# THE EFFECT OF A NOVEL COMPOUND ON YIELD AND FRUIT QUALITY IN HYDROPONICALLY GROWN TOMATOES

by

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# SUMMARY

**Key words:** Pheroids; fatty acids; *Lycopersicon esculentum*; Blossom-end rot; calcium; calcium deficiency; yield

A company, Elementol (Pty) Ltd, requested the evaluation of their novel product, Pheroids. Pheroids is apparently a micro-emulsion that has the ability to act as a vehicle transporting phytologically beneficial substances over membranes. They further claim that Pheroids alone, has plant growth promoting qualities. However, little information on Pheroids was provided, as its patent registration is still pending.

*Lycopersicon esculentum* (cv. Floridade) was used for this study as it is prone to developing a nutritional disorder, Blossom-end rot (BER), under circumstances that promote a calcium deficiency in fruits. It can reduce potential yield with up to 70%.

The plants were cultivated in a controlled greenhouse environment in a drip hydroponic set-up using complete- and calcium deficient nutrient media. The plants cultivated in the calcium deficient nutrient medium markedly developed BER. In an effort to reduce the occurrence of BER, these plants were treated with additional calcium using 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, as foliage sprays. The purpose of these treatments was to test the ability of Pheroids to act as a vehicle for the transport of additional calcium into the plants. Control plants cultivated in a complete nutrient medium were also treated foliarly with pure Pheroids to determine the possible stimulatory effect of Pheroids on plant growth.

Reduced yield, and the subsequent high incidence of BER, as a result of the calcium deficiency, was prevented by supplying calcium stressed plants with additional calcium in the form of the 1% and 2% $CaCl_2$ foliage sprays. These treatments improved yield above that of the calcium stressed plants, but failed to completely prevent the occurrence of BER.

Mixing Pheroids with these $CaCl_2$ solutions addresses its potential to transport additional calcium into the plants to improve cellular calcium concentrations. The 2% $CaCl_2$ Pheroids mixture specifically proved to be a very efficient treatment in reducing the effect of a calcium deficiency on yield and the development of BER. Although BER was not

completely prevented, the yield and income generated with this mixture compared favourably to that of control plants.

The efficiency of this 2% $CaCl_2$ Pheroids mixture as a preventative foliage spray for reducing the occurrence of BER, were also compared to a treatment where only the fruits of calcium stressed plants were treated with this mixture. Treating only the fruits reduced the occurrence of BER effectively, but yield and profit were markedly decreased, making it ineffective, unpractical and uneconomical.

A further aim of this study was to investigate the ability of Pheroids to act as a growth promoting substance by spraying control plants with Pheroids. The data obtained suggested that Pheroids stimulated plant growth in general as it stimulated yield. However, its potential stimulatory response also promoted the development of BER, and subsequently a reduction in net yield and profit. Since general plant growth was stimulated by Pheroids under control conditions, it is recommended that Pheroids should be extensively tested on a variety of crops to evaluate its growth stimulating potential.

In summary, it appeared that Pheroids has the potential to act as a growth promoting substance, but needs further detailed investigation. However, it did indeed act as a vehicle for the transportation of phytologically beneficial substances over membranes, especially in tomatoes grown under calcium stress conditions. Taking into consideration that most crops are grown in sub-optimal conditions, Pheroids might have numerous potential applications for the agricultural industry.

# OPSOMMING

**Sleutelwoorde:** Pheroids; vetsure*;* *Lycopersicon esculentum*; Blom-end-vrot; kalsium; kalsium-arm toestande; oesopbrengs

‘n Maatskappy, Elementol (Edms) Bpk, het die evaluering van hul produk, “Pheroids” onder gekontroleerde toestande aangevra. Pheroids is ‘n mikro-emulsie wat vermoedelik oor die vermoë beskik om die vervoer van fisiologies voordelige verbindings oor membrane te verbeter. Elementol (Edms) Bpk beweer verder dat Pheroids ook oor groeistimulerende eïenskappe beskik. Min inligting oor Pheroids is egter bekend aangesien die patentregistrasie nog nie afgehandel is nie.

*Lycopersicon esculentum* (cv. Floridade) is in die studie gebruik aangesien die vrugte geneig is om blom-end-vrot (BEV) onder toestande wat ‘n kalsiumtekort in vrugte bevorder, te ontwikkel. Die voorkoms van BEV kan oesopbrengs met soveel as 70% verminder.

Die plante is in ‘n kweekhuis verbou in hidroponiese drup sisteme met volledige- en kalsium-arm voedingsmediums. Daar is aangetoon dat ‘n hoë voorkoms van BEV by die plante wat in die kalsium-arm voedingsmedium verbou is, voorgekom het. In ‘n poging om die ontwikkeling van BEV te beperk, is die plante van addisionele kalsium voorsien in die vorm van blaarbespuitings met 1% en 2% $CaCl_2$ oplossings, alleen of gemeng met Pheroids. Die doel hiervan was om die vermoë van Pheroids om fisiologies voordelige verbindings oor membrane in die sel te vervoer, te ondersoek. Kontroleplante is addisioneel met Pheroids bespuit om die moontlike groeistimulerende eïenskappe daarvan op plantgroei te bepaal.

Kalsium-arm toestande het duidelik die oesopbrengs verlaag en die voorkoms van BEV verhoog. Daarenteen het behandeling van hierdie kalsium-arm plante met 1% en 2% $CaCl_2$ oplossings die oesopbrengs verbeter en die voorkoms van BEV gedeeltelik beperk. Hierdie behandelings kon egter nie die effek van kalsium-arm toestande op oesopbrengs en die voorkoms van BEV ten volle ophef nie.

Deur die $CaCl_2$ oplossings met Pheroids te vermeng, is die potensiaal van Pheroids om addisionele kalsium na plantselle te vervoer, aangespreek. Die Pheroids teenwoordig in die 2% $CaCl_2$-Pheroids-mengsel het moontlik die vervoer van addisionele kalsium verbeter aangesien dit die mees doeltreffende behandeling was om oesopbrengs te verhoog en die voorkoms van BEV beduidend te verminder. Alhoewel die voorkoms van BEV nie volledig met hierdie $CaCl_2$–Pheroids-behandeling onderdruk was nie, het die oesopbrengs en winsgrense baie goed vergelyk met díe van die kontroleplante.

Die doeltreffendheid van die 2% $CaCl_2$-Pheroids-mengsel as ‘n voorkomende blaarbespuiting om die voorkoms van BEV te beperk, is ook vergelyk met ‘n behandeling waar slegs die vrugte van kalsium-arm plante met díe mengsel behandel is. Die direkte behandeling van slegs die vrugte het ook die voorkoms van BEV doeltreffend verlaag, maar dit het ook oesopbrengs en gevolglike winste, merkbaar verminder. Dus, om slegs die vrugte van kalsium-arm plante met die $CaCl_2$-Pheroids-mengsel te behandel, is ondoeltreffend, onprakties en nie ekonomies nie.

‘n Verdere doel van die studie was om die groeistimulerende eïenskappe van Pheroirds te evalueer. Behandeling van kontroleplante wat reeds onder optimale voedingstoestande groei met Pheroids, het die oesopbrengs verbeter. Dit dui daarop dat Pheroids plantgroei in die algemeen kan bevorder. Pheroids het egter ook die voorkoms van BEV gestimuleer en gevolglik die oesopbrengs en winste verlaag. Daar word aanbeveel dat die groeistimulerende kapasiteit van Pheroids wat in hierdie studie waargeneem is, ook op ‘n aantal ander belangrike landbougewasse ondersoek word.

Dit blyk dus dat Pheroids die potensiaal het om plantgroei te stimuleer, maar dit vereis verdere ondersoeke. Dit blyk ook dat Pheroids die vervoer van ander fisiologiese voordelige verbindings oor membrane bevorder, veral by plante wat aan spanningstoestande blootgestel is. As in ag geneem word dat die meeste gewasse in elk geval in sub-optimale toestande verbou word, wil dit voorkom of Pheroids verskeie toepassings in die landbouindustrie het. Verdere studies op ‘n verskeidenheid van landbougewasse, is dus noodsaaklik.

# 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.

| | |
|---|---|
| **A** | Absorbance |
| **ABA** | Abscisic acid |
| **Acetyl-CoA** | Acetyl-coenzyme A |
| **ATPases** | Adenosine triphosphatases |
| **Avg** | Average |
| $Ba^{2+}$ | Barium cation |
| **BER** | Blossom-end Rot |
| **BHT** | Butylated hydroxytoluene |
| $B(OH)_3$ | Boric acid |
| **C** | Carbon |
| **Ca** | Calcium |
| $Ca^{2+}$ | Calcium cation |
| $Ca(NO_3)_2$ | Calcium nitrate |
| $CaCl_2$ | Calcium chloride |
| $[Ca^{2+}]_{apoplast}$ | Apoplastic calcium cation |
| $[Ca^{2+}]_{cyt}$ | Cytosolic calcium cation |
| $CaCO_3$ | Calcium carbonate |
| **CaM** | Calmodulin |
| **Cycle no** | Cycle number |
| $Cd^{2+}$ | Cadmium cation |
| **CDPK** | Calcium cation dependent protein kinase |
| **Cl** | Chlorine |
| $CuCl_2 . 2H_2O$ | Copper (II) chloride dihydrate |
| **cv.** | Cultivar |
| **DMAPP** | Dimethylallyl diphosphate |
| **Dm** | Dry mass |
| **DNA** | Deoxyribonucleic acid |
| **EDTA** | Ethylenediaminetetraacetic acid |

| | |
|---|---|
| **ER** | Endoplasmic reticulum |
| **$FADH_2$** | Reduced flavin adenine dinucleotide |
| **FBPase** | Fructose-1,6-bisphosphatase |
| **FeEDTA** | Ferric Ethylenediaminetetraacetic acid |
| **$FeSO_4.7H_2O$** | Iron(II) sulfate heptahydrate |
| **Fm** | Fresh mass |
| **GGPP** | Geranylgeranyl diphosphate |
| **$H^+$** | Hydrogen cation |
| **HACC** | Hyperpolarisation-activated channels |
| **IAA** | Indole acetic acid |
| **$IP_3$** | Inositol phosphates |
| **IPP** | Isopentenyl diphosphate |
| **JA-dependent** | Jasmonic dependent |
| **K** | Potassium |
| **$K^+$** | Potassium cation |
| **$K^+_{in}$** | Potassium inward-rectifying channel |
| **$K^+_{out}$** | Potassium outward-rectifying channel |
| **$KH_2PO_4$** | Potassium dihydrogenphosphate |
| **$KNO_3$** | Potassium nitrate |
| **LYCb** | Lycopene β-cyclase |
| **LYCe** | Lycopene ε-cyclase |
| **MEP pathway** | Methylerythritol phosphate pathway |
| **Mg** | Magnesium |
| **$Mg^{2+}$** | Magnesium cation |
| **$MgSO_4$** | Magnesium sulphate |
| **$Mn^{2+}$** | Manganese cation |
| **$MnCl_2. 4H_2O$** | Manganese(II) chloride tetrahydride |
| **MVA pathway** | Mevalonic acid pathway |
| **N** | Nitrogen |
| **Na** | Sodium |
| **$Na^+$** | Sodium cation |
| **$NAD^+$** | Nicotinamide adenine dinucleotide (Oxidized) |
| **NADPH** | Nicotinamide adenosine dinucleotide phosphate (Reduced) |
| **$Na_2EDTA$** | Disodium ethylene diamine tetra acetate |

| | |
|---|---|
| **$Na_2MoO_4$** | Sodium molybdate |
| **$NaNO_3$** | Sodium nitrate |
| **$NH_4^+$** | Ammonium cation |
| **$Ni^{2+}$** | Nickel cation |
| **$NO_3^-$** | Nitrate anion |
| **$O^-_2$** | Singlet oxygen |
| **P** | Phosphorus |
| **PAR** | Photosynthetic active radiation |
| **PDS** | Phytoene desaturase |
| **$PIP_2$** | Phosphatidylinositol bisphosphate |
| **PLD** | Phospholipase D |
| **ppm** | Parts per million |
| **PSY** | Phytoene synthase |
| **PVC** | Polyvinyl chloride |
| **R** | Rand |
| **RH** | Relative Humidity |
| **R-type** | Rapid transient anion channel |
| **S** | Sulphur |
| **SD** | Standard Deviation |
| **Sr** | Strontium |
| **S-type** | Slow-activating sustained anion channel |
| **SV** | Slow-activating vacuolar channel |
| **TCA-cycle** | Tricarboxylic acid cycle |
| **TSS** | Total soluble solids |
| **UDP-glucose** | Uracil-diphosphate glucose |
| **VICC** | Voltage-independent cation channel |
| **WAT** | Week after transplantation |
| **WOH** | Week of Harvest |
| **w/v** | Weight to volume |
| **XET** | Xyloglucan endotransglycosylases |
| **ZDS** | ζ-carotene desaturase |
| **$Zn^{2+}$** | Zinc cation |
| **$ZnCl_2$** | Zinc chloride |

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# CHAPTER 1

# INTRODUCTION

Tomatoes (*Lycopersicon esculentum*) are one of the most cultivated and important crops on the produce market today (http://businessafrica.net). The tomato industry has grown to include more than 7000 cultivars (http://en.wikipedia.org/wiki/Tomato).

Various health benefits have been attributed to the consumption of tomatoes because it is a valuable source of Vitamins C and E, as well as lycopene. Lycopene is a potent antioxidant that is responsible for the red colour of the fruits (Bramley, 2000). The consumption of tomatoes has been associated with a lowered risk in prostate cancer and heart diseases (Agarwal & Rao, 2000; Polder *et al.*, 2004).

Tomato yield is affected by a variety of factors such as the water content of the soil, uneven watering in greenhouses and tunnels, light intensity, temperature and the mineral composition of the soil or nutrient medium (Mahajan & Singh, 2006). Studies by Georgeta *et al.* (1977) showed that unfavourable nutritional conditions such as excess, deficiencies or the lack of a balanced nutrient composition, affected yield more negatively than environmental conditions such as light intensity and temperature. An example of a nutritional disorder that affects yield negatively, is Blossom-end rot (BER).

Blossom-end rot occurs widely among greenhouse- and field-grown tomatoes (Ho & White, 2005). Tomatoes that suffer from BER have sunken lesions at the blossom-end of the fruit (Ho & White, 2005). Up to 70% of a harvest can be lost due to BER, depending on cultivar, agricultural practices and environmental conditions (Taylor *et al.*, 2004).

Plants require 16 essential mineral elements for growth (Salisbury & Ross, 1992). These nutrient elements are divided into two groups, macro- and micronutrients. One of the macronutrients needed for normal growth and fruit production, is calcium. Factors such as a low calcium and phosphate supply, high magnesium-, nitrate- and potassium supply, high salinities, low and very high relative humidities and high light intensities and temperatures in the shoot environment (Ho *et al.,* 1993; 1999; José *et al.,* 1994; Nukaya *et al.,* 1995*a*&*b*), can cause a cellular calcium deficiency in the distal fruit tissue, which is the primary cause of BER (Ho *et al.,* 1993; Taylor *et al.,* 2004; Ho & White, 2005).

Several practices are utilised commercially to reduce the occurrence of BER. The most common practices are the addition of calcium to soil or the direct spraying of plants with calcium. Calcium deficient soils are treated with 567 to 1134 kg $CaCO_3$. $ha^{-1}$ several months before planting (http://www.ces.ncsu.edu; http://pubs.caes.uga.edu; http://vegedge.umn.edu). Alternatively, calcium can be sprayed directly onto established plants with either 0.25% calcium chloride ($CaCl_2$) or 0.5% calcium nitrate ($CaNO_3$) until the point of drip-off. Spraying starts as soon as the first symptoms of BER appears and is applied every seven to ten days for three or four applications (www.pubs.caes.uga.edu). Studies by Ho and White (2005) indicated that a weekly $CaCl_2$ spray of 0.5% (w/v) reduce the occurrence of BER with up to 40%.

A company, Elementol (Pty) Ltd, is in the process of registering a product under the name of Pheroids. Pheroids is a micro-emulsion that contains fatty acids and/or fatty acid derivatives. Elementol (Pty) Ltd claims that this emulsion is a vehicle for the delivery and translocation of phytologically beneficial substances over membranes. They further claim that Pheroids on its own, also stimulates plant growth in general. Patent registration for Pheroids is still pending and for this reason Elementol (Pty) Ltd disclosed very little information regarding the structure and nature of Pheroids. Elementol (Pty) Ltd requested the evaluation of Pheroids as a growth promoting substance as well as its ability to reduce or prevent disorders in crops by enhancing the translocation of substances.

The rationale for this study was to evaluate the claims made by Elementol (Pty) Ltd regarding Pheroids. This was done by growing tomatoes hydroponically under calcium deficient conditions to promote the incidence of BER. The calcium deficient plants were then sprayed with one percent and two percent $CaCl_2$ solutions, singly and mixed ("packed") with Pheroids. It is hypothesized that if Pheroids acted as a translocating molecule, it may improve the transport of calcium across the membranes, increasing the concentration of calcium cations ($Ca^{2+}$) in the plants and fruits, thereby improving yield and decreasing the occurrence of BER. A further aim was to determine whether Pheroids applied on its own, promotes plant growth and yield.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

Tomatoes are worldwide one of the most consumed food crops in the vegetable economy (Salunkhe *et al.,* 1974; Chapagain & Wiesman, 2004), due to their year-round availability and accessible prices (Abushita *et al.*, 1997). It is no different in South Africa where the size of the tomato industry is approximately 650 000 tons to the value of R1.3 billion per annum (www.nda.agric.za/docs/Cropsestimates).

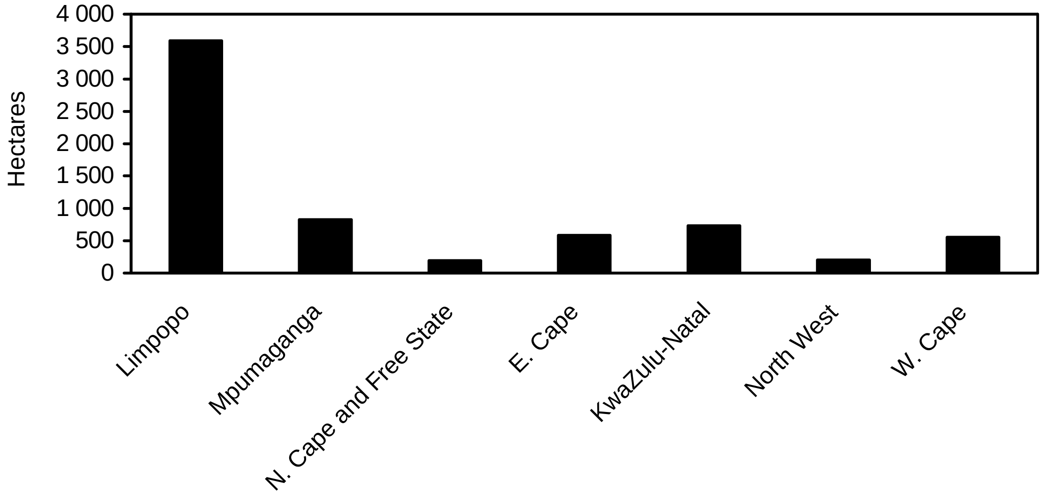


**Figure 2.1:** The areas committed to tomato production in the respective South African provinces for 2006 (www.nda.agric.za/docs/Cropsestimates).

Tomato yield in any given year is largely determined by two factors, namely the quality of the tomatoes and the ability to produce tomatoes outside of the normal growing season (www.nda.agric.za/docs/Cropsestimates).

The price of tomatoes increased from approximately R2000. ton$^{-1}$ for 2001, to approximately R2800. ton$^{-1}$ for 2005 (Table 2.1). In order to guarantee a producer price of approximately R4. kg$^{-1}$, the total local market supply should not exceed 16 000 tons per month (www.nda.agric.za/docs/Cropsestimates).

**Table 2.1:** Average vegetable prices in the South African fresh produce markets for the period 2001/02 to 2005/06 (http://www.nda.agric.za/docs/Cropsestimates).

| **Year:** | **2001/02** | **2002/03** | **2003/04** | **2004/05** | **2005/06** |
|---|---|---|---|---|---|
| | **R. ton$^{-1}$** | | | | |
| **Tomatoes** | 2 071,31 | 2 471,79 | 2 852,08 | 2 267,02 | 2 848,71 |
| **Onions** | 1 469,52 | 1 672,73 | 1 558,47 | 1 221,39 | 1 346,58 |
| **Green mealies** | 4 145,82 | 5 996,33 | 6 082,33 | 5 195,00 | 5 926,97 |
| **Cabbages** | 563,16 | 685,15 | 681,27 | 642,61 | 716,64 |
| **Pumpkins** | 689,44 | 874,74 | 775,71 | 876,17 | 864,71 |
| **Carrots** | 1 258,48 | 1 325,92 | 1 214,57 | 1 404,02 | 1 461,07 |
| **Other** | 1 604,84 | 1 998,25 | 2 194,80 | 2 046,90 | 2 347,52 |

## 2.2 TOMATOES (*Lycopersicon esculentum*)

The genus *Lycopersicon* is native to western South America where it is subjected to little rain, high relative humidities and temperatures ranging from 10°C to 24°C, but this photoperiod insensitive plant (Samach & Lotan, 2007) grows well practically everywhere (Salunkhe *et al.,* 1974).

### 2.2.1 PHYSIOLOGY OF FRUIT GROWTH

Tomato fruits are essentially swollen ovaries that contain associated flower parts. The development of fruits follows fertilization and occurs simultaneously with seed maturation (White, 2002). The cumulative growth pattern of a fruit can be divided into three phases (Wang, *et al*., 1993):

1. An initial slow growth period after anthesis that range from day 0 to day 14 of fruit development. Most of the cell division takes place during this period (Asahira, *et al*., 1968).

2. Day 10 to 40 of fruit development marks the fast growing period. Tomato fruits accumulate most of its dry matter, such as starch, during this period. The accumulation rate is maximal at day 20 (Wang *et al.,* 1993).
3. A maturation period. During this period the embryo matures, the seeds accumulate storage products, lose water and acquire desiccation tolerance (White, 2002) and the fruits ceases to import carbohydrates.

Initially, the fruit enlarges through cell division, which is then followed by cell expansion (Asahira, *et al*., 1968; Wang, *et al*., 1993). Cell expansion requires an increase in the plasma membrane, cell wall area and hydrostatic/turgor pressure. Turgor pressure is achieved by the accumulation of osmotically active solutes in vacuoles during the initial phase of cell expansion. Cytosolic and apoplastic calcium ions control the accumulation of osmotically active solutes (Ho & White, 2005).

