

**THE ABILITY OF A NOVEL COMPOUND TO ENHANCE
THE EFFECT OF UREA ON NITROGEN DEFICIENT
TOMATOES**

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UREA ON NITROGEN DEFICIENT TOMATOES**

by

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submitted in fulfillment of the requirements for the degree

MAGISTER SCIENTIAE

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Bloemfontein

November 2009

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ACKNOWLEDGEMENTS

I would like to thank the following persons who made it possible for me to complete this study:

I would like to thank Dr. G.P. Potgieter, of the Department of Plant Sciences, for his valuable advice and supervision. His enthusiasm and constructive comments made a real learning experience of the study.

I am indebted by the company, Elementol (Pty) Ltd, for giving me the opportunity to study and investigate their novel compound, Pheroids.

I would like to acknowledge the University of the Free State for providing me with financial support.

My sincere appreciation goes to my wife who made it possible for me to study by providing me with loving and moral support.

I am greatly indebted to my parents for enabling me to study and for their moral support and keen interest in my study.

I would also like to thank my friends and colleagues for their support and assistance.

Last but not least, I humbly thank the Lord for guidance, insight and determination. Without Him, nothing is possible.

LIST OF ABBREVIATIONS

This list of abbreviations does not include the accepted SI units and abbreviations or accepted abbreviations utilized for common language use. Symbols used to illustrate mathematical manipulations in the text are also included.

A₅₀₃	absorbance at 503 nm
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
B	Boron
BHT	Butylated Hydroxytoluene
C	carbon
C₅	carbon 5
Ca	calcium
Ca²⁺	calcium cation
CaCl₂	calcium Chloride
Ca(NO₃)₂	calcium nitrate
Cl	chloride
Cl⁻	chloride anion
CO₂	carbon dioxide
CO(NH₂)₂	urea
COOH	carboxyl group
Cu	copper
Cu⁺	copper (I) cation
Cu²⁺	copper (II) cation
cv	cultivar
Cys	cysteine
DMAPP	dimethylallyl diphosphate
DNA	deoxyribonucleic acid

DOXP	1-deoxy-D-xylose-5-phosphate
EC	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
ER	endoplasmic reticulum
Fe	iron
Fe²⁺	ferrous cation (Iron (II) cation)
Fe³⁺	ferric cation (Iron (III) cation)
GA-3-P	D-glyceraldehyde-3-phosphate
GDH	glutamate dehydrogenase
Glu	glutamate
Gln	glutamine
Gly	glycine
GOGAT	glutamine-2-oxyglutarate amino transferase
GS	glutamate synthase
H	hydrogen
H⁺	hydrogen cation
H₂O	water
H₂PO₄⁻	dihydrogen phosphate anion
H₃BO₃	boric acid
ha⁻¹	hectare
His	histadine
HPO₄²⁻	monohydrogen phosphate anion
HMG-CoA	3-hydroxy-3-methylglutaryl co-enzyme A
Ile	isoleucine
Leu	leucine
IPI	isopentenyl diphosphate isomerase
IPP	isopentenyl diphosphate pathway
K	potassium
K⁺	potassium cation
KNO₃	potassium nitrate
Lys	lysine
m:v	mass volume ratio

m/v	mass per volume
M	molar
Met	methionine
Mg	magnesium
Mg²⁺	magnesium cation
mM	milli molar
Mn	manganese
Mn²⁺	manganese cation
Mo	molebdenium
MoO₄²⁻	molybdate anion
MVA	mevalonic acid pathway
mw	molecular weight
N₂	nitrogen (gas)
Na(NO₃)₂	sodium nitrate
NADPH	nicotinamide adenosine dinucleotide phosphate (reduced)
NADP	nicotinamide adenosine dinucleotide phosphate (oxidized)
NH₂	amino group
NH₄NO₃	ammonium nitrate
NH₄SO₄	ammonium sulphate
Ni	nickel
Ni²⁺	nickel cation
NFT	nutrient film technique
NH₄⁺	ammonium cation
NO₂⁻	nitrile anion
NO₃⁻	nitrate anion
O₂	oxygen
OH	hydroxide
OH⁺	hydroxyl cation
P	phosphorous
P53	tumor protein 53
Phe	phenylalanine
PMSF	phenyl methylsulfonyl fluoride

ppm	parts per million
Pro	proline
PVC	polyvinyl chloride
Rb	retinoblastoma protein
RH	relative humidity
RNA	ribonucleic acid
ROS	reactive oxygen species
S	sulphur
Ser	serine
SO₄²⁻	sulphur-oxide anion
Std	standard
TCA	tricarboxyl cycle
Thr	threonine
TiP's	trans intrinsic proteins
Tris-HCl	tris hydrochloric acid buffer
Trp	tryptophan
TSS	total soluble solids
Tyr	tyrosine
UV	ultra violet
Val	valine
w/v	weight per volume
Zn	zinc
Zn²⁺	zinc cation

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SUMMARY

Key words: Elementol, Pheroids, *Lycopersicon esculentum*, hydroponics, nitrogen deficiency, urea, yield

A company, Elementol (Pty) Ltd, requested the evaluation of their novel product, Pheroids. Pheroids can apparently facilitate the transport of phytochemical beneficial substances over membranes. Information regarding the chemical attributes was withheld as patent registration is still pending. Pheroids is apparently a micro-emulsion containing free fatty acids (FFA's) and or fatty acid derivatives. It apparently encapsulates a substance and facilitates its transport over the membrane. The exact mechanism involving encapsulation, transport and release of the substances inside the cells is still vague due to little information available on it.

The aim of this study was to evaluate the ability of Pheroids to facilitate the transport of additional nitrogen, urea in this case, in tomatoes grown under nitrogen limiting conditions. Tomatoes (*Lycopersicon esculentum* cv. Rodade Star) were cultivated in a greenhouse using a circulating ebb and flow hydroponic setup, which supplied the plants with either a control- or nitrogen limiting nutrient solutions. The plants cultivated in the nitrogen limiting conditions showed a remarkable reduction in vegetative development and yield. To alleviate the effect of nitrogen limiting conditions on yield, the plants were foliarly sprayed with 0.5% and 1% urea solutions, singly or mixed with Pheroids, once every two weeks. The purpose of these foliar treatments was to determine whether Pheroids can further enhance the absorption and transport of urea across membranes of the leaves to alleviate the effect of limiting nitrogen supply. Plants grown under nitrogen adequate conditions (control) were also foliarly treated with a 0.5% urea solution, singly and mixed with Pheroids, to determine to which extent control plants react to the additional nitrogen supplied.

The reduction in yield, as a result of limited nitrogen supply, was partially alleviated by spraying nitrogen deficient plants with the 0.5% and 1% urea solutions. However, mixing the 0.5% and 1% urea solutions with Pheroids, not only improved vegetative growth and generative development, but also improved yield, suggesting that Pheroids indeed has the ability to improve the uptake of urea. The 0.5% urea / Pheroids solution specifically proved to have the best ability in alleviating the effect of nitrogen limiting conditions on yield without compromising fruit quality. Although the reducing effect was not completely alleviated, the yield and loss in income as a result of nitrogen limiting conditions was prevented to a large extent.

Spraying control plants with 0.5% urea, singly or mixed with Pheroids, also improved yield, without compromising fruit quality. In addition, Pheroids itself, without mixing it with any substance, also resulted in increased yields in both control- and plants grown under nitrogen limiting conditions.

In summary, it appeared that Pheroids has the ability to facilitate the transport of phytological beneficial substances, in this case urea, over plant membranes and enhances cellular nitrogen content, but this needs further detailed analyses. This phenomenon was more evident in plants grown under nitrogen limiting conditions than in plants grown under control conditions. Taking into consideration that most crops frequently may suffer from nitrogen limiting conditions in standard agricultural practices, Pheroids may have numerous potential applications in the agricultural industry.

OPSOMMING

Sleutelwoorde: Elementol, Pheroids, *Lycopersicon esculentum*, hidroponika, stikstofbeperking, ureum, oesopbrengs

'n Maatskappy, Elementol (Edms) Bpk, het die evaluering van hul produk "Pheroids" aangevra. Pheroids beskik vermoedelik oor die eïenskap om die vervoer van fitologiese voordelige verbindings oor membrane te fasiliteer. Inligting rakend die chemiese eïenskappe van Pheroids was weerhou aangesien patentregistrasie nog hangende was. Pheroids is vermoedelik 'n mikro-emulsie bestaande uit vry vetsure en afgeleide vetsure. Pheroids "verpak" die verbindings waarna hierdie Pheroids / verbindingskompleks deur die membraan beweeg. Die presiese meganisme rakend "verpakking", vervoer en vrystelling van verbindings in die sel is onduidelik, as gevolg van die beperkte inligting bekend oor die werking en samestelling van Pheroids.

Die doel van die studie was om die vermoë van Pheroids te ondersoek om die opname van voordelige verbindings, in dié geval ureum, in tamaties te verhoog deur dit onder stikstofbeperkende toestande te verbou. Tamaties (*Lycopersicon esculentum* cv. Rodade Star) was in 'n kweekhuis verbou met behulp van 'n sirkulerende "ebb and flow" hidroponiese stelsel. Die stelsel het die plante van kontrole- en stikstofbeperkende groeimedia voorsien. Die plante wat onder stikstofbeperkende toestande verbou was, het 'n sigbare verlaging in oesopbrengs getoon. Om die negatiewe invloed wat stikstofbeperkende toestande op oesopbrengs het te oorkom, is die plante elke twee weke met 0.5% en 1% ureum oplossings, alleen of vermeng met Pheroids, bespuit.

Die doel van hierdie blaarbespuitings was om vas te stel of Pheroids die opname van ureum bevorder en sodoende die invloed van stikstofbeperkende toestande op groei en oesopbrengs te beperk. Kontrole-plant was ook met 'n 0.5% ureum oplossing, alleen of met Pheroids vermeng, bespuit. Dit was gedoen om vas te stel of kontrole-plant ook op die addisionele stikstof-toedienings reageer.

Die afname in oesopbrengs, as gevolg van stikstofbeperkende toestande, is gedeeltelik opgehef wanneer plant met die 0.5% en 1% ureum oplossings bespuit word. Deur die 0.5% en 1% ureum oplossings met Pheroids te vermeng, kon die effek van stikstofbeperkende toestande op vegetatiewe groei, generatiewe ontwikkeling en oesopbrengs verder opgehef word. Dit dui daarop dat Pheroids wel oor die vermoë beskik om ureum-opname te verhoog. Die 0.5% ureum / Pheroids mengsel was die mees suksesvolle behandeling om die invloed van lae stikstofvlakke op groei en ontwikkeling te beperk. Alhoewel die negatiewe invloed nie ten volle opgehef kon word nie, was die verlies in oesopbrengs en gepaardgaande inkomste wel tot 'n groot mate beperk.

Kontrole-plant wat met 0.5% ureum, alleen of met Pheroids vermeng, bespuit is het ook positief op die bespuitings gereageer deur 'n hoër oesopbrengs te lewer. Pheroids alleen as behandeling het ook 'n toename in oesopbrengs tot gevolg gehad by die plant wat onder stikstofbeperkende toestande verbou was.

Dit blyk dus dat Pheroids wel die potensiaal besit om verbindings soos ureum se vervoer oor plantmembrane te fasiliteer, om sodoende waarskynlik die sellulêre stikstofvlakke te verhoog. Hierdie verskynsel benodig egter nog verdere ondersoek. Die invloed van Pheroids was egter meer opvallend by die plant wat onder stikstofbeperkende toestande verbou was. As daar in ag geneem word dat die meeste gewasse van tyd tot tyd stikstofbeperkende toestande ervaar, blyk dit dat Pheroids groot toepassingspotensiaal in die landboukundige industrie mag hê.

CHAPTER 1

INTRODUCTION

Plant growth and development involves a number of complex interactions between the plant and the environment. Nitrogen is the most abundant element in the atmosphere. Nitrogen is a critical component of all proteins which regulates plant metabolism and growth and plays an important role in forming compounds that is vital for plant metabolism and growth. Nitrogen is also an integral part of the chlorophyll molecule, which plays an important role during photosynthesis. (Salisbury & Ross, 1991; Layzell, 1990). Nitrogen is found in the environment in the following forms: N_2 (nitrogen gas or di-nitrogen), NO (nitric oxide), NO_2 (nitrogen dioxide), N_2O (nitrous oxide), NO_2^- (nitrite ion), NO_3^- (nitrate ion), NH_3 (ammonia), NH_4^+ (ammonium ion) and R-NH₂ (organic nitrogen including amino acids, proteins, alkaloid bases and urea).

Plants absorb nitrogen as NO_3^- or NH_4^+ (Layzell, 1990). Most soil nitrogen is in organic form and must first be oxidized by soil organisms into inorganic forms in order for plants to absorb the nitrogen (Salisbury & Ross, 1991). This is a complex interaction between plants and microorganisms (Verma *et al.*, 1986). Crop plants require large concentrations of nitrogen for growth and it frequently may become a limiting nutrient. Losses of nitrogen from the soil may result in low yields (Layzell, 1990).

To alleviate the losses of nitrogen, due to leaching and high absorption rates, urea is commonly used to supply additional nitrogen to crop plants, mostly in the form of a foliar spray. Several benefits have been identified in supplying crops with urea through their foliage (Gooding *et al.*, 1992). After urea is absorbed it is transported with the transpiration stream (Kirkby & Mengel, 1967). Galluci *et al.* (1971) suggested that urea can easily cross biological membranes without requiring protein-mediated transport, because it is an uncharged molecule

(Salisbury & Ross, 1991). However, plants also absorb urea actively by H⁺-urea co-transporter proteins. (Liu *et al.*, 2003). Most plants absorb foliarly applied urea rapidly (Wittwer *et al.*, 1963; Nicoulaud & Bloom, 1996) and hydrolyze the urea in the cytosol where it is further metabolized. However, when urea is applied as a foliar spray, urea may only be absorbed in low concentrations because not all urea is transported into the leaves by the transport proteins. These proteins may become saturated with urea, temperature may play a role or the urea mixture may evaporate from the leaf surface. All these factors may limit urea absorption (Liu *et al.*, 2003). Thus, an attempt has to be made to improve or enhance the absorption of urea in tomato leaves.

A company, Elementol (Pty) Ltd, is in the process of registering a novel compound under the name of Pheroids. Elementol (Pty) Ltd, disclosed very little information regarding the chemical attributes of Pheroids. Pheroids is apparently a micro-emulsion containing free fatty acids (FFA's) and / or fatty acid derivatives. Elementol (Pty) Ltd, propose that Pheroids is a vehicle for the delivery and translocation of phytologically beneficial substances over membranes. Elementol (Pty) Ltd. further propose that Pheroids itself has a stimulatory effect on plants. However, this has not been proven. Elementol (Pty) Ltd, therefore, requested the evaluation of Pheroids as a growth promoting substance as well as its ability to enhance the transport of beneficial substances over membranes.

The rationale for this study was to evaluate the ability of Pheroids to facilitate the additional uptake of urea in tomatoes grown hydroponically under nitrogen limiting conditions. The plants were sprayed with 0.5% and 1% urea solutions, singly and mixed ("packed) with Pheroids. This was done in an attempt to reduce the effect of nitrogen limiting conditions on vegetative growth and subsequent yield by enhancing the uptake of urea through the action of Pheroids. Another aim was to determine whether Pheroids itself can be promoted as a bio-stimulant. Hydroponics is an ideal technique to address this as nutrient supply to the plants can be accurately controlled.

CHAPTER 2

LITERATURE REVIEW

2. TOMATOES

Lycopersicon esculentum Miller. (Solanaceae).

2.1 TOMATO PRODUCTION AND USES

Tomatoes are one of the most important crops worldwide and the second most important crop in economic value, with an annual production of 40 million tons worldwide (Internet 1) in 2008. Taking tomato fruit and the processing of tomatoes into account, tomato is the second most popular vegetable per capita used in the United States (USDA, 2000). Tomatoes are consumed fresh or is used in various processed foods. More than 65% of the world tomato production is processed and used as flavourings, sauces, etc. Quality attributes of tomatoes vary depending on their intended use e.g. smaller fruit would be processed whereas larger fruit would be sold on the fresh market (Schuch & Bird, 1994). The market value of tomatoes are determined by fruit quality which includes size, shape, firmness, colour, taste and solids content. Market demand varies, particularly for fresh market tomatoes. While fruit quality has been improved by genetic manipulation and breeding, fruit quantity has particularly been improved by propagation using hydroponics (Ho & Hewitt, 1986).

Tomatoes are consumed fresh or in salads etc. Tomatoes have also been used in the making of soups, drinkable juices, tomato sauces and tomato pastes etc. Due to tomato fruit containing high levels of lycopene, dietary intake of tomatoes and tomato products has been shown to decrease the risk of chronic diseases like cancer and cardiovascular diseases (La Vecchia, 2002; Rao, 2002). Tablets and juices with high lycopene content are manufactured and sold as a daily

antioxidant supplement. These tablets and juices may also contain natural or synthetic lycopene products (Internet 3).

2.2 THE PLANT

Tomatoes have two growth tendencies, namely indeterminate and determinate growth characteristics. Although a distinguishment is made between these two growth patterns, both are actually determinate growers. Determinate growers may grow to a height of 2 m, are erect and have a restricted flowering and fruiting period. These determinate growers are best suited for field conditions, however, it can be grown in greenhouses with great ease. The indeterminate cultivars, however, are characterized by the main stem which grows upward indefinitely reaching more than 10 m per year. When the stem reaches the desired height, the stem is redirected which allows an indefinite growth tendency. These cultivars are ideally suited for greenhouse cultivation as well as hydroponic production and guarantees continued flowering and fruiting.

A detailed description of the growth, anatomy and histogenesis of the tomato is given by Hayward (1938). Leaves are arranged alternately with a 2/5 phyllotaxy. Leaf size is variable (Aung & Austin, 1971). Leaves lower in the stem may be small with few leaflets. Thereafter, leaves are typically 0.5 m long with a large terminal leaflet with up to eight lateral leaflets which may be compounded. Leaflets are usually petiolate and irregularly lobed with toothed edges. Tomatoes have a taproot system which can extend up to 1.5 m into the soil, but is often limited in hydroponic production (Hayward, 1938).

2.3 CULTIVARS

While field and greenhouse tomatoes share many characteristics, several requirements are specific for greenhouse production. A large number of tomato cultivars are available depending on the grower or consumer requirements. They are divided into cultivars specific for yield, resistance to specific diseases, fruit

type and size and whether the fruit will be used for processing or the fresh market (Ho & Hewitt, 1986). Some popular cultivars in South Africa include Floradade, Heinz 1370, Homestead, Manapal and Moneymaker (Gilbert & Hadfield, 1987). It must be kept in mind that each cultivar have different requirements and different yields, but some cultivars are great all-round cultivars with a high yield and good fruit quality, are easily managed and are suitable for greenhouse hydroponic production.

2.4 FLOWERING AND POLLINATION

Development of flowers is a prerequisite for the development of fruits as a result of pollination. Any factor preventing pollination will result in less fruit forming. Furthermore, any delay in flowering will cause a delay in subsequent fruit development. More flowers from more frequent flowering does not necessarily yield more fruit, but rather increases the potential for competition between fruit which may causes a reduction in fruit size (van Ravestijn & Molhoek, 1978). In both determinate and indeterminate growers, fruit production may be limited by the lack of pollination.

Several pollination related problems may be experienced when tomatoes are grown in a greenhouse. Fertilization of the ovules is very important with regard to the growth of seeded tomato fruit. Fruit weight is related to seed number, as a result of successful pollination and fertilization and fruit size is positively correlated with seed number (Rylski, 1979). All current tomato cultivars are self pollinated (Smith, 1935). Any factor influencing pollen production, pollen transfer, pollen germination and fertilization will result in no fruit being formed. Factors influencing the above mentioned include temperature, nutrient deficiencies, nutrient toxicities, relative humidity, absence of a draft inside the greenhouse and the absence of pollinators in cultivars which are not self pollinating (Rudich *et al.*, 1977, Internet 2).

2.5 TOMATO DISEASES

Hydroponic production of crops results in a physical environment which is favorable for pathogens. Ideal temperatures, high humidity and the supply of enriched nutrient solutions can be not only favorable, but ideal for pathogen development (Gullino & Garibaldi, 1994; Paulitz, 1997; Paulitz & Bélanger, 2001; van Assche & Vangheel, 1994). Biotic diseases of tomatoes favored by these ideal conditions include insects like cutworm, whitefly, erinose, tomato rust mite, white mite, red spider mite, nematodes and tomato russet mite, where the latter cause irreversible wilting of plants followed by chlorosis (yellowing of leaves), and finally necrosis (dying of leaves). Unfortunately, these organisms are often noticed only in a fairly colonized state. Other biotic organisms causing diseases include late rust, early rust, septoria leaf spot, Fusarium wilting, bacterial wilt and bacterial cancers.

2.6 THE FRUIT

A tomato fruit is a berry consisting of seeds within a fleshy pericarp developed from the ovary of the flower. The fruit is composed of flesh (pericarp walls and skin) and pulp (placenta and locular tissue including seeds). In general the pulp accounts for less than one third of the fruit fresh weight. Two types of fruits are distinguished, namely bilocular and multilocular fruits. Bilocular fruit have two locular cavities which contains the seeds (Fig. 2.1), whereas the multilocular fruit contains more than two locular cavities. The locular cavities are separated by the columella or the inner wall of the pericarp.

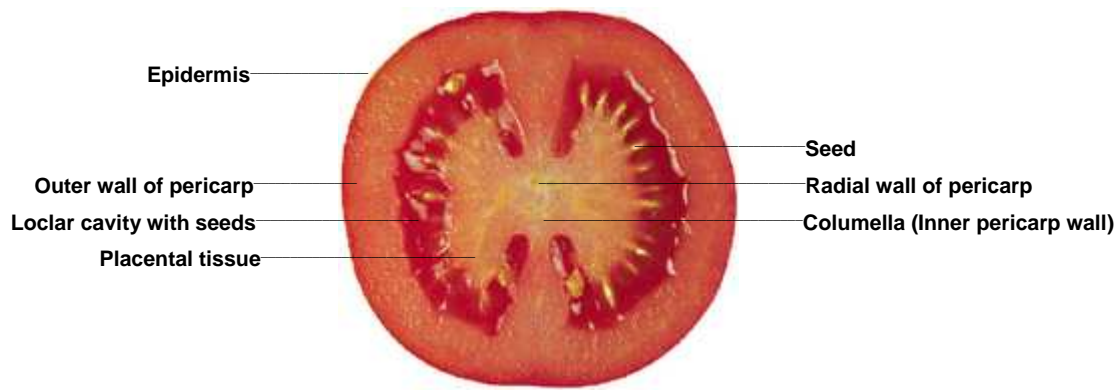


Figure 2.1 Bilocular tomato showing two locules separated by the pericarp which houses the placenta and locular tissue including seeds (Internet 8).

2.6.1 Fruit ripening and the role of ethylene

The development of a tomato fruit from a green to a fully ripe state involves dramatic changes in colour, composition, aroma and texture. These changes are highly coordinated. Fruit ripening is a complex metabolic regulated process. Physical, chemical and physiological changes take place during fruit ripening. Tomato fruit take 7 to 9 weeks to develop from a fertilized ovary to a ripe red fruit. This involves the physical changes in size, colour, softness and integrity.

Changes in chemical composition of fruits involve an increase in dry matter, glucose, fructose, starch and organic acid contents (Rudich *et al.*, 1977), an increase in electrical conductivity, as well as a decline in fruit pH (Domingos *et al.*, 1987). During ripening the chlorophyll concentration decreases, while carotenoids, especially lycopene, accumulate in the fruit causing the fruit to change colour (Laval-Martin *et al.*, 1975). Many environmental factors like light, CO₂ levels, temperature, water and nutrient supply play a role in fruit ripening.

A natural non-environmental factor which influences fruit ripening to a great extent is ethylene. Ethylene, as the gaseous hormone, plays an important role in fruit ripening in general (Alexander & Grierson, 2002) and was shown to stimulate ripening in green tomatoes (McGlasson *et al.*, 1978). Fruits exhibit

different ripening mechanisms and can be divided into two groups namely non-climacteric and climacteric fruit, where ripening in the latter is accompanied by an increase in respiration with a concomitant increase in ethylene production. In non-climacteric fruit no increase in respiration occurs, while ethylene production remains at a very low level. In tomato and other climacteric fruits, such as apple, melon and banana, the ethylene burst is a prerequisite for normal fruit ripening (Oeller *et al.*, 1991).

The role of ethylene in climacteric fruit ripening is divided into two separate stages of ethylene production. The first system is functional during normal vegetative growth where ethylene is auto-inhibitory and controlled on gene level, and is responsible for producing basal ethylene. The second system, however, occurs during ripening usually in one region of a fruit, spreading to neighboring regions as ethylene diffuses freely from cell to cell and initiates the ripening process throughout the fruit. However, ethylene is not the sole initiator for fruit ripening (Oeller *et al.*, 1991). Gene expression and climatic factors may also play a role.

2.6.2 Fruit colour

The development of a tomato fruit from a green to a fully ripe red state involves dramatic changes in colour. These changes occur within the plastids after the disappearance of chlorophyll from the fruit. These colour changes are highly coordinated and is a regulated multi step process. The two major groups of pigments found in tomato fruit are chlorophylls and carotenoids. The most noticeable change during ripening is the remarkable increase in the carotenoid content of the fruit (Laval-Martin *et al.*, 1975). During ripening the chlorophyll concentration decreases while carotenoids, especially lycopene, accumulate in the fruit (Laval-Martin *et al.*, 1975).

Tomato fruit follows a transition from partially photosynthetic to heterotrophic metabolism during development by the parallel differentiation of chloroplasts into

chromoplasts and the dominance of carotenoids and lycopene during ripening. (Fernando & Alisdair, 2006; Harris & Spurr, 1969).

2.6.3 Fruit ripening - softness, appearance and aroma

The softening and decline in fruit firmness during ripening is generally reported to result principally from disassembly of the primary cell wall and middle lamella. This causes a reduction in intercellular adhesion depolymerization, and solubilization of hemicellulosic and pectic cell wall polysaccharides and, in some cases, wall swelling (Brummell & Harpster, 2001). The ripening of fleshy fruits involves many physiological processes, including the production of aromatic compounds and nutrients, changes in colour, and softening of the flesh to an edible texture, which have evolved to attract animals to promote seed dispersal (Giovannoni, 2004). However, much less is known about the critical molecular components of fruit firmness and softening. In part, this reflects the difficulties in evaluating the many physical and sensory characteristics that determine texture (Harker *et al.*, 1997; Waldron *et al.*, 2003), a characteristic that, unlike colour or aroma, cannot be defined by a quantitative measurement of specific metabolites or by monitoring a particular biosynthetic pathway.

The softening and decline in fruit firmness are accompanied by the increased expression of numerous cell wall degrading enzymes, including polysaccharide hydrolases, transglycosylases, lyases, and other wall loosening proteins, such as expansin (Harker *et al.*, 1997; Rose *et al.*, 2003; Brummell, 2006). It has been reported that factors such as turgor and cell morphology contribute to aspects of texture (Lin & Pitt, 1986; Shackel *et al.*, 1991), and invariably attribute to fruit softening and disassembly of polysaccharide networks in the primary wall and middle lamella (Rose *et al.*, 2003; Brummell, 2006). Tomato flavour is influenced by sugars, acids and their interactions, which in turn is important in determining overall fruit sweetness, sourness and flavour. More than 130 volatile compounds are apparently responsible for overall fruit aroma (Kato *et al.*, 1984).

2.6.4 Fruit quality parameters

Tomato quality and flavour are influenced by the aromas of many chemical constituents. These include sugars (glucose, fructose, sucrose), alcohol insoluble solids (proteins, pectic substances, hemicellulose, cellulose), organic acids (citric and malic acid), minerals (mainly potassium, calcium, magnesium and phosphorus), lipids, dicarboxylic amino acids, pigments, ascorbic acid, volatiles, other amino acids, vitamins and polyphenols (Davies & Hobson, 1981). Sugars, acids and their interactions are the most important factors influencing sweetness, sourness and overall flavour (de Bruin *et al.*, 1971). The quality of tomato fruit is partially dependent on the flavour of the fruit. Factors affecting fruit flavour, aroma, appearance etc. will ultimately influence fruit quality. It is of utmost importance that in a good quality fruit, fruit taste must not be compromised.

2.6.4.1 % Brix (Brix index)

Total soluble solid concentration (TSS, measured as % Brix) of tomato fruits is an important variable which is used to determine fruit quality, because TSS is most commonly associated with sugar and organic acid concentrations (Stevens *et al.*, 1977; Young *et al.*, 1993). The % Brix index includes all soluble solids like sucrose, fructose, glucose, vitamins, amino acids, protein, organic acids, minerals and hormones. Starch, pectic substances and cellulose are water insoluble and do not contribute to the Brix index (Baxter *et al.*, 2005). Each % of Brix is equal to 1 gram of soluble solids in 100 g of fresh mass. A high Brix % normally indicates high sugar content (Baxter *et al.*, 2005).

2.6.4.2 Fruit electrical conductivity

Fruit electrical conductivity is an indication of the concentration of dissolved solids present in the fruit. These dissolved solids include sugars (glucose, fructose and sucrose), organic acids, and other soluble cellular constituents like amino acids. When these constituents are present within the fruit in larger

quantities, fruit puree has the ability to conduct electricity better than the puree of fruit with a low number of these soluble solids. By measuring fruit conductivity ($\text{milliSiemens.cm}^{-1}$), the number of charged soluble solids can be determined. Domingos *et al.* (1987) found that electrical conductivity increases during fruit ripening, thus total soluble solids, which greatly influences fruit taste. Electrical conductivity can be considered an important parameter in measuring fruit quality.

2.6.4.3 Fruit pH

Fruit pH plays an important role in fruit ripening and taste. As fruit ripening persists, a slight decline in pH was observed by Domingos *et al.* (1987). Citric acid seemed to be more important in fruit sourness than malic acid. Citric acid apparently accounts for more than half the acidity of the fruit (Davies & Hobson, 1981). A high acid content is generally required for best flavour in tomato fruit. The pericarp portion of tomato fruit contains low concentrations of organic acids opposed to the locular portion of the fruit (Stevens *et al.*, 1977).

Fruit pH and carbohydrate content are the main factors influencing fruit taste and the interaction of these two factors influence fruit taste dramatically. An example of this is the relationship or ratios between the different sugars and acids. Fructose and citric acid are more important in fruit sweetness and sourness than glucose and malic acid respectively (Davies & Hobson, 1981). A high sugar content, as well as a high acid content is required for the best flavour. A high acid and a low sugar content will produce a sharp-tasting tomato, whereas a high sugar content and a low acid content will result in a fruit with a bland taste. When sugar and acid contents are low, a tasteless fruit is normally the result. Therefore, the carbohydrates (sugars) present in tomatoes need to be discussed.

2.6.4.4 Carbohydrates (Sugars)

Carbohydrates (sugars) have a major influence on tomato flavour. Sucrose, glucose and fructose are the foremost carbohydrates, which accounts for 65% of fruit dry weight. Winsor (1979) found that tomato fruits with a high hexose accumulation being characteristic of the domesticated tomato. Together with quinic-, malic- and citric acid, these compounds are also the principal quality components for determining the total soluble solid content (TSS) or Brix index in tomato fruit (Davies & Hobson, 1981). Two main factors influence the carbohydrate content in fresh tomato fruits. These are 1) the environmental conditions during development and ripening and 2) the cultivar (Luckwill, 1943). Sugar content increases progressively during development and maturation of tomato fruit (Winsor *et al.*, 1962a; 1962b). Yelle *et al.* (1991) have reported that sugars can contribute up to 60% of the total dry weight of a fruit.

Sucrose is the major photo-assimilate transported from the leaves (source) to the tomato fruit (sink), where it is subsequently cleaved by sucrose synthase and invertase. These enzymes are the main enzymes responsible for the degradation of sucrose and greatly affect the levels of sucrose and overall fruit metabolism. Invertase cleaves sucrose into glucose and fructose. Plant invertases are separated into two groups on the basis of pH optima, namely acid and alkaline invertases. While an alkaline invertase activity has not been reported in tomato fruit, two types of acid invertases have been observed, namely a soluble and a particulate form (Klann *et al.*, 1996). According to Klann *et al.* (1996), plants with a low invertase activity had increased sucrose concentrations in the fruits, resulting in smaller fruits. This implies that small fruit may have a higher sucrose content than larger fruit.

