan-1-ol. *J. Agr. Food Chem.* 49(11), 5412-5417.

Nikolantonaki, M., Chichue, I., Teissedre, P.-L.L. & Darriet, P., 2010. Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. *Anal. Chim. Acta* 660(1), 102-109.

Omnia, 2015. Sulphur the fourth macro element. Online: <http://fertilizer.co.za/downloads/send/9-sulphur/23-sulphur-the-short-and-sweet-of-it> [accessed March 2017].

Oosterhuis, D., 2009. Foliar fertilization: Mechanisms and magnitude of nutrient uptake. Proceedings of the Fluid Forum, 15-17 February, Scottsdale, Arizona, USA

Ough, C., Stevens, D., Sendovski, T., Huang, Z. & An, D., 1990. Factors contributing to urea formation in commercially fermented wines. *Am. J. Enol. Vitic.* 41, 68-73.

Pambianchi, D., 2001. pHiguring out pH. Online: <https://winemakermag.com/article/547-phiguring-out-ph> [accessed March 2017]

Papadopoulou, D. & Roussis, I., 2001. Inhibition of the decline of linalool and α -terpineol in Muscat wines by glutathione and N-acetyl-cysteine. *Ital. J. Food Sci.* 13, 413-419.

Patel, P., Herbst-Johnstone, M., Lee, S., Gardner, R., Weaver, R., Nicolau, L. & Kilmartin, P., 2010. Influence of juice pressing conditions on polyphenols, antioxidants, and varietal aroma of Sauvignon blanc microferments. *J. Agr. Food Chem.* 58, 7280-7288.

Peacock, W.L., Christensen, L.P. & Hirschfelt, D.J. 1991. Influence of timing of nitrogen fertilizer application on grapevines in the San Joaquin Valley. *Am. J. Enol. Vitic.* 42(4), 2-6.

Perez-Harvey, J. & Witting, D., 2001. Influence of soil nitrogen fertilisation and artificial shading on the nitrogen and potassium levels in leaves and berries of Cabernet Sauvignon. GESCO (Gestion de l'eau Dans Le Vignoble), Montpellier, France.

Petering, J.E., Symons, M.R., Langridge, P. & Henschke, P.A., 1991. Determination of killer yeast activity in fermenting grape juice by using a marked *Saccharomyces* wine yeast strain. *Appl. Environ. Microbiol.* 57(11), 3232-3236.

Petrovic, G., Kidd, M. & Buica, A., 2019. A statistical exploration of data to identify the role of cultivar and origin in the concentration and composition of yeast assimilable nitrogen. *Food Chem.* 276, 528-537.

Peyrot des Gachons, C., Van Leeuwen, C., Tominaga, T., Soyer, J.P., Gaudillere, J.P. & Dubourdieu, D., 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L on Sauvignon blanc in field conditions. *J. Sci. Food Agr.* 85(1), 73-85.

Ramey, D. & Ough, C., 1980. Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agr. Food Chem.* 28, 928-934.

Ribéreau-Gayon, P., Glories, Y., Maujean, A. & Dubourdieu, D., 2006. *Handbook of enology: The chemistry of wine stabilization and treatments* (Vol. 2). John Wiley & Sons, Ltd, Chichester, West Sussex.

Robinson, J. & Harding, J., 2014. *The Oxford Companion to Wine*. Third. Oxford University Press, Oxford, UK.

Roland, A., Schneider, R., Razungles, A. & Cavelier, F., 2011. Varietal thiols in wine: Discovery, analysis and applications. *Chem. Rev.* 111(11), 7355-7376.

Roland, A., Vialaret, J., Razungles, A., Rigou, P. & Schneider, R., 2010. Evolution of S-cysteinylated and s-glutathionylated thiol precursors during oxidation of Melon B. and Sauvignon blanc musts. *J. Agr. Food Chem.* 58(7), 4406-4413.

