Improvement of the nitrogen content of grape must with fertilisation

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirely or in part submitted it at any university for a degree.

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SUMMARY

Stuck fermentation is currently a serious production associated problem in the South-African wine industry. This can mainly be attributed to insufficient levels of nitrogen in grape must, thus are not being able to supply in yeast demands. This study was undertaken to investigate whether the nitrogen content of grape must can be improved by fertilisation.

In order to achieve this three nitrogen application treatments (N_1C = 20 kg N/ha^{-1} post-harvest; N_2F = 20 kg N/ha^{-1} post-harvest, followed with 20 kg N/ha^{-1} after budbreak and fruitset respectively; and N_2V = 20 kg N/ha^{-1} post-harvest, followed with 20 kg N/ha^{-1} after budbreak and veraison respectively), were applied to six grape cultivars (Cabernet Sauvignon, Chenin blanc, Pinot noir, Weisser Riesling, Chardonnay and Pinotage) for two seasons (1999/2000 = 1st season and 2000/2001 = 2nd season). The vineyard is situated on a high potential loam soil, classified as a red-brown Oakleaf, with a mean organic matter content of 1.4%. The effect of these nitrogen application treatments on the growth characteristics, grape must composition, leaf nitrogen content, as well as the soil nitrate content, were measured.

Nitrogen applications during the vegetative phase had a positive effect on the shoot length and shoot elongation during both seasons, although more so during the 1st season. The pruning mass was significantly affected by cultivar during both seasons. Treatments receiving no nitrogen fertilisation during the vegetative phase (N_1C) resulted in higher free amino nitrogen (FAN) and FAN/°B ratio in grape must, indicating a negative reaction to nitrogen fertilisation for soils having a high organic matter content. No nitrogen fertilisation during the vegetative season resulted in significantly lower bunch and berry mass values than those receiving fertilisation. This might indicate a negative relationship between bunch and berry mass and the FAN content of grape must. The nitrate content of the soil differed for the different soil depths and was affected by sampling date and N treatments. Results indicated that 70% of the nitrate was available in the top 30 cm of soil.

Nitrogen fertilisation during the vegetative season on soils with an organic matter content of 1.4% should be strongly discouraged. This study therefore indicates that although high organic matter content and inorganic fertiliser applications may increase the nitrate content of the soil, this might not have the same effect on the nitrogen status of the vine.

KEY WORDS : Nitrogen fertilisation, FAN, cultivar, yield, soil nitrate, leaf analysis, shoot elongation

OPSOMMING

Slepende gisting is tans 'n ernstige produksieverwante probleem in die Suid-Afrikaanse wynindustrie. Dit kan hoofsaaklik toegeskryf word aan die lae vlakke van stikstof in druiwemos, wat dus nie aan die gistingsbehoefte kan voorsien nie. Hierdie studie is gevolglik onderneem om te ondersoek of die stikstofinhoud van druiwemos deur bemesting verhoog kan word.

Om hierdie doel te bereik is drie stikstof bemestingbehandelings (N_1C = 20 kg N/ha^{-1} na-oes; N_2F = 20 kg N/ha^{-1} na-oes, gevolg deur 20 kg N/ha^{-1} na bot en met vrugset onderskeidelik; en N_2V = 20 kg N/ha^{-1} na-oes, gevolg deur 20 kg N/ha^{-1} na bot en met deurslaan onderskeidelik) tot ses wyndruifcultivars (Cabernet Sauvignon, Chenin blanc, Pinot noir, Weisser Riesling, Chardonnay and Pinotage) toegedien oor twee seisoene (1999/2000 = 1ste seisoen en 2000/2001 = 2de seisoen). Die wingerd is geplant op 'n hoë potensiaal leem grond, geklassifeseer as rooi-bruin Oakleaf, met 'n organiese materiaal inhoud van 1.4%. Die invloed van hierdie drie stikstof bemestingbehandelings op groei-eienskappe, mossamestelling, stikstofinhoud van blare, asook die nitraatinhoud van die grond, is gemeet.

Stikstofbemesting gedurende die vegetatiewe groeifase het gedurende beide seisoene 'n positiewe effek op die lootlengte en lootverlenging gehad (wel meer so gedurende die eerste seisoen). Die tipe cultivar het (gedurende beide seisoene) die winterlootmassa betekenisvol beïnvloed. Behandelings waar geen stikstof gedurende die vegetatiewe groeifase toegedien is nie (N_1C) het tot hoër vry aminostikstof (FAN) en FAN/°B inhoud van die mos gelei. Dit dui op 'n negatiewe reaksie waar stikstofbemesting toegedien word op gronde met 'n relatief hoë organiese materiaal inhoud. Geen stikstof gedurende die vegetatiewe groeifase toegedien het ook betekenisvolle laer tros- en korrelmassas tot gevolg gehad. Dit mag op 'n negatiewe verwantskap tussen tros- en korrelmassa en die FAN-inhoud van druiwemos dui. Die nitraatinhoud het verskil oor die verskillende gronddieptes, datums van monsterneming en stikstofbehandelings. Resultate het aangedui dat 70% van die nitraat in die boonste 30 cm grondlaag beskikbaar was.

Die toediening van N bemesting tydens die vegetatiewe fase van die wingerd op gronde met 'n organiese materiaal inhoud van 1.4% of meer behoort dus ten sterkste afgeraai te word. Resultate van hierdie studie dui daarop dat, hoewel 'n hoë organiese materiaalinhoud van die

grond en anorganiese N bemesting die nitraatinhoud van die grond kan verhoog, dit nie noodwendig tot 'n verhoging in die N status van die wingerdstok sal lei nie.

SLEUTELWOORDE : Stikstof bemesting, FAN, cultivar, opbrengs, grond nitraatinhoud, blaarontledings, lootverlenging

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INDEX

CHAPTER 1

INTRODUCTION

Stuck fermentation is currently a serious production associated problem in the South-African wine industry. This can mainly be attributed to insufficient levels of nitrogen (N) in grape must which are not able to supply in yeast demands. As a result of this N insufficiencies di-ammoniumphosphate (DAP) is added during fermentation as a yeast supplement. Due to a possible negative effect of DAP on wine quality, increased resistance against this practice can be expected, which might effect the overseas marketing of South-African wines in future (Louw, 1998). If the natural N content of grape must can be raised with N fertilisation, fewer additions would have to be made in the cellar, making the wine more acceptable to the buyer. The worldwide trend is to produce more environmentally friendly wines, keeping the additives to the wine as few as possible.

As a result of the relatively large amounts of N needed by plants, due to its vital role in plant nutrition, it is probably the element in agricultural industry on which most research is done. This is also valid for grapevines, although the amount of N required is less than that for annual crops. Although the N requirements and seasonal uptake pattern of N in grapevines are largely known, Peacock, Christensen & Broadbend (1989) and Christensen, Bianchi, Peacock & Hirchfelt (1994) suggested that more research is needed to determine the effect of N application during the summer on ripeness, vegetative growth and the N content of berries. Research in this context was undertaken by Conradie (1998), who evaluated the effect of N application at different growth stages on the free amino nitrogen (FAN) content of Sauvignon blanc at two localities.

The objective of this study was to determine the effect of different N applications on the N content of grape must from various *Vitis vinifera* cultivars. Secondary objectives were to measure the effect of N fertilisation on the growth characteristics, grape must composition and leaf N content of various *Vitis vinifera* cultivars, as well as on the soil nitrate content.

CHAPTER 2

NITROGEN CONTENT OF GRAPE MUST: A REVIEW

2.1 THE ROLE OF NITROGEN DURING FERMENTATION

Yeasts need a vast amount of supplements during fermentation. Of these supplements N is one of the most important and no fermentation can take place in the absence of N (Vos, 1998). According to Rankine (1989) a N shortage in the must of grapes inhibits sugar uptake and may lead to lagging fermentation.

2.1.1 Flavours

Nitrogen in grape must also serve as an important source of precursors of aroma compounds (Kluba, Mattick & Hackler, 1978; Rapp & Versini, 1991). According to Marais (1998) little is known about the biochemical pathways of N containing aroma-components. Sub-optimal amounts of N in must may lead to lagging fermentation, minimum formation of esters, higher levels of higher alcohols and volatile acids, the development of H_2S and other related off-flavours (Rankine, 1989; Henschke & Jiranek, 1993; Rauhut, 2001). All these factors lead to a lower organoleptic quality of the wine. According to Ough & Bell (1980) fruity flavours in wine developed better when the N content in the must was higher. Amino acids promote the formation of compounds that are associated with wine aroma and flavour (Spayd, Wample, Evans, Stevens, Seymore & Nagel, 1994). According to Rapp & Versini (1991) different kinds of organic N containing compounds are found in must which could include amino acids, amines, amides, piridines and pyrazines. It seems that amides and amines do not have an affect on wine aroma or taste although amines may lead to a hard taste in beer. Piridine is associated with off-flavours while pyrazines are associated with asparagus, vegetative and gooseberry flavours. Higher arginine and urea concentrations are, however, frequently associated with high concentrations of ethylcarbamate in wines (Spayd *et al.*, 1994).

2.1.2 Nitrogen in grape must

According to Henschke & Jiranek (1993) grape must contains of all supplements needed for yeast growth. The main source of energy for growth is supplied by sugar and is usually available in excess. In contrast to sugar the N concentration in grape must vary and may be inhibiting to yeast growth. Amino acids and ammonia represent the group of N compounds that can be assimilated directly by yeast, *viz* the assimilable N (Vos & Gray, 1979; Vos, 1998). The determination of FAN is the most common method used to indicate the total available N in grape must (Monteiro & Bisson, 1991). The total N concentration of grapes consists of ammonium ions and organic N compounds (Table 2.1) while nitrate N only occurs in very small quantities (Treeby, Holzapfel, Walker & Nicolas, 1998; Vos, 1998).

TABLE 2.1 : Nitrogen containing compounds found in grape must (Henschke & Jiranek, 1993)

Nitrogen components	**Concentration (mg l^{-1})**				
	1	**2**	**3**	**4**	**5**
Ammonia	10 – 120	45 – 99	45 – 89	7 – 127	0 – 146
Amino acid	170–1120(a)	–	704–1 070(b)	19 – 144	–
Amine	–	101 – 168	46 – 81	14 – 176	15 – 182
Amide (c)	10 – 40	–	–	–	–
Humine (d)	5 – 20	–	–	–	–
Polypeptide	–	–	–	10 –70	38 – 132
Hexosamine	–	–	–	–	19 – 29
Protein	10 – 100	–	–	–	28 – 97
Residue	100 – 200	–	–	–	–
Total	**305 – 1 600**	**358 – 570**	**322 – 490**	**98 - 618**	**98 – 1130**

Number 1 – 5 indicate the number of the samples

(a) Excluding dipeptides

(b) Arginine, proline, serine and treonine

(c) Asparagine and glutamine

(d) Triptofan and tirosine

According to Winkler, Cook, Kliewer & Lider (1974) N in grape must contribute up to 20% of the total N in the berry while the rest is to be found in the seeds and berry skins. The

synthesis of organic N compounds occurs mainly in the last six to eight weeks of ripening (Kliewer & Lider, 1968; Winkler *et al.,* 1974; Rapp & Versini, 1991). Precursors of this organic N are found in the leaves but the synthesis of the compounds mainly occurs in the berry itself (Kliewer & Cook, 1974). The concentration of free amino acids in grapes can vary as a result of cultivar, orientation, ripeness, sample preparation, cultivation method and analysing method (Kluba *et al.,* 1978; Henschke & Jarinek, 1993; Louw, 1998). The concentration of FAN increases after the lag phase of berry development with further increases during ripening (Pandey, Rao & Singh, 1974; Vos & Gray, 1979; Rapp & Versini, 1991). According to Kluba *et al.* (1978) the initiation time and rate of this increase differ between cultivars and individual amino acids.

2.1.3 The correlation between chemical compounds in grape must

Significant correlation was found between FAN, H_2S and soluble solids (Vos & Gray, 1979). There was also significant correlation between protein N, titrable acid, turbidity and pH, as well as between non-protein N, turbidity and total N and in the last place between pH and titrable acid. The most significant correlations were between FAN and H_2S. Increasing the FAN is the most effective way of preventing the formation of H_2S (this also promotes fermentation). The formation of H_2S is thus indirectly caused by a N shortage in the must. According to Eschenbruch (1974) neither high nor low concentrations of amino acids in must played a significant role in the formation of H_2S, and FAN is the controlling factor. According to Vos & Gray (1979), the opposite relationship between FAN and H_2S shows that when assimilable N in must are low, proteolitic activities are stimulated and proteins and higher peptides are degraded to the form of assimilable N. During this process sulphur derivatives of the protein are released to form H_2S.

2.1.4 The addition of di-ammonium phosphate to grape must

According to Monteiro & Bisson (1991), Conradie (1998), the addition of an N source prior to fermentation is a common practice in the wine industry to ensure that the fermentation process completes successfully. By making the addition prior to fermentation it is ensured

that alcohol which forms during fermentation does not inhibit the uptake of ammonium. According to Louw (1998), the addition should be made prior to fermentation as most N is utilised before 5% alcohol is formed.

Ammonium and glutamate is the most accessible sources of N to yeast cells (Henschke & Jarinek, 1993; Louw, 1998). Di-ammonium phosphate is used most commonly in the industry to raise the N assimible for yeasts. It is a cheap source of N, is easy to apply and work effectively. Rankine (1989) and Louw (1998) suggest the addition of 10 - 20 g DAP hl^{-1} must before fermentation. Most cellars do not have the equipment to determine the FAN levels in must and apply between 30 - 75 g hl^{-1} of DAP before fermentation. By using this method there is always a shortage or a surplus of N in the must (Louw, 1998). Where shortages occur, more DAP is added and over saturation is induced.

This uncalculated addition of DAP to must can effect the wine quality negatively in many ways. Uncalculated addition may lead to the formation of urea, hydrogensulfide (H_2S) and ethylcarbamate (Monteiro & Bisson, 1992). According to Conradie (1998), wine quality is always better when the natural level of N in the must is high enough for fermentation. In Germany research showed that musts with a low FAN content do not mature as well (Conradie, 1998; Marais, 1998). When the iron content of musts was high, the addition of DAP may lead to tubidity of the must during fermentation. Although not yet in South-Africa, other countries have laws which regulate the amounts of DAP that may be added before fermentation. The European Union limits the amount of DAP that may be added before fermentation to 300 mg l^{-1} (Louw, 1998). Worldwide the addition of DAP prior to fermentation is discouraged and even limited in favour of natural sources of N (Conradie, 1998; Louw, 1998). According to Monteiro & Bisson (1992), the addition of DAP had little effect on the use of amino acids in must by yeast. When higher levels of DAP were added to must the degradation and utilisation of arginine were negatively affected.

2.1.5 Raising the nitrogen content of must with fertilisation

According to Ough & Bell, (1980), Conradie & Saayman, (1989a), Spayd *et al.* (1994) and Conradie (1998), the natural N content of must can be raised with correct N fertilisation. The total N content of grape must was raised by 50 mg l^{-1} when fertilising with 96 kg N ha^{-1} a^{-1} instead of 16 kg N ha^{-1} a^{-1} (Conradie & Saayman, 1989b). Nitrogen in the berry could be raised with N fertilisation but fertilisation above 112 kg N ha^{-1} a^{-1} had no effect on the N in the must (Ough & Bell, 1980). The biggest contrast was found between the control (0 kg N ha^{-1} a^{-1}) and a 112 kg N ha^{-1} a^{-1} fertilisation. Raising the N content of the must led to lower levels of higher alcohols in the wine. According to Spayd *et al.* (1994) FAN and total N concentrations in must doubled when applying 56 kg N ha^{-1} a^{-1} instead of 0 kg N ha^{-1} a^{-1}. Monteiro & Bisson (1991) found no clear correlation between N fertilisation at budbreak and the N content of must. Saayman & Conradie (1982) found no consistent logical pattern in the nutrient content of must. They ascribed the insensitivity of must analyses to fertiliser N treatments to the high inherent fertility of the specific soil and suggested that different results may be obtained under less favourable soil conditions.

It seems that the N content of must is a more sensitive indicator of the N fertilisation status of the vine than that in leaves and petioles on a sandy loam soil in the Stellenbosch area (Conradie & Saayman, 1989b). Total N, FAN, and ammonium in must were positively correlated with nitrate concentrations in petioles, of which concentrations at bloom showed the highest correlation (Spayd *et al.,* 1994). Low nitrate concentrations in petioles at bloom could, therefore, serve as an indicator of low N content of grape must at harvest.

2.2 FACTORS WHICH COULD EFFECT THE NITROGEN CONTENT OF MUST

The N content of must can vary as a result of climate, water status of the soil, organic matter content of the soil, crop load, cultivar, ripeness, sample preparation, cultivation method, analysing method as well as timing and quantities of N fertilisation (Kluba, *et al.,* 1978; Henschke & Jarinek, 1993; Conradie, 1998; Louw, 1998). According to Winkler, *et al.*

(1974), Vos & Gray (1979) and Monteiro & Bisson (1991), cultivar and time of harvest mainly effects the concentration of N and amino acids in the must. The N content of South African wines is generally low. According to Conradie (1997), this can be ascribed to excessive vegetative growth, which is induced by warm conditions during the early vegetative phase. This excessive vegetative growth disturbs the natural balance in the vine and less N is canalised to the bunches (Hunter, 1997).

During drought or when the water content of the soil nears wilting point, N uptake cannot proceed effectively and the FAN content of the must is usually lower (Hunter, 1997). According to Winkler *et al.* (1974), Coombe & Monk (1980), the concentration of proline increased when the vine experiences stress during ripening. When the soil has an inherent high organic matter content, it has a naturally higher N supplying capacity which leads to higher N contents in grape must (Conradie, 1994; Conradie, 1997; Conradie, 1998). In vines with a higher crop load, the N is distributed to more bunches, which leads to a lower N content of the must (Winkler *et al.,* 1974; Conradie, 1998). Additional N fertilisation usually cannot compensate for this.

According to Conradie (1998), different cultivation practices also have an effect on the N content of must. In Germany a decrease of the N content of must was found with an active growing cover crop during the vegetative phase. Where leguminous cover crops are sown during winter, the N content of must should be slightly higher. Clean cultivation in the vineyard should have no effect on the N content of grape must. The depth of initial soil preparation may also affect the N content of must. This can be ascribed to the fact that with effective soil preparation a larger soil volume is available for roots, which then can take up more water and nutrients.

According to Vos (1998), the N content of must also differs between different cultivars. Gewürztraminer and Pinotage have a high natural N content, while Chenin blanc has a medium and Cape Riesling and Weisser Riesling have lower levels of N. Current research is done on Sauvignon blanc and the effect of N on the FAN in grape must of other cultivars is not known (Personal communication, 1999: W.J. Conradie, Inftuitec-Nietvoorbij. Private

Bag X5026, Stellenbosch 7599). The ideal time when N should be applied is when active shoot growth has stopped. During this period the maximum amount of N should be canalised to the bunches (Conradie, 1997).

There are no fixed amount of N which should be applied to vineyards during the growing season (Conradie, 1994). Each situation is unique and vegetative growth should be used as a measure for N fertilisation (Du Toit, 1997). A range of 20-60 kg N ha^{-1} a^{-1} for the purpose of this research in the Stellenbosch region was suggested (Personal communication, 1999: W.J. Conradie, Inftuitec-Nietvoorbij. Private Bag X5026, Stellenbosch 7599).

According to Ough, Cook & Lider (1968), Winkler *et al.* (1974) and Treeby *et al.* (1998), rootstocks may also effect the N content of grape must. For example, St. George has higher N content than 99 R. Ough & Tabacman (1979) could not confirm these results. Huang & Ough (1989) found that more vigorous rootstocks induced higher concentrations of amino acids in must.

2.3 NITROGEN REQUIREMENTS AND UPTAKE OF THE VINE

2.3.1 Format of uptake

Nitrogen occupies a unique position amongst the elements essential for plant growth because of the large amounts required by most agricultural crops (Stevenson, 1986). According to Du Preez & Burger (1986) nearly all the inorganic N in the root zone can be utilised by the plant roots and should be taken into account during N fertilisation.

Before N is available to the vine it must first, through mineralisation and nitrification, be changed to ammonium and nitrate (Saayman, 1981). The vine can take up and metabolise both ammonium and nitrate ions. Due to the fast microbiological oxidation of ammonium to nitrate in well aerated soils, N uptake is usually in the nitrate form (Du Toit, 1997).

Nitrogen metabolism in the vine occurs, according to Saayman (1998), in three basic steps. During the first step inorganic N is converted to low molecular mass organic compounds.

Nitrate is reduced, through nitrate reductase, to nitrite in the meristemal tissue. In the chloroplast nitrite is further reduced by nitrite reductase to ammonia. The enzymes function in series to prevent buildup of toxic nitrites. If N is taken up in ammonium form, it is deprotonated at the plasma membrane and transported as ammonia over the chloroplast membrane. Ammonia is further assimilated by further enzyme action as low molecular mass amino acids, amides and amines.

During the second step, high molecular mass N compounds are formed, which include proteins and nucleic acid. The third step consists of the break-up of N macro molecules, through hydrolyzing enzymes, to soluble amino compounds. Nitrogen taken up by the vine is transported mainly as amino acids or nitrate through the xylem to the rest of the vine (Winkler *et al.*, 1974).

The amount of N released from applied and other soil organic matter will be determined by the efficiency of the mineralisation process (MacDuff & White, 1985) and can make an important contribution to the total N need of a crop (Addiscott, 1983). Greyling, Du Preez & Human (1990) found that significantly more mineralisable N occurred in the top 15 cm of soil than in deeper layers. This is in accordance with work by Soudi, Sbai & Chiang (1990), who found that the sharpest decrease in N mineralisation was in the 0-20 cm of soil depth, with a less rapid decrease for the 20-60 cm layer. Only 10% of the total mineralisation occurred in soil layers deeper than 40 cm. Cassman & Munns (1980) found that the relative contribution to the total N mineralised in a 108 cm deep soil profile, was respectively 42%, 18%, 25% and 15% for the 0-18 cm, 18-36 cm, 36-72 cm and 72-108 cm depths.