Fruit cells expand in response to hormones such as gibberellins and auxins (Cosgrove, 2000; Ho & White, 2005; Carrari & Fernie, 2006). These hormonal signals trigger specific changes in cytosolic calcium cations ($[Ca^{2+}]_{cyt}$), which in turn is responsible for the generation of the proper developmental responses for initiating cell expansion of the plasma membrane and the cell wall (Cosgrove, 2000; White & Broadley, 2003; Ho & White, 2005; Carrari & Fernie, 2006). Cell wall expansion is achieved by the bonding of wall components (Cosgrove, 2000). Concomitant to an increase in the cell wall during cell expansion, the plasma membrane also expands. Plasma membrane expansion is achieved through the incorporation of vesicles that contain the materials and enzymes required for membrane and wall construction, into the plasma membrane. Elevated levels of $[Ca^{2+}]_{cyt}$ influence the incorporation of these vesicles (Cosgrove, 2000; Ho & White, 2005). While the cell expands, the pectins in the cell wall become progressively de-esterified and branched through the activity of pectin methylesterases. Crosslinkage by $Ca^{2+}$ eventually halts cell expansion (Cosgrove, 2000; Ho & White, 2005).

In addition to cell expansion, the rate of starch accumulation (Wang *et al.*, 1993) and several other factors such as water availability in the root zone, agricultural practices like thinning, high light intensities and ambient temperatures also influence fruit growth (Grossman & DeJong, 1995; Thompson *et al.*, 1999; Ho & White, 2005). All these factors influence fruit growth either directly or indirectly by affecting hormone concentrations

(Taylor *et al.,* 2004; Ho & White, 2005), photosynthesis and/or the supply of photo assimilate to the fruits (Ho *et al.*, 1993).

### 2.2.2 PHYSIOLOGY AND BIOCHEMISTRY OF FRUIT RIPENING

Fruits can be divided into two basic groups based on their ripening mechanisms, namely climacteric and non-climacteric fruits. Tomatoes are an example of climacteric fruits. In climacteric fruits, ripening is initiated by ethylene synthesis and a subsequent increase in the respiration rate (White, 2002). In contrast to the climacteric fruit, the respiration rate and ethylene levels remain low in non-climacteric fruit during fruit development (Alexander & Grierson, 2002). According to various genetic and biochemical studies, climacteric fruit development is controlled by both ethylene-dependent and ethylene-independent regulatory cascades, which alters metabolism and gene expression with subsequent effects on fruit quality (Atherton & Rudich, 1986; White, 2002; Alexander & Grierson, 2002; Carrari & Fernie, 2006).

Ripening is a highly coordinated, complex and genetically programmed process that culminates in colour, composition, aroma, flavour and textural changes (Atherton & Rudich, 1986; White, 2002; Alexander & Grierson, 2002). Tomato fruit follows a transition from a partially photosynthetic- to a true heterotrophic fruit by the parallel differentiation of chloroplasts into chromoplasts and the ensuing dominance of carotenoids and lycopene, which are responsible for the red colour of tomato fruits (Carrari & Fernie, 2006). Ripening is non-uniform, as is evident in colour distribution (Polder *et al.,* 2004; Ramandeep & Savage, 2004), and is accompanied by fruit softening and large increases in hexoses and aromatic amino acids namely aspartate, lysine, methionine and cysteine (Alexander & Grierson, 2002; Carrari & Fernie, 2006).

Softening and textural changes during ripening are brought about by the partial disassembly of the fruit cell wall (Marín-Rodríguez *et al.*, 2002). As ripening progresses, the cell wall becomes increasingly hydrated as the pectin rich middle lamella is modified and partially hydrolysed (Bewley *et al.*, 2000; Marín-Rodríguez *et al.*, 2002; Alexander & Grierson, 2002). Cell wall loosening proceed through auxin-induced apoplastic acidification and the activation of endoglycosidases, xyloglucan endotransglycosylases (XET) and expansins that cleave the load-bearing bonds tethering the wall’s cellulose microfibrils to other polysaccharides (Cosgrove, 2000). The final texture of the ripe fruit is affected by the ease with which one cell can be separated from another, a process which is governed by changes in the cohesion of the pectin gel (Alexander & Grierson, 2002).

The chemical composition of fresh tomato fruits depends on factors such as environmental conditions, ripening, maturity, cultivar, soil fertility, irrigation, agricultural practices and storage conditions (Salunkhe *et al.*, 1974; Sahlin *et al.*, 2004). Tomato quality assessments are based on fruit colour, texture, moisture content (tomatoes can contain up to 94% moisture), fruit shape and size, nutrient value, taste and aroma (Salunkhe *et al.*, 1974). All these quality attributes is the result of various tomato fruit constituents and the concentrations in which they are found in the fruit.

### 2.2.3 COMPOUNDS THAT CONTRIBUTE TO THE BIOCHEMICAL QUALITY AND HEALTH BENEFITS OF TOMATOES

#### 2.2.3.1 Carbohydrates

The carbohydrate compounds found in tomato fruit determine the organoleptic quality of tomatoes (Salunkhe *et al.*, 1974; Islam *et al.*, 1996). The carbohydrate concentration is determined by two factors: the environmental conditions during development and ripening, and the cultivar (Islam *et al.*, 1996). The major sugars contained in tomato fruits are sucrose, glucose and fructose (Islam *et al.*, 1996). Sugars such as free D-glucose, D-fructose, trace amounts of sucrose, *a*-ketoheptose and raffinose account for 60% of the soluble solids in tomato fruits (Salunkhe *et al.*, 1974). Glucose and fructose are present in approximately equal amounts but fructose contributes more to the sweetness of tomatoes. In general, the sugar content of tomato fruit is a function of the stage of maturity and increases uniformly from green to mature fruit (Winsor *et al.*, 1959; Salunkhe *et al.*, 1974).

##### 2.2.3.1.1 *Fruit carbohydrate metabolism*

Fruits obtain sugars either directly from photosynthesis or indirectly through import from source leaves and stems (Obiadalla-Ali *et al.*, 2004). Obiadalla-Ali *et al.* (2004) found that the fruit's photosynthetic process contributes 15% to 20% of the fruit's total carbon content, even though the amount of chlorophyll in the fruit is 30 fold lower than that of green leaves. Fructose-1,6-bisphosphatase (FBPase) activity is an indicator of photosynthetically active fruit because this enzyme is present in green fruits but not in red fruits. Thus, reduced fructose-1,6-bisphosphatase activity indicates the transition from photosynthetically active green fruits to ripening (Obiadalla-Ali *et al.,* 2004). The transition is accompanied by the decomposition of starch and a subsequent increase in soluble sugars (glucose and fructose) and carotenoid synthesis (Büker *et al.,* 1998).

Early fruit development is characterized by symplastic sucrose uptake. Sucrose is the major photo-assimilate transported from photosynthetic leaves and stems to the developing fruits (Wang, *et al*., 1993). Tomato fruits are very strong sinks for carbohydrates (Wang *et al*., 1993). Sink strength is controlled by invertase (Islam *et al*., 1996) and is described as a function of size and activity. Sink size is a physical restraint that includes cell number and cell size (Wang *et al.*, 1993). The rate of sucrose import into developing fruits is regulated by the sucrose concentration gradient between the sink (fruit) and the source (leaves and stem; Wang *et al.*, 1993). Enzymes controlling this, keep the sucrose concentration in the fruits at a minimum to allow for the maintenance of a steep sucrose concentration gradient between the phloem and the surrounding cells (Islam *et al*., 1996). Thus, sucrose accumulation is determined by the balance between sucrose synthesis and degradation (Islam *et al*., 1996).

The initial step in sucrose metabolism takes place primarily via the action of sucrose synthase and results in the transient accumulation of starch (Miron *et al.*, 2002; Carrari & Fernie, 2006). Sucrose synthase converts sucrose into fructose and uracil-diphosphate glucose (UDP-glucose), which is then compartmentalized in the vacuole. This is the dominant enzyme in metabolizing imported sucrose (Islam *et al*., 1996) and is linearly related to the final fruit size (Wang *et al*., 1993).

In the next development stage, there is a transition from symplastic uptake to apoplastic sucrose uptake. Apoplastic acid invertase catalyzes the hydrolyses of sucrose to hexoses (Wang *et al*., 1993; Islam *et al*., 1996; Miron *et al*., 2002). The latter is then transported to the cytosol via energy-dependent plasmalemma hexose transporters (Miron *et al.*, 2002). The reducing sugar concentration gradually increases with a concomitant decrease in sucrose accumulation and sucrose synthase activity (Islam *et al*., 1996).

### 2.2.3.2 Organic Acids

Malic- and citric acid are the two major organic acids that contribute to the taste of tomato fruits (Salunkhe *et al.,* 1974). Rangnekar (1975*a*) has found that the leaves of tomato plants exposed to calcium deficient conditions for eight to ten days accumulated these organic acids, possibly due to reduced translocation of organic acids from the leaves. Other organic acids detected in tomato fruits are acetic acid, formic acid, trans-aconitic acid, lactic acid, fumaric acid, galacturonic acid and α-oxo acids (Salunkhe *et al.*, 1974).

According to Salunkhe *et al.* (1974), as tomato fruits change from green to red, acidity increases to maximum values during the pink stage (Winsor *et al.,* 1959) after which it decreases again towards the red stage (Salunkhe *et al.,* 1974). The acidity of tomato fruit is very important for flavour. It is also an important factor that processors need to keep in mind during the production of tomato products since butyric, thermophyllic and putrefactive anaerobic micro-organisms are repressed at pH values below 4.3 (Salunkhe *et al.*, 1974). Thus, care should be taken not to increase the pH value of tomato fruits during processing to levels above 4.3.

### 2.2.3.3 Carotenoids

Tomatoes are considered to be health stimulating fruits due to the antioxidant properties of their main compounds of which the most important are carotenoids, ascorbic acid, vitamin E, phenolic acids and flavonoids (Polder *et al*., 2004). Some carotenoids have been proven to alleviate age-related diseases when taken in sufficient quantities due to their powerful properties as lipophilic antioxidants. For example, zeaxanthin and lutein protect against macular degeneration and β-Carotene is known for its provitamin-A activity (Bramley, 2002; Sahlin *et al*., 2004).

There are 600 different types of carotenoids (Stahl & Sies, 1996). Carotenoids are isoprenoid molecules common to all photosynthetic tissues. The biosynthesis and accumulation of pigment carotenoids such as β-Carotene, lycopene, violaxanthin, neoxanthin and zeaxanthin proceed concomitant with the assembly of the light harvesting antennae and reaction centres in photosynthetic tissue (Francis & Cunningham, 2002), since they participate in the light harvesting process (Bramley, 2002; Botella-Pavia *et al.,* 2004). In plants, carotenoids are also precursors for the biosynthesis of abscisic acid (ABA) and play a vital role in the development of colour in flowers and fruits. Colour contributes to the survival of plants as it attracts animals that disperse pollen and seeds (Botella-Pavia *et al.,* 2004).

### **2.2.3.3.1** ***Lycopene***

The red colour of tomatoes is mainly due to the presence of its primary fat-soluble carotenoid, lycopene (Stahl & Sies, 1996; Davis *et al*., 2002; Bramely, 2000, 2002; Javanmardi & Kubota, 2006), which is named after the genus *Lycopersicon,* as tomatoes are one of the vegetables with the highest levels of lycopene (Agarwal & Rao, 2000; Stacewicz-Sapuntzkis & Bowen, 2005).

On average, 80% to 90% of the total carotenoid content in tomato fruits is made up of lycopene (George *et al*., 2004). Its antioxidant activity has recently been found to be more effective than that of β-Carotene, α-carotene, α-tocopherol or albumin–bound bilirubin (Abushita *et al*., 1997; Agarwal & Rao, 2000).

Lycopene and other plant carotenoids are synthesized in photosynthetic- and non-photosynthetic plastids (Francis & Cunningham, 2002). Carotenoids are structurally tetraterpenes derived from five carbon isopentenyl diphosphate (IPP) units and their isomers, dimethylallyl diphosphates (DMAPPs). These two 5-carbon isoprene compounds are the universal precursors of all isoprenoid compounds (Francis & Cunningham, 2002; Bramley, 2002; Botella-Pavia *et al.,* 2004).

Plants synthesise IPP and DMAPP via two independent pathways in two different compartments; the mevalonic acid (MVA) pathway (Figure 2.2), which produces cytosolic/endoplasmic reticulum IPP, and the pastidial methylerythritol phosphate (MEP) pathway (Francis & Cunningham, 2002; Bramley, 2002; Botella-Pavia *et al.,* 2004; Ahn & Pai, 2008). Isopentenyl diphosphate (IPP) and DMAPP are condensed in their respective compartments to yield prenyl diphosphates of increasing size that serve as the starting point for the multiple branches that lead to the final isoprenoid products (Figure 2.2). The enzyme, geranylgeranyl diphosphate (GGPP) synthase, catalyzes the reaction between three IPP units and one DMAPP unit to yield GGPP (Figure 2.2). Geranylgeranyl diphosphate (GGPP) is the immediate precursor for the first $C_{40}$ carotenoid, phytoene, as well as for the biosynthesis of gibberellins and the phytol tail of chlorophylls, phylloquinones and tocopherols.

The condensation of two molecules of GGPP forms 15-*cis* phytoene (Figure 2.2) and is catalyzed by phytoene synthase (PSY; Bramley, 2002). Two structurally similar membrane-bound enzymes, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), convert phytoene via ζ-carotene into an open hydrocarbon chain that contains 40 carbon atoms (Rao & Agarwal, 1999), namely lycopene (Figure 2.2). Lycopene contains 11 conjugated double bonds and two non-conjugated double bonds (Stahl & Sies, 1996; Agarwal & Rao, 2000).

There are basically two different forms of lycopene namely *cis*-lycopene and *trans*-lycopene (Wertz *et al.*, 2004; Lin & Chen, 2005). Light, thermal energy and chemical reactions induce *cis-trans* isomerisation in lycopene as a polyene chain (Agarwal & Rao, 2000). The bioavailability of *cis*-lycopene exceeds that of *trans*-lycopene *in vitro* and *in vivo.* (Wertz *et al.,* 2004; Lin & Chen, 2005). However, lycopene predominantly exists in the all-*trans* configuration, the thermodynamically more stable form (Stahl & Sies, 1996, Agarwal & Rao, 2000). The long chromophore in the polyene chain accounts for the red colour of lycopene (Bramley, 2000). In tomato fruit, lycopene has a half-life of about two to three days (Rao & Agarwal, 1999). The cyclisation of lycopene creates a series of carotenes that have either two β-rings or one β-ring and one ε-type. Lycopene β-cyclase (LYCb) adds two β-rings to the symmetrical lycopene substrate producing β-carotene (Figure 2.2) and eventually absisic acid (ABA). Lycopene ε-cyclase (LYCe) adds one ε-ring to the lycopene substrate that leads to the eventual production of lutein (Francis & Cunningham, 2002).

Lycopene and other carotenoids such as β-carotene and α-tocopherol, play a vital role in the protection of the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls, superoxide anion radicals and singlet oxygen (Rao & Agarwal, 1999; Agarwal & Rao, 2000; Bramley, 2002; Botella-Pavia *et al.*, 2004). The number of conjugated double bonds, and to a lesser extent the end groups, determine the quenching activity of carotenoids (Stahl & Sies, 1996). Singlet oxygen ($O^-_2$) quenching takes place via two mechanisms: physical and/or chemical quenching. The singlet-oxygen-quenching ability of lycopene is twice that of β-carotene and ten times that of α-tocopherol (Agarwal & Rao, 2000; Sahlin *et al*., 2004).

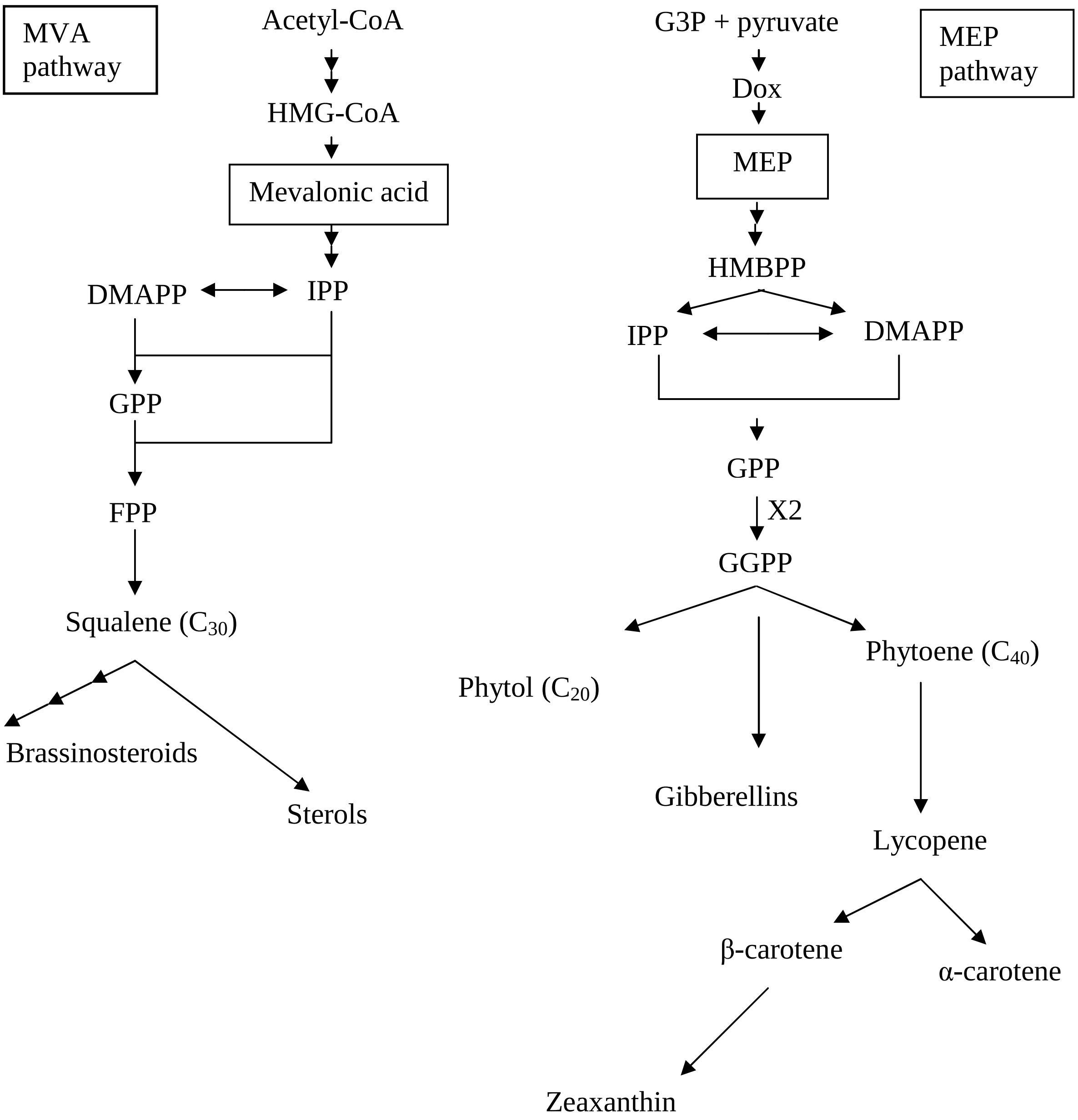


**Figure 2.2:** The MVA pathway in the cytosol and the MEP pathway in the stroma of photosynthetic- and non-photosynthetic plastids. These biosynthetic pathways produce tetraterpenes such as carotenoids. Carotenoids are derived from the five carbon unit isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP; Francis & Cunningham, 2002; Bramley, 2002; Botella-Pavia *et al.*, 2004).

Lycopene's biological activities in humans include singlet oxygen ($O^-_2$) quenching, scavenging of peroxyl radicals, induction of cell-to-cell communication and modulation of hormones, the immune system, growth and other metabolic pathways (Stahl & Sies, 1996; Gerster, 1997; Agarwal & Rao, 2000; Sahlin *et al*., 2004; Wertz *et al*., 2004). Lycopene may also protect against the oxidation of lipids, proteins and deoxyribonucleic acid (DNA; Agarwal & Rao, 2000; Bramley, 2002; Wertz *et al.,* 2004; Stacewicz-Sapuntzkis & Bowen, 2005). Oxidative DNA damage causes DNA mutations, which is implied in cancer initiation (Agarwal & Rao, 2000; Wertz *et al.,* 2004). A study by Stacewics-Sapuntzakis and Bowen (2005) concluded that the increased intake of tomato sauce may induce apoptosis in some tumour cells such as prostate cancer cells (Agarwal & Rao, 2000), mammary cancer cells (Agarwal & Rao, 2000), endometrial cancer cells and promyelocytic leukemia cells consequently arresting cancer progression (Giovannucci, 1999; Rao & Agarwal, 1999; Bramley, 2002; Wertz *et al*., 2004; Stacewicz-Sapuntzkis & Bowen, 2005). Evidence exists that suggest that lycopene is also beneficial for the prevention of coronary heart disease and cancers of the lung, stomach, pancreas, colon and rectum, esophagus, oral cavity, and skin (Giovannucci, 1999; Rao & Agarwal, 1999; Agarwal & Rao, 2000).

Whether the effects of lycopene on cancer arrest are singular or synergistic is still unknown. The reports published so far vary considerably. However, the possibility of a synergistic action with other phytochemicals such as glycoalkaloids, phenolic compounds, salicylates and carotenoids other than lycopene in tomatoes cannot be ruled out as it is well known that salicylates possess an anti-inflammatory action and quercetin inhibits the prostate androgen receptor (Stacewicz-Sapuntzkis & Bowen, 2005).

Factors such as environmental conditions, cultivar and ripening stage (Ramandeep & Savage, 2004; Javanmardi & Kubota, 2006) influence the lycopene concentration in tomatoes. In different tomato cultivars lycopene concentrations can range from 77 mg. $kg^{-1}$ to 150 mg. $kg^{-1}$. The choice of cultivar is therefore important when lycopene concentration is considered (Moraru *et al*., 2004). According to studies by Ilahy and Hdider (2007), lycopene accumulation in tomato pulp and skin is similar and both are affected by the ripening stage. They found that lycopene accumulation started after the yellow stage and increased until the red stage. Skin lycopene decreased sharply during the overripe stage (Ilahy & Hdider, 2007).

Studies by Sahlin *et al*. (2004) also showed that differences exist between the lycopene concentrations of field-grown- and greenhouse-grown tomatoes. Generally, field-grown tomatoes have been reported to have higher lycopene concentrations (52 mg. $kg^{-1}$ fresh mass to 230 mg. $kg^{-1}$ fresh mass) than greenhouse-grown tomatoes (10 mg. $kg^{-1}$ fresh mass to 108 mg. $kg^{-1}$ fresh mass), possibly due to differences in light intensity (Sahlin *et al*., 2004). A study conducted by Gautier *et al*. (2005), found that a drastic reduction in photosynthetic light (97%) reduced the β-carotene and lycopene content, and consequently the red colour of tomatoes, by 21% (Gautier *et al.,* 2005). Exposure to photosynthetic active radiation (PAR), and more specifically blue light, led to increased levels of lycopene and β-carotene (Grumbach, 1984; Gautier *et al*., 2005).

###### 2.2.3.3.1.1 *The effect of storage conditions and fruit processing operations on lycopene stability*

It appears that the lycopene concentration remains unchanged during the multi-step processing operations of juice and paste production (Agarwal *et al.,* 2001). However, storage does have an influence on the lycopene concentration of these juices and pastes. Lin and Chen (2005) have shown that the lycopene content of these juices and pastes were degraded under different storage conditions. They prepared tomato juice by pulverizing tomatoes at 82ºC, after which they autoclaved it at 121ºC for 40 seconds. The juice was then stored under dark and light conditions at 4ºC, 25ºC, and 35ºC for 12 weeks (Lin & Chen, 2005). Their results showed that light, increasing temperatures and long storage periods enhance the degradation and isomerisation of all *trans*- and *cis*-isomers of lycopene (Lin & Chen, 2005).