Early during tomato fruit development there is a transient increase in both sucrose synthase activity and starch levels, which is correlated with fruit growth and sink strength, suggesting a regulatory role for sucrose synthase in sugar import. Plants with reduced sucrose synthase activity showed a reduction in fruit

size, which also correlates with a reduction in starch during the early stages of the fruit development (Klann *et al.*, 1996). High starch levels in turn are well correlated with high levels of soluble solids in a number of tomato lines (Dinar & Stevens, 1981; Sun *et al.*, 1992), suggesting that sucrose synthase might have a controlling role in loading assimilates into the fruit. It was found that sucrose synthase activity is low in the first week after anthesis, reaching a peak early in development and then subsequently decline as the fruit matures (Demnitz-King *et al.*, 1997; Schaffer & Petreikov, 1997; Yelle *et al.*, 1988). The peak of sucrose synthase activity occurs when import of sugar into the fruit switches from a predominantly symplastic to an apoplastic mechanism (Patrick, 1997). The rate of sucrose import into tomato fruit is further reported to be regulated by the sucrose concentration gradient between the leaves and the fruits (Walker & Ho, 1977).

Fruit growth is thus mainly determined by the import of leaf assimilates. Most of the fruit dry matter is apparently derived from leaf assimilates. The import rate of assimilates like starch into tomato fruit is inversely related to the sucrose concentration in the fruit. From this it seems that the import of starch is regulated by sucrose hydrolysis (Walker *et al.*, 1978). The rate of starch accumulation during fruit development has a great effect on the final total soluble solid content. Starch content reaches a maximum of 1% dry matter at the mature green state, where after the starch content decreases. The decrease in starch is accompanied by an accumulation of reducing sugars (Dinar & Stevens, 1981).

2.7 LYCOPENE

Tomatoes have a high nutritional value, and are important sources of vitamins A, C, and especially lycopene. Lycopene is a very important nutritional source and are of utmost importance in determining overall tomato quality.

The change in colour during ripening is due to differentiation of chloroplasts to chromoplasts and the dominance of carotenoids like lycopene. Lycopene is responsible for the red colour in tomato fruit (Bramley, 2000; Salunkhe *et al.*, 1974). Salunkhe *et al.* (1974) have found a correlation between the colour index of tomato fruit and lycopene content, which implies that the most reddish fruit will have the highest level of lycopene.

2.7.1 Lycopene structure and attributes

Lycopene is a carotenoid (Nguyen & Schwartz, 1999), a forty carbon molecule and an acyclic isomer of β -carotene. It is a highly unsaturated aliphatic hydrocarbon chain consisting of 13 carbon-carbon double bonds, one of which is a conjugated bond and two which are un-conjugated double bonds (Bramley, 2000). The extended series of alternated double bonds and cyclic end groups are the key to the biological activity of lycopene. These bonds are arranged in a linear array. The molecule further consists of two central methyl groups arranged in the 1,6-position relative to each other. Other methyl groups in the molecule are in a 1,5 position relative to each other (Fig. 2.2).

Lycopene in plants primarily exists in an all-trans configuration, which is a relatively stable form (Bramley, 2000). Lycopene undergoes cis-trans isomerization induced by light (Nguyen & Schwartz, 1999; Xianquan *et al.*, 2005). Exposure to high temperatures, catalysts, active surfaces, oxygen, acids, catalysts and metal ions can cause seven of the double bonds of lycopene to isomerize to a less stable conformation (mono- poly- or *cis*- isomeration)

(Functional Foods, 1998). Thermodynamically, the all-trans configuration corresponds to the most stable configuration (Functional Foods, 1998).

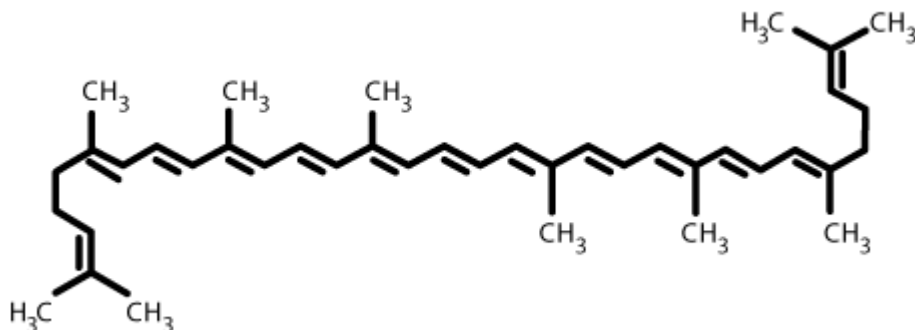


Figure 2.2 Lycopene structure indicating 13 double bonds of which 11 are conjugated and two are un-conjugated (Internet 3).

2.7.2 Lycopene synthesis

Carotenoids are a group of isoprenoids (terpenoids) which exist in plastids. Carotenoids are composed of five carbon building blocks, isoprene units that serve as precursors for the synthesis of other carotenoids and isoprenoid compounds. The isoprenoids, which constitute the most diverse group of natural products, serve numerous biochemical functions in plants. They play important roles as quinones in electron transport chains, as components of membranes (sterols), in sub-cellular targeting and regulation (prenylation of proteins), as photosynthetic pigments (carotenoids, side chain of chlorophyll), as hormones (gibberellins, brassinosteroids, abscisic acid, cytokinins), as plant defense compounds and as attractants for pollinators (monoterpenes, sesquiterpenes, and diterpenes; Harborne, 1991). Isoprenoids (terpenoids) are synthesized ubiquitously among prokaryotes and eukaryotes through condensation of the five-carbon intermediates isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Lange *et al.*, 2000).

Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are produced in two different localities (cytosol and chloroplasts) using two different pathways.

The first pathway is the mevalonate (MVA) pathway in the cytosol and the second pathway is the non-mevalonate or 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway in the chloroplast. Both pathways form the active C₅-unit isopentenyl diphosphate (IPP) as the precursor from which all other isoprenoids are formed via head-to-tail addition (Figure 2.3). The difference between the MVA and the DOXP pathways are the starting point of these pathways. The MVA pathway forms IPP outside the chloroplast, where after the IPP is transported into the chloroplast. The DOXP pathway forms IPP inside the chloroplast. IPP is therefore the condensation point for these two pathways.

The MVA pathway in the cytosol begins with acetyl-CoA and proceeds via a number of independent steps to form IPP, which is then reversibly converted to DMAPP in a reaction catalyzed by IPP isomerase (IPI) in the chloroplast (Bach *et al.*, 1999). The MVA pathway also contributes to the biosynthesis of sterols, sesquiterpenes, triterpenoids and ubiquinone (Laule *et al.*, 2003).

The DOXP pathway involves the condensation of pyruvate and glyceraldehyde-3-phosphate via 1-deoxy-D-xylulose 5-phosphate (in the chloroplast) as a first intermediate, which is used for the synthesis of isoprene, carotenoids, abscisic acid, and the side chains of chlorophylls and plastoquinone (Arigoni *et al.*, 1997; Lichtenthaler *et al.*, 1997; Lichtenthaler, 1999; Schwender *et al.*, 1997; Milborrow & Lee, 1998; Hirai *et al.*, 2000). The DOXP pathway in the chloroplast not only produce lycopene but like the MVA pathway, provides precursors for the biosynthesis of plastidic isoprenoids, such as carotenoids, phytol (a side-chain of chlorophylls), plastoquinone-9, isoprene, mono-, and diterpenes.

Although this subcellular compartmentation allows both pathways to operate independently in plants to form IPP in the chloroplast and cytosol, there is evidence that they cooperate in the biosynthesis of certain metabolites. For example, the chamomile sesquiterpenes are composed of two C₅ isoprenoid units formed via the MVA-dependent pathway, with a third unit being derived from the MVA-independent pathway (Adam *et al.*, 1999).

Environmental factors, cultivar type (Moraru *et al.*, 2003) and the specific stage of ripening (Ilahy & Hinder, 2007) all affect the level of lycopene in tomatoes (Ramandeep & Savage, 2004). Lycopene content can vary considerably depending on cultivar type resulting in some cultivars having higher lycopene content than others (Moraru *et al.*, 2003). A difference in lycopene content was distinguished between field grown tomatoes and greenhouse tomatoes. Depending on the quantity of light the fruit receives, lycopene content of field grown fruit differ from 52 mg. kg⁻¹ to 230 mg. kg⁻¹ fresh mass, whereas greenhouse grown tomatoes have lycopene contents of 10 mg. kg⁻¹ to 108 mg. kg⁻¹ fresh mass (Sahlin *et al.*, 2004). Bramley (2000) and Levy & Sharoni (2004) recorded that the average lycopene content of tomatoes grown in greenhouses to vary between 8.8 mg. kg⁻¹ to 42.0 mg. kg⁻¹ fresh mass.

Lycopene is an intermediate for the synthesis of other carotenoids like β -carotene (Fig. 2.3). The cyclization of lycopene is a key branch point in the pathway of carotenoid biosynthesis. Lycopene present in tomato fruit, (giving it a red colour), will attract animals, which will help with the dispersal of fruit and seed.

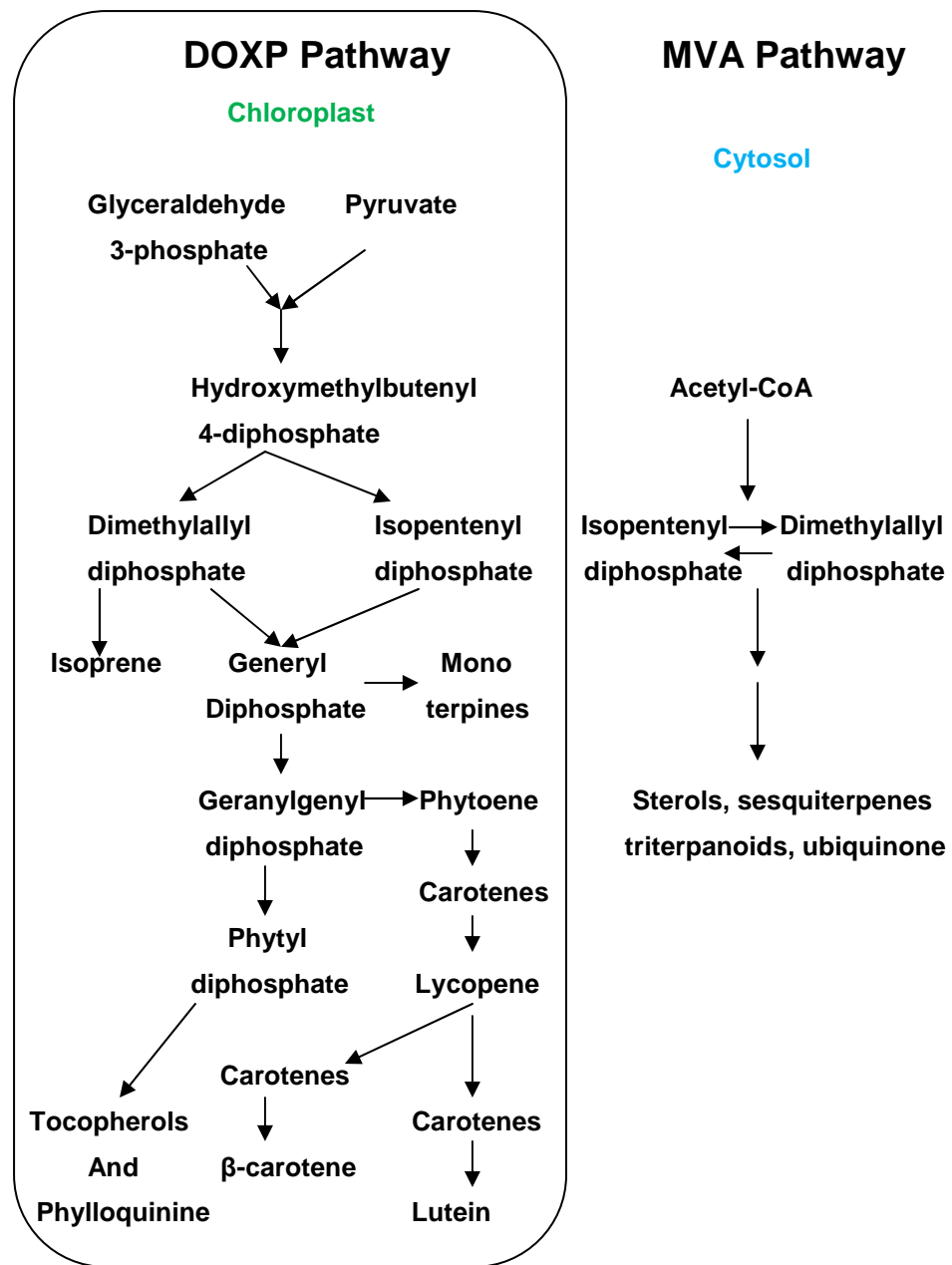


Figure 2.3 Isoprenoid (terpenoid) synthetic pathways (DOXP Pathway and MVA Pathway) localized to the cytosol and chloroplast (Laule *et al.*, 2003).

2.7.3 Role of Lycopene

Lycopene plays a major role in plants in being an anti-oxidant and prevents photosynthetic pigments to undergo photo-oxidation. Lycopene was also found to be an effective anti-oxidant in the human body. The role of lycopene can be divided into its role in plants, as well as the role of lycopene in the human diet.

2.7.3.1 Role of lycopene in the plant

Lycopene is one of the most important carotenoids present in tomatoes. Lycopene is present in both the skin and the pericarp of tomato fruit. Lycopene, with its cyclic end groups, is an essential photosynthetic component in all plants and serves different functions (Goodwin, 1980). Certain metabolic activities generate reactive oxygen species (ROS). These ROS react with cellular components and cause oxidative damage to lipids, proteins and DNA. Lycopene with its good anti-oxidant properties prevents these ROS to cause damage to cellular constituents (Conn *et al.*, 1991; Di Mascio *et al.*, 1989; Rice-Evans *et al.*, 1997).

Carotenoids are essential components of the photosynthetic membranes protecting chlorophyll against photo-oxidation and act as light absorbing pigments (Salisbury & Ross, 1991). These pigments harvest light for photosynthesis, and dissipate excess light energy absorbed by the antenna pigments. The chlorophyll is protected against photo-oxidation by quenching triplet chlorophyll, superoxide anion radicals and singlet oxygen (Agarwal & Roa, 1999). The quenching ability of lycopene is determined by the number of conjugated double bonds, as well as the end groups in the structure (Stahl & Sies, 1996).

2.7.3.2 Role of Lycopene in the human diet

The role of carotenoids, including lycopene, has received much attention in recent years in the prevention of diseases (Halliwell *et al.*, 1995; Sies & Stahl, 1995; Frei, 1994; Block, 1992; Steinmentz & Potter, 1996). Lycopene as an antioxidant, make it one of the most important carotenoids for human health. Lycopene can be obtained from a number of fruits like fresh tomato and tomato products, pink grapefruit, watermelon and guava (Holden *et al.*, 1999). When absorbed in the stomach, lycopene is transported in the blood by various lipoproteins and accumulates in the liver, adrenal glands and testes. Lycopene can also be incorporated into lipid micelles in the small intestine. These micelles are formed from dietary fats and bile acids, and help to make lycopene more soluble and enable it to permeate into the intestinal mucosal cells by a passive transport mechanism. Little is known about the liver metabolism of lycopene, but like other carotenoids, lycopene is incorporated into chylomicrons and released into the lymphatic system. In blood plasma, lycopene is eventually distributed into the very low and low density lipoprotein fractions (Stahl & Sies, 1996).

2.7.3.2.1 Method of action in the human body

Intake of lycopene has been shown to be correlated to a decreased risk of chronic diseases, like cancer and cardiovascular diseases (Rao & Agarwal, 2000), mostly caused by oxidative stress induced by reactive oxygen species (ROS).

Metabolic activities inside the human body generate reactive oxygen species (ROS). These ROS are highly reactive oxidant molecules and include superoxide, hydrogen peroxide, and hydroxyl radicals, which can cause damage to cellular constituents like lipids, proteins and DNA. Many forms of cancer are thought to be the result of reactions between free radicals and DNA, resulting in mutations that can adversely affect the cell cycle and potentially lead to malignancy that is a normal response of the human body to infection and damages to cell walls and DNA alterations (Princemail, 1995; Ames *et al.*, 1995;

Witztum, 1994; Halliwell, 1994). Lycopene, as a natural anti-oxidant in the human diet, can for example quench free radicals and reactive oxygen species, significantly delay or prevent oxidative damage by free radicals (Conn *et al.*, 1991; Di Mascio *et al.*, 1989; Rice-Evans *et al.*, 1997) and lower the risk of heart diseases (Kohlmeier *et al.*, 1997; Rao, 2002) and cancers (Canene-Adams *et al.*, 2005; Dorgan *et al.*, 1998; Giovannucci *et al.*, 1995).

In humans, the two mechanisms in which lycopene can be effectual is derived from the type of disease it prevents and can either be anti-carcinogenic or anti-atherogenic. These are also known as non-oxidative and oxidative mechanisms.

Lycopene is hypothesized to suppress carcinogen-induced phosphorylation of regulatory proteins such as tumor protein 53 (P₅₃) and retinoblastoma proetin (Rb) anti-oncogenes and stop cell division at the G₀-G₁ cell cycle phase, which then inhibits cancerous growth (Matsushima *et al.*, 1995). Astorg *et al.* (1997) proposed that lycopene-induced modulation of the liver metabolizing enzyme, cytochrome P450 2E1 (involved in the metabolism of xenobiotics) was the underlying mechanism of protection against carcinogen-induced pre-neoplastic lesions in rat liver.

Patients with prostate cancer were found to have low levels of lycopene and high levels of oxidation of serum lipids and proteins (Rao *et al.*, 1998). Preliminary *in vitro* evidence indicates that lycopene reduces cellular proliferation induced by insulin-like growth factors, which is potent mitogens, in various cancer cell lines (Levy *et al.*, 1995). Lycopene regulated intrathymic T-cell differentiation (immunomodulation) and is suggested to be the mechanism for suppression of mammary tumor growth (Nagasawa *et al.*, 1995; Kobayashi *et al.*, 1996).

In humans, lycopene or tomato-free diets resulted in loss of lycopene from blood serum causing an increase in lipid oxidation (Rao, 2002). Dietary supplementation of lycopene for 1 week increased serum lycopene levels and reduced endogenous levels of oxidation of lipids, proteins, lipoproteins and DNA (Agarwal & Rao, 1998), therefore decreasing the chance of coronary heart

disease. Lycopene has also been shown to act as a hypo-cholesterolemic agent by inhibiting HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, thereby limiting cholesterol synthesis and enhances cholesterol degradation (Fuhramn *et al.*, 1997; Arab *et al.*, 2000).

2.8 HYDROPONICS

In 1929, William Frederick Gericke termed early soilless agricultural gardening “aquaculture”, but later found that aquaculture was already applied to culture of aquatic organisms. In 1937 he then decided that the term aquaculture should be termed hydroponics. The word “Hydroponics” is derived from two Greek words, hydro- meaning water and ponos- meaning labour. Hydroponics is a method of cultivating plants without soil, using an inert support medium. Nutrients are supplied to the plants via a nutrient medium containing the correct concentration of nutrients.

Hydroponics has many advantages which include the following:

- Much higher crop yields can be obtained because hydroponics can be used in arid areas where ordinary agriculture is impossible.
- Crops produced hydroponically tend to grow faster combined with the absence of soil diseases, resulting in consistent crops, quality crops and improved yields.
- Reduction in growing area is a great advantage.
- Weed contaminants are eliminated and does not influence yield quality.
- Hydroponics is less labour effective, costs less and has minimal manual labour except harvesting of the crop. Most plants can be grown hydroponically out of normal growing season which guarantees continues yield.
- Nutrients are controlled more accurately and seldom become limiting which results in higher yields and better quality crops.

- The avoidance or elimination of pesticides is of utmost importance in growing healthy strong plants and minimizes soil contamination with pesticides.
- Plants are under continuous observation and diseases and deficiencies are detected early on before major plant damage and crop loss occurs.
- Hydroponic setups are environmentally friendly because no unnecessary nutrients are applied to the soil that can pollute the environment. In an environmentally conscious world, hydroponics is the way to go.

Hydroponics is a cultivation technique to maximize crop yield. Obtaining economic yields of high quality and quantity of tomatoes while minimizing the use of pesticides and other agri-chemicals has put hydroponics on the frontline of tomato production. Hydroponics provides optimum conditions for cultivation of tomatoes and has great potential for alleviation of environmental related problems (Howard, 2002). A review of the global commercial hydroponic industry revealed that the commercial hydroponics industry has grown four to five fold in recent years.

“Hydroponic gardening is the way of the future for environmentally controlled agriculture. By carefully controlling nutrient levels, light intensity and temperature, phenomenal yields (quantity and quality) can be achieved in relatively small space” (Internet 1). Moraru *et al.* (2003) has grown 10 different tomato cultivars for NASA Advanced Life Support Program in order to determine the best cultivar for outer space usage.

Hydroponic technology allows for growing crops where crops has never been grown before, whether it is underground, above ground, in space or under the oceans. Hydroponic technology will allow humanity to live where humanity chooses. If used for our own survival or our colonization, hydroponics is and will be a major part of the future (Winterborne, 2005).

2.8.1 Hydroponic systems

There are six basic hydroponic systems. These include the water culture technique, wick system, ebb and flow (flood and drain) system, nutrient film technique (NFT), drip system and aeroponics. The latter being the latest development. The most common and widely used hydroponic system used is the drip system (Jensen, 1999).

Since a re-circulating ebb and flow (flood and drain) system was used in this study for certain reasons, it will be discussed in more detail. An ebb and flow system is a fairly simple and reliable hydroponic setup. This setup consists of containers filled with an inert support media, which acts as a support material and act as a temporary reservoir for water and nutrients for the roots. In an ebb and flow setup the nutrient solution temporarily floods the containers to predetermined levels and cycles before it drains back to the nutrient reservoir. The roots are thus temporarily flooded with nutrient solution where after it is aerated again. Depending on the water hold capacity of the support media, some may require flooding of the trays more frequently than others.

Ebb and Flow systems have advantages, which includes the following: plants are rarely subjected to dry conditions. Depending on the design of the setup, some trays may have a small reservoir of nutrient solution left on the bottom of the tray after flooding the tray. This can act as a reserve of nutrients and water during cycles. The ebb and flow setups are cost effective, re-circulating and fairly easy to manage with low maintenance and labour.

Disadvantages include the following: root pathogens can spread easily, as all the roots share the same space and nutrient source. Excessive non-drained water may cause root rot.

2.8.2 Support media

One characteristic support media must have in common is that it must be inert and not supply the plant with any nutrients. It must be kept in mind that some media may work for some crops, while others may work better for other crops (Romer, 1997).

There are various support media which can be used. These media include rockwool, coconut fiber, perlite, vermiculite, sand, gravel or inert silica sand and many other synthetic and natural media. One of the most obvious decisions to take into account is which medium is the most applicable for a crop. Different media are appropriate for different crops.

Rockwool (mineral wool) is the most widely used medium in hydroponics. Rockwool is an inert substrate for both re-circulating and non-circulating systems. Rockwool is produced from molten mineral compounds (molten rock) which is aerated at a temperature of about 1600 °C, resulting in a light, sterilized and fibrous medium. Rockwool is not degraded by microbiological activity. Rockwool can hold large quantities of water and air that aids root growth and nutrient uptake and its fibrous nature provides a good mechanical structure to support the plant. Rockwool has a neutral pH (Internet 7).

Perlite is a volcanic rock that has been heated to 850 – 900 °C, which then expands into lightweight pebbles (± 1 mm). Perlite is a fusion of granite, obsidian, pumice and basalt. The expanded material is a brilliant white, very light substance (30 – 150 kg.m⁻³). Due to its low density and relatively low price, it is ideal for hydroponics. Perlite has similar properties to vermiculite, but generally holds more air and less water. Perlite has a neutral pH (Internet 7).

Like perlite, vermiculite is an inorganic mineral that has been heated to 1100 °C until it expands into light flakes. The expansion process is called exfoliation and it is routinely accomplished in purpose-designed commercial furnaces. Vermiculite is formed by hydration of certain basaltic minerals. Vermiculite is also very light

and is often used in hydroponics as support media. Vermiculite has a neutral pH of 7 and has a very good ability to regulate temperature around the root zone. However, vermiculite is not always available and may be expensive (Internet 7).

Sand (river or sea sand) or silica sand is cheap and easily available. However, it is heavy, thus impractical in some setups and it does not always drain well due to very small grains. Sand must often be sterilized by baking the sand in an oven at 200 °C between seasons or between handlings. Aeration may also pose a threat and cause root rot due to high water retention abilities. Sand is slightly acidic (pH < 7). Depending on the colour of the grains, grey or white, silica sand regulate temperature fairly good (Internet 7).

Gravel is a much more coarse support medium than sand, which makes it a very effective support medium for hydroponics. Gravel is inexpensive, easily kept clean and drains well. However, gravel is heavy due to its large (< 2 mm) grain size and some systems will be unpractical when gravel is used as support medium. A disadvantage of gravel is that if the system doesn't regularly receive water, the gravel dries out quickly. Gravel can be grouped into two different gravel types depending on the origin of the gravel namely silica gravel and crushed rock. Silica gravel comes from a silica rock (quartz) which is crushed into the desired particle size. After it has been crushed it undergoes a series of washes to clean it. This give the silica gravel it distinctive white appearance. This white appearance may influence temperature in the root zone. Crushed rock is limestone or dolomite which is crushed to a predetermined particle size. The crushed rock has a grey appearance which may cause the root zone to heat to levels above the desired temperatures. Both silica and crushed rock gravel is inert and has a neutral pH with relatively low water retention ability, but provides excellent aeration for roots (Internet 7).

Coconut fiber, also known as peat, coir or coco, is the fibers of a coconut husk which have been removed from the bolster (outermost shell) of the coconut. Raw coconuts are washed, heat-treated, screened and graded before being processed into coco peat products of various granularity and densities. Coconut

fiber is an organic medium which generally has an acidity in the range of pH - 5.5 to 6.5 and has a high water retention capacity (Internet 8).

2.8.3 Electrical Conductivity (EC)

The electrical conductivity (EC) of a nutrient solution change continuously. These changes in EC are a result of absorption or accumulation of dissolved salts within the medium. Therefore, the EC indicates that elements may become limiting or accumulate within the media. Electrical conductivity is measured as milliSiemens per centimeter (mS.cm^{-1}) or microSiemens per centimeter ($\mu\text{S.cm}^{-1}$) depending on the concentration of dissolved salts. The average EC required for crops is approximately 2.1 mS.cm^{-1} (Romer, 1997) to 2.6 mS.cm^{-1} (du Plessis, 2001). Measuring EC is a valuable tool in hydroponics to ensure a high yield and fruits of good quality and taste (Dorai *et al.*, 2001).

By adjusting and monitoring the EC of the nutrient solution at an optimal level, fruit quality can be maintained. At some point a high EC may limit yield. The concentration of nutrients available to plants influences fruit size and the dry matter content. Changes in size and dry matter content increases linearly with the EC of the nutrient solution. The exact rate of yield decline or incline varies between cultivars, environmental factors and nutrient solution composition. Electrical conductivities which are low (less than 2 mS.cm^{-1}) may result in a low yield, whereas an EC of $3.5 - 9.0 \text{ mS.cm}^{-1}$ may improve fruit quality (Dorai *et al.*, 2001).

2.8.4 pH

pH is used to measure the degree of acidity or alkalinity in a solution. Excessive H^+ ions in a solution will result in an acidic solution, whereas a solution with excess hydroxyl ions (OH^-) will be alkaline. A solution with a pH of 7 contains an equal number of hydrogen and hydroxyl ions, and is termed a neutral solution.

Solutions with a pH less than 7 are more acidic and solutions with a pH more than 7 are basic. The abbreviation, pH, stands for “Positive hydrogen”

When pH levels for a nutrient solution is not optimal, some elements may become limiting (du Plessis, 2001). This happens because the pH of the nutrient solution affects the availability of many nutrients. By increasing the pH (more alkaline) of the nutrient solution, the availability of phosphorous, boron, copper, iron, manganese and zinc decreases, whereas decreasing the pH (more acidic) the availability of molybdenum is affected. Changes in the absorption rates of ammonium and nitrates are strongly correlated with pH levels. If the pH influences the availability of these elements, then pH indirectly influences fruit yield and quality. pH is thus another important parameter to monitor the quality of the nutrient solution.

2.8.5 Hydroponics and Tomatoes

Tomatoes are produced on an enormous scale throughout the world and are estimated to be 40 million tons (Internet 1) of which 20 million tons are produced hydroponically per annum (Stern *et al.*, 2003). A large number of tomatoes are consumed fresh, used for tomato paste and other tomato containing products (Stern *et al.*, 2003). Sutherland & Sutherland (1987) reported that tomatoes are an excellent hydroponic crop and is cultivated with great ease and satisfaction.

2.8.5.1 Hydroponic requirements

Tomatoes are a summer crop, which requires both warm and cool temperatures. Plants should not be subjected to frost or very high humidity. Light affects the development of fruit colour and pigmentation (Herrmann, 1976; Lopez-Andreu *et al.*, 1986; Sahlin *et al.*, 2004). Growing tomatoes in greenhouses requires the regulation of environmental factors like light, temperature, humidity, CO₂ levels and pests. By making use of hydroponics, water and nutrients can be regulated

to almost perfection. Because nutrients play such an important role, a much more detailed discussion will be given.

2.8.5.2 Climatic requirements

Because tomatoes are a summer crop, tomatoes have a specific climatic requirement which should be controlled optimally for tomato production. Tomatoes grown in hydroponics are normally grown in a greenhouse where the environmental factors can be specifically manipulated and controlled for optimal production. These factors include temperature, relative air humidity and the light intensity.

2.8.5.2.1 *Temperature*

Tomatoes have an optimum temperature requirement of 21°C to 24°C during the day and 12°C to 18°C at night (Niederwieser, 2001). An exhaust fan can help to regulate air temperature in a greenhouse and also provide fresh air. Fruit set and development is markedly affected by temperature. Non-optimal temperatures may cause premature bud and flower abortion, limit inflorescence size, leaf growth, pollen germination and overall vegetative growth and fruit formation (Rudich *et al.*, 1977). Davies and Hobson (1981) have found that temperatures below 16°C and above 30°C inhibit lycopene production, while temperatures between 16°C and 21°C is optimal for lycopene synthesis.

2.8.5.2.2 *Relative Humidity*

The optimum relative humidity (RH) for tomato production ranges between 60 – 70 % (Buitelaar, 1983). However, relative humidity is difficult to control, and may vary in a greenhouse, depending on the season, sunny or rainy days, type of greenhouse construction and its associated ventilation. Yield is also indirectly affected by relative humidity, since relative humidity influences pollination

(Rudich *et al.*, 1977). Relative humidity influences the ability of certain pathogens to colonize the greenhouse and cause diseases (Buitelaar, 1983).

2.8.5.2.3 *Light Intensity*

Another environmental parameter, light intensity is difficult to control. Low light may be the single most important factor in limiting yield in greenhouses. This is due to the abortion of fruit trusses and lack of fruit set during low light periods. Low light may cause a lack of pollination and result in smaller fruit size. Artificial light sources can be used to control day length, as well as light quality and intensity.

In times of excessive light, shade cloth can be used to prevent excessive light which may induce damage like photo-oxidation. When tomatoes are grown in a greenhouse, ultra violet (UV) wavelengths are filtered by the green or plastic surfaces. Greenhouse cultivated tomatoes are thus less exposed to UV radiation than field grown tomatoes (Stewart *et al.*, 2000). McCollum (1954) also found that tomato fruits exposed to direct sunlight during development had a higher lycopene content than fruit which was exposed to lower light intensities during development and maturation. Similar results were demonstrated more recently by Sahlin *et al.* (2004), Bramley (2000) as well as Levy and Sharoni (2004).

2.8.5.3 Nutrient media and related parameters

Nutrition is the most important aspect of hydroponics. Modern tomato cultivars have the ability to produce very high yields. A huge yield requires the correct concentration of nutrients. There is no ideal or optimal nutrient formulation for hydroponics as each crop requirements differ. When nutrient concentrations are low, plant growth is reduced compared to when nutrients are available in a high concentration. The minimum concentration for a specific element is referred to as the critical concentration of that element (Salisbury & Ross, 1991). Elements in high demand become limiting first and need to be replaced more frequently than

other elements. When elements are not replaced periodically, elements become limiting resulting in nutrient deficiencies in the plant (Sutherland & Sutherland, 1987). Table 1 indicates the average concentration (ppm – parts per million) of each element required for most hydroponic set-ups (Sonneveld, 1995).

Table 2.1 Elements needed in a typical hydroponic nutrient solution. Macro- and micro- mineral elements concentrations are based on the concentrations needed.

Macro-elements	ppm
N	234
P	54
K	370
Mg	58
Ca	216
S	139
Micro-elements	ppm
Fe	0.8
Mn	0.55
Zn	0.3
B	0.3
Cu	0.05
Mo	0.05
Cl	0.02

Since nutrient minerals are an integral part of plant nutrition and hydroponics, it will be addressed in more detail in 2.9 (Essential Elements).