Environmental conditions like water supply and temperature, soil conditions like pH, inorganic N, structure and texture and cultivation methods may effect N mineralisation in a soil (Alexander, 1977; Campbell & Souster, 1982; Meyers, Campbell & Weier, 1982; Stevenson, 1886). Campbell (1978) showed that mineralisation is strongly effected by temperature. Ammonification and nitrification are negatively effectd by low soil temperatures, with optimum temperatures between 25°C and 35°C. Cassman & Munns (1980) reported an optimum temperatures for N mineralisation between 30°C and 35°C.

Greyling *et al.* (1990) found that this variation in optimum N mineralisation temperatures could mainly be attributed to variation in clay, organic carbon and total N content in soils.

George, Ladha, Buresh & Garrity (1993) measured cumulative amounts of nitrate as high as 155 kg nitrate N ha^{-1} a^{-1} in fertilisation trials. According to Linn & Doran (1984), Doran, Mielke & Power (1990) and Rochester, Constable & Macleod (1991) soil water and aeration have a major effect on nitrate levels and measurements just after heavy rains are likely to indicate lower nitrate amounts. Thompson & Thomas (1996) found a significant water and N interaction in soil. Microbe activity and N mineralisation are effectd in three ways by the water content of soil: (1) water stress inhibits microbial growth directly; (2) as water content increases, aeration decreases, thereby inhibiting microbial growth; (3) cycles of wetting and drying tend to increase the amount of available substrate for the soil microbes (Haynes, 1986). Higher water content and temperatures generally favour microbial growth and thus mineralisation (Jenkinson & Ayanaba, 1977; Thompson & Thomas, 1996). Cassman & Munns (1980) found N mineralisation to be maximal at a soil matrix potential of -30 kPa, with a sharp decrease between –30 kPa to -200 kPa. Irrigation, therefore, affects mineralisation through its effect on soil water regimes, soil aeration, and soil temperature. According to Harmsen & Van Schreven (1955), the decomposition of humus in arid climates is accelerated by irrigation. Nitrification, denitrification, and leaching are, however, dynamic processes and can occur simultaneously (George *et al.*, 1993). In a 4-year study Boman, Westerman, Ruan & Jojola (1995) found that the year with the lowest precipitation had the highest amount of soil residual nitrate N in the 45 - 60 cm soil layer.

2.3.2 The nitrogen need of the vine

According to Winkler *et al.* (1974) and Peacock *et al.* (1989), the N need of the vine is greatest during the period of rapid shoot growth, bloom and early berry development in Thompson Seedless. Under normal conditions vines do not show fertilisation deficiencies due to a well-developed root system which can effectively take up water and nutrients during a long vegetative season (Saayman, 1981; Conradie & Saayman, 1989a).

According to Du Toit (1997), the vigour of the vine should be used to determine N fertilisation. When applying N fertilisation for table grapes and mass-production wine grapes more vigorous growth should be induced (Conradie, 1994), while average growth should be induced for high-quality wine grapes (Table 2.2). Subjective to vigour, production can also be used as a norm when applying N fertilisation. According to Conradie (1997), 3.9 kg N ha^{-1} should be applied for each ton of grapes harvested. When using this method of fertilisation more than enough N is applied to meet the vine's needs as about half of the applied N used goes back into the soil via leaves and shoots (Conradie, 1994). Table 2.3 shows the utilisation of N by the different organs of the vine.

TABLE 2.2 : Nitrogen fertilisation norms for grapesvines under dryland or with irrigation (Conradie, 1994)

Physiological norm	**Vigour**		
	Poor	**Ideal**	**Vigorous**
Shootlength	Most shoots 30 – 70 cm	Most shoots 70 – 100 cm	Most shoots > 100 cm
Active growing points	None / few before veraison	None / few after veraison	Present at harvest
Length of internodes	< 5 cm	5 – 10 cm	> 10 cm
Leaf colour	Pale yellow-green	Dull light green	Shiny dark green
Shoot base diameter	< 1 cm	1 – 1.5 cm	> 1.5 cm
Fertilisation time	**kg N ha^{-1}**		
Post harvest After budbreak After bloom	20 – 40 10 – 20 10 - 20	20 - 40	0 – 20
Total kg N ha^{-1}	**40 – 80**	**20 - 40**	**0 – 20**

According to Winkler *et al.* (1974) and Saayman (1981), N fertilisation should be broadly applied and washed into the soil. Nitrogen applications should be made on the vine row with young and poor growing vines (Saayman, 1982). According to Saayman (1982), FSSA

(1997) and Du Toit (1997), there is less volatilisation when applying limestone ammoniumnitrate instead of ammoniumsulphate on neutral to alkaline and calcareous soils. Ammoniumsulphate is also the most acidifying and N fertilisation leaches slower into the soil. Urea volatilizes easily and should be washed in immediately after application (FSSA, 1997). According to Saayman (1981) half of the N in limestone ammoniumnitrate is immediately available after application. Limestone ammoniumnitrate can be applied on alkaline as well as acid soils and should enjoy preference when applying N to the soil.

TABLE 2.3: Amount of nitrogen utilised by the different plant organs of Chenin blanc / 99R, in a sand culture, for the production of 1 ton of grapes (Conradie, 1994)

Nitrogen	Plant organ					
	Grapes	**Roots**	**Trunk**	**Leaves**	**Shoots**	**Total**
kg	1.39	0.55	0.18	1.21	0.56	3.89
(%)	35.8	14.0	4.7	31.1	14.4	100

According to Saayman (1982) and Conradie (1997), N fertilisation could be applied in three increments during the season. The first of these increments should be made after budbreak followed by one at bloom and the final application in the post-harvest period. The first application should be made 3 - 4 weeks after budbreak (mid October) because the soil temperature in the Western Cape is too low for active root growth (Conradie, 1994; Personal communication, 2000: E. Archer, University of Stellenbosch. Private Bag X1, Stellenbosch 7599). The second application should be made just after bloom during the fruitset period (mid November). This application should be accompanied by irrigation to ensure that the N is washed into the soil. The post-harvest application should be applied within one month of harvest (Du Toit, 1997). According to Peacock *et al.* (1989), enough active growing leaves should still be available for photosynthesis at this stage. According to Boman *et al.* (1995), timing of N application had no effect on residual NH_4-N and little effect on residual NO_3-N concentration and distribution in the soil profile at the end of each cropping cycle.

The optimum soil pH (KCl) for vines varies between 5.5 - 6.5 (Conradie & Saayman, 1989a). Bramley & White (1989) found nitrification to be at an optimum rate at pH (H_2O) levels

between 5.2 and 6.3. Below and above these values, nitrification was slower. Ammonification is not as sensitive to pH as nitrification (Follet, Murphy & Donahue, 1981). Minimum reaction to N fertilisation on soils with a high organic matter content shows that the natural N supplying capacity of the soil is enough for the vine. When the organic matter content of the soil is ≥1.2%, little or no N fertilisation is needed (Conradie, 1994; Conradie, 1997). When the inorganic N content of the soil exceeds 15 mg kg^{-1} no further N fertilisation is needed (Conradie, 1994).

According to Saayman (1981), Conradie (1994) and Du Toit (1997), leaf analysis cannot be solely used to determine the N need of the vine but should be used in conjunction with soil analysis. Leaf analyses vary between seasons and are also effectd by rootstock, cultivar, climate, diseases and cultural practices (Conradie, 1994). Leaf samples could be taken at bloom, fruitset or at veraison. In practice leaf samples are usually taken at fruitset when berries are about 5 mm in diameter (last week in November). Leaves as well as petioles can be sampled to serve as an indicator of N status. According to Saayman & Conradie (1982) there are little similarity in the N content of leaf blades and petioles on a fertile soil. According to Conradie (1997) petioles are more sensitive and usually a better indicator of N status. Table 2.4 can serve as a guideline for the N content of leaves and petioles and Table 2.5 for the nitrate content of petioles. Nitrogen is rarely applied as a leaf supplement because of the small quantities utilised by leaves as well as the higher cost aspect (Conradie, 1998).

TABLE 2.4 : Norms for the nitrogen content of grapevine leaves and petioles (Adapted from Conradie, 1994)

Element	Leaf				Petioles			
	Fruitset		Veraison		Fruitset		Veraison	
	Min	Max	Min	Max	Min	Max	Min	Max
N (%)	1.6	2.7	1.5	2.4	0.6	0.98	0.50	0.95

TABLE 2.5 : Norms for nitrate content of grapevine petioles, sampled at full bloom, as guidelines for nitrogen fertilisation at fruitset (Conradie, 1994)

Nitrate – nitrogen (mg kg^{-1})	**Nitrogen status**	**Nitrogen fertilisation (kg LAN ha^{-1})**	
		Production of 10 – 20 ton ha^{-1}	**Production of > 20 ton ha^{-1}**
0 – 300	Serious deficiency	100	150
300 – 700	Slight/Mild deficiency	75	100
700 – 1000	Normal	50	75
> 1000	Over supplied	-	-

2.3.3 Reaction of the vine to nitrogen fertilisation

According to Spayd, Wample, Stevens, Evans & Kawakami (1993) and Spayd *et al.* (1994) the impact of N fertilisation on the crop mass, vigour, must and wine composition depends on the N status of the soil prior to manipulation, the climate, the cultivar and other viticultural practices. Conflicting research results with N fertilisation may have resulted because of differences in canopy density (vigour) and crop mass of the vine (Spayd *et al.*, 1994). According to Saayman (1981) and Jackson & Lombard (1993), N is the element most associated with excessive vegetative growth (disturbing the leaf canopy : crop ratio), which may induce higher humidity and reduce light penetration to the inner leaves and bunches. In spite of a significant reaction of the vine to N fertilisation, it is not known whether this reaction is direct or indirect (Jackson & Lombard, 1993). According to Peacock *et al.* (1989) and Christensen *et al.* (1994) more research is needed to determine the effect of N application during the summer on ripeness, vegetative growth and the N content of berries.

Nitrogen concentration in specific organs was positively correlated with an increase or decrease of dry matter content (Marocke, Balthazard & Huglin, 1977; Conradie, 1985). Increases in N fertilisation led to more vegetative growth, high N content in petioles, higher acid concentration in berries and more anthocianine in the berry skin (Jackson & Lombard,

1993). When using soil, leaf and must analyses, and shoot and crop mass (yield) as indicators of N fertiliser responseon a fertile soil, a tendency towards improved shoot mass was found, but no significant differences between crop mass (Saayman & Conradie, 1982). Results of Freeman, Lee & Turkington (1979) indicated that yield is directly related to the vigour of vines as measured by shoot diameter and shoot length. Research done by Spayd *et al.* (1993) showed that Thompson Seedless vines with low nitrate content in petioles had an increase in crop mass with N fertilisation. When petioles had a high nitrate content, a decrease in crop mass occurred with increased N fertilisation. According to Conradie & Saayman (1989a), N fertilisation increased crop mass and vegetative growth on a sandy loam soil with 1.1% organic matter, but lowered the pH of the soil. Within a given year, N applications did not affect yield, yield components or pruning mass of vines (Morris, Spayd & Clawthon, 1983). Only minor effects on vine size, cane periderm, and yield were noted and no consistent year-to-year pattern was evident for these parameters (Reynolds & Wardle, 1989).

Morris *et al.* (1983) found that irrigation increased yields and was beneficial in attaining acceptable quality levels and maintaining vine size, compared to N fertilisation, which had little effect on yields or vine size but tended to increase the percentage soluble solids and pH and to reduce titratable acids of the must. Jackson & Lombard (1993) found that fruit ripeness and must pH was not affected by N fertilisation. Spayd *et al.* (1994) however, found that pH showed a linear increase with increased N fertilisation. According to Christensen *et al.* (1994), soluble solids were the most negatively affected by an increase in N fertilisation. Soluble solids were lower with increased N fertilisation (Saayman, 1981; Spayd *et al.*, 1994). Thompson Seedless ripened more slowly with increasing N fertilisation, which could partly be ascribed to the increase in crop mass and canopy density. According to Spayd *et al.* (1994) N fertilisation does not effect the total acid or organic acid of must. This is not the same as the findings of Saayman (1981) and Jackson & Lombard (1993), which indicates higher acid concentration in berries with increased N fertilisation. According to Jackson & Lombard (1993) it is known that N fertilisation gives Semillon a more spicy flavour.

Increased N fertilisation may increase the vine's susceptibility to bunch rot, especially when applied at veraison (Jackson & Lombard, 1993; Christensen *et al.,* 1994). Nitrogen fertilisation at veraison had the least effect on crop mass (Jackson & Lombard, 1993; Christensen *et al.,* 1994). Increasing veraison fertilisation may lead to a lower crop mass, lower total acids, more bunch rot and less nutrients to the vegetative parts due to a higher demand from the bunches (Christensen *et al.*, 1994).

According to Conradie & Saayman (1989b) there was a 16% increase in shoot mass of Chenin blanc when fertilised with 56 kg N ha^{-1} a^{-1} instead of 16 kg N ha^{-1} a^{-1}. Saayman & Conradie (1982) concluded that there were no significant differences in the mean shoot mass of grafted vines receiving different N applications in any particular year. This was in spite of an obvious tendency for unfertilised vines to have a lower shoot mass than vines receiving fertiliser. Bravdo, Hepner, Loinger, Cohen, & Tabacman (1984) found that pruning mass was positively correlated with leaf area and linked to the capacity of the vine to ripen a specific crop, due to the high fertility of grapevines compared to that of other fruit trees. According to Ewart & Kliewer (1977) N fertilised vines had significantly better fruitset, higher total shoot length, longer internodes, higher shoot growth rate, petiole nitrate content and fruit acidity, compared to unfertilised vines.

2.3.4 Nitrogen content of leaves

According to Perez & Kliewer (1982), Christensen (1984) and Christensen *et al.* (1994), the nitrate accumulation in leaves and petioles are significantly affected by the cultivar. Malbec had nearly double the amount of nitrate in leaves and petioles compared to Chardonnay and Zinfandel. Christensen (1984) found that total N was highest in leaves but did not differ significantly between cultivars. According to Christensen *et al.* (1994), the inherent property of cultivars to have different nitrate contents in leaves can be ascribed to genetic differences in N metabolism. Cultivars may also differ in rate of reductase activities.

Perez & Kliewer (1982) found that the nitrate content of leaves and petioles were higher at lower light intensities for all cultivars tested. This means that N is more readily translocated

from the roots to the leaves at low light intensities. Nitrate reductase activities were lowest in leaves under low light intensities and differed between cultivars. With optimum light intensity, no significant differences were found in the nitrate content of petioles when applying different amounts of N.

According to Conradie & Saayman (1989b), the addition of N fertilisation at 16, 56 and 96 kg N ha^{-1} a^{-1} resulted in marginal increases in the N content of leaves and petioles. Leaf and petiole analysis at bloom and veraison were not a good indicator when applying different amounts of N at different times (Christensen *et al.*, 1994). The N content usually increased with increased N fertilisation. Leaves of vines that had N applications nearest to the date of sampling had the highest N content (Conradie & Saayman, 1989b). Leaves analysed at bloom showed the highest N content when N was applied at budbreak and during the post-harvest period. Leaves at veraison showed the highest N content when fertiliser was applied at fruitset. In some leaf samples, fertiliser applications in the post-harvest period induced the same or higher total N content in leaves at bloom than when applied at budbreak. Fertilisation at veraison had the least impact on petiole N content.

According to Perez & Kliewer (1982), nitrate reductase activities was negatively correlated to nitrate concentrations in the leaves and petioles. Other factors, which may effect nitrate content and nitrate reductase activities, are temperature and water availability. Soil, rootstock and climatic conditions can also effect the nitrate content of leaves of different cultivars (Christensen, 1984). According to Perez & Kliewer (1982) cultivars with inherent fruitset problems usually accumulate more nitrate in petioles and flower parts. When the nitrate content in petioles was highest, the arginine concentration was lowest and *vice versa* (Christensen, 1984). The nitrate content of petioles can, however, not be used to predict potential arginine concentrations in must. Nitrate concentration of petioles was highly correlated with proline concentrations in must. According to Christensen *et al.* (1994), N studies showed a positive correlation between N utilisation and partitioning during different phenological stages and the accumulation of degree-days.

According to Christensen (1984) the N content in leaves were highest in the early vegetative period, decreased slowly during bloom towards fruitset and the end of the season increased only slightly at veraison. The nitrate content of petioles rose prior to bloom after which it decreased during the rest of the season. Spayd *et al.* (1993) found that nitrate concentrations in petioles were the same when taken at veraison and harvest while applying the same amount of N fertilisation during the same year to all manipulations. According to Christensen (1984), nitrate concentrations peaked prior to bloom and decreased during veraison for most of the 26 cultivars, that were evaluated. A higher positive correlation was found between total N and nitrate than total N and ammonium. Nitrate N also made a bigger contribution to the total N than ammonium. Nitrate N of petioles was also more sensitive to N applications than ammonium.

2.4 NITROGEN METABOLISM IN THE VINE

According to Conradie (1990), about 80% of N is used in the year of application. Nitrogen distribution and metabolism of the vine can be affected by the inherent vigour of a cultivar, soil fertility and the rootstock. The vine mainly stores reserve N as soluble, low molecular mass compounds (amino acids), but soluble and insoluble proteins can also be used (Conradie, 1985) with the rootstock trunk having greater amounts of soluble N (Conradie, 1990). Low soil pH can slow down nitrification, according to Peacock *et al.* (1989). According to Christensen *et al.* (1994), initial N is needed for shoot and leaf development, but as the season progresses the demand from the bunches increases (Archer, 1981b). When N is applied in the post harvest period, it is stored in the permanent parts of the vine and distributed for growth during the early vegetative stage of the following season (Peacock *et al.*, 1989). Stassen, Terblanche & Strydom (1981) found similar results on peach trees. The dry mass and total N content of the vine showed a linear increase from the end of rapid shoot growth to harvest (Conradie, 1985). It seems that the pattern of N distribution is genetically determined and applications at veraison and during spring are distributed in the same way Marocke *et al.* (1977), Conradie (1985) and Christensen *et al.* (1994) concluded that N concentration in specific organs was correlated with an increase or decrease of dry matter content.

2.4.1 Postharvest and winter

Except during veraison, dry root mass increase steadily until leaf fall (Conradie, 1985). The amount of spring applied N increased with 23% and 46% in the medium and fine roots during the period from harvest to the end of leaf fall (Conradie, 1990). During autumn N is in both the soluble and insoluble form. According to Conradie (1985), the total N concentration in shoots showed an increase until leaf fall, while leaves showed an increase to harvest. This indicates a simultaneous influx and efflux of N (Conradie, 1985; Conradie, 1990). Leaves and shoots can thus serve as a part-time reservoir of N from the roots. The great amount of N that flows through these organs is initially in the form of proteins. The research of Glad, Farnineau, Regnard & Morot-Gaudry (1994) showed that xylem sap excretion during the winter consisted of 4% post-harvest applied N, while 40% of this came from spring-applied N. The dry mass and absolute N of the vine showed an increase until after leaf fall. A positive correlation therefore, excisted between the dry mass and total N content of the vine. Marocke *et al.* (1977) and Conradie (1985) also reported that N concentration in specific organs was correlated with an increase or decrease of dry matter content. This supports the view that changes in N concentration does not necessarily indicate an influx or efflux of N. The N concentration showed a decrease in all parts of the vine until harvest after which it increased and only showed decreases at the beginning of the next growing season.

The N content of the trunk stayed the same during the season (Williams & Biscay, 1991). During this period the N concentration in the roots decreased until harvest, after which it nearly doubled until winter. The insoluble N concentration in the shoots stayed constant, which indicates the hydrolyses of proteins (Christensen *et al.,* 1994). During the post-harvest period the permanent structure and roots are important points of demand for N, which are remobilized for vegetative growth during the next season. According to Glad *et al.* (1994), about 30% of N that was applied in the post-harvest period is stored in the permanent parts of the vine while 17% of spring applied N are stored in the permanent parts. Prior to budbreak

it was found that high concentrations of nitrate and total amino acids (especially glutamine) were translocated in the sap through the xylem (Glad *et al.*, 1994).

According to Conradie (1990), spring applied N migrates from the leaves in the post-harvest period to shoots and permanent parts of the vine. The N increase in the shoots is only short lived, after which the N also moves to the permanent parts. Conradie (1985) found that after harvest, spring applied N was evenly distributed in the permanent parts of the vine.

2.4.2 Budbreak and spring

After winter Conradie (1985) found equal amounts of spring applied N in the roots, rootstock and parts above the graft. During this time N assimilated by the roots was not able to supply in the demand of the vegetative parts (Winkler *et al.*, 1974). According to Christensen *et al.* (1994) and Glad *et al.* (1994), roots and other permanent structures supply reserve-N for initial vegetative growth, even if ample N is available in the soil. At budbreak the vine uses reserve-N nearest to the point of demand, while N from the roots is used more from bloom onwards. Of the spring-applied N, 83% was used for new growth (Glad *et al.*, 1994). Both N-reserves and assimilated N are responsible for new shoot growth until bloom. According to Conradie (1991) significant feeding of flower bunches only starts a few weeks after budbreak and is mainly dependent on N-reserves in the permanent parts of the vine. When N (ammonium sulphate) is applied at budbreak, uptake was not fast enough to show significant differences during bloom (Conradie, 1990).

Conradie (1990) also found that, while spring applied N that was stored as reserves is only utilised during the bloom of the next season, the summer-applied N was immediately utilised at the beginning of the next growing season. With spring applied N, leaves assimilated nearly double the amount of that of N compared to the shoots (Conradie, 1991). According to Glad *et al.* (1994), the "Physiological coulure" phenomenon, *viz* the abortion of flowers during bloom (even when climatic conditions are favourable), can be related to the N budget of the vine over the whole growth period. Research on Pinot noir showed a sharp increase in the N pool during bloom, feeding flower bunches during this period.

According to Conradie (1990), N that was applied during spring was also translocated from the permanent parts of the vine to the bunches. Vast amounts of spring applied N is stored in the permanent parts in soluble form (Conradie, 1985). Even when enough N is available for the vine, part of the spring-applied N is also stored as proteins in the roots.