In a separate study by Javanmardi and Kubota (2006), fresh fruit from hydroponically-grown tomato plants were stored at 5°C and 12°C for two consecutive weeks. Storage at 5°C inhibited weight loss and increased the lycopene concentration, total soluble solids and antioxidant activity compared to the fruits stored at 12ºC. In contrast, the fruits stored for seven days at room temperature (control), displayed enhanced weight loss and loss of lycopene concentration and antioxidant activity (Javanmardi & Kubota, 2006).

## 2.3 BLOSSOM-END ROT

### 2.3.1 INTRODUCTION

First described by Galloway in 1888 as black-rot (Taylor *et al.,* 2004), blossom-end rot (BER) is a non-infectious nutritional disorder of *Lycopersicon esculentum* (tomatoes), *Capsicum annuum* (pepper fruits), *Solanum melongena* (eggplants) and *Citrullus lanatus* (watermelon). Fifty-six years later, in 1944, Raleigh and Chucka were the first to find evidence that calcium is involved in the occurrence of BER (Taylor *et al.,* 2004). Blossom-end rot may cause substantial yield and financial losses (Taylor *et al.,* 2004; Ho & White, 2005). Up to 70% of a tomato yield can be lost to BER depending on the cultivar, environmental conditions and agricultural practises (http://www.ipm.uiuc.edu/diseases; Taylor *et al.,* 2004).

Blossom-end rot is caused by a local calcium deficiency during the first few weeks after anthesis when fruit development enters the stage of rapid fruit growth (Sonneveld & Voogt, 1991; Ho *et al.,* 1999; Marcelis & Ho, 1999; Taylor *et al.,* 2004), and the vegetative parts of a plant are unable to meet the fruit cells' calcium demands. Calcium is a structural component of cell walls and membranes, serves as a cytosolic signal that regulates the process of cell expansion and serves as a counter-cation in enlarging vacuoles (Ho *et al.,* 1993; White & Broadley, 2003).

However, excess calcium can be just as damaging as a deficiency (Jiang & Huang, 2001). Excess calcium might lead to the inhibition of germination and a reduction in growth rates. Symptoms of excess calcium in cultivated tomatoes lead to the development of calcium oxalate crystals that appear as small, yellow flecks in the cell wall around the calyx and the shoulders of the fruit (Nukaya *et al.,* 1995*a*&*b*; White & Broadley, 2003). Excess calcium may also cause a Mg or $K^+$ deficiency (http://www.cartage.org).

Studies by Ho and White (2005) found that BER has not been reported for wild tomato species. However, a wide spread occurrence of BER was found amongst greenhouse- and field-grown tomatoes in all areas of the world, even though these tomato plants are cultivated in soil/nutrient media with adequate calcium concentrations. These results suggest that certain cultivation and environmental conditions disrupt the balance between calcium supply and calcium demand in distal fruit tissue, especially during periods of rapid fruit expansion (Marcelis & Ho, 1999). Calcium nutrition is therefore neither a primary, nor an independent factor in the development of BER (Marcelis & Ho, 1999; Ho & White, 2005).

### 2.3.2 SYMPTOMS AND OCCURRENCE OF BER

The hypothesis for the induction of BER in tomatoes is based on the fact that all environmental and genetic factors that influence the occurrence of BER, either affect the rate of cell expansion, or the delivery of calcium to young tomato fruit (Ho *et al.*, 1993; Marcelis & Ho, 1999). Blossom-end rot only occurs in distal fruit tissue one to three weeks after anthesis when fruit growth is marked by a phase of rapid growth before the development of any locular tissue. Rapid cell expansion and vacuolation is characteristic of this phase (Sonneveld & Voogt, 1991; Marcelis & Ho, 1999; Ho *et al.*, 1999; Taylor *et al.*, 2004). Vacuoles enlarge during rapid cell expansion and sequestrate calcium. During periods of limited calcium supply, the enlarging vacuole’s sequestration of calcium could starve the cytoplasm or the apoplast of $Ca^{2+}$. A reduction in apoplastic calcium cations ($[Ca^{2+}]_{apoplast}$) and $[Ca^{2+}]_{cyt}$ can result in impaired cell wall properties, structural weakness, precocious cell expansion, alterations in the plasma membrane permeability, unregulated solute fluxes and aberrant responses to environmental or developmental signals, leading ultimately to uncontrolled cell death. This causes the formation of watery, discoloured brown necrotic tissue at the blossom-end of the fruits (Figure 2.3), symptoms characteristic of BER (Suzuki *et al.*, 2000; Ho & White, 2005; http://www.uvm.edu). Thus, the calcium concentration in BER affected fruits are not necessarily lower than in unaffected fruit tissue since the vacuolar calcium concentration could still be high (Ho & White, 2005). Consequently, predicting and preventing the occurrence of BER from measuring the calcium status in plants is not effective, since BER occurs in plants and fruits with apparently adequate tissue calcium concentrations.

Blossom end rot also manifests in internal symptoms. Internal BER, also known as black seeds, entail the development of necrotic regions in the parenchyma tissue surrounding the young seeds and the distal placenta (Ho & White, 2005). These internal symptoms are an earlier phase in the development of BER, or evidence of a milder case.

**Figure 2.3:** A tomato fruit displaying external symptoms of BER. The collapse of cells in the epidermis and subepidermal parenchyma, the disruption of the plasma membrane and tonoplast, a wavy shaped cell wall, broken mitochondrial membranes and endoplasmic reticulum (ER), the degeneration of organelles and swollen plastids etc. (Suzuki *et al.*, 2000), lead to the appearance of a sunken lesion at the blossom-end of the fruit (Suzuki *et al.*, 2000; Ho & White, 2005; Photographs by Dr. GP Potgieter).

The length of the asymptomatic period is not affected by the growing stage of the plant during which a calcium stress is experienced. It does however, have a significant effect on the severity of the BER symptoms (Sonneveld & Voogt, 1991).

### 2.3.3 DEVELOPMENTAL AND ENVIRONMENTAL FACTORS INFLUENCING THE INCIDENCE OF BER

Any factor disturbing the relationship between calcium demand and supply has the ability to induce BER (Ho & White, 2005). Calcium demand is determined by the requirements of the fruits and leaves, while calcium supply is dependent on calcium uptake by the roots and the transportation thereof through the plant. Calcium is taken up by the roots from the soil solution and delivered to the shoots via the xylem. Calcium may enter the roots either through the $Ca^{2+}$-permeable channels located in the plasma membrane of the cells, or through the spaces between the cells (White, 2001; White & Broadley, 2003). Calcium movement through the plant and its accumulation in fruits are correlated to the transpirational movement of water (Sonneveld & Voogt, 1991; Ho *et al.,* 1999; Marcelis & Ho, 1999; Taylor *et al.,* 2004), suggesting that rapidly growing transpiring leaves or stems, that have a higher surface area than fruits, act as competing sinks with fruits for the directional flow of calcium and water (Taylor *et al.,* 2004).

Ho *et al*. (1993) proved that the physiological basis for the susceptibility of tomatoes to BER, is a relationship between fruit development and environmental conditions (Marcelis & Ho, 1999). The extent to which environmental conditions influence the induction of BER varies with different cultivars and the susceptibility of the cultivars to BER.

Cultivars have specific fruit shapes and sizes that influence the induction of BER. For example, plum tomatoes are more susceptible to BER than round tomatoes. Based on these observations, the susceptibility of cultivars to BER is apparently influenced by the distribution of their xylem network (Ho *et al.,* 1993). During fruit expansion, the density of the xylem vessels decreases resulting in fewer and narrower xylem vessels at the blossom-end of the fruit in comparison to the proximal end. This in turn decreases the xylem:phloem ratio towards the distal end of the fruit (Ho *et al.,* 1993). After the phase of rapid fruit expansion has been completed, only two single functioning strands of xylem remain in the placental tissue even though the xylem network increases in the pericarp (Ho & White, 2005). Cultivars less susceptible to BER have better developed xylem networks and higher calcium concentrations in the distal ends of their fruits when compared to the fruits of susceptible cultivars (Ho *et al.,* 1993; Marcelis & Ho, 1999).

Several environmental factors such as relative humidity (Ho *et al.*, 1999), nutrient composition, salinity/osmotic strength of the soil solution/nutrient media, root zone temperature, anoxia, drought, uneven watering and the interactions between light and temperature on fruit enlargement, may influence the uptake and portioning of calcium to the fruits and therefore the incidence of BER (Faust, 1980; Ho *et al.*, 1993; Saure, 2001; Adams, 2002; Ho & White, 2005; Napier & Combrink, 2006).

Each leaf requires a certain minimum transpiration rate to ensure a sufficient calcium supply (Adams & Ho, 1993). The minimum transpiration rate of older, more mature leaves is greater than that of younger leaves and fruits (Adams & Ho, 1993; Taylor *et al.,* 2004).

Therefore, under conditions of very high (> 90%) and low relative humidities, fruits and younger leaves are by-passed, as they represent weak transpiration sinks. Consequently, a local calcium deficiency develops in the fruits and young leaves of the plants exposed to these conditions, eventually leading to the development of BER (Adams & Ho, 1993; Taylor *et al.,* 2004) and tipburn (Berghoef, 1986).

As mentioned earlier, nutrient composition and the calcium concentration in the soil/nutrient media affect the uptake of calcium. Certain mineral stresses that inhibit the uptake of calcium or promote the synthesis of inorganic acids that chelate calcium, might exacerbate a period of limited calcium supply. An increase in ions such as $NH_4^+$ (Nukaya *et al.*, 1995*b*), $Na^+$, $Mg^{2+}$ (José *et al.*, 1994) and $K^+$ antagonize $Ca^{2+}$ uptake since ion uptake decreases as the valence of the ions increase (Taylor *et al.,* 2004). However, the addition of $NO_3^-$ to soil/nutrient medium stimulates calcium uptake, but high concentrations may also promote BER as this nutrient stimulates vegetative growth, thus promoting a large canopy which subsequently increases transpiration rates (Taylor *et al.,* 2004; Ho & White, 2005).

Other factors that also influence the uptake of calcium are salinity or osmotic stress, low root zone temperature, anoxia and drought. All of these factors reduce the uptake of water and therefore also the uptake of calcium as the uptake of the latter is directly proportional to water uptake (Ho *et al.,* 1995; 1999; Adams, 2002; Taylor *et al.,* 2004; Ho & White, 2005).

Uneven watering also proves to be detrimental to calcium distribution in plants. Moisture fluctuations during periods of rapid plant and fruit growth may create a moisture stress and an imbalance between the supply of calcium to the fruits and the calcium demand of the fruits, limiting calcium distribution to the fruits (Taylor *et al.,* 2004; Ho & White, 2005).

A relationship also exists between the occurrence of BER, average daily light intensity and temperature during periods of rapid fruit growth and cell expansion (Ho *et al.,* 1993; Marcelis & Ho, 1999). High light intensities and temperatures during the early stages of fruit development, which is characterized by rapid fruit growth, cause a local cellular calcium deficiency in the fruit. These environmental factors create a large calcium demand during a period of rapid fruit growth by increasing the metabolic functioning and growth rate of rapidly expanding fruit cells. When the rate of fruit growth creates a calcium demand that exceeds the rate of calcium supply, BER will develop, even in the presence of sufficient quantities of calcium. In addition to the relationship between light intensity, temperature and rapid cell growth during the early stages of fruit development, very high and low light intensities, relative humidities and temperatures can cause the preferential distribution of the transpiration stream to stronger transpiration sinks such as mature leaves, by-passing the weaker sinks such as the fruits (Ho *et al.,* 1993; Marcelis & Ho, 1999).

### 2.3.4 PREVENTION OF BER

In order to prevent or reduce the occurrence of BER, good management practices that optimise $Ca^{2+}$ uptake and accumulation in young fruits, should be used. One such practice is the optimisation of the mineral composition of the nutrient media/soil by avoiding high salinities and adjusting the pH to values between 6.5 to 6.8 or by avoiding excessive $NH_4^+$, $K^+$ and $Mg^{2+}$ concentrations while maintaining adequate $Ca^{2+}$ concentrations (http://www.wsutoday.wsu.edu). Rapidly growing plants with large fruit loads require large amounts of $NO_3^-$, $K^+$ and increased $Ca^{2+}$concentrations. In order to avoid a temporary reduction in the uptake of $Ca^{2+}$ from the soil/nutrient solution, the $K^+$: $Ca^{2+}$ ratio should never exceed 2:1. A ratio larger than two will favour the uptake of $K^+$ (Ho *et al.,* 1999). Furthermore, the maintenance of adequate P levels is also important since low P fruit levels hamper the movement of $Ca^{2+}$ to the distal end of the fruit. Phosphorus (P) in the nutrient/soil solution should be maintained between 40 ppm and 50 ppm (Ho *et al.,* 1999).

It is recommended that soil analyses should be done prior to planting. Calcium deficient soil should be treated with 567 kg. $ha^{-1}$ to 1134 kg $CaCO_3$. $ha^{-1}$ several months in advance of planting (http://www.ces.ncsu.edu; http://pubs.caes.uga.edu). José *et al.* (1994) found that the addition of protein hydrolysates to $CaCO_3$ increases the efficacy of $CaCO_3$ to reduce the occurrence of BER. Protein hydrolysates are essentially amino acids and peptides of low molecular weight, which is easily absorbed by plant roots and transported to other organs. Amino acids also strengthen cell walls and increase membrane permeability. They found that fertigation of 35-day-old tomato seedlings with protein hydrolysates 5 and 15 days after transplantation, increased the calcium concentration and Ca: Mg ratio of fruits (José *et al.,* 1994).

Other examples of good management practices are the maintenance of healthy roots and water levels, the prevention of extreme root temperatures and root pruning (http://www.uvm.edu).

Amongst established tomato plants displaying BER symptoms, BER can be reduced by spraying young tomato crops with cations such as $Ca^{2+}$ or Sr, which replenish the calcium stores or stabilizes the membrane structure (Ho *et al.,* 1999; Ho & White, 2005). According to industry specifications, and studies conducted by Ho and White (2005), spraying should start as soon as the first symptoms of BER appear. Blossom-end rot is treated on a weekly basis for three or four applications with 0.25% to 0.5% $CaCl_2$ or $CaNO_3$, respectively (Ho & White, 2005; www.pubs.caes.uga.edu). Ho and White (2005) found that a weekly spray of 0.5% (w/v) $CaCl_2$ reduced the occurrence of BER up to 40% (Ho & White, 2005).

Other management practices that aim to improve calcium accumulation in fruits, optimise calcium delivery to the fruits in the transpiration stream. One example of such a management practice, is de-leafing greenhouse-grown tomatoes. This technique involves the removal of leaves from behind the flower truss in the upper areas of the plant during the initial establishment phase of the crop as the leaves compete with the developing truss for assimilates. Removal of the leaves will therefore direct more moisture and assimilates into the developing truss, consequently promoting generative development and fruit growth (Ho *et al.,* 1999). The older leaves at the bottom of the plants can be removed under conditions of low and high relative humidities. This will allow the partial regulation of $Ca^{2+}$ movement by directing more $Ca^{2+}$ towards the fruits and younger leaves. Moreover, the bottom leaves contribute little to the photosynthetic gain of the plant (Subrahmanyam & Rathore, 2005). During de-leafing there is a risk of *Botrytis* infection. To minimise the risk of *Botrytis*, de-leafing should take place during the morning to allow enough time for the wounds to dry (Ho *et al.,* 1999). Another method of improving calcium delivery to the fruits of greenhouse-grown tomatoes, is by avoiding light intensities and temperatures that will stimulate high transpiration rates. Partial shading for example, will reduce the light intensity and temperature in the canopy. Selecting the light intensity at which to start shading will depend on the vigour of plant growth and how far into production the crop has progressed (Ho & White, 2005).

## 2.4 CALCIUM

In addition to good management practices and an understanding of the environmental factors that influence the induction of BER, knowledge of fruit calcium physiology at cellular level is necessary to aid in the prevention of BER. For example, knowledge regarding the role of calcium in cell functioning aids in selecting cultivars that are resistant to BER (Ho & White, 2005; http://www.wsutoday.wsu.edu). This selection might consider a range of growth characteristics such as (Ho & White, 2005):

- Fruit shape.
- Fruit size.
- Fruit: leaf ratio.
- The response of fruit cell expansion to light and temperature.
- The efficiency of calcium transport to and within the fruit.

### 2.4.1 THE FUNCTIONS OF CALCIUM IN PLANTS

Calcium, as an essential macronutrient, fulfils a variety of important roles in plant cells (Supanjani *et al.,* 2006). In addition to its structural roles, it is essential for cell division, cell expansion, $[Ca^{2+}]_{cyt}$ perturbations, which is necessary for cell signalling, and it functions as a counter cation for inorganic and organic anions in the vacuole (Jiang & Huang, 2001; White, 2001; Supanjani *et al.,* 2006). Studies by Jiang and Huang (2001) showed that calcium increases antioxidant and enzyme activities. They found that calcium increased the activity of catalase, ascorbate peroxidase and glutathione reductase during heat stress in *Festuca arundinacea* and *Poa pratensis.* Calcium is also required for carbohydrate translocation, protein and nucleic acid synthesis and basipetal auxin transport (Poovaiah & Leopold, 1973; Terry & Huston, 1975; Gossett *et al.,* 1977; Fuente, 1984). Evidence has been found that suggests that calcium affects abscission. Studies have shown that the application of calcium solutions to bean petioles can inhibit or prevent the progress of abscission (Poovaiah & Leopold, 1973). The effect of calcium on abscission is ascribed to the formation of salt bridges in the membrane between pectic components, and in large part, to the delay of senescence development (Poovaiah & Leopold, 1973).

Calcium also regulates guard cell tugor and stomatal opening (Jiang & Huang, 2001; White, 2001). Transpiration is a major determinant of plant productivity and stress management and takes place through the stomata (Murata *et al.,* 2001; Dietrich *et al.,* 2001). The opening and closure of stomatal pores are associated with large changes in the $K^+$ levels of the guard cells (Irving *et al.,* 1992). Opening of the guard cells is induced by light, low carbon dioxide concentrations, low humidity levels, indoleacetic acid (IAA), fusicoccin, cytokinins and an increase in the $K^+$ concentration of the guard cells (Irving *et al.,* 1992). Elevated levels of carbon dioxide, darkness, increased humidity and ABA induce the closure of stomatal openings (Irving *et al.,* 1992; Murata *et al.,* 2001; Prokić *et al.,* 2005). The closure of stomata is associated with the movement of $K^+$ out of the vacuole across the plasma membrane and a rise in $[Ca^{2+}]_{cyt}$. Cytosolic $Ca^{2+}$ elevations activate slow-activating sustained- (S-type; Schroeder & Hagiwara, 1989; Murata *et al.,* 2001) and rapid transient (R-type; Hedrich *et al*., 1990) anion channels (Prokić *et al.,* 2005). These channels mediate the release of anions from the guard cells to depolarise them (Prokić *et al.,* 2005). In turn, the change in membrane potential deactivates inward-rectifying $K^+$ ($K^+_{in}$) channels and activates outward-rectifying $K^+$ ($K^+_{out}$) channels (Schroeder *et al*., 1987), causing a net ion release of $K^+$ and a tugor reduction that closes the stomata (MacRobbie, 2000; Murata *et al.,* 2001; Prokić *et al*., 2005). The regulation of the opening and closing of stomata by calcium, allows the uptake of sufficient quantities of carbon, which is essential for photosynthesis (Jiang & Huang, 2001; White, 2001).

Strong evidence has also been found for the importance of calcium for the process of photosynthesis. Studies of photosynthesis and the photosynthetic efficiency of salt stressed rice leaves showed that the addition of $Ca^{2+}$ increased the chlorophyll content, proline- and soluble sugar concentration of the leaves (Skórzyńska-Polit *et al.,* 1998; Zhu *et al*., 2004). Similar results were found when heat stressed grasses were treated with calcium. Calcium treatment of these grasses improved the quality, water- and chlorophyll content of grasses studied. These results are consistent with that found for several other plant species (Jiang & Huang, 2001). Moreover, photosystem II efficacy is promoted when the intrathylakoid calcium is high (Brand & Becker, 1983; Skórzyńska-Polit *et al.,* 1998). Evidence supporting calcium’s function in the water-splitting complex and the reaction centre of photosystem II have also been found (Brand & Becker, 1983). Calcium cations ($Ca^{2+}$) also appear to regulate the distribution of energy between photosystems I and II, the thermal stabilization of photosynthetic membranes, the binding of plastocyanin to membranes, thylakoid stacking and stimulation of electron transport activities (Brand & Becker, 1983). Calcium may also modulate the phosphatase enzymes in the carbon reduction cycle, as well as the regulation of chloroplast $NAD^+$ kinase activity through calmodulic like protein (Brand & Becker, 1983). A calcium deficiency apparently seems to impair photosynthesis by damaging the chlorophyll lamellae (Terry & Huston, 1975; Rangnekar, 1975*a*). A study conducted on tomato leaf tissue, found a 50% loss of chlorophyll after eight to ten days of calcium deprivation and a 30% reduction in the photosynthetic rate (Rangnekar, 1975*b*).

In addition to influencing photosynthesis and consequently carbohydrate production, calcium is also important for the selective uptake of ions, especially under saline conditions when $NO_3^-$ uptake is inhibited (Rains, 1972; Ward *et al.*, 1986). The addition of $Ca^{2+}$ to soil/nutrient solutions under saline conditions enhance $NO_3^-$ uptake. It increases the activity of $NO_3^-$ transporters and reduces the time period needed by the $NO_3^-$ transporters to reach constant rates of $NO_3^-$ transport (Ward *et al.*, 1986).

Calcium cation perturbations also link specific environmental and developmental stimuli to the appropriate physiological responses (White, 2001; Supanjani *et al.*, 2006). Calcium's ability to couple a large array of signals and responses enable wound signalling and the activation of the plant's defence system against attempted microbal invasion and pathogen infection (Reymond *et al.*, 1996; León *et al.*, 1998; 2001; Blume *et al.*, 2000; Lecourieux *et al.*, 2006).

### 2.4.2 CALCIUM UPTAKE AND MOVEMENT TO THE SHOOT

For a $Ca^{2+}$ to move from the soil solution to the tracheary elements or root stele, the ion must move through the epidermis, exodermis, central cortex and endodermis. However, the Casparian band of the endodermis is impenetrable to ions. Thus, the development of the Casparian band influences the movement of $Ca^{2+}$ from the soil into the xylem (Cholewa & Peterson, 2004). The endodermis cells develop via three different states. During the first two states of development, calcium can move freely through the endodermal cells, but during state three, calcium delivery to the xylem through the enodermal cells is severely restricted even though the cells are still connected to the cortex and stele via plasmodesmata (White, 2001). Calcium destined for the xylem, then enters via the calcium ion permeable channels on the cortical side of the Casparian band, from where it is actively exported by the plasma membrane $Ca^{2+}$-ATPases or $Ca^{2+}/H^+$ antiporters of cells within the stele. Regulating the expression and activity of these transporters will allow the cell to deliver calcium selectively to the xylem at a rate consistent with the requirements of the shoot (White & Broadley, 2003). This method of calcium uptake is termed, the symplastic route.