2.8.5.4 Foliar fertilization

It has been known that plants can absorb essential elements through their foliage (Swietlik & Faust, 1984; Gooding & Davies, 1992; Bondada *et al.*, 2001; Johnson *et al.*, 2001). Foliar feeding is a very useful technique to supply the plant with additional nutrients and correcting nutrient deficiencies. The mobility of a nutrient is determined by the ability of the nutrient to be transported in the plant. Tomatoes grown hydroponically are already under optimal nutrient conditions but have been shown to have an improved yield and fruit quality when sprayed with a micro-nutrient complex on a weekly basis (El-Naggar & Awad, 1986).

2.9 ESSENTIAL ELEMENTS

An element is essential, firstly, if the plant cannot complete its life cycle in the absence of that element. Secondly, an element is essential if it forms part of any molecule or constituent of the plant that is itself essential to the plant (e.g. nitrogen in proteins and magnesium in chlorophyll; Epstein, 1972).

Seventeen such chemical elements are known to be important for plant growth and development. These elements are divided into non-mineral elements and mineral elements. The mineral elements are further divided into two groups namely macro-elements (macro-nutrients) and micro-elements (micro-nutrients).

2.9.1 Non-mineral elements

Non-mineral elements include oxygen (O), available in the form of O₂, H₂O and CO₂, hydrogen (H), available in the form of H₂O and carbon (C), available as carbon dioxide (CO₂). Oxygen, hydrogen and carbon accounts for more than 96% of a plants dry weight (Salisbury & Ross, 1991).

2.9.2 Mineral elements

In addition to the non-mineral elements (oxygen, hydrogen and carbon) plants require a great variety of other elements in various chemical forms. The mineral elements are usually divided into two groups namely macro-elements (macro-nutrients) and micro-elements (micro-nutrients) based on the quantities required by the plants. These elements are absorbed from the soil as nutrient ions. However, absorption of these elements is influenced by the availability of the particular element, soil pH, and the concentration of the element available (Atherton & Rudich, 1986). Ions are usually associated with chelating agents which helps with the availability of the particular ion because ions are easily precipitated by other nutrients.

2.9.2.1 Macro-nutrients, their related roles and deficiency symptoms

Macro-nutrients are nutrients required in larger concentrations by the plants. These elements become deficient whenever the specific element cannot be absorbed in the required concentration due to factors limiting absorption. Plants thus require a continues supply of these elements to sustain growth and development. It should be emphasized that many of the symptoms accounted with a deficiency are differently expressed in different plants, and the concentration of the element resulting in a deficiency may be different in different species.

Table 2.2 The available forms, role and estimated critical concentrations of macro-elements and deficiency symptoms in tomatoes.

Element	Symbol	Available form	Functions in plants	Deficiency symptom(s)	Optimum range ppm
Nitrogen	N	NO_3^- ; NH_4^+	Part of living cells, proteins, chlorophyll, metabolic processes.	Stunted growth, chlorosis, and small. Plants with low yield. Deficiency if less than 200 ppm (see 2.9.2.1.1)	234
Phosphorus	P	H_2PO_4^- ; HPO_4^{2-}	Synthesis of oils, sugars, starches. Root growth, flowering and photosynthesis.	Similar to nitrogen deficiency. Dark green leaves, delays maturity. Deficient below 45 ppm	54
Potassium	K	K^+	Protein synthesis, photosynthesis, disease resistance.	Chlorotic, necrotic, curly leaves, premature fruit drop, stunted growth. Deficient below 70 ppm	370
Calcium	Ca	Ca_2^+	Cell wall structure, absorption of other elements.	Slight chlorosis, scorching of leaves, blossom end rot. Deficient below 150 ppm	216
Magnesium	Mg	Mg_2^+	Part of chlorophyll. Photosynthesis, enzyme activator.	Interveneal chlorosis, necrosis slow growth. Deficient below 20 ppm	58
Sulfur	S	SO_4^{2-}	Synthesis of proteins, chlorophyll and vitamins. Cold tolerance.	Like nitrogen deficiency, woody and elongated stems. Deficient below 70 ppm	139

(Atherton & Rudich, 1986).

The macro-nutrients accounts for approximately 4% of a plants dry weight (Salisbury & Ross, 1991). Macro-nutrients are needed in larger quantities (100mg.kg^{-1} dry matter) than micro-nutrients (Trace elements; 1mg.kg^{-1} dry matter). Nitrogen (N), Phosphorus (P) and Potassium (K) are the most important macro-nutrients and are needed in the largest amounts. These elements are often limited or deficient due to a high uptake by plants (Salisbury & Ross, 1991). The remaining macro-nutrients include calcium (Ca), magnesium (Mg) and sulfur (S). These elements are needed in lower quantities than nitrogen, phosphorus and potassium. Because nitrogen is needed in such high concentrations, it frequently becomes limiting.

2.9.2.1.1 Nitrogen deficiencies

Nitrogen deficiency is the most common type of deficiency due to its high requirement by plants. Nitrogen deficiency is first observed in older leaves because nitrogen is mobile in plants and moves with the transpiration stream (Kirkby & Mengel, 1967).

Three visual deficiencies can be detected in plants. i) The synthesis of chlorophyll decreases and the plants become chlorotic. ii) Retarded or stunted growth can occur in new shoots and especially lateral shoots. Because nitrogen is important for cell division, deficient nitrogen levels will limit and retard growth. iii) When nitrogen is needed in younger leaves, available nitrogen will be translocated from older leaves to younger leaves. When this nitrogen requirement is not replenished, the older leaves become chlorotic. Stems of leaves become shorter and slender and in severe nitrogen deficiency, leaves can become necrotic and fall off.

Nitrogen is part of all amino acids, proteins, coenzymes, chlorophyll, RNA and DNA and when nitrogen is deficient, it can influence all processes involving these metabolites (Devlin, 1969).

Nitrogen deficiency rarely occurs under hydroponic conditions but is frequently observed in field trials. To reduce nitrogen deficiency, additional nitrogen must be added to soils. This however is not always a solution because the nitrogen can again become limiting due to factors influencing nitrogen uptake.

2.9.2.2 Micro-nutrients, their related roles and deficiency symptoms

Micro-nutrients are needed in only very small concentrations (1mg.kg^{-1} dry matter), but are still essential for plant growth and development (Salisbury & Ross, 1991). These nutrients include copper (Cu), Boron (B), Iron (Fe), Manganese (Mn), chloride (Cl), zinc (Zn) and molybdenum (Mo) and recently Nickel (Ni) (Brown *et al.*, 1987).

The micro-nutrients usually serve in catalytic processes in plants. Although micro-nutrients are widely distributed in soils, certain micro-nutrients may be absent due to the absence of base the rock. In addition to this, soil pH, other solutes and soil oxygen levels affect the solubility of the elements or the plant's ability to absorb these micro-nutrients.

Table 2.3 Available forms, role and estimated critical concentrations of micro elements and deficiency symptoms in tomatoes.

Element	Symbol	Available form	Functions in plants	Deficiency symptom(s)	Optimum range. ppm
Copper	Cu	$\text{Cu}^+ ; \text{Cu}^{2+}$	Reproductive growth, root metabolism, protein utilization.	Leaf wilt, chlorosis, necrosis, fruit split. Rare. Deficient below 0.02 ppm	0.05
Boron	B	H_3BO_3	Uptake of other elements, partial production of sugars.	Slight chlorosis, reduced growth, thick leaves. Fruit crack. Deficient below 0.2 ppm	0.3
Iron	Fe	$\text{Fe}^{3+} ; \text{Fe}^{2+}$	Formation of chlorophyll, frequently underestimated.	Chlorotic to white, small leaves. Deficient below 0.3 ppm	0.8
Manganese	Mn	Mn^{2+}	Breakdown of sugars, nitrogen metabolism, enzyme catalyst.	Immature chlorotic leaves, poor flowering, premature leaf drop. Deficient below 0.28 ppm	0.55
Chloride	Cl	Cl^-	Photosynthesis, specific oxidation reactions (PSII).	None known. Possible lower photosynthetic rate. Low yield.	0.02
Zinc	Zn	Zn^{2+}	Sugar transformation and breakdown, enzyme catalyst.	Chlorotic molting, short internodes, small leaves. Deficiency occurs below 0.18 ppm	0.3
Molybdenum	Mo	MoO_4^{2-}	Nitrogen metabolism, breakdown of purines.	Cupped, thick leaves, necrosis, rare. Deficient below 0.03 ppm	0.05
Nickel	Ni	Ni_2^+	Essential catalyst for Urease.	Inability to metabolize urea. Possible chlorosis and stunted growth.	< 0.01

(Atherton & Rudich, 1986).

2.9.3 Toxicity symptoms

Once nutrient deficiencies have been identified, nutrient deficiencies can easily be corrected by supplying the plant with additional nutrients. However, caution must be taken not to over supply plants with nutrients. Overdose or nutrient toxicities may permanently damage the plant without correction. Nutrient toxicities are seldom found in soils due to correct natural buffering of soil nutrients by microbes, pH management, leaching of nutrients and temperature effects (Roorda van Eysinga & Smilde, 1981).

In hydroponics, toxicity rarely occurs because of a regulated nutrient supply. When such toxicity occurs, it can easily be corrected by replacing or modifying the nutrient solution. Because nitrogen is such an important macronutrient, it is the most likely element to become toxic. Toxicity symptoms of nitrogen can be detrimental to the plant by producing lush looking plants with few blossoms and a poor fruit set. Very excessive concentrations of nitrogen can cause generalized or complete necrosis, poorly coloured tomato fruit with a puffy appearance due to limited pollination. Fruit may also split as they ripen (Roorda van Eysinga & Smilde, 1981; Salisbury & Ross, 1991).

2.9.4 Physiological Disorders

There are many symptoms in tomatoes which are not caused by nutrient deficiencies, but rather by environmental factors. These disorders are termed physiological disorders and are mainly caused by abiotic factors. Only the most common physiological disorders in tomatoes will be briefly listed.

Table 2.4 Abiotic induced physiological disorders in tomato fruit, visual effects and possible causes of the related symptoms.

Symptom	Visual effects	Probable cause
Concentric cracking	Cracked fruit from calyx to bottom.	Varying water supply, high growth rates and temperatures.
Splitting	Skin of fruit crack, causing markings on fruit. Complete fruit splitting when ripe.	Varying temperatures, excessive nitrogen.
Catfacing	Scarring, malformation, cracking of fruit, holes in fruit.	Extreme variation in temperatures during fruit set.
Zipper Scar	Vertical scar on the side of the fruit.	Anther, genetic causes.
Blotchy ripening	Blotchy, brown markings on fruit, uneven ripening.	Various environmental factors (temperature, water etc.).
Blossom end rot	Brown or black sunken area at the blossom end of the fruit.	Insufficient supply of calcium.
Puffiness	Angular appearance of fruit. Absence of seed and suspension gel in locules causing a smaller fruit.	Any factor influencing pollination.

(Atherton & Rudich, 1986).

2.10 NITROGEN

The importance of nitrogen to plants is emphasized by the fact that only carbon (C), oxygen (O) and hydrogen (H) are more abundant in them. Because nitrogen plays such an important role in plants, and is needed in relative large quantities, nitrogen may frequently become limiting. Many factors can influence the availability and uptake of nitrogen from soils. These include low soil pH, the type and availability of nitrogen ions, presence of other macro nutrients like potassium (K), temperature and the presence of nitrogen reducing microbes. Tomato plants need approximately $170 \text{ mg nitrogen.plant}^{-1}.\text{day}^{-1}$ during the flowering stage (Adams & Winsor, 1979). Whenever this concentration is not available, nitrogen becomes limiting and may cause reduced yield and fruit quality. The role of nitrogen in plants is of utmost importance in maintaining an optimum metabolism controlling critical pathways in plants.

2.10.1 The role of nitrogen in the plant

Whenever nitrogen becomes limiting, the metabolic activities in the plant which depends on nitrogen cannot commence fully. Nitrogen is important in overall metabolism and growth, which ultimately determines yield.

Nitrogen is a component of all plant proteins which is responsible for plant metabolism. Additionally, nitrogen is found in purines, pyrimidines and coenzymes. Purines and pyrimidines are found in nucleic acids (RNA and DNA) which are essential for protein synthesis. Nitrogen is also part of the porphorin structure found in chlorophyll and cytochrome enzymes which are very important metabolic compounds involved in photosynthesis and respiration. Coenzymes are essential for the function of many enzymes. Nitrogen is thus a key factor in vegetative growth and chlorophyll formation. Nitrogen is also an intrinsic part of many vitamins (Devlin, 1969).

2.10.1.1 Enzymes, proteins and amino acids.

Nitrogen is an intrinsic part of all enzymes, proteins and amino acids (Salisbury & Ross, 1991). The rate and type of metabolic pathways in cells are controlled by enzymes. Enzymes are very specific of the reactions they catalyze. Nearly all enzymes have a protein as a major part of the structure and many enzymes are made up of only protein.

Proteins are polypeptide chains, each made up of hundreds of amino acids. The type and size of a protein is determined by the number of amino acids added together (peptide bonds). There are twenty different kinds of amino acids known. Amino acids are the building blocks of proteins and some proteins have a full complement of twenty amino acids. Amino acid chains are held together by non-covalent bonds, ionic bonds and hydrogen bonds. The amino acid building blocks in proteins are represented as:

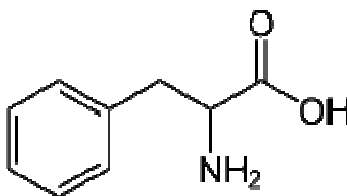


Figure 2.4 Amino acid. R indicates a remainder of the molecule. This is different for each amino acid. The -NH_2 is the amino group and the -COOH is the carboxyl group (Redrawn from Salisbury & Ross, 1991).

The -R groups adds to the overall properties of amino acids, determining for example, its water solubility, and polarity. Amino acids can either be bound to each other in enzymes and proteins but it may also occur in minor concentrations of unbound molecules (Salo-Väänänen & Koivistoinen, 1996).

Amides are amino acids but the –R group is connected to the carbonyl carbon. Amides are formed from glutamic and aspartic acids. Amides are structural parts of many proteins and are important in the transport of nitrogen from one part of the plant to another and acts as a reservoir in which excess nitrogen can be stored (Salisbury & Ross, 1991).

The nitrogen content in proteins is approximately 16% (Rubner, 1885; Atwater & Bryant, 1899; Jones, 1931; Browne, 1944). Jones (1931) proposed that by measuring protein content, the concentration of nitrogen can be determined by dividing the concentration of protein measured by 6.25 (or multiply by 16%). Salo-Väänänen & Koivistoinen (1996), however, found that by using the conversion factor of 6.25 (16 %) overestimates the true protein content of biological materials. This is because the protein composition in biological material differ in terms of amino acids compositions. The percentages of nitrogen of all 20 amino acids are given in the table below as well as the basic amino acid (α amino acid).

Table 2.5 Nitrogen content of different amino acids.

Amino Acid	Abbreviation	% Nitrogen as part of amino acid structure
Alanine	Ala	15.72
Arginine	Arg	32.16
Asparagine	Asn	21.20
Asparagine	Asp	10.52
Cysteine	Cys	11.56
Glutamate	Glu	9.52
Glutamine	Gln	19.17
Glycine	Gly	18.65
Histidine	His	27.08
Isoleucine	Ile	10.68
Leucine	Leu	10.68
Lysine	Lys	19.16
Methionine	Met	9.39
Phenylalanine	Phe	8.48
Proline	Pro	12.17
Serine	Ser	13.33
Threonine	Thr	11.76
Tryptophan	Trp	13.72
Tyrosine	Tyr	7.73
Valine	Val	12.01
Basic amino acid (α amino acid)		18.66
Average nitrogen content of amino acids		14.92

Taking the percentage nitrogen present in all the amino acids gives an average value of 14.92 %. It must be kept in mind that not all nitrogen present in plant material is present in amino acids, but may be in ionic forms such as nitrates,

nitrites, ammonia, ammonium and molecules like atmospheric nitrogen, chlorophyll, nucleic acids and other cell constituents. This nitrogen also adds to the total concentration of nitrogen present within the plant material. Thus, when protein concentrations are known, the nitrogen content can be estimated as 16% of the protein content.

2.10.2 Sources of nitrogen

Nitrogen can be taken up through plant roots from the soil in highly oxidized forms but must be reduced by energy dependant processes before it can be metabolized and incorporated into cellular constituents. Nitrogen can alternatively be obtained from symbiotic relationships with soil bacteria. The fixation of atmospheric nitrogen (N_2) by microbes yields nitrate (NO_3^-) and ammonium (NH_4^+). Many factors can influence the symbiotic relationship between plants and soil bacteria (James *et al.*, 2002), thus also affecting the concentration of available nitrogen.

Plants can utilize any form of nitrogen ions, ammonium and nitrate, respectively. If both of these ions are available, plants will metabolize any one (Salisbury & Ross, 1991) although nitrate (NO_3^-) are metabolized more commonly. In agriculture, nitrogen sources include rainwater (source of NO_3^-), alfalfa meal, blood meal, compost, feather meal, fish meal, animal manure, plant residues and fertilizers.

2.10.3 Nitrogen Metabolism

Once absorbed, nitrates (NO_3^-) are reduced to nitrites (NO_2^-) via nitrate reductase and is further reduced to ammonium (NH_4^+) by nitrite reductase. The ammonium (NH_4^+) formed is utilized by plants by combining ammonium into organic forms via glutamate. Glutamate synthetase and glutamate synthase is the most important enzymes converting ammonium and glutamate into the amino acid

glutamine. Originally, glutamate dehydrogenase (GDH) was thought to be the enzyme of ammonia assimilation in plants. However, an alternative pathway of ammonia assimilation, involving glutamine synthetase (GS) and an NADPH-dependent glutamine-2-oxoglutarate amidotransferase (GOGAT) also referred to as glutamine synthase was shown to be operating when ammonia was present (Tempest *et al.*, 1970). This is the main route by which ammonia enters plant metabolism. The NH_2 group is then used in the formation of other carbon skeletons to produce other amino acids via aminotransferases.

Amino acids may also be synthesized by reverse transamination of NH_2 from glutamate to other carbon skeletons. Aminotransferase can transfer NH_2 groups from amino acids onto α -keto acids like oxaloacetate. Oxaloacetate is a Krebs cycle intermediate and in conjunction with aminotransferase is used to synthesize amino acids. Other metabolic intermediates formed include -ketoglutarate, phosphoenol pyruvate, erythrose, ribose-5-phosphate, 3-phosphoglycerate and pyruvate. Essential amino acids that can be formed from the above mentioned intermediates include glutamine, glutamate, proline, arginine, asparagine, aspartate, methionine, threonine, lysine, isoleucine, tryptophan, phenylalanine, tyrosine, histidine, serine, glycine, cysteine, alanine, valine and leucine (Vance & Griffith, 1990).

2.10.4 Mobility of nitrogen in the plant

The mobility of an element is determined partially by the solubility of the element and how well it can enter various tissues when transported (e.g. phloem sieve tubes). If an element can be loaded into translocating phloem cells the element will be mobile within the plant. The ability of an element to be mobile in a plant and the rate at which the element is mobile determines the time in which the element is metabolized. Thus, when an element is only partially mobile or very slow moving in the plant the deficiency of the element in the tissue where it is needed may cause irreversible damage even if element levels are optimal.

Elements that have been classified as mobile include potassium, phosphorus, chlorine and nitrogen. Partially mobile elements include zinc, copper, manganese, molybdenum and magnesium. Immobile or very slowly mobile elements include boron, calcium, sulfur and iron (Salisbury & Ross, 1991).

Nitrogen is absorbed from the soil in a highly oxidized form and must be reduced by energy-dependant processes before it can be incorporated into proteins and other cellular constituents (Salisbury & Ross, 1991). Nitrogen move readily from older to younger leaves, then to storage organs (Salisbury & Ross, 1991; Ireland, 1990). This is why nitrogen deficiency is first observed in older leaves, due to a high demand for nitrogen in younger leaves (see nitrogen deficiencies, 2.9.2.1.1). Another very effective way of translocating nitrogen in the plant is by means of amino acid transport (Okumoto *et al.*, 2004). Once nitrogen has been incorporated into amino acids in the cytosol, these amino acids can be freely transported in the plant, for example to where new growth commences like in new shoots or leaves. Nitrogen no longer needed in older tissue will be transported via the transpiration stream (Kirkby & Mengel, 1967; Palta *et al.*, 1991) to new growth areas and older leaves become nitrogen deficient first. To prevent nitrogen deficiency, plants need optimal concentrations of nitrogen which is approximately $170 \text{ mg nitrogen} \cdot \text{plant}^{-1} \cdot \text{day}^{-1}$ for tomatoes during the flowering stage (Adams & Winsor, 1979).

2.10.5 Nitrogen in nutrient salts and fertilizers used in hydroponics

Plants need the correct concentration and ratio of nutrients to grow. Fertilizers applied to the soil may be less effective than that applied in hydroponics. Only a small concentration of fertilizer is taken up by a crop and the rest either remains in the soil, leaches out, is physical washed off, fixed by soil bacteria or can be released into the atmosphere by microbial activity (Hera, 1996). Nitrogen is especially prone to these cases and easily become a limiting nutrient, thereby lowering yields.

However, in hydroponics, these problems can be prevented by optimal management of the nutrient solution. In hydroponics, nitrogen is obtained by plants directly from the nutrient solution. Inorganic salts such as calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3), sodium nitrate ($\text{Na}(\text{NO}_3)_2$), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) and urea ($\text{CO}(\text{NH}_2)_2$) are commonly used to supply optimal nitrogen concentrations to the plants (Layzell, 1990). Hydroponics provides nitrogen in optimal concentrations because it eliminates the factors limiting nitrogen availability.

2.11 UREA

Due to frequent nitrogen limiting conditions in agricultural practices, urea is commonly used as an additional source of nitrogen for many crops, including tomatoes. Urea is widely applied as a foliar fertilizer. However, soil urea usually is low because of the reduction of urea by soil microbes. Other urea products (Black Urea) have been developed that limits urea from being depleted too fast (Internet 4).

Urea is a white crystalline inorganic salt ($\text{CO}(\text{NH}_2)_2$), which contains 46% nitrogen per mass. Urea can be supplied to the soil (Vavrina & Obreza, 1993) or used as a foliar spray (Wittwer *et al.*, 1963; Yamanda *et al.*, 1965; Knoche *et al.*, 1994; Bondada *et al.*, 2001). Urea is the most frequent used nitrogen fertilizer globally and is the most important additional nitrogen source for vegetable production in the field (Vavrina & Obreza, 1993).

Urea has been successfully applied to tomato leaves to reduce a nitrogen deficiency (Maximum yield, 2003). The application of urea fertilizer to the leaves of plants has given responses approximately equal to that of fertilizer applied to the soil (Nicoulaud & Bloom, 1996; Maximum yield, 2003).

When urea is applied as a foliar spray, it may cause burning of leaves when the concentration is too high, mostly above 2%, due to the presence of biuret. Biuret is a condensation compound of urea, equivalent to two molecules of urea, but with one molecule of ammonia absent from the molecule ($C_2O_2N_3H_5$; Krogmeier *et al.*, 1989). Attempts should rather be made to apply lower concentrations of urea (0.5% - 1%) more frequently. Most plants absorb foliar applied urea rapidly (Wittwer *et al.*, 1963; Nicoulaud & Bloom, 1996) where urease hydrolyzes urea in the cytosol where it is then further metabolized.

2.11.1 Foliar absorption and metabolism of urea

Urea is able to enter leaves through stomata or have specific translocators located in the leaves, as demonstrated in *Arabidopsis* (Liu *et al.*, 2003). A gene, AtDUR3 apparently encodes for a high affinity urea/ H^+ symporter in *Arabidopsis* (Liu *et al.*, 2003), which is an energy requiring process (Wilson *et al.*, 1988).

Urea can easily pass through the phospholipids bilayer of membranes, due to its non-polar properties (Salisbury & Ross, 1991; Galluci *et al.*, 1971). Wittwer *et al.* (1963), as well as Nicoulaud and Bloom (1996), stated that most plants absorb foliar applied urea rapidly and complete absorption of foliar applied urea occurred within 24 hours. Wilson and Walker (1988) suggested that urea was taken up by both high- and low affinity transport systems. Krogmeier *et al.* (1989) reported that after leaves were sprayed with urea, urea accumulated within the leaf, suggesting that urea enters the leaf in an intact form. Hine and Sprent (1988) obtained similar results, but with urea supplied to the roots.

It has been shown that foliar applied urea (^{15}N) was transported to potato tubers within 48 hours, followed by a more gradual redistribution within the plant (Witte *et al.*, 2002). This redistribution of urea derived nitrogen has been observed in hydroponically grown tomatoes as well (Tan *et al.*, 1999). In fact, it has been shown by Shelp and Shattuck (1986) that urea fed to tomatoes via the foliage

can be transported directly to the fruit, suggesting that urea entered the transport tissue. When urea is sprayed onto the leaf, it enters the cell through an urea specific symporter (urea + H⁺ → urea + H⁺) peripheral protein (AtDUR3), where it is immediately or quickly available for metabolic processes (Dilley & Walker, 1961; Liu *et al.*, 2003). This reaction is catalyzed by urease and is essential to make nitrogen derived from urea accessible to plants. Urease, a nickel-dependant enzyme that occurs in almost all organisms is responsible for the degradation of urea once inside the cytosol (Gerendas *et al.*, 1999). This reaction is as follows:

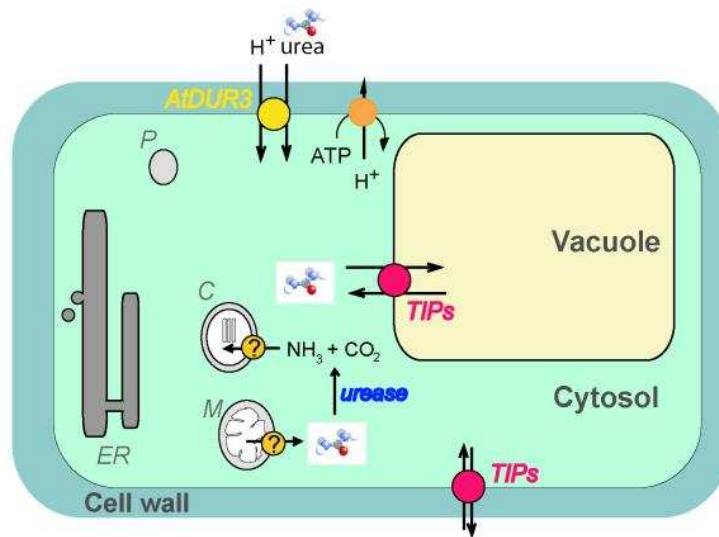
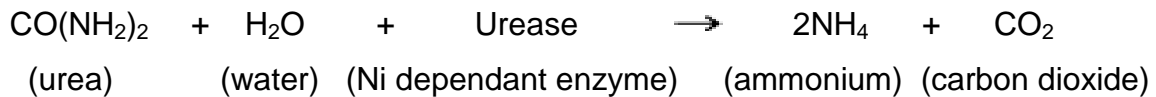


Figure 2.5 Transport of urea into plant cells (through high affinity urea/H⁺ peripheral symporter protein). AtDUR3 is the gene encoding for high affinity urea/H⁺ peripheral symporter protein in *Arabidopsis* (Liu *et al.*, 2003). (C = chloroplast, M = mitochondria, ER = endoplasmic reticulum, P = protein, TIP's = tonoplast intrinsic proteins or aquaporins) .

When urea is applied as a foliar spray, urea may only be absorbed in low concentrations because not all urea is transported into the leaves by the transport proteins. These proteins may become saturated with urea, temperature may play a role or the urea mixture may evaporate from the leaf surface. All these factors may limit urea absorption. Thus, an attempt has to be made to improve or enhance the absorption of urea in tomato leaves (Liu *et al.*, 2003).

Urea is not only supplied to a plant, but is also formed inside the plant during arginine breakdown via arginase in the urea cycle (Polacco & Holland, 1993). The ammonium formed is further metabolized into other cellular constituents.

2.12 PLANT MEMBRANES

Membranes regulate the flow of dissolved substances in and out of the cells. The term used for plant membrane structure is referred to as the fluid mosaic model (Singer & Nicolson, 1972). The cell membrane of plants consists mainly of a phospholipid bilayer, which in turn consists of a three carbon glycerol backbone to which two long fatty acids is estrified. A phosphate group is attached to the remaining carbon. Phospholipids are arranged with the polar phosphate heads on the outside, which is hydrophilic, and the fatty acid chains located on the inner sides, which are hydrophobic. These phospholipids are not anchored and can move freely among each other. This gives rise to the term “Fluid Mosaic membrane”. This also allows the membranes to stretch and change in shape (Singer & Nicolson, 1972; Brock 2003).

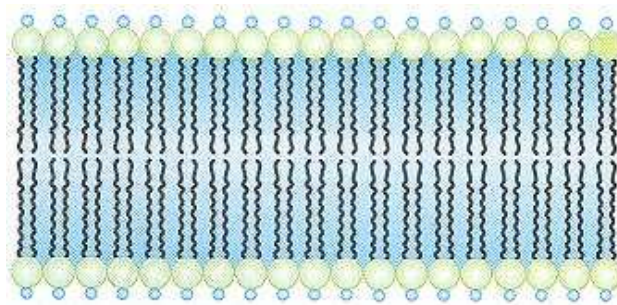


Figure 2.6 A simplified Fluid Mosaic membrane. The lipid like inner part (blue) indicates a hydrophobic region whereas the outer part (green) indicates a hydrophilic region (Brock, 2003).

Additional to the phospholipids, glycolipids, sterols and proteins are intrinsic parts of plant membranes. Proteins may be only on one side of the phospholipid bilayer, peripheral or extrinsic proteins, or can extend across the bilayer as integral or intrinsic proteins, or be attached on the surface of the membranes. These proteins control the transport of substances in or out of the cell. Proteins with carbohydrate groups attached are termed glycoproteins. Like the phospholipids, these proteins can move freely within the mosaic phospholipid layer and can change position within the membrane (Singer & Nicolson, 1972).

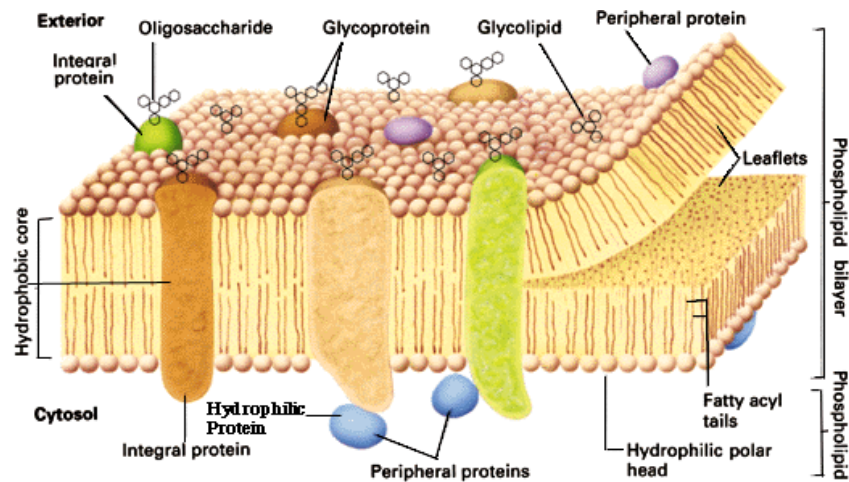


Figure 2.7 The phospholipids accompanied by membrane associated proteins, glycoproteins and glycolipids (Singer & Nicolson, 1972; Brock, 2003)

Transport of substances through membranes can occur in two different ways. Firstly, by simple or passive transport (diffusion). This involves the simple movement of an uncharged molecule over the membrane with a concentration gradient. Such molecules include water (H_2O), carbon dioxide (CO_2), small amounts of atmospheric nitrogen (N_2), oxygen (O_2) and urea. Active transport proteins in the membrane regulate the substances entering and exiting the cell, but do not influence the direction of substances.

Secondly, by means of active transport where molecules are transported against a concentration gradient. This, however, are energy (ATP) dependant transport systems. These molecules are transported by transport proteins, channel proteins or carrier proteins (Sussman & Harper, 1989). Active transport proteins can further be categorized into uniporter, antiporter and symporter proteins, based on the direction of movement of the substances (Fig. 2.8). In uniporter transporters, only one molecule or ion is transported in one direction. In antiporter transporters, a molecule or ion is passed through the membrane in exchange for another molecule or ion. In the case of symport transport, two molecules or ions are transported over the membrane in the same direction at the same time (Brock, 2003).