2.4.3 Fruitset to veraison

According to Archer (1981b) and Saayman (1981), the vine has a huge demand for N during the period from the end of rapid shoot growth until veraison (phase 2 of berry development). Christensen *et al.* (1994) found that higher N fertilisation led to a higher total N content at veraison. Conradie (1990) applied ^{15}N was applied at the end of rapid shoot growth (8 mm berry size). At veraison 52% of this N occurred in the vegetative parts, 28% in the bunches and 20% in the permanent structure. There were, 43% of the N in the shoots, 23% of the leaves and 35% of the permanent structure translocated to the bunches at veraison.

2.4.4 Veraison to harvest

According to Conradie (1985), bunches showed higher concentrations of N than leaves and shoots from veraison until harvest, indicating the dominant role of bunches during this period. According to Williams & Biscay (1991), the insoluble N concentration in the permanent parts of the vine reached maximum values from veraison to harvest. The dry mass and total N content of the vine showed a linear increase from the end of rapid shoot growth to harvest (Conradie, 1985). It seems that the pattern of N distribution are genetically determined and applications at veraison or during spring are similarly distributed. Translocation of N to shoots and bunches was highest with summer N applications and low with spring N applications, while the opposite applied to roots.

According to Glad *et al.* (1994), a large amount of spring applied N was first translocated to leaves and shoots, after which a turnover occurred and the N was exported to the bunches. At harvest, bunches contained 46%, vegetative growth 41% and the permanent structure 13%

of labelled N (Conradie, 1991). It seems that this distribution stayed the same in different cultivars with N absorbed during the period from the end of rapid shoot growth to veraison.

CHAPTER 3

METHODS AND PROCEDURES

3.1 EXPERIMENTAL TERRAIN

Vineyard: This trial was done in a 14-year-old cultivar collection block in the vineyards of Elsenburg Agricultural College near Stellenbosch, Republic of South-Africa. The trial vineyard is situated on a northern slope with an east-west row direction. The vines were planted in 1986 and had optimum yield and vigour. The vines are trained onto a Five Strand Lengthened Perold trellising system as described by Zeeman (1981), with a planting distance of 3 x 1.5 m.

Soil: The vineyard is situated on a high potential loam soil, classified as an red-brown Oakleaf (Soil Classification Working Group, 1991). Prior to planting the soil was double delve-ploughed to an effective depth of 80 cm. For the three layers sampled, the soil has a mean pH of 5.6, clay content of 20% and organic matter content of 1.4% (using a value of 1.7 to convert from organic carbon to organic matter). The soil has sufficient to high concentrations of elements (Table 3.1) and is uniform as far as classification and general chemical and physical properties are concerned.

TABLE 3.1 : General chemical properties of the Oakleaf soil in the nitrogen fertilisation trial; Elsenburg, Stellenbosch

Soil depth	Texture	pH (KCl)	C (%)	Resistance (ohms)	P	Ca	Mg	K	Na
					(mg kg^{-1})				
0-15 cm	Loam	5.58	0.95	1643	45.6	706	75.6	165.9	11.8
15-30 cm	Loam	5.61	0.81	1731	29.5	670	64.8	147.6	13.3
30-60 cm	Loam	5.63	0.68	1587	18.5	594	60.00	135.6	14.2

Climate: Stellenbosch is situated in the coastal region of the province, Western Cape receiving 692 mm of rain annually (Saayman, 1981). About 30% of the annual rainfall occurs during the summer months. Vineyards are farmed with or without irrigation. As a result of relatively low yields, farmers concentrate on the production of grapes for quality wine production.

In this study the 1999/2000 season is referred to as the first and the 2000/2001 season as the second growing season. Because of the complex seasonal climatic conditions, the effect it has on soil and vine variables, and the translocation effect of nitrogen in the permanent parts of the vine, the first and second growing seasons are discussed separately. Mean monthly temperature, rainfall and wind conditions that prevailed during the two seasons of the trial, are given in Table 3.2.

TABLE 3.2 : Mean climatic conditions, measured at the Elsenburg weather station, during the two growing seasons of the nitrogen fertilisation trial; Stellenbosch

Climatic parameters	**Season**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Mean**
Average temperature (°C)	**1999/ 2000**	13.0	18.2	19.0	23.8	22.7	22.3	20.8	**20.0**
	2000/ 2001	13.3	16.8	19.5	19.7	21.0	22.8	19.8	**19.0**
Average windspeed ($m\ s^{-1}$)	**1999/ 2000**	2.5	3.0	2.8	2.7	3.1	2.8	2.6	**2.8**
	2000/ 2001	2.4	2.8	2.8	3.1	2.9	2.9	2.7	**2.8**
Total rainfall (mm)	**1999/ 2000**	100.8	9.4	36.0	7.6	28.2	0.4	24.4	**206.8 (Total)**
	2000/ 2001	86.6	18.4	19.8	10.4	17.2	14.4	4.2	**171.0 (Total)**

3.2 TREATMENTS

Cultivars : The plant material used was *Vitis vinifera* L. var. Chenin blanc, Weisser Riesling, Chardonnay, Pinotage, Pinot noir and Cabernet Sauvignon, which were all grafted on 99 Richter as rootstock. Abbreviations used for each cultivar are given in Table 3.3.

TABLE 3.3 : Abbreviations used for different cultivars in a nitrogen fertilisation trial; Elsenburg, Stellenbosch

Cultivars	Abbreviation
Cabernet Sauvignon	CS
Chenin blanc	SN
Pinot noir	PN
Weisser Riesling	WR
Chardonnay	CY
Pinotage	PT

Nitrogen treatments : Three N treatments were applied as indicated in Table 3.4. The N_1C treatment served as a control since that is usually regarded as a standard N fertilisation recommendation for wine grapes in the Western Cape. The vines in the trial received only a post-harvest N fertilisation of 30 kg N ha^{-1} a^{-1} (LAN) for at least the previous five years. No fertilisation was applied during the vegetative season. Treatments N_2F and N_2V were applied to determine whether it is possible to improve the nitrogen content of grape must by fertilising at different growth stages.

The aim with these three treatments was to apply differential amounts of N when all cultivars were in generally the same phase of berry development and root activity. All the treatments had a 20 kg N ha^{-1} post-harvest application to ensure a good reserve status of the vine before the next vegetative season. The budbreak application of 20 kg N ha^{-1} in the case of the N_2F and N_2V treatments were applied about 4 weeks after budbreak to avoid leaching and to

ensure that the mean soil temperature is above 10 °C and active root growth has commenced. This application should have resulted in a good nitrogen status of the vine before bloom.

TABLE 3.4 : Treatments applied to determine the effect of nitrogen fertilisation on the FAN content of grape must, soil nitrogen, vine performance, and nitrogen content of vine and must; Elsenburg, Stellenbosch

Nitrogen treatments	**Time and amout of nitrogen fertilisation (kg ha^{-1})**				
	Budbreak	**Fruitset**	**Veraison**	**Post-harvest**	**Total nitrogen**
N_1C	-	-	-	20	20
N_2F	20	20	-	20	60
N_2V	20	-	20	20	60

- N_1C = N applied during the post harvest period
- N_2F = N applied as for N_1C with additional applications of 20 kg at budbreak and fruitset
- N_2V = N applied as for N_1C with additional applications of 20 kg at budbreak and veraison

An additional 20 kg N ha^{-1} was applied during phase II of berry development (referred to as the fruitset N application) in the case of the N_2F treatments and during phase III (referred to as the veraison nitrogen application) in the case of the N_2V treatments. The application of the additional 20 kg N ha^{-1} in the case of the N_2F treatment and the N_2V treatment coincides with, or directly precedes a phase of strong berry development that should ensure good translocation of N into the berry. At these stages there should also be enough active root growth to utilise the applied N as indicated in Figure 3.1.

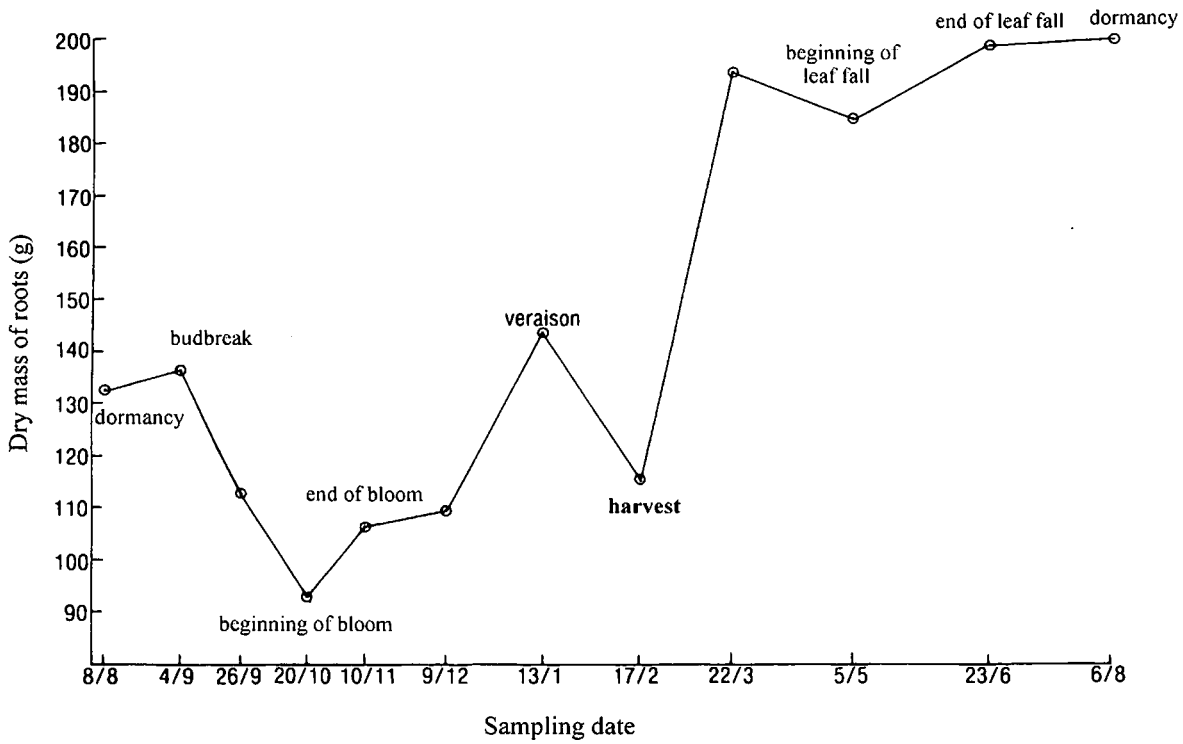


FIGURE 3.1 The root growth pattern of Chenin blanc; Nietvoorbij, Stellenbosch (Archer, 1981b)

Clean cultivation was practiced to prevent any nitrogen utilisation by the cover crop. Limestone ammonium nitrate (28% N) was used as nitrogen source as it is easily available to the plant with low volatilisation properties. A split randomized block was used as experimental design and the trial layout is given in Table 3.5. There was at least one buffer row and vine between plots. Each repetition consisted of one vine receiving treatments. The N treatments were randomly applied to all six cultivars and repeated four times for each cultivar.

TABLE 3.5 : Layout of to the split randomized block design; Nitrogen fertilisation trial, Elsenburg, Stellenbosch.

	Treatment					
Block	**SN**	**WR**	**CY**	**PT**	**PN**	**CS**
1	N_2F	N_1C	N_2F	N_1C	N_2V	N_2V
	N_2V	N_2F	N_1C	N_2F	N_1C	N_1C
	N_1C	N_2V	N_1C	N_2V	N_2F	N_2F
2	N_1C	N_2V	N_2V	N_2V	N_2V	N_2V
	N_2V	N_1C	N_2F	N_2F	N_1C	N_1C
	N_2F	N_2F	N_1C	N_1C	N_2F	N_2F
3	N_2V	N_2V	N_2V	N_2V	N_2F	N_1C
	N_1C	N_1C	N_1C	N_2F	N_2V	N_2F
	N_2F	N_2F	N_2F	N_1C	N_1C	N_2V
4	N_2F	N_2F	N_2F	N_1C	N_1C	N_2V
	N_1C	N_2V	N_2V	N_2V	N_2F	N_1C
	N_2V	N_1C	N_1C	N_2F	N_2V	N_2F

These applications were broadcast by hand and raked into the topsoil before irrigation (Figure 3.2). Micro-sprinkler irrigation was used, with micro-jets spaced 2 meters apart and 3 meters between rows. Irrigation was applied directly after each nitrogen application. Soil water levels were monitored weekly with tensiometers at 300 mm and 600 mm of soil depths.

FIGURE 3.2 : Nitrogen applications being raked into the topsoil and washed in with an irrigation immediately thereafter; Nitrogen fertilisation trial, Elsenburg, Stellenbosch

3.3 COLLECTION OF DATA

Soil and leaf samples: Soil samples were taken before commencing differential fertilisation in the trial to determine the general fertility and chemical properties of the soil (Table 3.1). These samples were analysed for : pH (1.0 M KCl), P (Bray no.2), Na, K, Ca, and Mg (extracted with 1.0 M ammonium acetate) and organic C (The Non-affiliated Soil Analysis Work Committee, 1990). During the trial soil samples were taken 2 weeks after each nitrogen application for the increments 0 – 15 cm, 15 – 30 cm and 30 – 60 cm using procedures described by Bundy & Malone (1988). Composite soil samples were made up by combining two randomly chosen replications of each treatment at each cultivar for analysis (Figure 3.3). These samples were thoroughly mixed and analysed for the different nitrogen components, using the methods of the Non-affiliated Soil Analysis Work Committee (1990). Tensiometers were installed for the 30 cm and 60 cm depth intervals and measurements were taken once a week.

Leaf samples were collected, during the second season only, between veraison and harvest by taking undamaged leaves opposite bunches. Bulk samples for analysis were made up by combining samples from two randomly chosen replications of each treatment combination. As described by Conradie (1994), the leaf blades and petioles were immediately separated and placed in plastic bags (Figure 3.3) for ammonium and nitrate analysis. Total nitrogen content was determined on leaf blades and petioles mixed together, using a Leco Nitrogen Determinator.

A **B**

FIGURE 3.3 : Soil samples (A) were taken two weeks after nitrogen application and leafblade and petiole samples (B) taken at veraison

Crop load and pruning mass: Crop mass was controlled by winter pruning and suckering of vines during early summer. Vines were pruned to 0, two node spurs, per vine spaced approximately 12 cm apart. Pruning was done as near to the 15 th of July as possible. The shoot mass of each vine was measured following pruning each season. Vines were suckered to 2 shoots per spur at 5 – 30 cm shootlength (Figure 3.4).

FIGURE 3.4 : Vines suckered to 2 shoots per spur to control harvest mass and to ensure good light penetration into the canopy; Nitrogen fertilisation trial, Elsenburg, Stellenbosch

Cluster and berry mass: Plots were harvested as near as possible to 22 °B as determined by refractometer. At harvest mass and number of bunches were recorded on an individual vine basis. To determine the average berry mass, 100 berries were randomly selected from each vine and weighed. The number of berries per bunch was calculated by dividing average cluster mass by mean berry mass.

Shoot growth and canopy manipulations: Two shoots per vine were randomly selected prior to the first N application and total length was measured at 7 day intervals to determine shoot growth. All trial vines were simularly manipulated to avoid differences in canopy density and excessive growth removed (Figure 3.5). Lateral shoots in the harvesting zone were removed at the beginning of December to improve light penetration. Except for shoots marked to measure shoot growth, all shoots were topped to 1.1 m at the beginning of December.

FIGURE 3.5 : Uniform canopy of the vines at veraison; Nitrogen fertilisation trial, Elsenburg, Stellenbosch

Shoots were positioned continually during the growth season. A normal precautionary spraying program against diseases was followed according to IPW (Anonymous, 1998) guidelines.

Harvest and must analysis: The bunches of each plot were crushed and pressed twice to 1.2 kPa. The must was collected and analysed for soluble solids, titratable acid, pH and FAN. Soluble solids were determined by refractometer and expressed as degree Brix (°B). Titratable acid (expressed in g l^{-1}) and pH were determined by titration to pH 7, while the FAN concentrations were determined by means of an automated ninhydrin method described by Lie (1973).

Statistical analysis : Data were analysed by analysis of variance, to determine the significance of differences between means of treatments using the SAS (Statistical Analysis Systems, SAS Institute Inc.) program and Tukey's test.

CHAPTER 4

THE EFFECT OF NITROGEN FERTILISATION ON THE GROWTH CHARACTERISTICS OF *Vitis vinifera*

Abstract

A N fertilisation study was carried out on a loam soil with an organic matter content of 1.4 % at Elsenburg, Stellenbosch, RSA. Nitrogen fertilisation was applied after budbreak, fruitset and veraison to *Vitis vinifera* L. cv. Chenin blanc, Weisser Riesling, Chardonnay, Pinotage, Pinot noir and Cabernet Sauvignon. This was done during the 1999/2000 (1st season) and 2000/2001 (2nd season) seasons and shoot length, shoot elongation, pruning mass and bunch mass were measured. Results indicated that N applications had a significant effect on shoot length, shoot elongation and bunch mass, but not on pruning mass. Cultivar also had a significant effect on shoot length, shoot elongation, bunch and pruning mass. It seems that the general distribution of N was the same for the six cultivars although variables like soil and climatic conditions, phenological stages of the vine and competition from bunches might all have affected the shoot length and elongation patterns of individual cultivars and between the two seasons.

KEY WORDS : Nitrogen fertilisation, cultivar, shoot length, shoot elongation, pruning mass, bunch mass

4.1 INTRODUCTION

Nitrogen is the element most likely to be associated with vigorous growth, that might alter the leaf: fruit ratio, increase canopy humidity, and reduce sunlight penetration to inner leaves and berries. It seems that the pattern of N distribution are genetically determined and applications at veraison and during spring are distributed in the same way (Conradie, 1985). Marocke *et al.* (1977), Conradie (1985) and Christensen *et al.* (1994) concluded that N concentration in specific organs was correlated to an increase or decrease of dry matter content.

Within a given year, N applications did not have a significant affect on yield, yield components or pruning mass of vines (Morris *et al.*, 1983; Spayd *et al.*, 1993). In spite of an obvious tendency for unfertilised vines to have a lower shoot mass than vines receiving fertiliser Saayman & Conradie (1982) could, however not find significant differences in the mean shoot mass of vines receiving different N applications in any particular year. This may indicate that the natural supply of N from the soil was enough for the vine without any inorganic applications. According to Conradie & Saayman (1989a), N fertilisation increased crop mass, while they also measured a 16% increase in shoot mass when fertilising with 56 kg N ha^{-1} a^{-1} instead of 16 kg N ha^{-1} a^{-1}. Results of Freeman, Lee & Turkington (1979) indicated that yield is directly related to vine vigour as measured by shoot diameter and shoot length. According to Bravdo *et al.* (1984), the effect of the yield on the growth of vines and quality of the fruit and the wine was not always found to be consistent.

Conflicting research results with N fertilisation may be related to differences in canopy density (vigour) and crop mass of the vine (Spayd *et al.*, 1994). According to Conradie (1990), Spayd *et al.* (1993), Percival, Fisher & Sullivan (1994) and Spayd *et al.* (1994), the impact of N fertilisation on the crop mass, vigour, and must and wine composition depends on the N status of the soil prior to manipulation, climate, cultivar, rootstock and other viticultural practices. During this study, vines were manipulated to ensure uniform canopy densities and number of bunches per vine. Cultivars were planted on a uniform soil and grafted on the same rootstock.

The primary objective of this study was to measure the effect of N applications during the growing season on the growth characteristics of six cultivars. This was done by measuring shoot length, shoot elongation, bunch and pruning massand testing the significance of means for treatment combinations.

4.2 RESULTS AND DISCUSSION

4.2.1 Shoot length

A summary on the analyses of variance that were done to establish the effect of the three N treatments on the shoot length of the six different vine cultivars, is presented in Table 4.1. In both seasons there were no significant interaction between N treatments and vine cultivar at any time of measurement, except for the 13th November measurement in the 1st season, therefore N and cultivarmain effects are discussed seperately. However, the N treatments affected shoot length significantly during the 1st season at almost every measurement, which was not the case during the 2nd season. A similar trend was observed with shoot length between the vine cultivars with far more significant differences in the 1st than 2nd season. The detail effects of the N treatments and vine cultivars on shoot length are discussed in the following two sections, respectively.

TABLE 4.1 : Summary of the effect of nitrogen fertilisation on the mean shoot length of different cultivars over two seasons; Elsenburg, Stellenbosch

Time of shoot measurement		Nitrogen treatments		Cultivar		Nitrogen treatments x Cultivar	
1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
30-Oct	29-Oct	ns	ns	✓	✓	ns	ns
06-Nov	05-Nov	✓	ns	✓	✓	ns	ns
13-Nov	12-Nov	✓	ns	✓	✓	✓	ns
20-Nov	19-Nov	✓	ns	✓	ns	ns	ns
27-Nov	29-Nov	✓	ns	✓	ns	ns	ns
04-Dec	05-Dec	✓	ns	✓	ns	ns	ns
11-Dec	13-Dec	✓	ns	✓	ns	ns	ns
18-Dec	20-Dec	✓	ns	✓	ns	ns	ns
25-Dec	28-Dec	✓	✓	✓	ns	ns	ns
01-Jan	4-Jan	✓	✓	✓	ns	ns	ns

- Significance indicated within (LSD Tukey ≥ 0.05)

  ✓ = indicating significant differences

  ns = indicating no significant differences

4.2.1.1 Nitrogen treatments

The effect of N treatments on the shoot length of the different vine cultivars for both seasons are summarised in Table 4.2. Differences as a result of N treatments were far more prominent in the 1st than in the 2nd season (Figure 4.1).

