

Quantifying the effect of commercial nitrogen formulations BlackUrea™ / BlackDAP™ as alternative nitrogen source on fruit tree physiology, yield and fruit quality of two apple cultivars

By

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*Thesis presented in partial fulfilment of the requirements for the degree of Master of Science
in Horticultural Science at the Stellenbosch University*

1918 · 2018

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March 2018

DECLARATION

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Date: March 2018

ACKNOWLEDGEMENTS

I would like to take this opportunity to acknowledge the following people who have enabled me to complete this thesis:

- Dr. Elmi Lötze – You are a true mentor, thank you for all your inputs and help during the past few years. It was a privilege to go through this journey together, you are a definite ambassador for not only the department, but horticulture in general.
- Mr. Jakkie Stander – your inputs and efforts are highly appreciated, thank you.
- Mr. André Britz – Your continued support, from start to end.
- Dr. Caren Jarman – Your inputs and training regarding FruitLook®
- Mr. Gustav Lötze and his team – all your help in the lab and field, a truly golden team.
- The Department of Horticulture – For aiding in teaching me the true fundamentals of not only horticulture, but science in general.
- The Top of the Lötze group – A bunch of great colleagues, helping each other grow, never stop the good work.
- My Friends and Family...especially my Wife, Rulandi. – For believing, supporting and standing by me.
- Profert (Pty) Ltd – For the funding of this project.

DEDICATION

To my mom “Anna”

*... for always believing in me, and reminding me that nothing is impossible as long as you put
in the necessary effort...*

Table of Contents

DECLARATION	i
ACKNOWLEDGEMENTS	ii
DEDICATION	iii
Summary	v
Opsomming	vi
General introduction	viii
Literature study: The effect of soil applied N on fruit tree physiology.....	1
Paper 1: Evaluating the effect of three commercial nitrogen formulations on tree physiology as well as quality and yield of ‘Rosy Glow’ fruit (<i>Malus domestica</i> Borkh.)	24
Paper 2: The effect of two commercial nitrogen formulations on tree performance of bearing ‘Golden Delicious’ (<i>Malus domestica</i> Borkh) trees.....	42
Paper 3: Evaluating the suitability of FruitLook® to quantify the response of ‘Golden Delicious’ (<i>Malus domestica</i> Borkh.) trees on two commercial soil-applied nitrogen formulations.	54
General Conclusion.....	68

Summary

The relationship between plant and soil is very complex and understanding it is fundamental to optimal use of fertilizers for a profitable yield, without compromising the environment. Nitrogen (N) fertilizers plays an important role in ensuring high-quality apples while sustaining high yields. N fertilizer products face challenges regarding loss of efficacy due to leaching and volatility. Understanding the mode of action of the available commercial N formulations is necessary to maximize the efficiency of N applications under different environmental conditions.

To determine the effect of applied N, three commercial N fertilizer formulations: BlackUrea™, BlackDAP™ and UreaHB, were applied as the only N source on ‘Rosy Glow’ and ‘Golden Delicious’ cultivars, on sandy loam soils in Ceres, South Africa. Different parameters, including physiological and vegetative measurements, were recorded to determine the effect of different N formulations after soil applications on three dedicated phenological stages. The reaction of root growth dynamics on the different N formulations were also studied in the ‘Rosy Glow’ orchard. Lastly, the use of satellite imaging (FruitLook®) to quantify the effect N formulations on biomass and N levels in trees was evaluated on the ‘Golden Delicious’ orchard.

The results from this study proved that urea could be used as an alternative N source to other ammonium formulations, under these conditions, without any negative effects on fruit quality or yield, during this short-term study of two seasons. However, the potential acidification of urea as only N source over time has been reported under different conditions and needs to be considered. No significant (ns) differences were reported between treatments in the ‘Rosy Glow’ trial, which may partly be due to the cultivar choice. Nevertheless, the positive trend in an increase in yield with the BlackUrea™ treatment compared to UreaHB in the ‘Rosy Glow’ was confirmed in an increase (ns) in yield after one season of application in the ‘Golden Delicious’ trial.

White root growth showed differences between treatments in the ‘Rosy Glow’ trial. UreaHB and BlackDAP™ treatments had more white roots compared to BlackUrea™ which had a more evenly distributed white root count. This did not affect the leaf N concentrations or other vegetative parameters during the two seasons but indicated an effect of formulation. A pot trial with tomatoes showed contrasting results compared to those in the apples, with more growth (ns) and higher leaching of ammonium and nitrates in the BlackUrea™ than UreaHB

treatment. This may be partly due to the effect of the coating of the BlackUrea™ that underperformed under conditions of high irrigation and inert sand as medium.

FruitLook® was able to identify differences in biomass and N levels between treatments, providing accurate additional information of treatments in addition to field measurements, derived from spectral data.

Opsomming

Die verhouding tussen plante en grond is baie kompleks en ‘n goeie begrip daarvoor is van kardinale belang vir die optimale gebruik van kunsmis vir ‘n winsgewende opbrengs, sonder om die omgewing te benadeel. Stikstof (N) bemesting speel ‘n belangrike rol in die verbouing van hoë kwaliteit appels en handhawing van ‘n volhoubare hoë opbrengs. Die gebruik van N kunsmis produkte het sekere uitdagings weens die verlies van N via loging en vervlugtiging. ‘n Begrip van die wyse van werking van beskikbare kommersiële N formulasies is noodsaaklik om die effektiwiteit van N toedienings onder verskillende omgewingstoestande te optimaliseer.

Ten einde die effek van die toegediende N kunsmis te bepaal, is drie kommersiële N kunsmis formulasies: BlackUrea™, BlackDAP™ and UreaHB, as enkel N bron toegedien op ‘Rosy Glow’ en ‘Golden Delicious’ kultivars, op ‘n sandleem grond in Ceres, Suid Afrika. Verskillende parameters, insluitende vegetatiewe en fisiologiese metings, is bestudeer om die effek van die verskillende N formulasies as grondtoedienings op drie fenologiese stadia, te kwantifiseer. Die reaksie van wortel groei dinamika op die verskillende N formulasies is ook bestudeer in die ‘Rosy Glow’ boord. Laastens is die gebruik van satelliet beelde (FruitLook®) ingespan om die effek van N formulasies op biomassa en N vlakke in die bome te kwantifiseer in die ‘Golden Delicious’ boord.

Resultate van die studie het bewys dat onder hierdie omstandighede, ureum as alternatiewe N bron, gebruik kan word sonder negatiewe effekte op vrug kwaliteit of opbrengs, oor die kort tydperk van twee seisoene. Die potensiaal van ureum om as enigste N bron oor tyd tot versuring van grond onder ander omstandighede te lei moet in gedagte gehou word.

Geen betekenisvolle verskille tussen behandelings is gevind in die ‘Rosy Glow’ proef nie. Dit mag gedeeltelik te wyte wees aan die kultivar keuse. Desnieteenstaande is die positiewe tendens in toename in opbrengs met die BlackUrea™ behandeling teenoor die UreaHB in die ‘Rosy Glow’ proef bevestig met ‘n nie-betekenisvolle toename in opbrengs na een seisoen se toediening in die ‘Golden Delicious’ proef.

Witwortelgroei het verskille getoon tussen behandelings in die ‘Rosy Glow’ proef. Die UreaHB en BlackDAP™ behandelings het meer wit wortels in vergelyking met die BlackUrea™ behandeling wat meer gelyke verspreiding van witwortels getoon het. Dit het geen effek gehad op die blaar N konsentrasies of ander vegetatiewe parameters gedurende die twee seisoene nie, maar daar is aanduidings van ‘n moontlike formulaseffek.

‘n Potproef met tamaties het kontrasterende resultate met die van die appels getoon, beter groei (nb) en ‘n meer logging van ammonium en nitrate in diewas verkry by die BlackUrea™ behandelings versus UreaHB. Dit kan gedeeltelik toegeskryf word aan die effek van die BlackUrea™ wat onderpresteer het in die omgewing van hoë besproeiingsfrekwensies en die inerte sandmedium.

FruitLook® was in staat om verskille in biomassa en N vlakke tussen behandelings te kwantifiseer vanaf spektrale data en daardeur akkurate asook addisionele inligting rakende die behandelings toe te voeg wat nie deur veldmetings gekwantifiseer was nie.

General introduction

Optimising profitable yields requires the inputs of fertilizers to ensure sustainable yields under commercial production conditions (Brunetto et al., 2015). Due to soils not always having adequate amounts of all essential mineral nutrients required for growth and production, fertilizers are applied in the commercial production of crops (Taiz and Zeiger, 2010). Nitrogen (N) is one of these fertilisers often associated with realising of high yields as well as high quality in commercial apple production (Wrona, 2011).

One of the challenges in applying N fertilizers, is the loss of unutilised N via leaching and volatilization, posing a threat to the environment (Vitosh et al., 1995). Understanding the mode of action of N as a fertilizer can improve the efficiency of application and reduce possible losses to the environment (Masclaux-Daubresse et al., 2010).

N fertilizers all originate from a single N source, Ammonia (NH_3), from which several forms of N fertilizers are produced (Misstof Vereniging Suid Afrika (MVSA), 2007). Different forms of N fertilizers are used in the agricultural sector due to the economic impact of the choice of fertilizer that will affect the overall profitability. Therefore, fertilizers companies started producing different fertilizer formulations to try to enhance the overall efficiency of N as a fertilizer.

BlackUrea™ and BlackDAP™ were produced by Profert (Pty) Ltd with this focus in mind (Nyati, 2014). BlackUrea™ is produced from granular urea (high biuret) and coated with an enriched coating and BlackDAP™ (a di-ammonium-phosphate consisting of both N and P). The term Black™, refers to an organic compound mixture that is used to completely coat the UreaHB and DAP granule, by means of drenching. This coating is reported to consist of a unique combination of biostimulants and nutrient biocatalysts derived from humic acid, plant hormones, vitamins and minerals, thus creating a dynamic biological management system within the soil on application (Nyati, 2014). This biological system could possibly enhance the biological availability N to the plant, while minimizing the losses often associated with urea-based fertilizers. Higher yields were reported in three different grain cultivars when BlackUrea™ was applied compared to no fertilizer application (Nyati, 2014). Overall, the efficiency of BlackUrea™ was reported to be higher when using less N, compared to standard N applications (Nyati, 2014).

The use of BlackUrea™ and BlackDAP™ as alternative N sources to UreaHB, have not been evaluated on apples under South African conditions. This study evaluated the effect of two N formulations as urea soil applications on aspects of tree physiology, yield and quality of two apple cultivars ('Golden Delicious' and 'Rosy Glow'), grown in sandy-loam soils in Ceres. In addition, white root growth patterns were quantified for each treatment in the 'Rosy Glow' trial. FruitLook® was used on the 'Golden Delicious' site to determine the possible use of satellite imaging as alternative tool to determine biomass as well as upper leaf N and whole tree N on a hectare basis. FruitLook® is a web-based system, integrating satellite images to generate data regarding different growth parameters (Jarmain, 2012).

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Literature study: The effect of soil applied N on fruit tree physiology

1.0 Introduction

Nitrogen (N), the mineral element that is required in the highest amount by plants and is incorporated in many plant cell components such as amino acids, nucleic acids and plant proteins (Taiz and Zeiger, 2010). N is a natural constituent of air with about 80% of the atmosphere being covered by N gasses (Lindemann et al., 1990). N nutrition plays a major role in controlling vegetative and reproductive growth of deciduous trees (Tagliavini et al., 2005). N is a mobile element and could easily be transferred between the different areas of the plant according to the desired needs (Tagliavini et al, 2005). External N inputs into a orchard system are mostly made by the application of N fertilizers (Kotze, 2001). N can exit the orchard system via plant uptake as well as losses due to volatilization and leaching (Cameron et al., 2013). Losses of N can have a negative impact on the environment, as a possible greenhouse gas (volatilization of ammonia) and/or possible pollution of water (leaching of nitrates) posing an indirect health risk for all forms of life (Cameron et al., 2013). Due to these losses of N, it is of great importance that the available N should be managed to the optimal level, in the orchards. Optimal management systems for applied N is needed to develop a system for sustainable fruit production. Understanding the effect of different rates of fertilizer and managing these rates according to how it is applied and when it is applied, are included in the process of understanding and improving additional N application. It is also important to acknowledge and understand the different N sources involved in fruit tree growth and physiology (Tagliavini et al., 2005).

Apple yield and quality as influenced by applied N may differ regarding tree vigour. Vigorous rootstocks had shown to result in apple trees displaying no effect following applied N (Ernani et al., 2008). In a study done by Ernani et al (2008) it was found that over a 6-year period, N applied had no effect on fruit yield. This result was explained as a possible effect of N release from the soil and senescent leaves. 110kg.ha⁻¹ of N is annually released from the top 30cm of soil due to organic matter decay (Ernani et al., 2008). This implicated that on soils with very high organic matter, applied N may have little or no effect on fruit yields and fruit tree growth. Although the applied N had an effect on the overall vigour of trees, there was no significant effect on the quality of the fruit. No significant effect was noted in regard to the nutrient composition of the fruit, with no change in quality or ability to store the fruit after harvest.

Therefore, it is important to approach additional applications of N with caution and ensure all factors impacting on efficient uptake and allocation in the plant are taken in consideration before recommendations are made. The most important factors reported in literature will now be discussed.

2.0 Nitrogen sources

2.1 Air

In the biosphere, N can be present in different forms, but not all forms are readily available to living organisms. N gas (N_2), a very stable triple bond of two N molecules, needs to be broken to produce ammonia (NH_3) or nitrate (NO_3^-) to be completed. The production of NH_3 and NO_2^- through the breaking of these stable bonds is known as N fixation and can exist in both with natural and under industrial conditions. Under industrial conditions, known as the Haber-Bosch process, N_2 combines with hydrogen to form ammonia. The Haber-Bosch process is considered as the starting point from which many other agricultural manufacturing of N containing products developed. Natural processes of N-fixation can be either lightning, photochemical- or biochemical reactions. Biochemical reactions involve cyanobacteria fixing N_2 into ammonia, which then dissolves into water to form ammonium (NH_4^+). From an agricultural standpoint, biological N fixation is critical, because industrial production of N fertilizers seldom meets agricultural demand (FAOSTAT 2009). In short N forms a cycle which entails cycling thorough the atmosphere, changing from a gas to reduced ions before it is incorporated into organic compounds, which form part of living organisms. Both ammonium (NH_4^+) and nitrate (NO_3^-) ions which are generated through fixation or released via decomposition of organic matter in the soil will become the object of competition among both plants and microorganisms.

2.2 Soil

There are two major sources of N contributing to vegetative growth and reproduction in the fruit trees namely root uptake and internal cycling (Millard, 1996). These two major sources can originate from either mineralization of organic matter or from external sources such as applied N fertilizer (Millard, 1996). Perennial plants, such as apples, can use the internal cycling of N (N reserves in permanent tissues) as a strategy to promote plant growth without, or with less N taken up by the roots (Quinones et al., 2007). Reasons for this strategy can be either to initiate growth in the start of new season, or, when fruit are maturing, or when the N

demand is higher than normal. The storage of N, after harvest (during winter), occurs in the roots and bark of deciduous trees (Nielsen et al., 2001). N utilised during bloom in the spur leaves of apples (Nielsen et al. 2001) is derived from recycling of stored N, while applied N taken up via roots are transported to the leaves of developing shoots. Remobilization of N in apple trees are normally complete within 40 days from bud burst (Tagliavini et al., 2005). In some cases this process, can continue as long as 60 days depending on whether N stored is sufficient (Tagliavini et al., 2005). As soon as the remobilisation is terminated, uptake by the roots will serve as the main source of N during the rest of the season.

The uptake of N depends on many factors, including applied N concentration, soil conditions as well as current available photosynthates that can be translocated to the roots. If we thus understand the internal N cycle, the timing and concentration of soil applied N can be managed more efficiently.

Plants have developed mechanisms to be competitive, are therefore able to compete efficiently for N ions within the soil and soil solutions. Under increased soil concentrations that often occur post fertilization, both ammonia and nitrate absorptions via the roots may exceed the plants capability to assimilate these ions, resulting in a build-up within the plant tissue. It should also be noted that elevated levels of nitrate or ammonium not being assimilated can be highly toxic to both plants and animals. Plants can counter these effects by either assimilating ammonium near the site of absorption and store any excess ammonium in their vacuoles, and in doing this, avoiding the toxic effect on both the membranes and the cytosol (Taiz and Zeiger, 2010). Plants can also manage high levels of nitrate by either storing it or translocating it from one tissue to another. Most natural and agricultural ecosystems show dramatic gains in productivity after fertilization with inorganic N, assisting to the importance of this element (Taiz and Zeiger, 2010).