The delivery of calcium to the xylem is restricted to the root tip where lateral roots are initiated (White, 2001; White & Broadley, 2003). Due to the presence of lateral roots, the Casparian band between the endodermal cells are absent and/or the endodermal cells surrounding the stele are unsuberized (White, 2001; Cholewa & Peterson, 2004; Supanjani *et al.,* 2006), therefore allowing the transport of water and divalent cations to the shoot. The transport of water and cations via the xylem is known as the apoplastic route.

Both the symplastic- and apoplastic route have certain distinct disadvantages and advantages. Cells avoid $Ca^{2+}$ toxicity by maintaining low cytosolic concentrations of free $Ca^{2+}$ (Jiang & Huang, 2001). However, the shoot of the plant requires large quantities of calcium, which would imply toxic levels of cytosolic calcium concentrations since it is impossible for the symplastic route to maintain low $[Ca^{2+}]_{cyt}$ for cell signalling and supply the aerial parts of the plant with enough calcium to maintain healthy growth (Clarkson, 1984). The functional separation of apoplastic $Ca^{2+}$ fluxes for transfer to the shoot, and symplastic $Ca^{2+}$ fluxes for cell signalling, will enable the roots to fulfil the calcium demand of the shoots without compromising intracellular $[Ca^{2+}]_{cyt}$ signalling (White 2001; White & Broadley, 2003; Cholewa & Peterson, 2004). At low transpiration rates, transmembrane solute fluxes will dominate because less $Ca^{2+}$ are transported via the apoplastic route (White, 2001).

A disadvantage of the apoplastic route, is its non-selectivity for divalent cations. This route runs the risk of accumulating toxic levels of solutes in the shoot. In contrast, one of the great advantages of the symplastic route is its selectivity and the ability to control the rate of calcium transport to the shoot (White, 2001; White & Broadley, 2003).

### 2.4.3 CALCIUM MOVEMENT IN THE CELLS: CALCIUM WAVES

Once inside the cells, calcium is not freely mobile in the cytoplasm and binds easily to the many proteins that are attached to the cytoskeleton or membrane surfaces (Trewavas, 1999). Thus, calcium movement through the cell is established through calcium waves. The discovery of inositol phosphates ($IP_3$) provided clarification regarding calcium movement by means of wave formations (Trewavas, 1999).

Phospholipase C is bound to the plasma membrane and activated by cell signals. According to Trewavas (1999), phosphatidylinositol bisphosphate ($PIP_2$) is the substrate for phospholipase C. Upon the activation of phospholipase C, $PIP_2$ is hydrolyzed to diglyceride and inositol phosphate ($IP_3$). Inositol phosphate ($IP_3$) is mobile in the cytoplasm and can bind to an $IP_3$-channel. The binding of $IP_3$ to an $IP_3$-channel briefly exposes a calcium-binding site. In the absence of calcium, the channel rapidly deactivates, but in the presence of calcium, the binding site is occupied and the channel opens. Inositol phosphate ($IP_3$) channels therefore need both $IP_3$ and calcium to open (Trewavas, 1999). The binding of calcium only delays the eventual $IP_3$-dependent channel inactivation via protein phosphorylation (Trewavas, 1999). Mobilization of calcium through an $IP_3$ dependent channel is brief and self-limiting, effectively guarding against spontaneous opening.

The calcium concentration surrounding $IP_3$-dependent channels increases upon opening, subsequently causing the opening of nearby surrounding $IP_3$-dependent channels. Calcium is therefore needed to induce the release of more calcium. This action forms the basic structure of a calcium wave. A wave is not the forward movement of calcium, but rather a forward release of calcium (Trewavas, 1999). Inositol phosphate ($IP_3$)-sensitive channels are located in the vacuole, ER and plasma membranes, suggesting the movement of calcium across these membranes (Trewavas, 1999).

Calcium cations ($Ca^{2+}$), for calcium waves, are either imported from the apoplast across the plasma membrane or from intracellular calcium stores (vacuoles, vesicles, cell wall, ER, mitochondria and possibly the Golgi vesicles) through $Ca^{2+}$ permeable channels. The remaining calcium in the cell, after protein binding and transport to organelles, is termed ‘resting’ calcium (Trewavas, 1999).

The storage of calcium in organelles creates electrochemical calcium concentration gradients between the cytoplasm and the cell wall and the cytosol and organelles (Trewavas, 1999). Calcium-channel proteins allow the flow of calcium from the cytosol to other cellular compartments in response to a variety of developmental and environmental signals. Calcium from the various calcium stores enters the cytosol down the electrochemical gradient. Different types of channels are responsible for the flow of calcium between the cytosol and the various calcium stores. Membrane potential or secondary messengers activate these channels. After an increase in the cytoplasmic calcium levels, the channels immediately close and dependent calcium-ATPases transport the calcium back to intracellular stores and the cell wall, restoring $[Ca^{2+}]_{cyt}$ to resting levels (Trewavas, 1999). The transient elevations of calcium may last anything from a few seconds to a few minutes.

### 2.4.3.1 The calcium transporters in cellular membranes that contribute to the generation of calcium waves

#### 2.4.3.1.1 *$Ca^{2+}$ efflux from the cytosol through $Ca^{2+}$-ATPases and H/$Ca^{2+}$-antiporters*

After an elevation in $[Ca^{2+}]_{cyt}$, calcium levels return to a resting state by the removal of $Ca^{2+}$ from the cytosol against an electrochemical gradient either to the apoplast or the intracellular organelles (Supanjani *et al.*, 2006). The removal of $Ca^{2+}$ requires active transport and the expenditure of cellular energy and is catalysed by $Ca^{2+}$-ATPases and $H^+/Ca^{2+}$ antiporters (White & Broadley, 2003).

The calcium pumps and antiporters most likely form a multiplex system within the membrane (Harper, 2001). Calcium cation-ATPases, which has a high affinity but a low capacity for $Ca^{2+}$ transport, are responsible for maintaining $[Ca^{2+}]_{cyt}$ homeostasis in unstimulated cells, while the $H^+/Ca^{2+}$-antiporters, which has a low affinity, but a high capacity for $Ca^{2+}$ transport, are responsible for the removal of $Ca^{2+}$ from the cytosol during $[Ca^{2+}]_{cyt}$ signalling (White & Broadley, 2003). The $H^+/Ca^{2+}$-antiporters usually require a stoichiometry of at least three ($3H^+/1Ca^{2+}$; Sanders *et al.*, 2002). The removal of $[Ca^{2+}]_{cyt}$ to either the apoplast or organelles makes normal metabolic functioning possible by:

- Maintaining low $[Ca^{2+}]_{cyt}$ levels in unstimulated cells for appropriate cytoplasmic metabolism (Sanders *et al.*, 2002; White & Broadley, 2003).

- Restoring $[Ca^{2+}]_{cyt}$ to resting levels after calcium perturbations (White & Broadley, 2003).
- Replenishing intracellular and extracellular $Ca^{2+}$ stores,which permits $[Ca^{2+}]_{cyt}$ signalling and the generation of local $[Ca^{2+}]_{cyt}$ oscillations through the interplay of calcium pumps and antiporters with calcium cation channels (Sanders *et al.,* 2002; White & Broadley, 2003).
- Providing the ER with $Ca^{2+}$ making normal secretory system functioning possible (Sanders *et al.*, 2002; White & Broadley, 2003).
- Removing divalent cations such as $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$ or $Zn^{2+}$ from the cytosol to support the specialized functioning of certain organelles as well as preventing mineral toxicity (White & Broadley, 2003).

#### 2.4.3.1.2 *Calcium influx to the cytosol: calcium channels*

An elevation is $[Ca^{2+}]_{cyt}$ requires the influx of calcium to the cytosol through $Ca^{2+}$ permeable channels. The principle role of these channels is cell signalling, which is made possible by the presence of diverse classes of $Ca^{2+}$ permeable channels in the membrane allowing physiological flexibility (White, 2000; White & Broadley, 2003). Calcium permeable channels are divided into three different classes based on their voltage–dependence (White & Broadley, 2003):

1) Depolarisation-activated cation channels (DACC). Several types have been observed, but all are permeable to both monovalent and divalent cations that contribute to the uptake of essential-, as well as toxic cations. Three types of pharmacologically distinct DACCs have been observed of which the slow activating vacuolar (SV) type is the most common (Sanders *et al.,* 2002; White & Broadley, 2003). It has been proposed that DACCs act in tandem with cytoskeletal re-arrangements to aid chilling-resistant plants in their acclimatisation to low temperatures (White & Broadley, 2003).

2) Hyperpolarisation-activated cation channels (HACC). These channels are permeable to many divalent cations including $Ba^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Cd^{2+}$, and $Zn^{2+}$ (Sanders *et al.,* 2002; White & Broadley, 2003). Elicitor-activated HACCs raise $[Ca^{2+}]_{cyt}$ to

initiate cellular responses to pathogens. Hyperpolarisation-activated cation channels also contribute to the closure of stomata in response to water stress (White & Broadley, 2003).

3) Voltage-independent cation channels (VICC). It is unlikely that voltage-gated pathways comprise the only route for $Ca^{2+}$ entry across the plasma membrane. Channels that discriminate poorly between monovalent and divalent cations and exhibit at best only weak voltage-dependence, are termed voltage-independent cation channels (Sanders *et al.*, 2002). Voltage-independent cation channels open at physiological voltages and are generally insensitive to cytoplasmic modulators. In most plants, these VICCs are the only channels open at a resting potential (White & Broadley, 2003). White and Broadley (2003) suggested that $Ca^{2+}$ influx through VICCs is required to balance the perpetual $Ca^{2+}$ efflux through $Ca^{2+}$-ATPases and $H^+/Ca^{2+}$-antiporters to maintain $[Ca^{2+}]_{cyt}$ homeostasis in an unstimulated cell.

#### 2.4.3.2 The evolution of the 'signature' of a calcium wave ($[Ca^{2+}]_{cyt}$ perturbation)

It is essential for $[Ca^{2+}]_{cyt}$ in plant cells to increase in response to developmental and environmental stimuli such as mechanical perturbations, cold shock, heat shock, acute salt stress, hyper-osmotic stress, anoxia, exposure to oxidative stress and absisic acid to produce physiological responses (Miller & Sanders, 1987; Knight *et al.,* 1997; Romeis *et al.,* 2001; Fasano *et al.,* 2002; White & Broadley, 2003; Kim *et al.,* 2003; Supanjani *et al.,* 2006). To ensure the correct physiological response to a particular stimulus, each calcium wave has a unique $[Ca^{2+}]_{cyt}$ signature evoked by a specific type of stimulus (Miller & Sanders, 1987; Evans *et al.*, 2001; White & Broadley, 2003; Romeis *et al*., 2003; Lecourieux, *et al.*, 2006). A stimulus activates phospholipase C uniquely, the primary mobilising calcium agent, through $IP_3$ dependent channels enabling cellular $IP_3$ concentrations to quantify the strength of a signal proportionately to the strength of the stimulus (Miller & Sanders, 1987; Evans *et al*., 2001; White & Broadley, 2003; Romeis *et al*., 2003; Lecourieux, *et al.,* 2006). However, even though elevated $[Ca^{2+}]_{cyt}$ is necessary for signal transduction, a prolonged increase in $[Ca^{2+}]_{cyt}$ is lethal as sustained high levels of $[Ca^{2+}]_{cyt}$ might cause apoptosis (White & Broadley, 2003).

The uniqueness of a signature manifests in the sub-cellular location and/or the kinetics of the calcium wave or $[Ca^{2+}]_{cyt}$ perturbation (White & Broadley, 2003). Wave kinetics can be defined by alterations in the rate of change in the $[Ca^{2+}]_{cyt}$, maximum reached by $[Ca^{2+}]_{cyt}$ and/or the duration of $[Ca^{2+}]_{cyt}$ above a certain threshold (White & Broadley, 2003). The type, cellular location, channel state and density of $Ca^{2+}$ channels regulate the spatial characteristics of calcium waves, since the low diffusion rate and high buffering of $Ca^{2+}$ within the cytoplasm result in the rapid dissipation of $[Ca^{2+}]_{cyt}$ after ion channel closure. It is for this reason, that the proteins responding to the changes in $[Ca^{2+}]_{cyt}$ are associated with the $Ca^{2+}$ channels, or tethered closely to the membranes (White & Broadley, 2003). Intracellular store replenishment, secondary messengers, and the age and state of cell development also regulate the spatial characteristics of calcium waves (Trewavas, 1999).

### 2.4.3.3 Responding to cytosolic calcium waves

Different calcium sensors are responsible for the initial perception of a calcium wave. These sensors can be divided into two groups namely sensor relays and sensor responders (Sanders *et al.*, 2002).

#### 2.4.3.3.1 *Sensor Relays*

Sanders *et al*. (2002) define sensor relays as proteins that function through bi-molecular interactions. Sensor relays, such as calmodulin (CaM), CaM-like proteins and calcineurin B-like proteins (White & Broadley, 2003; Kim *et al.*, 2003), bind $Ca^{2+}$ using a helix-loop-helix structure termed the EF hand. The latter binds a single $Ca^{2+}$ molecule with high affinity (Strynadka & James, 1989) altering the structural or enzymatic properties of the protein sensor. These changes influence the interactions of these proteins with target proteins and therefore ultimately influence solute transport, enzymatic activities, cytoskeletal orientation, protein phosphorylation cascades and gene expression. It is said that these changes result in stress tolerance and/or a developmental change (Strynadka & James, 1989; White & Broadley, 2003).

#### 2.4.3.3.2 *Sensor Responders*

Whereas sensor relays function through bi-molecular interactions, sensor responders are defined as proteins that function through intramolecular interactions (Sanders *et al.,* 2002). Sensor responders undergo a calcium-induced conformational change that alters the protein's own activity or structure. An example of such a protein is $Ca^{2+}$-dependent protein kinases (CDPKs) that function as a sensor as well as a kinase (Sanders *et al.,* 2002; White & Broadley, 2003; Kim *et al.,* 2003). Calcium cation-dependent protein kinase can function as a sensor that decodes and translates the elevation of cytosolic free calcium into biochemical and genetic consequences through the phosphorylation of diverse target proteins including membrane solute transporters, ion and water channels, NADPH oxidases, enzymes involved in carbon and nitrogen metabolism, cytoskeletal proteins, proteases and DNA binding proteins (Romeis *et al.,* 2001; Sanders *et al.,* 2002; White & Broadley, 2003; Kim *et al.,* 2003). It is possible that the possession of many CDPKs with contrasting calcium affinities and target proteins allow plant cells to respond appropriately to specific calcium waves or $[Ca^{2+}]_{cyt}$ perturbations (White & Broadley, 2003). Other protein kinases responding to $[Ca^{2+}]_{cyt}$ are less well characterized than the CDPKs. Calcium-dependent protein kinases (CDPKs) are implicated in pollen development, control of the cell cycle, ABA signal transduction, light-regulated gene expression, gravitropism, thigmotropism, nodulation, cold acclimation, salinity tolerance, drought tolerance and responses to pathogens (Jiang & Huang, 2001; Romeis *et al.,* 2001; Kim *et al.,* 2003; White & Broadley, 2003).

Several other proteins are also capable of binding $Ca^{2+}$ (White & Broadley, 2003). For example phospholipase D (PLD) activity, which is regulated by $[Ca^{2+}]_{cyt}$ through a $Ca^{2+}$/phospholipid binding site, termed the 'C2 domain' (White & Broadley, 2003), is implicated in a variety of activities such as cellular responses to ethylene and ABA, α-amylase synthesis in aleurine cells, stomatal closure, pathogen responses, leaf senescence and drought tolerance (White & Broadley, 2003).

Another example is annexins, which is located at the cell periphery and is especially abundant in highly secretory cell types (White & Broadly, 2003). The binding of $Ca^{2+}$ to annexins enables the association of annexins to membranes allowing the formation of cation channels. They may have a role in membrane fusion, membrane trafficking and/or secretion (White & Broadley, 2003).

## 2.5 PHEROIDS

In addition to investigating the functions and importance of calcium in the plant and the role it plays in preventing or reducing BER, the other important substance of interest, was Pheroids.

A private company, Elementol (Pty) Ltd, is marketing a novel substance under the name of Pheroids. It is a micro-emulsion containing free fatty acids and/or fatty acid derivatives. The fatty acid base of this emulsion apparently acts like a micro sponge in an aqueous carrier, allowing it to transport phytologically beneficial substances across membranes. Furthermore, Elementol (Pty) Ltd claims that it has a stimulatory effect on plant growth in general. For Pheroids to have a stimulatory effect on plant growth, it must enhance cellular functioning. Very little information was given regarding the components and their structures within Pheroids since patent registration is still pending.

In order to investigate the translocation properties of Pheroids mixed with other substances across plasma membranes, membrane structure and the movement of molecules across membranes must be highlighted. The structure of all membranes is based on S.J. Singer and G. L. Nicolson's fluid mosaic model from 1972, which states that all cell membranes are made up of glycoproteins embedded in a phospholipid bilayer (Alberts *et al.,* 1989; Wolfe, 1993). Generally, non-polar molecules diffuse passively through the lipid component of membranes more readily than polar or charged molecules (Alberts *et al.,* 1989; Wolfe, 1993). The rate of diffusion of non-polar molecules is directly proportional to the concentration of the substance (Wolfe, 1993). Polar molecules and ions are transported with specificity, either passively or actively, by means of membrane proteins across cell membranes (Alberts *et al.,* 1989; Wolfe, 1993). The properties of both the lipid and the protein components of the membrane determine the rate at which polar molecules and ions are transported (Alberts *et al.,* 1989; Wolfe, 1993).

There are two major classes of membrane transport proteins, namely carrier proteins/transporters and channel proteins (Alberts *et al.,* 1989).

Carrier proteins/transporters bind to a specific substance or group of substances to induce a conformational change that will enable the proteins to transport the polar molecule/ion across the membrane. Channel proteins on the other hand, form water-filled gated channels that extend across the lipid bilayer. When open, the channels allow certain molecules, usually inorganic ions of the appropriate size, to pass through the membrane (Alberts *et al.*, 1989; Raven *et al.*, 1992).

The two primary mechanisms underlying ionic and molecular movement across cell membranes are passive and active transport. Passive transport depends on concentration- or electrical gradients and does not require the expenditure of cellular energy (Alberts *et al.*, 1989). In contrast to passive transport, active transport requires the expenditure of cellular energy since molecules are transported across cell membranes against electrochemical or concentration gradients (http://ccgb.umn.edu; Alberts *et al.*, 1989).

Some molecules, especially large molecules and molecular complexes, move into the cell through the fusion of vesicles with the cell membrane. This method of transport is termed endocytosis and the outward movement is termed exocytosis.

The principle aim of this study was to investigate the claims made by Elementol (Pty) Ltd regarding the properties of Pheroids for commercialisation purposes as a vehicle for transport of phytologically beneficial substances over membranes and as a stimulant of plant growth in general. If Pheroids is a vehicle for phytologically beneficial substances, it must be “packed” with these substances and enter plant cells either as polar or non-polar complexes through transport proteins, diffusion or endocytosis.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 MATERIALS

- Chemicals of the highest quality and purity for analytical and nutrient media purposes were purchased from Merck.
- Elementol (Pty) Ltd provided Pheroids.
- Inert coconut fibre and Dr Fisher's Multifeed Classic (Plaaskem (Pty) Ltd) were purchased from a local merchant.

## 3.2 METHODS

### 3.2.1 GREENHOUSE CONDITIONS

The temperature, relative humidity and light intensity were recorded once a week at 12:00. These greenhouse conditions were also recorded once a month at two hour intervals from 08:00 to 18:00 to illustrate seasonal changes during the experimental period.

#### 3.2.1.1 Light Intensity

A Li-Cor photometer (Model LI-185A) was used to record light intensity (µE. $m^{-2}$. $s^{-1}$). Light intensities were recorded at six different positions within the experimental layout at a height of 1.8 m. The average of these six values was used to quantify the light intensity.

#### 3.2.1.2 Temperature and Relative Humidity

The temperature of the greenhouse was set to vary between 16ºC and 25ºC. The temperature and relative humidity (%) were recorded weekly using a swirl Manson hygrometer. In addition, a thermohygrograph was used for constant temperature and relative humidity recordings during the entire study.

### 3.2.2 HYDROPONIC SET-UP

A drain-to-waste hydroponic drip system was used. White, silica gravel of approximately 550 g was placed in the bottom of 24, 7 l plastic potholders. The pots were then filled with inert coconut fibre as a support and rooting medium and placed on gravel-filled plastic drip trays. Green microtubes were connected to the drip trays to drain excess nutrient media into a waste bin. Excess nutrient media amounted to approximately 260 $cm^3$. $plant^{-1}$. $day^{-1}$.

The nutrient media were delivered to the plants through a network of PVC piping and drippers from a 70 l darkened reservoir. The flow rate was approximately 17 $cm^3$. $min^{-1}$. $dripper^{-1}$. The nutrient media were replaced once every two weeks to replenish the nutrients in the media and to prevent the build up of harmful substances. An electronic timer controlled the rate of nutrient media supply to the plants at pre-programmed times (Table 3.1).

**Table 3.1:** The nutrient media were supplied through eight cycles. The length of these cycles was adapted to the changes in day temperatures and plant requirements as the study progressed.

| | **Development Stage** | **Time of day** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cycle No.** | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| | | 08:00 | 10:00 | 11:00 | 12:00 | 13:00 | 14:00 | 15:00 | 17:30 |
| | | minutes. $cycle^{-1}$ | | | | | | | |
| A | Seedlings. No generative growth visible | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |
| B | Seedlings with Buds and Flowers | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 |
| C | Plants with small Buds, Flowers and Fruits | 4 | 4 | 4 | 4 | 5 | 3 | 3 | 3 |
| D | Large Plants with Green Fruit | 4 | 4 | 4 | 5 | 6 | 5 | 5 | 4 |
| E | Large Plants during the Early Harvest Season | 5 | 5 | 5 | 5 | 6 | 5 | 5 | 4 |
| F | Large Plants in full Production | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 6 |

The 24 pots were divided into four groups of six pots each (also see figure 3.2). The plants were 45 cm apart in a row, and the distance between two rows, was 30 cm. The plant density in the experimental layout was 4.25 plants. $m^{-2}$, which amounts to approximately 43 000 plants. $ha^{-1}$.

### 3.2.3 ESTABLISHMENT AND TRANSPLANTATION OF SEEDLINGS

Tomato seedlings (*Lycopersicon esculentum*; cv. Floridade) were germinated and established in seed trays filled with potting soil at 25°C and a constant light intensity of 200 µE. $m^{-2}$. $s^{-1}$. The cultivar used for this study, Floridade, is a determinate grower.

The seedlings were kept moist by mist spraying it with quarter strength Multifeed Classic nutrient medium until week eight of development.

#### 3.2.3.1 Transplantation of seedlings

The adhering potting soil was carefully removed from the roots of the eight-week-old seedlings with running tap water. The seedlings were then carefully blotted dry and weighed before it was transplanted to the moist coconut fibre of the hydroponic set-up. The seedlings were immediately watered to establish a plant/fibre/water continuum before the nutrient cycles were started.