TABLE 4.2 : The effect of nitrogen fertilisation at different growth stages on the mean shoot length (cm) of six different cultivars during two seasons; Elsenburg, Stellenbosch

Time of shoot measurement	Nitrogen treatments			LSD
	N_1C	N_2F	N_2V	
	1st Season (1999/2000)			
30-Oct	57.90 a	59.23 a	58.85 a	3.17
06-Nov	74.00 a	77.67 b	77.06 b	2.60
13-Nov	89.98 a	94.58 b	92.94 b	2.93
20-Nov	106.15 a	115.40 b	110.58 a	4.79
27-Nov	116.58 a	132.00 b	124.50 c	6.69
04-Dec	126.15 a	148.00 b	137.52 c	7.70
11-Dec	133.58 a	161.13 b	150.52 c	9.23
18-Dec	139.17 a	169.40 b	157.75 b	10.37
25-Dec	143.98 a	177.73 b	165.84 c	11.14
01-Jan	146.90 a	184.81 b	175.04 b	11.09
	2nd Season (2000/2001)			
29-Oct	74.87 a	76.40 a	75.73 a	5.02
05-Nov	91.64 a	90.19 a	90.39 a	8.50
12-Nov	110.26 a	106.77 a	105.98 a	11.41
19-Nov	122.72 a	119.14 a	116.27 a	13.03
29-Nov	130.03 a	133.19 a	127.73 a	17.48
05-Dec	133.70 a	143.52 a	137.14 a	17.86
13-Dec	136.71 a	149.13 a	144.15 a	19.43
20-Dec	142.47 a	158.30 a	150.79 a	20.75
28-Dec	143.44 a	165.63 b	157.14 ab	22.16
4-Jan	145.01 a	169.98 b	163.60 ab	22.80

- Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

In the 1st season, from about 10 days before the fruitset, N application until about 3 weeks after the veraison N application, the shoots of the N_2F and N_2V treatments were significantly

longer than the shoots of the N1C treatments (Figure 4.1). From about 10 days after the fruitset N application until about 3 weeks after the veraison N application, the shoots of the N2F treatments were also significantly longer than the shoots of the N2V treatments (exceptions occurred on the 18th of December and the 1st of January.

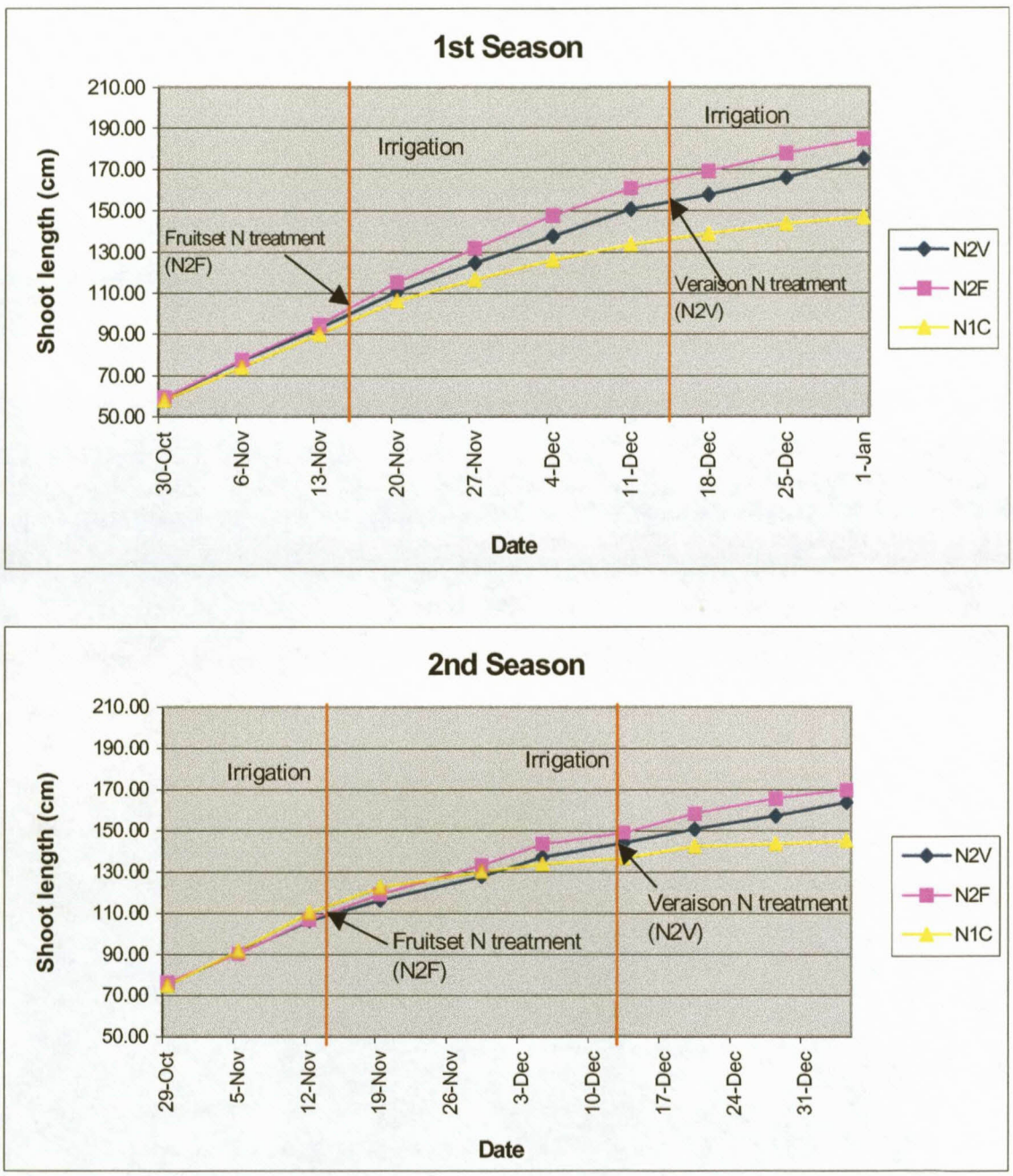


FIGURE 4.1 : The effect of nitrogen fertilisation at different growth stages on the shoot length of vines during the two seasons; Elsenburg, Stellenbosch

In the 2nd season there were no significant differences in shoot length until the last two weeks of measurement. During this period the shoots of the N1C treatments were generally shorter than the shoots of both the N2F and N2V treatments, N2F tending to be longer than that of N2V. However, from about 10 days after the fruitset N application the general pattern of shoot length in this season is similar to that in the 1st season.

The positive response of shoot length to the application of 20 kg N ha^{-1} after budbreak corresponds to the findings of Glad *et al.* (1994). This response was observed relatively late in the 1st season, from about 10 days before the fruitset N application, possibly as a result of high water and low nitrate contents in the soil which retarded vegetative growth (Conradie, 1997). In the 2nd season this response was observed even later. This could possibly be the result of better reserve buildup in the shoots of the N1C treatments at the end of the 1st season because of less vegetative growth. In contrast the strong vegetative growth of the N2F and N2V treatments might have resulted in less reserve buildup in the 1st season and poorer vegetative growth in the initial part of the 2nd season (Winkler *et al.*, 1974; Glad *et al.*, 1994). This late response is not surprising since Conradie (1990) reported that when N was applied at budbreak, uptake was not quick enough to result in significant differences at bloom.

The results also indicate that the application of 20 kg N ha^{-1} at fruitset resulted in longer shoots during the 1st season, while the application of 20 kg N ha^{-1} at veraison did not have the same effect. This phenomenon can be attributed to the fact that the vegetative growth of the vine is much stronger at fruitset than at veraison (Van Zyl, 1981; Kliewer *et al.*, 1983) and vines were therefore able to use the applied N more efficient during fruitset than at veraison.

4.2.1.2 Cultivar

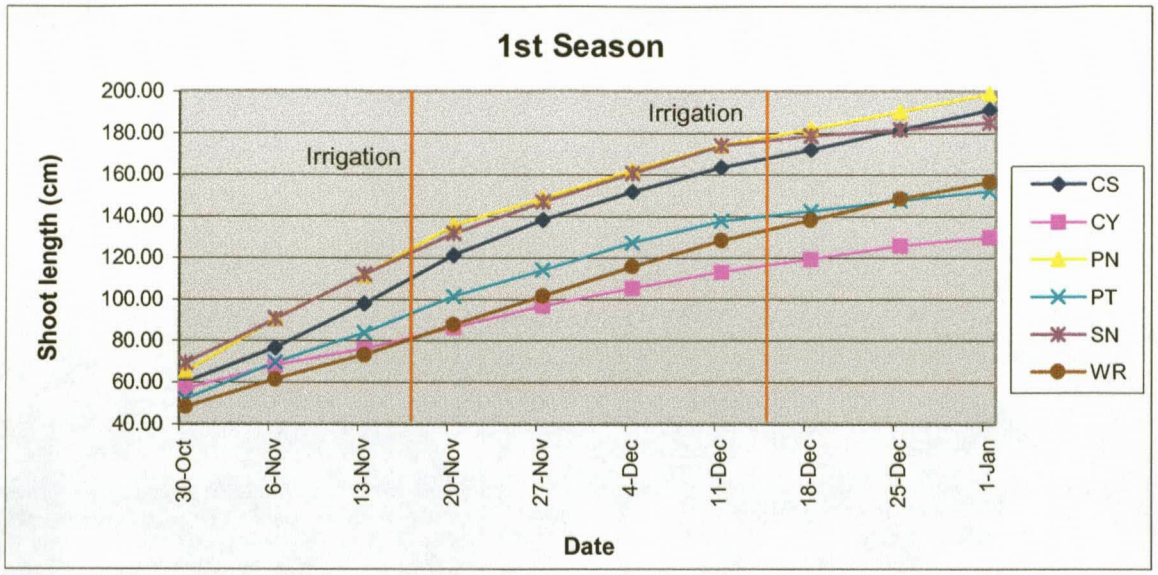
The shoot length of different cultivars during the two seasons are summarised in Table 4.3. In the 1st season, although initially shorter, the shoot of Chenin blanc, Cabernet Sauvignon, Pinotage and Pinot noir were eventually longer than in the 2nd season. This was not the case for Chardonnay and Weisser Riesling.

TABLE 4.3 : The shoot lengths (cm) of six different cultivars in a nitrogen fertilisation trial during two seasons; Elsenburg, Stellenbosch

Date	Cultivar						LSD
	CS	CY	PN	PT	SN	WR	
	1st Season						
30-Oct	60.04 ab	57.04 bc	65.21 ad	52.13 ce	69.17 d	48.38 e	5.35
06-Nov	76.54 a	68.17 b	90.79 c	69.83 b	90.54 c	61.58 d	5.08
13-Nov	97.83 a	76.17 b	111.79 c	84.00 d	111.92 c	73.29 b	7.29
20-Nov	121.29 a	86.38 b	135.54 c	101.58 d	131.75 c	87.71 b	8.06
27-Nov	138.21 a	96.88 b	148.50 a	114.17 c	146.83 a	101.58 bc	13.35
04-Dec	151.71 a	105.63 b	162.25 a	127.50 c	160.71 a	115.42 bc	16.77
11-Dec	163.25 a	113.17 b	174.17 a	137.83 c	173.83 a	128.21 bc	20.88
18-Dec	171.92 a	119.25 b	182.29 a	142.42 c	178.54 a	138.21 bc	21.31
25-Dec	181.42 a	125.88 b	190.58 a	147.58 b	181.40 a	148.25 b	22.43
01-Jan	191.17 a	129.88 b	199.00 a	152.00 bc	185.00 a	156.46 c	22.61
	2nd Season						
29-Oct	68.19 a	89.46 c	81.00 bc	61.15 a	71.58 ab	82.60 bc	11.69
05-Nov	81.40 ab	102.19 c	98.74 bc	72.61 a	86.52 abc	102.96 c	17.71
12-Nov	95.73 ab	120.42 c	116.13 bc	89.08 a	100.21abc	124.44 c	24.35
19-Nov	113.77 abc	132.67 bc	129.17 bc	98.50 a	104.25 ab	137.90 c	29.11
29-Nov	130.96 ab	144.04 b	142.38 b	105.96 a	112.92abc	145.64 b	34.59
05-Dec	146.75 ab	148.83 ab	149.65 ab	115.56 b	116.29 ab	151.64 a	35.53
13-Dec	159.17 a	153.83abc	151.46abc	120.96 bc	118.33 c	156.24 ab	37.84
20-Dec	168.56 a	158.69 ab	158.13 ab	129.75 ab	124.29 b	163.69 ab	39.75
28-Dec	175.65 a	164.15 ab	161.04 ab	135.44 ab	125.96 b	170.17 a	43.91
4-Jan	182.25 a	167.21 ab	164.92 ab	140.35 ab	129.42 b	173.04 ab	44.91

Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

Cabernet Sauvignon, Pinot noir and Chenin blanc had simular general growth patterns during the the 1st season (Figure 4.2). The shoot lengths of Pinot noir and Chenin blanc did not differ significantly during this period. Weisser Riesling and Pinotage also followed similar growth patterns, but with no consistency with regard to differences in shoot length. Initially the shoot lengths of Chardonnay were longer than that of Weisser Riesling, until the 1st irrigation, after which the opposite prevailed. From the 2nd irrigation, the growth rate of Pinotage and Chenin blanc shoots declined relative to that of the other four cultivars.



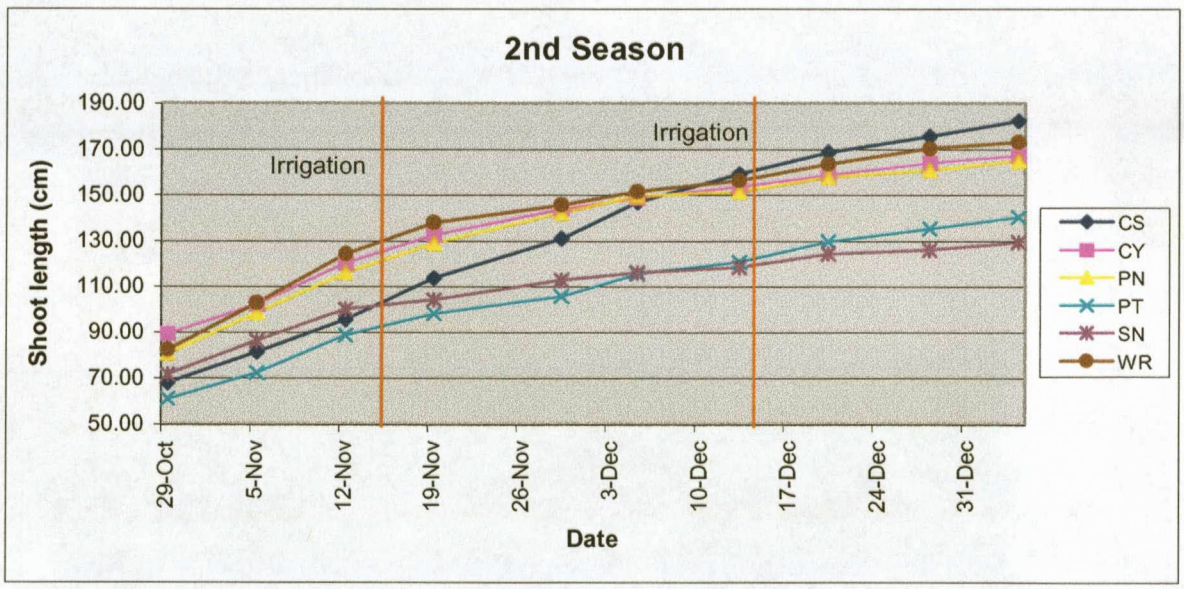


FIGURE 4.2 : The response of shoot length of different cultivars on nitrogen treatments over two seasons; Elsenburg, Stellenbosch

During the 2nd season the growth pattern of Chardonnay, Pinot noir and Weisser Riesling were very similar and their shoot lengths never differed significantly from each other. The shoot lengths of Pinotage and Chenin blanc also did not differ significantly from each other

during the whole period but were shorter compared to that of the other four cultivars. Compared to Cabernet Sauvignon, the shoot growth of all other cultivars declines significantly after the 1st irrigation.

These above results indicate that the six grapevine cultivars differ in vigour and growth pattern, confirming similar reports by Orffer (1979), Carstens, Burger & Kriel (1981) and De la Harpe & Visser (1985). General soil and climatic conditions during the 1st season seemed to have been more favourable for vegetative growth when compared to the 2nd season. Seasonal differences might have a major effect on the vegetative and reproductive characteristics of the vine.

Results also indicate that the shoot growth of Pinotage and Chenin blanc were retarded during both seasons, *viz.* from the 2nd irrigation in the 1st season and from the 1st irrigation in the 2nd season. Winkler *et al.* (1974), Freeman *et al.* (1979), Conradie, 1990 and Christensen *et al.* (1994) found that yield is negatively related to the vigour of the vine as measured by shoot length. This was not the case with Cabernet Sauvignon, especially in the 2nd season. Orffer (1979) and De Villiers (1986) describe Cabernet Sauvignon as a much more vigorous cultivar compared to the other five cultivars. Yield had no major effect on the growth characteristics of Cabernet Sauvignon in this study.

4.2.2 Weekly shoot elongation

A summary on the analyses of variance that were done to establish the effect of the three N treatments on the weekly shoot elongation of the six different vine cultivars is presented in Table 4.4. In both seasons there were no significant interaction between N treatments and vine cultivar at any time of measurement, except for the 13th November measurement in the 1st season, therefore N and cultivar main effects are discussed seperateley. The N treatments affected weekly shoot elongation during the 1st season significantly at almost every measurement, which was not the case during the 2nd season. These trends are similar to that described for the effect of N treatments on shoot length. However, the weekly shoot elongation rate was almost at every measurement in both seasons significantly affected by

cultivar. The detail effects of the N treatments and grape cultivars on weekly shoot elongation is discussed in the following two sections.

TABLE 4.4 : Summary of the effect of nitrogen on the weekly shoot elongation over two seasons of six different grape cultivars; Elsenburg, Stellenbosch

Time of shoot measurement		Nitrogen treatment		Cultivar		Nitrogen treatment x Cultivar	
1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
30-Oct	29-Oct	ns	ns	✓	✓	ns	ns
06-Nov	05-Nov	✓	ns	✓	✓	ns	ns
13-Nov	12-Nov	ns	ns	✓	✓	✓	ns
20-Nov	19-Nov	✓	ns	✓	✓	ns	ns
27-Nov	29-Nov	✓	ns	✓	✓	ns	ns
04-Dec	05-Dec	✓	ns	ns	✓	ns	ns
11-Dec	13-Dec	✓	✓	ns	✓	ns	ns
18-Dec	20-Dec	✓	✓	✓	✓	ns	ns
25-Dec	28-Dec	✓	✓	✓	ns	ns	ns
01-Jan	4-Jan	✓	✓	✓	✓	ns	ns

- Significance indicated within colomns (LSD Tukey ≥ 0.05)
  ✓ = indicating significant differences
  ns = indicating no significant differences

4.2.2.1 Nitrogen treatments

The effect of N treatments on the weekly shoot elongation of the different vine cultivars for two seasons are summarised in Table 4.5. As already mentioned, differences as a result of N treatments are far more prominent in the 1st than the 2nd season (Table 4.4).

TABLE 4.5 : The effect of nitrogen fertilisation on the weekly shoot elongation (cm) of six grapevine cultivars during the two seasons; Elsenburg, Stellenbosch

Time of shoot measurement	Nitrogen treatments			LSD
	N_1C	N_2F	N_2V	
	1st Season			
30-Oct	12.25 a	13.25 a	12.44 a	1.51
06-Nov	16.10 a	18.02 b	17.79 b	1.63
13-Nov	15.98 a	16.92 a	15.88 a	2.57
20-Nov	16.17 a	20.81 b	17.65 ab	3.37
27-Nov	11.69 a	17.23 b	13.92 ab	3.69
04-Dec	9.56 a	16.04 b	13.02 ab	4.25
11-Dec	7.92 a	13.13 b	13.00 b	3.04
18-Dec	5.15 a	7.90 b	7.83 ab	2.71
25-Dec	4.77 a	8.33 b	8.09 b	1.71
01-Jan	2.92 a	7.08 b	9.20 c	1.99
	2nd Season			
29-Oct	13.40 a	16.10 a	13.25 a	3.07
05-Nov	16.18 a	15.67 a	15.08 a	4.98
12-Nov	18.46 a	16.50 a	15.88 a	4.22
19-Nov	13.02 a	12.37 a	10.29 a	4.05
29-Nov	9.08 a	14.05 a	13.22 a	6.29
05-Dec	6.48 a	10.52 a	9.51 a	4.43
13-Dec	5.43 a	8.01 b	6.76 a	2.54
20-Dec	4.49 a	8.84 b	6.97 ab	2.66
28-Dec	3.01 a	7.33 b	6.34 b	1.87
4-Jan	1.30 a	4.52 b	6.68 c	2.00

- Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

The pattern of elongation rate were generally the same for N treatments as described for shoot length in the 1st season, *viz.* the weekly shoot elongation of the N_2F and N_2V treatments being more than that of the N_1C treatments (Figure 4.3). However, the N_2V treatment, in most cases, did not differ significantly from either the N_1C or the N_2F treatment. An exception occurred four weeks after the veraison nitrogen application when, during both seasons, a significantly higher elongation rate occurred compared to that of the N_2F and N_1C treatments.