Roots actively absorb nitrate from the soil solution via several low- and high-affinity nitrate-proton cotransporters (Crawford et al., 2002). Nitrate is assimilated via the shoots and roots, with small amounts being assimilated in the roots and as nitrate supply increase, being assimilated in shoots as well. Nitrate is converted to nitrite, which is highly active and a possible toxic ion and thus immediately converted to ammonium (Taiz and Zeiger, 2010). The conversion of nitrate to ammonium is regulated by nitrate and nitrite reductase enzymes (Crawford et al., 2002). This reduction of nitrate to ammonium takes place in the chloroplasts

(Taiz and Zeiger, 2010). Plant cells assimilate ammonium via transamination reactions into amino acids such as glutamine and glutamate, in turn avoiding ammonium toxicity. Biological fixation also plays an important role, fixing most of the ammonium formed from atmospheric N. Several types of N-fixing bacteria form symbiotic associations with higher plants (Taiz and Zeiger, 2010). N-fixing bacteria, depending on the bacteria, require anaerobic conditions (Taiz and Zeiger, 2010).

Biological N fixation is the conversion of N gas (N_2) into plant available N (Lindemann and Glover, 1990). Certain plants, like legumes, act as a host for certain N fixating bacteria (Lindemann and Glover, 1990). N fixating nodules will form via the rhizobium bacteria growing on the roots of the specific legume, in turn receiving all necessary energy from the plant (Lindemann and Glover, 1990). Legumes, depending on the specific cultivar, can fix between 50 and 250 kg of N per hectare but the return of N to the soil cannot be seen as correlating with this fixing of N (Lindemann and Glover, 1990). N return, from legumes, to the soil is mostly via decomposition of the plant parts after the growth period (Lindemann and Glover, 1990). The known role of bacteria in the fixation of ammonium puts an emphasis on the role that organic matter and overall biological health of different soils plays in ensuring this fixation is optimised to ensure optimum availability to plant roots (Cameron et al., 2013). Organic matter, mostly made up from humus, can contain as much as 907 kg organic N per 1 % of soil organic content (Lamb et al, 2014). Although the conversion rate of organic N is very slow, for every 1 % of soil carbon content, up to 22 kg N per hectare can be available for plant uptake (Lamb et al, 2014).

3.0 Nitrogen metabolism in the plant

With most crop species, the plant life cycle can be divided into a sink and source stadium (Hawkesford and Barraclough, 2011). The sink stadium is primarily occurring during vegetative growth, when most of the growing leaves and shoots assimilates most of the inorganic N mostly in the form of nitrate. This N is then used in both amino acid and protein synthesis (Hirel et al., 2007). The source stadium occurs primarily during the remobilization phase, when organic compounds like carbohydrates and amino acids are translocated from older plant parts to younger parts, still in the sink phase (Hirel et al., 2007). Acquiring a better understanding about these metabolic processes, how they interact and how nitrates controlled

within these processes, plays a crucial role in understanding the use of crop applied fertilizer (Hirel et al., 2007).

The principal N source taken up by most plants, is nitrate. Nitrate is taken up via specific nitrate transporters situated in the root cell membrane. Nitrate is then reduced to nitrite via nitrate reductase, with nitrite being reduced to ammonium via nitrite reductase. Ammonium can also be taken up directly via root specific ammonium transporters (Taiz and Zeiger, 2010) or generated within the plant, via different metabolic pathways such as photorespiration, propanoid metabolism, utilization of N transport compounds and amino acid interconversion during the process of N recycling after protein hydrolysis. Regardless of the metabolic origin, ammonium will be converted into organic forms via glutamine synthetase glutamine, which is then processed to amino acids and other proteins. This inorganic pathway of N assimilation is therefore one of the important limiting factors controlling crop production (Hirel et al., 2007). Both ammonium and nitrate can be toxic to the plant. Unassimilated ammonium can dissipate the cell proton gradient, resulting in a loss of electron transport, from both photosynthesis and respiration, this being detrimental to plant. Plants store excess ammonium in their vacuoles, ensuring that the potential negative effect of excess ammonium is avoided.

4.0 Physiological effect of nitrogen in the plant

4.1 Photosynthesis.

N fertilizers are often applied to growing trees to promote not only growth, but also tree survival (Nicodemus et al., 2008). N is a component of proteins, amino acids, RNA, DNA, as well as other essential molecules in plants (Taiz & Zaiger, 2010). N also has a great, possibly one of the greatest, impacts on plants nutrition in the way it plays a role in photosynthesis. The largest portion of N allocated to leaves is invested in ribulose 1,5-biphosphate carboxylase-oxygenase (RUBISCO). N is also a necessary component of other enzymes involved in carbon assimilation reactions of photosynthesis and in the light-energy harvesting machinery of pigment complexes (Lawlor, 1994). The source of N applied, can have a strong influence on the energy needed for N uptake, transport and utilizing it in plant proteins (Masclaux-Daubresse et al., 2010). N is available in soil, in inorganic form as nitrate (NO_3^-) or ammonium (NH_4^+) (Lips et al., 1990). During the energy-requiring reactions, ammonium is converted into glutamine as well as glutamate, catalysed by glutamine synthetase and glutamate synthase

(Lawlor, 1994). In contrast to ammonium, nitrate must first be converted to ammonium via nitrate reductase before it can be used during energy requiring reactions. Nitrate, unlike ammonium, can be transported to the leaves before it is assimilated, where the reaction that convert it into amino acids take place using the direct products of the light reactions of photosynthesis, reducing energy costs to the plant (Lawlor, 1994). The amount of photosynthetic energy allocated to growth and N assimilation will be determined by the N source used by the plant. Although plants take up inorganic N as nitrate, it must be noted that plants can also take up N as ammonium. In short, the nitrate is reduced to nitrite via nitrate reductase enzymes. The nitrite is then transported to the chloroplast where it is reduced even further to ammonium. The coordinated action of the glutamine and glutamate synthetase assimilates ammonium into amino acid pools (Moorhead et al., 2003). Both the glutamine and glutamate will then function as N donors for the synthesis of not only amino acids, but also nucleic acids and other secondary metabolites.

In a study by Nicodemus et al. (2008), unfertilized plants was compared to low and high fertilized plants. Plants that received N fertilizer, compared to unfertilized plants, showed increased photosynthesis of 96.2 % and 96.1 % with chlorophyll a and b increasing with N addition (Nicodemus et al., 2008). Applied N source did affect photosynthesis significantly (Nicodemus et al., 2008). Photosynthesis was affected in both upper and lower leaves. Higher net photosynthesis in upper than in lower leaves suggest channelling of more N to upper growing points, which act as sinks for nutrients (Nicodemus et al., 2008). Interestingly, at 800 mg N, fertilized as Ammonia-nitrate and nitrate, significantly higher levels of chlorophyll-a were noted in comparison to jus ammonium. However, at 1600mg N, fertilized as ammonia-nitrate and ammonium, increased chlorophyll-a was noted in comparison to just nitrate (Nicodemus et al., 2008). These findings may mean that it is rather a combination of nitrate and ammonium that can play the enhancing role regarding photosynthesis. Furthermore, this increase in chlorophyll when applying N fertilizer indicates that more N is effectively allocated to the light harvesting complex (LHC). More N allocated to LHC means possible increase in N allocated to RUBISCO, and thus more carbondioxide used by the plant. Nicodemus et al. (2008) furthermore found that N fertilizer could improve growth as well as photosynthetic responses. With regard to the optimum type of N source a mixed source of NH_4NO_3 showed better physiological responses over NO_3^- or NH_4^+ applied separately. However, additional

factors such as the use of nitrification inhibitors to sustain ammonium in mixed fertilizers should be considered under field conditions (Nicodemus et al., 2008).

For crops species such as apples characterized by high yields, nutrient uptake from the soils must be adequate to meet the nutrient demand (Mengel, 2002). In the case of N, enzyme-based metabolic processes leading to increases in vegetative and reproductive growth and yield are totally dependent on its adequate supply (Lawlor, 2002). Both field and laboratory investigations have demonstrated that increasing the supply of N fertilizer increases growth and photosynthesis (Prsa et al, 2007). Decrease in photosynthesis as a result of enzymatic and non-enzymatic components of the photosynthetic complex can partially be related to N deficiency.

In the case of N being deficient, it was shown that CO₂ assimilation had also decreased, and decreased CO₂ assimilation lead to a decrease in RUBISCO products as well as a decrease in synthesis of several other enzymes involved in the Calvin-Benson Cycle (Prsa et al., 2007). Lower rates of photosynthesis under conditions of N limitation can also be attributed to the reduction of chlorophyll content (Prsa et al., 2007). Total RUBISCO activity increased linearly with leaf N content (Prsa et al., 2007). In Fuji apples, the chlorophyll content decreased with decreasing leaf N content (Prsa et al., 2007). Environmental conditions, cultivar development and the stage of plant growth could be a big depending factor for the effect of applied N on photosynthesis. Due to the mobility of N, leaf N levels were found to be lower at harvest compared to when samples were taken after full bloom the reason for this decreasing leaf N level can be explained by referring to N and its allocation towards the roots and trunk of trees for storing purposes. Leaf N levels, although decreasing after harvest was still higher in the fertilized treatments than in the control. In contrast, low or high soil N levels did not influence leaf N (Nielsen et al., 2001). This finding from Nielsen et al. (2001) showed that leaf N levels are less response towards applied N when soils have already high soil available N. Chlorophyll content showed to be higher in treatments that received N than control treatments that received no N fertilizer. Increasing chlorophyll was also higher in leaves that are thicker, and thus N can't be singled out as the only factor influencing chlorophyll levels. In the study done by Prsa et al. (2007) it was shown that higher rates of applied N resulted in higher carbon dioxide (CO₂) saturated photosynthetic rates. However, no effect was found on the carboxylation efficiency in regard to applied N and although leaf N was increased with applied N, the existing pool of

N in control plants did not limit the activity of RUBISCO. Thus, a limited effect of extra N can be expected in plants that do not suffer from N deficiency (Prsa et al., 2007).

N fertilizer makes a smaller contribution towards leaf growth in mature trees than in young trees (Prsa et al., 2007). In addition, the cultivar demand should be evaluated and used to efficiently manage the application of N fertilizer. N demanding processes, such as photosynthesis and organic matter biosynthesis requires small amounts of soil mineral N, result in a certain link between the N- and carbon cycles within soils (Iversen et al., 2008). Over time, limitation of available N may have certain restrictions in regard to carbon storage (Iversen et al., 2008). Applied N resulted in a sudden increase of inorganic N availability with fertilized trees showing a sudden increase directly after fertilization and again after leaf senescence. Theoretically whole-canopy photosynthesis is maximised when the N within the leaves is distributed towards the leaves receiving the highest irradiance resulting in these leaves also having the highest levels of N (Iversen et al., 2008).

4.2 Respiration

Key metabolic, chemical and structural attributes of leaves of higher plants are often related to each other in similar and predictable ways across the angiosperms, largely independent of growth form, plant functional type or biome (Iversen et al., 2008; Reich et al. 2008). One of these relationships is the relationship between dark respiration rate, also known as specific respiration rate, and mass-based N content (Reich et al., 2008). Considerably fewer research have been made of this relationship but still results suggest that there is consistent coupling of leaf respiration rate and mass-based N content among species worldwide (Reich et al. 2008; Wright et al. 2004). Furthermore Reich et al. (2008) found that although the total respiration of the entire plant scales isometrically with the total amount of N per plant, whole plant data is scares (Reich et al. 2008). Ryan (1991), emphasised that there should rather be a general relationship between rate of respiration and mass-based N for all plant-organs and plants. With this in mind, Reich et al did analysis to determine whether there is a relationship between rate of respiration and N content. The results showed that among all the 287-species measured the rate of respiration increased with increased tissue N, with two-thirds of all variation within the rate of respiration were associated with variation in N leves (Reich et al. 2008).

Strong relations, per unit N increasing with tissues with higher metabolic rates, were also found for leaves, stems and fine roots with rate of respiration. This is mostly due to the higher turnover rate of proteins, solute gradient maintenance and ion transport in more metabolic active tissue (Reich et al., 2008). The results further also showed a disproportionate increase in N in metabolically active pools relative to structural N with increasing tissue N concentration (Reich et al., 2008). Results further showed no consistent difference in organ specific rate of respiration – mass N slopes (R-N Slopes) but did however show greater R-N Slope for leaves than for roots. Leaf respiration, at certain mass-based N, was consistently lower than stem and root rate of respiration (Reich et al. 2008). The reason for this lower rate of respiration in the leaves would be because most of the N in leaves are utilised for functions other than respiration. Leaf N is involved in both respiration and photosynthesis with 50 – 75% of N being distributed within photosynthetic operations (Reich et al., 2008). In stems and roots most N is used to drive the metabolic processes such as storing, transporting and converting non-structural carbohydrates (Reich et al., 2008). In general, it was found that any decline in N resulted in a decline in the rate of respiration in leaves, stems and roots.

5.0 Nitrogen application on fruit trees

5.1 Vegetative effects

N applications will not only have an effect on reproductive, but also on vegetative growth of apple trees. Apple growth is not only limited to just N applications, but when N is seen as the only variant it will increase vegetative growth with increasing soil available N (Brunetto et al., 2015). Under conditions of proper soil management, the effect of only applied N will not always have significant effects on vegetative growth (Wrona, 2011). Trees fertilized with at least 100 kg of N per season showed more vigorous growth than unfertilized trees, but was not significant (Wrona, 2011). When soils are managed to its full potential, seasonal build-up of especially N can result in differences in N supply to have less of an effect regarding plant reaction as to other mineral elements (Wrona, 2011). It should be noted, that excessive N application can have a negative effect on vegetative growth. Over application of N will result in excessive vegetative growth, posing a risk to not only the quality of the fruit, but also the overall health of the trees (Wrona, 2011). Too much vegetative growth can result in shoot not hardening off quick enough, posing a risk for amongst others, frost damage (Wrona, 2011). Post-harvest N applications plays an important role in ensuring enough available N is stored for the new seasons vegetative growth (Toselli et al., 2000). Soils, that show N as a limiting

factor, will benefit from spring N applications, increasing not only the vegetative but also reproductive growth of apples (Toselli et al, 2000). Applied N will not necessarily be stored in the roots, but rather the older woody parts of the tree, enabling remobilization during spring with N uptake from soil being active via the roots (Toselli et al., 2000). Balance between vegetative and reproductive growth is key and understanding N application and the timing thereof plays an important role in ensuring this balance.

5.2 Reproductive effects

N containing fertilizers have been commonly applied to fruit trees to increase vigour and yield (Raese et al., 2007). N is a principle factor that influences leaf and fruit colour of apple trees (Raese et al., 2007). The effect of different N rates on biennial bearing were tested over four seasons (Drake et al, 2002). Higher N rates, 227 g N per tree applied during mid-summer, reduced the effect of biennial bearing with higher numbers of return bloom in the following season (Drake et al, 2002). However, N treatments are not the only factor influencing biennial bearing (Drake et al, 2002). In contrast to annual crops, there is a poor correlation between applied N rates and yield, with only slightly bigger yields reported when applying higher rates of N fertilizer to perennial crops (Drake et al., 2002). Neither fruit size, nor trunk girth was significantly affected by different N fertilizer rates. Shoot extensions however, was increased with the higher rates of applied N. Leaf size increased slightly with the number of nodes on a shoot being more for higher N rates. Internode length was not affected (Raese et al., 2007).

Fruit quality was also affected by different rates of applied N. The peel of the fruit was greener with each added increment of N application (Raese et al., 2007). In terms of fruit firmness, titratable acid and sugar concentration, all three parameters were influenced negatively when excessive N levels were applied. Lower N fertilizer applications affected fruit quality positively in some cultivars (Drake et al., 2002). Lower rates also showed to have a more desirable effect on cold hardiness of apples (Rease et al., 2007).