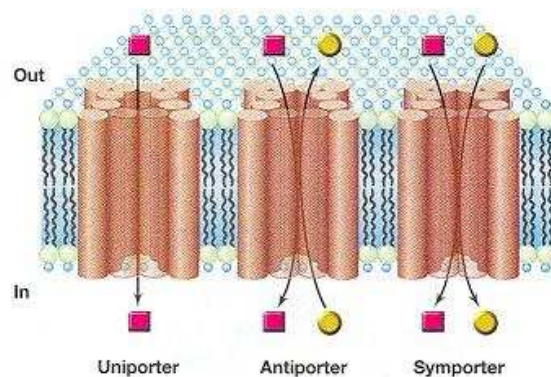


Figure 2.8 The three different types of transport events (Brock, 2003).

Although urea can easily pass through the biphospholipid layer it can be transported into the cell by transport proteins (Liu *et al.*, 2003). Urea is an uncharged molecule and would therefore be translocated over a biphospholipid either by simple diffusion or by means of a symporter transport system (Fig. 2.5; Liu *et al.*, 2003).

2.13 PHEROIDS

Pheroids is manufactured by ELEMENTOL (Pty) Ltd, and is apparently a “natural organic lipid like molecule” extracted from soya beans. Due to its lipid like structure, it is claimed that Pheroids easily associates with plant membranes and may enhance the transport of phytological beneficial substances over membranes. Due to pending patent registration, little is known about the chemical attributes of Pheroids. It is presumed that Pheroids “encapsulate” or “pack” substances and facilitate the transport of substances over membranes. Once inside the cell, the Pheroids / substance complex is metabolized, thereby releasing the substance. Whether the breakdown and “unpacking” of Pheroids is energy dependant or enzyme linked is unclear and requires further investigation. Due to the natural organic properties of Pheroids, it is claimed that Pheroids itself may stimulate plant growth. Pheroids is effective at very low concentrations (8 mL^{-1}).

2.14 RATIONALE FOR THIS STUDY

Elementol (Pty) Ltd, requested the evaluation of their novel compound, Pheroids, as a possible vehicle for the transport of phytological beneficial substances over membranes and as a bio-stimulant in general.

Field grown tomato plants may regularly experience nitrogen limiting conditions. Urea is commonly used as an additional source of nitrogen for many crops in the form of a soil fertilizer (Vavrina & Obreza, 1993) or as a foliar spray. Urea has been successfully applied to tomato leaves to reduce a nitrogen deficiency. Urea can easily pass through the phospholipids bilayer of membranes (Salisbury & Ross, 1991; Galluci *et al.*, 1971). Wittwer *et al.* (1963) as well as Nicoulaud and Bloom (1996) stated that most plants absorb foliar applied urea rapidly. When urea is applied as a foliar spray, urea may only be absorbed in low concentrations because not all urea is transported into the leaves by the transport proteins. These proteins may become saturated with urea, temperature may play a role or the urea mixture may evaporate from the leaf surface. All these factors may limit urea absorption. Thus, an attempt has to be made to improve or enhance the absorption of urea by tomato leaves.

The rationale of this study was to determine whether Pheroids has the ability to enhance the effect of foliar applied urea, an already fairly successful treatment, to reduce the effects of nitrogen limiting conditions on vegetative development and yield. This was addressed in a study conducted in a hydroponic setup where nitrogen levels could accurately be controlled. Plants were grown under optimal nitrogen control conditions and nitrogen limiting conditions. The plants were treated with different urea concentrations, either singly or mixed (“packed”) with Pheroids to determine Pheroids capacity to facilitate the additional uptake urea and to which extent. Another aim was to determine if Pheroids has any bio-catalytic properties by itself. In this case Pheroids was singly sprayed onto plants grown under nitrogen limiting conditions.

CHAPTER 3

MATERIAL AND METHODS

3.1 MATERIAL

Tomato plants (*Lycopersicon esculentum* Miller. cv. Rodade Star (Solanaceae)) was purchased from a local nursery.

All chemicals used were of the highest purity available. Most chemicals were purchased from Merck[®]. Urea was purchased from BDH AnalaR[®], Butylated Hydroxytoluene (BHT) from ICN Biomedicals, Inc. Pheroids was kindly provided by ELEMENTOL (Pty) Ltd. Nutrient mixtures for hydroponics were obtained from Hygrotech, South Africa.

3.2 METHODS

3.2.1 Cultivation

3.2.1.1 Hydroponic setup

Eight week old tomato seedlings were transplanted to an ebb and flow hydroponic system. Four identical experimental layouts were used (Fig. 3.1). The piping of each setup was constructed from 15 mm black PVC pipes, which in turn was connected to a 70 liter dark reservoir via a pump. Each layout consisted of two containers (900 mm X 200 mm X 200 mm) with a volume of 36 dm³. Each container was filled with white silica gravel (3 mm – 4 mm) to 30 mm from the rim. This resulted in a 30 dm³ support volume. Three plants were transplanted to each container 200 mm apart, resulting in a support volume of 10 dm³ for each plant. The containers were placed 180 mm apart. Each container contained a pipe feeding back to the reservoir to complete the circulating ebb and flow setup.

The total area for each setup was approximately 7 m². This gives an average of 3.5 plants.m⁻² (35 000 plants.ha⁻¹). The setups were flooded with the applicable nutrient solution seven times a day for five minutes, every two hours from 06:00. This is equivalent to 3.5 L.min⁻¹, which gives a volume of 17.5 L.cycle⁻¹ for each setup. After this, the nutrient solution was allowed to drain back into the dark reservoir (recirculation) which took approximately 10 minutes.

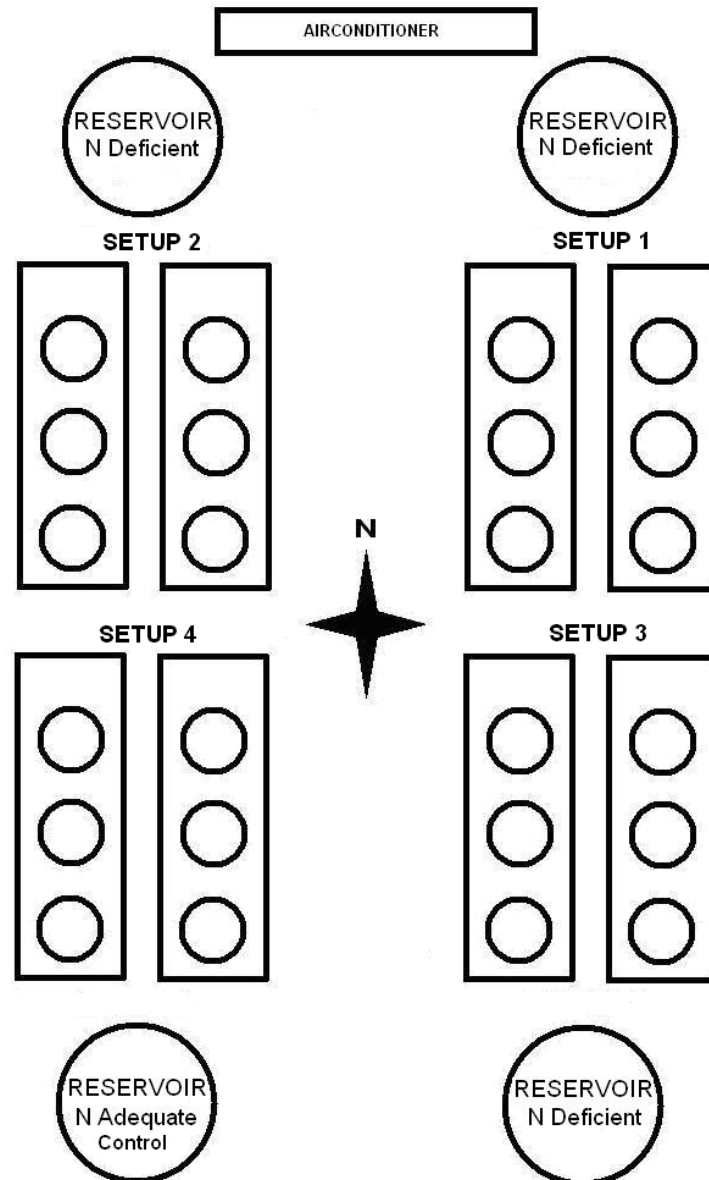


Figure 3.1 Experimental layout. Setup 1, 2 and 3 were nitrogen limiting whereas setup 4 was supplied with an adequate concentration of nitrogen (control).

3.2.1.2 Pruning and supporting

Where necessary, pruning was done in the early stages of vegetative development before any flowers and fruit set occurred. Some side branches below the first flower truss were removed to allow air circulation between the plants. All plants were pruned evenly. Care was taken not to remove all bottom leaves to allow for the translocation of nitrogen from older to younger leaves (Salisbury & Ross, 1991; Ireland, 1990) (see mobility of nitrogen, 2.10.4).

The evenly spaced tomato plants were additionally supported using twine tied to a supporting frame as part of the hydroponic setup. To prevent damage to the plant during vegetative growth care was taken not to wine the twine too tightly around the stems of the plants.

3.2.2 Greenhouse conditions

Greenhouse conditions, optimized for the production of tomatoes, were measured three times a week at midday (12:00), at three different localities within the greenhouse. Controlling greenhouse factors like temperature, relative humidity and light intensity are of utmost importance in obtaining and managing optimum yield.

3.2.2.1 Temperature and Relative Humidity (% RH)

The temperature was controlled within the greenhouse. Temperature (°C) was measured and monitored constantly throughout the trial. Temperature was measured with a stagnant thermometer, thermo hydrograph and a swirl hygrometer at three different localities within the greenhouse.

Relative humidity (% RH) was measured with a stagnant thermo hydrograph and additionally with a swirl hygrometer. The values (wet and dry bulb values) measured with the swirl hygrometer were used to obtain the percentage relative humidity (% RH).

3.2.2.2 Light intensity

Light intensity was measured with a LiCor LI – 185A quantum/radio/photometer ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Light measurements were taken at three different locations within the greenhouse and the average thereof was taken as the existing light intensity for the experimental layout.

3.2.3 Nutrient medium

3.2.3.1 Nutrient composition

Two nutrient media were used. A nitrogen adequate medium (control) and a nitrogen limiting medium. The control medium contained 210 ppm nitrogen, whereas the nitrogen limiting medium contained only 70 ppm nitrogen.

The nutrient media were prepared using Hygrotech's nutrient formula from two different stock mixtures (A & B). The first mixture (A) consisted of all the essential elements and the second mixture (B) consisted of $\text{Ca}(\text{NO}_3)_2$. The first mixture contained only 70 ppm nitrogen. When combined, the two mixtures resulted in a complete nutrient solution of 210 ppm nitrogen. The control medium consisted of both mixtures, whereas the nitrogen limiting solution consisted only of mixture A.

Table 3.1 Nutrient concentrations in the nitrogen adequate and limiting media.

Nutrient element	Nitrogen adequate medium control (ppm)	Nitrogen limiting medium (ppm)
Nitrogen (N)	210	70
Phosphorus (P)	43	43
Potassium (K)	214	214
Magnesium (Mg)	30	30
Sulphur (S)	64	64
Iron (Fe)	1.25	1.25
Copper (Cu)	0.02	0.02
Zinc (Zn)	0.149	0.149
Manganese (Mn)	0.3	0.3
Boron (B)	0.37	0.37
Molybdenum (Mo)	0.037	0.037

Calcium (Ca)	183	182
Chloride (Cl)	0	325

Due to the mixing ratios between the two mixtures, the nitrogen limiting medium was also deficient of calcium. Calcium was additionally added in the form of calcium chloride (CaCl_2) to ensure the same concentration of calcium as in the control medium.

The nitrogen adequate nutrient solution (control) contained three times more nitrogen (210 ppm) than the nitrogen limiting medium (70 ppm). This represents a ratio of 3:1 nitrogen between the control- and nitrogen limiting media.

3.2.3.2 Replacement of nutrient media

The nutrient media were replaced every two weeks to ensure fresh, accurate and precise levels of nutrients to the plants. The nutrients were also replaced to avoid acidification of the media and to limit the circulation of possible diseases.

3.2.3.3 Electrical Conductivity (EC)

The Electrical Conductivity (EC) of the nutrient media were constantly monitored using a Philips PW 9526 Digital Conductivity meter (mS cm^{-1}). This was done to ensure that accurate and correct levels of nutrients were obtained.

3.2.3.4 pH

The pH of the nutrient solutions were constantly monitored using a Radiometer Copenhagen PHM85 Precision pH Meter.

3.2.4 Treatments

The different treatments are listed in Table 3.2. It entails that both control plants and plants grown under nitrogen limiting conditions were additionally sprayed with 0.5% urea solution, singly or mixed (“packed”) with Pheroids. In addition, the nitrogen deficient plants were also sprayed with a 1% urea solution, singly and mixed with Pheroids. Pheroids only was also a treatment sprayed on the plants grown under nitrogen limiting conditions. The 0.5% urea solution was prepared by dissolving 2 g urea in 400 ml distilled water. The 1% urea solution was prepared by dissolving 4 g urea in 400 ml distilled water. In order to “pack” Pheroids with urea, 2 g urea was dissolved in 400 ml distilled water (0.5% urea solution), where after 2 ml of Pheroids was added and stirred on a magnetic stirrer for 30 minutes. To obtain a 1% urea Pheroids mixture, 4 g of urea and 2 ml of Pheroids was dissolved in 400 ml distilled water and stirred on a magnetic

stirrer for 30 minutes. The Pheroids treatment consisted of 2 ml Pheroids in 400 ml distilled water.

Table 3.2 Foliar applications of urea solutions, singly or mixed with Pheroids, to tomato plants grown under control- and nitrogen limiting hydroponic conditions.

Treatments	Foliar treatment
Nitrogen adequate (control) (210 ppm)	
Control	None
0.5 % Urea	2 g Urea dissolved in 400 ml dist. H ₂ O
0.5 % Urea / Pheroids	2 g Urea + 2 ml Pheroids dissolved in 400 ml dist. H ₂ O
Nitrogen limiting (70 ppm)	
Nitrogen limiting	None
0.5 % Urea	2 g Urea dissolved in 400 ml dist. H ₂ O
1 % Urea	4 g Urea dissolved in 400 ml dist. H ₂ O
0.5 % Urea / Pheroids	2 g Urea + 2 ml Pheroids dissolved in 400 ml dist. H ₂ O
1 % Urea / Pheroids	4 g Urea + 2 ml Pheroids dissolved in 400 ml dist. H ₂ O
Pheroids	2 ml Pheroids in 400 ml dist. H ₂ O

3.2.5 Pest Control

Hydroponically grown tomatoes are exceptionally prone to insects due to the favorable micro–environment inside the greenhouse. As soon as pests or diseases were noticed, applicable pest control measures were taken. Metasystox were used to control aphids, Kelthane for russet mites as well as White fly and Funginex for fungal infections. The insecticides were prepared according to the recommendations of the manufacturer and are given in Table 3.3.

Table 3.3 Insecticides used to control diseases.

Insecticide	Biotic disease	Concentration used
Metasystox	Aphids	2.5 ml.L ⁻¹
Kelthane AP	Russet mite, White fly	2 g.L ⁻¹
Funginex	Fungal pathogens	1.5 ml.L ⁻¹

3.2.6 Physical Parameters

Nitrogen is a key element in overall plant metabolism and when it becomes limited, vegetative development and yield of the plants are affected. Physical parameters such as plant height, fruit and flower formation, fruit mass and diameter as well as generative development of the plants were recorded weekly to determine the effect of a nitrogen deficiency and the subsequent corrective treatments on vegetative growth, generative development and yield.

3.2.6.1 Vegetative growth (plant height)

Plant height was measured on a weekly basis using a measuring tape. The distance from the origin of the stem (gravel level) to the utmost point of the apical bud was taken as parameter for plant height. Measurements were discontinued when the plants reached the roof of the greenhouse (± 2 m) which prevented further accurate measurements.

3.2.6.2 Generative development (bud, flower and fruit formation)

To measure generative development of the plants, the time span between transplantation and the formation of the first buds, open flowers, fruits appearing and first fruits harvested were recorded. Buds were recorded when it was 5 mm in length. Flowers were recorded when the centre of the flower was visible or fully open. Appearing fruit were recorded when a size of 5 mm was reached.

The time span between transplantation and when the first fruits were harvested was finally recorded.

3.2.6.3 Yield (number of fruit, mass and size)

The number of fully developed red fruits, consumer ready, were harvested and recorded on a weekly basis for the duration of the harvest period. Each fruit was accurately weighed on a Shimadzu AUW 320 analytical balance. The individual fruit harvested for a treatment were combined to give the total yield for the week, and finally for the harvest period.

Fruit size (diameter) was accurately measured to the nearest millimeter using a caliper (micrometer). The average of three consecutive diameter readings was taken to represent average fruit size.

3.2.7 Quality Parameters

Like physical parameters, quality parameters are also influenced by available nitrogen. Leaf protein content gives a direct indication of nitrogen content in the plant (Maynard & Loosli, 1969). Further, quality parameters are also of crucial importance in determining overall fruit quality and taste. Fruit quality is determined by fruit acidity (pH; Verkerke & Kersten, 2000), electrical conductivity, soluble solid content (Ya Ling & Stanghellini, 2001), sugar content (% Brix) (Monforte *et al.*, 2000) and moisture content. Moreover, as an anti-oxidant, lycopene content adds to overall fruit quality (Blum *et al.*, 2005). These quality parameters were used to determine the effect of a nitrogen deficiency and the subsequent corrective treatments on leaf protein content and fruit quality.

3.2.7.1 Extraction and determination of protein content of leaves

Leaf material was sampled from both the bottom, older leaves, and the top, younger leaves, of each plant. Two grams of leaf material was homogenized in 10 ml of ice cold (4°C) extraction buffer (m:v ; 1 g : 5 ml) for 30 seconds using a Polytron electric homogenizer. The extraction buffer consisted of 12.5 mM Tris-HCl (pH 6.8), containing 2 mM EDTA, 14 mM β -2-Mercapto-ethanol and 2 mM PMSF. The latter two were added before each protein extraction procedure. An aliquot of the homogenate was then centrifuged in a cooled bench centrifuge at 12 000 r.p.m for 10 minutes. The clear supernatant was used for protein assays. The protein concentration of the extracts was determined using the method of Bradford (1976). A clean micro-plate was prepared as follows:

Table 3.4 Preparation of a micro-plate for protein assays. Gamma globulin (0.5 mg.ml⁻¹) was used as standard.

	Blank (µl)	STD (µl)	Sample (µl)
H₂O	160	150	150
Standard	-	10	-
Sample	-	-	10
Bio-Rad	40	40	40
Total volume	200	200	200

After addition of the Bio-Rad dye, the plate was mixed on a rotational shaker and left for 10 minutes before absorbance values was recorded at 595 nm with a Bio-Rad (Model 3550) micro plate reader.

3.2.7.2 Leaf and fruit moisture content

The fresh mass of 15 discs, cut from leaves with a 1 cm diameter stainless steel borer, was recorded. These discs were then dried to a constant mass at 72 °C for 72 hours in a Labotec oven, where after the final dry mass of the leaf discs were recorded.

Fresh tissue slices of approximately 10 g, representative of the fruits overall condition, were obtained. These slices were then dried to a constant mass at 72 °C for at least 72 hours in a Labotec oven. The final dry mass of the slices were then recorded.

The moisture content of the leaf and fruit tissue were calculated using the following equation:

$$\{(Fresh\ mass - Dry\ mass) \div Fresh\ mass\} \times 100$$

= % Moisture content

Leaf dry mass content was used to express leaf protein content. Fruit dry mass content was used to express lycopene content.

3.2.7.3 Fruit pH, EC, % Brix

Some parameters were used to determine fruit quality. These parameters were measured on a two weekly basis from the onset of harvest for a period of 16 weeks. These parameters were pH, EC and Brix index (% total sugars or TSS). Fruit were chosen randomly from each plant and all quality parameters were obtained from the same fruit.

Fresh fruit were sliced in half and were homogenized for 30 seconds, using a Polytron electric homogenizer to obtain a liquid puree, which was kept on ice.

The pH of the resultant puree was measured with a Radiometer Copenhagen PHM85 Precision pH Meter. The puree was mixed on a magnetic stirrer until a constant pH was recorded.

The EC of the puree was recorded using a Philips PW 9526 Digital Conductivity meter with a cell constant of 1.52 cm^{-1} . The puree was mixed on a magnetic stirrer until a constant EC (mS.cm^{-1}) was obtained.

The % Brix of 60 μl puree samples was determined using an ATAGO refractometer by following the instructions of the manufacturer. The refractometer was calibrated using distilled water. Each % Brix is taken as the equivalent of 1 g sugar and other solids per 100 g of juice. A high Brix % will indicate a high sugar and other solid content of the puree (Baxter *et al.*, 2005).

3.2.7.4 Lycopene

Lycopene content was an additional biochemical parameter used to measure fruit quality. Lycopene content was also determined on a two weekly basis from the same fruit used to determine pH, EC, % Brix (see 3.2.7.3).

The lycopene content of the fruits was determined according to the reduced volume method of Davies *et al.* (2002). Ten g of fruit tissue was added to a test tube containing 10 ml of distilled water (1:1; m/v). The content was homogenized for 30 seconds, using a Polytron electric homogenizer. An aliquot containing 0.5 g of the puree was then added to a test tube containing a mixture of 5 ml 95 % ethanol, 10 ml hexane and 5 ml acetone, containing 0.05 % (w/v) butylated hydroxytoluene (BHT). The tubes were sealed with para-film, thoroughly mixed on a bench vortex and extracted on an orbital shaker at 180 rpm for 15 minutes.

After rotation, 3 ml de-ionized water was added to the tubes and replaced on the orbital shaker for 5 minutes. After extraction, the tubes were left at room temperature (22°C) for 15 minutes in the dark for phase separation to occur before the clear yellowish supernatant (hexane layer) was collected. The tubes were kept in the dark on ice until all samples were extracted. This was done to avoid the possible cis-trans isomerization induced by light (Nguyen & Schwartz, 1999; Xianquan *et al.*, 2005).

The supernatant (hexane upper layer) was used to determine the absorbance at 503 nm using a Phillips Pye Unicam SP8 – 400 UV/VIS Spectrophotometer.

Taking all variables of this specific extraction procedure into account, the lycopene concentration of the tissue was calculated using the following equation (Davies *et al.*, 2002):

$$\begin{aligned}
 \text{Lycopene} &= \frac{A_{503}}{17.2 \times 10^4 \text{ M.cm}} \times \frac{536.9 \text{ g}}{\text{mole}} \times \frac{1 \text{ l}}{10^3 \text{ ml}} \times \frac{10^3 \text{ ml}}{1 \text{ g}} \times \frac{10 \text{ ml}}{\text{kg tissue}} \\
 &= \frac{A_{503} \times 0.0312}{\text{kg tissue}} \\
 &= \frac{A_{503} \times 31.2}{\text{g tissue}} \\
 &= \text{mg lycopene.g}^{-1} \text{ tissue}
 \end{aligned}$$

Where A_{503} is the absorbance at 503 nm and $17.2 \times 10^4 \text{ M.cm}^{-1}$ the molar absorbance coefficient for lycopene. The molecular weight (mw) of lycopene is $536.9 \text{ g.mole}^{-1}$ (Davies *et al.*, 2002)

CHAPTER 4

RESULTS

Eight week old tomato seedlings were transplanted into an ebb- and flow hydroponic setup consisting of nitrogen adequate (210 ppm) and nitrogen limiting conditions (70 ppm). The trial was conducted in a greenhouse in hydroponic setup to accurately control nitrogen levels. The effect of nitrogen limiting conditions on vegetative- , generative growth and yield were recorded. Moreover, plants were sprayed with 0.5% and 1% urea solutions, singly or mixed with Pheroids, as corrective treatments to alleviate the effect of nitrogen limiting conditions (see 3.2.4). Furthermore, the changes in greenhouse conditions were deemed important and were measured and recorded over the entire trial period.

4.1 GREENHOUSE CONDITIONS

Plants grown in a greenhouse are still subjected to external factors affecting overall plant growth. These factors include temperature, light intensity and relative humidity. By controlling these environmental factors within limits, allow that the effect of the different treatments could be monitored accurately.

The study was conducted in a greenhouse under controlled conditions, optimum for tomato production. Different parameters, namely light intensity, temperature and relative humidity (% RH), were measured to follow variations in these parameters during the experimental period.

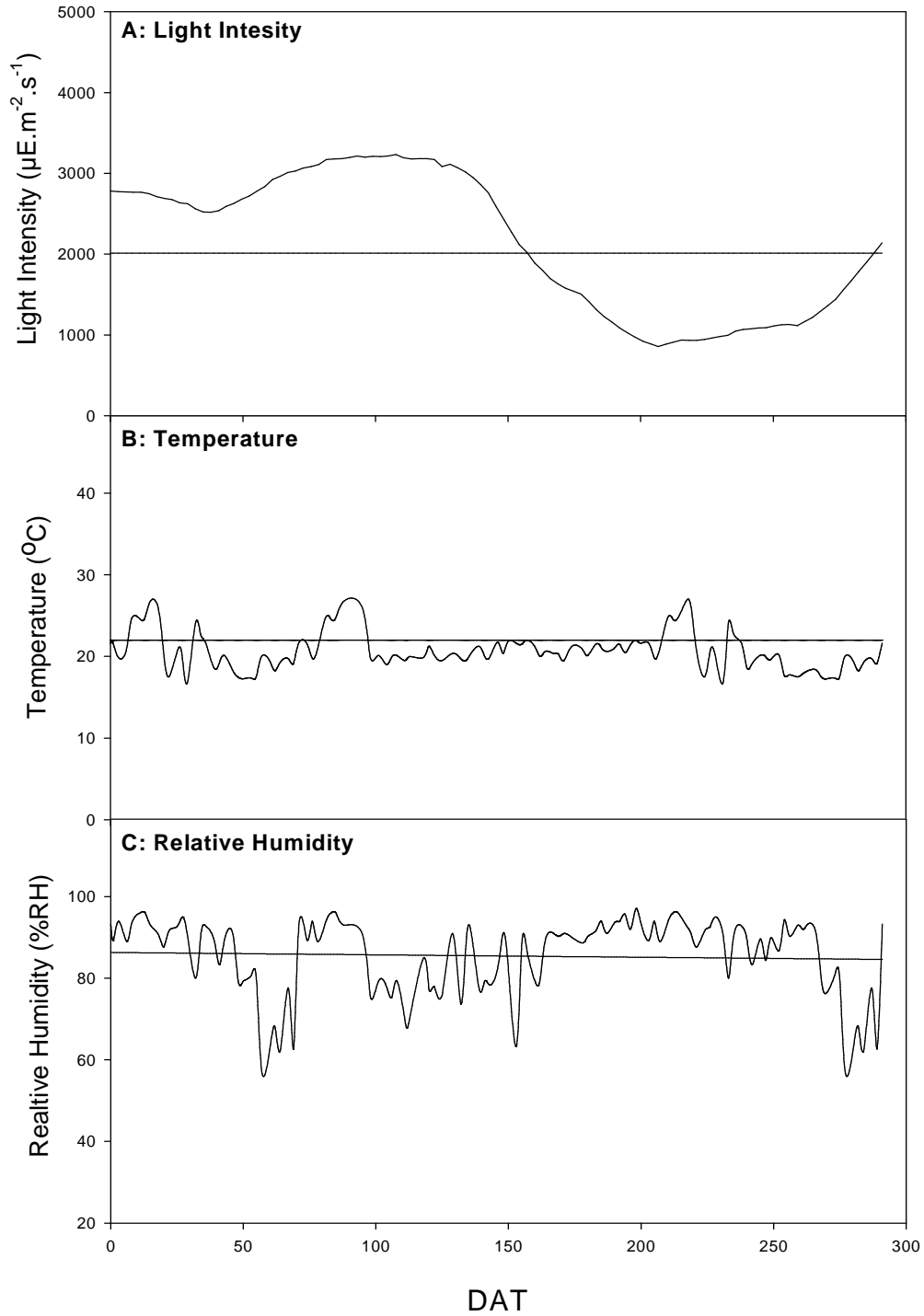


Figure 4.1 Changes in light Intensity (A), Temperature (B) and Relative Humidity (C) in the greenhouse during the duration of the trial period.
DAT = Days after transplantation.

Light intensity was measured every two days at 12:00 at three different localities to obtain an average light intensity within the greenhouse during the trial. The light intensity remained relatively constant at approximately $3000 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for the first half of the trial (summer) and decreased as winter approached. It is expected that solar radiation is less during winter due to the trajectory of the sun in relation to the earth. The start of spring from day 200 is obvious (Fig. 4.1 A). All the plants were subjected to these fluctuations in light during the trial period.

The temperature and relative humidity was also measured every two days at 12:00 at three different localities within the greenhouse. The temperature proved to be relatively constant throughout the trial period as it was regulated between 17°C (night) and 27°C (day). The average temperature at 12:00 was approximately 22°C for the duration of the experimental period (Fig. 4.1 B). Temperature fluctuations were small and varied within margins.

The relative air humidity (%RH) at 12:00 inside the greenhouse varied between 55% and 96%. The average relative humidity at 12:00 was approximately 86% for the duration of the experimental period (Fig. 4.1 C). A clear relationship between % RH and air temperature recorded at 12:00 is apparent as the periods with above average % RH correlates with the periods of above average temperatures and *vice versa*. All the plants were subjected to these fluctuations in temperature and humidity during the experimental period. Relative humidity fluctuations were small and varied within margins.

4.2 HYDROPONIC SETUP

4.2.1 Electrical Conductivity (EC) of nutrient media

Variation in electrical conductivity (EC) is an important parameter used to determine the availability of dissolved salts in the nutrient media, which inevitably affects overall plant growth, metabolism, fruit yield and quality (Dorai *et al.*,

2001). The EC for all the reservoirs were measured after both replenishment and replacement of nutrient media (see 3.2.3.2).

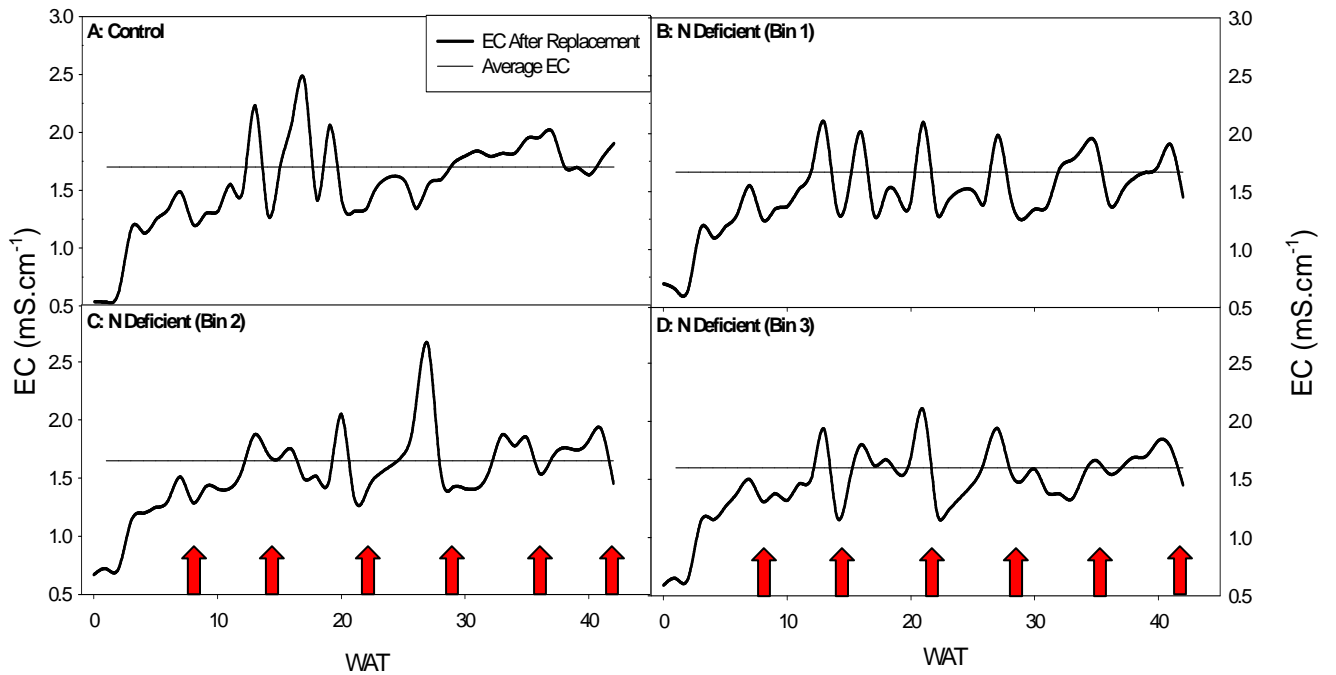


Figure 4.2 Fluctuations in electrical conductivity (EC) over the trial period. A = Control; B – D = Nitrogen limiting media in three different reservoirs. Arrows (↑) indicate time of complete nutrient replacement. WAT = Weeks after transplantation.

The EC of the nutrient media varied between 1.4 and 2.5 milliSiemens.cm⁻¹, with an acceptable average EC of approximately 1.7 milliSiemens.cm⁻¹ for both nitrogen deficient- and control media. The initial EC values were low due to half strength used for the first two weeks after transplantation.