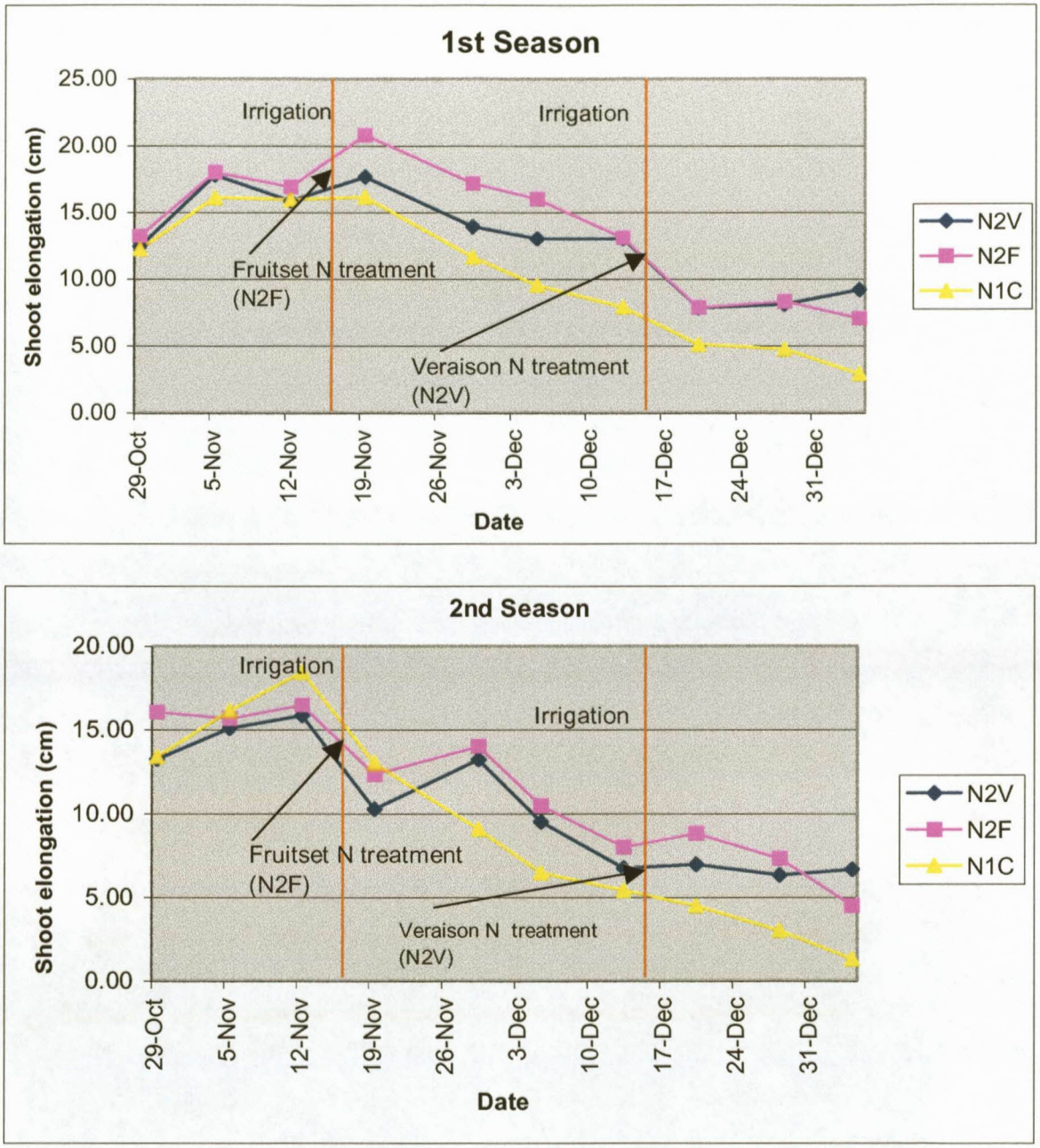


FIGURE 4.3 : Effect of nitrogen treatments on the weekly shoot elongation during the two seasons; Elsenburg, Stellenbosch

The reaction of the weekly shoot elongation to N treatments during the 2nd season was generally the same when compared to that of shoot length. In spite of the effect of N applications and irrigation, the weekly shoot elongation of all treatments reached a peak about a week after the fruitset N application in the 1st season, compared to a week before the fruitset N application in the 2nd season. Thereafter the weekly shoot elongation declined through the rest of the season.

In both seasons the elongation rate seemed to be in a downward trend when the fruitset and veraison N applications were applied. After the N applications and irrigations the elongation rate of the N_2F and N_2V shoots showed an increase (in the case of the fruitset N application) or stabilised (in the case of the veraison N application). The elongation rate of shoots of the N_1C treatment did not show this positive reaction after N applications and irrigations. After the veraison N application the weekly shoot elongation followed exactly the same pattern of significance during both seasons. It is important to note that the application of 20 kg N ha^{-1} during veraison resulted in the significantly higher elongation rate of N_2V when compared to N_2F for the last time of measurement. This is in contrast with findings of Christensen *et al.* (1994) that fertilisation at veraison may have little effect on vegetative growth due to a higher demand from the bunches. The reasons for the vegetative growth reaching a peak later in the 1st season than the 2nd season, were already discussed in the previous section.

The results also indicate that irrigation had a more positive effect on the weekly shoot elongation when vines received N fertilisation during the vegetative season, confirming similar suggestions of Van Zyl (1981) and Morris *et al.* (1983).

4.2.2.2 Cultivar

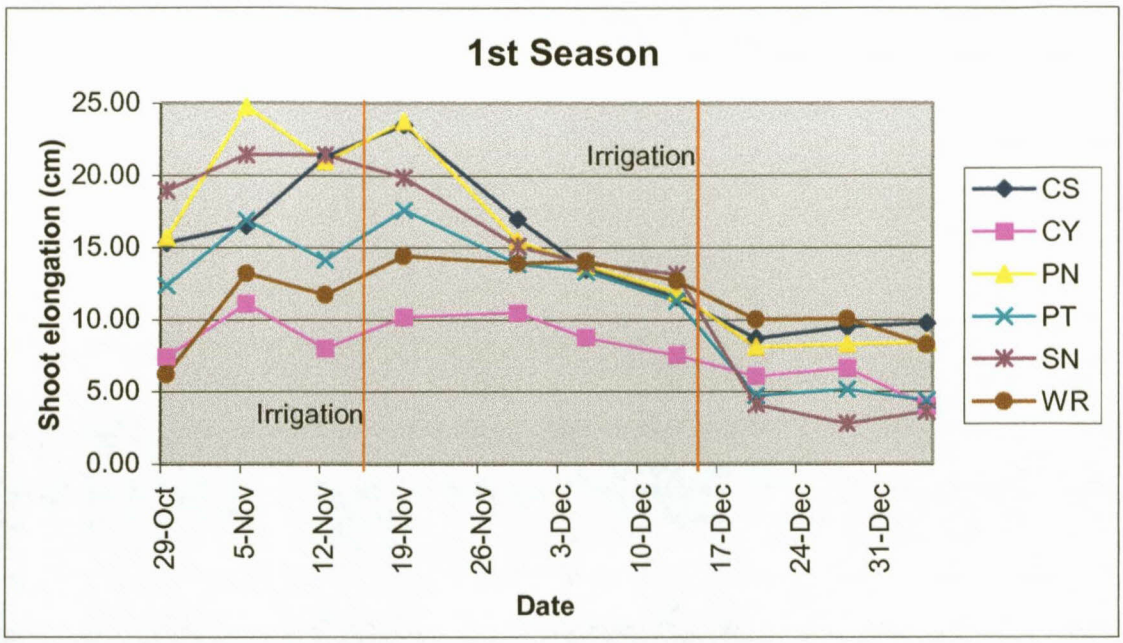
The shoot elongation rate of the different cultivars during the two seasons is summarised in Table 4.6. In the 1st season, although initially slower, the weekly shoot elongation of Chardonnay, Cabernet Sauvignon, Weisser Riesling and Pinot noir were faster than that of the 2nd season at the last time of measurement. This was not the case with Chenin blanc and Pinotage.

TABLE 4.6 : Weekly shoot elongation (cm) of six different cultivars in a nitrogen fertilisation trial during the two seasons; Elsenburg, Stellenbosch

Date	Cultivar						LSD
	CS	CY	PN	PT	SN	WR	
	1st Season						
30-Oct	15.33 bc	7.42 d	15.67 b	12.38 c	18.92 a	6.17 d	3.02
06-Nov	16.50 a	11.13 b	24.75 c	16.88 a	21.38 d	13.21 b	3.25
13-Nov	21.29 a	8.00 c	21.00 a	14.17 b	21.38 a	11.71 bc	4.19
20-Nov	23.46 ab	10.21 b	23.75 a	17.58 cd	19.83 bc	14.42 d	3.84
27-Nov	16.92 a	10.50 b	15.46 ab	13.83 ab	15.08 ab	13.88 ab	6.37
04-Dec	13.56 a	8.75 a	13.75 a	13.33 a	13.88 a	14.04 a	6.37
11-Dec	11.54 a	7.54 a	11.92 a	11.29 a	13.13 a	12.67 a	5.99
18-Dec	8.67 ab	6.08 bc	8.13 ab	4.75 c	4.13 c	10.00 a	3.19
25-Dec	9.50 a	6.63 bc	8.29 ab	5.17 cd	2.77 d	10.04 a	2.46
01-Jan	9.75 a	4.00 b	8.42 a	4.42 b	3.61 b	8.21 a	1.99
	2nd Season						
29-Oct	16.50 ab	11.70 bc	13.30 bc	14.10 abc	11.00 c	18.60 a	5.04
05-Nov	14.80 abc	15.60 abc	17.70 ab	11.40 c	13.70 bc	20.30 a	6.25
12-Nov	14.70 ab	18.23 ab	17.39 ab	16.49 ab	13.35 b	21.48 a	7.70
19-Nov	18.05 a	12.25 ab	12.20 ab	9.40 ab	6.00 b	13.45 ab	8.79
29-Nov	20.21 a	10.54 b	14.04 ab	8.21 b	8.33 b	11.34 b	8.31
05-Dec	15.79 a	6.50 b	5.02 b	10.25 ab	6.73 b	8.73 b	6.13
13-Dec	12.42 a	5.00 bc	4.06 c	7.27 b	5.08 bc	6.55 bc	3.16
20-Dec	9.30 a	4.80 bc	6.60 abc	8.70 a	3.70 c	7.00 ab	3.20
28-Dec	7.25 a	5.45 a	2.92 a	7.02 a	4.21 a	6.52 a	4.62
4-Jan	6.60 a	3.06 b	3.88 b	4.92 ab	3.46 b	3.08 b	2.68

- Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

Although not always significant, cultivars followed the same general pattern of weekly shoot elongation (Figure 4.4). Cabernet Sauvignon and Pinot noir did not differ significantly from each other from the 1st irrigation onwards. From the 2nd irrigation the weekly elongation rate of Pinotage and Chenin blanc declined relatively to the other four cultivars.



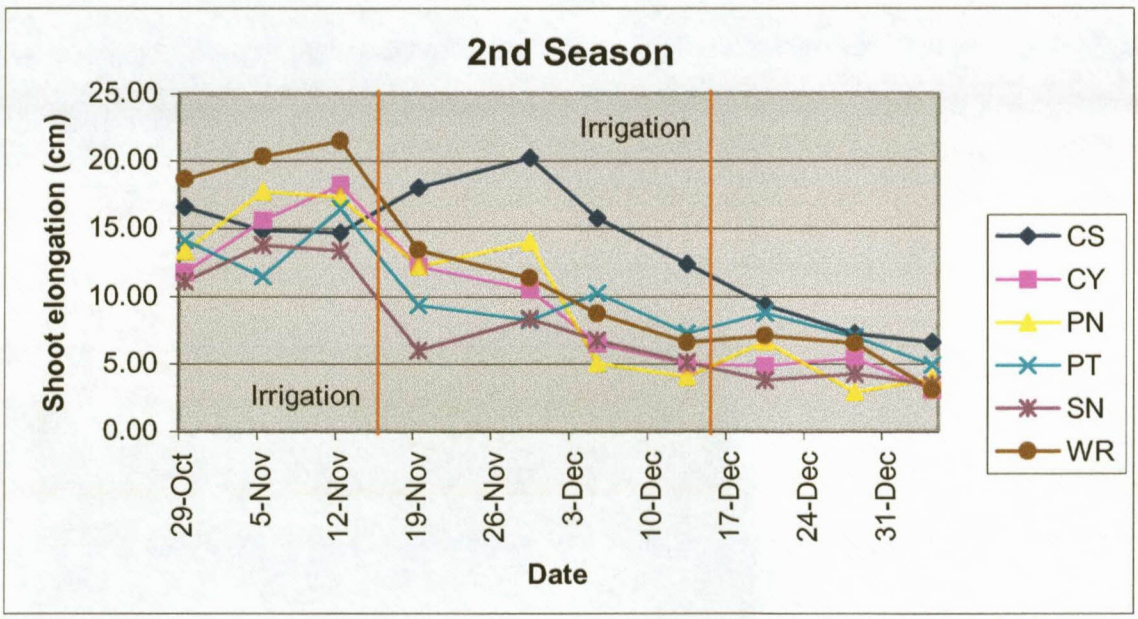


FIGURE 4.4 : The weekly shoot elongation of the different cultivars of two seasons; Elsenburg, Stellenbosch

With the exception of Cabernet Sauvignon, the weekly shoot elongation of cultivars in the 2nd season also followed the same general growth pattern. There were no significant

differences between the elongation rates of Chardonnay, Pinot noir and Weisser Riesling from about two weeks before the 1st irrigation until the end of measurement. The shoot elogation of Pinotage and Chenin blanc never differed significantly from each other during the whole 2nd season (last date of measurement excluded).

In contrast with the 1st season, Weisser Riesling had the highest weekly elongation until the 1st irrigation in the 2nd season. Between the 1st and 2nd irrigation all cultivars except Cabernet Sauvignon showed a steep downward trend in weekly shoot elongation. Although the significance between means of cultivars may differ, for these above mentioned results imply that the conclusions reached of shoot length are also applicable to weekly shoot elongation.

The steep downward trend in the weekly elongation rate between the two irrigations was not surprisingly when the rate of berry development during phase II is taken into account (Archer, 1981a) and therefore confirm findings of Conradie (1991) and Winkler *et al.* (1974) that the feeding of flowerbunches act as a major sink during the above mentioned period and may have a negative effect on vegetative growth (Freeman *et al.*, 1979; Christensen *et al.*, 1994).

4.2.3 Pruning mass

A summary on the analyses of variance that were done to establish the effect of the three N treatments on the pruning mass of the six different cultivars is presented in Table 4.7. In both seasons there were no significant interaction between N treatments and cultivars, and N and cultivar effects could be looked at seperately. The pruning mass of the vines were significantly affected by the different cultivars during both seasons. In contrast the N treatments had no significant effect on the pruning mass.

TABLE 4.7 : Summary of the effect of nitrogen fertilisation and cultivar on the mean pruning mass of six grapevine cultivras over two seasons; Elsenburg, Stellenbosch

Variable	1st Season	2nd Season
Cultivar	✓	✓
Nitrogen treatments	ns	ns
Cultivar x Nitrogen treatments	ns	ns

4.2.3.1 Nitrogen treatments

Results presented in Table 4.8 indicate that N treatments did not have a significant effect on the pruning mass of the six cultivars in any particular year. Simular results were found by Saayman & Conradie (1982), Morris *et al.* (1983) and Spayd *et al.* (1993).

TABLE 4.8 : Effect of nitrogen fertilisation on the pruning mass (g) of six grapevine cultivars over two seasons; Elsenburg, Stellenbosch

Season	Nitrogen treatments			LSD
	N_1C	N_2F	N_2V	
1st season	0.873 a	0.911 a	0.840 a	0.108
2nd season	0.887 a	0.859 a	0.862 a	0.103

- Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

4.2.3.2 Cultivar

The effect of cultivar on the pruning mass of the six grapevine cultivras is summarised in Table 4.9. During both seasons the pruning mass of Cabernet Sauvignon was significantly higher than that of the other cultivars.

TABLE 4.9 : The pruning mass (g) of six different cultivars in a nitrogen fertilisation trial over two seasons; Elsenburg, Stellenbosch

Cultivar	1st Season	2nd Season
Cabernet Sauvignon	1.443 a	1.408 a
Chardonnay	0.723 b	0.759 bc
Pinotage	0.731 b	0.690 bc
Pinot noir	0.862 b	0.828 bc
Chenin blanc	0.742 b	0.653 c
Weisser Riesling	0.746 b	0.878 b
LSD	**0.193**	**0.221**

- Values within rows, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

Cabernet Sauvignon, Weisser Riesling and Pinot noir were always the three cultivars with the highest pruning mass. The pruning mass of Chenin blanc was significantly lower compared to Weisser Riesling and Cabernet Sauvignon during the 2nd season. When the results are compared to the pruning mass quoted for these cultivars by De Villiers (1985; 1986; 1987), Weisser Riesling should have the highest shoot mass followed by Cabernet Sauvignon and Chenin blanc. The reason for the much higher pruning mass of Cabernet Sauvignon and the lower pruning mass of Chenin blanc can be also related to their vegetative growth characteristics discussed in previous sections. These confirm results of Kliewer *et al.* (1989) that indicated that cane pruning mass was related to shoot growth. Although not always significant, Pinotage and Chenin blanc had a lower pruning mass during both seasons.

4.2.4 Bunch mass

Except for Pinot noir and Cabernet Sauvignon, the bunch mass of all cultivars differed significantly during the 1st season with no significant interaction (Table 4.10). Chenin blanc

and Pinotage had the highest bunch mass, while that of Cabernet Sauvignon and Weisser Riesling were the lowest.

TABLE 4.10 : The effect of cultivar in a nitrogen fertilisation trial on the mean bunch mass during the 1999/2000 season; Elsenburg, Stellenbosch

Cultivar	Bunch mass (g)
CS	143 b
CY	176 c
PN	145 b
PT	221 d
SN	320 e
WR	118 a
LSD	**19.38**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

The N1C treatments resulted in significantly lower bunch mass compared to the N2F and N2V treatments (Figure 4.5). However, the bunch mass of the N2F and N2V treatments did not differ significantly from each other.

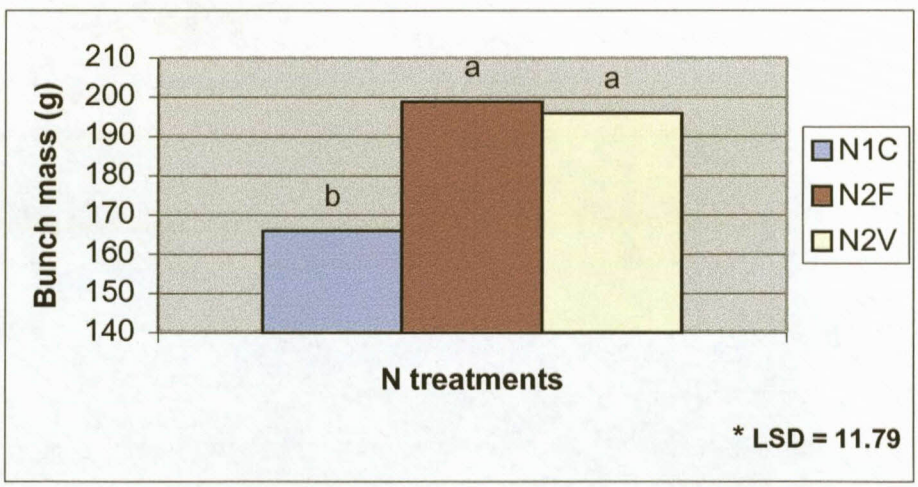


FIGURE 4.5 : The effect of nitrogen fertilisation on the mean bunch mass of six wine grape cultivars during the 1999/2000 season; Elsenburg, Stellenbosch

- Bars with simular letters do not differ significantly from each other (LSD Tukey ≥ 0.05)

The effect of the three N treatments on the bunch mass of the six cultivars in the 2nd season is presented in Table 4.11 with significant interaction between N treatments and cultivars. Nitrogen treatments did not have a significant effect on the bunch mass of Cabernet Sauvignon, Chenin blanc and Weisser Riesling. The N_1C treatments resulted in significantly lower bunch mass for Chardonnay, Pinot noir and Pinotage. For these cultivars, the N_2F and N_2V treatments did not differ significantly.

TABLE 4.11 : The effect of nitrogen fertilisation on the mean bunch mass of six different cultivars during the 2000/2001 season; Elsenburg, Stellenbosch

Cultivar	Nitrogen treatments	Bunch mass (g)	Significance
CS	**N_1C**	170.07	d c
CS	**N_2F**	154.73	d e
CS	**N_2V**	166.53	d c
CY	**N_1C**	155.27	d
CY	**N_2F**	175.52	c
CY	**N_2V**	176.43	c
PN	**N_1C**	95.72	g
PN	**N_2F**	127.75	f
PN	**N_2V**	115.93	f
PT	**N_1C**	183.87	c
PT	**N_2F**	215.02	b
PT	**N_2V**	209.19	b
SN	**N_1C**	267.34	a
SN	**N_2F**	280.81	a
SN	**N_2V**	281.77	a
WR	**N_1C**	123.10	f
WR	**N_2F**	134.82	f e
WR	**N_2V**	133.58	f
	LSD	**19.973**	

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

When comparing the bunch mass of cultivars for a specific N treatment in the 1st season (eg. N_1C treatments) Weisser Riesling and Pinot noir did not differ significantly for the N_2F and the N_2V treatments. The bunch mass of Cabernet Sauvignon, Chardonnay, Pinotage and Chenin blanc differed significantly from each other as well as from the other two cultivars for the N_2F and the N_2V treatments. This was only the case for Pinot noir, Pinotage, Chenin blanc and Weisser Riesling with the N_1C treatments. The bunch mass of Chenin blanc was the highest followed, by Pinotage, while Pinot noir had the lowest bunch mass followed by Weisser Riesling for the N_1C, N_2F and N_2V treatments.

Results of this study confirm that of Spayd *et al.* (1993; 1994) that the bunch mass differ significantly between cultivars. Chenin blanc had the highest bunch mass while Weisser Riesling (1st season) and Pinot noir (2nd season) had the lowest bunch mass, which is in accordance with the descriptions of Orffer (1979).

The N_1C treatments resulted in a lower bunch mass compared to the N_2F and N_2V treatments. Cabernet Sauvignon was an exception to this trend, where N_1C resulted in the heaviest bunches in the 2nd season. Although Morris *et al.* (1983) suggested that N fertilisation had little effect on yield, results of this study confirm results of Maroche *et al.* (1977) and Conradie (1985), who concluded that increased N fertilisation also increases the dry matter content of specific organs of the vine.

4.3 CONCLUSIONS

It seems that an application of 20 kg N ha^{-1} at budbreak has resulted in a positive response in terms of shoot length. This response was observed relatively late in the 1st and even later in the 2nd season. The application of 20 kg N ha^{-1} at fruitset resulted in longer shoots, while the application of 20 kg N ha^{-1} at veraison had little effect on shoot length.

The six grape cultivars differed in vigour and growth pattern. The general soil and climatic conditions during the 1st season seemed to have been more favourable for vegetative growth when compared to the 2nd season. The shoot growth of Pinotage and Chenin blanc were

retarded less than the other four cultivars during both seasons. This might be ascribed to higher yields (bunch mass) compared to the other cultivars. Cabernet Sauvignon was a much more vigorous cultivar compared to the other cultivars.