Placement of fertilizers is more important for immobile nutrients than mobile nutrients, such as N., e.g. band placing fertilizers in or near the row of planting with more mobile nutrients poses a risk of damaging the seeds or seedlings (Cooke, 1982). When N is added through the water (fertigation) in the form of drip irrigation, the total N concentration in the root zone can be predicted from the N concentration of irrigation water (Nielsen et al., 1998). The application

time of N may affect the contribution of root supplied N (Weinbaum et al., 1984). During a 4-year trial period, soil applied N had no effect on fruit size or yield in year one or two but had a positive effect in year three.

N can also be applied by broad casting granular formulations. Ensuring that the most efficient fertilizer practises are followed positively effects both the yield and profitability of crops, and it can also lower the risk of environmental contamination (Aguirre et al., 2001). In a recent study to determine the effect of application timing on N uptake, it was found that higher levels of N were taken up during spring than fall applications (Rufat and De Jong, 2000). Trees growing in colder regions, may be able to take up more of the soil applied N in the spring, rather than fall, due to higher soil temperatures in spring.

Fertilization should be aimed at the level at which N is required to maintain tissue N status (Rufat and De Jong, 2000). N concentrations in fruit are similar to concentrations in foliage (Rufat and De Jong, 2000). A nitrogen fertilizer is usually applied as a soil application and released in the soil over time, where it is then utilised by plants.

Foliar applied N has a shorter metabolic effect compared to soil applied N (Mudau et al., 2013). Recent found that foliar applied N can be used as an efficient mean of improving fruit set in midseason orange (Mudau et al., 2013). Every plant has its own kinetics and should be used to optimize the N requirements per season.

N availability and the effect on vegetative and reproductive organs of fruit trees is crucial in understanding the relationship between fertilization and fruit tree growth and crop management (Rufat and DeJong, 2000). N fertilizer recovered from N starved orange trees was much more than that of trees supplied with sufficient N throughout season (Rufat and DeJong, 2000). During field studies, done by Rufat and De Jong (2000), high N treatments (HN) were compared with low N treatments (LN). Photosynthetic carbon assimilation and stored carbohydrates supply the required carbon for respiration and growth. Low levels of applied N showed a reduced rate of photosynthesis, as well as a reduced light interception (Rufat and DeJong, 2000). According to Rufat and De Jong (2000), trees fertilized with N bloomed earlier than non-fertilized trees but was harvested later than non-fertilized trees. It should be expected that lower photosynthetic rates will have the effect of reduced crop growth rate, but instead

trees from non-fertilized trees grew more than fertilized tree during last stage of fruit maturation, that resulted in a longer fruit growth period for fertilized trees. Although fruit in non-fertilized trees were bigger, fertilized trees had more fruit or a bigger crop load than non-fertilized trees. Rufat and De Jong (2000) furthermore found that leaves and fruit demanded more N than perennial tissues. Calculated net assimilation rates of N were lower in low-N than high-N trees (Rufat and DeJong, 2000). The potential vegetative growth for low applied N was less than that of high N applications because of lower dry mass to vegetative growth partitioning in response to competition between vegetative and reproductive growth (Rufat and DeJong, 2000).

Fruit quality can be affected by several pre-harvest orchards cultural practises, particularly N fertilizer (Fallahi et al., 2006). Urea was applied to the soil as N source to determine the influence of applied N on fruit quality and yield of Early Spur Rome apples. Furthermore, the use of a humic substance to enhance the effect of N was added to urea. Humic substances affects N mineralization release rate (Fallahi et al., 2006). During the study, it was found that treatments receiving N and humic substances had higher leaf N levels, as well as higher yields. Regarding fruit quality, it was shown that medium N applications resulted in bigger fruit than the control. High applications of N showed a lower fruit firmness, as well as less red colour of fruit. The treatment receiving the humic-N combination also showed greater water retention in the root zone in comparison with the control. This finding may play an important role in the future of water conservation and become a greater part of the management strategy of crops every season.

6.0 Factors influencing nitrogen uptake

6.1 Xylem

Saplings of hybrid poplar trees were fertilized with either high or low N levels to study the effect of N levels on xylem structure and function (Plavcová et al, 2013). In the study done, N was used to perturb xylem phenotype as well as investigate the corresponding changes in gene expression with N having a profound effect on poplar growth and development including xylogenesis (Plavcová et al, 2013). Firstly, to refer to xylem anatomy it should be noted that the xylem plays an enormous role in long distance transport of water and solutes, acting as mechanical support system for the plant as well too (Taiz and Zeiger, 2010). Results showed that the availability of N can affect the xylem structure and function in the hybrid poplars used.

Applied N fertilizer showed both an enhancement of height and diameter during growth. Results further showed that adequate levels of applied N as well as high levels of applied N had an enhanced effect on vessels and fibre lumens of the xylem. Higher levels of N showed definite wider vessel and fibre lumens with vessel element length being longer in high N levels applied. One of the main effects of high levels of applied N fertilizer was fibre lengths and thick secondary cell walls (Plavcová et al., 2013). This study also showed that the thicker secondary cell wall resembled a gelatinous type layer as a result of these higher applied levels of N. Areas in the stem with thicker secondary cell walls accompanied by the thick gelatinous layer formed a distinct band around the stem (Plavcová et al, 2013). Although N fertilizer can lead to gelatinous layer production, it can also negatively affect the formation of regular secondary cell wall formation (Plavcová et al, 2013). These changes, as described above, in the structure of the xylem had a parallel correlation with differences in the hydraulic properties which thus have important implications for plant water use. In treatments receiving high levels of applied N, plants with larger vessel diameters transported water much more efficiently than the treatments receiving only adequate levels of applied N. It is important to remember that although treatments that received higher levels of applied N transported water more efficiently, these treatments were more sensitive to drought stress than treatments that received adequate applied levels of N.

The availability of N showed to evoke transcriptional changes in developing xylem (Plavcová et al, 2013). Gene expression profiling was done on developing xylem isolates from both treatments that received high levels of applied N as well as adequate levels of applied N. In terms of N metabolism results showed that certain amino acid transporter genes were down regulated in treatments that received high levels of applied N. Several genes involved in the organic acid metabolism were up-regulated in treatments that received high levels of applied N. The genes that were found to be up regulated included two genes that encode glucose-6-phosphate dehydrogenase of the pentose phosphate pathway as well as several other enzymes that is related to the organic acid metabolism of the plant cells (Campbell, 2002). These patterns that was observed in the gene expression, showed that the treatments receiving high levels of applied N had an increased need for N assimilation as well as amino acid synthesis with relatively more carbon being channelled towards organic acid synthesis (Plavcová et al, 2013).

The transcription of genes, involved in carbon metabolism, showed extensive changes, as revealed by microarray analysis (Plavcová et al, 2013). Both sucrose synthase as well as plant neutral invertase were up regulated by high levels of applied N. During faster growth of plants that received high levels of applied N, it was shown that not only enhanced expression of sucrose metabolism but also the up regulation of three trehalose-phosphatases was noted. Furthermore, gene that is potentially involved in the cell wall polysaccharide metabolism was also up regulated following high applied levels of N. Results further showed that only a few polysaccharide biosynthetic genes and genes not related to the synthesis of cellulose were down-regulated in plants that received high levels of applied N. This finding is surprising, taking in consideration, the thinner cell walls accompanied with high applied N levels. These thinner cell walls could be related to the faster growth of the plant cell due to higher levels of applied N.

Other findings were that in plants with higher levels of N, programmed cell death was down regulated and thus suppressing cell maturity and cell death. The study thus showed that when fertilizing with N, both changes in growth, anatomy and properties of the xylem (Plavcová et al, 2013). High levels of N thus elevate the formation of secondary xylem.

The composition of the xylem sap can reveal useful information on the storage, mobilization, and movement of nutrients in the plant (Subasinghe, 2007) and thus it can be used as an indicator of nutritional status in several plant species (Subasinghe, 2007). Nitrate (NO_3^-) to total N ratio of the xylem sap depends on the ratio of NO_3^- reduction in the root, which varies with species (Subasinghe, 2007). In most plant species, NO_3^- appears to be reduced in both shoots and roots, though the shoot is the primary site (Subasinghe, 2007). If plants were treated with sufficient amounts of N, the nitrate concentration of the xylem sap decreased with decreasing available N (Subasinghe, 2007). Nitrate concentrations within the xylem sap are a good indicator of external N status (Subasinghe, 2007). This means that applied N has a definite role in controlling the nitrate concentrations within the xylem sap and thus the translocation of macro and micronutrients. In the case of N dropping to “stress” levels, extreme low nitrate concentration was shown within the xylem sap. It is thus clear that N levels should be managed to ensure that N stress does not occur. If the best representative age and time of collection are determined, xylem sap composition could be a useful estimator of fertilizer requirements (Subasinghe, 2007).

6.2 Root zone pH

N from fertilizers comes in many forms that can be taken up by the plant. The three most common forms of N fertilizers are ammonium (NH_4^+), nitrate (NO_3^-) and urea ($(\text{NH}_2)_2\text{CO}$). Some fertilizers mixes can contain a mix of one or more of these three forms. As soon as N forms are added to a growing medium, certain natural processes take place that enable one form of N to be converted to another. Urea is converted to ammonium in less than two days (Mattson et al., 2009). Ammonium-based N is a term used to describe when urea and ammonium are grouped together. In a study done by Mattson et al (2009) it was found that by understanding the different sources of N applied, you can manage the root pH zone and thus avoid the toxic build-up of ammonium. Both ammonium and nitrate are charged molecules and when they are taken up by the roots, the roots typically release oppositely charged molecules thus maintaining a balanced pH in the plant cell. This means that because ammonium is charged positively and nitrate negatively, the substrate pH can be altered. The uptake of ammonium results in the release of one hydrogen (H^+) ion into the medium solution, thus lowering the pH over time. Plants take up nitrate coupled by the release of a negatively charged hydrogen ion (OH^-), thus potentially increasing the pH of the medium solution over time. In a study done on rose plants, the effect of N form on the characterising of the growing medium showed that higher percentages of ammonium in the hydroponic solution resulted in lower pH of the growing medium (Mattson et al., 2009). Selecting the right mix of ammonium N and nitrate thus plays an important part in determining the rate at which the substrate pH can change. The pH changing property of a fertilizer is known as the fertilizers potential acidity (Mattson et al., 2009)

6.3 The rhizosphere

The rhizosphere plays an important role in the efficiency of nutrient uptake in crops through being the interface between roots and soil thus meaning that nutrient availability in the specific rhizosphere will determine the uptake of these nutrients by the plant (Hawkesford and Barraclough, 2011). Regarding nutrient transport and the proportion of nutrients demanded by the plant, only a small amount of this demand can be taken up in the immediate vicinity of the roots. The amount of N required by the plant and taken up through interception can be as small as 2% (Hawkesford and Barraclough, 2011). Nutrient movement from the rest of the soil area is thus crucial in supplying the plant with adequate nutrients. The nutrient concentration in the rhizosphere is mostly determined by the ratio of nutrient movement to the roots and nutrient

uptake by the roots (Hawkesford and Barraclough, 2011). In the event of nutrients movement towards the roots being more than required by the plant, nutrient accumulation will occur with the opposite (nutrient depletion) being when the movement is less than plant demand. The soil pH plays a big role in nutrient availability and thus also the nutrient efficiency of plants (Hawkesford and Barraclough, 2011).

Plant roots can release CO₂ via root respiration as well as release or utilise of H⁺ and result in the change of the pH in the rhizosphere (Hawkesford and Barraclough, 2011). The balance between cation and anion uptake via the roots is responsible for greater changes in the rhizosphere pH. Cell cytoplasm charge maintenance is controlled via the acidification (efflux of protons) or alkalization (influx of anions) into the root cells (Hawkesford and Barraclough, 2011). N can be taken up in two different forms: Ammonium (NH₄⁺) or nitrate (NO₃⁻) and is taken up in large amounts by the plant thus having the greatest effect on rhizosphere pH in comparison to other nutrients (Hawkesford and Barraclough, 2011). Rhizosphere acidification, as a result of plants supplied with NH₄⁺ showed an increase in phosphorous availability over plants fertilized with nitrate (NO₃⁻) (Hawkesford and Barraclough, 2011). N mineralization or the transformation of N has a greater effect on soil pH and microorganisms. High microbial activity in the rhizosphere can result in a high rate of N mineralization (Hawkesford and Barraclough, 2011). The transformation of organic N to ammonium, or known as ammonification, will increase the rhizosphere pH whereas nitrification will result in decreased pH (Hawkesford and Barraclough, 2011). Although the findings made that supplied N in the form of ammonium or nitrate will have a definite effect on rhizosphere pH it should be noted that the acidification by use of ammonium may be limited to the use of applied ammonium with nitrification inhibitor due to the fact that in many soils ammonium is reduced to nitrate (Hawkesford and Barraclough, 2011). N mineralization is higher in the rhizosphere due to the release of easily decomposable substrates (Hawkesford and Barraclough, 2011). Respiration in the roots and microbial biomass create anaerobic microsites in the rhizosphere resulting in higher denitrification rates in the rhizosphere (Hawkesford and Barraclough, 2011).

Biofilms on the root surface of rice, because of ammonia-oxidizing bacteria, has been shown to improve the uptake of nitrate taken up directly by roots (Hawkesford and Barraclough, 2011). It should, however, be noted that microorganisms may also immobilize the N in their microbial biomass thus resulting in a decreased N availability to plants (Hawkesford and

Barracough, 2011). The interaction between microorganisms and N availability can be explained by the C:N ratio. Lower C:N ratios (<15), in the case of microorganisms, require larger amounts of N than higher C:N ratios (20+), such as plants. If these high C:N ratios break down it will result in N uptake into the microbial biomass exceeding N mineralization (Hawkesford and Barracough, 2011). The reduction of carbon availability leads to partial death of microbial biomass thus releasing nutrients, but it is still important to remember that net N immobilisation will still temporarily reduce N availability to plants (Hawkesford and Barracough, 2011). N used from the soil and the efficiency thereof could be increased by increasing the overall microbial action or activity in the soil, therefore increasing N mineralization bearing in mind that microbial biomass should also be stimulated to ensure the release of available immobilized N (Hawkesford and Barracough, 2011). Most soils, in the case of agriculture, are nitrate rich as ammonium is not readily because of the nitrification within soils. However, in soils occupied by plants rich in mycorrhizal activity an inorganic form of N is more susceptible (Simard et al., 2003). Mycorrhizal plants can form mycorrhizas with large amounts of fungi that can interconnect many plants known as a mycorrhizal network. The mycorrhizal network plays a role in providing a pathway for nutrient mobility, such as carbon and N (Simard et al., 2003).

6.4 Carbon

N and its relationship to carbon can play an important role towards the N cycling process, especially in higher rainfall areas. The relationship of Carbon to N is mostly dependant on the specific climate (temperature, rainfall), productivity as well as type of microorganisms that plays a role in composition within the soil (Adams et al., 2004). In a study done on forest soils it was found that a C:N ratio greater than 25 will have little effect on nitrate denitrification and leaching but C:N below 20 will have a definite effect with lower ratios resulting in more leaching than higher ratios. When N is over applied, the excess N can result in certain soil microorganisms becoming inactive, leading to a reduced decomposition of the soil substrates such as plant litter (Adams et al., 2004). When N is overapplied, leaching, especially into water sources, can have a negative effect on water quality of specific sources involved. It is important to understand the interaction of climate, N levels as well as the carbon status within different soils in order to fully grasp the interaction between N and carbon and the role it plays in effective utilization of N within the soil. Recent work done on N and carbon metabolism

showed that there is a definite relationship between N and carbon as N assimilation requires a carbon skeleton (Moorhead et al., 2003).

7.0 Nitrogen application and sustainability

The main driver for crop improvement over the 20th century has been yield (Hawkesford and Barraclough, 2011). During the 20th century, yield improvement did not increase only due to plant breeding but also due to the use of applied mineral fertilizers (Hawkesford and Barraclough, 2011). Of all the fertilizers, N is the most limiting factor in regard to crop production (Hawkesford et al., 2011). The problem with this extensive use of N is the result that the diversity as well as functioning of many non-agricultural bacterial -, animal-, and plant ecosystems has been damaged to severe extent including the carbon absorbing capacity of the biosphere (Hawkesford and Barraclough, 2011). Extensive evidence showed that by using applied fertilizer intensively, the overall microbial carbon utilization as well as N mineralisation is promoted, thus resulting in the depletion of soil organic matter (Hawkesford and Barraclough, 2011). Adding on to this problem is also the fact that applied N fertilizer can result in the release of nitrous oxide, reacting with the ozone and forming ammonia, a toxic gas (Hawkesford and Barraclough, 2011). It is thus clear to state, that with increasing productions, due to the increasing demand for food security, the sustainable use of especially applied N fertilizers is of utter importance.