#### 3.2.3.2 Staking, Pruning, and de-leafing

Two weeks after transplantation, the seedlings were staked using black, ultra violet resistant plastic twine. The twine was firmly attached to the base of the plant, wound around the main stem of the plant and attached to the supporting structure above the plants.

The plants were pruned for quality and hygienic purposes. Two methods of pruning were used to improve the quality of the yield and the hygienic conditions in the greenhouse. The first method of pruning involved the pinching off of all lateral branches (suckers ± 5 cm in length) except the one directly below the first cluster of flowers. This type of pruning may enhance the balance between vegetative- and reproductive growth and was done only once (Kemble *et al.*, 2000). This may result in larger and more uniform fruit of higher quality. The second type of pruning involved the removal of dead leaves, leaves touching the coconut fibre or leaves that constricted air movement between the plants. This was done to promote photosynthesis and reduce the spreading of diseases.

### 3.2.4 TREATMENTS

Eight treatments were used in this study. The treatments can be divided into two groups based on the nutrient media supplied to the plants (Figure 3.1). The first group (A) was cultivated in a complete Hoagland's nutrient medium and the second group (B) was cultivated in a modified Hoagland's nutrient medium deficient of calcium.

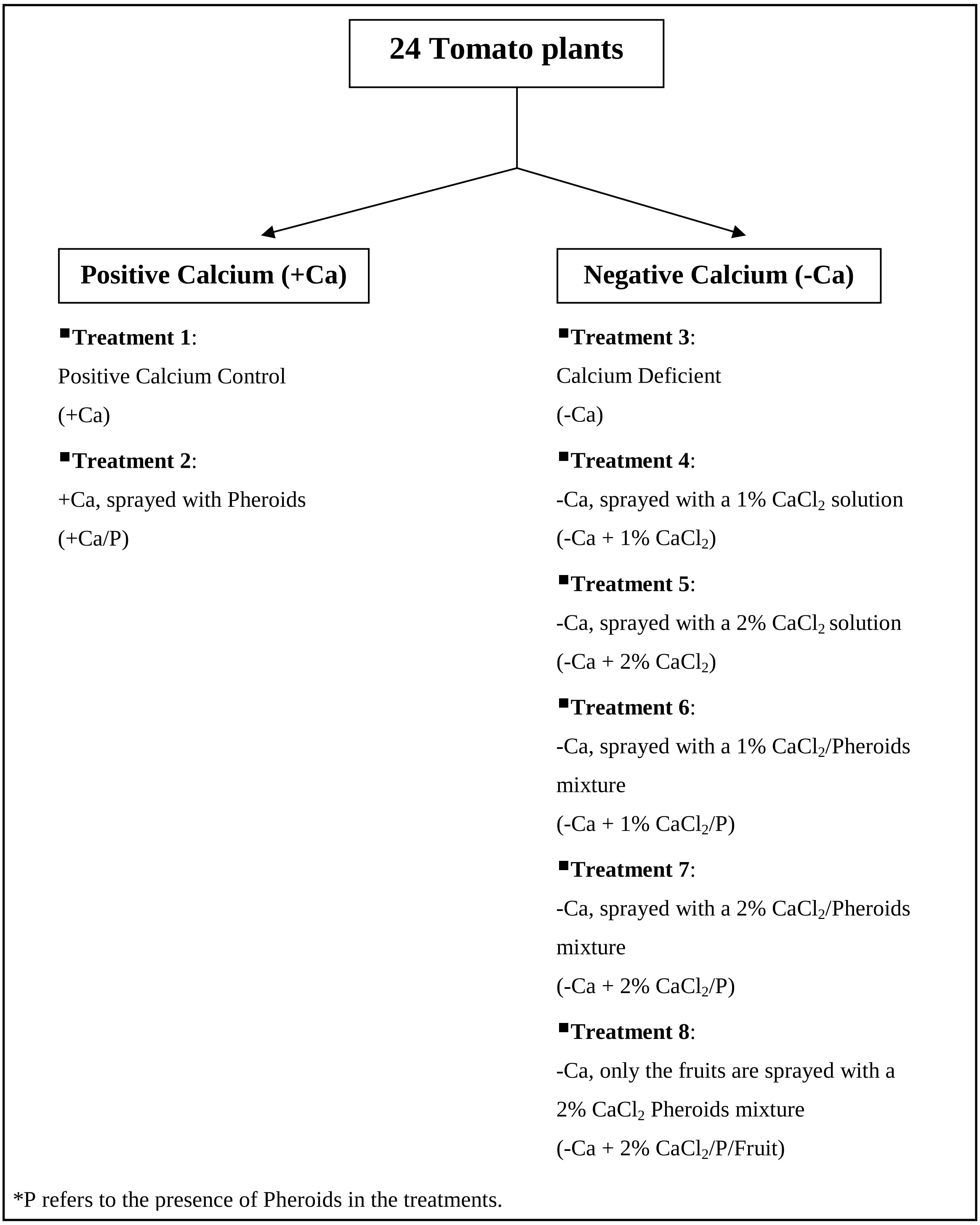
**Group A** was further subdivided into two treatments:

- **Treatment one (+Ca)** represented the control plants. These plants were cultivated in a complete Hoagland's nutrient medium and received no additional treatments.
- **Treatment two (+Ca/P)** was control plants additionally sprayed foliarly with Pheroids over the entire surface of the plant.

**Group B** was subdivided into six different treatments:

- **Treatment three (-Ca)** represented the plants cultivated in a modified Hoagland's nutrient medium deficient of calcium.
- **Treatments four (-Ca + 1% $CaCl_2$)** and **five (-Ca + 2% $CaCl_2$)** were calcium deficient plants treated foliarly with external calcium in the form of 1% and 2% $CaCl_2$ sprays.
- **Treatments six (-Ca + 1% $CaCl_2$/P)** and **seven (-Ca + 2% $CaCl_2$/P)** were calcium deficient plants, which were sprayed foliarly with 1% and 2% $CaCl_2$ solutions mixed with Pheroids.
- **Treatment eight (-Ca + 2% $CaCl_2$/P/Fruit)**. Only the fruits of calcium deficient plants were sprayed with a 2% $CaCl_2$ Pheroids mixture.

Foliage- and fruit treatments commenced as soon as the plants displayed the first symptoms of BER. Thereafter, for the duration of the experimental period, the plants were treated every two weeks.



**24 Tomato plants**

**Positive Calcium (+Ca)**

- **Treatment 1**:
  Positive Calcium Control
  (+Ca)
- **Treatment 2**:
  +Ca, sprayed with Pheroids
  (+Ca/P)

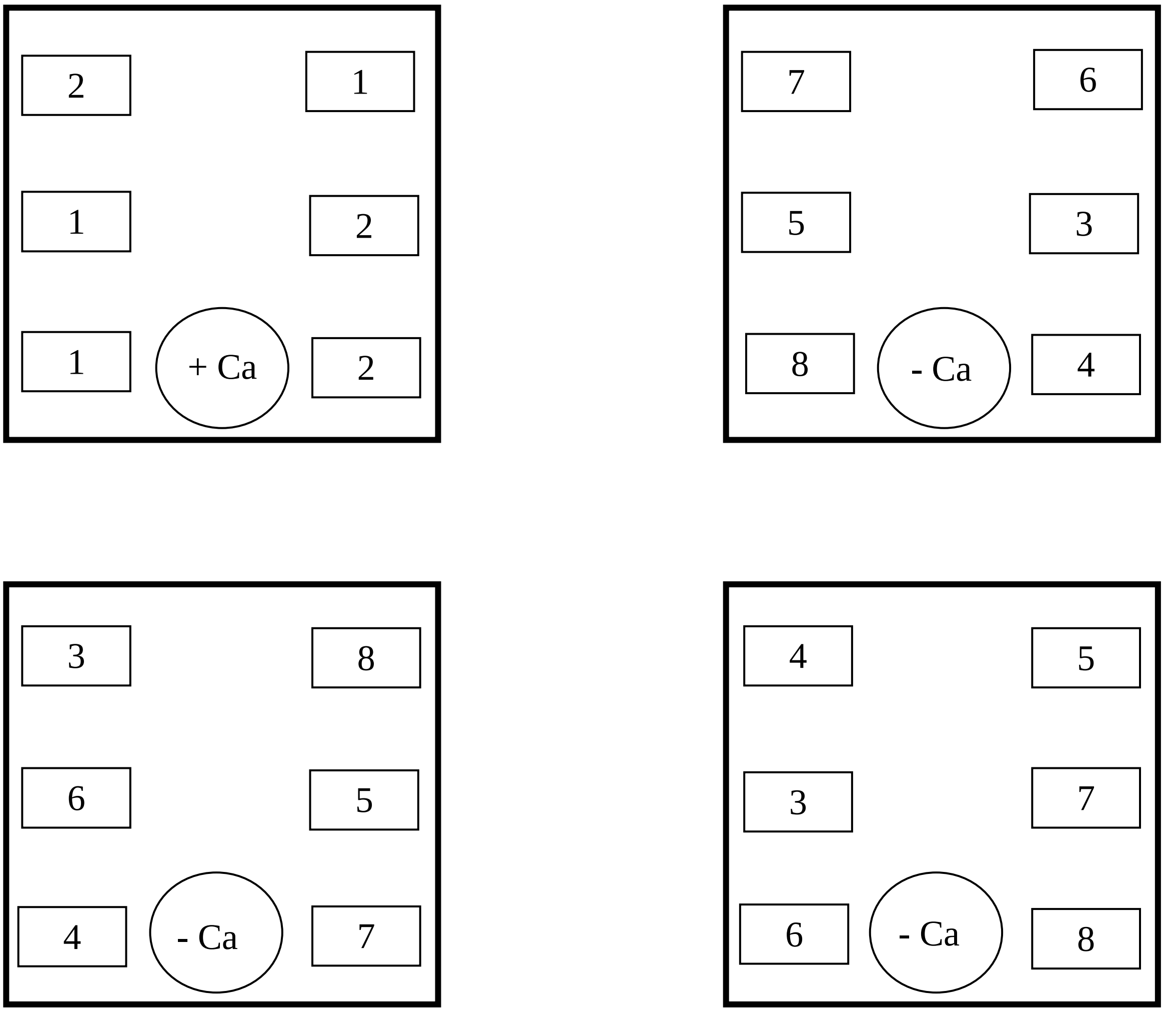
**Negative Calcium (-Ca)**

- **Treatment 3**:
  Calcium Deficient
  (-Ca)
- **Treatment 4**:
  -Ca, sprayed with a 1% $CaCl_2$ solution
  (-Ca + 1% $CaCl_2$)
- **Treatment 5**:
  -Ca, sprayed with a 2% $CaCl_2$ solution
  (-Ca + 2% $CaCl_2$)
- **Treatment 6**:
  -Ca, sprayed with a 1% $CaCl_2$/Pheroids mixture
  (-Ca + 1% $CaCl_2$/P)
- **Treatment 7**:
  -Ca, sprayed with a 2% $CaCl_2$/Pheroids mixture
  (-Ca + 2% $CaCl_2$/P)
- **Treatment 8**:
  -Ca, only the fruits are sprayed with a 2% $CaCl_2$ Pheroids mixture
  (-Ca + 2% $CaCl_2$/P/Fruit)

*P refers to the presence of Pheroids in the treatments.

**Figure 3.1:** A schematic illustration of the eight treatments used to investigate the effect of a calcium deficiency and the subsequent corrective/preventative treatments on the occurrence of BER and fruit quality. Treatments two and four to seven were applied foliarly, while treatment eight entailed the spraying of the fruits only.

The treatments were placed randomly within the hydroponic set-up. Figure 3.2 illustrates the experimental layout of the test trial.



| **Treatments:** | |
|---|---|
| **1** | +Ca |
| **2** | +Ca/P |
| **3** | -Ca |
| **4** | -Ca + 1% $CaCl_2$ |
| **5** | -Ca + 2% $CaCl_2$ |
| **6** | -Ca + 1% $CaCl_2$/P |
| **7** | -Ca + 2% $CaCl_2$/P |
| **8** | -Ca + 2% $CaCl_2$/P/Fruit |

**Figure 3.2:** The randomized layout of the eight treatments used in this study. The circles indicate the reservoirs containing the different nutrient media.

The aim of the layout was to ensure that all the treatments received approximately the same amount of sunlight within the experimental set-up.

The $CaCl_2$ concentrations used, were based on industry specifications and previous studies (Ho *et al.*, 1999; Ho & White, 2005). In all instances the solutions and mixtures were prepared at room temperature and applied at an average greenhouse temperature of ±24ºC till the point of "drip-off", which entails the formation of droplets large enough to fall to the floor.

For the 1% and 2% $CaCl_2$ solutions, 10 g and 20 g of $CaCl_2$ were dissolved in 1 l distilled water, respectively. To "pack" Pheroids with the respective $CaCl_2$ concentrations, Pheroids was added in a ratio of 2 $cm^3$ to 100 $cm^3$ $CaCl_2$ solution where applicable. In the instance of the pure Pheroids spray treatment, 2 $cm^3$ was added to 100 $cm^3$ distilled water. The mixtures were left to stir on a mixer for 30 minutes. The plants and fruits were initially sprayed with a volume of 150 $cm^3$. As the plants increased in size, the volume was increased to 250 $cm^3$. $plant^{-1}$.

In addition to the 1% and 2% $CaCl_2$ concentrations, a concentration of 4% $CaCl_2$ was also considered for the study. However, pre-test trials indicated that a 4% $CaCl_2$ solution burned the plants and it was therefore considered an unsuitable treatment for calcium deficient plants.

### 3.2.5 NUTRIENTS

The nutrient media employed for the purposes of the study were based on Hoagland's nutrient medium as this medium lends itself to manipulation (Hoagland & Arnon, 1950). A complete nutrient medium (control) and a modified medium, deficient of calcium, were prepared from one molar stock solutions (Table 3.2).

The FeEDTA stock solution was prepared by dissolving 5.57 g $FeSO_4$. $7H_2O$ in 250 $cm^3$ distilled water, to which was added, 7.45 g $Na_2EDTA$, which was dissolved in 250 $cm^3$ warm distilled water. This mixture was made up to 1 l and aerated until a brownish colour appeared. This solution was stored in a dark container.

The micronutrient stock solution was made up by dissolving 2.86 g $B(OH)_3$, 1.81 g $MnCl_2$. $4H_2O$, 0.11 g $ZnCl_2$, 0.05 g $CuCl_2$. $2H_2O$ and 0.025 g $Na_2MoO_4$ in distilled water. The mixture was made up to a final volume of 1 l and also stored in a dark container.

**Table 3.2:** Volumes used of 1 molar stock solutions to prepare complete (control)- and calcium deficient nutrient media of required final concentrations (See Table 3.3).

| **Stock solution:** | **Complete (control)** | **Calcium deficient media** |
|---|---|---|
| | **$cm^3. l^{-1}$** | **$cm^3. l^{-1}$** |
| 1 M $Ca(NO_3)_2$ | 10 | — |
| 1 M $KNO_3$ | 10 | 10 |
| 1 M $MgSO_4$ | 4 | 4 |
| 1 M $KH_2PO_4$ | 2 | 2 |
| 1 M FeEDTA | 2 | 2 |
| Micro elements | 2 | 2 |
| 1 M $NaNO_3$ | — | 20 |

All stock solutions were stored at room temperature in dark glass containers.

The nutrient formulae were applied at half strength (50%) for the first six weeks after transplantation after which it was increased to full strength (100%) for the remainder of the experimental period.

Changes in the pH and electrical conductivity (EC) of the nutrient reservoirs were measured using a PHM 85 Precision pH meter and a PW 9526 digital electrical conductivity meter before and after every refill. When necessary, the pH of the nutrient media was adjusted to values between 5.5 and 6.5 via the acid based titration method, using 98% phosphoric acid (Rosenfeld, 1999).

**Table 3.3:** The final concentrations of the macronutrients (ppm) at half (50%)- and full (100%) strength Hoagland's nutrient media

| **Elements** | **Control medium** | | **Calcium deficient nutrient medium** | |
|---|---|---|---|---|
| | **ppm** | | **ppm** | |
| | **Half strength** | **Full strength** | **Half strength** | **Full strength** |
| **N** | 210 | 420 | 210 | 420 |
| **P** | 31 | 62 | 31 | 62 |
| **K** | 234 | 468 | 234 | 468 |
| **Mg** | 48 | 96 | 48 | 96 |
| **S** | 61 | 122 | 61 | 122 |
| **Ca** | 200 | 400 | — | — |

## 3.2.6 VEGETATIVE AND GENERATIVE DEVELOPMENT OF TOMATO SEEDLINGS

The vegetative and generative development of the seedlings will ultimately determine the yield potential of the plants. It was therefore important to establish the effect a calcium deficiency and the subsequent corrective/preventative treatments have on the vegetative and generative development of the seedlings.

### 3.2.6.1 Vegetative development: Plant height and canopy diameter

Plant height and canopy diameter were accurately recorded weekly using a measuring tape until plant density and plant height (2 m = roof height) prevented further measurements.

### 3.2.6.2 Generative development: Buds, Flowers and Fruits

To determine the effect of the two different nutrient media on the generative development of the seedlings, the time span between transplantation, the appearance of the first buds (10 mm in length), fully opened yellow flowers and fruits (5 mm in diameter), was recorded.

### 3.2.7 DETERMINATION OF YIELD RELATED PARAMETERS

To determine the effect of a calcium deficiency and the subsequent corrective/preventative treatments on yield, the number of fruits harvested as well as the average fruit mass and size, was recorded.

Firm, red, tomato fruits (“consumer ready”) were picked weekly and the number of fruits harvested, as well as the number of fruits showing BER symptoms, were recorded for the individual plants from the respective treatments. An analytical balance was used to record fruit weight. A calliper was used to measure fruit diameter. The average of three different readings was taken to represent fruit diameter.

### 3.2.8 DETERMINATION OF FRUIT QUALITY RELATED PARAMETERS

Several biochemical parameters were used to determine fruit quality. These included: pH, electrical conductivity (EC), moisture content, lycopene concentration and Brix index.

#### 3.2.8.1 pH and EC

The fruits were homogenized for 30 seconds with a Polytron homogenizer to obtain a liquid puree, which was kept on ice. The pH and EC values of these purees were recorded using a PHM 85 Precision pH meter and a PW 9526 digital conductivity meter, respectively. To ensure repeatability, the pH and EC measurements for each of the samples were done in triplicate.

#### 3.2.8.2 Moisture Content

Tissue slices of approximately 10 g, representative of the fruit’s overall condition, were obtained. These slices were then dried to a constan t mass at approximately 68°C for six days. The moisture content was calculated using the following equation:

$$\% \text{ moisture content} = 100 - (\text{Dry mass}/ \text{Fresh mass} \times 100)$$

### 3.2.8.3 Lycopene concentration

The lycopene concentration of the fruits was determined in triplicate at two-week intervals.

The reduced volume extraction method (Davis *et al.*, 2002) was used to extract and determine the lycopene content of the fruits. Approximately 10 g tissue slices were sampled from the fruits. The tissue was then homogenized for more or less 30 seconds in 10 $cm^3$ double distilled water using a Polytron homogenizer. The resulting tissue purees were kept on ice in the dark as lycopene is oxidized by light (Sharma & Le Maguer, 1996; Sahlin *et al.*, 2004; Ramandeep & Savage, 2004).

Following homogenization, 0.5 g of the resulting purees were added to clean test tubes containing 5$cm^3$ of 0.05% (w/v) butylated hydroxytoluene (BHT) dissolved in acetone, 5 $cm^3$ 95% ethanol and 10 $cm^3$ hexane. The test tubes were sealed with parafilm and placed in a dark, ice filled container after which the purees and the chemicals were rotated at 180 rpm for 15 minutes on an orbital shaker to allow for the extraction of lycopene from the puree. After rotation, 3 $cm^3$ de-ionised water was added to each test tube and rotated for another five minutes. After this step, the test tubes were incubated at room temperature (±22ºC) in the dark for five minutes to allow for phase separation. The absorbance of the hexane (upper) layer was measured spectrophotometrically at 503 nm. The lycopene concentration in this hexane layer was calculated using the following equations (Davis *et al.*, 2002):

$$\text{Lycopene (mg. kg}^{-1}\text{ tissue)} = \frac{A_{503}}{17.2 \times 104/M \times cm^3} \times \frac{1\ l}{10^3\ cm^3}$$

$$\times \frac{10.0\ cm^3}{kg\ tissue}$$

$$= \frac{A_{503} \times 5.81 \times 10^{-8}}{kg\ tissue}$$

$$\begin{aligned}\text{Lycopene (mg. kg}^{-1}\text{ tissue)} &= \frac{A_{503}}{17.2 \text{ x } 10^{4}/\text{M x cm}^{3}} \text{ x } \frac{536.9 \text{ g}}{\text{mole}} \\ &\text{x } \frac{1 \text{ l}}{10^{3} \text{ cm}^{3}} \text{ x } \frac{10^{3} \text{ mg}}{1 \text{ g}} \text{ x } \frac{10.0 \text{ cm}^{3}}{\text{kg tissue}} \\ &= \frac{A_{503} \text{ x } 0.0312}{\text{kg tissue}} = \frac{A_{503} \text{ x } 31.2}{\text{g tissue}}\end{aligned}$$

The molar extinction coefficient for lycopene is 17.2 x $10^{4}$ l. $\text{mol}^{-1}$. $\text{cm}^{-1}$ and the molecular mass for lycopene is 536.9.

### 3.2.8.4 Brix Index

Brix is a measure of the percentage total soluble solids (TSS) in a given weight of plant juice. Each degree of Brix (%) is taken as the equivalent of 1 g sugar and other solids per 100 g of juice. The higher the value, the higher the sugar and other solids content of the produce (Mirza & Chen, 2005).

The Brix index of 20 µl puree samples, as prepared in 3.2.8.1, were determined using an Atago refractometer following the instructions of the manufacturer.

## 3.2.9 FRUIT DETERIORATION

To determine the effect of a calcium deficiency and the subsequent corrective/preventative treatments on fruit deterioration after harvesting, fresh (“consumer ready”) fruits were stored in dark cabinets at 12°C in a relative humidity of 75%. The fruits were placed in rows on steel racks in storage cabinets. After three weeks of storage, the fruits were removed and all the physical and quality parameters were determined as was described for fresh fruits.

# CHAPTER 4

# RESULTS

## 4.1 GREENHOUSE CONDITIONS

Environmental factors may have a profound effect on the vegetative and generative development of plants. For example, environmental factors such as light intensity, influences processes like photosynthesis, transpiration and fruit development in plants. Therefore, possible changes in the controlled greenhouse conditions were deemed important and were monitored on a weekly and monthly basis.

### 4.1.1 MONTHLY GREENHOUSE CONDITIONS

The greenhouse conditions were monitored from 08:00 to 18:00 at two hour intervals for selected months considered representative of autumn, winter and spring during the experimental period.

The light intensity of all the seasons peaked at approximately 12:00, after which it decreased (Figure 4.1A). Although the light intensity patterns were the same for autumn, winter and spring, the intensities differed for the months. The intensity at 12:00 decreased from autumn (May) to winter (July), after which it increased markedly during spring (October) as summer approached (Figure 4.1A).