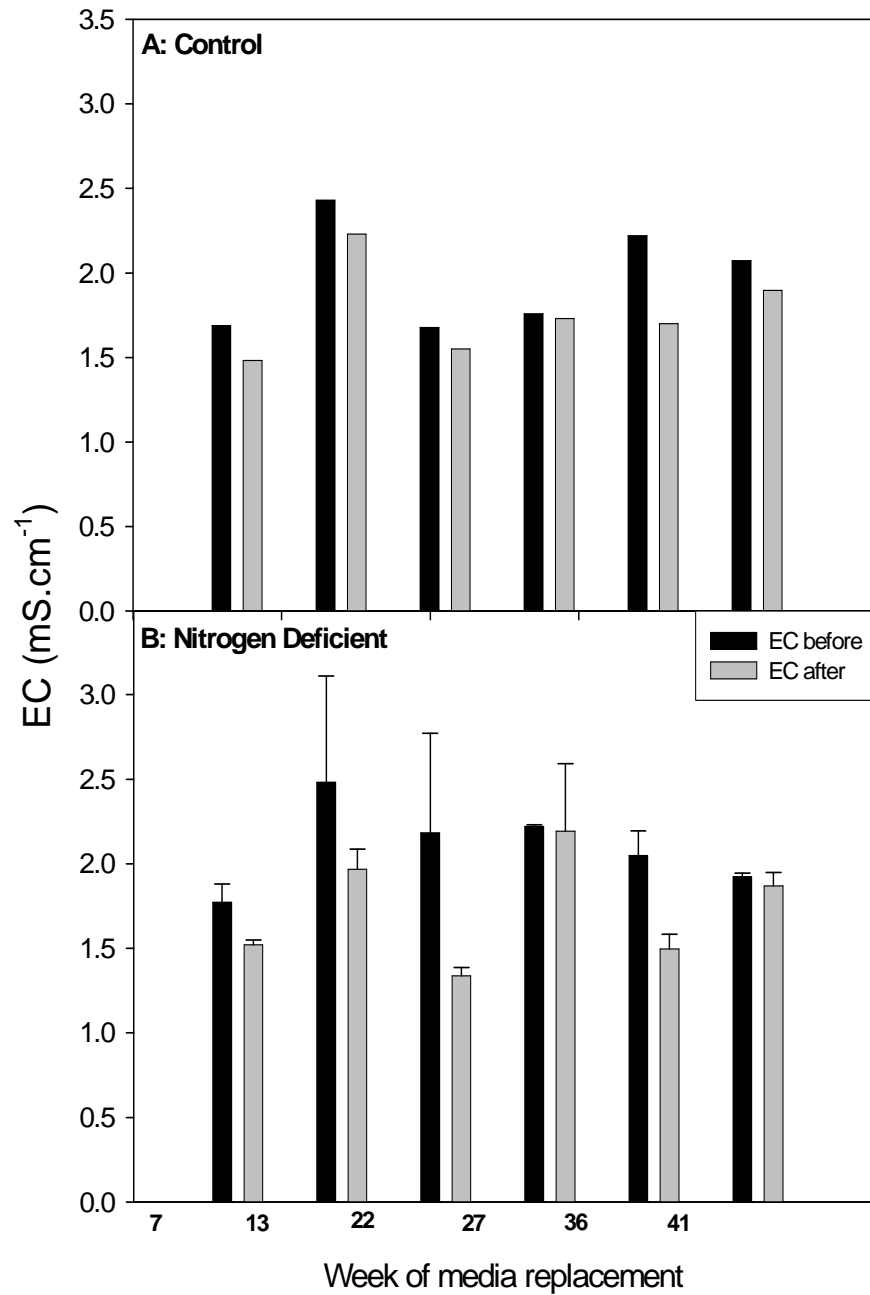


Figure 4.3 Average EC of the control- (A) and nitrogen limiting media (B) before and after complete replacement at weeks 7, 13, 22, 27, 36 and 41 after transplantation.

The nutrient media were not only replenished, but also completely replaced at weeks 7, 13, 22, 27, 36 and 41 to dispose of any possible harmful wastes. After complete replacement of the nutrient media the EC of the nutrient media was lower than before replacement. This possibly indicated an accumulation of dissolved charged nutrients and other molecules over time before replacement, due to a high transpiration rate or a possible buildup of charged contaminants (Fig. 4.3).

4.2.2 pH of nutrient media

Changes in pH is another parameter used to determine the quality of the nutrient media, as pH affects the solubility of nutrients in solution. The pH was measured after both replenishment and complete replacement of the nutrient media.

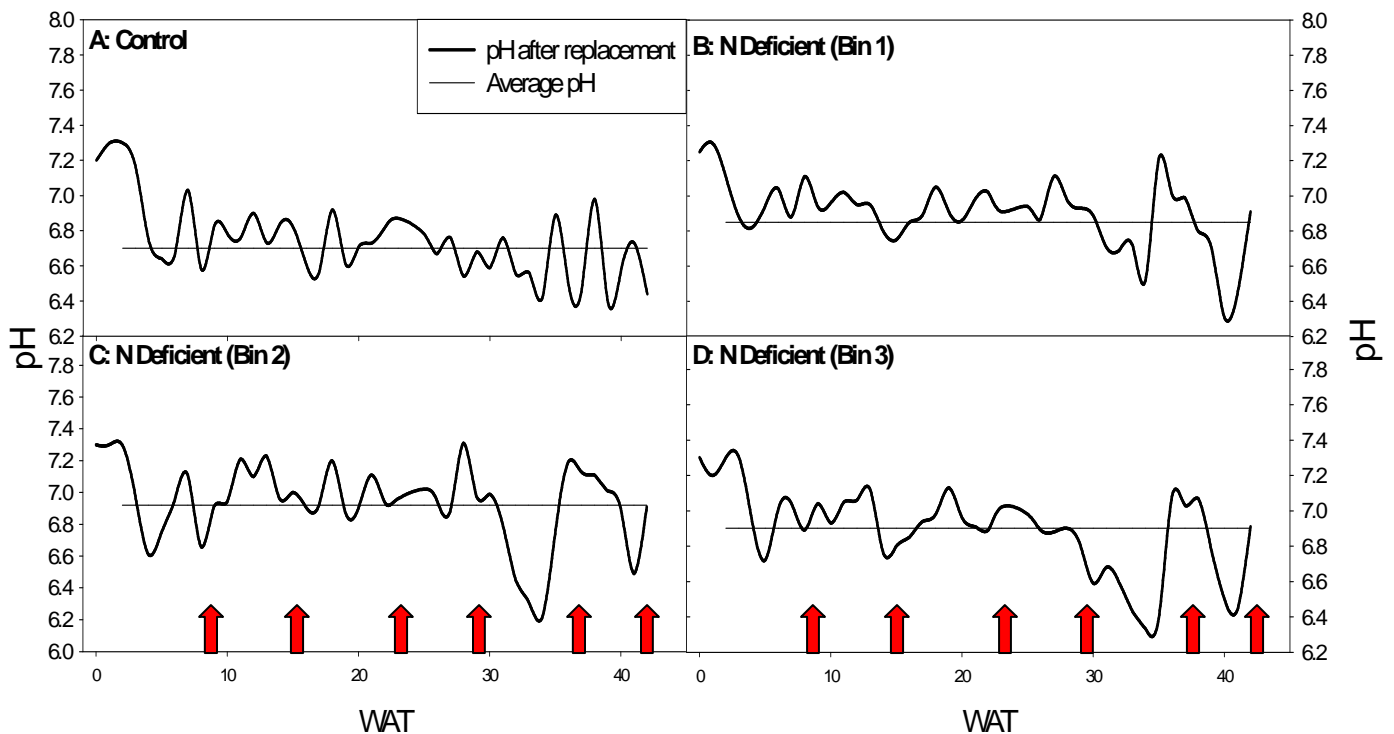


Figure 4.4 Variation in the pH of the nutrient media after replenishment and complete replacement. (A = Control; B – D = Nitrogen limiting media. Arrows (↑) indicate the time of complete nutrient replacement). WAT = Weeks after transplantation.

The pH varied between pH 6.2 and pH 7.3. The average pH of the control nutrient medium was approximately 6.7 and for the nitrogen limiting media approximately 6.8 for the greater part of the trial period. In addition, the pH of all the media used tends to decline slightly during the trial.

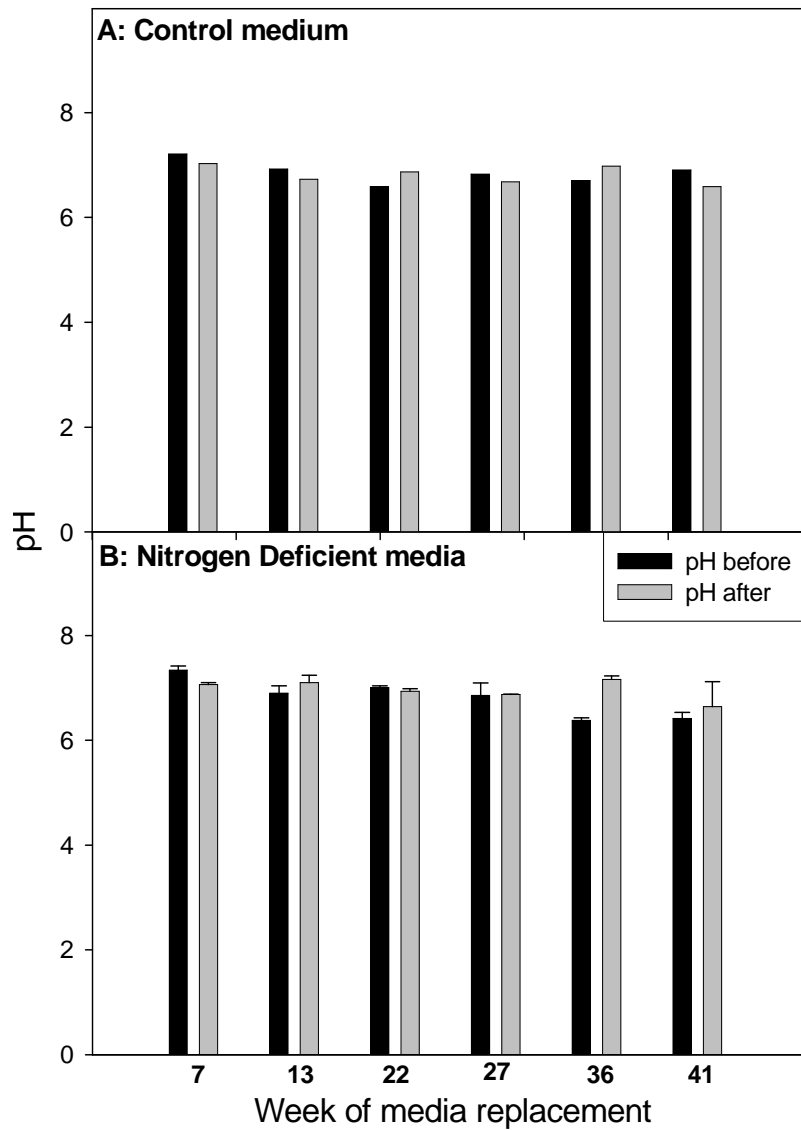


Figure 4.5 Average pH of the control- (A) and nitrogen limiting media (B) directly before and after replacement at weeks 7, 13, 22, 27, 36 and 41 after transplantation.

Moreover, it was found that the pH directly after replacement of the nutrient media was not lower than before replacement at the specified time intervals during the trial (Fig. 4.5), as was observed for EC (Fig. 4.3). No definite pattern in the variation in pH before and after replacement was evident (Fig. 4.5).

4.2.3 Nutrient media consumed

The exact volume of nutrient media replenished and replaced, were recorded over the experimental period of 44 weeks. The volume of nutrient media consumed during the trial by the control plants and plants grown under nitrogen limiting conditions was subsequently calculated (Fig. 4.6).

During the initial stages of the experimental period, up to five weeks after transplantation, no difference in the volume of nutrient media consumed was observed between the control- and nitrogen deficient plants. Thereafter, the control plants consumed substantially more nutrient media during the remainder of the experimental period than the nitrogen deficient plants. Control plants consumed approximately 315 liter media.plant⁻¹, compared to the 236 liter media.plant⁻¹ for the plants grown under nitrogen limiting conditions. This was approximately 79 liter media.plant⁻¹ more for a control plant over the period of 44 weeks.

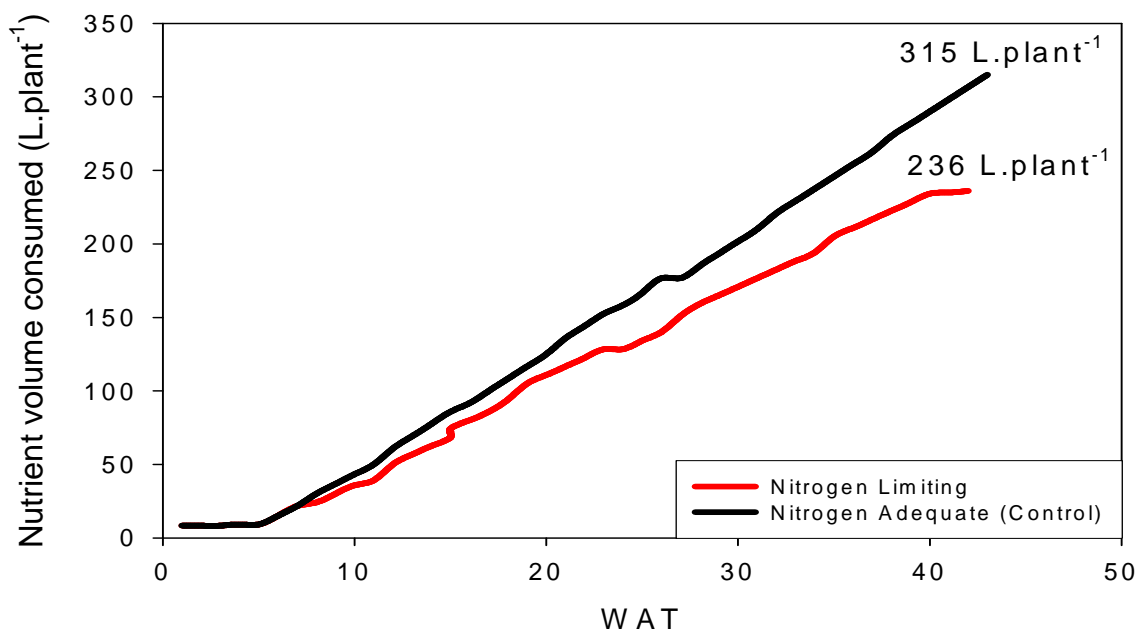


Figure 4.6 Nutrient consumption by control – and nitrogen deficient plants during the trial. WAT = Weeks after transplantation.

4.3 VEGETATIVE DEVELOPMENT OF PLANTS GROWN UNDER CONTROL- AND NITROGEN LIMITING CONDITIONS.

To determine the effect of nitrogen limiting conditions, using a controlled hydroponic setup, the initial vegetative development of the plants, prior to harvest was compared. Control plants were grown under optimum nitrogen levels of 210 ppm, while nitrogen deficient plants were grown under nitrogen limiting levels of 70 ppm.

The aim of the study was to alleviate the effect nitrogen limiting conditions may have on growth, development and yield by treating nitrogen deficient plants with additional nitrogen using 0.5% and 1% urea foliar sprays. Another aim was to possibly enhance the absorption of urea by mixing it with Pheroids before application. Plant height and leaf protein content was used as parameters to monitor vegetative development.

4.3.1 Plant height

To determine the effect of nitrogen limiting conditions and the subsequent corrective treatments on vegetative development, the height of the plants was recorded for 20 weeks from transplantation, until the structure (roof height) of the greenhouse limits growth, and therefore accurate measurements.

Both the control- and nitrogen deficient plants increased in height linearly throughout the trial period of 20 weeks (Fig. 4.7). During the initial stages of establishment, no differences between the control- and nitrogen deficient plants could be observed. However, during the phase of active fruit development, from week 8 onwards, the control plants outperformed the nitrogen deficient plants (Fig. 4.7 A & Fig. 4.8 B) in terms of vegetative growth.

Spraying nitrogen deficient plants with 0.5% and 1% urea solutions, either singly or mixed with Pheroids, have no obvious stimulatory effect on vegetative growth (plant height) of the plants during the initial phases, as well as during the period of active fruit development (Fig. 4.7 B & Fig. 4.8 A & B).

In addition, spraying nitrogen deficient plants with Pheroids only appear to impair vegetative growth during the later stage, when active fruit development occurred. Spraying control plants with a 0.5% urea solution, singly or mixed with Pheroids, had no stimulatory effect on vegetative growth of these plants under conditions of optimum nitrogen supply (Fig. 4.7 B & Fig. 4.8 B).

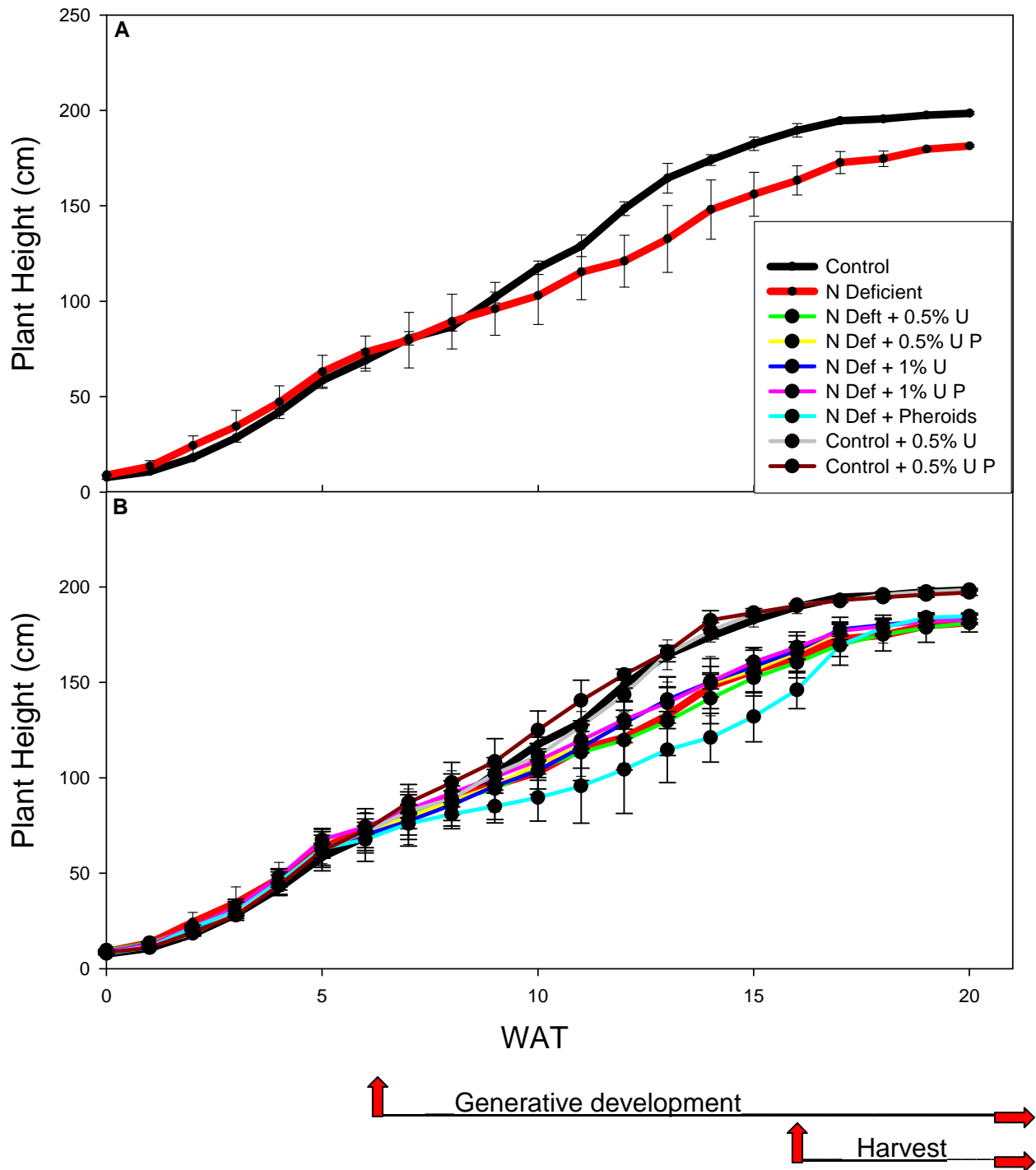


Figure 4.7 Changes in plant height over the experimental period. The arrows indicate when generative development and subsequent harvest period occur, as shown in Table 4.2.

WAT = Weeks after transplantation.

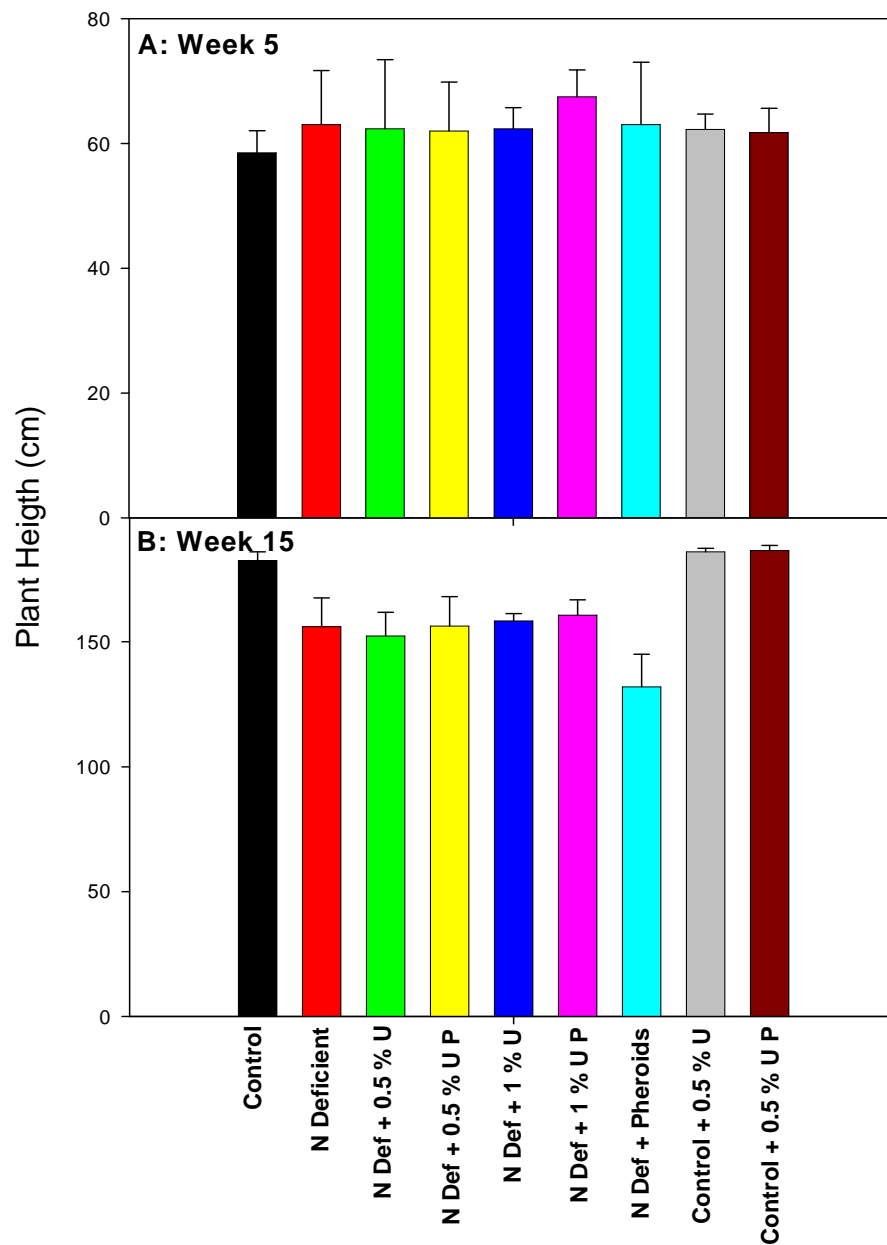


Figure 4.8 Average height of plants during early development (A: week 5) and the start of the harvest period (B: week 15).

4.3.2 Initial protein content of leaves

The protein content of both the top and bottom leaves was recorded for the first 8 weeks after transplantation. Protein content can be a measure of the nitrogen content (Maynard & Loosli, 1969), because nitrogen makes out a large part of proteins (Salisbury & Ross, 1991). When nitrogen is limited in supply, the nitrogen already present in the plant is translocated from the older to the younger active growing tissues in the form of amino acids (Salisbury & Ross, 1991; Ireland, 1990). As a result of this, nitrogen deficiency symptoms are first observed in older tissues (Gilbert & Hadfield, 1987, Sutherland & Sutherland, 1987).

Thus, by determining the protein content in the bottom and top leaves will give a good indication of the re-allocation of nitrogen within the plant, as well as the nitrogen content present in different parts of the plant. By monitoring these protein levels in leaves, the adverse effects of nitrogen on plant metabolism (vegetative and generative growth) can be determined.

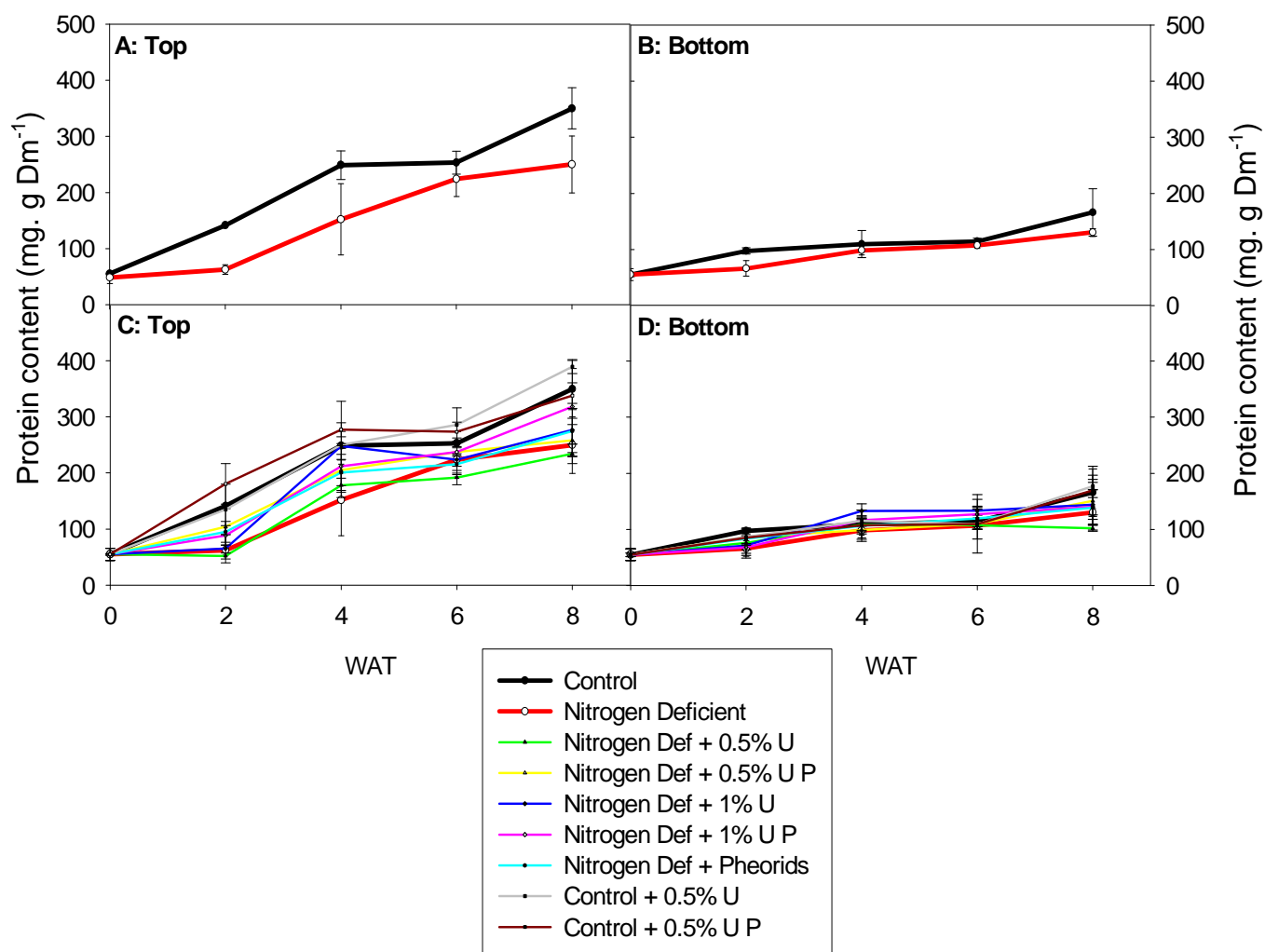


Figure 4.9 Protein content of top and bottom leaves early in vegetative development. WAT = Weeks after transplantation.

The protein content of both top and bottom leaves of control– and nitrogen deficient plants increased during this trial of 8 weeks, more so in the control plants than in the nitrogen deficient plants (Fig. 4.9 A & B). Moreover, the protein content of the top leaves from control plants is also visibly higher than that of the nitrogen deficient plants (Fig. 4.9 A). This difference in protein content was less pronounced for the bottom leaves of these plants (Fig 4.9 B). In addition, the top leaves had a considerably higher protein content than the bottom leaves (Fig. 4.9 A & B).

Spraying nitrogen deficient plants with 0.5% and 1% urea solutions, to supply these plants with additional nitrogen, resulted in an increase in the protein content for all the leaves, but these solutions failed to fully alleviate the effect a nitrogen deficiency had on the protein content of top (Fig. 4.9 C) and bottom leaves (Fig 4.9 D). Mixing these 0.5% and 1% urea solutions with the novel compound, Pheroids, resulted in a slightly higher leaf protein content, opposed to the plants treated with urea only. This stimulation is, however, very marginal. Treating nitrogen deficient plants with Pheroids only, gave similar results than when it was mixed with the urea solutions. Thus, it appeared that Pheroids is ineffective in enhancing the transport and absorption of urea, and the subsequent stimulation of vegetative growth.

Spraying control plants, which was grown under 210 ppm nitrogen levels, with a 0.5% urea solution, singly or mixed with Pheroids, resulted in a small increase in the protein content of the top leaves (Fig 4.9 C), but had no visible effect on the protein content of the bottom leaves (Fig 4.9 D). This is indicative that even under conditions of optimum nitrogen supply, control plants reacted to the additional source of nitrogen, supplied by means of the 0.5% urea and 0.5% urea / Pheroids foliar sprays.

Table 4.1 A comparison in leaf protein content for top- and bottom leaves for the different treatments from transplantation (week 0) to week 8.

Treatments	Protein content mg.g Dm ⁻¹ (a)		% Increase in protein content (b)		Ratio Bottom : Top(c)	Control : Treated Plants (d)	
	Bottom	Top	Bottom	Top		Bottom	Top
Week 0 (a)	48	55	-	-			
Week 8							
Control	165	349	344	634	1 : 2.11	1	1
N Deficient	130	249	271	453	1 : 1.92	0.79	0.71
N Def + 0.5 % Urea	102	234	212	425	1 : 2.29	0.62	0.67
N Def + 0.5 % U / P	150	258	312	469	1 : 1.72	0.91	0.74
N Def + 1 % Urea	144	276	300	502	1 : 1.92	0.87	0.79
N Def + 1 % U / P	140	318	292	578	1 : 2.27	0.85	0.91
N Def + Pheroids	138	274	287	498	1 : 1.98	0.84	0.79
Control + 0.5 % Urea	176	389	367	707	1 : 2.21	1.07	1.11
Control + 0.5 % U / P	169	337	352	612	1 : 1.99	1.02	0.97

a) Initial protein content of leaves at week 0 before any treatments

b) Increase (%) in leaf protein content from week 0 to week 8

c)Ratio in protein content between bottom- and top leaves after 8 weeks. Ratio in week one: 1 : 1.14.

d) Ratio in protein content between bottom- and top leaves of treated plants compared to control pants.

The protein content of both bottom- and top leaves of control plants increased from week 0 to week 8, an increase of approximately 344% and 634% respectively. The plants grown under nitrogen limiting conditions had a lower increase in protein content, 271% and 453% when compared to the control plants. Spraying the plants grown under nitrogen limiting conditions with additional nitrogen in the form of the 0.5% and 1% urea solutions, singly or mixed with Pheroids, gave mixed results. However, it appeared that applying the 1% urea solution mixed with Pheroids resulted in the highest levels of protein in the leaves of plants grown under nitrogen limiting conditions. Thus, it appeared that spraying the nitrogen deficient plants with 1% urea, singly and mixed with Pheroids, are the best treatments to alleviate the effect of nitrogen limiting conditions on the protein content between the top and bottom leaves.