The over application of N not only increases the production cost, but may also affect fruit quality (Dong et al., 2005). A Study done by Dong et al. (2005) showed that application of N in the autumn had no effect on shoot length and total tree biomass. Trees that received summer applications of N resulted in having the highest total tree biomass. In accordance with the study done by Fallahi and Simons (Dong et al., 2005) it was found that trees receiving soil applied N showed better shoot growth as well as higher tree biomass than trees receiving leaf applied N. Regarding root growth, it was noted that trees receiving soil applied N, in the summer, had more extension roots than trees receiving leaf applied N. Trees that received leaf applied N showed more feeder root growth. Extension roots are known to have higher IAA synthesis ability than feeder roots (Dong et al., 2005). More extension roots present in trees receiving soil applied N in the summer resulted in more IAA synthesis and thus more vegetative growth. More vegetative growth resulted in higher shoot/root ratio. Growth and biomass partitioning is influenced by the timing of N applied with summer applications influencing autumn

responsiveness of trees. In the one-year study done by Liakos et al. (2013) it was found that overall yield was improved by making use of variable applied N on apple crops. The over application of especially N, can lead to not just over growth (bigger trees) and lower yields but also contribute to increased groundwater pollution. The management of N in orchard is of very high importance; however, not a lot of studies have been done on the importance thereof. By determining the amount of N removed by the crop, the correct amount of N can be applied by making use of variable N applications. Using precision maps, based on the nutrients removed, the efficient amount of fertilizer need could be determined and thus applied on that specific area. The application of this technology in research is still being developed with more work required.

8.0 Conclusions

N, and the application thereof should be viewed more holistically in the context of crop production. The overall effect of not only different N sources, but different application times and rates of N fertilizer should receive further attention. Commercial N applications not only needs to meet the economical demands of the producer, but most important, is the physiological needs of the specific crop involved. Understanding the different mode of actions when different N sources are used, will be a key factor in understanding the overall efficiency of different N sources. It should be noted, that although soil applied N are very important regarding crop production, the role of foliar applications of N should never be underestimated. The different timing of not only soil applied, but also leaf applied N should always be taken into account when deciding on the pending season's fertilizer program. The balance between adequate vegetative growth and maintenance of productive high-quality fruit will be pivotal in ensuring optimal yields are achieved. N, as stated earlier, should be seen in the larger context as part of the total plant relationship.

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Paper 1: Evaluating the effect of three commercial nitrogen formulations on tree physiology as well as quality and yield of ‘Rosy Glow’ fruit (*Malus domestica* Borkh.)

Abstract

Optimal management of applied nitrogen (N) in a commercial orchard is required to produce export fruit quality and deliver a sustainable, high yield. Understanding the effect of different rates of fertilizer and managing these rates, according to amount and timing of application, are two aspects key to the consideration and improving the commercial practice of the additional application of N. Two major formulations of N, viz. nitrate (NO_3^-) and ammonium (NH_4^+), are currently applied in apple (*Malus domestica* Borkh.) orchards, but all forms of N are not equal regarding their mode of action. This study evaluated the effect of three different N formulations as soil applications on tree physiology, yield and quality of ‘Rosy Glow’ fruit, on a loamy clay soil. A commercial, bearing orchard was selected as experimental site in Ceres, South Africa. Fertilizers were applied at recommended dates on three phenological stages, viz. budburst to full bloom, six weeks after full bloom and post-harvest. Two types of granular urea fertilizers (BlackUrea™ and BlackDAP™) were compared with standard urea high biuret (UreaHB) as the control. No significant differences in yield or internal or external fruit quality were found between treatments. BlackDAP™ showed increased photosynthesis on two selected dates, but, although significant, it would be inaccurate to describe this difference to just the fertilizer formulation. The strongest reaction to the application of the different formulations of NH_4^+ was noticed in the number of white roots. In the UreaHB and BlackDAP™ treatments, more white roots, with a prominent peak during both seasons, were reported compared to the BlackUrea™ treatment. This could not be explained in the scope of the study but should be further investigated in future.

Key words

Ammonium, fertilizer, photosynthesis, white root growth, yield efficiency

Introduction

The daily demand for high quality fruit by consumers and suppliers is growing at a steady pace (Bright, 2005). Together with citrus (*Citrus* spp.) and grape (*Vitis vinifera* L.), apple (*Malus domestica* Borkh.) is one of the main fresh fruit export products from South Africa, thus best management practices are constantly being evaluated in different cultivars to optimise fruit yield and income (Kotze, 2001). The profitable production of a specific apple production unit not only calls for greater physical inputs such as labour, but also much needed specialised attention regarding the overall nutritional status of individual units, or trees. It is important to note that, for an orchard to be successful, it should be profitable, relating to yield and fruit quality.

Nitrogen (N) plays an intricate role in the sustainable production of these profitable units, as it is often over applied and contributes to contamination of ground water (McIsaac, 2003). The effective use of N is a key factor regarding the sustainable use of N fertilizers to avoid N over-fertilization (Zhang et al., 2012). Over the years, N fertilizers were, to some extent, neglected regarding the correct timing and use of N (Zhang et al., 2012). The overuse of N was the direct result of increased use of N due to its impact on enhancing crop yields (McIsaac, 2003). Limited time and resources often led to fertilizers being applied according to time availability rather than plant requirement and led to less N utilised by the plant, and more N prone to leaching and other forms of losses to environment (Randall et al., 2008).

Nitrogen fertilizers are mostly formulated as nitrate (NO_3^-) or ammonium (NH_4^+) (Tagliavini et al., 2005). The main goal behind different N formulations is to optimize the total uptake through the roots (Mattson et al., 2009), primarily taken up as NO_3^- or NH_4^+ . Currently, the three major forms of soil applied N are urea, ammonium and nitrate (Kotze, 2001).

Over application of N can be negative for plant production and although plants can store most of the excess N, too much N in the plant, will result in cell damage (Mattson et al., 2009). An increase in N does not necessarily result in an increased yield with bigger fruit. A reduction of internal quality was observed when trees that received too much N was compared to trees receiving no fertilizer (Wargo et al., 2003). Furthermore, the effect of different rates of applied N that did not have significant effects on photosynthesis of apple trees and, although lower

rates of N showed lower rates of photosynthesis, at optimum N levels, no significant differences regarding photosynthesis was reported (Fallahi et al., 2001).

All forms of N are not equal (Mattson et al., 2009). Urea is a white crystalline granular fertilizer and contains $460 \text{ g}\cdot\text{kg}^{-1}$ N (Overdahl et al., 2017). Soil applied urea caused a significant increase in both weight and yield when applied at certain phenological stages (Hasani et al., 2016). The advantage of Urea is that it has a neutral charge when compared to other N-containing fertilizers until it is hydrolysed to NH_4^+ and can be taken up either directly, via urease transport proteins, or as NH_4^+ or NO_3^- (Witte, 2011). Understanding urea, and the metabolism thereof within the plant could assist in helping in the overall enhancement of not only the use of Urea as a sole N source but understand N metabolism within plants as a whole (Witte, 2011).

Although ammonium, as well as nitrates, will play a contributing role in the vegetative growth, reproduction and overall vigour and yield of fruit trees, some N fertilizers like urea still faces challenges like volatilization and leaching (Du Preez, 1984). Due to the biochemical properties of ammonium-based fertilizers (NH_4^+), it is more susceptible to volatilization. The availability of ammonium is sometimes delayed, due to the microbial interaction required in the soil to reduce the ammonium to nitrate, before the roots can take up the N. Other limitations include leaching of both ammonia and nitrates in very sandy soils (Du Preez, 1984).

In most studies, a combination, rather than just one formulation, was used with trials resulting in not only higher yields, but also better-quality fruit at balanced N levels over four years (Drake et al., 2002). Internal quality and external fruit colour were also influenced positively when N was applied at the correct dosage (Drake et al., 2002). The combination of both ammonium and nitrate also showed that starch content and the rate of photosynthesis was only influenced when deficiencies regarding N were observed, thus an increase in N will not necessarily increase starch content or the rate of photosynthesis in apples (Prsa et al., 2007).

With volatilization and leaching in mind, different fertilizer formulations were introduced into agriculture. BlackUrea™ and BlackDAP™ was introduced to add value by increasing the efficiency of N according to Nyati (2014). BlackUrea™ comprise granular urea high biuret

(HB) coated with the enriched coating, whereas BlackDAP™ refers to a di-ammonium-phosphate comprising both N and phosphate (P) coated with the same coating as BlackUrea™.

The term Black™ is derived from an organic compound mixture used to coat the urea and DAP granule, drenching the granules to the point where it is totally coated by the humic and fulvic rich coating. The coating is reported to consist of a unique combination of biostimulants and nutrient biocatalysts, derived from humic acid, plant hormones, vitamins and minerals, creating a biological management system within the soil. This nutrient enhanced coating could possibly improve the biological release of available N to the plant, while minimizing the losses often associated with urea-based fertilizers (leaching and volatilisation).

The use of BlackUrea™ and BlackDAP™ as alternative N sources to UreaHB, has not been evaluated on apple orchards under South African soil and climate conditions. This paper evaluated the effect of three urea formulations as soil applications on tree physiology, yield and quality of ‘Rosy Glow’.

Material and methods

Orchard selection and layout

A commercial, bearing orchard was selected on Alhambra, situated in the Bo Swaarmoed Valley, Ceres (33°21’16.92” S; 19°32’7.87” E) in the Western Cape Province of South Africa, approximately 1076 m above sea-level. The trial was conducted on the apple cultivar ‘Rosy Glow’ grafted on M793 rootstock. The orchard was planted in 2009, in loamy soils with some clay deposits, at a planting density of 1481 trees·ha⁻¹. Micro-irrigation was used with granular fertilizer being applied by means of a mechanical spreader or by hand. The trial layout was a randomised complete block design with eight replications of five-tree experimental units.

Treatment applications

Fertilizers were applied at three phenological stages: bud burst to full bloom (26 % of total N); six weeks post full bloom (31 % of total N) and post-harvest (43 % of total N). Three types of urea granular fertilizers were applied, viz. BlackUrea™ (Profert (Pty) Ltd, Potchefstroom, South Africa), BlackDAP™ (Profert (Pty) Ltd, Potchefstroom, South Africa) and the standard Urea HB (Profert (Pty) Ltd, Potchefstroom, South Africa) fertilizer as the control treatment. Treatments were all applied at the same N rate of 140.4 kg N per hectare, as recommended by

the commercial fertilizer consultant. Fertilizer timing and applied rates are summarised in Table 1. No additional N was applied to the orchard during this time with the N present in soil, not being substantially different between BlackUrea™ and UreaHB treatments (Table 2). The fertilizers, were applied by hand to plots of five trees, with a buffer tree between treatments.

Physiological parameters

In all treatments, the third tree per plot was used for physiological measurements. Measurement to determine total photosynthetic capacity was carried out using a Li-Cor Li-6400 infra-red gas analyser (IRGA) (LiCor Biosciences, Lincoln NE) set with the following fixed parameters: CO₂ at 380 ppm; active radiation (PAR) at 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; flow rate at 500 $\mu\text{mol}\cdot\text{s}^{-1}$ and leaf temperature at 25°C. Three leaves, exposed to the sun and proximately at shoulder height, were selected for measured. Measurements were performed two weeks after treatments were applied.

White root growth dynamics

Root dynamics were quantified with a CI – 600 In Situ Root Scanner (CID Bio-Science, INC.) at a resolution of 300 DPI. Transparent poli-tubes were installed in the root zone to enable the roots to grow around the tubes during the period of August 2015 to August 2017 when MR scans were performed at monthly intervals. Two tubes were randomly installed per treatment next to 1 tree per plot. The tubes were installed at a 45° angle, making use of a soil auger, representing approximately 600 mm soil depth (Van Zyl., 2016). Four scans per tube were used to construct a complete image of the soil profile per treatment (Figure 1). This was used to determine the total number of white root tips per experimental unit by manually counting the number of white root tips per image.

Vegetative parameters

A LID-3100C leaf area scanner (LiCor Biosciences, Lincoln NE) was used to scan the leaves to determine the average leaf area (cm²) per treatment. Leaf weight (g) and trunk circumference (cm) was measured at harvest, using an electronic scale and a measuring tape respectively. Trunk circumference were used in accordance with yield (kg) per tree to calculate the overall yield efficiency (kg.cm).

Approximately 20 healthy leaves from each treatment per block, were sampled according to standard procedures in February each season for mineral analysis (SGS laboratories South Africa Pty Ltd., South Africa).

Yield and internal fruit quality

Yield was determined by harvesting all fruit from three individual trees per plot to calculate an average value. Twenty fruit per plot, of similar size, were sampled on shoulder height to evaluate internal quality at the laboratory of the Dept. of Horticultural Science, University of Stellenbosch. Internal quality parameters determinations included background and % pink colour, using both the Green- as well as 'Pink Lady' colour charts (Unifruco Research Services, Belville, R.S.A) (Figure 2). Starch break down (% starch conversion) was measured by cutting fruit diagonally, staining the one half with a 1 % iodine solution and evaluating the total percentage starch break down, using a starch conversion chart (Unifruco Research Services, Belville, R.S.A). Total soluble solutes, as determined by juicing the other half of the sample (composite sample), were determined with a digital refractometer (Model N1, Atago, Tokyo, Japan) as % Brix. Fruit diameter, mass and firmness were recorded using a flesh texture analyser (Guss electronic model GS 20, Strand, R.S.A).

Tomato Pot Trial

To determine the N nutrient balance in terms of leaching of the N, 20 tomato seedlings were planted in individual pots and filled with Console acid washed sand (Builder's Warehouse, Stellenbosch). The pots were exposed to external climate conditions when kept at the Welgevallen Experimental Farm, Stellenbosch, South Africa (33°56'53.54" S; 18°51'59.24" E) located approximately 116 m above sea level. Two treatments (UreaHB or BlackUrea™) were applied at 20g.plant⁻¹ (Table 3). The trial layout was a randomised complete block design, irrigated every second day, for twenty minutes per day, with drip irrigation making sure that the plants have adequate water throughout the trial. All excess water was collected using trays per individual pot. After two months, a composite water sample per pot, the sand per pot and the whole tomato plant was analysed for N (NH₄⁺ and NO₃⁻). Analysis was done by SGS laboratories (SGS South Africa (Pty) Ltd, South Africa).

Statistical analysis

SAS Enterprise 5.1 were used for all analysis. Both trials were analysed according to a Two-way ANOVA with significance determined at $P < 0.05$ (Clewer and Scarisbrick, 2001). Significance was determined for $P < 0.05$ unless stated otherwise. Root counts were analysed using the means and standard errors analysed in Excel, Microsoft Office.

Results and Discussion

Physiological parameters

The only significant differences in photosynthetic rate between treatments were found on 15/01/2016 and 01/03/2016 (Table 4). UreaHB and BlackDAP™ differed significantly from one another, but these treatments did not differ significantly from the BlackDAP™ treatment. These differences did not seem to be related to the application dates or the total photosynthetic capacity of the trees. Furthermore, the rate of photosynthesis was within the optimum range of $0 - 18 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for deciduous fruit, for all treatments, indicating optimum N levels in the leaves (Farquhar and Sharkey, 1982). Thus, although N plays an important role in photosynthesis (Taiz and Zeiger, 2010), neither the time of application or mode-of-action of the different urea formulations in this trial increased photosynthesis substantially amongst one another during the season, confirming results of Fallahi et al. (2001).

White root growth dynamics

White root numbers showed variation between tubes (natural tendency) and treatments (Figure 3; Figure 4). During both seasons, the same trend was observed between treatments for the average number of white roots per tube (Figure 3; Figure 4). The highest average numbers were recorded the UreaHB and BlackDAP™ treatments, followed by the BlackUrea™ treatment, with lower numbers. There was a declining number of white roots from the peak around September and October towards March, followed by an increase again towards June and July, for both seasons. This confirms the bi-annual growth pattern of white roots in apple reported by Van Zyl (2016). However, the timing of the main peaks seems to differ from those reported in the Elgin-Vyeboom-Villiersdorp (EGVV) area, where the winter peak occurs in July and declines towards September. The summer peak in the Elgin-Vyeboom-Villiersdorp area also started earlier, in November, and decline towards January. This may either be due to area differences (Ceres vs EGVV) or the prolonged drought experienced in Ceres during the last two years.