No distinct differences between monthly temperatures were detected during the experimental period since it was regulated between 16°C (Night) and 26°C (Day). Although regulated, the temperature did peak at approximately 26°C between 12:00 and 14:00 for all the months (Figure 4.1B). The peak for July however, was slightly lower than that of May and October. These peaks coincided with the light intensity peak observed at the same time of the day (Figure 4.1A).

The relative humidity (% RH) for autumn (May) and winter (July) varied between 65% and 90% while during spring (October), it decreased from 80% in the early mornings to 45% in the late afternoon (Figure 4.1C).

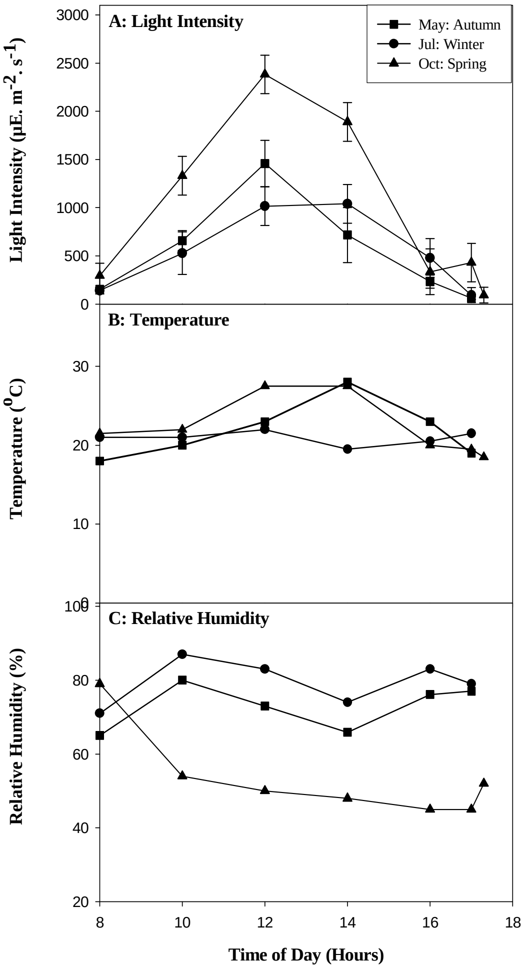


**Figure 4.1:** Changes in daily greenhouse conditions for selected months representing autumn, winter and spring (A: Light intensity; B: Temperature; C: Relative humidity).

### 4.1.2 WEEKLY GREENHOUSE CONDITIONS

Based on the changes measured for monthly light intensities (Figure 4.1A), temperature- (Figure 4.1B), and relative humidity (Figure 4.1C), changes in the greenhouse conditions were also recorded on a weekly basis at 12:00 for the duration of the experimental period.

The light intensity (Figure 4.2A) decreased from a maximum of approximately 1800 µE. m$^{-2}$. s$^{-1}$ during autumn to 1000 µE. m$^{-2}$. s$^{-1}$ during winter, whereafter it gradually increased during spring to approximately 3000 µE. m$^{-2}$. s$^{-1}$.

Temperatures at 12:00 varied between 20°C and 27°C (Figure 4.2B), indicating a controlled greenhouse environment.

The relative humidity (% RH) at 12:00 varied between approximately 55% and 85% (Figure 4.2C) for the greatest part of the experimental period. During the last ten weeks of the experimental period, it gradually fell below 60%. This decrease in relative humidity coincided with the increase in light intensity that marked the approaching summer (Compare figures 4.2A&C).

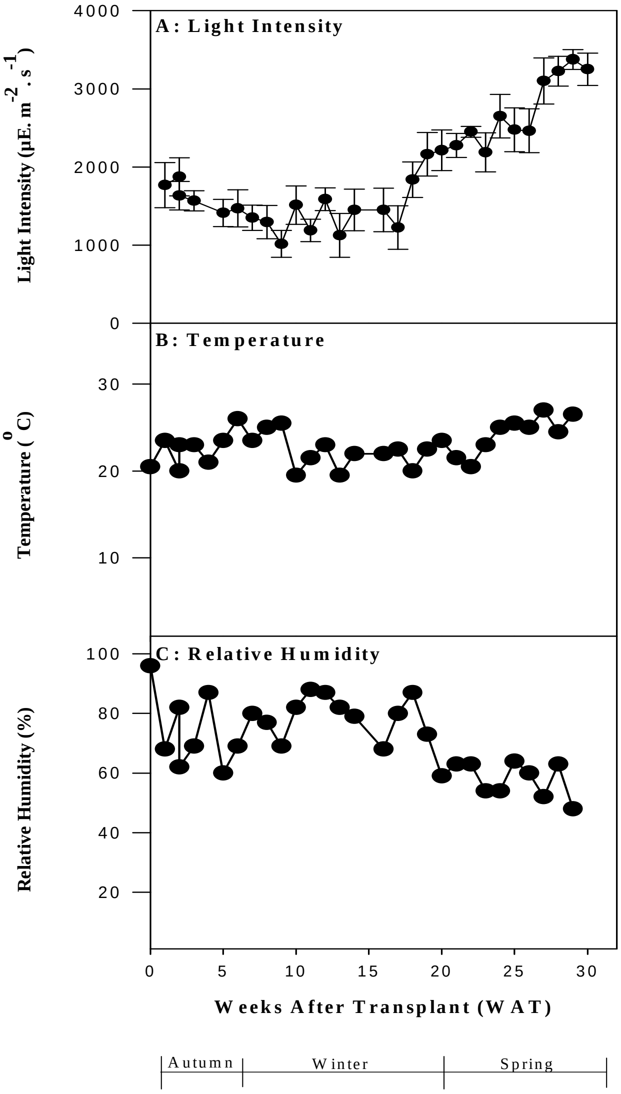


**Figure 4.2:** Weekly changes in greenhouse conditions measured at 12:00 over the entire experimental period (A: Light Intensity; B: Temperature; C: Relative Humidity).

## 4.2 EFFECT OF A CALCIUM DEFICIENCY ON THE VEGETATIVE DEVELOPMENT OF TOMATO PLANTS

To investigate the effect of a calcium deficiency on the vegetative development of tomato seedlings, plants were cultivated hydroponically in a complete (control)- and a calcium deficient nutrient medium. Changes in plant height and canopy diameter were used as parameters to determine the effect that a calcium deficiency had on early vegetative development.

A lack of calcium appeared to impede vegetative growth (Figure 4.3). The increase in canopy diameter (Figure 4.3A) and plant height (Figure 4.3B) for the plants cultivated in the calcium deficient nutrient medium was clearly less than that of the control plants. Measurement of canopy diameter was terminated after six weeks because plant density prohibited further accurate measurements.

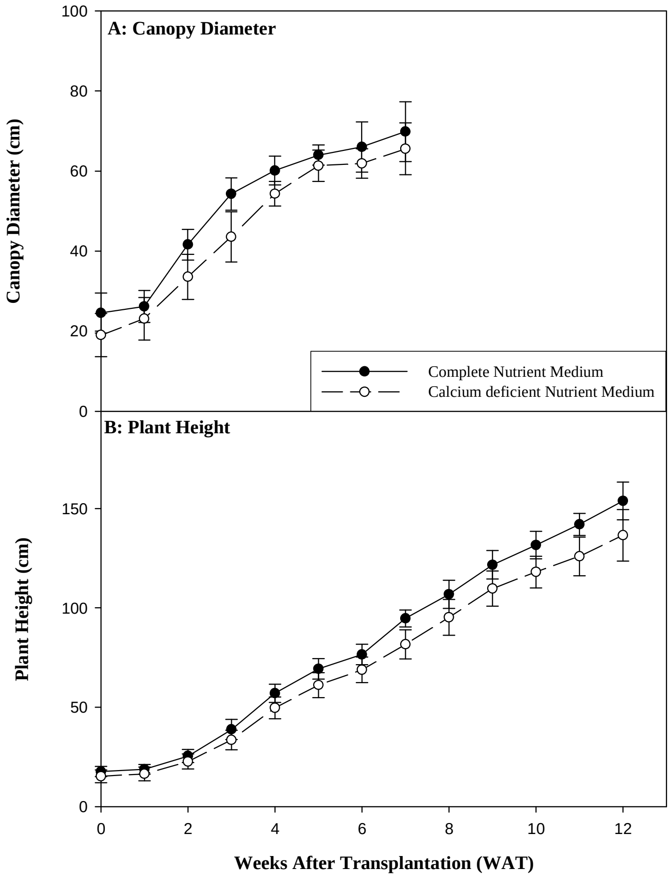


**Figure 4.3:** Effect of a calcium deficiency on the vegetative development of tomato seedlings (A: Canopy Diameter; B: Plant Height).

## 4.3 EFFECT OF A CALCIUM DEFICIENCY ON THE GENERATIVE DEVELOPMENT OF TOMATO PLANTS

The time it took for the first buds and subsequent flowers and fruits to develop, was recorded to determine the possible effect of a calcium deficiency on the generative development of tomato plants.

On average, the control plants took 19.83 days after transplantation, compared to the 22.5 days of the calcium deficient plants, to develop their first buds (Table 4.1). Approximately 13 days lapsed for both the control and calcium deficient plants for their buds to develop into flowers. It then took 12 and 12.5 days respectively, for the flowers of the control- and calcium deficient plants to develop the first fruit of 5 mm or bigger (Table 4.1). It appeared that a calcium deficiency only delayed bud formation with approximately three days during the early stages of generative development.

**Table 4.1:** Effect of a calcium deficiency on the generative development of tomato plants in a drip hydroponic system.

| **Treatment:** | **Average developmental time span from:** | | |
|---|---|---|---|
| | Transplantation to Bud (1 cm) | Bud to Flower (fully open) | Flower to Fruit (5 mm) |
| | Days | | |
| **Control Plants** | 19.83 ± 6.57 | 13.33 ± 0.82 | 12 ± 5.18 |
| **Calcium deficient Plants** | 22.5 ± 7.27 | 13.39 ± 2.12 | 12.5 ± 8.38 |

## 4.4 EFFECT OF A CALCIUM DEFICIENCY ON YIELD

The effect of a calcium deficiency on yield was determined by recording the number of fruits harvested as well as their respective masses and sizes.

The control fruits produced one of the highest yields in terms of mass during the experimental period of 16 weeks. The period between week three and nine of harvest showed the highest rate of mass accumulation was followed by a slight decrease (Figure 4.4A).

A calcium deficiency clearly had a negative effect on yield in terms of the fruit mass accumulated during the experimental period (Figure 4.4A). After 16 weeks of harvest, only 1.02 kg. plant$^{-1}$ was harvested from the calcium deficient plants compared to the 2.5 kg. plant$^{-1}$ harvested from the control plants. This represents a 59% decline in yield due to the calcium deficiency.

The corrective/preventative treatments of foliage sprays with 1% and 2% $CaCl_2$, singly and mixed with Pheroids, alleviated the severe effect a calcium deficiency had on yield only to a certain extent (Figure 4.4A). However, none of these treatments could completely alleviate the negative effect a calcium deficiency had on yield.

Where only the fruits of calcium stressed plants were sprayed with a 2% $CaCl_2$ Pheriods mixture, the yield (fruit mass) of these plants was even lower than that of the calcium deficient plants, making this the least effective treatment of all the corrective/preventative treatments (Figure 4.4A).

Spraying the control plants additionally with Pheroids had no clear effect on the yield (mass) of these plants (Figure 4.4A).

Although major differences were observed between the control- and calcium deficient plants for fruit mass harvested (Figure 4.4A), little difference was recorded for the total number of fruits harvested during the same period (Figure 4.4B). After a 16-week harvest period, approximately 65 fruits were harvested for both these treatments (Figure 4.4B). However, the rate at which the fruits were produced by the control plants exceeded that of the plants grown under calcium deficient conditions for the greatest part of the 16-week harvest period.

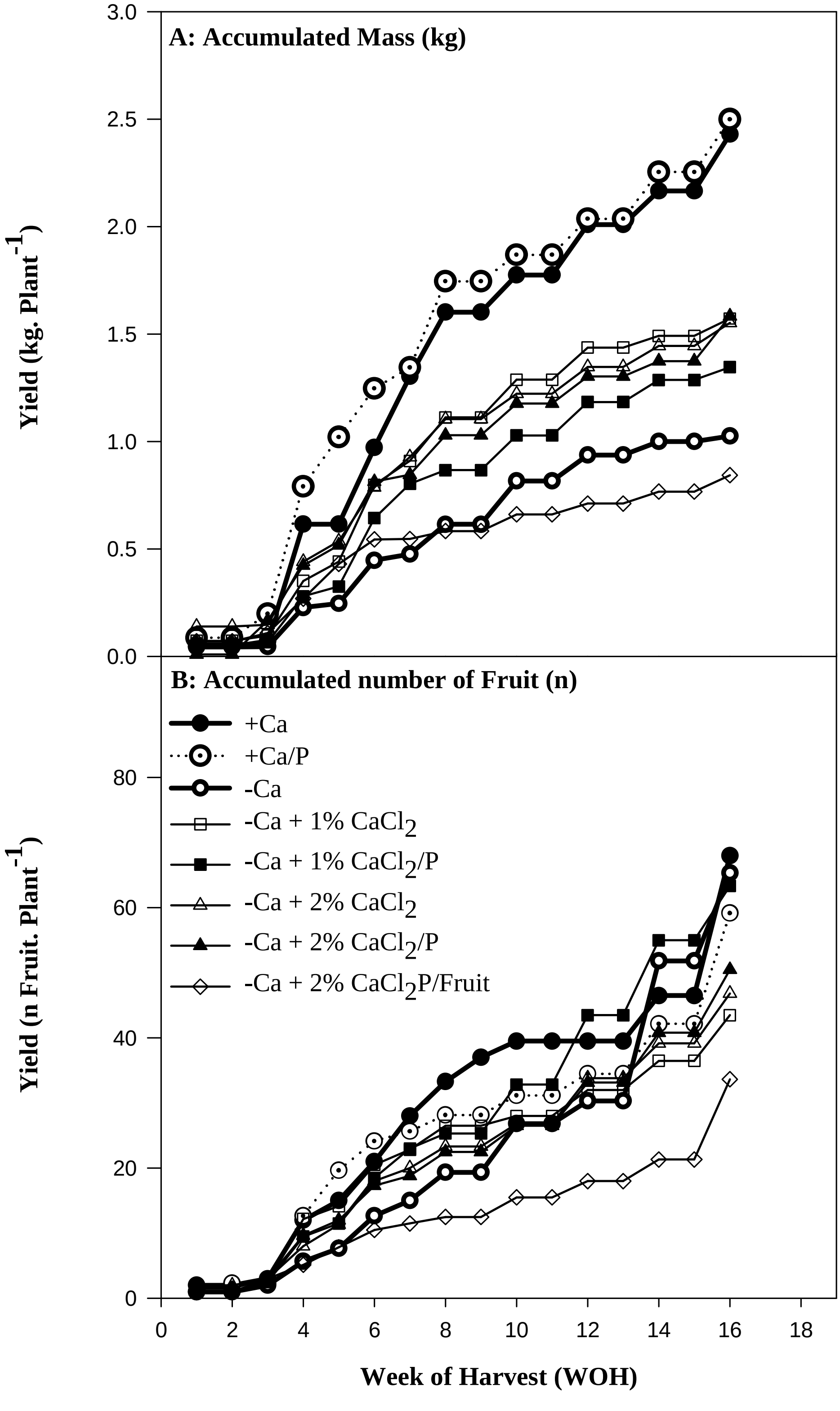


**Figure 4.4:** The effect of a calcium deficiency and the subsequent corrective/preventative treatments on the accumulated fruit mass and number of fruits harvested during the experimental period.

Spraying calcium deficient plants with a 1% $CaCl_2$ Pheroids mixture was the only corrective/preventative treatment that resulted in the same number of fruits harvested as that of the control- and calcium deficient plants (Figure 4.4B). None of the other treatments could produce a similar amount of fruits during the harvest season. Treating only the fruits of calcium stressed plants with a 2% $CaCl_2$ Pheroids mixture, resulted in the production of the least number of fruits of all the treatments (Figure 4.4B). This correlated with the low yield (mass) produced by these plants (Figure 4.4A).

The discrepancy between the number of fruits produced (Figure 4.4B) and the resulting fruit mass (Figure 4.4A) for the different spray treatments, suggests varying fruit sizes and masses. Despite variances in the fruit size and mass of the different treatments, a clear decreasing trend in fruit size and mass was evident during the experimental period (Figure 4.5A&B). The average fruit diameter (Figure 4.5A) and mass (Figure 4.5B) harvested from the calcium stressed plants were clearly less than that of the control plants, hence the lower yield observed for the plants (Figure 4.4A).

Moreover, the early and middle stages of the harvest period were the most apt for harvesting in terms of fruit size and mass. As the harvest season progressed, fruit size and mass decreased irrespective of the applied treatments. All the preventative $CaCl_2$ treatments, singly and mixed with Pheroids, resulted in bigger and heavier fruits than that of the calcium deficient plants (Figure 4.6A&B).

This is further illustrated by comparing the average fruit diameter and mass for the entire experimental period over a 16-week period (Figure 4.7A&B). Both the fruit diameter (Figure 4.7A) and fruit mass (Figure 4.7B) of calcium deficient plants were on average smaller than that of the control plants. The fruit sizes and masses obtained after the application of the corrective/preventative treatments, all exceeded that of the calcium deficient plants (Figure 4.7A&B). Mixing Pheroids with 1% and 2% $CaCl_2$ solutions as foliage sprays, also had no clear effect on the average fruit diameter and mass under calcium deficient conditions. However, spraying control plants additionally with Pheroids resulted in the marked stimulation of both the average fruit diameter and mass. This treatment clearly yielded on average the largest and heaviest fruits. Treating only the fruits of calcium stressed plants with the 2% $CaCl_2$ Pheroids mixture, resulted in fruits similar in size and mass to that of the calcium deficient plants (Figure 4.7A&B).

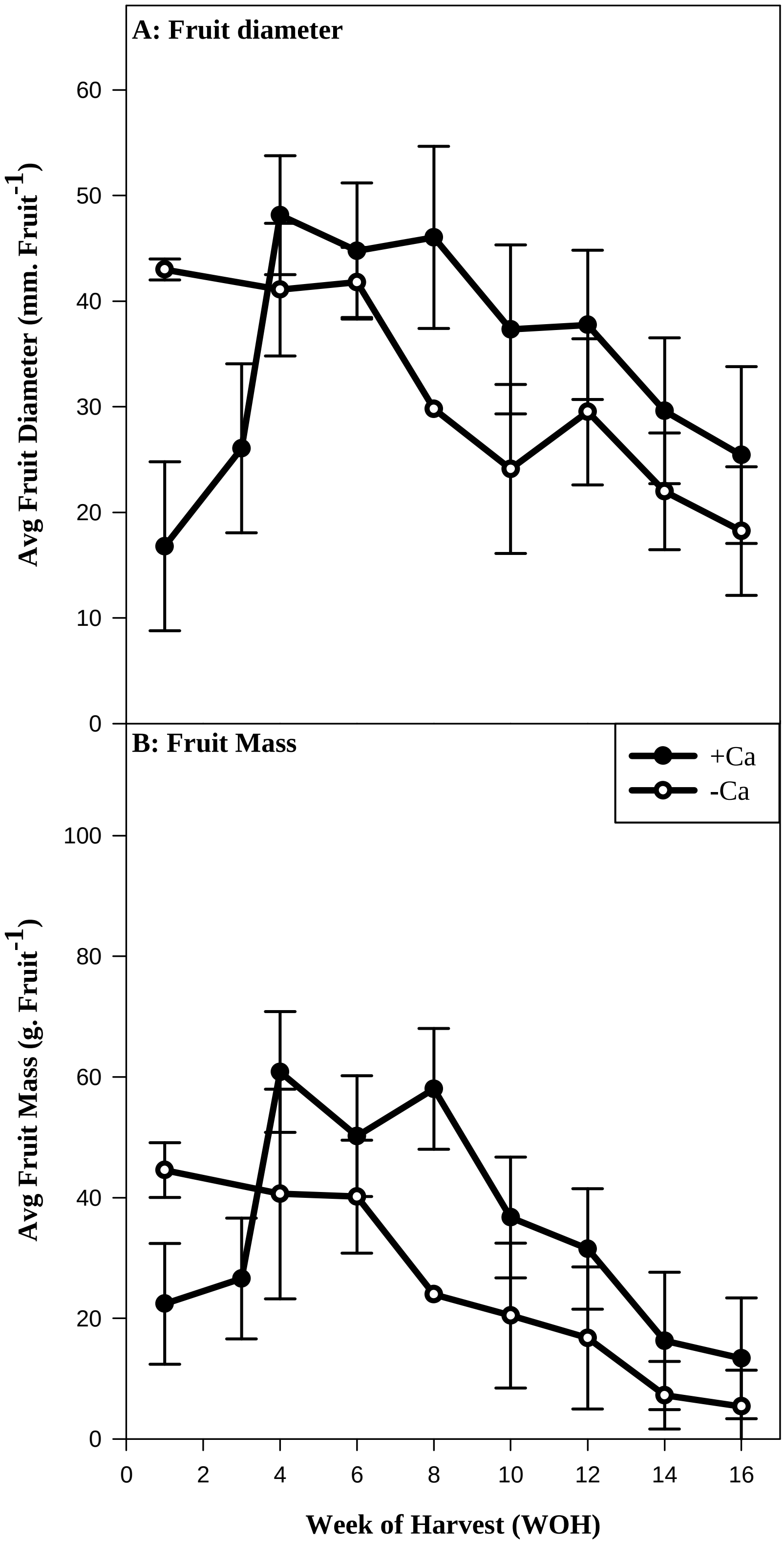


**Figure 4.5:** Effect of a calcium deficiency on average fruit size (A) and mass (B) over a 16-week harvest period.

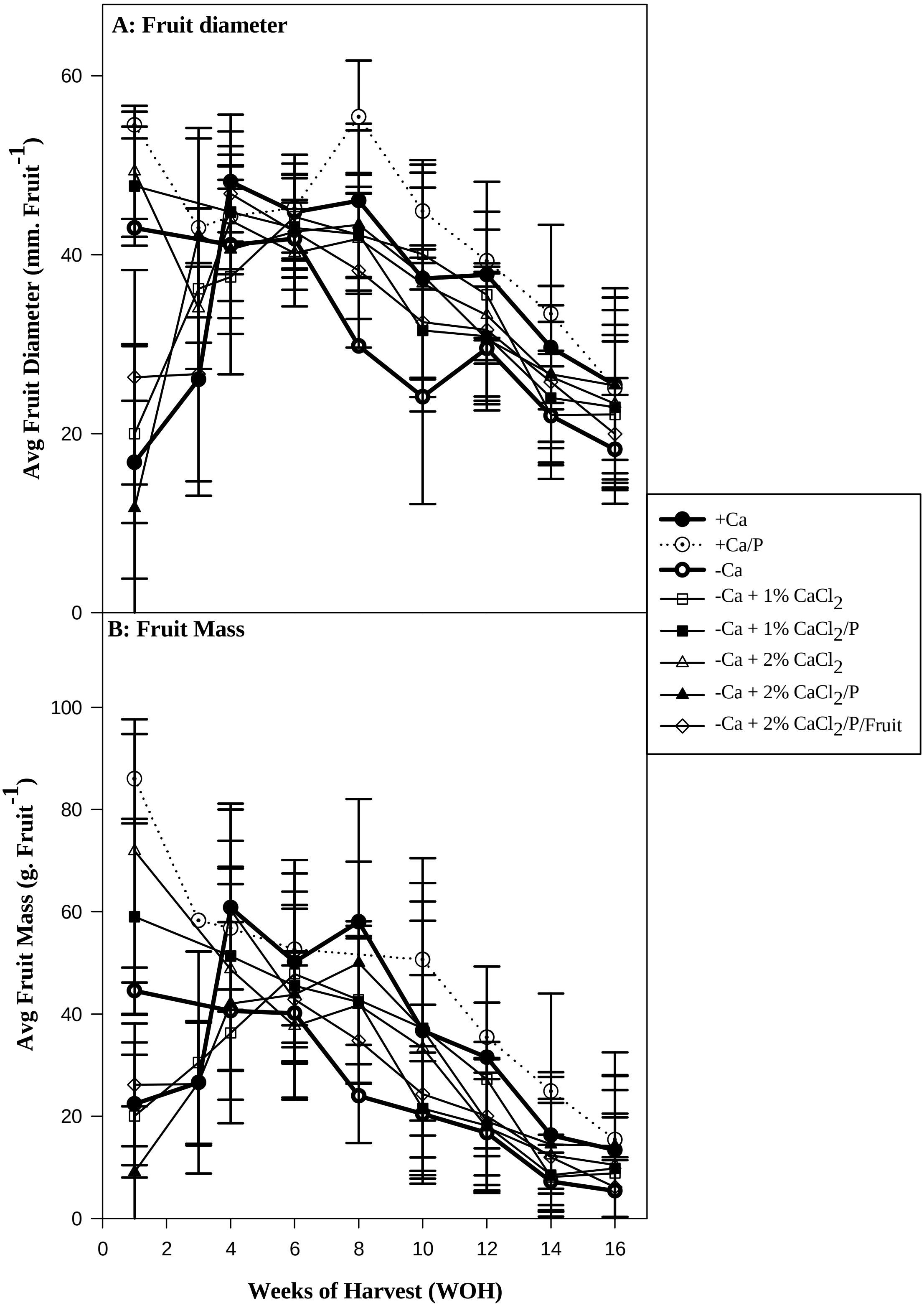


**Figure 4.6:** Changes in average fruit size (A) and mass (B) in response to different $CaCl_2$ preventative treatments, singly and mixed with Pheroids, over a 16-week harvest period.