When the control plants were sprayed with a 0.5% urea solution, an increase in leaf protein content was observed, especially in the top leaves. However, mixing the 0.5% urea solution with Pheroids resulted in a reduction in the protein content of the top leaves and only a marginal increase in the protein content of the bottom leaves occurred.

The ratio in the protein content between the bottom- and top leaves indicated a translocation of nitrogen from the bottom to the top leaves. A ratio greater than 1 : 1.14 in week 8 implied that nitrogen was translocated within the plant more effectively as in week 0. If the ratio exceeds 1 : 2.11, nitrogen was translocated more effectively in that specific treatment than in the control plants and *vice versa*.

Comparing leaf protein content of the different treatments to the leaf protein content of the control plants, ratios lower than 1 was found for all treatments. However, most of the corrective treatments showed a ratio higher than the 0.79 and 0.71 for the bottom and top leaves respectively, suggesting that these corrective treatments were partially successful in alleviating the effect nitrogen

limiting conditions had on the protein content of the leaves. The control plants which were sprayed with the 0.5% urea solution, singly or mixed with Pheroids, showed similar but slightly higher leaf protein content than the control plants.

4.4 GENERATIVE DEVELOPMENT

The generative development of the plants was recorded for a period until the first fruits were harvested. This was done to determine the effect of a nitrogen deficiency and the subsequent corrective treatments on generative development of the plants. A delay in generative development, caused by sub-optimal supply of nitrogen, may ultimately affect yield and net income.

Table 4.2 Generative development as influenced by a nitrogen deficiency and the addition of extra nitrogen through urea and Pheroids foliar applications.

Treatments	Time lapse from transplantation to the phase of the first:						
	buds	open flowers		fruits (5mm)		fruits harvested	
	DAT	DAT	DFPD *	DAT	DFPD *	DAT	DFPD *
Control	27 ± 0	35 ± 2	8	47 ± 5	12	112 ± 9	65
Nitrogen Deficient	30 ± 3	39 ± 4	9	55 ± 4	16	118 ± 0	63
N Def + 0.5% Urea	28 ± 1	35 ± 2	7	48 ± 4	13	114 ± 8	66
N Def + 0.5% U / P	28 ± 0	37 ± 2	9	52 ± 4	15	109 ± 8	57
N Def + 1% Urea	28 ± 0	37 ± 3	9	52 ± 8	15	109 ± 8	57
N Def + 1% U / P	28 ± 0	35 ± 3	7	48 ± 8	13	114 ± 8	66
N Def + Pheroids	28 ± 1	35 ± 2	7	48 ± 4	13	109 ± 8	61
Control + 0.5% Urea	28 ± 0	36 ± 0	8	50 ± 0	14	105 ± 0	55
Control + 0.5% U / P	28 ± 0	36 ± 0	8	50 ± 0	14	112 ± 0	62

* DFPD = Time lapse from previous phase of development

DAT = Day(s) after transplantation

The first buds appeared 27 days after transplantation on control plants (Table 4.2). It appeared that a nitrogen deficiency affected generative development as the first buds on the plants grown under nitrogen limiting conditions appeared only 30 days after transplantation. Compared to control plants, a nitrogen deficiency thus delayed the formation of the first buds by three days. Spraying these nitrogen deficient plants with 0.5% and 1% urea solutions, singly or mixed with Pheroids, all stimulated bud formation, as the first buds appeared one day earlier, at day 28, for these treated plants (Table 4.2).

The time for the first buds to develop into open flowers took 8 days for the control plants, while this process took one day longer in the nitrogen deficient plants. Spraying these nitrogen deficient plants with 0.5% urea, 1% urea mixed with Pheroids and Pheroids alone all stimulated the process and resulted in open flowers after seven days, outperforming the control plants by one day. Spraying nitrogen deficient plants with 0.5% urea mixed with Pheroids and 1% urea had no effect on this process. Similar results were found by applying additional 0.5% urea, singly or mixed with Pheroids, to control plants. Thus, applications of urea singly, or mixed with Pheroids had no obvious effect on the time lapse from bud formation to opening of the flowers.

It took 12 days from open flowers for the first fruits of 5 mm in diameter to form in control plants. This developmental process was retarded by four days in plants grown under nitrogen limiting conditions. Spraying these nitrogen deficient plants with the additional nitrogen corrective sprays all stimulated the formation of fruit. All these treatments took at least two days less than the nitrogen deficient plants to develop fruit. Foliar application of 0.5% urea, singly or mixed with Pheroids, to control plants grown under optimal nitrogen conditions failed to further stimulate fruit formation.

A time lapse of 65 days occurred before the fruits developed to a stage when it was harvested in control plants. In contrast, it took only 63 days before the fruits were harvested from nitrogen deficient plants. Treating nitrogen deficient plants with a 0.5% urea solution or a 1% urea solution mixed with Pheroids delayed fruit maturation by three days in nitrogen deficient plants. However, applying 0.5% urea solution mixed with Pheroids and 1% urea solution to the nitrogen deficient plants shortened the period of fruit maturation from 63 days to 57 days. When control plants were treated with additional nitrogen, a similar shortening of fruit maturation period was seen. Treating control plants with 0.5% urea mixed with Pheroids shortened fruit maturation period from 65 days to 62 days. Applying 0.5% urea singly to control plants shortened this period by 10 days.

Taking the total time lapse from transplantation to fruit harvest into account, control plants took 112 ± 9 days before the first fruits were harvested. In contrast, it took 118 days before any fruits could be harvested from the nitrogen deficient plants. All the corrective treatments used shortened the time lapse from transplantation to harvest by at least four days. Applying 0.5% urea mixed with Pheroids, 1% urea and Pheroids singly shortened this period with nine days, which is three days less than in the control plants. In addition, an improvement of seven days was observed when control plants were sprayed with 0.5% urea.

In Table 4.3, the time gain or loss by the different treatments are summarized. All treatments which supplied additional nitrogen to nitrogen deficient plants reduced the time from transplantation to harvest, indicating that these corrective treatments markedly alleviated the negative effect nitrogen limiting conditions had on generative development.

Table 4.3 Time gain and loss when control- and nitrogen deficient plants were sprayed with 0.5% and 1% urea solutions, singly or mixed with Pheroids.

Treatments	Time from transplantation to harvest (Days)	Time gain / loss i.t.o Control plants *
Control	112 ± 9	0
Nitrogen Deficient	118 ± 0	+ 6
N Def + 0.5% Urea	114 ± 8	+ 2
N Def + 0.5% Urea / P	109 ± 8	- 3
N Def + 1% Urea	109 ± 8	- 3
N Def + 1% Urea / P	114 ± 8	+ 2
N Def + Pheroids	109 ± 8	- 3
Control + 0.5% Urea	105 ± 0	- 7
Control + 0.5% Urea / P	112 ± 0	0

* Positive values indicate a delay in time lapse from transplantation to fruit harvest. Negative values indicate a gain in time from transplantation to harvest when compared to control plants.

4.5 YIELD

The number, mass and size of fruits harvested were the parameters used to determine the effect of a nitrogen deficiency on yield. The principle aim of the study was to determine whether the application of additional nitrogen in the form of foliar sprays of urea solutions, singly or mixed with the novel compound, Pheroids, was successful in alleviating the possible negative effect a nitrogen deficiency might have on yield. Furthermore, by applying these urea solutions, singly or mixed with Pheroids, to control plants may result in an even higher yield.

4.5.1 Number, mass and size of fruits harvested

Fruit number, mass and size was recorded over the harvest period of 25 weeks.

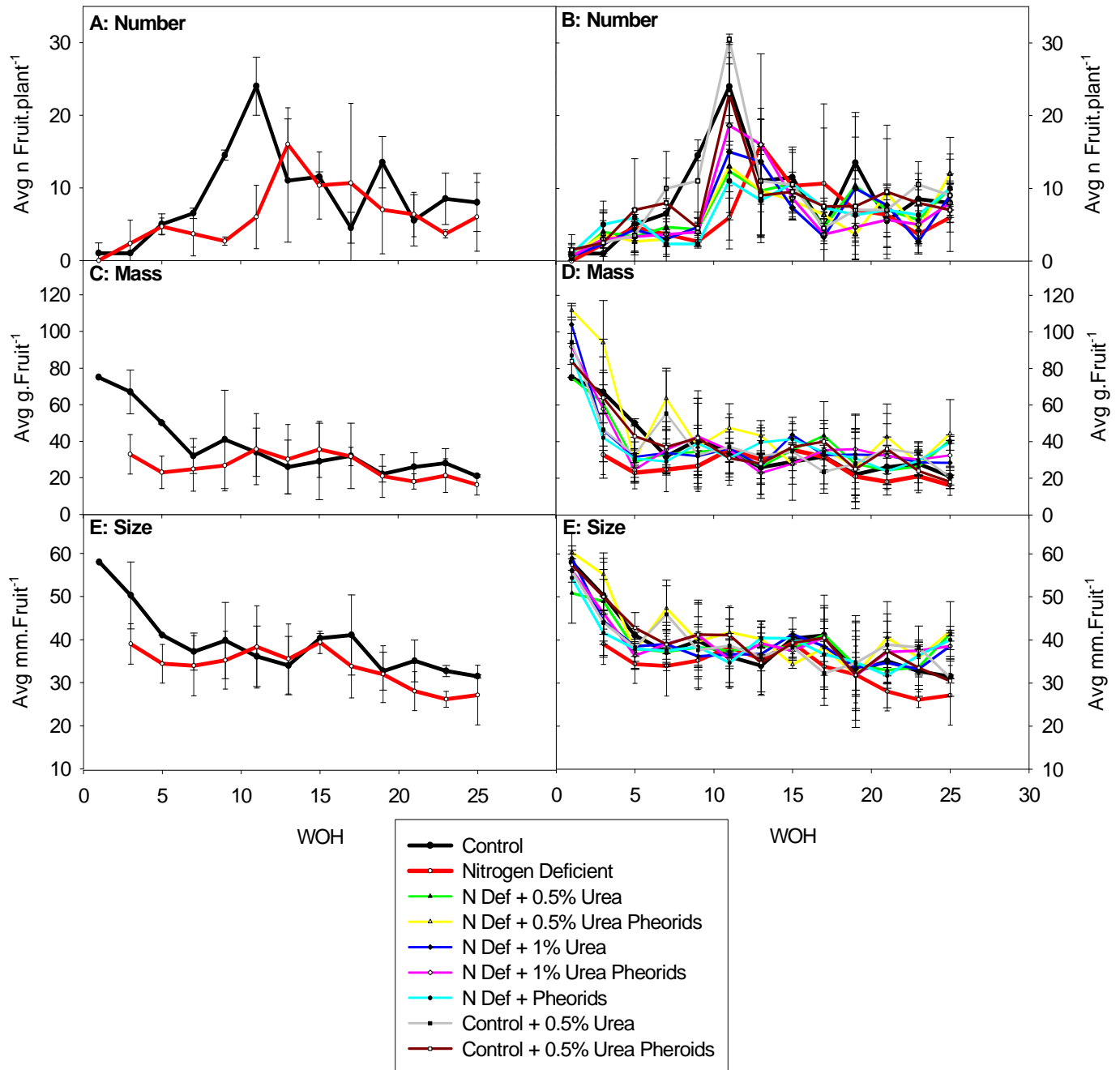


Figure 4.10 Average weekly number of fruit harvested (A & B), fruit mass (C & D) and fruit size (E & F) over a trial period of 25 weeks.

The average number of fruit harvested from control plants steadily increased up to week 11 after transplantation, followed by a decline in the number of fruit harvested for the remainder of the harvest period. Fruit from nitrogen deficient plants were initially harvested a week later and produced the highest number of fruit harvested in week 13, two weeks later than the control plants. In addition, the number of fruit produced during these periods is less in the plants grown under nitrogen limiting conditions than in the control plants. Thus, the control plants produced the highest number of fruit earlier in the harvest period than the nitrogen deficient plants (Fig. 4.10 A).

By applying urea solutions, singly or mixed with Pheroids, to nitrogen deficient plants, the highest number of fruit was harvested at the same time as the control plants. This implies that the foliar application of urea solutions, singly or mixed with Pheroids, improved and shortened the time lapse from transplantation to the time when the highest number of fruit were harvested (Fig. 4.10 B).

An overall decline in fruit mass harvested was observed for fruit from control plants as the harvest period progressed (Fig. 4.10 C). This appeared to be a natural occurrence in tomato plants where bigger fruit are harvested at the start of the harvest period with a subsequent decline in mass towards the end of the harvest period. This also corresponds to a reduction in fruit size (Fig. 4.10 E). In contrast, the fresh mass of the fruits harvested from the plants grown under nitrogen limiting conditions remained fairly constant during the harvest period.

Moreover, on average the control plants produced heavier fruit (Fig. 4.10 C), but not necessarily bigger fruits (Fig. 4.10 E), than the plants grown under nitrogen limiting conditions (Fig 4.10 C). Spraying these nitrogen deficient plants with the 0.5% and 1% urea solutions, singly or mixed with Pheroids, marginally enhanced the mass of the fruit harvested (Fig. 4.10 D), but failed to completely alleviate the effect of nitrogen limiting conditions.

The control plants had slightly bigger fruit than the nitrogen deficient plants (Fig. 4.10 E). Spraying these nitrogen deficient plants with urea, singly or mixed with Pheroids, slightly improved fruit size (Fig. 4.10 F). However, spraying control plants with additional 0.5% urea, singly or mixed with Pheroids, had no clear effect on fruit size.

The total yield (accumulated number and mass) are all the fruits harvested from a plant during the harvest period. The total accumulated yield of the plant gives a true representative effect the nitrogen limiting levels and the subsequent corrective treatments had on yield. Control plants showed a much higher yield in both the number of fruit harvested and its mass than the plants grown under nitrogen limiting conditions after a harvest period of 25 weeks (Fig. 4.11 A & B).

Spraying these nitrogen deficient plants with 0.5% and 1% urea solutions improved yield in terms of mass (Fig. 4.11 C), but had little to no effect on the number of fruit produced (Fig. 4.11 D). However, mixing these 0.5% and 1% urea solutions with Pheroids resulted in a further enhancement on fruit mass, but still had little effect on the number of fruit produced (Fig. 4.11 D). Spraying nitrogen deficient plants with Pheroids only, also increased yield (mass), similar to when it was mixed with a 1% urea solution (Fig. 4.11 C). Spraying nitrogen deficient plants with the 0.5% urea / Pheroids mixture was the most successful treatment in preventing the effect a nitrogen deficiency had on yield in terms of mass. However, none of the corrective foliar sprays used could completely alleviate the effect of nitrogen limiting conditions on the mass and number of fruits harvested over a harvesting period of 25 weeks.

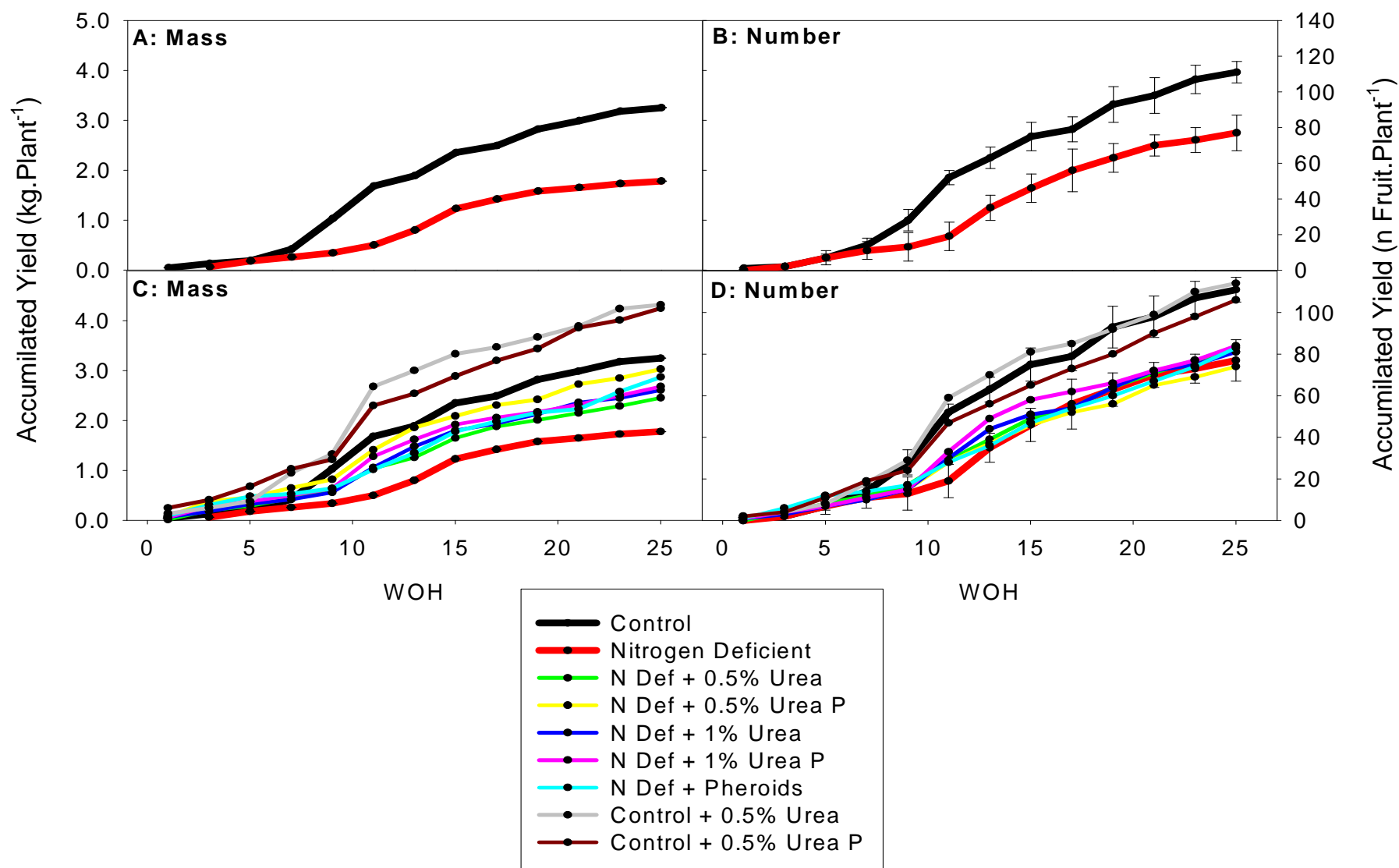


Figure 4.11 Accumulated yield for mass (A & C) and number (B & D) of fruit harvested over a period of 25 weeks.

WOH = Weeks of harvest.

Spraying control plants with a 0.5% urea solution, singly and mixed with Pheroids, markedly improved the yield (mass) under conditions of optimal nitrogen supply (Fig. 4.11 C). However, the effect of these treatments was not as marked on the number of fruits harvested. When the 0.5% urea solution was mixed with Pheroids and sprayed onto the control plants, the number of fruits harvested from control plants was reduced (Fig. 4.11 D).

Summarizing yield after a harvest period of 25 weeks, the average total accumulated yield, in terms of number and mass, can be compared between the different treatments (Table 4.4). The nitrogen deficient plants produced much less fruit, approximately 30%, than the control plants. Spraying the plants grown under nitrogen limiting conditions with 0.5% and 1% urea solutions, singly or mixed with Pheroids, had no apparent effect on the number of fruit harvested. The same effect was observed when control plants were sprayed with 0.5% urea, singly and mixed with Pheroids.

The plants grown under nitrogen limiting conditions produced approximately half the yield (mass) compared to control plants. However, spraying these plants with 0.5% and 1% urea solutions, all improved yield. When these urea solutions were mixed with Pheroids, an even higher improvement in yield was observed, specifically in the plants sprayed with the 0.5% urea/Pheroids mixture, where a yield approximately equal to control plants was achieved. Increasing the urea solution to 1% and spraying these plants, singly or mixed with Pheroids, also improved yield, but not as distinctly as the 0.5% urea/Pheroids treatment. It appeared that the yield in terms of mass of the control plants could further be improved by spraying these plants with 0.5% urea, singly and mixed with Pheroids.

Table 4.4 Average fruit number, mass, size and yield after 25 weeks of harvest.

Average:	Control Plants	Nitrogen deficient Plants +						Control Plants +	
		No foliar treatment	0.5% Urea	0.5% Urea P	1% Urea	1% Urea / P	Pheroids	0.5% Urea	0.5% Urea / P
n Fruit.plant⁻¹	108 ± 44	78 ± 21	81 ± 14	76 ± 4	81 ± 11	82 ± 11	81 ± 5	115 ± 8	108 ± 3
g.Fruit⁻¹	31 ± 7	26 ± 11	35 ± 12	42 ± 11	39 ± 11	38 ± 12	38 ± 12	39 ± 10	40 ± 7
mm.fruit⁻¹	39 ± 4	33 ± 5	39 ± 4	42 ± 4	39 ± 4	39 ± 4	38 ± 4	39 ± 4	41 ± 4
kg.plant⁻¹	3.35 ± 0.53	2.08 ± 0.05	2.83 ± 0.44	3.19 ± 0.24	3.16 ± 0.22	3.12 ± 0.92	3.08 ± 0.93	4.48 ± 0.52	4.32 ± 0.17

The control plants also produced considerably more fruit than the nitrogen deficient plants for the harvest period (Table 4.4). Spraying these nitrogen deficient plants with 0.5% and 1% urea solutions, singly or mixed with Pheroids, as corrective treatments, had no apparent effect on the number of fruits harvested. Approximately the same number of fruits were harvested from the nitrogen deficient treated with different corrective treatments. In contrast, it appeared that by spraying control plants with a 0.5% urea solution increased the number of fruit harvested from 108 to 115 fruits. This was not apparent when the 0.5% urea solution was mixed with Pheroids.

The average fruit mass and size varied considerably over the harvest period for all the treatments (Fig. 4.10). A marked difference between fruit size and mass of the control plants and nitrogen deficient plants were observed. However, all the different corrective treatments could fully alleviate the effects nitrogen limiting conditions had on average fruit size and mass. In fact, all the corrective treatments resulted in bigger and heavier fruit than the control plants. Thus, foliar applications of urea, singly and mixed with Pheroids, all fully alleviated the effect nitrogen limiting levels had on fruit size and mass. The best results were obtained when nitrogen deficient plants were sprayed with the 0.5% urea/Pheroids mixture. Moreover, spraying the control plants with both the 0.5% urea and 0.5% urea/Pheroids solutions also increased fruit size, but had no clear effect on mass (Table 4.4).

The individual mass of the fruits harvested were combined to give a total accumulated yield for each treatment (Fig. 4.11). The control plants produced a higher total accumulated yield than the plants grown under nitrogen limiting conditions. It appeared that all the corrective treatments was fairly successful in alleviating the effects a nitrogen deficiency had on total yield. Total yields of these treated plants were only slightly lower than the yield for the control plants. Spraying control plants, which was already growing under optimal conditions,

with a 0.5% urea solution, singly and mixed with Pheroids, further improve the total yield of these plants?

4.5.2 Market implication

The total yield of a crop directly influences income. Thus, a reduction in yield will inevitably result in a lower income due to the market price obtained. A nitrogen deficiency may result in a low yield, which inevitably will reduce net income. The effect of nitrogen limiting conditions and the subsequent corrective treatments on market value were subsequently calculated (Table 4.5). The experimental setup used in this study (Fig. 3.1) of 3.5 plants.m⁻² represent approximately 35 000 plants.ha⁻¹. Using total yield after 25 weeks of harvest (Table 4.4), the potential yield per hectare was calculated. In addition, using the current prices, gross income per hectare can be calculated. These estimates are summarized in Table 4.5.

Nitrogen limiting conditions ultimately reduced gross income (Table 4.5). Treating these plants with additional nitrogen alleviated the reducing effect of a nitrogen deficiency on yield and potential loss in income to a certain extent.

Cultivating plants under control conditions resulted in an income of approximately R 526 452.ha⁻¹. This is substantially higher than the R 326 872.ha⁻¹ obtained for the plants grown under nitrogen limiting conditions. This corresponds to a 37.91% reduction in gross income over a period of 25 weeks.

Spraying 0.5% and 1% urea solutions onto nitrogen deficient plants resulted in a marked improvement by reducing the loss in income from approximately 38% to 15% and 5% respectively. Moreover, mixing these 0.5% and 1% urea solutions with Pheroids further improved the income and reduced losses to only 4% and 6% respectively less than the income from the control plants. The latter treatments thus resulted in nearly equal incomes to that of the control plants. In

addition, spraying the nitrogen deficient plants with Pheroids only also markedly increased the gross income from R 326 872.ha⁻¹ to R 484 020.ha⁻¹, limiting losses to a level which is only 8% less than the income from the control plants. Thus, Pheroids itself, without mixing it with urea, are successful in limiting losses in income in plants grown under nitrogen limiting conditions.

Control plants sprayed with a 0.5% urea solution, singly or mixed with Pheroids, responded positively to the additional nitrogen. It was found that when control plants were sprayed with a 0.5% urea solution the income increased from R 526 452.ha⁻¹ to R 704 032.ha⁻¹, an increase of nearly 34% in income. However, mixing this 0.5% urea solution with Pheroids as a treatment proved to be less effective. The income increased from R 526 452.ha⁻¹ to only R 678 888.ha⁻¹, an increase of approximately 29% (Table 4.5).

Table 4.5 The effect of a nitrogen deficiency and the subsequent corrective treatments on yield and gross income over a harvest period of 25 weeks.

Treatments	Yield (kg.plant ⁻¹)	Yield Ton.ha ⁻¹ (est.) (a)	Income R.ha ⁻¹ (b)	% loss / gain (c)
Control	3.35 ± 0.53	117.25	R 526 452	0
Nitrogen deficient	2.08 ± 0.05	72.80	R 326 872	- 37.91
N def + 0.5 % U	2.83 ± 0.44	99.05	R 444 734	- 15.52
N def + 0.5 % U / P	3.19 ± 0.24	111.65	R 501 308	- 4.77
N def + 1 % U	3.16 ± 0.22	110.60	R 496 594	- 5.67
N def + 1 % U / P	3.12 ± 0.92	109.20	R 490 308	- 6.68
N def + Pheroids	3.08 ± 0.93	107.80	R 484 020	- 8.06
Control + 0.5 % U	4.48 ± 0.52	156.80	R 704 032	+ 33.73
Control + 0.5 % U / P	4.32 ± 0.17	151.20	R 678 888	+ 28.95

(a) 35 000 plants.ha⁻¹ x yield (kg.Plant⁻¹) ÷ 1000 = Ton.ha⁻¹

(for 35 000 plants.ha⁻¹ – see 3.2.1.1).

(b) Ton.ha⁻¹ x R 4.49 / kg⁻¹ (Trade in Feb 2009) = R.ha⁻¹

(c) % loss/gain when compared to control plants.

4.6 FRUIT QUALITY

Nitrogen limiting conditions may not only impair yield, but may also cause a reduction in fruit quality. Various quality parameters were used to determine fruit quality and were recorded for the first 16 weeks of the 25 week harvest period. These parameters include fruit pH, electrical conductivity (EC), % Brix index, moisture content, as well as lycopene content. Each of these parameters may individually be influenced by low nitrogen conditions and the subsequent corrective treatments used. Each parameter has its unique characteristic to describe fruit taste, integrity and overall appearance (quality).

4.6.1 pH, Electrical conductivity, % Brix and moisture content of the fruit

pH is a very important parameter in determining fruit taste (Dorai *et al.*, 2001) and may vary depending on cultivar type, etc. Tomato fruit are naturally acidic fruit and therefore have a relatively low pH (pH 4; Atherton & Rudich, 1986). The pH was recorded for the first 16 weeks of the harvest period (Fig. 4.13 A & B).

Fruits from the control plants had a pH which varied between 4.0 and 4.2 for most of the harvest period (Fig. 4.12 A & B). The pH of fruit from the nitrogen deficient plants remained fairly constant at a pH of 4.0, lower than that recorded for control fruits (Fig. 4.12 A). The slight differences in pH of fruit harvested from control plants and those harvested from nitrogen deficient plants will have no noticeable influence on fruit taste. As a result of the small differences in pH of the control- and nitrogen deficient plants, applications of the 0.5% and 1% urea solutions, singly or mixed with Pheroids, had no obvious effect of the pH of the fruit.

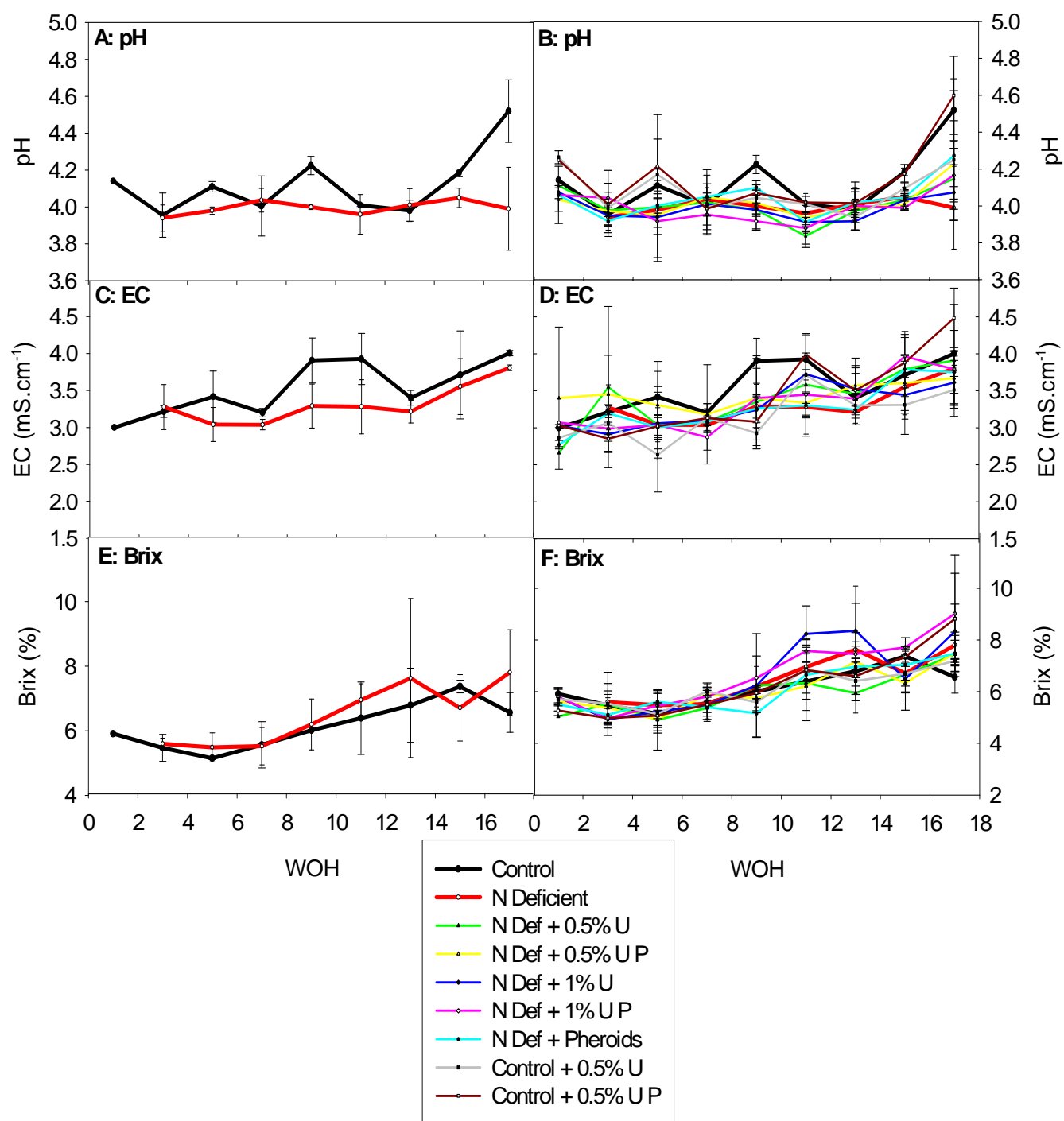


Figure 4.12 Changes in fruit pH (A & B), EC (C & D) and % Brix (E & F) between the different treatments for the duration of the harvest period.

Fruit electrical conductivity is another important parameter used to determine fruit taste and overall fruit quality (Dorai *et al.*, 2001). By measuring electrical conductivity, the number of dissolved salts, which affects overall fruit taste, was determined.

No marked differences in fruit EC between fruits from control- and nitrogen deficient plants could be observed (Fig. 4.12 C & D). Moreover, an increase in fruit EC for all fruits was observed as the harvest period progressed, with a slightly higher EC for fruit from control plants than that from the nitrogen deficient plants (Fig. 4.12 C). As a result of the small differences in EC between the control- and nitrogen deficient plants, applications of 0.5% and 1% urea solutions, singly or mixed with Pheroids, had no clear effect on the EC of the fruit.