Roujou de Boubée, D., Van Leeuwen, C. & Dubourdieu, D., 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agr. Food Chem.* 48(10), 4830-4834.

Roussis, I., Lambropoulos, I. & Papadopoulou, D., 2005. Inhibition of the decline of volatile esters and terpenols during oxidative storage of Muscat white and Xinomavro red wine by caffeic acid and N-acetyl cysteine. *J. Agr. Food Chem.* 93, 485-492.

Saerens, S., Delvaux, F., Vertrepene, K., Van Dijck, P., Thevelein, J. & Delvaux, F., 2008. Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Appl. Environ. Microbiol.* 74, 454-461.

Sala, C., Busto, O., Guasch, J. & Zamora, F., 2004. Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazine content in must and wines from *Vitis vinifera* variety Cabernet Sauvignon. *J. Agr. Food Chem.* 52, 3492-3497.

Sarrazin, E., Sinkharuk, S., Pons, M., Thibon, C., Bennetau, B. & Darriet, P., 2010. Elucidation of the 1,3-sulfanylalcohol oxidation mechanism: An unusual identification of the disulfide of 3-sulfa-nylhexanol in Sauternes botrytized wines. *J. Agr. Food Chem.* 58, 10606-10613.

Singleton, V., Salgues, J., Zaya, J. & Trousdale, E., 1985. Caftaric acid disappearance and conversion to products of enzymatic oxidation in grape must and wine. *Am. J. Enol. Vitic.* 36, 50-56.

Spayd, S.E., Wample, R.L., Evans, R.G., Stevens, R.G., Seymour, B.J. & Nagel, C.W., 1994. Nitrogen fertilization of white Riesling grapes in Washington. Must and wine composition. *Am. J. Enol. Vitic.* 45(1), 34-42.

Spayd, S.E., Wample, R.L., Stevens, R.G., Evans, R.G. & Kawakami, A.K., 1993. Nitrogen fertilization of white riesling grapes in Washington: Nitrogen seasonal effects on bud cold hardiness and carbohydrate reserves. *Am. J. Enol. Vitic.* 44(8), 159-167.

Stines, A., Grubb, J., Gockowiak, H., Henschke, P., Høj, P. & Van Heeswijk, R., 2000. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. *Aust. J. Grape Wine Res.* 6(2), 150-158.

Šuklje, K., Antalick, G., Buica, A., Langlois, J., Coetzee, Z.A., Gouot, J., Schmidtko, L.M. & Deloire, A., 2016. Clonal differences and impact of defoliation on Sauvignon blanc (*Vitis vinifera* L.) wines: A chemical and sensory investigation. *J. Sci. Food Agr.* 96(3), 915-926.

Šuklje, K., Lisjak, K., Baša Česnik, H., Janeš, L., Du Toit, W., Coetzee, Z., Vanzo, A. & Deloire, A., 2012. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (*Vitis vinifera* L.). *J. Agr. Food Chem.* 60(37), 9454-9461.

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A. & Pretorius, I.S., 2005. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* 11, 139-173.

Swiegers, J.H., Francis, I.L., Herderich, M.J. & Pretorius, I.S., 2006. Meeting consumer expectations through management in vineyard and winery. *Aust. NZ Wine Ind. J.* 21(1), 34-43.

Téa, I., Genter, T., Naulet, N., Morvan, E. & Kleiber, D., 2003. Isotopic study of post-anthesis foliar incorporation of sulphur and nitrogen in wheat. *Isot. Environ. Health S.* 39, 289-300.

Thurston, P., Taylor, R. & Ahvenainen, J., 1981. Effects of linoleic acid supplements on the synthesis by yeast of lipids and acetate esters. *J. I. Brewing* 87, 92-95.

Tominaga, T., Baltenweck-Guyot, R., Des Gachons, C.P. & Dubourdieu, D., 2000. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am. J. Enol. Vitic.* 51(2), 178-181.