BlackUrea™ also seemed to have a more even white root growth pattern compared to the other two treatments. This may indicate a difference in N availability between products, either in the actual N concentration available to the plant, or the period that the N is available. Low available N levels can result in the apple tree producing more roots to ensure increased uptake of available N (Hou et al., 2004). However, this was contrary to our observations, where less white roots were found in the BlackUrea™ treatment. The even growth of white roots together with the lower number of total white root growth could indicate that more available ammonium was converted and utilised by the roots in turn, producing less white roots due to more available N in the surrounding root growth area. From personal observations while installing the probes, there were differences in soil texture between the plots, which is typical in the South African fruit industry. Differences in soil type and microbial interactions within the soil should, however, also be taken into account and thus, this statement should be researched further.

Vegetative parameters

No significant differences were found between treatments regarding leaf N for either of the seasons (Table 5). The values were also within industry norms for apple (Kotze, 2001), confirming that the N status of the leaves were optimal. Leaf area index and dry weights of leaves did not differ significantly between treatments (Table 5). This further supported the lack of significant differences between treatments with regard to average shoot length after two seasons, confirming the balanced application of N in this trial. This lack of response to applied N, concur with findings of Prsa et al. (2007) who reported that leaf N concentration is not influenced when N is applied at adequate rates.

Yield and fruit quality

There was no significant difference for yield efficiency between treatments for either of the seasons, although yield efficiency was lower during the second season (Table 6). Although not significant, only the BlackDAP™ treatment maintained the same, high, yield efficiency during the second season and this may merit future observations.

With regard to fruit quality, no significant differences between treatments were found in either of the seasons, but no negative effect of urea on fruit quality (including pink colour) was noticed either (Table 7). Neither of these quality parameters were influenced negatively by the urea treatments either, confirming no negative impacts of urea as N formulation on fruit quality.

Varying rates of N did not affect yield significantly when applied within the industry norms, but higher rates, over a five-year period, could have a negative effect on especially apple colour (Fallahi et al., 2001). This confirms the importance of a longer evaluation time when this type of research is conducted. No storage trials were conducted due to the lack of significant differences in fruit quality between treatments.

Tomato pot trial

The tomato pot-trial resulted in no significant differences between treatments, in neither the plant nor the soil parameters, although the plant weight of the UreaHB was lower than the weight of the BlackUrea™ (Table 8). In contrast, there was a significant difference between treatments with reference to the ammonium concentrations in the leachate. Tomato results showed a higher concentration of water soluble ammonium in BlackUrea™ than UreaHB (Table 8). The increased amount of ammonium in the BlackUrea™ pots indicated that more ammonium leached from this treatment. The UreaHB pots had less ammonium in the water, but it should be noted that volatilization was not measured as volatility of UreaHB will result in less N being leached through to the water. In a trial conducted by Murdoch-Brown (2014) urea (HB) showed 38% more volatilization than urea coated with Launch™, the coating used to produce BlackUrea™ (Murdoch-Brown, 2014). These findings are however only speculative and the effect of volatility on different N formulations should be researched further. Thus, the question was raised whether increased effective uptake of the BlackUrea™ treatment resulted in less ammonium being taken up by the plants due to an increased effectiveness of this formulation, resulting in the higher ammonium in the leachate. It is also possible that the plant medium (sand) was not conducive to optimum performance of the microbial organisms in the coated ammonium. This however is only a speculative question and more research needs to be done for this question to be answered.

Conclusions

Results from the 'Rosy Glow' trial showed no significant differences between treatments for yield efficiency, fruit quality, vegetative parameters or photosynthesis (for most dates). This agrees with existing findings that moderate N soil applications does not result in substantial changes in bearing trees in commercial orchards where no N deficiency occurs. Thus, it is possible that the rate of N will override the effect different formulations in resulting in significant changes. Furthermore, we were not able to substantiate the claim of a different mode

of action of BlackUrea™ BlackDAP™ (a slower N release than UreaHB) with improved plant performance with this protocol.

The application of different nitrogen formulations resulted however in differences in white root growth between treatments, with UreaHB and BlackDAP™ showing more white roots and more fluctuation compared to BlackUrea™, for both seasons. This did not result in significant differences in leaf N concentrations or other vegetative parameters in the trees. However, white root dynamics indicated a possible effect of formulation under these conditions. Although not conclusive, the BlackDAP™ treatment showed a completely different dynamic to those of the other two treatments, posing the question as to the role of the additional P in this formulation on root behaviour.

The potted trial showed conflicting results, with more growth (ns) with higher leaching of ammonium and nitrates in the BlackUrea™ (coated) than UreaHB. It is possible that the coated urea underperforms under conditions of high irrigation and inert sand as medium, resulting in excessive leaching in pots. In addition, the volatilisation of the two formulations were not quantified in this trial and may have explained some of the variation.

Lastly, this study indicated that under certain conditions, ammonium-based fertilizers could well be used as alternative nitrogen source, without compromising yield or fruit quality of 'Rosy Glow'.

Acknowledgments

This trial was funded by Profert Pty Ltd, South Africa.

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Tables and Figures

Table 1: Fertilizer dates and rates as applied on the ‘Rosy Glow’, Alhambra, Ceres for two consecutive seasons 1 (2015/16) and 2 (2016/17).

Treatment	bud break – full	6 weeks post-full	post-harvest
	bloom (08/10/2015)	bloom (19/11/2015)	(28/04/2016)
UreaHB (46%N)	50 g.tree ⁻¹	63 g.tree ⁻¹	82 g.tree ⁻¹
BlackUrea™ (46%N)	50 g.tree ⁻¹	63 g.tree ⁻¹	82 g.tree ⁻¹
BlackDAP™ (18%N)	127 g.tree ⁻¹	158 g.tree ⁻¹	172 g.tree ⁻¹
	(13/10/2016)	(29/11/2016)	
UreaHB (46%N)	54 g.tree ⁻¹	64 g.tree ⁻¹	133 g.tree ⁻¹
BlackUrea™ (46%N)	54 g.tree ⁻¹	64 g.tree ⁻¹	133 g.tree ⁻¹
BlackDAP™ (18%N)	137 g.tree ⁻¹	165 g.tree ⁻¹	335 g.tree ⁻¹

Table 2: Soil N analysis at two depths (SGS South Africa Pty Ltd., South Africa) indicating N levels for the BlackUrea™, BlackDAP™ and UreaHB plots before any soil applications were made.

Soil N Analysed	Treatment		
	BlackUrea™	UreaHB	BlackDAP™
Soil total NH ₄ ⁺ (mg/kg) 0- 30 cm	7.57	6.17	5.52
Soil total NO ₃ ⁻ (mg/kg) 0 – 30 cm	2.75	3.25	2.75
Soil total NH ₄ ⁺ (mg/kg) 30 – 60 cm	4.74	4.52	1.20
Soil total NO ₃ ⁻ (mg/kg) 30 – 60 cm	0.94	0.68	0.32

Table 3: Fertilizer applications dates and rates in a potted trial to determine N nutrient balance, Welgevallen farm, Stellenbosch during 2016.

Treatments	fertilizer application dates.		
	18/10/2016	25/10/2016	10/11/2016
Urea HB (46%N)	20g.plant ⁻¹	20g.plant ⁻¹	20g.plant ⁻¹
BlackUrea™ (46%N)	20g.plant ⁻¹	20g.plant ⁻¹	20g.plant ⁻¹

Table 4: Photosynthetic rate of ‘Rosy Glow’, Alhambra, Ceres for two consecutive seasons.

Measurement date	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			
	BlackUrea™	UreaHB	BlackDAP™	Pr>F
05.11.2015	11.44	11.58	11.21	0.7579 ^{ns}
12.11.2015	13.52	12.92	13.07	0.5960 ^{ns}
19.11.2015	8.94	9.37	9.10	0.8636 ^{ns}
03.12.2015	11.61	10.52	10.98	0.1216 ^{ns}
18.12.2015	16.29	16.22	16.10	0.9490 ^{ns}
15.01.2016*	16.62 ^{ab}	15.11 ^b	16.87 ^a	0.0275
05.02.2016	12.79	12.29	12.31	0.6642 ^{ns}
01.03.2016*	9.30 ^{ab}	8.07 ^b	10.11 ^a	0.0258
06.12.2016	10.95	11.10	10.68	0.7189 ^{ns}

*significant differences.

Table 5: Leaf analysis including Leaf Area Index (LAI) and dry weight in ‘Rosy Glow’ on Alhambra, Ceres for two seasons.

Sample date	Treatments			
	BlackUrea™	UreaHB	BlackDAP™	Pr \geq F
<u>Nitrogen percentage per treatment (% N)</u>				
16.03.2016	2.69	2.69	2.61	0.5854 ^{ns}
10.03.2017	2.50	2.51	2.55	0.5084 ^{ns}
<u>Leaf area index (LAI)</u>				
16.03.2016	34.46	35.35	33.84	0.2994 ^{ns}
<u>Dry Leaf weight (g)</u>				
16.03.2016	12.56	13.22	13.22	0.1001 ^{ns}
10.03.2017	11.09	11.00	10.77	0.7720 ^{ns}

*Leaf nitrogen content (%) norms for industry: 2.1 – 2.6 % (Kotze, 2001).

Table 6: Yield data for 'Rosy Glow', Alhambra, Ceres for season 1 (2015/2016) and 2 (2016/2017).

Season	Harvest data per treatment			
	BlackUrea™	UreaHB	BlackDAP™	Pr ≥ F
<u>Yield (kg.tree⁻¹)</u>				
2016	76.01	74.52	73.87	0.8939 ^{ns}
2017	65.00	63.88	76.00	0.1126 ^{ns}
<u>Circumference (cm)</u>				
2016	28.49	29.13	25.59	0.6537 ^{ns}
2017	29.84	30.17	29.23	0.4967 ^{ns}
<u>Yield efficiency (kg.cm⁻¹)</u>				
2016	2.70	2.60	2.60	0.7440 ^{ns}
2017	2.18	2.13	2.62	0.0684 ^{ns}

Table 7: Fruit quality parameters recorded for 'Rosy Glow', as produced on Alhambra, Ceres for two consecutive seasons.

Season	External fruit quality parameters.			
	BlackUrea™	UreaHB	BlackDAP™	Pr ≥ F
<u>Firmness (kg)</u>				
2016	8.71	8.63	8.70	0.6966 ^{ns}
2017	8.46	8.60	8.30	0.2466 ^{ns}
<u>Diameter (mm)</u>				
2016	70.41	70.19	70.9	0.7597 ^{ns}
2017	71.70	71.19	69.95	0.1466 ^{ns}
<u>Mass (g)</u>				
2016	138.12	136.62	140.64	0.7107 ^{ns}
2017	151.31	149.81	140.18	0.1152 ^{ns}
<u>Background green</u>				
2016	3.27	3.23	3.21	0.5436 ^{ns}
2017	3.10	3.14	3.10	0.3964 ^{ns}
<u>% Pink colour</u>				
2016	8.6	8.34	8.52	0.5766 ^{ns}
2017	10.42	10.61	9.79	0.0798 ^{ns}
<u>Starch breakdown (%)</u>				
2016	39.36	41.31	42.75	0.5155 ^{ns}
2017	58.39	55.00	60.50	0.0780 ^{ns}
<u>Total soluble solids (%)</u>				
2016	14.79	14.74	14.67	0.8832 ^{ns}
2017	14.42	14.59	14.09	0.2774 ^{ns}

Table 8: Tomato potted trial mineral analyses (N-balance) and growth parameters performed on Welgevallen Experimental Farm during 2016.

Parameters	Treatment		
	BlackUrea™	UreaHB	Pr \geq F
Plant total nitrogen (%)	3.33	3.16	0.4931 ^{ns}
Plant dry weight (g)	57.31	50.78	0.1916 ^{ns}
Water total NH ₄ ⁺ (mg/kg)*	58.13 ^a	33.75 ^b	<.0001
Soil total NH ₄ ⁺ (mg/kg)	11.39	9.26	0.3507 ^{ns}

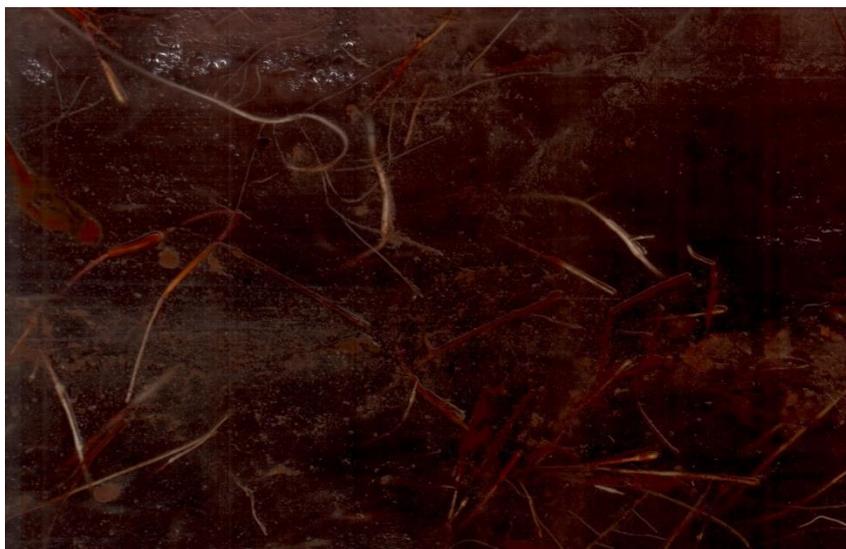


Figure 1: An In Situ root scanner image of white root growth as was used for quantification during a trial of ‘Rosy Glow’ apple, evaluating three N formulations on tree physiology, also including root performance.



Figure 2: Apple ‘Pink Lady’ colour chart (Unifruco Research Services, Belville, R.S.A) showing the colour spectrums that was used for quantification of over colour of ‘Rosy Glow’.

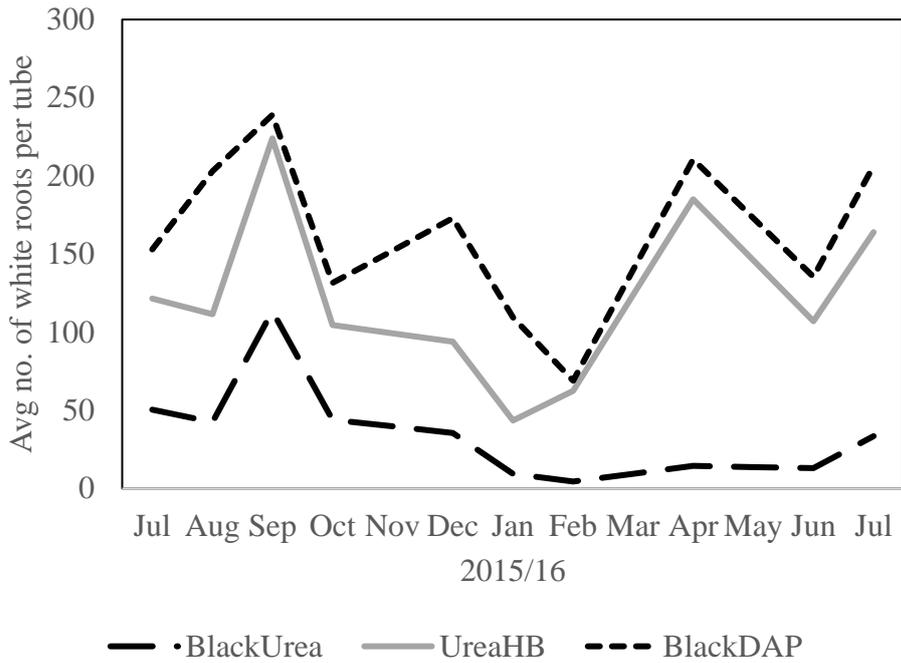


Figure 3: Average number of white roots per tube for the apple cultivar 'Rosy Glow' during 2015/16 as recorded at Alhambra, Ceres, when subjected to three different soil applied N formulations.