The % Brix index gives an indication on total dissolved soluble solids (TSS) within the fruit. The Brix index may be the single most important parameter in determining fruit quality and taste. The fruit Brix index, like fruit EC, also increased for fruits as the harvest period progressed (Fig. 4.12 E & F). No clear differences in Brix index between the fruits from control- and nitrogen deficient plants, as well as all nitrogen deficient plants treated with the corrective treatments could be observed (Fig. 4.12 E & F). Thus, foliar application of the urea solutions, singly or mixed with Pheroids, had no clear effect on the fruit Brix index.

Using the averages obtained for pH, EC and % Brix of the fruit over the entire harvest period, a comparison can be made on which treatments resulted in the overall best quality fruit.

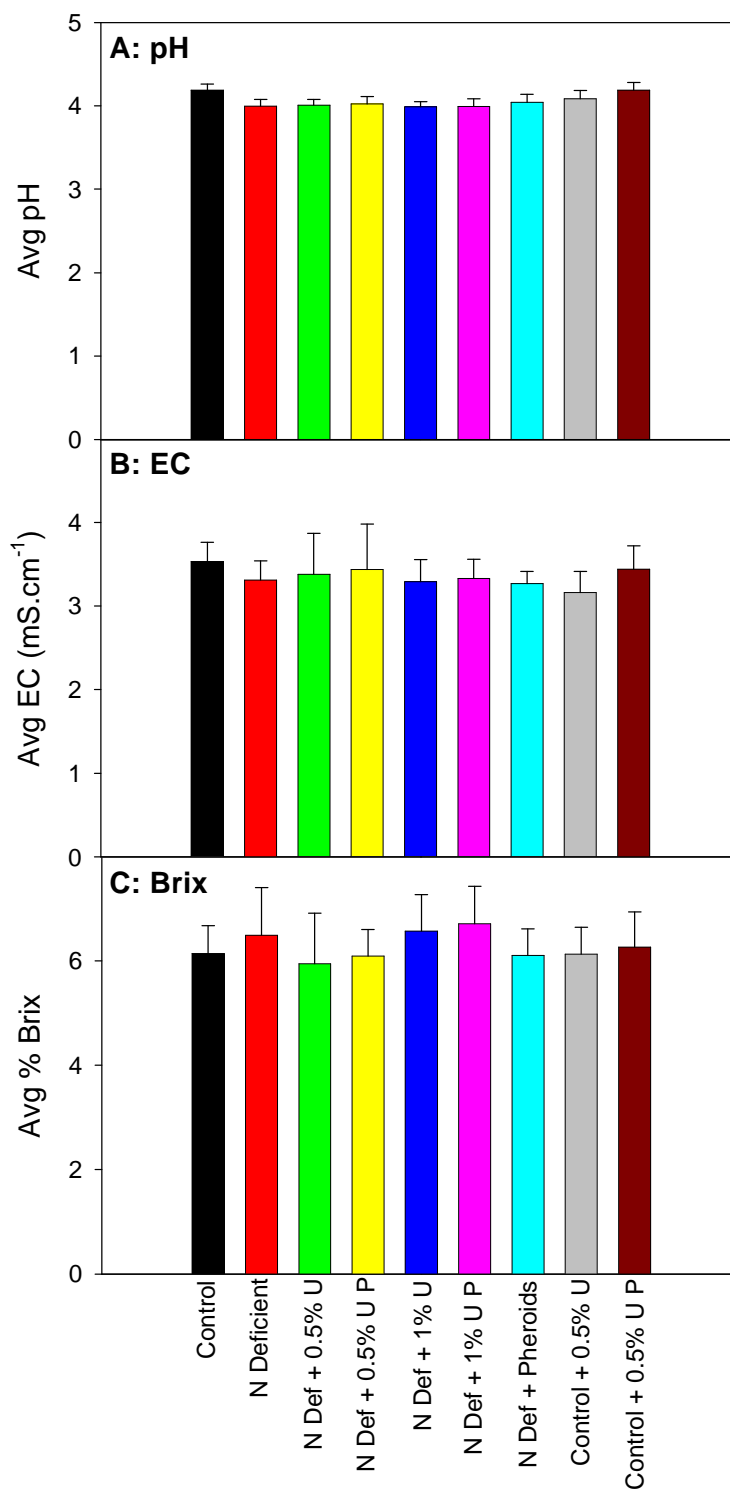


Figure 4.13 Average pH (A), EC (B) and % Brix (C) for fruits harvested over the harvest period of 16 weeks.

On average, the pH of the fruits from control plants was 4.2 for the experimental period. This is slightly higher than the pH 4.0 obtained for the fruits from nitrogen deficient plants. Spraying the nitrogen deficient plants with additional nitrogen in the form of the 0.5% and 1% urea solutions, singly or mixed with Pheroids, have little to no effect on the average pH of the fruit. Similar results were found by spraying control plants with 0.5% urea, singly or mixed with Pheroids (Fig. 4.13 A).

Nitrogen limiting conditions reduced the average fruit EC marginally, when compared to control conditions (Fig. 4.13 B). Spraying these nitrogen deficient plants with a 0.5% urea solution improved the fruit EC levels slightly. This could further be improved by mixing the 0.5% urea solution with Pheroids, resulting in fruit EC levels similar to those found for control plants (Fig. 4.13 B). In contrast, spraying the plants with a 1% urea solution, singly or mixed with Pheroids, as well as with Pheroids alone, had no effect on the EC levels. Moreover, spraying control plants with a 0.5% urea solution, singly and mixed with Pheroids, have an inhibitory effect by decreasing the fruit EC of the control plants. However, the effect of a nitrogen deficiency and the corrective treatments on fruit EC is so marginal that no clear conclusion could be made from these results.

Fruits from nitrogen deficient plants had a higher average % Brix index than the fruit from the control plants (Fig 4.13 C). Spraying nitrogen deficient plants with a 0.5% urea solution, singly or mixed with Pheroids, reduced the % Brix index to a level lower than that of the control plants. In contrast, using a urea solution of 1%, singly or mixed with Pheroids, had no effect on the % Brix index of nitrogen deficient plants. Applying Pheroids only also, like in the 0.5% urea and 0.5% urea/Pheroids treatments, reduced the % Brix index to a level lower than that of the control plants. Spraying control plants with the 0.5% urea solution, singly or mixed with Pheroids, have no effect on the fruit % Brix index levels of these plants.

Other parameters which may affect fruit quality and taste is the deposition of organic molecules (dry mass accumulation) and the moisture content of the fruit (Ertan, 1990).

Changes in the fresh- and dry mass of the fruit were recorded for the first 16 weeks of the 25 week harvest period from which the moisture content and the Fm:Dm ratio were calculated (Fig. 4.14). As mentioned previously (Fig. 4.10 C & D), a natural tendency occurred where the fresh mass of the control fruit decreased as the harvest season progressed (Fig. 4.14 A & B) while the fresh mass of the fruit from the nitrogen deficient plants remained fairly constant during this period. The fresh mass of the fruit from control plants was consistently higher than that of the nitrogen deficient plants (Fig. 4.14 A). None of the foliar treatments used could alleviate the effect a nitrogen deficiency had on the fresh mass of the fruit (Fig. 4.14 B).

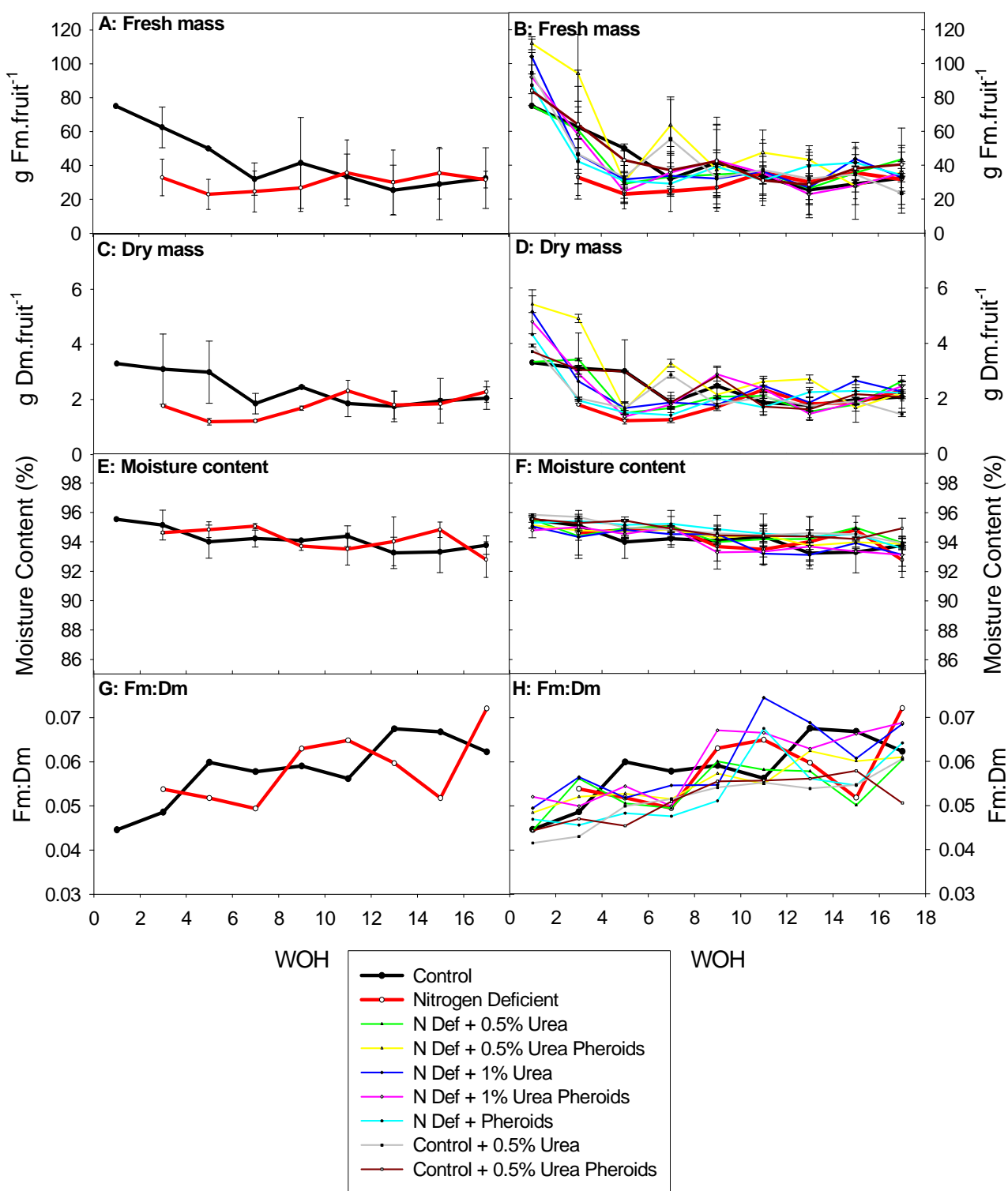


Figure 4.14 Changes in the fresh mass (A & B), dry mass (C & D) and moisture content (E & F) of the fruit and the fresh to dry mass ratio (Fm:Dm). (G & H) over a harvest period of 16 weeks.

Similar results were obtained when the fruit dry mass for the different treatments were recorded (Fig. 4.14 C & D). It also appeared that nitrogen limiting conditions had little effect on the deposition of organic molecules (dry mass), as no clear differences between fruits from control- and nitrogen deficient plants were observed (Fig. 4.14 C). Moreover, no marked changes were observed with the different corrective foliar treatments used in this study (Fig. 4.14 D).

As a result of this, the moisture content of both the control fruits and fruits from nitrogen deficient plants decreased from approximately 95% at the start of the harvest period to approximately 93% at the end of week 16 (Fig. 4.14 E). No clear differences in moisture content between control- and nitrogen deficient fruits could be observed (Fig. 4.14 E). For this reason the different corrective treatments used was ineffective in affecting the moisture content of the fruits (Fig. 4.14 F).

The Fm:Dm increased for both control fruit and the fruits from nitrogen deficient plants as the harvest period progressed. No clear distinction could be made between the different treatments during the first 16 weeks of harvest due to varying fruit mass (Fig. 4.14 A & B) and moisture content (Fig. 4.14 E & F). Although the Fm:Dm increased over the first 16 weeks of harvest, no marked differences between control- and nitrogen deficient fruits (Fig. 4.14 G) were observed. No clear effect on this Fm:Dm was observed when nitrogen deficient plant were sprayed with the different corrective treatments (Fig. 4.14 H).

The average fresh mass, dry mass and moisture content, as well as the average Fm:Dm were recorded over the experimental period of 16 weeks.

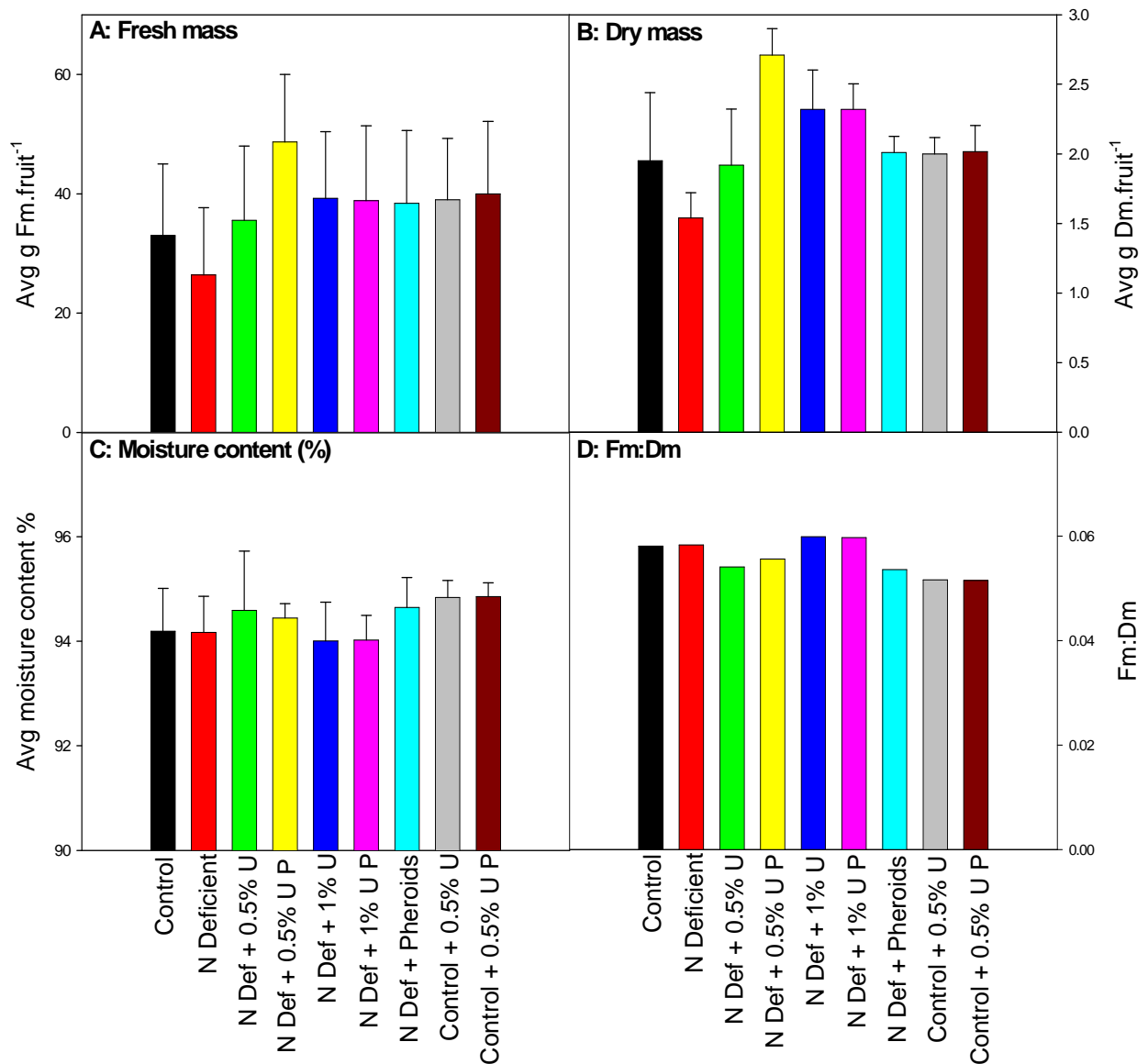


Figure 4.15 Differences in average fruit fresh mass (A), dry mass (B), moisture content (C) and Fm:Dm (D) after the first 16 weeks of harvest.

The average fresh mass of the fruits from the control plants were noticeably higher than that from the nitrogen deficient plants (Fig. 4.15 A). Treating these nitrogen deficient plants with the 0.5% and 1% urea solutions, singly or mixed with Pheroids, all improved the fresh mass of the fruit from nitrogen deficient plants. In fact, it completely alleviated the effect of nitrogen limiting conditions on

the fresh mass (Fig. 4.15 A). Spraying control plants with 0.5% urea, singly and mixed with Pheroids, also increased the fresh mass of the fruit.

The acquisition of dry mass in fruit is due to deposition of organic molecules. A difference in dry mass was observed between fruit from control plants and those of plants grown under nitrogen limiting conditions (Fig. 4.15 B). Spraying nitrogen deficient plants with a 0.5% urea solution improved fruit dry mass from $\pm 1.5 \text{ g Dm.fruit}^{-1}$ to $\pm 2.0 \text{ g Dm.fruit}^{-1}$. Mixing this 0.5% urea solution with Pheroids, further improved fruit dry mass from $\pm 1.5 \text{ g Dm.fruit}^{-1}$ to $\pm 2.7 \text{ g Dm.fruit}^{-1}$. Increasing the urea concentration to 1% also improved dry mass. However, no further increase in dry mass could be achieved by mixing this 1% urea solution with Pheroids. Spraying control plants with a 0.5% urea solution, singly and mixed with Pheroids, had no visible effect on fruit dry mass (Fig. 4.15 B). Thus, the different foliar treatments all alleviated the effect of nitrogen limiting conditions on fruit dry mass.

No difference in fruit moisture content was observed between fruits from control- and nitrogen deficient plants (Fig. 4.15 C). Applying 0.5% urea, singly or mixed with Pheroids, increased the moisture content to levels above that of the control fruits. In contrast, spraying plants with 1% urea, singly and mixed with Pheroids, reduced the moisture content of the fruits. The best improvement in moisture content under nitrogen deficient conditions was achieved when the plants were sprayed with Pheroids only. Spraying control plants with the 0.5% urea solution, as well as the 0.5% urea Pheroids solution, resulted in fruits with higher moisture contents (Fig. 4.15 C).

By calculating the fruit Fm:Dm for the entire harvest period of 16 weeks, no marked differences in Fm:Dm was observed between the fruits from the control plants and those from the nitrogen deficient plants (Fig. 4.15 D). A slight decrease in the Fm:Dm of the fruit was observed when the nitrogen deficient plants were sprayed with a 0.5% urea solution, singly or mixed with Pheroids.

Increasing this 0.5% urea solution to 1%, resulted in a slight increase in the Fm:Dm ratio. Spraying the plants grown under nitrogen limiting conditions with Pheroids singly, resulted in a reduction in the Fm:Dm. Spraying control plants with 0.5% urea, singly and mixed with Pheroids, caused a decline in the Fm:Dm.

4.6.2 Lycopene content of fruit

Lycopene content, which determines the colour intensity of the fruit and because of its anti-oxidant properties (Bramley, 2000; Salunkhe *et al.*, 1974), is another important quality parameter used to measure fruit quality. Salunkhe *et al.* (1974) found that the red colour of tomatoes is directly related to lycopene content. The lycopene content of the fruits varied throughout the harvesting period. The average lycopene content for all treatments was approximately 150 mg.g Dm⁻¹. No clear distinction could be made in the lycopene content between fruits from control- and the nitrogen deficient plants (Fig. 4.16 A). Spraying nitrogen deficient plants with 0.5% or 1% urea solutions, singly or mixed with Pheroids, did not affect lycopene content markedly. Spraying control plants with 0.5% urea, singly or mixed with Pheroids, showed similar results (Fig. 4.16 B).

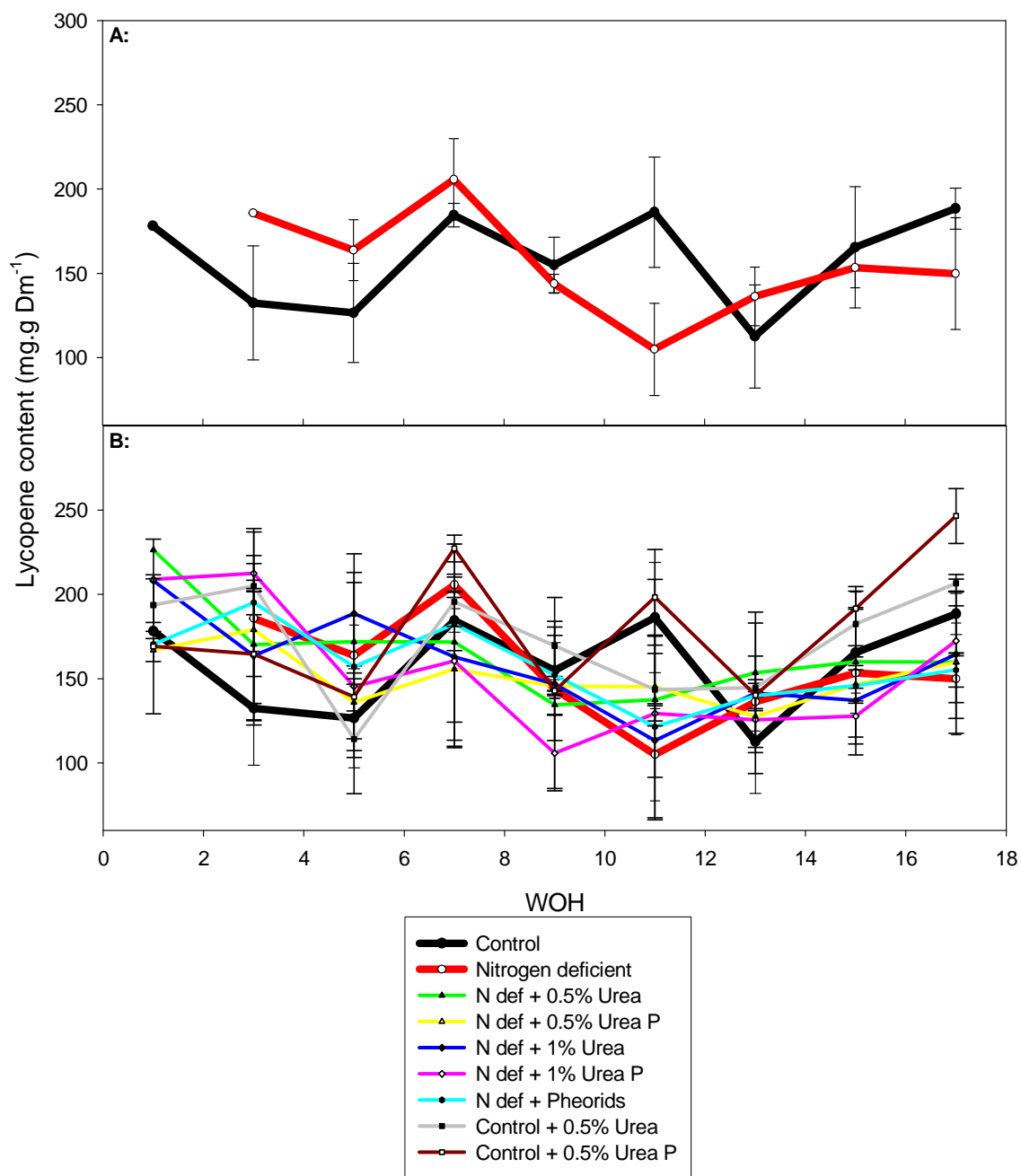


Figure 4.16 Changes in lycopene content of the fruit over a harvest period of 16 weeks.

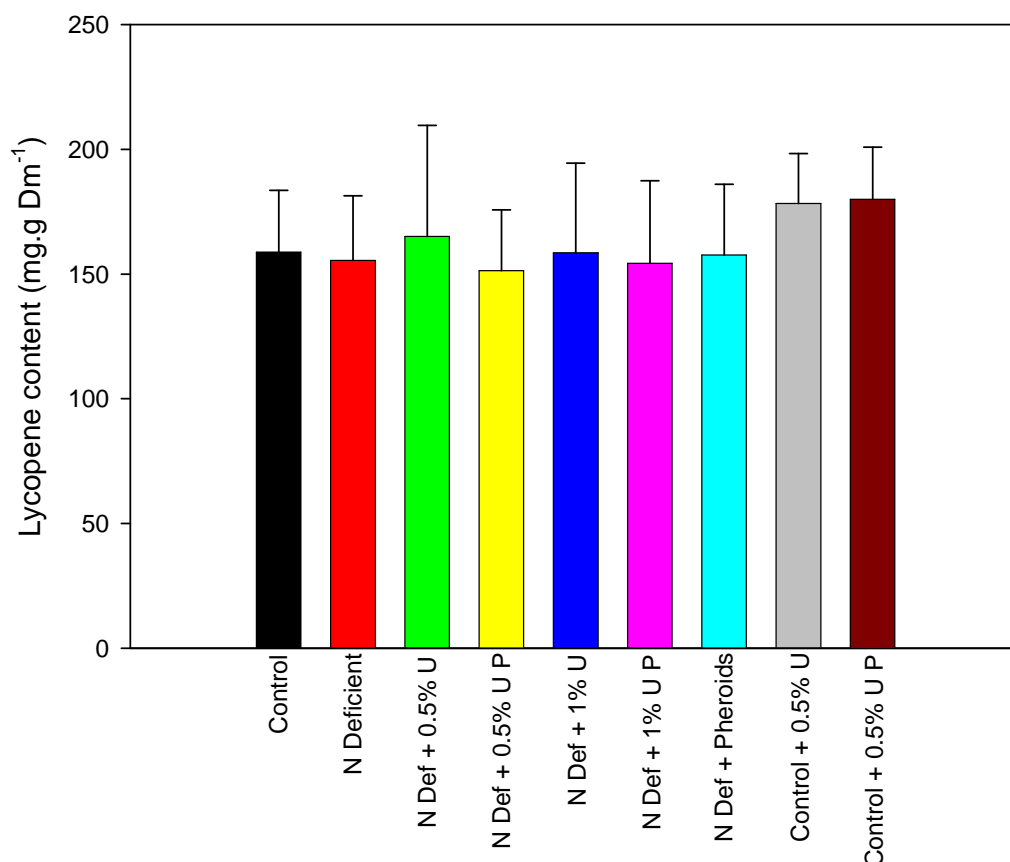


Figure 4.17 Average Lycopene content for fruits from the different treatments over a harvest period of 16 weeks.

No difference in the average lycopene content between fruits from control- and nitrogen deficient plants was observed (Fig. 4.17). Spraying nitrogen deficient plants with a 0.5% urea solution, improved the lycopene content slightly. It can be concluded that none of the other urea solutions used, singly or mixed with Pheroids, had an effect on fruit quality, since none of the treatments used had an effect on the average lycopene content of the fruit over a harvest period of 16 weeks.

Spraying control plants with 0.5% urea, singly or mixed with Pheroids, both increased fruit quality slightly by increasing the average lycopene content of the fruit harvested from these plants (Fig. 4.17).

CHAPTER 5

DISCUSSION

5.1 RATIONALE FOR THIS STUDY

Tomatoes are considered to be one of the most important fruit producing crops in the world. Tomatoes can either be consumed fresh or processed in many products. Yield and quality attributes of tomatoes vary considerably. Factors affecting yield and fruit quality include cultivar type, cultivation method (field or greenhouse), availability of nutrients, subjection to diseases and environmental factors. Among these factors, availability of nutrients probably play the single most important role in determining yield and quality.

For a crop to be healthy and have a high yield, it requires optimal growing conditions. These conditions include light (amount and intensities), humidity, temperature, water and nutrients. Therefore, the study was conducted in a greenhouse where all of these factors were controlled as accurately as possible. The plants were grown in a circulating ebb and flow hydroponic setup, not only to supply the plants with optimal water, but to accurately control nutrient levels.

Plants utilize three major non-mineral elements, namely carbon (C), hydrogen (H) and oxygen (O), which contributes up to 96% of the dry weight of the plant. The remaining 4% of a plants dry weight is made up of different mineral elements. Although only 4% of the plants dry mass consists of mineral elements, these elements are of critical importance in plant metabolism. Mineral elements can be divided into two groups depending on the concentrations required by the plants, namely macro-nutrients and micro-nutrients or trace elements.

Of these nutrients, nitrogen (N) is considered to be the most important macro-nutrient due to its critical role in metabolism and due to its requirement by plants it may become deficient first (Salisbury & Ross, 1991; Atherton & Rudich, 1986). Nitrogen is usually absorbed from the soil, or obtained from mutualistic plant-microbial relationships, as nitrate (NO_3^-) and ammonium (NH_4^+) ions. Once absorbed, nitrogen is metabolized in various metabolic pathways to yield a vast number of cellular constituents (Salisbury & Ross, 1991). Low levels of nitrogen are most frequently responsible for a low yield.

Nitrogen deficiency is a common phenomenon, due to its role in plant metabolism. Nitrogen in soils may be utilized by soil microbes or may be leached out of the soils, which easily leads to a nitrogen deficiency which can persist. A nitrogen deficiency is first observed in older leaves because of the mobility of nitrogen in plants (Salisbury & Ross, 1991). It moves with the transpiration stream (Kirkby & Mengel, 1967) to younger tissues where a higher input of metabolites is required due to a higher metabolic rate in these tissues. Visually, a nitrogen deficiency is observed as chlorosis in older leaves and retarded or stunted growth in new shoots. In severe cases the leaves finally become necrotic and fall off (Devlin, 1969).

In order to correct nitrogen deficiencies, nitrogen can be supplied additionally to plants, either through the soil or as foliar treatments. One common practice is to spray plants with urea, which contains 46% nitrogen per weight (Wittwer *et al.*, 1963; Yamanda *et al.*, 1965; Knoche *et al.*, 1994; Bondada *et al.*, 2001). However, urea may cause burning of leaves when applied at concentrations exceeding 2%. Therefore, urea is more frequently applied with lower concentrations at 0.5% or 1% (Atherton & Rudich, 1986). Applications of urea as a foliar spray have given responses in yield approximately equal to that when fertilizer was applied to the soil (Nicoulaud & Bloom, 1996; Maximum yield, 2003).

When urea is applied to the leaves, it may be absorbed rapidly (Wittwer *et al.*, 1963; Nicoulaud & Bloom, 1996). However, not all urea is absorbed (Lui *et al.*, 2003). The transport proteins may become saturated with urea, temperature may play a role or the urea mixture may evaporate from the leaf surface. Thus, an attempt has to be made to improve or enhance the absorption of urea by the leaves (Lui *et al.*, 2003).

Pheroids is marketed by Elementol (Pty) Ltd, which proposes that this lipid like molecule apparently facilitates the transport of beneficial substances or molecules over membranes. When Pheroids is mixed with a substance, it apparently encapsulates the substance and “disguises” its chemical attributes. Due to its lipid like structure, Pheroids is readily absorbed by the leaves, thereby facilitating the transport of the encapsulated substance over the membranes. Elementol (Pty) Ltd, requested the evaluation of Pheroids as a possible vehicle for the transport of phytolegically beneficial substances over membranes.

The rationale for this study therefore was i) to evaluate whether Pheroids, when mixed with urea, can improve the known effects of urea in reducing the effect nitrogen limiting conditions had on yield and fruit quality and to ii) determine whether Pheroids on its own can be promoted as a bio-stimulant. It must be kept in mind that a foliar application of urea is already a successful treatment in alleviating the effects of a nitrogen deficiency (Nicoulaud & Bloom, 1996). The principle aim was therefore to determine whether the positive effect of urea may further be improved by “packing” it with Pheroids.

This was done by exposing tomato plants to optimum- (control) and nitrogen limiting conditions using a controlled ebb and flow hydroponic set-up. Additional nitrogen was then supplied to the plants in the form of the 0.5% and 1% urea solutions sprayed every two weeks onto the vegetative parts. This was done, firstly to measure the effect of nitrogen limiting conditions on vegetative growth, yield and fruit quality under controlled hydroponic conditions.

Secondly, the capacity of urea, as an additional source of nitrogen, to reduce the effect of nitrogen limiting conditions was subsequently determined.

To investigate the ability of Pheroids to further enhance the absorption and transport of urea into leaves, the 0.5% and 1% urea solutions were “packed” or encapsulated with Pheroids, before these mixtures were sprayed onto the plants. In addition, to determine whether Pheroids itself has any bio-stimulatory ability, Pheroids singly was sprayed onto plants grown under nitrogen limiting conditions.