Tominaga, T., Darriet, P. & Dubourdieu, D., 1996. Identification de l'acétate de 3-mercaptopropanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis* 35(4), 207-210.

Tominaga, T., Furrer, A., Henry, R. & Dubourdieu, D., 1998a. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Frag. J.* 13(3), 159-162.

Tominaga, T., Murat, M.-L. & Dubourdieu, D., 1998b. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agr. Food Chem.* 46(3), 1044-1048.

Tominaga, T., Peyrot des Gachons, C. & Dubourdieu, D., 1998c. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agr. Food Chem.* 46(12), 5215-5219.

Ugliano, M., Henschke, P.A., Herderich, M.J. & Pretorius, I.S., 2007. Nitrogen management is critical for wine flavour and style. *Aust. NZ Wine Ind. J.* 22(6), 24-30.

Ugliano, M., Kwiatkowski, M., Vidal, S., Capone, D., Siebert, T., Dieval, J.-B., Aagaard, O. & Waters, E.J., 2011. Evolution of 3-mercaptopropanol, hydrogen sulfide, and methyl mercaptan during bottle storage of Sauvignon blanc wines. Effect of glutathione, copper, oxygen exposure, and closure-derived oxygen. *J. Agr. Food Chem.* 59(6), 2564-2572.

Van Leeuwen, C., Friant, P., Soyer, J.-P., Molot, C., Chone, X. & Dubourdieu, D., 2000. Measurement of total nitrogen and assimilable nitrogen in grape juice to assess vine nitrogen status. *J. Int. Sci. Vigne Vin* 34(2), 75-82.

Van Schalkwyk, H. & Archer, E., 2000. Optimum Ripeness in Wine Grapes. Online: <http://www.wineland.co.za/optimum-ripeness-in-wine-grapes/>. [accessed March 2017]

Van Wyngaard, E., 2013. Volatiles playing an important role in South African Sauvignon blanc wines. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Verdenal, T., Spangenberg, J.E., Zufferey, V., Lorenzini, F., Dienes-Nagy, A., Gindro, K., Spring, J.L. & Viret, O., 2016. Leaf-to-fruit ratio affects the impact of foliar-applied nitrogen on N accumulation in the grape must. *J. Int. Sci. Vigne Vin* 50(1), 23-33.

Verdenal, T., Spangenberg, J.E., Zufferey, V., Lorenzini, F., Spring, J.L. & Viret, O., 2015a. Effect of fertilisation timing on the partitioning of foliar-applied nitrogen in *Vitis vinifera* cv. Chasselas: A ¹⁵N labelling approach. *Aust. J. Grape Wine Res.* 21(1), 110-117.

Verdenal, T., Zufferey, V., Lorenzini, F., Dienes-Nagy, Á., Jorge E. Spangenberg, J.-L.S. & Viret, O., 2015b. Distribution de l'azote dans la vigne: impact de la période d'application de l'urée foliaire Thibaut. *Rev. Suisse Vitic. Arboric. Horticult.* 47(2), 96-101.

Watson, B., Godard, M. & Chen, H., 2000. Manipulating soil moisture and nitrogen availability Part II: Effects on Pinot noir must and wine composition. in: Proc. Troisième Colloque International Bourgogne-Californie-Oregon: Terroir et Qualité. Dijon, France, November 13-15

Wilson, C.L., 2017. Chemical evaluation and sensory relevance of thiols in South African Chenin blanc wines. Thesis, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Wilton, N., 2015 (2nd ed). Stikstof en gisvoeding. In: Die Vinlab handboek., Stellenbosch. pp. 24 – 28.

Wolf, T.K & D'Attilio, D., 2014. Assessment of grapevine nitrogen status and optimized nitrogen fertilization practices. Virginia Wine Board

WOSA, South African Wine Styles. Online: <https://www.wosa.co.za/The-Industry/Varieties-and-Styles/Wine-Styles/> [accessed March 2017]