Conclusions made for shoot length were in most cases also applicable to the weekly shoot elongation. The weekly shoot elongation of all treatments reached a peak near the fruitset N application and declined through the rest of the season. Although water stress may play a role in timing of this turning point it appeared from this study rather that combined effects of soil, climate, yield and inherent cultivar characteristics were dominant. In contrast with expectations, the application of 20 kg N ha^{-1} during veraison eventually resulted in a higher elongation rate when compared to 20 kg N ha^{-1} at fruitset. Irrigation seems to have a positive effect on the shoot elongation rate of vines receiving N fertilisation during the vegetative season. A downward trend in the elongation rate between fruitset and veraison N applications, imply that flower bunches acted as a major sink.

It was confirm that pruning mass was related to shoot growth, with the pruning mass of Cabernet Sauvignon higher than that of all other cultivars during both seasons. Nitrogen treatments did not effect the pruning mass of cultivars in any particular year. Nitrogen applied only in post-harvest, resulted in lower bunch mass, compared to additional N applied at fruitset and veraison. Increased N fertilisation therefore also increased the bunch mass of the vine.

CHAPTER 5

THE EFFECT OF NITROGEN FERTILISATION ON GRAPE MUST COMPOSITION

Abstract

In an effert to increase the nitrogen content of grape must nitrogen fertilisation were applied during two seasons at budbreak, fruitset and veraison to *Vitis vinifera L.* cvs. Chenin blanc, Weisser Riesling, Chardonnay, Pinotage, Pinot noir and Cabernet Sauvignon. Musts were analysed for FAN, °B, titratable acid and pH and berry mass was determined. Results showed that cultivars differ in the composition of their must and their reaction to N fertilisation. Additional nitrogen fertilisation at fruitset and veraison had a negative effect on the FAN content of grape must, but increased berry mass. Nitrogen fertilisation at these stages should thus be discouraged on soils with organic matter contents $\geq$ 1.4%. Climate turned out to be a determining factor in grape and must composition.

KEY WORDS: Stuck fermentation, nitrogen fertilisation, berry mass, cultivar reaction

5.1 INTRODUCTION

Grape must contain all supplements needed for yeast growth. The main source of energy for growth is supplied by sugar, usually available in excess. Sub-optimal amounts of N in must inhibit sugar uptake and may lead to lagging fermentation, minimum formation of esters, higher levels of higher alcohols and volatile acids, the development of H_2S and other related off-flavours (Rankine, 1989; Louw, 1998; Vos, 1998; Rauhut, 2001).

Partitioning of N to shoots and bunches was highest with summer treatments (Conradie, 1991). According to Conradie (1991) and Glad *et al.* (1994) a large amount of spring applied N was first translocated to leaves and shoots, after which turnover occurred and the N was exported to the bunches. Increasing veraison fertilisation may lead to a lower crop mass, lower total acids, more bunch rot and less nutrients to the vegetative parts due to a higher demand from the bunches (Christensen *et al.*, 1994). Saayman (1981) and Jackson & Lombard (1993) indicated higher acid concentration in berries with increased N fertilisation. Nitrogen fertilisation at veraison had the least effect on crop mass (Jackson & Lombard, 1993; Christensen *et al.*, 1994). The concentration of free amino acids in grapes can vary as

a result of cultivar, ripeness, sample preparation, cultivation method and analysing method (Kluba *et al.*, 1978; Henschke & Jarinek, 1993; Louw, 1998). According to Winkler *et al.* (1974), Vos & Gray (1979) and Monteiro & Bisson (1991) cultivar and time of harvest mainly affect the concentration of N and amino acids. According to Kluba *et al.* (1978), the initiation time and rate of this increase differ between cultivars and individual amino acids.

According to Peacock *et al.* (1989) and Christensen *et al.* (1994), more research is needed to determine the effect of N application during the summer on ripeness, vegetative growth and the N content of berries. The primary objective of this study was to measure the effect of N fertilisation during the vegetative season on the must of six grape cultivars. This was done by testing the means obtained for FAN, FAN/°B, titratable acid, pH and berry mass for significance for the six cultivars that received differential N fertilisation.

5.2 RESULTS EN DISCUSSION

A summary on the analyses of variance that were done to establish the effect of the three N treatments on the grape must and bunch analysis of six cultivars is presented in Table 5.1. The interaction between N treatments and cultivars were in both seasons significant for FAN, FAN/°B and berry mass. A significant interaction was also present between cultivars and N treatments for pH and bunch mass during the 2nd season. The titratable acid content of grape must was significantly affected by cultivar during both season.

TABLE 5.1 : Summary of the effect of nitrogen fertilisation on the must and bunch analysis of six different grape cultivars over two seasons; Elsenburg, Stellenbosch

	Season	FAN	FAN/°B	Titratable acid	pH	Berry mass
Cultivar	1st season	✓	✓	✓	✓	✓
	2nd season	✓	✓	✓	✓	✓
Nitrogen treatments	1st season	✓	✓	ns	ns	✓
	2nd season	✓	✓	ns	ns	✓
Cultivar / N treatments	1st season	✓	✓	ns	ns	✓
	2nd season	✓	✓	ns	✓	✓

- Significance indicated within colomns (LSD Tukey ≥ 0.05)

✓ = indicating significant differences

ns = indicating no significant differences

5.2.1 FAN

Although not always significant the FAN contents of the must from the N_1C treatments was the highest, followed by that from the N_2V and then the N_2F treatments for Chardonnay, Pinotage, Chenin blanc and Weisser Riesling in the 1st season (Table 5.2). The FAN content of Cabernet Sauvignon and Weisser Riesling were not significantly affected by the different N treatments in the 1st season. For Pinot noir the N_2F treatments resulted in the highest FAN values, followed by N_2V, with N_1C having the lowest values.

In the 2nd season the N_1C treatments resulted in the highest FAN values for Cabernet Sauvignon, Pinotage and Weisser Riesling, while N_2V resulted in the 2nd highest values for Pinotage and Weisser Riesling. The N treatments had no significant effect on the FAN content of Cabernet Sauvignon, Pinot noir and Weisser Riesling in the 2nd season. For Pinot noir the N_2F treatments resulted in the highest FAN values, followed by N_2V with N_1C having the lowest values. The N_1C treatments applied to Pinotage resulted in the highest and the N_2F treatments applied to Weisser Riesling resulted in the lowest FAN content of the must.

When comparing the FAN content of cultivars for a specific N treatment in the 1st season (e.g. N_1C treatments), Chenin blanc had the lowest FAN content of all cultivars. Chardonnay had the highest FAN contents of all cultivars for the N_1C and the N_2V treatments, while Cabernet Sauvignon had the highest FAN content for the N_2F treatments.

A comparison of the FAN content between cultivars of specific N treatments in the 2nd season, indicated that Pinotage had the highest value of all cultivars. Although not always significant, Weisser Riesling had the lowest FAN contents regardless of N treatments. The FAN contents between N treatments of Cabernet Sauvignon, Pinot noir and Weisser Riesling did not differ significantly. The FAN content of Chardonnay and Pinot noir were comparable for the N_1C and the N_2V treatments.

TABLE 5.2 : The effect of nitrogen fertilisation on the FAN content of must of six different cultivars during two seasons; Elsenburg, Stellenbosch

Cultivar	Nitrogen treatments	FAN (mg l^{-1})	
		1st Season	2nd Season
CS	N1C	1645.00 edc	1130.50 g f
CS	N2F	1740.00 bdc	998.80 gh
CS	N2V	1520.00 egdf	922.50 gh
CY	N1C	2112.50 a	2100.00 c
CY	N2F	1580.00 edf	1842.50 ed
CY	N2V	1942.50 ba	2137.80 c
PN	N1C	1250.00 gh	2052.50 cd
PN	N2F	1605.00 edf	2190.00 c
PN	N2V	1392.50 eghf	2110.00 c
PT	N1C	1910.00 bac	3170.00 a
PT	N2F	1290.00 gh	2270.00 cb
PT	N2V	1345.00 ghf	2465.00 b
SN	N1C	662.50 kj	1322.50 f
SN	N2F	305.00 l	1752.50 e
SN	N2V	490.00 kl	1822.50 ed
WR	N1C	1125.00 ih	951.30 gh
WR	N2F	852.50 ij	854.80 h
WR	N2V	910.00 ij	876.30 gh
LSD		**283.90**	**254.81**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

This study could not confirm findings of Ough & Bell, (1980), Conradie & Saayman, (1989a), Spayd *et al.* (1994) and Conradie (1998) that the N content of must can be raised with N fertilisation. During the 1st season, the N1C treatments had the highest FAN values followed by N2V treatments and then by the N2F treatments for Chardonnay, Pinotage, Chenin blanc and Weisser Riesling. During the 2nd season the N1C treatment resulted in the

highest FAN values for Cabernet Sauvignon, Pinotage and Weisser Riesling, followed by N_2V for Pinotage and Weisser Riesling. In addition, this study could not confirm findings of Conradie (1998) that N application at veraison could induce maximum canalisation of N to bunches. With regard to FAN values, and on soils with caracteristics described in this study, Cabernet Sauvignon and Weisser Riesling can thus be classified as cultivars with little reaction to N fertilisation with the reaction of Pinot noir differing between seasons.

According to Sponholz (2000), grape must with FAN values higher than 800 mg l^{-1} should not experience fermentation problems. Based on this norm, no fermentation problems should have occurred for any of the cultivars (Chenin blanc excluded). The lower FAN values of Chenin blanc during the 1st season can be ascribed to faulty laboratory procedures. These results, therefore, confirm recommendations of Conradie (1994), Conradie (1997) and Du Toit (1997) that no N fertilisation is needed when the organic matter content of soil reaches 1% or above. The conclusion can thus be made that the differences in FAN values between cultivars are determined genetically and stuck fermentation may occur more frequently on less N-rich soils with cultivars with lower natural FAN values.

All considered, Pinotage, Pinot noir and Chardonnay were the cultivars with the highest FAN contents while Chenin blanc and Weisser Riesling had the lowest values. These results show that the FAN content of must does not only differ between white and red grape varieties as suggested by Löhnertz (2001), but also between cultivars in white and red grape varieties, thus confirming simular findings of Winkler *et al.* (1974), Kluba *et al.* (1978), Vos & Gray (1979), Monteiro & Bisson (1991), Henschke & Jarinek (1993) and Louw (1998). Cabernet Sauvignon, Pinotage and Pinot noir showed major differences between seasons in the FAN contents of their must.

This study indicates that different cultivars differ in their sensitivity towards N fertilisation and the FAN content of their must. Like the genetic differences between cultivars found for the FAN content of their must, differences in their reaction to N fertilisation also seems to be related to the genetical properties of the cultivars.

5.2.2 FAN / °B

In spite of minor differences in significance, the general trends for the effect of N fertilisation on the FAN/°B ratio of the six cultivars are exactly the same as discussed for FAN (Table 5.3).

In the 2nd season the N_1C treatments resulted in the highest FAN/°B ratio for Cabernet Sauvignon, Pinotage, Chardonnay and Weisser Riesling while the N_2V treatment resulted in the 2nd highest values for Pinotage and Chardonnay. The N treatments had no significant effect on the FAN/°B of Cabernet Sauvignon, Pinot noir and Weisser Riesling in the 2nd season. For Pinot noir the N_2F treatments resulted in the highest FAN/°B ratio followed by N_2V with the N_1C treatments having the lowest values. The N_1C treatments applied to Pinotage resulted in the highest and the N_2V treatments applied to Weisser Riesling resulted in the lowest general FAN/°B ratio of the must.

When comparing the FAN content of cultivars at a specific N treatment in the 1st season (e.g. N_1C treatments) Cabernet Sauvignon and Pinot noir did not differ significantly for the FAN/°B ratio of treatments. The FAN content of Chardonnay and Pinotage also did not differ significantly for the N_1C and the N_2F treatments. Apart from these differences the discussion for the FAN content of grape must is also applicable to the FAN/°B ratio in the 1st season. When comparing the FAN/°B ratio of specific N treatments between cultivars in the 2nd season the trends and results discussed for the FAN content of must are also applicable to the FAN/°B ratio.

According to Vos (1998) the optimal N content of must is determined by the relationship between FAN and soluble solids (°B). When compared to the industry norm of 43.9:1 for the FAN/°B ratio, most cultivars and N treatments in this trial were far above the minimum value. Exceptions occurred for Chenin blanc (all the N treatments) and Weisser Riesling (N_2F treatments) in the 1st season and for Weisser Riesling (N_2F and N_2V) in the 2nd season.

TABLE 5.3 : The effect of nitrogen fertilisation on the mean FAN/°B ratio of six different grape cultivars during the two seasons; Elsenburg, Stellenbosch

Cultivar	Nitrogen treatments	FAN/°B ratio	
		1st Season	2nd Season
CS	N_1C	67.45 e c d	52.82 g f
CS	N_2F	70.72 b c d	45.20 g h
CS	N_2V	63.55 e c d	41.75 g h
CY	N_1C	86.05 a	90.27 c
CY	N_2F	66.95 e c d	77.69 e d
CY	N_2V	81.28 b a	89.64 c
PN	N_1C	57.40 e f	88.21 c d
PN	N_2F	73.04 b c	93.93 c
PN	N_2V	63.40 e c d	90.21 c
PT	N_1C	82.96 b a	138.84 a
PT	N_2F	57.49 e f	99.11 c b
PT	N_2V	60.51 e f d	107.52 b
SN	N_1C	29.36 i h	58.31 f
SN	N_2F	13.56 j	72.91 e
SN	N_2V	22.08 i j	75.26 e
WR	N_1C	49.76 g f	44.52 g h
WR	N_2F	38.06 g h	39.70 h
WR	N_2V	41.07 g h	39.56 h
LSD		12.486	

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

The results indicate that the FAN and FAN/°B showed the same trends. The possible fluctuation in soluble solids (°B) content between cultivars and N treatments therefore did not have a major effect on the FAN contents. This study also confirm suggestions of Conradie (1998) and Vos (1998) that the FAN/°B ratios could be used as an indicator for the N content of grape must. All other results discussed for the FAN content of grape must are also applicable to the FAN/°B ratio.

5.2.3 Titratable acid

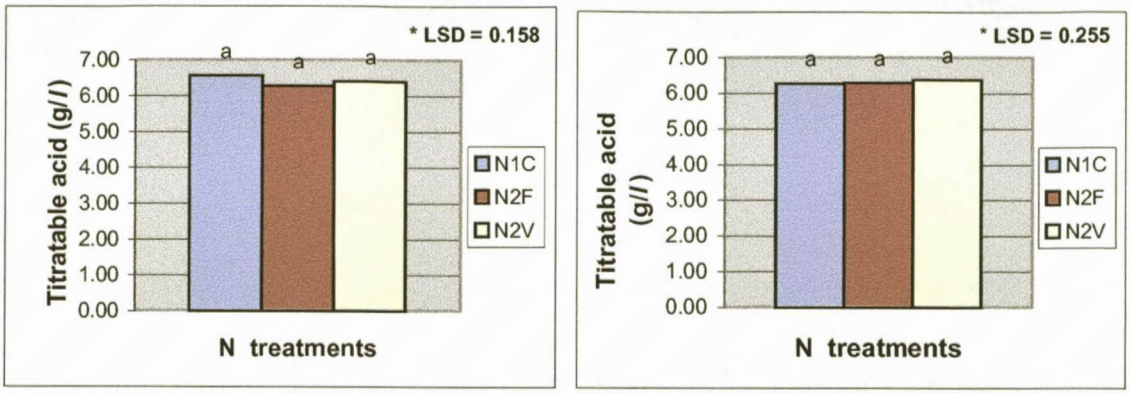
Differences in titratable acid content between the different cultivars is presented in Table 5.4. In both seasons Pinotage, Chardonnay and Pinot noir had the highest titratable acid contents in the must and Weisser Riesling, Chenin blanc and Cabernet Sauvignon the lowest. According to Saayman (1981) and Jackson & Lombard (1993), acid concentration in berries increase with N fertilisation, while Christensen *et al.* (1994) found that increased veraison fertilisation may lead to lower titratable acid concentration in must.

TABLE 5.4 : The effect of grape cultivars in a nitrogen fertilisation trial on the titratable acid content (g l^{-1}) of must during two seasons; Elsenburg, Stellenbosch

Cultivar	Season	
	1st season	2nd season
PT	7.62 a	7.66 a
PN	7.23 b	6.69 c
CY	6.73 c	7.24 b
SN	6.23 d	5.88 d
WR	5.63 e	5.74 d
CS	4.52 f	5.31 e
LSD	**0.21**	**0.29**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

No significant differences between the N treatments were found in both seasons (Figure 5.1). Spayd *et al.* (1994) and Löhnertz (2001) also indicated that N fertilisation does not effect the titratable acid content of must.



1st season 2nd season

FIGURE 5.1 : The effect of nitrogen fertilisation on the titratable acid content of must of six different cultivars during the two seasons; Elsenburg, Stellenbosch

- Bars with simular letters do not differ significantly from each other (LSD Tukey ≥ 0.05)

5.2.4 pH

Nitrogen treatments did not significantly affect the pH of grape must in the 1st season (Table 5.1). The effect of cultivars on pH is illustrated in Figure 5.2. The pH of Cabernet Sauvignon, Chardonnay and Chenin blanc did not differ significantly from each other while the pH of Pinotage was significantly lower than that of the other five cultivars.

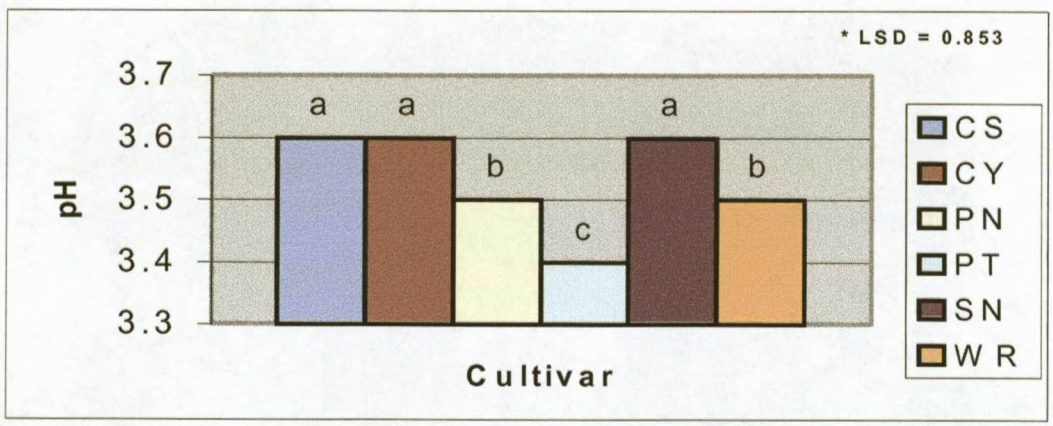


FIGURE 5.2 : The effect of grape cultivars in a nitrogen fertilisation trial on the pH of the must during the 1999/2000 season; Elsenburg, Stellenbosch

- Bars with simular letters do not differ significantly from each other (LSD Tukey ≥ 0.05)

The combined effects of cultivar and N fertilisation in the 2nd season, is shown in Table 5.5. The pH in the must of Cabernet Sauvignon, Chardonnay, Pinot noir, Chenin blanc and Weisser Riesling was not affected by N treatments. The pH of Cabernet Sauvignon and Chenin blanc did not differ from each other but was significantly higher than that of Chardonnay, Pinot noir and Weisser Riesling. It was only for Pinotage that the pH of must from the N_1C treatments was significantly higher than that for the N_2F and N_2V treatments.

TABLE 5.5 : The effect of nitrogen fertilisation on the pH of must of different grape cultivars during the 2000/2001 seasons; Elsenburg, Stellenbosch

Cultivar	Nitrogen treatments	pH
CS	**N_1C**	3.63 a
CS	**N_2F**	3.63 a
CS	**N_2V**	3.65 a
CY	**N_1C**	3.50 b
CY	**N_2F**	3.50 b
CY	**N_2V**	3.50 b
PN	**N_1C**	3.53 b
PN	**N_2F**	3.53 b
PN	**N_2V**	3.55 b
PT	**N_1C**	3.65 a
PT	**N_2F**	3.43 c
PT	**N_2V**	3.43 c
SN	**N_1C**	3.65 a
SN	**N_2F**	3.68 a
SN	**N_2V**	3.68 a
WR	**N_1C**	3.50 b
WR	**N_2F**	3.53 b
WR	**N_2V**	3.55 b
LSD		**0.0594**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

Except for the N_1C treatent applied to Pinotage, N treatments did not have a significant effect on the pH of grape must. The pH and titratable acid differed between seasons, with the 1st season having higher values for titratable acid and lower values for pH. This can be ascribed to the cooler climatic conditions in the 2nd season that induced overall higher acids and lower pH values (Personal communication, 2001: B. Stipp, Vinpro S.A., P.O. Box 528, Suider–Paarl, 7624). These results confirm that titratable acid may vary as a result of cultivar and year, which in turn effect the pH of the must and concur with similar findings of Winkler *et al.* (1974).

5.2.5 Berry mass

The combined effect of cultivar and N treatment in the two seasons are presented in Table 5.6. The N_1C treatment resulted in significantly lower berry mass than the N_2F and N_2V treatments for all cultivars in both seasons. Except for Pinot noir (both seasons) and Chenin blanc (2nd season), there were no significant differences between the berry mass of the N_2F and the N_2V treatments. When comparing the berry mass of cultivars for a specific N treatment in the 1st season, Cabernet Sauvignon and Pinotage did not differ significantly from each other for the N_1C and for the N_2V treatments. For the N_2F treatments, the berry mass of Pinot noir and Cabernet Sauvignon did not differ significantly from each other. The berry mass of Chardonnay, Pinotage and Weisser Riesling did not differ from each other for the N_1C treatments. The berry mass of all other cultivars differed significantly from each other for the different treatments. Chenin blanc had the highest and Pinot noir in the lowest berry mass for all treatments.