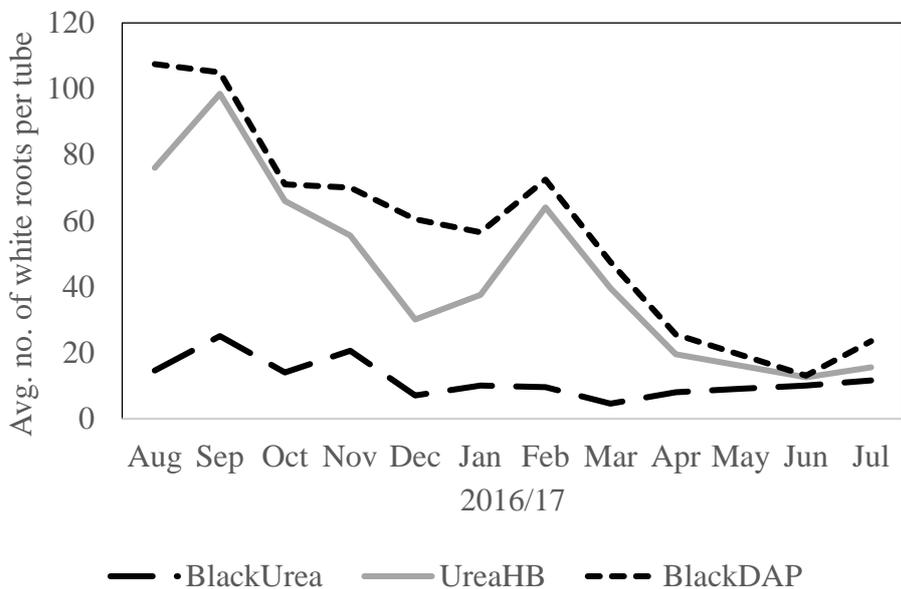


Figure 4: Average number of white roots per tube for the apple cultivar 'Rosy Glow' during 2016/17 as recorded at Alhambra, Ceres, when subjected to three different soil applied N formulations.

Paper 2: The effect of two commercial nitrogen formulations on tree performance of bearing ‘Golden Delicious’ (*Malus domestica* Borkh) trees.

Abstract

Fertilization of apple (*Malus domestica* Borkh.) trees with nitrogen (N) influences fruit yield and quality. Different rates of N have different effects on not only rate of leaf photosynthesis, but also on the quality of apple fruit. Urea can, under certain conditions, serve as a single source of N fertilization. BlackUrea™ is an N formulation alternative to high biuret Urea (UreaHB). The effect of both these N formulations has not been evaluated in apple orchards under South African conditions. This study evaluated the effects of two different formulations of urea as soil applications, on photosynthesis, leaf N levels, fruit yield of ‘Golden Delicious’ apples produced in Ceres, South Africa. No differences were found between treatments for any of the parameters after one season. However, there was an indication that BlackUrea™ increased yield per hectare substantially. No negative effects regarding tree performance was noticed on either of the urea formulations when applied on a ‘Golden Delicious’ orchard. Planted in a sandy loam soil.

Key Words

Fruit quality, nitrogen, urea fertilizers, yield

Introduction

Apple (*Malus domestica* Borkh.) production is not only influenced by environmental factors, but certain technological factors, is also key to optimise apple production (Prsa et al., 2007). Increasing fruit yield, while obtaining optimal fruit quality, is a big challenge in a growing apple industry (Wang et al., 2001; Kotze, 2001) and is partially addressed with balanced N nutrition.

The two major N formulations currently applied in commercial apple production are ammonium and nitrate (Lamb et al., 2014; Kotze 2001). The importance of fruit quality parameters, like starch breakdown and colour especially, plays an important role in cultivars with colour (Wang et al., 2011). In addition, both vegetative and reproductive growth is determined by adequate N supply via enzyme-based metabolic processes (Prsa et al., 2007). Decreasing leaf N levels towards harvest in ‘Golden Delicious’ proved that N plays an essential role as part of vegetative growth and fruit development (Prsa et al, 2007). Excessive N levels, applied as a combination of ammonium-nitrate (NH_4NO_3), at a rate of $105.4 \text{ g} \cdot \text{tree}^{-1}$ throughout the growing season, had a negative effect on fruit colour, with the effect on starch breakdown being more complex (Wang et al., 2011). Although negative correlations were obtained between N and fruit colour, increasing levels of NH_4NO_3 increased both yield and fruit size (Wang et al., 2011). Plants that received no N, had significantly lower levels of leaf N compared to plants that were treated with N at a rate of $80 \text{ kg} \cdot \text{ha}^{-1}$, as well as $250 \text{ kg} \cdot \text{ha}^{-1}$ calcium-ammonium nitrate (Prsa et al., 2007). Urea fertilizer is a well-known source of ammonium-N and is used on a global scale for fruit production (Witte, 2011).

Urea can enter the plant via active urea transporters as ammonium or nitrate, depending on the metabolic interactions in the soil and plant (Witte, 2011). Laboratory studies that eliminated microbial hydrolysis of urea, as well as nitrification, revealed that urea could be used as a single N source for plant growth (Witte, 2011). For a plant to utilise urea, a few factors, such as the combination of the nutrition medium plays a critical role (Du Preez, 1984). In the soil, urea is hydrolysed to ammonium which, in turn, is assimilated to nitrate through nitrate reduction (Hawkesford et al., 2014). The importance of the formulation of the specific N fertilizer used is therefore of great importance to ensure optimal fruit production (Witte, 2011). The combination of both ammonium and nitrates ensures optimal utilisation of N within the plant (Witte, 2011). All forms of inorganic N are metabolised to nitrates and thus, no single form of

N can be seen as more important than the others (Lamb et al., 2014). The use of urea-based fertilizers has some limitations regarding the loss of product through volatilization, as well as leaching in some soils (Overdahl et al., 2017). Sole-sourced, urea-based N nutrition may lead to N starvation and reduced growth if not utilised effectively by plants (Witte, 2011).

The two main concerns regarding soils that are less ideal for urea hydrolyses are i) leaching of urea in the lower parts of the profile and ii), volatilization in the upper part of the specific soil (Du Preez, 1984). It is important to bear in mind that the organic composition, as well as the specific texture of the cultivated soil, play an intricate part in ensuring the optimal availability of N to the plant (Du Preez, 1984). Soils containing more coarse fragments, like sandy soils, may lead to higher N losses by leaching, as opposed to soils with a high clay content due to the water holding capacity being lower in more coarse soils (Lamb, 2014). Soils containing higher amounts of organic matter may result in more available organic N due to soil mineralization of organic matter to NH_4^+ (Lamb et al., 2014).

To address the limitations of urea, two new formulations, viz. BlackUrea™, were introduced to increase the efficiency of N in the soil (Nyati, 2014). BlackUrea™ consists of granular urea high biuret (HB) coated with enriched coating. The term Black™, is derived from an organic compound mixture that is used to coat the Urea granule and drench the granules to the point where it is totally coated by the humic and fulvic rich coating (Nyati, 2014). The coating is reported to consist of a unique combination of bio stimulants and nutrient biocatalysts derived from humic acid, plant hormones, vitamins and minerals, creating a biological management system within the soil. This enriched coating could possibly enhance the biological control of available N to the plant, while minimizing the losses often associated with urea-based fertilizers.

This paper evaluated the effect of urea formulation, BlackUrea™ versus UreaHB, as soil application on performance of ‘Golden Delicious’ in a loamy clay soil in Ceres. The effect of the two urea formulations was quantified for photosynthesis, as well as fruit quality and yield.

Material and methods

Plant material and experimental site

Experiments were conducted on ‘Golden Delicious’ trees budded onto M7 rootstock, in a commercial bearing orchard on the Alhambra farm in the Bo-Swaarmoed Valley in Ceres (33°22’57.41” S; 19°31’51.61” E), in the Western Cape Province of South Africa. The trees were planted in 2014, in a predominantly loam-to-sandy soil at a plating rate of 1481 trees per hectare (ha). ‘Granny Smith’ trees were used as cross-pollinators. The trees were irrigated using a micro-irrigation system according to standard practices. Granular fertilizer was applied with a mechanical spreader, or by hand. Commercial N fertilizer was applied at three stages, namely budburst to full bloom (28 % of total N); 6 weeks after full bloom (51 % of total N) and during the post-harvest period (22.4 % of total N) (Table 1). All commercial N applications were made at a rate of 107.84 kg N per ha, as recommended by the farm fertilizer consultant. The commercial N formulation applied was Limestone ammonium nitrate (LAN). Both the UreaHB and the BlackUrea™ were applied at the same total N rate as the rest of the orchard (107.8 kg N) with both treatments being applied by hand. The applications mentioned above were the only N applied during the season.

Trial layout

The orchard was divided into two equal blocks of 0.5 ha each to represent the two treatments. In each block, 10 trees were randomly selected to determine the means and standard errors using Microsoft Excel in MS Office, as it formed part of a bigger trial involving satellite imaging that required this layout.

Treatments

Two treatments consisting of BlackUrea™ and UreaHB respectively were applied to two separate 0.5ha sized blocks. BlackUrea™ [Profert (Pty) Ltd., Potchefstroom, South Africa] consisted of 45.6 % N, compared to UreaHB [Profert (Pty) Ltd., Potchefstroom, South Africa] that consisted of 46 % N. The total N per hectare was divided by number of trees per hectare to determine the amount of fertilizers to be applied per tree. Standard fertiliser applications, other than N, was administered by the farm.

Physiological measurements

Total rate of photosynthesis was determined with the Li-Cor Li-6400 infra-red gas analyser (IRGA) (LiCor Biosciences, Lincoln NE, USA). Three leaves per tree, exposed to sunlight and proximately at shoulder height, were measured to obtain the average photosynthesis. Measurements were carried out on 07 Dec. 2016 and 23 Mar. 2017, from 08:00 to 11:00 am (Table 2). Fixed settings on the IRGA were used to determine the photosynthetic capacity of the different treatments: carbon dioxide (CO₂) supply was set at 380 ppm; photosynthetically-active radiation (PAR) at 1500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; flow rate of 500 $\mu\text{mol}\cdot\text{s}^{-1}$ and leaf temperature of 25°C.

N infiltration measurements using wetting front detectors (WFD)

The statement that BlackUrea™ reduced leaching of N (Nyati, 2014) was investigated in a pilot trial using WFDs. A pair of wetting front detectors was installed in the BlackUrea™ treatment, to quantify the N concentrations in the 30 cm and 60 cm root zone areas (Agriplas, 2016). A WFD works on a simple basis of three components: the funnel, filter and float with the funnel filling up to the point of saturation. When the funnel is filled, the float is pushed out as an indicator that the WFD is filled, thus ready for extraction of the water contents (Zwane, 2017). WFDs were checked at weekly intervals, after each N application, to determine if any leachate accumulated. All leachate present was then extracted with of a medical syringe. A composite seasonal sample, from the day of installation to the day of extraction, was collected and analysed for both depths, to determine the ammonium and nitrogen levels in the leachate (Table 3). A commercial laboratory (Integral laboratories, Zandwyk Park, Paarl, South Africa) was used to determine the ammonium and nitrate contents.

Vegetative measurements

Trunk circumference was measured using a measuring tape at harvest to calculate yield efficiency.

A leaf analysis was performed close to harvest (Table 4). Approximately 20 healthy leaves were sampled per tree, at shoulder height both sides of the tree making sure the leaves were free from any mechanical, insect or climatic damage at the beginning of February 2017 (Kotze, 2001) and send to a commercial laboratory (SGS South Africa Pty Ltd., South Africa) to determine the N concentration.

Biomass increase during the season, for both treatments, were quantified using satellite imaging (Fruitlook[®]) and will be discussed in detail in the following paper.

Yield and fruit quality

Yield and yield efficiency was determined by harvesting and weighing all the fruit of the individual trees and extrapolated to $\text{ton}\cdot\text{ha}^{-1}$ and yield efficiency calculated (Table 5). Twenty fruits per tree, of similar size, were sampled randomly on shoulder height from each tree to determine treatment effect on the following fruit quality parameters: external quality - background colour with a colour chart (Unifruco Research Services, Belville, R.S.A), internal quality - total starch breakdown and total soluble solid concentration (TSS) (Table 6 and 7). Starch breakdown was determined by cutting each fruit in half, and dyeing with an (1 %) iodine solution and comparing the degree of colouring from dye using a specific starch breakdown chart (Unifruco Research Services, Belville, R.S.A). Total soluble solids (TSS) content was determined from a composite juice sample (Model N1, Atago, Tokyo, Japan). Individual fruit diameter, mass and firmness was also determined with flesh texture analyser (Guss electronic model GS 20, Strand, R.S.A).

Results and Discussion

Physiological measurements

The rate of photosynthesis was similar on the first evaluation date, with a slight increase in the BlackUrea[™] compared to the UreaHB treatment during the second (23.03.2017) date. However, as this was not consistent during the season, it cannot be directly due to the possible slower release of N in the BlackUrea[™] treatment at this stage.

N infiltration measurements using wetting front detectors (WFD)

In a trial conducted on tomato seedlings (Paper 1), more N leached from the sand at the BlackUrea[™] compared to the UreaHB trial, but no other differences were observed regarding the plant, fruit weight or mineral analysis. Although this comparison was not continued in the apple trial, results from the WFD at both depths confirmed leaching of N after BlackUrea[™] application in the sandy soil as well. The high occurrence of NH_4^+ in the WFD data (Table 3) could indicate slower nitrification due to the coating of BlackUrea[™], indicating a possible effect on slower release of N over time, but should be researched further to determine the exact effect of the coating on the conversion of NH_4^+ to plant available NO_3^- . No significant

differences were found, over two seasons, between these two formulations on ‘Rosy Glow’ planted on a loamy soil, as discussed in Paper 1. In contrast, trends in the ‘Golden Delicious’ trial shows potential with the application in BlackUrea™ on the sandier soil and justifies future research under these circumstances. It also confirms the higher potential of N leaching in sandy soils.

A carbon content of 0.86 % C was present in the upper layer (0 – 30cm) of the soil (Table 8). This is typical of a sandy soil and relative low. Leaching is a combination of different factors including soil texture, irrigation and soil carbon content (Lamb et al, 2014). Although soil carbon is related to more N present in the soil, it is not the sole factor for more N release, as it forms part of a more complex system that is dependable on a vary of different factors including soil moisture temperature (Horneck et al., 2011).

Vegetative measurements

Leaf N was higher in both treatments when compared to the same time in the previous year (Table 9) but did not differ between treatments (Table 4). The N levels of both treatments represented the higher threshold values for leaf N according to industry norms. This confirmed findings by Prsa et al. (2007) that leaf N did not differ significantly between treatments fertilized with adequate amounts of N (Prsa et al., 2007).

Yield and fruit quality

No significant differences were found between BlackUrea™ and UreaHB, for any of the parameters measured (Tables 5 - 8). Although no significant differences were found, no negative impacts with application of BlackUrea™ on yield, tree physiology and quality of the fruit harvested compared to UreaHB. The BlackUrea™ treatment resulted a higher yield efficiency than the UreaHB treatment (ns) and should be researched further to determine the significance of these findings. On a practical side, the higher yield noted in the BlackUrea™ treatment resulted in a 5 t.ha⁻¹ increase, including not only bigger (ns) , also but firmer (ns) fruit. This represents a substantial increase on the averaged 20 t.ha⁻¹ yield of the orchard and equates to an economical advantage for the producer.

Conclusions

The BlackUrea™ treatment did not result in increases in the rate of photosynthesis, fruit quality or yield of ‘Golden Delicious’ compared to the control (UreaHB). Nevertheless, the ‘Golden Delicious’ trial indicated a positive reaction towards BlackUrea™ compared to UreaHB, which was more prominent than with similar treatments performed on ‘Rosy Glow’ (Paper 1), over a longer period. Thus, it is possible that cultivars react differently to urea as N source. In addition, the contribution of the different soil types towards the perceived increase in yield in the ‘Golden Delicious’ BlackUrea™ treatment was not quantified in this study and may have played an important role in this trial. With ‘Golden Delicious’, the increase in yield (ns) experienced with the BlackUrea™ treatment, when extrapolated to a hectare basis, resulted in a substantial increase in income, justifying further research with this formulation.

No negative effects on tree performance was noted with either of the urea treatments, indicating that this N formulation was suitable as alternative to existing N formulations, for ‘Golden Delicious’ production, quantified after one season. The long-term effect of replacing N application with a urea formulation still needs to be evaluated before such a recommendation can be made.

Acknowledgments

This trial was funded by Profert Pty Ltd, South Africa.

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Tables and figures

Table 1: ‘Golden Delicious’ fertilizer applications, applied by hand, for the 2016/17 season on a commercial orchard at Alhambra farm, Ceres.