5.2 PHYSICAL ENVIRONMENT OF CULTIVATION

Light intensity, temperature and relative humidity all affect overall plant metabolism (Salisbury & Ross, 1991), fruit formation and fruit quality (Atherton & Rudich, 1986). The study was conducted inside a greenhouse to control light intensity, temperature and relative humidity as accurately as possible (Fig. 4.1 A - C). The plants were placed randomly in the hydroponic setup to ensure that all the plants were equally exposed to these environmental factors. Greenhouse cultivated tomato plants are usually grown at a light intensity of approximately $2.0 \mu\text{E.m}^{-2}.\text{s}^{-1}$ (Atherton & Rudich, 1986). Light intensity, which is considered to be one of the most important factors in ensuring optimal growth (Atherton & Rudich, 1986), varied between acceptable norms of $3200 \mu\text{E.m}^{-2}.\text{s}^{-1}$ and $800 \mu\text{E.m}^{-2}.\text{s}^{-1}$ (Fig. 4.1 A) at midday during the experimental period.

The temperature varied between 17°C and 27°C in the temperature controlled greenhouse (Fig.4.1 B). The relative humidity strongly correlated with changes in temperature and varied between acceptable norms of 55% and 96% (Fig. 4.1 C). The optimum relative humidity (RH) for tomato production in greenhouses ranges between 60% and 70% (Buitelaar, 1983). It has also been shown that a high humidity had no marked effect on yield (Welles, 1985). The above mentioned environmental factors all varied between acceptable norms. All the plants were

subjected equally to these factors. Thus, any changes measured in vegetative growth, generative development, yield and fruit quality were attributed to the different treatments used.

By growing tomato plants in a hydroponic setup, nitrogen levels could be accurately controlled. The plants were grown in nitrogen adequate (control) and nitrogen limiting nutrient media. The control medium contained 210 ppm nitrogen, while the nitrogen limiting medium contained 70 ppm nitrogen. Nitrogen accounts for approximately 100 mg.kg⁻¹ (100 ppm) and above of dry matter. Thus, any supply of nitrogen less than 100 ppm is considered to be limiting (Atherton & Rudich, 1986). The control medium also contained three times more (3 : 1) nitrogen than the nitrogen limiting medium. The quality of both media was constantly monitored throughout the study.

The EC of both the control- and nitrogen limiting media was approximately 1.7 mS.cm⁻¹ (Fig. 4.2 A – D), within the acceptable values of 2.1 mS.cm⁻¹ (Romer, 1997) to 2.6 mS.cm⁻¹ (du Plessis, 2001) reported for tomatoes. The pH of the nutrient media also ranged within acceptable norms for tomatoes (du Plessis, 2001), namely between 6.2 and 7.3 for both the control- and nitrogen limiting media (Fig. 4.4 A – D).

5.3 PREVENTION OF THE REDUCING EFFECT OF NITROGEN LIMITING CONDITIONS ON VEGETATIVE AND GENERATIVE DEVELOPMENT IN TOMATOES

During the initial stages of establishment, from transplantation up to six weeks thereafter, before any generative development commences, no differences in the rate of vegetative growth (plant height) was observed between the control plants and the plants grown under nitrogen limiting conditions (Fig. 4.7 A & B). However, a clear reduction in growth was observed in the nitrogen deficient plants as soon as flowering and fruit set commenced (Fig. 4.7 A). This may imply

that plants grown under nitrogen limiting conditions divert their available nitrogen (metabolites) towards fruit formation and less nitrogen is used to sustain vegetative growth, whereas control plants could sustain vigorous growth during the different stages of generative development.

A high growth rate not only requires a high input of nutrients, but also a sufficient supply of water. It was observed in this study that control plants consumed more nutrient medium than the plants grown under nitrogen limiting conditions (Fig. 4.6). The control plants consumed approximately 315 liters of nutrient medium per plant opposed to the 236 liters by a plant grown under nitrogen limiting conditions (Fig. 4.6). Thus, 79 liters more nutrient medium was consumed by a control plant. This could not have been due to a higher transpiration rate as all plants were subjected to the same environmental factors. This higher nutrient medium uptake indicates a higher metabolic activity as was evident in the differences in plant height observed (Fig. 4.7).

Plants with a high protein content will have the ability to sustain a high metabolic rate in general. Protein consists of approximately 16% nitrogen (Salisbury & Ross, 1991; Rubner, 1885; Atwater & Bryant, 1899; Jones, 1931 and Browne, 1944). Jones (1931) proposed that by measuring protein content, the concentration of nitrogen can be determined by dividing the concentration of protein measured by 6.25. Thus, protein content indirectly indicates the concentration of nitrogen inside the plants (Maynard & Loosli, 1969).

Protein content of the leaves was used in an attempt to indicate metabolic capacity and possibly the translocation of nitrogen between the bottom and the top leaves.

When nitrogen becomes limiting, plants apparently transport nitrogen, in the form of NO_3^- , NH_4^+ and/or amino acids, from older to the younger, more active growing tissues (Salisbury & Ross, 1991; Ireland, 1990). If nitrogen is limited in a plant, the bottom leaves will have a lower nitrogen content because of the translocation of nitrogen to the top leaves.

It was found that the protein content of the top and the bottom leaves increased for both the control- and the nitrogen deficient plants (Fig. 4.9 & Table 4.1) for the first eight weeks after transplantation. The highest differences in protein concentrations were observed in the top leaves between the control- and nitrogen deficient plants. The control plants had the highest concentration and the increase in leaf protein content suggested that these plants had a higher growth rate than the plants grown under nitrogen limiting conditions, as was clearly evident in the differences in plant height (Fig. 4.7) and the rate of nutrient consumption (Fig. 4.6). Moreover, the protein content in the leaves from nitrogen deficient plants was also less than in the control plants. This suggested that the plants grown under nitrogen limiting conditions probably have a lower metabolic rate which resulted in the retarded and slow growth as was observed.

Spraying the plants grown under nitrogen limiting conditions with 0.5% and 1% urea solutions, singly and mixed with Pheroids, in an attempt to alleviate or reduce the nitrogen deficiency, not only stimulated vegetative growth, but also increased the protein content in both the bottom and the top leaves. It also positively affects the ratio in protein content between the bottom and the top leaves.

The ratio of protein concentrations between the bottom and top leaves can be used to indicate whether any translocation of nitrogen between the leaves occurred. The plants grown under nitrogen limiting conditions appear to have a lower translocation of nitrogen from the bottom to the top leaves, than the control plants. This possibly indicates a deliberate transport of nitrogen in the control

plants to supply the top leaves with nitrogen to maintain high vegetative growth. This translocation of nitrogen from the bottom to the top leaves in control plants is high, since the control plants have higher concentrations of nitrogen available in the bottom leaves to transport, than found in the bottom leaves of the nitrogen deficient plants.

It can be postulated that the control plants, which clearly has the highest rate of vegetative development, would also have a higher rate in generative development and subsequent yield, than the plants grown under limiting nitrogen conditions. Generative development entails the development of reproductive structures like buds, flowers and ultimately the fruit. During generative development available nitrogen in the plant has to be divided between vegetative and generative growth. Reduced nitrogen supply clearly affected generative development (Table 4.3) in delaying the formation of buds, flowers and fruit, subsequently delaying yield. Limited nitrogen supply also negatively affected the time lapse between the developmental stages of these structures, as was clearly demonstrated in this study. Low nitrogen levels caused a delay of at least six days from transplantation to fruit harvest (Table 4.3). These plants were clearly unable to sustain a high vegetative growth, as well as generative development.

Spraying nitrogen deficient plants with the 0.5% and 1% urea solutions, singly and mixed with Pheroids, as corrective treatments, all shortened the time span from transplantation to fruit harvest. In some cases fruit from these treated nitrogen deficient plants could be harvested even before that from the control plants.

When Pheroids alone was sprayed onto plants grown under nitrogen limiting conditions, the time lapse from transplantation to harvest was also shortened, indicating that Pheroids itself also contributes to generative development. Although Pheroids doesn't directly contribute to nitrogen levels, it stimulates the plant in some way which reduces the effect of nitrogen limiting conditions on

generative development. Pheroids contains free fatty acids or free fatty acid derivatives which can only be postulated to be metabolized via cellular respiration to release extra energy. This, however, must be investigated in future detailed studies.

When control plants were additionally sprayed with a 0.5% urea solution, the time span was also shortened from transplantation to harvest by at least a week. This clearly indicates that the plants grown under optimal nitrogen conditions actively respond to the addition of additional nitrogen in the form of the foliar application of urea.

It can be concluded that by spraying control- and nitrogen deficient plants with these urea solutions, singly or mixed with Pheroids, not only stimulated vegetative growth (Fig. 4.7), but also generative development in that the development of buds, flowers and fruits are sooner completed which then shortens the time span from transplantation to harvest.

5.4 PREVENTION OF THE REDUCING EFFECT OF NITROGEN LIMITING CONDITIONS ON YIELD

Gul *et al.*, (1967), as well as Atherton and Rudich (1986), showed that yield was directly influenced by different concentrations of available nitrogen. This is because nitrogen forms an integral part of all proteins, enzymes, co-enzymes RNA, DNA and chlorophyll (Salisbury & Ross, 1991). Thus, any factor affecting any of the above mentioned will affect yield.

Assimilates and water contribute to fruit size. Thus, the plants which have the highest assimilate and water incorporation into the fruit will produce the largest and heaviest fruits (Winsor, 1979). Shelp and Shattuck (1986) found that urea applied foliarly to tomatoes was transported directly to the fruit where it contributed in producing larger fruit. Plants subjected to adequate nitrogen levels

would have higher concentrations of proteins, chlorophyll etc. enabling it to produce more assimilates, resulting in more and larger fruit. Metabolism may be lower in plants grown under nitrogen limiting conditions due to a lower concentration of proteins (Fig. 4.9). The incorporation of assimilates like sucrose may also be low which will result in a smaller fruit (Wang *et al.*, 1993). The reduced movement of nitrogen within the plant from leaf tissue to fruits may also contribute to less and smaller fruit forming under nitrogen limiting conditions.

Control plants also produced substantially more fruit than the plants grown under nitrogen limiting conditions (Fig. 4.10 & Table 4.4). It was also observed that the average number of fruits harvested per week for control plants increased till week 11 of harvest, where after it declined. This was also true for the plants grown under nitrogen limiting conditions, but this phenomenon was delayed by at least two weeks. Moreover, the highest average number of fruit harvested in the nitrogen deficient plants was also less than in the case of the control plants (Fig. 4.10).

Spraying the nitrogen deficient plants with the 0.5% urea and 1% urea solutions, singly and mixed with Pheroids, had a beneficial effect in that the time when the highest number of fruit being produced were advanced by two weeks. This was similar to the phenomenon observed in control plants. Although the fruits were harvested sooner in these treated plants, the number of fruit harvested did not necessarily increased (Table 4.4) which clearly are reflected in the final yield obtained (Fig. 4.11).

Fruit mass and size are strongly correlated (Atherton & Rudich, 1986). It was observed that fruit size and mass of both control- and nitrogen deficient plants declined as the harvest period progressed. This is apparently a natural phenomenon. Although there was a decline, the average mass and size of fruits from the control plants was higher than that from nitrogen deficient plants. Thus, not only the number of fruit harvested from nitrogen deficient plants was

retarded, the fruits were also smaller with less weight when compared to that from the control plants.

Spraying these nitrogen deficient plants with the corrective treatments, all appeared to increase both fruit mass and size of these plants (Fig. 4.10; Table 4.4). In fact, these corrective treatments were so successful that on average the fruit mass and size exceed that from the control plants after a harvest period of 25 weeks. Moreover, control plants growing under optimum conditions also reacted to the additional nitrogen when sprayed with the 0.5% urea solution, singly and mixed with Pheroids, in that the average number of fruits harvested, as well as their mass increased (Table 4.4). This was possibly due to an increase in metabolic rate related to elevated nitrogen levels.

Although small differences were observed in the average fruit mass and number of fruits harvested between control- and nitrogen deficient plants, marked differences were obtained when the total accumulated yields were compared. The control plants had a much higher final yield than the plants grown under nitrogen limiting conditions (Fig 4.11; Table 4.4). This was also found by Shelp and Shattuck (1986). Plants grown under nitrogen limiting conditions produced about half the number of fruit and mass when compared to the control plants (Fig. 4.11; Table 4.4) over a harvest period of 25 weeks. The urea applied to the foliage to supply the nitrogen deficient plants with additional nitrogen, was apparently not sufficiently absorbed to completely alleviate the negative effect of nitrogen limiting conditions on fruit development. The relieving effect of urea could be improved by mixing it with Pheroids. This combined mixture further enhanced yield in the nitrogen deficient plants.

This indicated that Pheroids indeed facilitated the further uptake of urea by the leaves. Although an improvement in yield was observed, all the corrective treatments could still not completely alleviate the negative effect of nitrogen limiting conditions on fruit development and subsequent yield. Moreover,

Pheroids itself also markedly improved yield in these plants suggesting that the lipid like composition of Pheroids itself has an alleviating effect regarding nitrogen limiting conditions.

Fruit number and especially mass determines yield, which eventually affects income. A low yield would result in a low income and *vice versa*. Based on a hypothetical case where yield is projected on a per hectare basis, it is clear that plants grown under nitrogen limiting conditions resulted in a $\pm 38\%$ reduction in yield and income when compared to the control plants (Table 4.5). The hypothetical income from control plants was R 526 452.ha⁻¹, whereas the income from plants grown under nitrogen limiting conditions was only R 326 872.ha⁻¹. This is clearly the result of less fruits harvested, which was lighter and smaller than the fruit from the control plants (Fig. 4.11; Table 4.4).

This marked reduction in yield was partially prevented by spraying the nitrogen deficient plants with the 0.5% and 1% urea solutions, singly and mixed with Pheroids, as corrective treatments. Although the 0.5% and 1% urea solutions was successful in reducing the loss in potential income to only 15.5% and 5.6% less than that of the control plants respectively, the best result was obtained when the 0.5% urea solution was mixed (“packed”) with Pheroids. This treatment almost completely reduced the effect nitrogen limiting conditions had on yield (mass). With this treatment a hypothetical income of R 501 308.ha⁻¹ was obtained, which was only 4.8% less than the R 526 452.ha⁻¹ for control plants. In fact, it represents an income of R 174 436.ha⁻¹ more than the R 326 872.ha⁻¹ obtained for the non-treated nitrogen deficient plants.

Moreover, application of Pheroids alone also substantially reduced the loss in potential income. This treatment resulted in a hypothetical income of only 8% less than in the control plants. This clearly demonstrates that Pheroids itself act as a bio-stimulant and has the capacity to reduce the effect of nitrogen limiting conditions on yield in hydroponically grown tomatoes. In addition, the income in

control plants was also enhanced when sprayed with the 0.5% urea solution, singly and mixed with Pheroids. These treatments resulted in an increase in yield and potential income of 33.7% and 28.95% respectively. Control plants, which already grow under optimal conditions, clearly reacted to the additional urea applied as a source of nitrogen in that the yields increased. Both the 0.5% urea and the 0.5% urea / Pheroids solutions have great agricultural potential, even under optimum growing conditions.

It is thus clear that nitrogen limiting conditions had a reducing effect on yield, similar to the findings of Gul *et al.* (1967). It is not only a reduction in the number of fruit harvested, but also a reduction in mass and size, which eventually reduces total yield and the subsequent income. It was clearly demonstrated in this study that spraying nitrogen deficient plants with 0.5% and 1% urea solutions, singly and mixed with Pheroids, as corrective treatments prevented losses in yield and income. Although these corrective treatments did not completely alleviate the effect nitrogen limiting conditions had on growth and subsequent yield, it markedly prevented the effect of nitrogen limiting conditions in these plants.

5.5 PREVENTION OF THE REDUCING EFFECT OF NITROGEN LIMITING CONDITIONS ON FRUIT QUALITY

Another factor, apart from yield, which will affect the market value is overall fruit quality like appearance, aroma and the taste of the fruit (de Bruin *et al.*, 1971; Davies & Hobson, 1981). Different parameters were used in this study to describe fruit quality and to determine the effect of limited nitrogen supply, as well as the corrective treatments used, on this quality. These parameters include pH, electrical conductivity, % Brix, moisture content, as well as the lycopene content of the fruit. All these parameters were measured from the same fruit. Fruit quality parameters were measured for the first 16 weeks of the 25 week harvest period. The fruits were allowed to develop to maturity on the plants and

were harvested at the red, consumer ready stage. The fruits from the control plants were considered to represent high quality fruit, since these plants were cultivated under optimal hydroponic conditions.

Fruit pH in tomatoes varies considerably between cultivars (Dominigos, *et al.*, 1987). Gul *et al.* (1967) found that different nitrogen levels did not influence pH considerably and varied in a range of 4.24 to 4.26, similar to the pH measured in this study (Fig. 4.12 A & B; Fig. 4.13 A). Fruit pH remained fairly constant throughout the trial (Fig. 4.12 A & B). The pH of the fruit from the control plants had a slightly higher pH than that from the plants grown under nitrogen limiting conditions (Fig. 4. 12 A). This difference in pH is however insignificant. None of the corrective treatments used had a clear visible effect on the fruit pH either (Fig. 4.12 B & Fig. 4.13 A). It can be concluded that neither nitrogen limiting conditions, nor the different corrective treatments used, affected fruit pH and thus fruit quality.

Although fruit pH remained fairly constant, fruit EC and % Brix increased as the harvest period progressed (Fig. 4.12 C & D). Fruit EC may be affected by the EC of the nutrient solution. Dorai *et al.* (2001) have found that higher EC values of the nutrient solution resulted in better quality fruit, but it reduced yield. However, the EC of both the control- and nitrogen limiting media used in this trial was similar and remained fairly constant at 1.7 mS.cm^{-1} (Fig. 4.2). The fact that both the EC and % Brix of the fruit increased as the harvest period progressed, can be seen as a natural phenomenon. This inevitably will affect fruit quality. Control plants had a slightly higher average fruit EC than the fruits from the plants grown under limiting nitrogen conditions (Fig. 4.13 B), suggesting a slightly better quality fruit.

Measuring the % Brix of the fruit, the concentration of total soluble solids in the fruits can be estimated (Young *et al.*, 1993). These total soluble solids include sugars such as sucrose, fructose and glucose, as well as other substances like

vitamins, organic acids etc. which all affects fruit taste and aroma (Stevens *et al.*, 1977). The concentration of the total soluble solids (TSS) was found by Huett and Dettman (1988) to increase as the concentration of nitrogen increases. It can thus be postulated that plants grown under nitrogen limiting conditions will produce fruit with less total soluble solids (TSS). However, the % Brix measured in this study between the control- and nitrogen deficient plants, was similar. No clear differences were observed (Fig. 4.12 & Fig. 4.13).

Changes in fruit pH, EC and % Brix are, however, dependent on the moisture levels within the fruit. When the moisture content within the fruit is low, the fruit will be more concentrated, resulting in increased EC and % Brix values and *vice versa*. Thus, by measuring the fruit moisture content, the changes in the above mentioned parameters can be further evaluated, as will be addressed later.

As mentioned previously, it was observed that fruit mass and size in both control- and nitrogen deficient plants declined as the harvest period progressed (Fig. 4.10 C – F & Fig. 4.14 A & B). This phenomenon was also evident for the dry mass of the fruits (Fig. 4.14 C & D). Most of the fruits dry matter is the result of leaf assimilates accumulating in the fruits during development and maturation. More than 65% of the total uptake of nitrogen is apparently accumulated in the fruit (Tanake *et al.*, 1974). Fruit growth is thus mainly determined by the import of leaf assimilates. The fact that fruit size and mass (Fig. 4.10 C – E) and dry mass (Fig. 4.14 C) declined as the harvest period progressed, while the number of fruit produced increased in the first half of this period (Fig. 4.10 A), could be ascribed to the distribution of leaf assimilates to all the new fruits formed. This phenomenon was more evident in the control plants than in the nitrogen deficient plants, where changes in fresh- and dry mass remained fairly constant (Fig. 4.14 A - D), but on average was lower than that of the control plants (Fig. 4.15 A & B). Nitrogen limiting conditions reduced vegetative development (Fig. 4.7) and thus the subsequent synthesis of leaf assimilates through photosynthesis. Less assimilates lead to the eventual reduced deposition of these assimilates in the

fruits, as suggested by the lower EC values (Fig. 4.13 B) measured for these fruits. The decrease in fruit dry mass (solids) may also be due to a decrease in metabolic rate as the plant matures (Atherton & Rudich, 1986).

It must also be kept in mind that fruit nearing maturity are mostly situated further down on the plant while the developing fruit are higher up on a plant. When the plants experience a nitrogen deficiency, the nitrogen in the bottom parts of the plants may be translocated to the upper parts of the plant (Salisbury & Ross, 1991), resulting in lower concentrations of nitrogen to be available in the bottom parts of the plants (Fig. 4.9 & Table 4.1). This may possibly contribute to fruits having a high rate of assimilate deposition and a subsequent high dry mass at the start of the harvest period when only a few fruit are to be found on a plant, opposed to the fruit harvested later when many fruit were found on the plant.

Another factor which also may influence fruit dry matter content is the physical position of the fruit on the truss. Photosynthetic assimilates originating in the leaves are transported to the fruits. Fruit situated at the origin of the truss may utilize assimilates, subsequently lowering the assimilate concentration available to fruits situated at the tip of the truss. Thus, fruit nearer to the origin of the truss may be larger and may also contain higher levels of assimilates than fruit at the end of the truss. Detailed studies, however, are required to prove this.

Similar to fruit dry mass, the fruit moisture content also decreased, but only marginally (Fig. 4.14 D & F). No clear differences were obtained for fruits from the control- and nitrogen deficient plants. For both plants, the moisture content decreased from approximately 95% to 93%. This is indicative that the fruits not only became smaller and lighter, but also more concentrated as the harvest period progressed. This correlates with the increase in EC and % Brix which was observed (Fig. 4.12 C & E). Differences in the fruit moisture content of less than 2% were found not to affect fruit taste greatly (Ertan, 1990). Moreover, comparing the ratio between fruit dry mass and fresh mass (Fm:Dm), showed a clear

increase throughout the harvest period (Fig. 4.14 G & F). This is indicative of the import of leaf assimilates and its deposition during fruit development and maturation. Thus, the fruits became more concentrated with carbon skeletons and this is most probably the reason why the average moisture content decreased during this period (Fig. 4.14 E). On average, no clear differences in Fm:Dm ratio was observed between fruits from the control plants and the plants grown under nitrogen limiting conditions.

Taking all the parameters used to measure fruit quality into account, it appeared that nitrogen limiting conditions had little to no effect on fruit quality. Only minor differences in these parameters between the fruits from control- and nitrogen deficient plants were measured. Although the fruit became smaller, lighter and more concentrated, it can be seen as a natural phenomenon occurring, irrespective whether tomato plants are grown under control- or nitrogen limiting conditions. Thus, fruit quality is not compromised when the plants experience a sub-optimal supply of nitrogen.

Moreover, none of the 0.5% and 1% urea solutions used, singly or mixed with Pheroids, to supply nitrogen deficient plants with additional nitrogen, had any further effect on the changes in the above mentioned quality parameters. No marked changes were observed for the pH, EC, % Brix (Fig. 4.12 & Fig. 4.13), moisture content and Fm:Dm (Fig. 4.14 & Fig. 4.15) of the fruits.

However, all the corrective treatments used in this study could completely alleviate the effect nitrogen limiting conditions had on the fresh- and dry masses of the fruit. In fact, the average fresh- and dry masses obtained with these corrective treatments exceeded the averages for the control fruit (Table 4.4 & Fig 4.15 A & B). The fresh- and dry mass of fruits can, however, also be seen as parameters to quantify yield (Table 4.4). It was clearly demonstrated that nitrogen limiting conditions had an enormous effect on yield in terms of mass as was also observed by Gul *et al.* (1967).

In addition, treating control plants additionally with the 0.5% urea and 0.5% urea / Pheroids treatments, also had no marked effect on fruit quality. None of the parameters used to qualify fruit quality was visibly affected by these treatments. The same results were obtained when nitrogen deficient plants were sprayed with a Pheroids solution only. Pheroids as a bio-stimulant also had no effect on fruit quality under nitrogen limiting conditions.

Another parameter used to describe fruit quality was the lycopene content of the fruits. Lycopene does not contain nitrogen. However, the metabolic pathways responsible for producing carotenoids like lycopene involves many enzymes, which contains nitrogen. Nitrogen levels may affect the synthesis of lycopene. During fruit maturation, a vast number of metabolites are produced and converted from a partial photosynthetic to a heterotrophic metabolism in the fruits, where lycopene finally becomes the dominant carotenoid (Carrari & Fernie, 2006).

Carotenoids, like lycopene, protect chlorophyll pigments against photo-oxidation during photosynthesis (Salisbury & Ross, 1991). Lycopene dissipates excess light energy absorbed by the antenna pigments, and thus protects chlorophyll against photo-oxidation by quenching triplet chlorophyll, superoxide anion radicals and singlet oxygen (Rao & Agarwal, 1999). Lycopene also plays a role in the human diet in preventing diseases like chronic-, cancer- and cardiovascular diseases (Agarwal & Rao, 1999), which are caused by oxidative stress induced by reactive oxygen species (ROS) (Halliwell *et al.*, 1995; Sies & Stahl, 1995; Frei, 1994; Block, 1992; Steinmentz & Potter, 1996). Lycopene as an anti-oxidant, makes it an important molecule for human health.

It has been shown that high nitrogen levels may increase the lycopene content in tomato fruit, but seasonal variations in climate and light intensities also influenced its content (Bruulsema *et al.*, 2004). Furthermore, lycopene levels were also found to be affected by the availability and concentration of nitrogen (Grusak *et al.*, 1999; Brandt & Molgaard, 2001). Plants will produce primary and secondary metabolites, like lycopene, relative to the abundance and availability of nitrogen (Stamp, 2003). Thus, it can be assumed that the plants grown under control conditions will have the higher lycopene concentration.

The lycopene content of the fruits from both the control- and nitrogen deficient plants remained fairly constant throughout the trial (Fig. 4.16 A & B). This is in contrast to the findings of Bruulsema *et al.* (2004) who found a direct correlation between high lycopene content and a high nitrogen supply. Although no marked differences in the lycopene content were seen, the average lycopene content of the fruit for both the control- and nitrogen deficient fruits was approximately 150 mg.g dm⁻¹. Bramley (2000), as well as Levy and Sharoni (2004), found the average lycopene content for hydroponic greenhouse tomatoes varied between 84 mg.g dm⁻¹ and 420 mg.g dm⁻¹.

Like in the case of the other quality parameters used, spraying nitrogen deficient plants with the 0.5% and 1% urea solutions, singly and mixed with Pheroids, also had no clear effect on the lycopene content of the fruit. Thus, these plants did not respond to the additional nitrogen in the synthesis of more lycopene. When the control plants were treated with the 0.5% urea solution, singly and mixed with Pheroids, a small improvement in the lycopene content of the fruit was observed (Fig. 4.17). This indicated that the control plants, which are growing under optimal nitrogen levels, could react to the additional nitrogen supply by stimulating the synthesis of more lycopene. This may be due to enhanced metabolic activities (DOXP and MVA pathways) in these plants, which is then able to sustain a high rate of lycopene synthesis. This can have an effect on the

health attributes of tomato fruits. However, this increase in the lycopene content is only marginal to be of any significance.

To summarize, it appears that neither nitrogen limiting conditions had any apparent effect on fruit quality, nor did the foliar application of 0.5% and 1% urea, singly and mixed with Pheroids as corrective treatments. The changes measured are so marginal that it can be concluded that fruit quality is not compromised under nitrogen limiting conditions, as well as when the different corrective treatments were used to prevent the effect of the nitrogen limiting conditions.

5.6 CONCLUDING REMARKS

Crops may experience a limited supply of nitrogen from time to time. Urea, which contains 46% in its structure, is commonly used to treat and prevent nitrogen limiting conditions (Wittwer *et al.*, 1963; Yamanda *et al.*, 1965; Knoche *et al.*, 1994; Bondada *et al.*, 2001). It was found that urea concentrations in excess of 2% may burn the leaves. Therefore, producers use concentrations lower than 2%, which is mostly applied as a foliar spray to crops. Moreover, due to a number of reasons, not all of the urea applied is absorbed (Liu *et al.*, 2003). Thus an attempt has to be made to improve or enhance the absorption of urea by plants.

The principle aim of the study was to evaluate Pheroids as a potential vehicle to facilitate the transport of substances over membranes. To evaluate this, tomato plants were cultivated in a circulating ebb and flow hydroponic setup to simulate nitrogen deficient soil conditions. Plants grown under nitrogen limiting conditions were then sprayed with 0.5% and 1% urea solutions, as an additional source of nitrogen. In an attempt to possibly enhance the absorption of urea, these urea solutions were mixed (“packed”) with Pheroids, before this mixture were sprayed onto the plants.

Results confirmed that nitrogen is of critical importance for growth and development. Low nitrogen levels reduced vegetative growth, delayed fruit formation, reduced the number of fruit harvested, mass and size and finally yield. Yield was reduced by approximately 38%, when compared to plants grown under control conditions. Although nitrogen deficiencies caused a reduction in yield, it had no apparent effect on the quality of the fruit.

Spraying nitrogen deficient plants with the 0.5% urea and 1% urea solutions, the mass and size of the fruits harvested and subsequently overall yield, had little effect on fruit quality. The foliar application of these urea solutions also improved the hypothetical income, as these plants produced a substantially higher yield than the non-treated nitrogen deficient plants. Although these foliar applications improved yield, it could not fully alleviate the reducing effect of low nitrogen levels on yield.

To address the main objective of the study the 0.5% and 1% urea solutions were then mixed (“packed”) with Pheroids and sprayed onto the foliage of the plants. It was postulated that this treatment would enhance the further uptake of urea into the leaf cells, elevating the nitrogen levels and thereby reducing the effect of nitrogen limiting conditions. Spraying nitrogen deficient plants with these urea/Pheroids further alleviated the reducing effect of low nitrogen levels on vegetative and generative growth, as well as on yield. Although the number of fruit produced was not increased, the individual fruit size and mass was improved which eventually further improved total yield. This indeed supports the assumption made that Pheroids can facilitate and improve the absorption of urea, thereby increasing cellular nitrogen levels. Moreover, mixing the 0.5% urea solution with Pheroids, limited losses in yield and potential income to as little as 4% when compared to control plants, making it the most successful treatment in preventing the effect of nitrogen limiting conditions on yield. Using this treatment inevitably increased the profit margins of the producer.

Spraying nitrogen deficient plants with Pheroids only, also alleviated the effects of low nitrogen levels on yield, but to a lesser extent than when it was mixed with urea. Although Pheroids, consisting of free fatty acids or fatty acid derivatives, did not directly contribute to cellular nitrogen levels, it still alleviated the effects of nitrogen limiting conditions on yield. Therefore, Pheroids itself can be marketed as a bio-stimulant in general.

The apparent encapsulating ability of Pheroids allows it to facilitate the transport of phytochemical important substances, like urea, over membranes without making use of concentration dependant diffusion or membrane protein co-transporters. Due to the lipid like attributes of Pheroids, it can be postulated that the urea/Pheroids complex easily associates with membranes and readily move into the cells, where after it is metabolized. The urea is metabolized by urease to yield NH_4^+ and CO_2 , which both can contribute to metabolism in improving growth and eventually yield. After the urea has been metabolized, Pheroids is most probably also metabolized giving it its ability to be a bio-stimulant. Pheroids apparently consist of free fatty acids and/or fatty acid derivatives. For example, a fatty acid of 16 carbons (Palmitic acid) can be metabolized via β -oxidation in the peroxisomes to yield sucrose and eventually a minimum of 72 ATP molecules (Salisbury & Ross, 1991). Assuming Pheroids consists of many such fatty acid chains, it is postulated that its breakdown can contribute to cellular ATP levels, which will stimulate metabolism, ultimately improving growth. This is most probably the reason why Pheroids have bio-stimulating characteristics, as was measured in the plants grown under nitrogen limiting conditions.

Thus, Pheroids show a lot of promise as a potential vehicle for the transport of substances over membranes, as well as a bio-stimulant in general, but this requires further detailed investigations. It is recommended that this study should be performed on a larger scale, including field trials, to accurately assess the input costs, and the different parameters used to measure yield and quality on a higher population of plants. It can be considered to spray plants more frequently,

for example once a week, instead of every two weeks. A vast number of crops may suffer from nitrogen deficiencies and it is recommended that more crops should be included in a study to get a clearer indication of the potential of Pheroids.

In conclusion, it appears that Pheroids has the ability to facilitate the transport of substances, like urea, over membranes of tomato plant cells grown under control- and nitrogen limiting conditions. Pheroids also exhibits good growth promoting characteristics in general and can be promoted as a bio-stimulant. Pheroids, singly or mixed with beneficial substances, show great promise for use in the agricultural industry.

CHAPTER 6

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