When comparing cultivars the N_1C treatment within the 2nd season, the berry mass of Chenin blanc and Pinotage did not differ significantly from each other, while the berry mass of Chardonnay and Pinot noir also did not differ significantly from each other. Like the 1st season, Pinot noir had the lowest berry mass for the different N treatments. In contrast the berry mass of Weisser Riesling (N_1C treatment) and Chenin blanc (N_2F and N_2V treatments) was significantly higher than that of the other cultivars. The berry mass of cultivars reacted in the same way to the N_2F and N_2V treatments. The berry mass of Pinotage and Cabernet

Sauvignon did not differ from each other, while the berry mass of Chenin blanc and Weisser Riesling also did not differ significantly from each other. In both seasons Chenin blanc and Weisser Riesling had the higher and Pinot noir always the lowest berry mass for all N treatments.

TABLE 5.6 : The effect of nitrogen fertilisation on the mean berry mass of six different grape cultivars during two seasons; Elsenburg, Stellenbosch

Cultivar	Nitrogen treatments	Berry mass (g)	
		1st Season	2nd Season
CS	N_1C	1.48 i h	1.77 f
CS	N_2F	1.68 f e	1.96 d c
CS	N_2V	1.64 f e g	1.99 c
CY	N_1C	1.60 f e g	1.44 h
CY	N_2F	1.81 d	1.73 f
CY	N_2V	1.84 d	1.78 f e
PN	N_1C	1.33 j	1.42 h
PN	N_2F	1.57 h g	1.56 g
PN	N_2V	1.47 i	1.49 h g
PT	N_1C	1.54 i h g	1.72 f
PT	N_2F	1.69 e	2.04 c
PT	N_2V	1.69 e	2.01 c
SN	N_1C	1.83 d	1.75 f
SN	N_2F	2.08 b a	2.31 a
SN	N_2V	2.15 a	2.20 b
WR	N_1C	1.59 f g	1.87 d e
WR	N_2F	1.97 c	2.23 b a
WR	N_2V	1.98 b c	2.18 b
LSD		**0.0986**	**0.0977**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

The berry mass of the N_1C treatment was the lowest for all cultivars during both seasons. This confirm results of Marocke *et al.* (1977) and Conradie (1985), *viz* that increased N

fertilisation was positively correlated with an increase in the dry matter content of specific organs of the vine. The berry mass of the N_2F and N_2V treatments did not follow a clear pattern for the different cultivars although they did not differ significantly from each other in most cases. This study further shows that N fertilisation led to an increase in berry mass, which seems to be negatively correlated to the FAN content of berries in a specific season. Results imply that berry mass also differ between seasons confirming the results of Spayd *et al.*, 1993.

5.3 CONCLUSIONS

This study could not confirm that the N content of must can be raised with N fertilisation. Overall, the N_1C treatment induced the highest FAN values, followed by the N_2V and then by the N_2F treatments. In addition, this study could not confirm that N application at veraison induce maximum canalisation to bunches. With regard to FAN values of cultivars, Cabernet Sauvignon and Weisser Riesling can be classified as cultivars with little reaction to N fertilisation, while Pinot noir reacted different between seasons. Based on industrial norms, no fermentation problems should have occurred with any of the cultivars. No N fertilisation is, therefore, needed on a soil with an average organic matter content of $\geq 1.4\%$. The conclusion can thus be made that differences in FAN values between cultivars are genetically determined and that stuck fermentation may occur more frequently on less N-rich soils with cultivars with lower natural FAN values.

Pinotage, Pinot noir and Chardonnay were the cultivars with the highest FAN contents while Chenin blanc and Weisser Riesling had the lowest values. Cabernet Sauvignon, Pinotage and Pinot noir showed major differences between seasons in the FAN contents of their must. These genetic differences between cultivars should be kept in mind by the winemaker during the fermentation process, with special attention to cultivars with a lower natural FAN content.

The FAN and FAN/°B showed the same trends implying that soluble solids (°B) content between cultivars and N treatments did not fluctuate significantly or had a major effect on the FAN contents. No significant differences in titratable acid content of must occurred between

N treatments for both seasons. N fertilisation did not have a significant effect on the pH of grape must but pH and titratable acid differed between seasons, with the 1st season inducing the higher values for titratable acid and lower pH values. Titratable acid and pH also varied as a result of cultivar.

In both seasons Chenin blanc and Weisser Riesling had the higher and Pinot noir always the lowest berry mass for all N treatments. The berry mass of the N_1C treatments was the lowest for all cultivars during both seasons. Increased N fertilisation was, therefore, positively correlated with an increase in the berry mass. The berry mass of the N_2F and N_2V treatments did not follow a clear pattern for the different cultivars and did not differ significantly from each other in most cases. This study further showed that an increase in berry mass as a result of N fertilisation can in most cases be negatively correlated to the FAN content of berries in a specific season. With more wine farmers turning to mechanisation and the resulting changes in canopy micro-climate and the bunch and berry mass, the effect of these practices on the FAN and FAN/°B content of grape must for different cultivars need further research. Future research should also examine the effect of climate on the FAN content of grape must.

CHAPTER 6

THE EFFECT OF NITROGEN FERTILISATION ON SOIL NITRATE AND *Vitis vinifera* LEAF NITROGEN CONTENT

Abstract

Under normal conditions vines do not easily show nutrient deficiencies due to a well-developed root system that effectively take up water and nutrients during a long vegetative season. This study was carried out on a loam soil with an organic matter content of 1.4% during the 1999/2000 (1st season) and 2000/2001 (2nd season) growing seasons. Results indicate that on soils with a high organic matter content and with irrigation, seasonal fluctuation and organic matter content may play a determining role in nitrogen mineralisation and hence nitrate availability in the soil. The nitrate content of the soil showed a cumulative pattern during both seasons with excessive nitrate leaching during winter. Seventy percent of total nitrate was available in the top 30 cm of soil. N fertilisation had a significant influence on the ammonium content of leaf blades and the total N content of the whole leaves, while the nitrate content of petioles showed significant differences between cultivars.

KEY WORDS : Nitrogen, grapevine, application time, soil nitrate, leaf nitrate.

6.1 INTRODUCTION

Nitrogen occupies a unique position amongst the elements essential for plant growth because of the large amounts required by most agricultural crops (Stevenson, 1986). According to Du Preez & Burger (1986) nearly all the inorganic N in the root zone can be utilised by the plant roots and should be taken into account during N fertilisation. According to Winkler *et al.* (1974) and Peacock *et al.* (1989), the N need of the vine is greatest during the period of rapid shoot growth, bloom and early berry development.

According to Perez & Kliewer (1982), Christensen (1984) and Christensen *et al.* (1994), the nitrate accumulation in leaves and petioles are significantly affected by cultivar. The inherent property of cultivars to have different nitrate contents in leaves can be ascribed to

genetic differences in their N metabolism (Christensen *et al.*, 1994). Leaves were highest in N when sampled close to time of applications (Conradie & Saayman, 1989b). According to Conradie (1997), petioles are more sensitive and usually serve as a better indicator of N status of vines. Leaf analysis varies between seasons and is also affected by rootstock, cultivar, climate, diseases and cultural practices (Conradie, 1994). According to Saayman (1981), Conradie (1994) and Du Toit (1997), leaf analysis cannot be solely used to determine the N need of the vine, but should be used in conjunction with soil analysis.

The amount of N released from soil organic matter will be determined by the efficiency of the mineralisation process (MacDuff & White, 1985) and can make an important contribution to the total N need of a crop (Addiscott, 1983). Greyling *et al.* (1990) found that significantly more mineralisable N occurred in the top 15 cm of soil than in deeper layers. Environmental conditions like water and temperature, soil conditions like pH, inorganic N, structure and texture and cultivation methods may effect N mineralisation in a soil (Alexander, 1977; Campbell & Souster, 1982; Meyers *et al.*, 1982; Stevenson, 1886). Soil organic matter, including the microbial biomass, is however, a dynamic nutrient reservoir that functions both as a source of and sink for N through the competition effects of mineralisation and immobilisation (Boone, 1990; Du Toit & Du Preez, 1993; George *et al.*, 1993). Harmsen & Van Schreven (1955) and Chiang, Soudi & Moreno (1983) found that light rainfall occurring after a period of summer drought, may lead to enhanced mineralisation of organic N, which is evident by an increased ammonium content, followed by an increase in nitrate. According to Linn & Doran (1984), Doran *et al.* (1990), Rochester *et al.* (1991) and Thompson & Thomas (1996), soil water and aeration also have a major effect on nitrate levels and measurements just after heavy rains are likely to indicate lower nitrate amounts.

The primary objective of this study was to measure the effect of N applications during the growing season on the soil nitrate content at three different depth intervals, *viz.* 0-15 cm, 15-30 cm and 30 – 60 cm. In the 2nd season only, the ammonium and nitrate content of the leaf blades and petioles, and the total nitrogen content of whole leaves of the six cultivars were measured to also determine the significance of N application thereon.

6.2 RESULTS EN DISCUSSION

6.2.1 Soil nitrate content

A summary on the analyses of variance that were done to establish the effect of N treatments and sampling dates on soil nitrate content at different depth intervals are presented in Table 6.1. In both seasons and at all soil depth intervals, N treatment and sampling date had a significant effect on the nitrate content of the soil. Interaction between N treatments and sampling date were present for all depth intervals for both seasons, therefore the effects of N treatments, sampling dates and soil depth on soil nitrate content are discussed in combination.

TABLE 6.1 : Summary of the effect of nitrogen fertilisation and sampling dates on the soil nitrate content at different depth intervals for both seasons; Elsenburg, Stellenbosch

Depth interval (cm)	Treatment	1st Season	2nd Season
0 – 15 cm	N treatment	✓	✓
	Sampling date	✓	✓
	N treatment x Sampling date	✓	✓
15 – 30 cm	N treatment	✓	✓
	Sampling date	✓	✓
	N treatment x Sampling date	✓	✓
30 – 60 cm	N treatment	✓	✓
	Sampling date	✓	✓
	N treatment x Sampling date	✓	✓

- Significance indicated within columns (LSD Tukey ≥ 0.05)

  ✓ = indicating significant differences

  ns = indicating no significant differences

The effect of N treatments on the soil nitrate content at different soil depths for two seasons, are summarised in Table 6.2. The general effect of N treatments on the soil nitrate content were the same at the different soil depths in the 1st and the 2nd season (Figure 6.1). Soil and climatic conditions seems to have been more favourable for mineralisation during the 1st season as evidenced by the much higher soil nitrate content of the 1st season compared to that of the 2nd season.

In the 1st season the N_1C treatments resulted in a decrease in the soil nitrate content from budbreak to the fruitset and an increase towards veraison at all three depth intervals. According to Winkler *et al.* (1974), Conradie (1985) and Peacock *et al.* (1989), the N need of the vine is greatest during the period of rapid shoot growth, bloom and early berry development, which might be the reason for the decrease in soil nitrate at fruitset for the N_1C treatments. Differences were, however, never significant. This pattern was not repeated in the 2nd season and the N_1C treatments resulted in a reduction in soil nitrate from fruitset to the veraison sampling date at all depth intervals. In both seasons the 30 - 60 cm soil layer had the lowest nitrate content, followed by the 15 - 30 cm layer, while the 0 - 15 cm layer had the highest nitrate content. Greyling *et al.* (1990) and Soudi *et al.* (1990), found significantly more mineralisable N in the top 20 cm of soil. According to Cassman & Munns (1980), the contribution of the soil layers to the total N mineralised was respectively 42% (0-18 cm); 18% (18-36 cm); 25% (36-72) and 15% (72-108 cm). In this study 70 % of the absolute amount of nitrate N was found in the top 30 cm of soil.

During both seasons the N_2F treatments resulted in an increase in the nitrate content from the budbreak to the fruitset and a decrease toward veraison. The sharp increase in soil nitrate of the N_2F and N_2V treatments from budbreak to fruitset, may be ascribed to the positive effect of the budbreak fertilisation and favourable conditions for mineralisation. An exception to the N_2F pattern occurred in the 30-60 cm soil layer which showed a cumulative effect from the budbreak to veraison, possibly as the result of leaching of nitrate from the upper layers.

TABLE 6.2 : The effect of nitrogen treatments and sampling dates on the soil nitrate content (NO_3-N mg kg^{-1}) at different depth intervals for both seasons

Depth interval (cm)	Nitrogen treatment	Sampling date			Mean
		Budbreak	Fruitset	Veraison	
		1st Season			
0 – 15 cm	N1C	8.40 d	7.78 d	9.26 d	**5.81**
	N2F	16.70 d	54.02 a	43.28 b	**38.00**
	N2V	14.73 d	27.74 c	47.02 ab	**29.83**
	Mean	**13.28**	**29.84**	**33.19**	**24.54**
15 – 30 cm	N1C	11.55 cd	7.38 d	9.79 cd	**9.39**
	N2F	17.10 cb	42.84 a	35.88 a	**31.94**
	N2V	13.74 cd	23.83 b	36.85 a	**24.81**
	Mean	**14.13**	**24.68**	**27.33**	**22.05**
30 – 60 cm	N1C	10.26 cd	5.77 d	7.02 d	**7.68**
	N2F	16.80 cb	28.87 a	31.18 a	**25.61**
	N2V	12.74 cbd	18.49 b	28.65 a	**19.99**
	Mean	**13.27**	**17.71**	**22.28**	**17.75**
		2nd Season			
0 – 15 cm	N1C	1.80 d	9.48 c	5.71 hij	**5.66**
	N2F	3.63 d	21.34 b	20.94 bc	**15.30**
	N2V	2.77 d	17.16 b	30.96 a	**16.96**
	Mean	**2.73**	**15.99**	**19.20**	**12.64**
15 – 30 cm	N1C	1.46 e	8.35 d	3.95 ij	**4.59**
	N2F	3.13 e	18.32 b	15.32 de	**12.26**
	N2V	2.43 e	14.15 c	28.07 a	**14.88**
	Mean	**2.34**	**13.61**	**15.78**	**10.57**
30 – 60 cm	N1C	1.27 c	7.18 cb	3.08 c	**3.84**
	N2F	2.39 c	13.87 b	21.64 a	**12.63**
	N2V	1.96 c	11.36 b	24.91 a	**12.74**
	Mean	**1.87**	**10.80**	**16.54**	**9.73**

- Values for each depth, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

The N_2V treatments resulted in an accumulation of soil nitrate from budbreak to veraison. This is in accordance with results of George *et al.* (1993) who found cumulative amounts of nitrate in fertilisation trials.

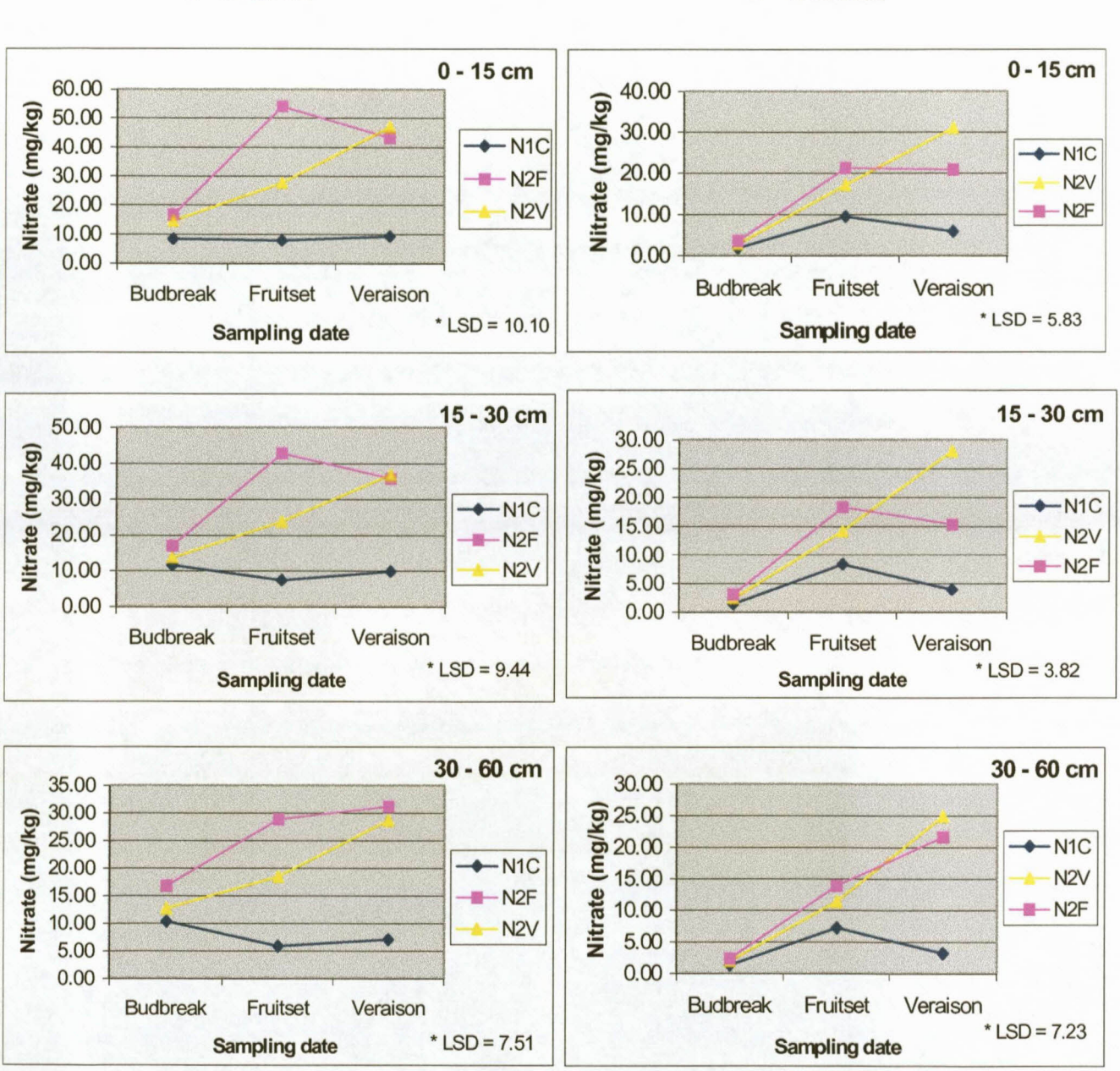


FIGURE 6.1 : The effect of nitrogen fertilisation and sampling date on the soil nitrate content at different depths during two seasons; Elsenburg, Stellenbosch

For the different N treatments the nitrate content of the soil did not differ significantly over the different depth intervals when soil samples were taken after the budbreak application (Table 6.2). The lower and more uniform soil nitrate content in the beginning of each season may be ascribed to leaching during the winter and low soil temperatures resulting in lower microbe activity (Haynes, 1986). Results, therefore, imply that neither irrigation nor the mineralisation of organic matter significantly affected the nitrate content of the soil. Significant differences occurred between the different depth intervals for the samples taken nearest to a specific N application (e.g. N2F and the fruitset sampling and N2V and the veraison sampling). Results imply that N treatments applied at fruitset and veraison had a positive effect on the nitrate content of the soil. Especially in the 2nd season, N applications of the N2F and N2V treatments after budbreak failed to result in significant differences at the budbreak sampling date. This could be ascribed to slower mineralisation because of high soil water contents and low soil temperatures after the winter. The nitrate content of the soil, therefore, differed for the different soil layers and was affected by sampling date with reaction to the N treatments not always the same.

During the 1st season much higher soil nitrate values occurred than during the 2nd season (Figure 6.1). This is in accordance with findings of Alexander (1977), Campbell & Souster (1982), Meyers *et al.* (1982), Stevenson (1986) and Du Toit & Du Preez (1993) that environmental conditions, such as climate, may effect N mineralisation in a soil.

Mineralisation of organic soil N can make a large contribution to the total N need of a crop (Addiscott, 1983). Bearing in mind the recommendation made by Conradie (1994), *viz* that no further N fertilisation is needed when the soil nitrate content exceeds 15 mg kg, sufficient N was available at most measurements when applying N during the 1st season, while this was not the case in the 2nd season. Environmental conditions can, therefore, play a determining role in nitrate availability, even in soils with a high organic matter content under irrigation conditions. The FAN content of grape must showed a negative relationship to N fertilisation (Chapter 5). This fact can also be applied to the difference in soil nitrate content between years. The lower soil nitrate content of the 2nd season led to higher FAN concentrations of

grape must, in contrast to the higher soil nitrate content in the 1st season with lower FAN concentrations in must.