Treatment	Bud break to full bloom (13/10/2016)	6 Weeks after full bloom (24/11/2016)	Post-harvest (09/03/17)
UreaHB	45 g.tree ⁻¹	83 g.tree ⁻¹	34 g.tree ⁻¹
BlackUrea™	45 g.tree ⁻¹	83 g.tree ⁻¹	34 g.tree ⁻¹

Table 2: Infra-Red Gas Analyser (IRGA) measurements to determine the rate of photosynthesis on a commercial bearing ‘Golden Delicious’ block for the 2016/17 season in Alhambra, Ceres, approximately two weeks after N application.

Measurement date	BlackUrea™	UreaHB
07.12.2016	14.29±0.57	14.61±0.57
23.03.2017	11.39±1.11	9.90±0.73

Table 3: The N content (ammonium and nitrate) in the leachate of the BlackUrea™ treatment, by use of wetting front detectors at two different soil depths: 0 – 30 cm and 30 – 60 cm, in a commercial bearing ‘Golden Delicious’ orchard, for the 2016/17 season, at Alhambra, Ceres.

Type of N(mg/L)	Soil Depth	
	0 - 30 cm	30 - 60 cm
Ammonia (NH ₄ ⁺)	140.00	0.77
Nitrate (NO ₃ ⁻)	114.00	26.10

Table 4: Leaf analysis results for N at the beginning of February 2017, in a commercial bearing ‘Golden Delicious’ orchard, on the farm Alhambra, Ceres.

Parameters	Treatments	
	BlackUrea™	UreaHB
*Nitrogen percentage per treatment (% N)	2.74±0.02	2.77±0.04
Leaf Wet Weight (g)	24.28±0.62	23.49±0.60
Leaf Dry Weight (g)	11.75±0.22	11.18±0.22

*Leaf nitrogen content (%) norms for industry: 2.1 – 2.6 % (Kotze, 2001).

Table 5: Harvest data for the 2016/17 season for ‘Golden Delicious’ on Alhambra, Ceres.

Parameters	Treatments	
	BlackUrea™	UreaHB
Yield (kg.tree ⁻¹)	18.66±0.68	15.07±0.72
Circumference (cm)	17.60±0.50	18.85±0.47
Yield efficiency (kg.cm ⁻¹)	1.09±0.15	0.80±0.04

Table 6: Post harvest external fruit measurements for ‘Golden Delicious’ harvested during the 2016/17 season on Alhambra, Ceres.

Parameters	Treatments	
	BlackUrea™	UreaHB
Firmness (kg)	8.08±0.15	7.96±0.11
Diameter (mm)	76.03±0.47	75.61±0.94
Mass (g)	172.04±3.41	168.59±6.24
Background colour (green)	3.19±0.13	3.16±0.12

Table 7: Post harvest internal fruit measurements for ‘Golden Delicious’ harvested during the 2016/17 season on Alhambra, Ceres.

Parameters	Treatments	
	BlackUrea™	UreaHB
Starch breakdown (%)	9.85±1.41	11.99±1.01
Total sugar content (Brix)	13.82±0.29	13.16±0.24

Table 8: The total carbon content (C %) for 2014 and 2017 of the sandy to loam soil in the commercial bearing ‘Golden Delicious’ orchard on Alhambra, Ceres.

Year	Sample Depth (cm)	Soil	C %
2014	0 - 30	Sand	0.52
2017	0 - 30	Sand	0.87

Table 9: Leaf N % as part of the producer's (Alhambra) results from the 2015/16 season leaf analysis taken in February 2016 and analysed at Bemblab laboratories (Bemblab, Van der Berg Crescent 16, Gant's Centrum, Strand).

Orchard	Cultivar	N %
Jo44 - 45A	Golden Delicious	2.66

Paper 3: Evaluating the suitability of FruitLook® to quantify the response of ‘Golden Delicious’ (*Malus domestica* Borkh.) trees on two commercial soil-applied nitrogen formulations.

Abstract

The growing demand for high quality apple (*Malus domestica* Borkh.) production draws attention to more detail regarding the efficiency of every production unit. The use of technology to determine certain parameters and develop a better understanding of production unit efficiency is becoming a major role-player in agriculture. Satellite captured images within the visible, near thermal- and infra-red ranges are currently used as part of complex spatially explicit models to determine certain parameters or datasets regarding growth, water use efficiency and nitrogen (N) content. These images are analysed *via* geographical information systems (GIS) - adding to manage and better understand certain parameters like weekly tree biomass accumulation, water use efficiency and leaf nitrogen (N) dynamics during the season. With the aid of the Western Cape Government (Department of Agriculture), FruitLook®, a web-based system was developed by eLEAF® to provide producers in the Western Cape Province of South Africa, with spatially explicit information on growth and water relations of agricultural production units. Analysing apple fields with different formulations of N fertilizers applied in FruitLook®, showed differences in biomass production and N status of the specific N treatments. Higher biomass accumulation, as well as tree total N, were observed when comparing this data over two seasons (ns) after applying different N formulations. This indicated a possible effect of the treatments (different N formulations) in addition to quantifying the effect of the natural increase in growth of the trees over the two seasons.

Key Words

Precision farming, biomass, leaf nitrogen, satellite imaging

Introduction

Crop growth and leaf N content are correlated, with increasing N levels showing a positive, but not indefinite, effect on biomass accumulation (Hou et al., 2004). Adequate N supply plays a key role in apple (*Malus domestica* Borkh.) production, with the use of technological measurements being pivotal in ensuring the optimal timing and rate of applications, of N (Prsa et al., 2007). Due to economic and environmental pressure regarding the use of N fertilizers, and the negative effect of leaching and volatilisation on the environment, it is of high importance that the specific N source is used with maximum efficacy thus minimizing possible losses to the environment (Lamb et al., 2014).

Precision farming and the effective use thereof is becoming a part of everyday farming (Goddard, 1997). The goal of precision farming is to use a combination of agricultural and climatic datasets to enable more informed decisions on the specific farm (Goddard, 1997). Precision farming will thus not necessarily make farming easier, rather more efficient and economically viable (Goddard, 1997). Integrating the various data sets generated *via* precision agriculture will generate new information that can be used as an aiding tool in strategic production decisions (Whitting et al., 2006).

Different methods are currently available to quantify leaf N. Tissue analysis of different plant structures has been used as the primary reference for determining N levels of leaves and plants (Munuz-Huerta et al., 2013) Although very effective, tissue analysis's main drawback is the destruction of either the whole plant, or parts thereof, to determine plant N levels. Furthermore, it is costly and logistically difficult to perform on a weekly basis and thus in most cases are only done annually.

Leaf chlorophyll measurements is another technique for determining N levels in plants. This is a non-destructive method that is relatively easy to perform, can be carried out within a short period of time and is affordable once the equipment (SPAD meter) has been acquired. However, this is an indirect measurement, extrapolated from the chlorophyll content in the tissue and cannot indicate whether a plant is fertilized at too high or too low rates, neither can it distinguish between different N forms in the plant (Munuz-Huerta et al., 2013). Often it is also necessary to determine a chlorophyll reaction curve that is plant species specific and a based on destructive, laboratory analysis.

Another “in-field” method is canopy data processing, focusing on processing electromagnetic reflectance, to determine parameters like biomass and leaf area index with the use of hand held sensors. This measurement requires constant calibration and sufficient sunlight (Munuz-Huerta et al., 2013). Like with the chlorophyll measurements, it can be repeated often due to the measurement being non-destructive as well as economically viable once the equipment is obtained.

However, all of the above measurements are performed on an individual plant structure, with the assumption that this measurement of the plant/leaf/structure is representing a whole orchard or field. This assumption can influence the reliability of the results when the area under observation is heterogeneous, as is often the case.

Drone technology and the use of drones in agriculture are fairly recent advances in precision agriculture. Drone technology focuses on the principle of high resolution “photos” of a certain area that is then used to determine and analyse certain plant physiological, yield and maturity analysis *via* the use of specific models or algorithms (SenseFLy, 2017). Image processing software is used to process photographs taken by the drones using algorithms to convert it e.g. into the Normalized Difference Vegetation Index (NDVI) map to detect certain stress related differences within orchards. Furthermore, drones can also be used for general scouting, of pests and stress areas, and counting, more on the livestock side, functionality rendering this technology another effective tool in precision agriculture (SenseFly, 2017).

FruitLook[®] is a web based portal funded by the Western Cape Government (Department of Agriculture) and provides spatially explicit data which is generated using the ETLook model together with satellite imagery and spatially extrapolate weather data (eLeaf, 2017). The data is provided at a 20m spatial resolution and at weekly time intervals throughout the main deciduous fruit production season (eLeaf, 2017). These images are used to generate data sets regarding growth, water use of fruit crops and the nitrogen status (Roux et al., 2014). A total of nine data components including: Biomass production, upper leaf N and total plant N are made available weekly *via* the FruitLook[®] website (www.fruitlook.co.za) (Eleaf, 2017). FruitLook[®] is currently used for different crops, including grapes and deciduous fruit (personal communication N. Kapp, Soil2Root Technologies), initially focusing mainly on the improvement of water use efficiency (eLeaf, 2017).

The main difference between satellite imaging and drone technology is the fact that drone is not fully limited by cloud cover and, depending on the camera used, provide very high-resolution, sub-metre resolution, images (SenseFly, 2017). Drones can, in certain cases, be requested on a relative short notice and can be deployed on a small area, e.g. one row in an orchard. In contrast, satellite images cover a bigger range, typically orchards, and is depended on the source/supplier that will determine the temporal -and spatial resolution (pixel size) of each image.

Making the decision on a specific method depends on the specific outcome required. Choosing between in-orchard organ or leaf based methods and spatial methods (drones or satellites) will mainly be determined by the specific measurement outcomes required. Therefore, the method chosen for determination of N content of leaves/plants should be based on specific requirements, with an acquit awareness of the advantages and disadvantages of each system.

To date, no study has investigated the use of Fruitlook[®] data to determine the effect of soil applied N fertilizers on tree performance of ‘Golden Delicious’. This paper evaluates the suitability of FruitLook[®] to quantify the response of ‘Golden Delicious’ trees on two commercial soil-applied nitrogen formulations (BlackUrea[™] and UreaHB) in a commercial orchard in Ceres, South Africa.

Material and Methods

Orchard selection layout

A one hectare commercial, bearing ‘Golden Delicious’ orchard on M7 rootstock was selected on the Alhambra farm, in the Bo-Swaarmoed valley, Ceres (33°22’57.41” S; 19°31’51.61” E), in the Western Cape, South Africa. ‘Golden Delicious’ was planted (4.5 x 1.5m) on sandy, to loamy sand soil, with ‘Granny Smith’ as cross pollinator. The orchard floor was clean in the tree row, with some light annual grass cover between rows (Figure 1). The grass cover observed was uniform over the two seasons. Micro-irrigation was used, with fertilizers being applied by hand, at three different growth stages during 2016/17 (Table 1): budburst to full bloom (28 % of total N); 6 weeks after full bloom (51 % of total N) and post-harvest (22.4 % of total N) (Table 1). In the two different treatments, fertilizer was applied at the same N rate, at 107.84 kg N per hectare, according to recommendation from the commercial fertilizer consultant. The orchard was divided in two half hectare blocks, one treated with BlackUrea[™] and the other

left as the control, receiving UreaHB. BlackUrea™ (Profert (Pty) Ltd., Potchefstroom, South Africa) consist of 45.6 % N compared to UreaHB (Profert (Pty) Ltd., Potchefstroom, South Africa) consisting of 46 % N. Although both formulations consist of closely the same amount of N it should be noted that the BlackUrea™ formulation differs from the UreaHB and thus the reason for the two products as explained in paper 2. This trial formed part of a bigger trial (Paper 2) and reference to additional growth parameters will be made where applicable.

Field data

Actual leaf N concentrations at harvest and yield efficiency of the two blocks were collected as part of the trial and data are presented in Paper 2 and referred to in the discussion. Vegetative growth was not quantified during this trial, since similar treatments on ‘Rosy Glow’ showed no differences between treatments. This was partly due to the N dosages applied being according to industry standards, with no treatment with excessive N included, to maintain fruit quality.

FruitLook® data

The specific ‘Golden Delicious’ block polygon was drawn on the Fruitlook® website to determine the growth parameters quantified by the software (Biomass accumulation, leaf/tree N content). Growth parameters were calculated weekly by eLeaf and was made available on the Fruitlook® web-based portal. In the case of adverse weather, the specific week’s data was not available due to cloud cover influencing satellite images negatively. Seasonal data was extracted, from the raw data generated, to a spread sheet (Microsoft Excel, MS Office 2010) to calculate the mean and standard error for each treatment of the specified parameters.

Growth parameter calculations

Biomass production (kg. ha⁻¹)

Biomass production refers to the total above and below ground dry matter production, including not only the main crop, but also other vegetation like the cover crops or weeds. Total biomass production provided through FruitLook® is calculated per week (Table 2) in kilogram growth per hectare (kg. ha⁻¹ per week).

Leaf/tree nitrogen content

FruitLook[®] directly calculates the total amount of N for the whole tree growth above the soil, currently present in the tree, and this can be defined as upper leaf and whole tree N content (Table 2). eLeaf has developed chlorophyll index that considers the green, red and infra-red spectral bands thus obtaining an index that corresponds to high and low chlorophyll content within the plant (eLeaf, 2017). Upper leaf N are calculated *via* this chlorophyll content and upper leaf N calculated as a result of integrating leaf N with leaf area index (eLeaf, 201). Both N content parameters are calculated as kg. ha⁻¹ per week

Results and discussion

Field data

Actual leaf N concentrations at harvest showed no difference between treatments and were in the upper norms recommend for apple leaf N concentration. BlackUrea[™] had a slightly lower N% (2.74 %) than the UreaHB treatments (2.77 %) and although different in values, these values did not differ significantly. Yield efficiency varied between the treatments, with the BlackUrea treatment showing a higher yield efficiency (1.09 kg.cm⁻¹) (ns) compared to the UreaHB treatment (0.8 kg.cm⁻¹) (Paper 2). This was a positive result on a per tree basis. No information was obtained regarding vegetative growth in the different treatment blocks.

FruitLook[®] data

Apple tree growth normally represents a sigmoidal growth pattern, but young trees growing under favourable conditions, follow an expo-linear growth curve (Lakso et al., 1995). Furthermore, biomass production for apple trees under optimum growing conditions has been reported to follow a bell-shaped curve referring to the weekly biomass production, with a s-curve reported when the weekly values are added up over a monthly period (FruitLook[®], 2016).

The bell-shaped curve for biomass production for both treatments in this trial, during the 2016/17 season, confirmed the expected growth patterns as described *via* Fruitlook[®] (2016). Differences between the two treatments were noticed throughout the season. Biomass, leaf N and whole tree N were higher in the UreaHB than BlackUrea[™] treatment (Figs. 2 to 4).

During the 2015/16 season, before treatment commenced, the biomass production was lower compared to the biomass of the 2016/17 season (Figure 2). The higher biomass during 2016/17

is partly due to the increase in vegetative growth associated with the age of the orchard, as well as the higher yield accompanying this growth. The biomass trends for both seasons and treatments also agreed with Lakso et al. (1995), showing a linear increase of biomass early in the season (October) until harvest (February), before declining after the crop is harvested (March) and leaf senescence commences (April). Thus, FruitLook[®] was able to determine biomass accumulation from satellite images accurately, representing the physical growth of this orchard during the season. Although both N formulations were the same applied amounts, small differences (ns) were observed between the two treatments indicating the possible use of Fruitlook[®] to determine in-orchard differences between growth and N status of the trees. Yields differed between the two seasons, with the orchard average increasing from 7.76 ton. ha⁻¹ (2015/16) to 28.29 ton. ha⁻¹ (2016/17). The difference in yield could be a combination of factors e.g. higher rates of fertilizer applied in 2016/17 due to the higher expected yield with young orchards, as well as increased overall growth during the 2016/17 season, bearing in mind that this is a young orchard that has still not reached its full potential.

The Fruitlook[®] biomass trends for 2016/17 indicated treatment differences. The UreaHB treatment showed a higher biomass (ns) than the BlackUrea[™] treatment. This difference was noticeable from September and continued until April 2017. These findings confirmed the difference between the treatments that was observed in actual leaf N (Paper 2) and should be investigated further.