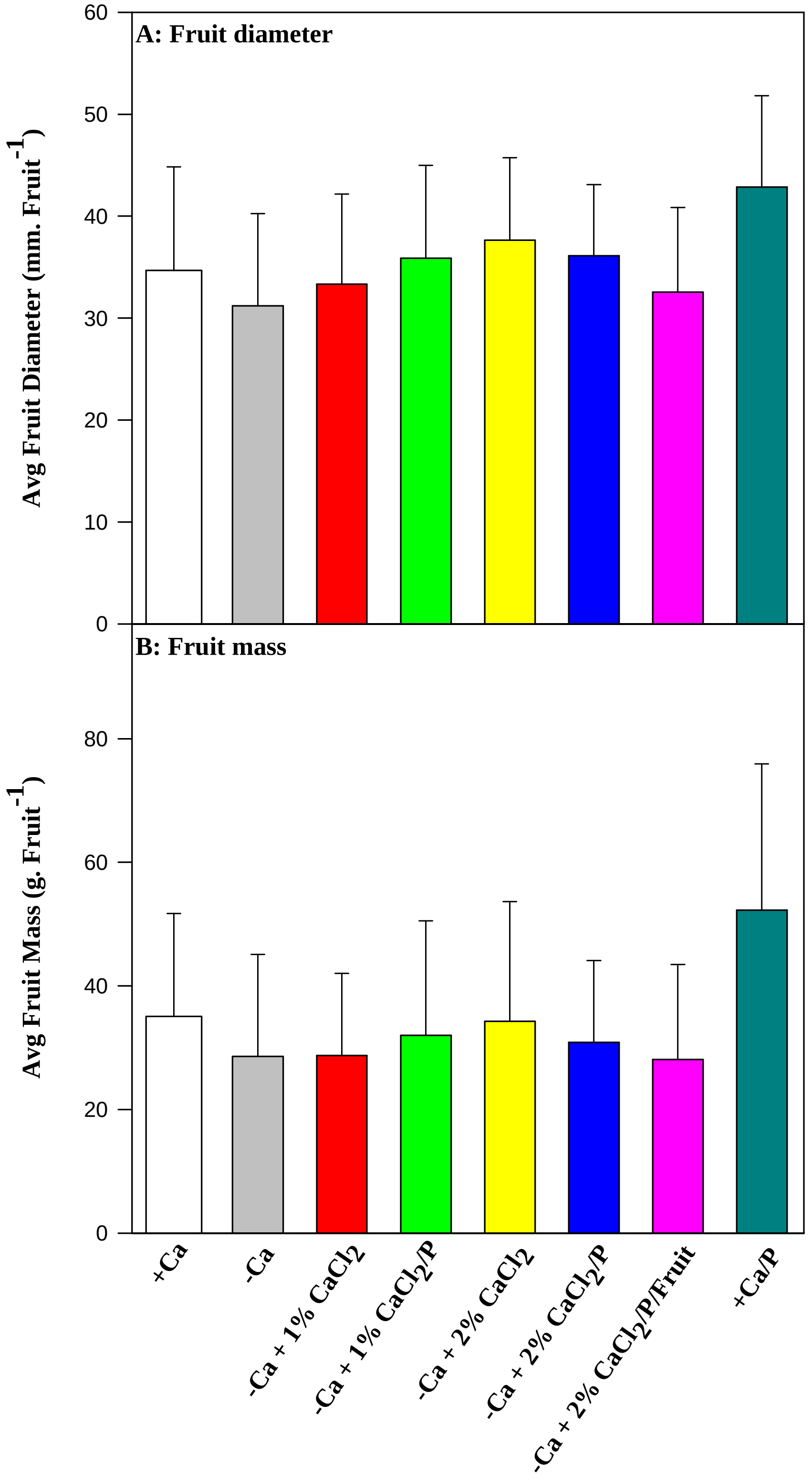


**Figure 4.7:** The average fruit size (A) and mass (B) at the end of a 16-week harvest period for the control-, calcium stressed plants and the plants of the corrective/preventative treatments.

## 4.5 EFFECT OF A CALCIUM DEFICIENCY ON THE QUALITY OF TOMATOES

Tomato quality is defined by factors such as: size, intended use, attractiveness, flavour, nutritional value and undamaged condition (Polder *et al*., 2004). To determine the effects of a calcium deficiency and the subsequent corrective/preventative treatments on fruit quality, several biochemical parameters, namely electrical conductivity (EC), dry mass (Dm), Brix index, moisture content and lycopene concentration were measured.

### 4.5.1 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON MOISTURE CONTENT, DRY MASS, BRIX INDEX AND ELECTRICAL CONDUCTIVITY OF THE FRUITS

Under control conditions, the average fruit mass (Figure 4.8A) and the dry mass:fresh mass ratio of the control fruits decreased  as the harvest season progressed, indicating that the average fruit size and fresh mass of the fruits decreased (Figure 4.8A&B). Concomitant to the decrease in the dry mass:fresh mass ratio, the moisture content of the fruits also decreased indicating that a decrease in moisture was one of the factors responsible for the decrease in fruit mass and fresh mass and that less moisture were accumulated in the fruits as the harvest season progressed (Figure 4.8C). Closer investigation of the results for average fruit mass and moisture content established that a positive relationship existed between these two parameters since a decrease in fruit mass was accompanied by a decrease in moisture content irrespective of when the fruits were harvested (Figure 4.9). To establish whether changes in assimilate accumulation contributed to the decrease in average fruit mass, the dry mass of the fruits were plotted (Figure 4.8D). The dry mass in the control fruits were the highest and remained relatively constant throughout the harvest season (Figure 4.8D). Thus, even though the average fruit mass, fresh mass and moisture content of these fruits decreased, dry mass accumulation remained constant. It is possible that fruits import and accumulate the same amount of carbon compounds, irrespective of average fruit mass and the stage of harvest. If this is the case, then an increase in fruit compounds is expected as the harvest season progresses. The Brix index and the EC values of these fruits increased as the harvest season progressed. Moreover, the relationship between these two quality parameters and fruit mass, were negative, indicating that as average fruit mass decreased, Brix index (Figure 4.11) and fruit EC (Figure 4.10) increased. Thus, the fruits seemed to become more concentrated as the harvest season progressed.

A similar decrease in fruit mass (Figure 4.8A), dry mass:fresh mass ratio (Figure 4.8B) and moisture content (Figure 4.8C) was also observed for the fruits from the calcium deficient plants. In addition, a calcium deficiency had no effect on the Brix index (Figure 4.8F) and EC of the fruits (Figure 4.8E), but these fruits did however, accumulate slightly less dry mass compared to the control fruits (Figure 4.8D).

Spraying calcium deficient plants with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, had no clear effect on the decrease in fruit mass, moisture content, dry mass accumulation or the increase in the Brix index and EC of the calcium deficient fruits (Figure 4.8).

The fruits of the control plants sprayed additionally with Pheroids gave results similar to that of the control fruits (Figure 4.8). However, the fruits of this treatment had a slightly higher moisture content and a dry mass:fresh mass ratio compared to the control fruits (Figure 4.8C). Furthermore, these fruits also accumulated less dry mass than the control fruits (Figure 4.8D), suggesting that the foliage application of additional Pheroids under control conditions resulted in more hydrated and less concentrated fruits during the harvest period.

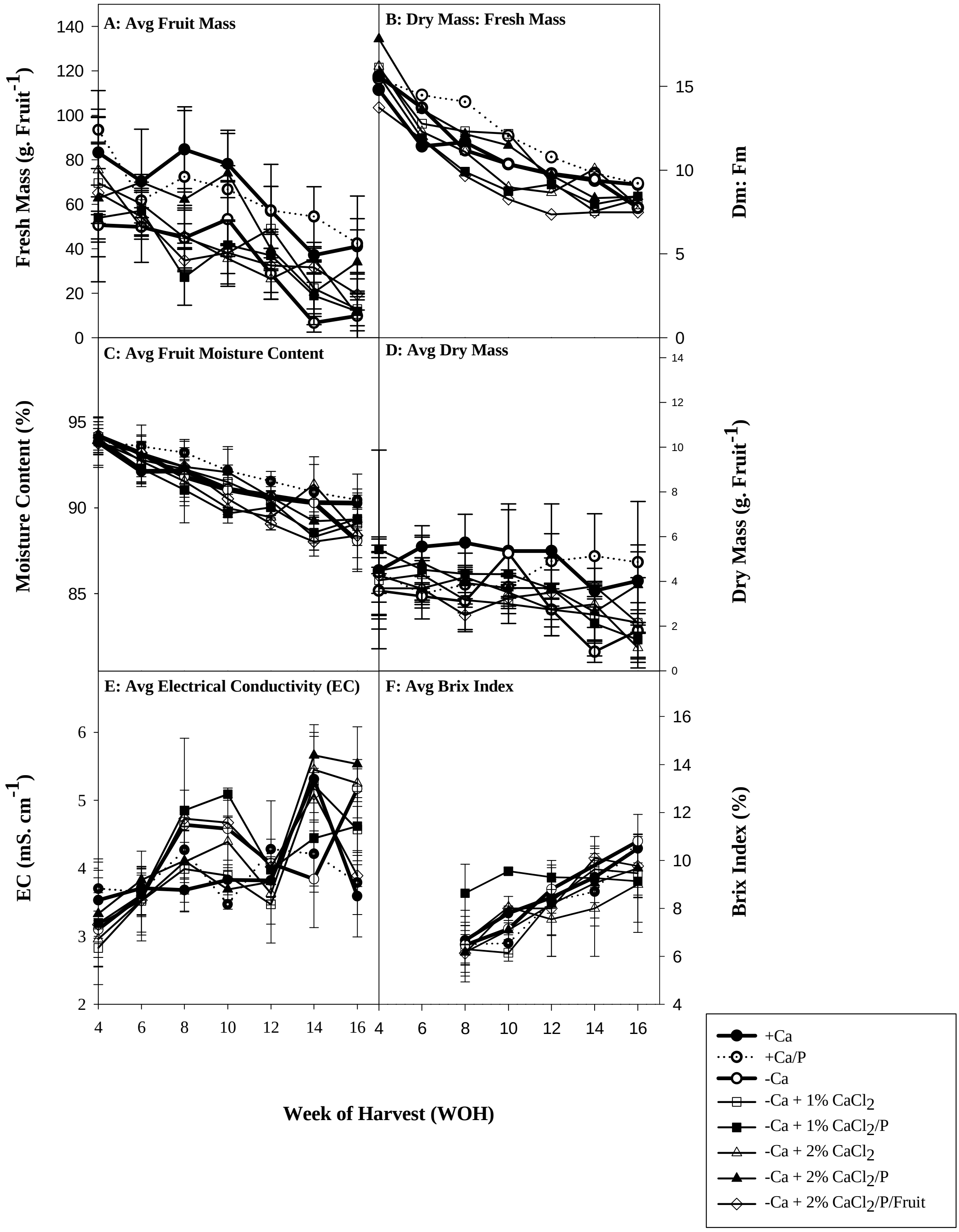


**Figure 4.8:** Effect of a calcium deficiency and the subsequent corrective/preventative treatments on the different parameters used to determine fruit quality

(A: Average fruit mass; B: Dry mass:Fresh mass; C: Average moisture content; D: Average dry mass; E: Average electrical conductivity; F: Average Brix index).

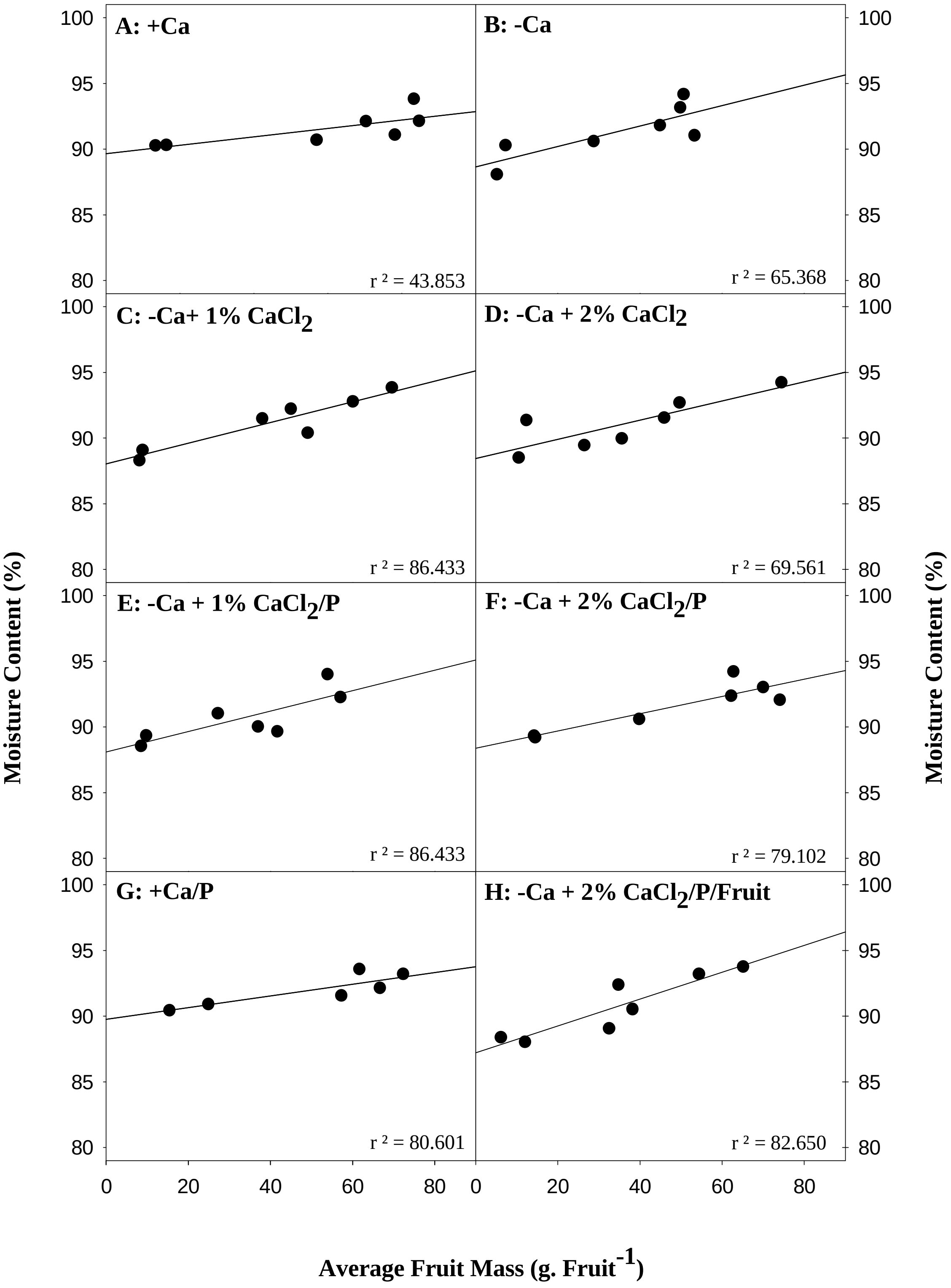


**Figure 4.9:** The relationship between average fruit mass (x axis) and average moisture content (y axis) for the fruits of the control-, calcium stressed- and treated plants during a 16-week harvest period.

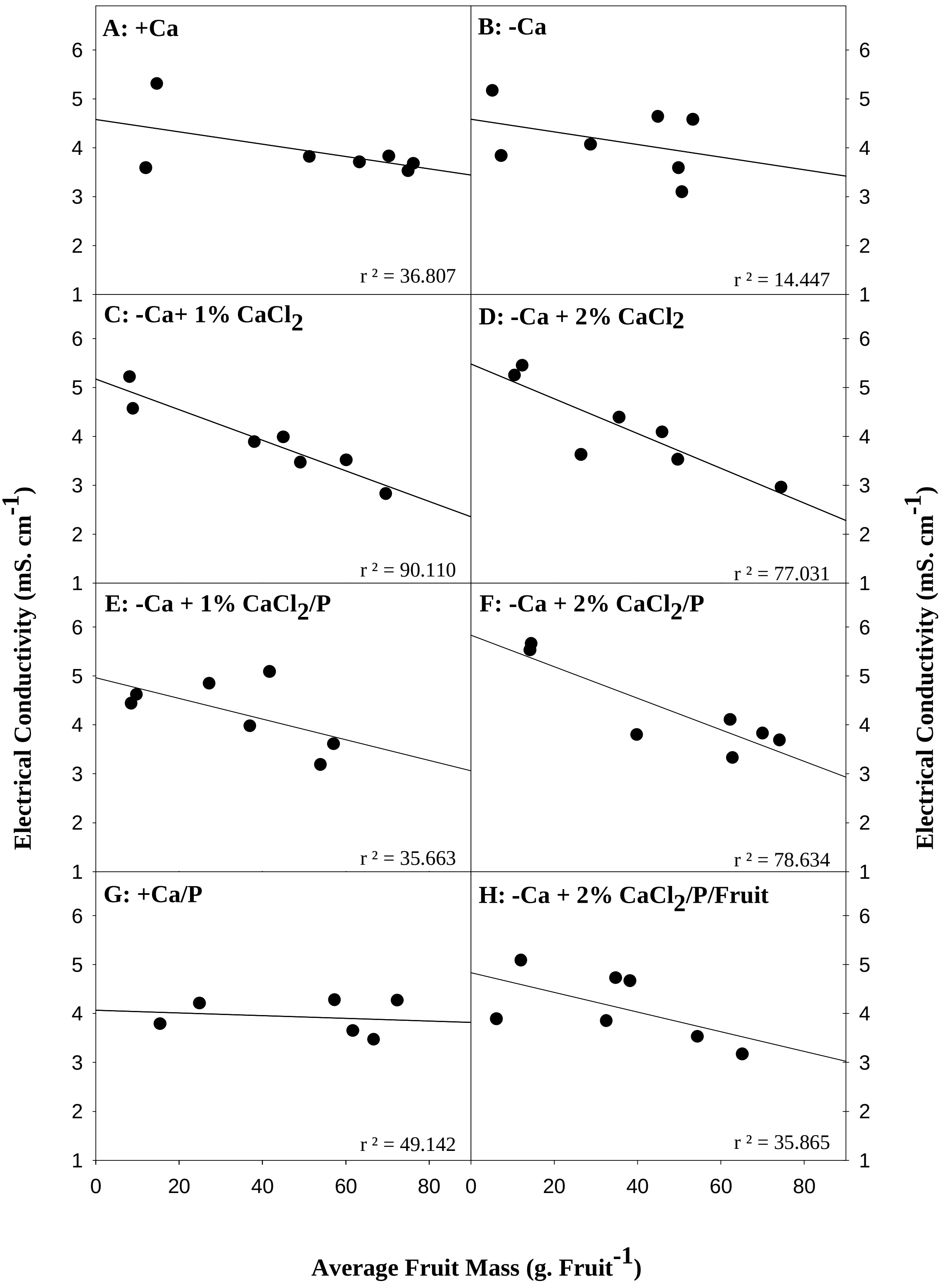


**Figure 4.10:** The relationship between average fruit mass (x axis) and electrical conductivity (y axis) for the fruits of the control-, calcium stressed- and treated plants during a 16-week harvest period.