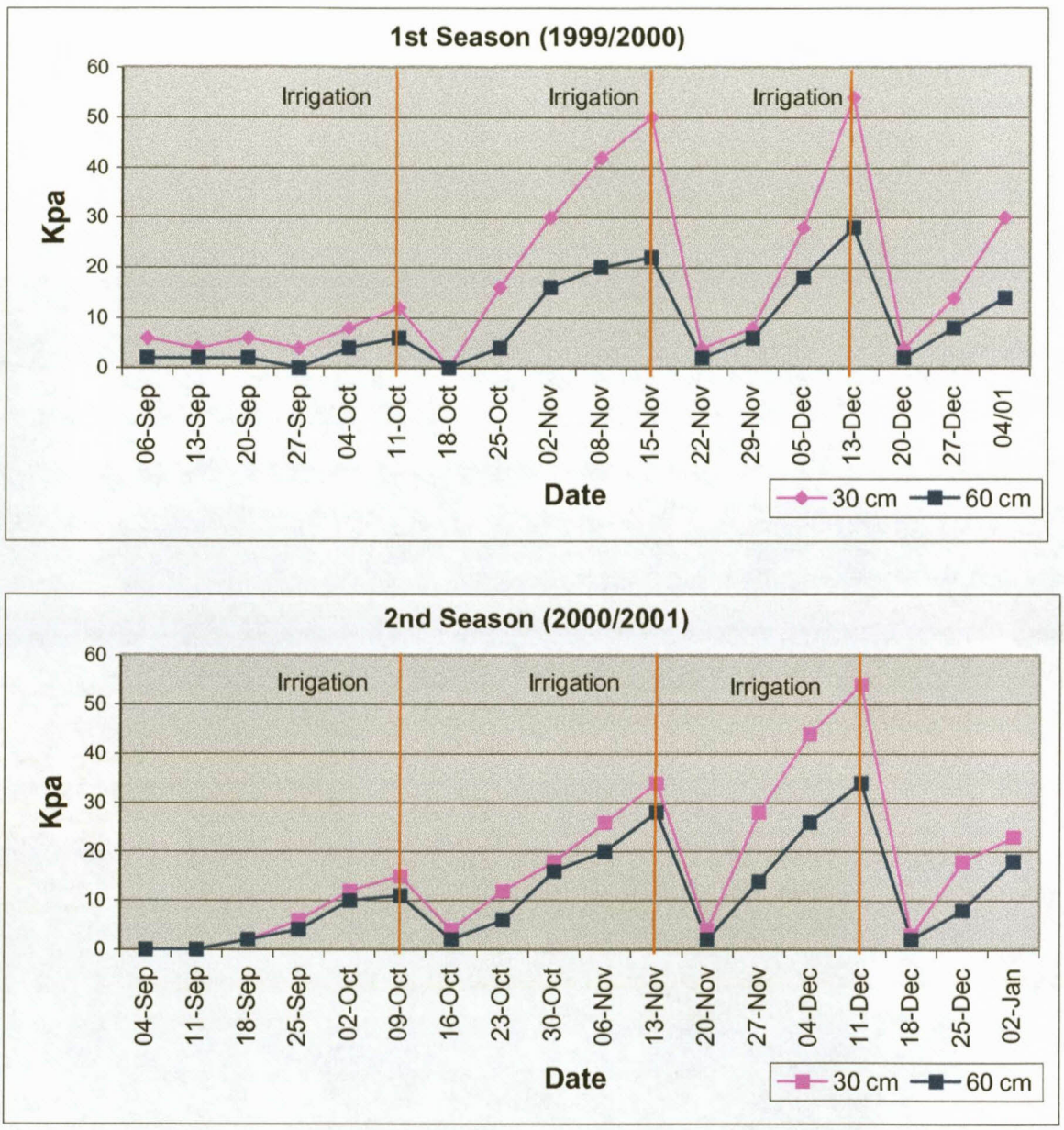


FIGURE 6.2 : Tensiometer readings at 30 cm and 60 cm soil depth during two seasons in the nitrogen fertilisation trial vineyard; Elsenburg, Stellenbosch

Rainfall and temperature effect the organic C and total N contents of a soil (Du Toit & Du Preez, 1993). During the period from budbreak until the last sampling date, the 1st season

had a higher average temperature and precipitation compared to the 2nd season (Table 3.2). This confirms the results of Boman *et al.* (1995) that the year with the lowest precipitation had the highest amount of soil residual nitrate N. These cycles of wetting and drying also tend to increase the amount of available substrate for the soil microbes and therefore mineralisation (Jenkinson & Ayanaba, 1977; Haynes, 1986). Tensiometer readings through the seasons (Figure 6.2), confirm that the cycles of wetting and drying were more extreme during the 1st than during the 2nd season. Cassman & Munns (1980) found N mineralisation to be maximal at a soil matrix potential of -30 kPa.

6.2.2 Leaf nitrogen content

A summary of the effect that cultivars and N treatments had on either the ammonium and nitrate contents of the leaf blades and petioles, and the total nitrogen content of the whole leaves are given in Table 6.3. Only the nitrate content of the petioles differed significantly between the cultivars. However, the N treatments significantly affected the ammonium content of the leaf blades and the total nitrogen content of the whole leaves. A significant interaction between cultivars and N treatments was manifested only for total N content of the whole leaves.

TABLE 6.3 : Summary of the effect that cultivar and nitrogen fertilisation had on the leaf nitrogen content

	NH_4-N (Blades)	NH_4-N (Petioles)	NO_3-N (Blades)	NO_3-N (Petioles)	Total N (whole leaves)
Cultivar	ns	ns	ns	✓	ns
Nitrogen treatments	✓	ns	ns	ns	✓
Nitrogen treatments x Cultivar	ns	ns	ns	ns	✓

- Significance indicated within columns (LSD Tukey ≥ 0.05)

  ✓ = indicating significant differences

  ns = indicating no significant differences

6.2.2.1 Ammonium and nitrate content of leaf blades and petioles

6.2.2.1.1 Nitrogen treatments

The N_2V treatments resulted in significantly higher ammonium content of the leaf blades than the N_1C treatments (Table 6.4). This was not the case for the ammonium content of the petioles.

TABLE 6.4 : Effect of nitrogen fertilisation on the ammonium and nitrate content of post veraison leaf blades and petioles of six different cultivars; Elsenburg, Stellenbosch

Nitrogen treatments	NH_4-N (mg kg^{-1})		NO_3-N (mg kg^{-1})	
	Blades	**Petioles**	**Blades**	**Petioles**
N_1C	231 b	217 a	2636 a	1719 a
N_2F	252 ab	271 a	2273 a	1622 a
N_2V	265 a	233 a	2386 a	1622 a
LSD	**24**	**87**	**371**	**213**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

Neither the nitrate content of the leaf blades nor that of the petioles was significant effect by differential N applications. Perez & Kliewer (1982) also found no significant differences in the nitrate content of petioles when applying different amounts of N. It should, however, be noted that there were indications that the nitrate contents of the leaf blades and petioles from the N_1C treatments were higher than those from the N_2F and N_2V treatments in spite of no N fertilisation during the season. This is in accordance with research by Saayman & Conradie (1982), Christensen (1984) and Conradie (1997) stating that petioles are more sensitive than leaf blades to N fertilisation and that nitrate usually serve as a better indicator of N status than ammonium.Results in general confirm recommendations of Saayman (1981), Conradie (1994) and Du Toit (1997) that leaf analysis should not be solely used to determine the N need of the vine.

6.2.2.1.2 Cultivar

The effect of cultivar on the ammonium and nitrate content of leaf blades and petioles are presented in Table 6.5. In contrast to data reported by Christensen (1984), these results confirm findings of Perez & Kliewer (1982) and Christensen *et al.* (1994), *viz* that nitrate accumulation in petioles is affected to some extent by cultivar.

TABLE 6.5 : Effect of cultivar on the ammonium and nitrate content of post-veraison leaf blades and petioles of vines in a nitrogen fertilisation trial; Elsenburg, Stellenbosch

Nitrogen treatments	NH_4-N (mg kg^{-1})		NO_3-N (mg kg^{-1})	
	Leaf blades	Petioles	Leaf blades	Petioles
PT	283 a	243 a	2394 a	2060 a
CY	268 a	148 a	2326 a	1259 b
SN	253 a	279 a	2285 a	1320 b
PN	250 a	180 a	2804 a	2185 a
CS	229 a	314 a	2482 a	1783 a
WR	213 a	279 a	2300 a	1320 b
LSD	**71**	**219**	**1075**	**410**

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

There were no differences in the ammonium content of the leaf blades and petioles between cultivars. The nitrate content in the petioles of Pinotage, Pinot noir and Cabernet Sauvignon was significantly higher than for Chardonnay, Chenin blanc and Weisser Riesling. In the evaluation of the FAN contents of grape must, Pinotage and Pinot noir (2^{nd} season) proved to be sensitive to N fertilisation, which induced a high FAN content in the must. The nitrate content of petioles at veraison might, therefore, be a good indicator of the FAN content of grape must. This aspect however, calls for further research. According to Christensen *et al.* (1994), the inherent property of cultivars to have different leaf nitrate contents can be ascribed to genetic differences in their N metabolism.

6.2.2.2 Total nitrogen content of whole leaves

The effect of cultivars and N treatments on the total N in the leaves is presented in Table 6.6, and also illustrated in Figure 6.3. Results indicate that Chardonnay and Pinotage were responsible for the interaction. In the case of the other four cultivars, Cabernet Sauvignon, Pinot noir, Chenin blanc and Weisser Riesling the total N content of the leaves was the highest with the N_2F treatment. The other two treatments, *viz.* N_1C and N_2V resulted in lower total N content of the leaves for these four cultivars. The N_1C and N_2V treatments did not differ with respect to the total N content in the leaves for Chardonnay, Cabernet Sauvignon, Pinot noir and Chenin blanc. The lower values at N_1C can be ascribed to the lack of N fertilisation during the growing season, while the lower values at N_2V can be ascribed to lower root activity and a possible lower mineralisation rate after the veraison N application. According to Winkler *et al.* (1974) and Conradie (1991), flower clusters act as a major sink for N from veraison.

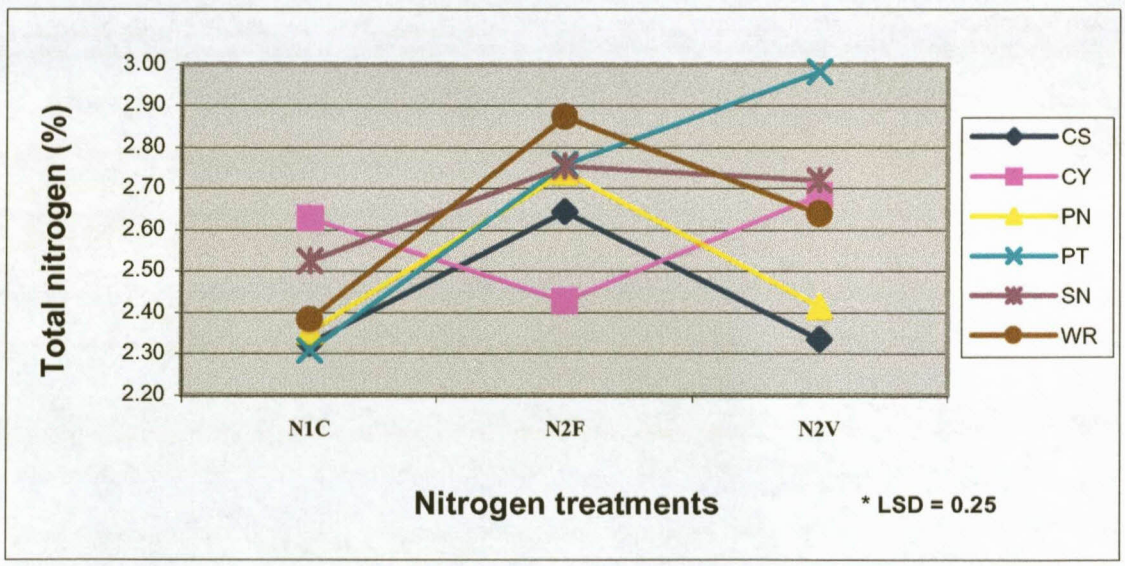


FIGURE 6.3 : The effect of cultivar and nitrogen fertilisation on the post-veraison total nitrogen content of the whole leaves; Elsenburg, Stellenbosch, 2001

For Chardonnay the N_2F and N_2V treatments resulted respectively in lower and higher total N in the leaves than the N_1C treatment. When compared to the FAN content during the 2nd

season, a positive relationship existed for this cultivar between the FAN contents of the must and the total N content in leaves at veraison. For Pinotage the N_1C treatment had the lowest total N content in the leaves at veraison, followed by the N_2F and N_2V treatments respectively. When compared to the FAN content of the must during the 2nd season, a negative relationship between the FAN content and the total N content becomes evident for this cultivar. Perez & Kliewer (1982), Christensen (1984) and Christensen *et al.* (1994) ascribe the differences of N accumulation in the leaves of cultivars to genetic differences in their N metabolism which, in some cases, might be a good indicator of the potential FAN content of their must.

TABLE 6.6 : The effect of cultivar and nitrogen fertilisation on the post-harvest total nitrogen content of the whole leaf; Elsenburg, Stellenbosch, 2001

Cultivar	Nitrogen treatments	Total nitrogen (%)	Significance
CS	N_1C	2.33	f
	N_2F	2.65	bcd
	N_2V	2.34	f
CY	N_1C	2.63	bcde
	N_2F	2.43	def
	N_2V	2.69	bc
PN	N_1C	2.36	f
	N_2F	2.74	abc
	N_2V	2.42	def
PT	N_1C	2.31	f
	N_2F	2.76	abc
	N_2V	2.99	a
SN	N_1C	2.53	cdef
	N_2F	2.76	abc
	N_2V	2.72	bc
WR	N_1C	2.39	ef
	N_2F	2.88	ab
	N_2V	2.64	bcd
LSD		0.26	

- Values within columns, followed by the same letter, do not differ significantly from each other (LSD Tukey ≥ 0.05)

6.3 CONCLUSIONS

On soils with a relatively high organic matter content and where irrigation is applied, seasonal fluctuation in climate may play a determining role in nitrogen mineralisation and hence nitrate availability. Higher nitrate levels prevailed in the 1st season than in the 2nd season, which can be ascribed to the higher average rainfall and temperature in the 1st season when compared to the 2nd season.

Excessive nitrate seemed to leach below 60 cm during the winter of both seasons. In both seasons the mean soil nitrate content declined with depth, 70% of the nitrate being in the top 30 cm of soil. The application of pre-harvest N treatments had a positive effect on the nitrate content of the soil. Plots receiving N applications after budbreak, however, did not show in significant increases in soil nitrate content compared to plots receiving no pre-harvest fertilisation. This was applicable to all three soil depths and for both seasons. The mean nitrate content of the loam soil showed a cumulative effect during both seasons, with the lowest values at budbreak and the highest at veraison.

It seems that the N content of leaves at veraison might be a good indicator in predicting the potential FAN contents of grape must for some cultivars. Further research is, however, needed to determine parameters for the N content of leaves at veraison and the potential FAN content of the must for specific cultivars. Because of lower root activities and less favourable conditions for mineralisation at veraison, the effect of N foliar sprays during this period should be investigated as a means to increase the N content of the must.

Cultivars also differed in their reaction to N fertilisation. Generally N fertilisation after budbreak and at fruitset resulted in the lowest FAN content of grape must, with fertilisation after budbreak and at veraison in 2nd place and no N fertilisation in the pre-harvest period resulting in the highest FAN content of grape must. This study, therefore, indicates that, although high organic matter content through mineralisation, together with nitrogen fertilisation, may increase the nitrate content of a soil, this might not have the same effect on the N status in the vine.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Stuck fermentation is one of the most serious production associated problems in the South-African wine industry today. This can mainly be ascribed to insufficient levels of N in grape must to support yeast growth. Di-ammonium phosphate is usually added during fermentation as a yeast supplement, but increased resistance against this practice can be expected, which might effect the overseas marketing of South-African wines in future. If the native N content of grape must can be raised with N fertilisation, lower additions of DAP can be made in the cellar, making the wine more acceptable to the international buyer. The importance of correct N application has been emphisised out in the past in the light of the negative effects of both under and over-fertilisation with N on quality and yield. Apart from the economic implications of over-fertilisation with N, nitrate pollution of the environment is also an ever increasing problem.

This study was undertaken to compliment current research on the topic and also to assist grape farmers in making better decisions when fertilising different grape cultivars to ensure sufficient N in grape must. In order to achieve this, three nitrogen application treatments (N_1C = 20 kg N ha^{-1} post-harvest; N_2F = 20 kg N ha^{-1} post-harvest, followed with 20 kg N ha^{-1} at budbreak and fruitset respectively; and N_2V = 20 kg N ha^{-1} post-harvest, followed by 20 kg N ha^{-1} at budbreak and veraison respectively) were applied to six grape cultivars (Cabernet Sauvignon, Chenin blanc, Pinot noir, Weisser Riesling, Chardonnay and Pinotage) for two seasons (1999/2000 = 1st season and 2000/2001 = 2nd season). The effect of these nitrogen applications on the growth characteristics, grape must composition and leaf nitrogen content of the cultivars as well as the soil nitrate content, were measured.

7.1 THE EFFECT OF NITROGEN FERTILISATION ON THE GROWTH CHARACTERISTICS OF *Vitis vinifera*

Pre-harvest nitrogen applications had a positive effect on shoot length during both seasons, although more so during the 1st season. Apart from increasing shoot growth, pre-harvest N applications also postponed the turning point in vegetative growth. Shoot length was also significantly affected by cultivar. Although cultivars followed the same general growth pattern, slight variations occurred as a result of vigour and phenological properties of individual cultivars. The positive effect of irrigation and N fertilisation clearly played a bigger role during the 2nd season, resulting in higher crop loads and less favourable conditions for vegetative growth. The higher bunches of Pinotage and Chenin blanc had a strong effect on their growth characteristics. The higher bunch mass of cultivars during the 2nd season, when compared to the 1st season, also had a negative effect on their growth characteristics. Cultivars reached a turning point in vegetative growth each season as the result of a combined effect of soil and climatic conditions and also because of differences in bunch mass and inherent cultivar characteristics. After this turning point the balance between vegetative and reproductive growth for each cultivar kept on playing a role in shoot growth during the rest of the season. Further research is needed to determine the ideal vegetative to reproductive growth ratio for each cultivar and how the vegetative growth curve is affected by differences in crop and bunch mass.

The conclusions for shoot length are in most cases also applicable to weekly shoot elongation (growth rate). The weekly shoot elongation of all treatments reached a peak near the fruitset nitrogen application and declined through the rest of the season. Although water stress may play a role in what time this turning point is reached, this study rather points to a combined effect of soil and climatic conditions between seasons and also to yield and inherent cultivar characteristics. The downward trends in the elongation rate between the fruitset and veraison N applications imply that flower clusters acted as a major sink. More research is needed, however, to differentiate between the effects of soil, climatic, genetic and phenological variables on the performance of vine growth for individual cultivars.

Bunch mass differed between cultivars with Chenin blanc having the highest bunch mass while Weisser Riesling (1st season) and Pinot noir (2nd season) had the lowest bunch mass. Additional pre-harvest N fertilisation increased the bunch mass of vines. The pruning mass was significantly effectd by cultivar in both seasons with the pruning mass of Cabernet Sauvignon being significantly higher than all other cultivars during both seasons. Nitrogen applications had no significant effect on the pruning mass in any particular year, with no consistent year-to-year pattern.

7.2 THE EFFECT OF NITROGEN FERTILISATION ON THE GRAPE MUST COMPOSITION

Parameters like FAN, FAN/°B, titratable acid, pH and berry mass were all effected by the cultivar. Pinotage, Pinot noir and Chardonnay were the cultivars with the highest FAN contents, while Chenin blanc and Weisser Riesling had the lowest values. Cabernet Sauvignon, Pinotage and Pinot noir showed major differences between seasons in the FAN contents of their must. These genetic differences between cultivars should be kept in mind during the fermentation process, with special attention to cultivars with a low native FAN content. Nitrogen applications also significantly effectd FAN, FAN/°B and berry mass. Grapes from the NiC treatments were significantly higher in FAN and FAN/°B, indicating a negative reaction to pre-harvest N fertilisation on soils having relativeley high organic matter content. There were indications that N fertilisation at fruitset had a more negative effect on FAN and FAN/°B than N at veraison. According to these findings N fertilisation during the growing season on soils with an organic matter content of ≥1.4% should be strongly discouraged.

The NiC treatment induced lower berry mass compared to fertilisation during the growing season. This might indicate a direct relationship between bunch and berry mass and the FAN content and FAN/°B ratio of grape must. With more wine farmers turning to mechanisation and the effect of these measures on canopy micro-climate and the bunch and berry mass, the effect of these practices on the FAN and FAN/°B content of grape must for the different cultivars calls for further research.

The cooler climate of the 2nd season induced higher FAN, FAN/°B and bunch and berry mass values when compared to the 1st season. It also led to lower acid and pH contents in grape must. Stuck fermentation may, therefore, be a bigger problem during warmer seasons with more sensitive cultivars on less fertile soils. Future research should further examine the effect of climate on the FAN content of grape must. This should also be of concern when examining the effect of different pruning systems on the FAN content of grape must because of differences in microclimate.

7.3 THE EFFECT OF NITROGEN FERTILISATION ON SOIL NITRATE AND *Vitis vinifera* LEAF NITROGEN CONTENT

In both seasons and at all soil depths studies, N fertilisation and time had a significant effect on the nitrate content of the soil. Interaction between N treatment and sampling date were however, also present for all depths for both seasons. The effect of N treatments on the soil nitrate content stayed the same for different soil depths in the 1st and the 2nd season. Soil and climatic conditions seems to have been more favourable for mineralisation during the 1st season. In both seasons the 30 - 60 cm soil depth had the lowest nitrate content, followed by the 15 - 30 cm depth, while the 0 - 15 cm had the highest nitrate content. The relative contribution of the nitrate content, 70% of the absalute nitrate N being present in the top 30 cm of soil.

During both seasons additional N at fruitset resulted in an increases in the soil nitrate content from budbreak to the fruitset and a decrease towards veraison. In most cases no pre-harvest N did not result in significant differences in the soil nitrate content between the three samplings dates. This indicate that neither irrigation nor the mineralisation of organic matter significantly effectd the nitrate contents of the soil. Additional N at veraison (N_2V) resulted in accumulation of soil nitrate from budbreak to the veraison. For the different N treatments the nitrate content of the soil did not differ significantly between the different soil depths.

Significant difference in soil nitrate occurred between the soil depths for the samples taken nearest to a specific N application. Nitrogen applications at fruitset and veraison failed to result in significant differences in soil nitrate at the budbreak sampling date. This could be ascribed to slower mineralisation because of high soil water contents and low soil temperatures after the winter. The FAN content of grape must in most cases showed a negative relationship to increased nitrate content (N fertilisation) in this soil was also true concerning seasons. The lower soil nitrate content of the 2nd season led to higher mean FAN concentrations in grape must in contrast to the higher soil nitrate content of the 1st season with lower FAN concentrations in must.

Only the nitrate content of the petioles differed significantly between the cultivars, while N treatments significantly effected the ammonium content of leaf blades and total nitrogen content of whole leaves. The nitrate content of leaf blades and petioles from the N_1C treatment were higher than those from the N_2F and N_2V treatments, in spite of no N fertilisation during the pre-harvest period. This indicates a positive relationship between the FAN content of grape must and the nitrate content of leaf blades and petioles.

The nitrate content of the soil can be raised with N fertilisation but this does not necesserily end up in the must of grapes. The availability of nitrate from inorganic and organic sources, however, varies between seasons as the result of differences in soil and climatic conditions. The reaction of the grapevine to this available soil nitrate also varies as the result of internal balances in the grapevine as well as genetic differences between cultivars. This study, therefore, emphasised how these variables may effect the eventual reaction of the vine on nitrogen fertilisation.

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