The relationship between whole tree N, upper tree N and biomass during the season (2016/17) (Figures 2 – 4) confirmed the findings by Hou et al. (2004) that biomass is correlated with tree N. These findings also support the results from Paper 1 in a study with ‘Rosy Glow’, indicating no significant increase of leaf N with the use of BlackUrea[™] compared to UreaHB as N source. The BlackUrea[™] treatment had a lower biomass, whole tree N and upper leaf N, but higher yield efficiency (Paper 2) (ns) than UreaHB and indicated towards a possible higher efficiency of this formulation of N as far as N accumulation in the tree is concerned. Lower white root numbers were observed in the ‘Rosy Glow’ trial (Paper 1) (ns), with a higher yield efficiency (ns), with application of BlackUrea compared to the UreaHB and BlackDAP[™] treatments. This poses the question whether the BlackUrea[™] treatment indeed represents a more efficient uptake of N (mode-of-action) than the alternative treatments. If so, this resulted in the trees

utilising less N, more effectively, that increased yield efficiency in ‘Rosy Glow’ (Paper 1) and in ‘Golden Delicious’ (Paper 2).

N levels increased in both whole tree N (Figure 3), as well as upper leaf N (Figure 4), after each application of N. This confirmed a tree response on N treatment that could not be quantified with the destructive measurements performed in Papers 1 and 2. These N trends also differed from those determined for 2015/16, when N applications were managed differently according to the growth of the trees. This indicated a positive response of trees when the alternative approach of three N applications was followed. Slight decreases in whole tree and leaf N (Figure 3 and 4), before harvest, confirmed the findings by Prsa et al. (2007), that the N levels decrease a few weeks after full bloom until harvest.

Conclusions

Fruitlook[®] could quantify certain vegetative trends and differences in a commercial ‘Golden Delicious’ orchard that confirmed horticultural growth principles widely recognised. Data showed differences between two commercial N soil application treatments on an orchard basis, which supplemented individual tree observations in a previous trial (Paper 2).

Early detection of sub optimal growth, as well as N levels within the tree, can play a pivotal role in optimising the effective use of N application e.g. correct application times and quantities. This information could be provided by Fruitlook[®]. Thus, FruitLook[®] data could be used to quantify average biomass accumulation during the season and this will allow changes in management practices should tree performance be unsatisfactory. In addition, data indicated a reduction or an increase in biomass and N levels, as well as showed the effect of applied N after treatment, on both parameters, with the N status increasing after applications. This effect could not be detected with the use of ad hoc field measurements (Papers 1 and 2). Thus, it was possible to confirm that the applied treatments resulted in an increased uptake of N at these application times showing an increase in both the upper leaf N as well as whole tree N.

FruitLook[®] confirmed findings from both Paper 1 and 2 that urea, as a sole N source, could be applied under these conditions, without a detrimental effect on tree performance during time span of the trial (two seasons). The differences in tree performance between the UreaHB and

BlackUrea™ treatments indicated a possible difference in the mode of action of the two formulations, that should be investigated further.

The potential of FruitLook® and the effective implementation thereof, with reference to tree performance, depends largely on the understanding and correct interpretation of the data. This paper confirmed an additional use of FruitLook® data for N management in orchards, as well as validated tree performance measured in the field. In addition, utilizing satellite images increased the data basis of the research conducted to a bigger scale and supplied valuable additional information that contributed towards the final conclusion of the bigger study.

Acknowledgments

This trial was funded by Profert Pty Ltd, South Africa. Fruitlook® supplied the data and Dr Caren Jarman was a valuable collaborator that assisted with interpretation of the information.

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Tables and Figures

Table 1: Fertilizer dates and rates for the 2016 / 17 season in the ‘Golden Delicious’ orchard, Alhambra, Ceres with permission from Paper 2.

Treatment	Bud break – Full bloom (13/10/2016)	6 Weeks post-full bloom (24/11/2016)	Post-harvest (09/03/2017)
UreaHB (control)	45 g.tree ⁻¹	83 g.tree ⁻¹	34 g.tree ⁻¹
BlackUrea™	45 g.tree ⁻¹	83 g.tree ⁻¹	34 g.tree ⁻¹

Table 2: Biomass production and N status of ‘Golden Delicious’ on Alhambra, Ceres, generated through Fruitlook® for the 2016 / 17 season. Values below indicate averages over the season from August 2016 to April 2017.

Season	Treatments	
	BlackUrea™	UreaHB
Biomass (kg.ha ⁻¹ per week)	648.01±44.32	706.41±48.37
Total plant N (kg.ha ⁻¹)	15.5±0.60	16.23±0.63
Upper leaf N (kg.ha ⁻¹)	18.26±1.02	20.07±1.20



Figure 1: Picture of the orchard representing the ‘Golden Delicious’ trees with typical orchard floor covered with annual grasses for the FruitLook® study conducted on at Alhambra, Ceres.

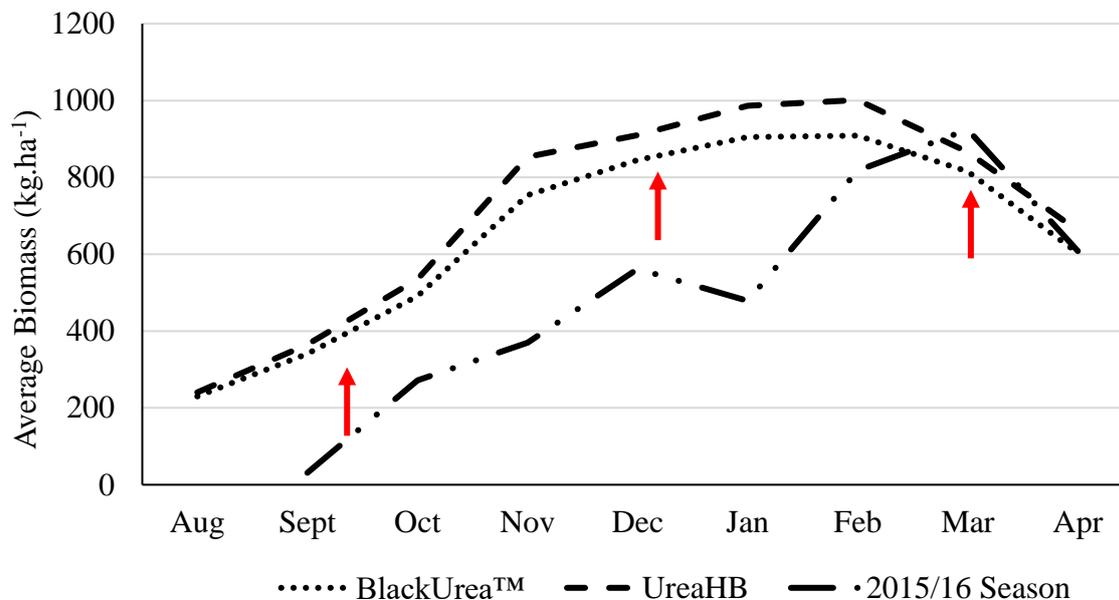


Figure 2: Monthly trend of average biomass of ‘Golden Delicious’ from August 2016 to April 2017 as generated by Fruitlook® in comparison with the 2015/16 biomass production before treatments were applied. Red arrows indicate 2016/17 season fertilizer application dates.

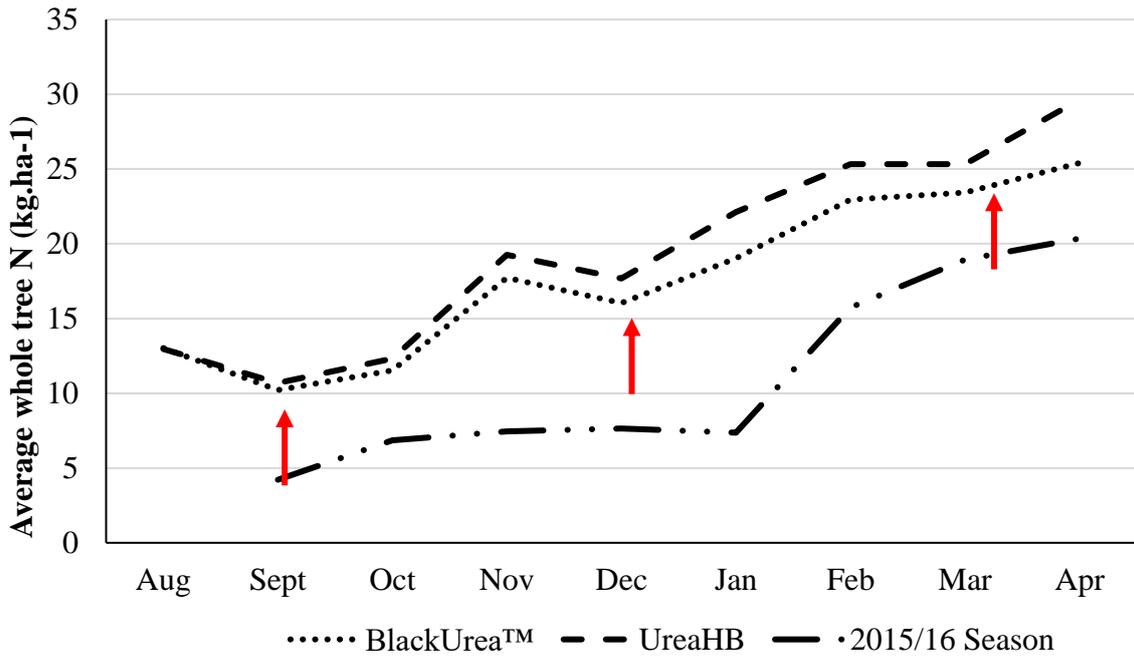


Figure 3: The monthly trend for average whole tree N levels for 'Golden Delicious' from August 2016 to April 2017 as calculated by Fruitlook[®] satellite imagery. Red arrows indicate timing of N fertilizer application dates for the 2016/17 season.

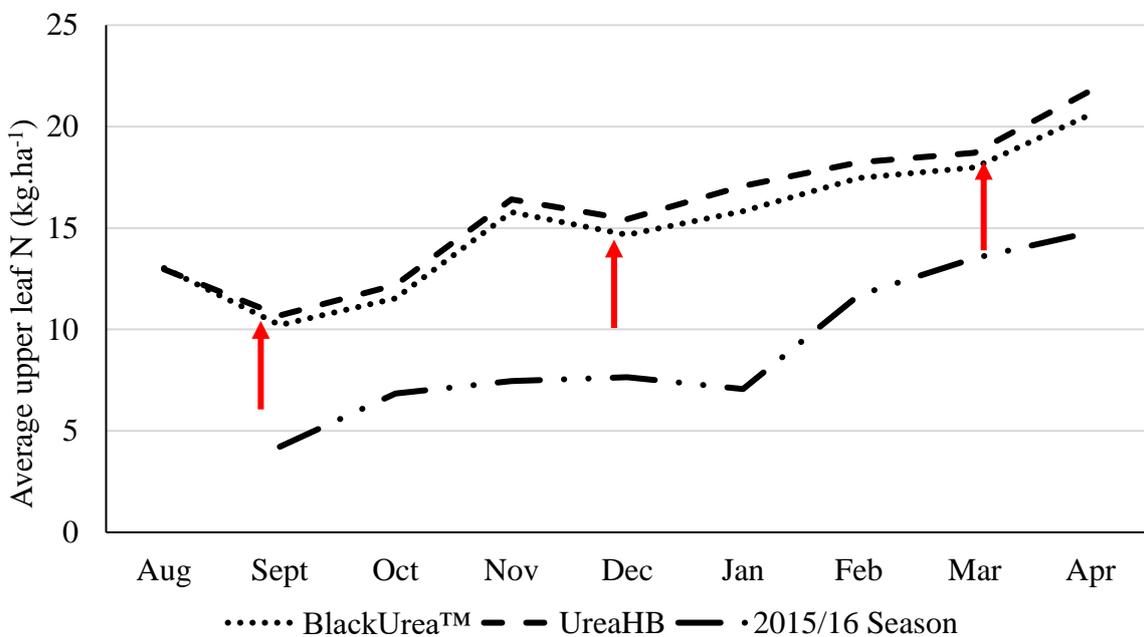


Figure 4: The monthly trend for average upper tree N levels for 'Golden Delicious' from August 2016 to April 2017 as calculated by Fruitlook[®] satellite imagery. Red arrows indicate timing of N fertilizer application dates.

General Conclusion

Nitrogen (N) plays an integral role in commercial apple production (Kotze, 2001). However, it is one of the major contributors to environmental pollution, due to the fact that applied N leaches easily and is often over applied (Cameron et al., 2013). One possible solution to this problem is an increase in efficiency of applied N. This can be achieved by precision application, based on the N requirements of the tree (Rufat and De Jong, 2000), applying different N formulations during the season and utilising N formulations that allow for slow or controlled release (Cameron et al., 2013).

In this study, two alternative granular urea fertilizers (BlackUrea™ and BlackDAP™) were compared with standard high biuret urea (UreaHB) as the control. The alternative formulations are coated with humic and fulvic products, a combination of bio stimulants and nutrient biocatalysts, claimed to create a more biological environment in the soil (Nyati, 2004). This allows a more controlled release of N during the season.

The quantification of the effect of these alternative formulations against the UreaHB was performed during two consecutive seasons (2015/16 and 2016/17) on a commercial farm, Alhambra in Ceres, on ‘Rosy Glow’. During 2016/17, the trial was repeated on ‘Golden Delicious’, with two treatments: BlackUrea™ and UreaHB, as the control. Tree performance on single trees were evaluated in relation to physiology, yield and fruit quality. In addition, the effect of the treatments were quantified with regard to white root growth dynamics in the ‘Rosy Glow’ trial and tree performance, on an orchard basis was quantified with satellite imaging, using FruitLook® software, in the ‘Golden Delicious’ trial.

No significant differences were found between treatments regarding physiology, yield or quality of both ‘Rosy Glow’ and ‘Golden Delicious’. However, in both trials there was an indication of higher yields in the BlackUrea™ treatment compared to the UreaHB that justifies further studies. Significant differences regarding photosynthesis in the ‘Rosy Glow’ trial was not consistent and could not be attributed to treatment. This confirms previous research (Fallafi et al, 2001; Hou et al, 2004; Prsa et al, 2007) stating that fertiliser application is only one of the numerous factors influencing plant performance. Furthermore, the applications of N in these trials were optimal, compared to a fertilised control, thus resulting in subtle changes in

physiology, also confirming existing literature (Prsa et al, 2007). Lastly, it is often stated that these types of trials require long term applications of fertilizers to establish significant differences (Fallahi et al., 2001) and thus, the two seasons' application at optimum rates, may not be sufficient to show significant differences in orchards with optimum N levels, albeit trends showed promise for the alternative N sources.

Trends in white root growth patterns indicated that the different formulations may influence white root growth and should be researched further, confirming above ground observations.

The use of technology platforms, like FruitLook[®], that uses satellite technology to interpret physiological changes in vegetation, can be beneficial for orchard management on a bigger (orchard) scale. This can provide information about the uniformity of the orchard as well as quantify additional parameters not always available to the producer. In the 'Rosy Glow' trial, FruitLook[®] data were not used due to in-orchard variations (soil type) being too great and thus the repeat of the trial on a bigger scale ('Golden Delicious' trial). FruitLook[®] data generated with the 'Golden Delicious' trial showed correlations between biomass and growth curves and confirmed studies done regarding the growth of 'Golden Delicious' (Lakso et al., 1995), as well as biomass accumulation over a season (FruitLook[®], 2016). The findings regarding biomass showed not only differences between treatments, but differences between seasons as an indication of growth within the specific orchard. The use of FruitLook[®] parameters and the correct implementation of the data generated should be researched further to help understand and quantify the role of FruitLook[®] in precision agriculture and application thereof in research.

In conclusion, this study confirmed the important role of site selection, cultivar selection, time of the trial and orchard mineral nutrient status on results. As trials were performed under commercial conditions, environmental impact on measurements compared to laboratory or pot trials were expected but is often required from producers to evaluate commercial products for implementation, leading to a compromise for the researchers. Nevertheless, preliminary results showed a possible impact of the coated urea formulations on white root growth ('Rosy Glow') and biomass and N level ('Golden Delicious') in a sandy loam soil which has not been reported in apple before. This may justify ongoing evaluation. Lastly, one aspect not addressed in this study due to logistics and expertise, is the possible effect of the coated urea formulations on

soil biology and the effect thereof on plant physiology. This may be a suggestion for future studies in the view of the trends reported.

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