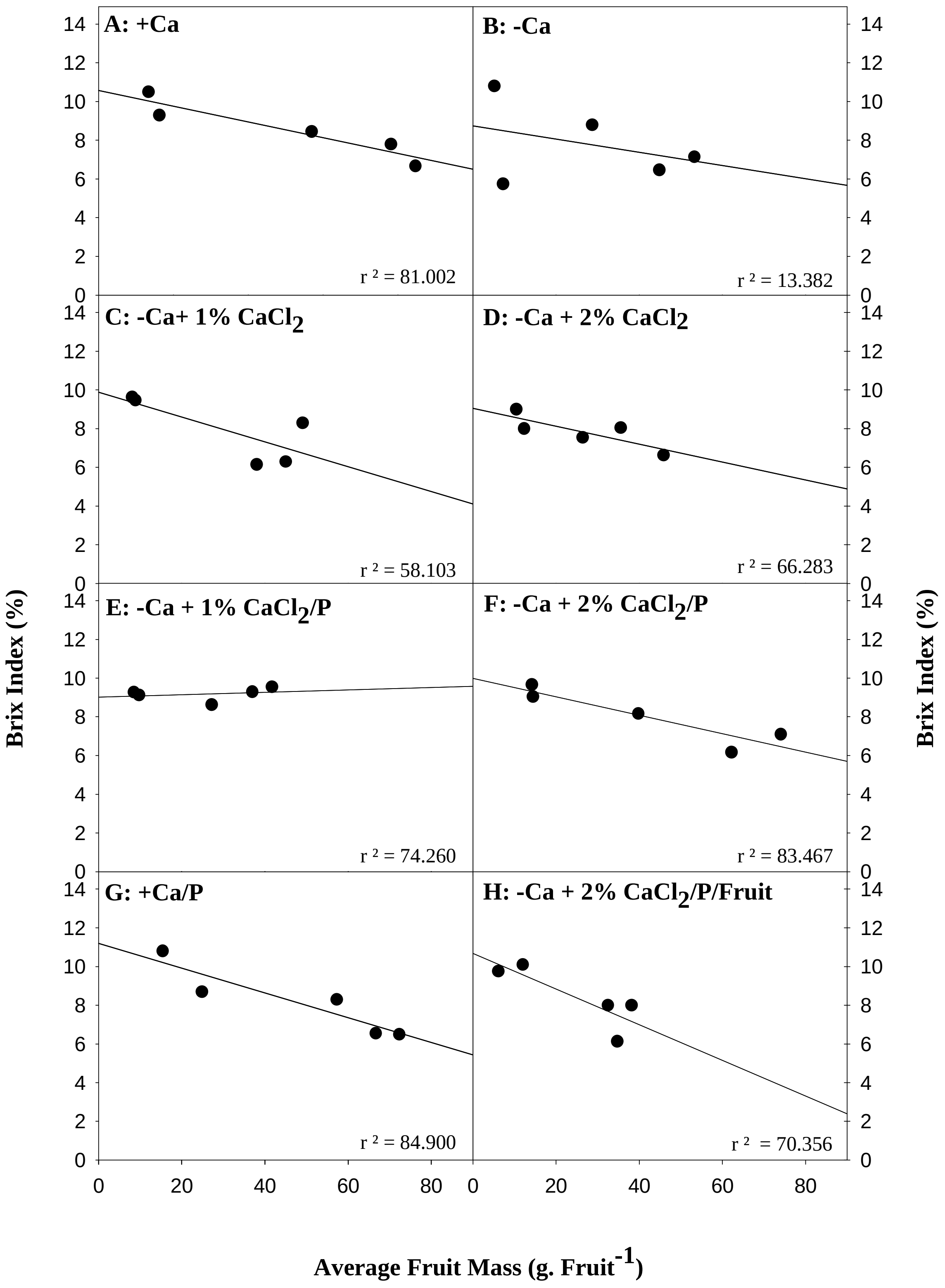


**Figure 4.11:** The relationship between average fruit mass (x axis) and Brix index (y axis) for the fruits of the control-, calcium stressed- and treated plants during a 16-week harvest period.

### 4.5.2 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON THE LYCOPENE CONCENTRATION OF FRUITS

Another quality parameter measured, was lycopene concentration (Figure 4.12A). Lycopene is a fat-soluble component and was therefore expressed on a dry mass basis to ensure an accurate depiction. Under control conditions, the lycopene concentration of the fruits decreased as the harvest season progressed (Figure 4.12A). The lycopene:fruit mass ratio remained relatively constant for the greatest part of the harvest season after which it increased during the last four weeks of the harvest season (Figure 4.12B).

The fruits of the calcium deficient plants had a higher lycopene concentration than that of the control plants and also displayed a decrease in their concentration during the harvest period (Figure 4.12A). Moreover, the calcium deficiency had no clear effect on the lycopene:fruit mass ratio (Figure 4.12B)

Spraying additional calcium onto the calcium stressed plants as 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, also resulted in a decrease in the lycopene concentration, but at concentrations comparable to that of the control fruits (Figure 4.12A). This indicates that the corrective/preventative treatments might have relieved the calcium stress to a certain extent, since it succeeded in lowering the lycopene concentration comparable to that of the control fruits (Figure 4.12A). However, no clear distinction could be made between the efficacies of these treatments to relieve the calcium stress in these fruits or the effect thereof on the lycopene:fruit mass ratio (Figure 4.12B).

When pure Pheroids were applied foliarly to control plants, the decreasing trend in the lycopene concentration was still evident during the harvest season (Figure 4.12A). This treatment also had no clear effect on the lycopene:fruit mass ratio of the fruits. It did, however, result in the highest lycopene concentrations. This may suggest that Pheroids stimulated the accumulation and synthesis of lycopene in these fruits under control conditions.

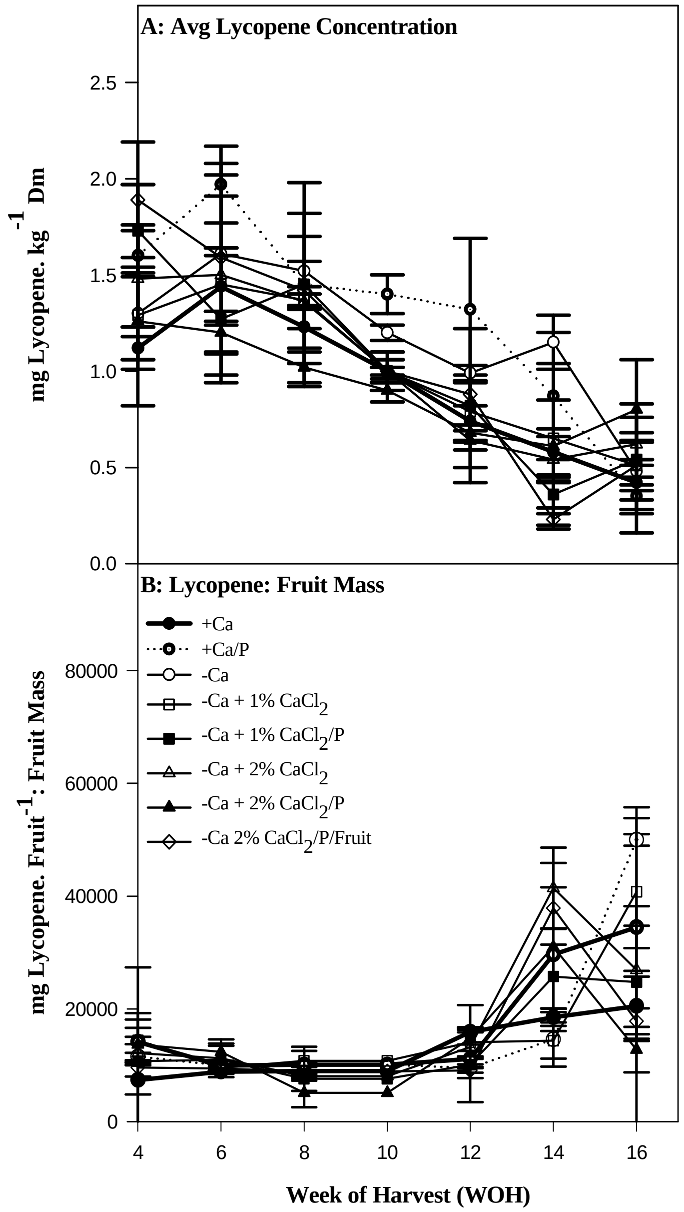


**Figure 4.12:** Effect of a calcium deficiency and the subsequent corrective/preventative treatments on the lycopene concentration of tomato fruits during a 16-week harvest period.

## 4.6 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON THE OCCURRENCE OF BLOSSOM-END ROT (BER)

Fruit displaying BER symptoms are unattractive and not suitable for human consumption. Preventing the occurrence of BER will therefore improve yield and the subsequent profit generated by the produce. The most likely causes of BER are a combination of various factors such as the interactions between light and temperature on fruit enlargement, inadequate xylem development in fruit and competition between the leaves and fruits for calcium (Ho *et al.,* 1993), which creates a local calcium deficiency in distal fruit tissue causing BER (Ho *et al.,* 1993; Taylor *et al.,* 2004; Ho & White, 2005).

To determine the effect of a calcium deficiency on the incidence of BER, the number of fruits with BER symptoms were recorded and compared on a weekly basis. Moreover, the number of fruits developing BER after applying the different corrective/preventative treatments were also recorded and compared to control- and calcium stressed fruits to determine the efficacy of these treatments to reduce or prevent BER.

A calcium deficient nutrient medium promoted the incidence of BER. Plants grown under these conditions lost ten times more fruit to BER than the control plants over a harvest period of 16 weeks (Figure 4.13).

In an effort to reduce the occurrence of BER, plants were treated with additional calcium by spraying them foliarly with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids. In an additional treatment, only the fruits of calcium stressed plants were sprayed with a 2% $CaCl_2$ Pheroids mixture. All the corrective/preventative treatments reduced the fruit mass (kg. $ha^{-1}$; Table 4.2) lost due to a calcium deficient nutrient medium and BER, but only the 2% $CaCl_2$ solution and the 2% $CaCl_2$ Pheroids mixture succeeded in reducing the number of fruits lost to BER. Thus, treating calcium stressed plants foliarly with an additional 2% $CaCl_2$ solution, singly and mixed with Pheroids, proved to be successful (Table 4.2).

When only the fruits of the calcium stressed plants were treated with a 2% $CaCl_2$ Pheroids mixture, the least number of fruits were lost to BER (Figure 4.13A&B). Thus, it proved to be the most efficient corrective/preventative treatment in reducing the number of fruits lost to BER (Figure 4.10A&B), but logistically it is the least viable technique in a commercial set-up.

It appeared that Pheroids on its own under optimum growth conditions, may have increased the occurrence of BER, because where control plants were treated with Pheroids alone, a slight increase in the occurrence of BER was observed (Figure 4.13A&B).

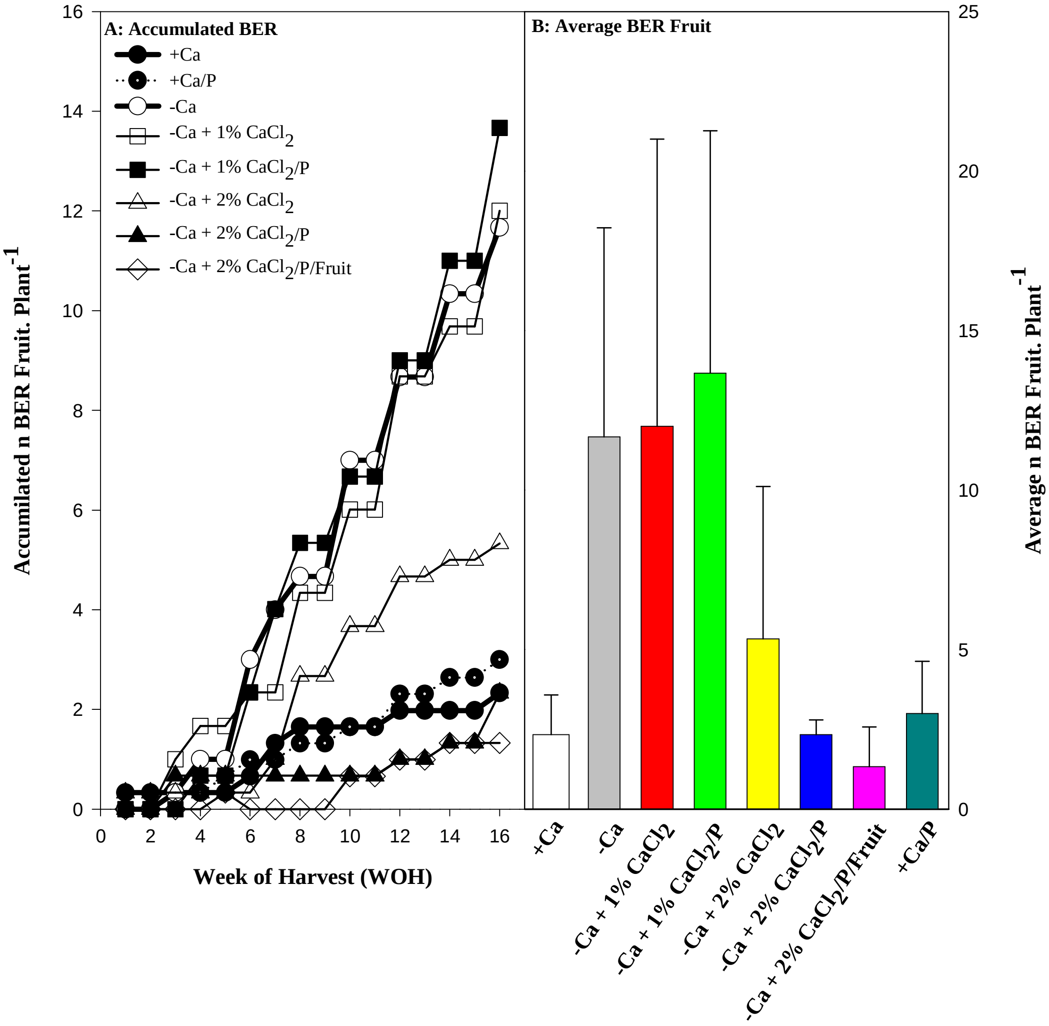


**Figure 4.13:** The effect of a calcium deficient nutrient medium and the subsequent corrective/preventative treatments on the incidence of BER during the harvest period (A) and the total number of fruits lost to BER (B) after an experimental harvest period of 16 weeks.

### 4.6.1 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON NET INCOME

Blossom-end rot (BER) clearly affected marketable yield (Figure 4.13A-C), making the prevention of this nutritional disorder essential for the generation of a profitable net income.

The aim of the different corrective/preventative treatments was to reduce the incidence of BER thereby improving marketable yield and profit margins. The effect of BER on marketable yield was determined for the early (week five), middle (week ten) and late (week 16) stages of the harvest season.

The higher incidence of BER under calcium deficient conditions clearly lowered yield (Figure 4.14A-C and Table 4.2). Treating the calcium stressed plants additionally with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, reduced the effects of a calcium deficiency and the occurrence of BER on yield, as all of the treated plants produced higher total- and marketable yields (Figure 4.13C). Even the 1% $CaCl_2$ Pheroids mixture, which was the least effective of the foliage treatments to reduce the number of fruits lost to BER (Figure 4.13A&B), produced a higher yield and net income than the calcium stressed plants (Table 4.2&4.3 and Figure 4.14C). Moreover, the 2% $CaCl_2$ solution, especially when mixed with Pheroids, proved to be the most efficient treatments to use since it resulted in the highest marketable yield and net income. This warrants the use of 2% $CaCl_2$ above 1% $CaCl_2$ as a foliage spray in the reduction or prevention of BER.

Another treatment that was effective in reducing the incidence of BER fruits, was when only the fruits of calcium stressed plants were sprayed with a 2% $CaCl_2$ Pheroids mixture (Figure 4.13B). However, this treatment resulted in the lowest yield (Figure 4.14C) and thus a reduced income compared to the different foliage treatments. Therefore, the lowered yield and extra expenses related to the labour and time of spraying each fruit individually, makes this the least successful treatment in reducing BER in calcium stressed plants.

Spraying control plants foliarly with Pheroids resulted in higher yields (Figure 4.14A-C) and Table 4.2) during the early (week five) and middle (week ten) stages of harvest, while it also reduced the occurrence of BER (Figure 4.14A&B). However, after 16 weeks of harvest this treatment appeared to be counter productive. The total yield was similar to that of the non-treated control plants, while the occurrence of BER was apparently stimulated by the addition of Pheroids (Figure 4.14A-C), which resulted in yield and profit losses due to BER (Table 4.2&4.3).

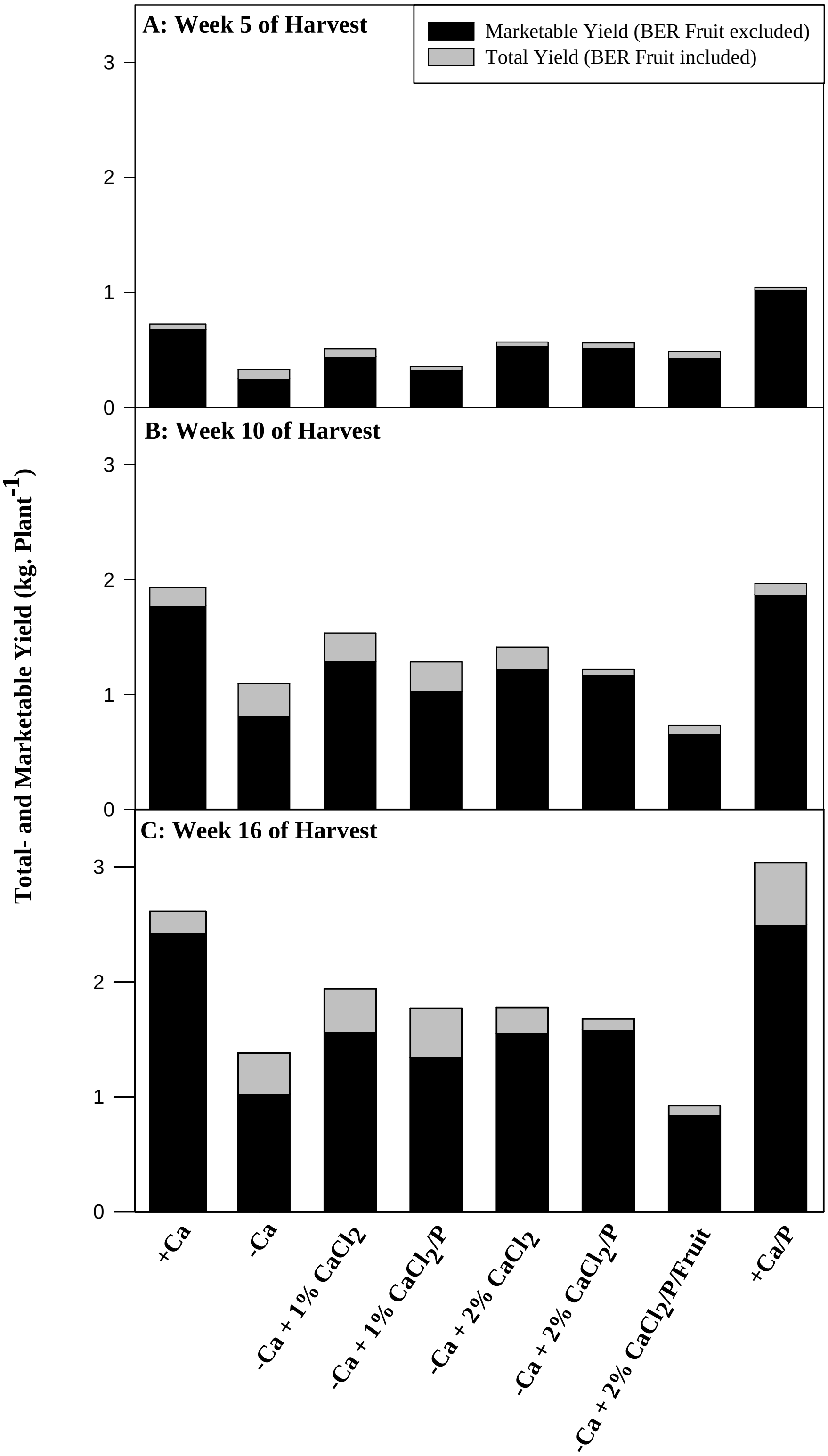


**Figure 4.14:** The effect of calcium deficient nutrient conditions and the subsequent corrective/preventative treatments on total- and marketable yields (kg. plant$^{-1}$) at different stages of the harvest period.

**Table 4.2:** Effect of a calcium deficiency and the subsequent corrective/preventative treatments on total- and marketable yields after a harvest period of 16 weeks.

| **Treatments** | **Yield** | | | | **Yield lost due to Ca/BER**** |
|---|---|---|---|---|---|
| | **Total (kg. Plant$^{-1}$)** | **Marketable (kg. Plant$^{-1}$)** | **Total (kg. ha$^{-1}$)** | **Marketable (kg. ha$^{-1}$)** | **kg. ha$^{-1}$** |
| **+Ca** | **2.43** | **2.24** | **104 421** | **96 354** | **0** |
| +Ca/P | 2.5 | 1.96 | 107 509 | 84 381 | 11 973 |
| **-Ca** | **1.03** | **0.67** | **44 084** | **28 707** | **67 648** |
| -Ca + 1% $CaCl_2$ | 1.57 | 1.2 | 67 592 | 51 735 | 44 619 |
| -Ca + 1% $CaCl_2$/P | 1.35 | 0.92 | 57 889 | 39 444 | 56 911 |
| -Ca + 2% $CaCl_2$ | 1.55 | 1.33 | 66 754 | 57 100 | 39 255 |
| -Ca + 2% $CaCl_2$/P | 1.59 | 1.49 | 68 237 | 64 255 | 32 100 |
| -Ca + 2% $CaCl_2$/P/Fruit | 0.84 | 0.76 | 36 245 | 32 762 | 63 593 |

* - Based on the plant density of the experimental setup = 43 000 plants. ha$^{-1}$

** - Based on marketable yield (kg. ha$^{-1}$) of control plants

**Table 4.3:** The effect of a calcium deficiency and the subsequent corrective/preventative treatments on the gross- and net income of tomatoes after a harvest period of 16 weeks.

| **Treatments** | **Yield (kg. Plant$^{-1}$)** | | **Income [a] (R. Plant$^{-1}$)** | | **Income generated[aa] (R. ha$^{-1}$)** | | **Income lost due to Ca/BER***** |
|---|---|---|---|---|---|---|---|
| | **Total*** | **Market**** | **Total*** | **Market**** | **Gross** | **NET** | **R. ha$^{-1}$** |
| **+Ca** | **2.43** | **2.24** | **9.71** | **8.96** | **417 685** | **385 418** | **0** |
| +Ca/P | 2.5 | 1.96 | 10 | 7.55 | 430 034 | 337 524 | 47 893 |
| **-Ca** | **1.03** | **0.67** | **4.1** | **2.67** | **176 338** | **114 827** | **270 590** |
| -Ca + 1% $CaCl_2$ | 1.57 | 1.2 | 6.29 | 4.81 | 270 367 | 206 942 | 178 476 |
| -Ca + 1% $CaCl_2$/P | 1.35 | 0.92 | 5.39 | 3.67 | 231 557 | 157 776 | 227 642 |
| -Ca + 2% $CaCl_2$ | 1.55 | 1.33 | 6.21 | 5.31 | 267 019 | 228 399 | 157 019 |
| -Ca + 2% $CaCl_2$/P | 1.59 | 1.49 | 6.35 | 5.98 | 272 947 | 257 020 | 128 398 |
| -Ca + 2% $CaCl_2$/Fruit | 0.84 | 0.76 | 3.37 | 3.05 | 144 979 | 131 047 | 254 370 |

[a] - Based on tomato prices of 2005: R4. kg$^{-1.}$ See chapter 2 section 2.1

[aa] - Based on the plant density of the experimental setup = 43 000 plants. ha$^{-1}$

* - Total Yield: All fruit harvested (BER fruit included)

** - Market (Marketable) Yield: All the harvested fruit that can be sold commercially (BER fruit excluded)

*** - Income lost to a Ca deficiency and/or BER = Net income of control plants - Net income of treated plants

## 4.7 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON POST HARVEST TOMATO DEGRADATION DURING STORAGE

The aim of this specific investigation was to determine the effect of a calcium deficiency and the subsequent corrective/preventative treatments on fruit deterioration (shelf life) after harvest. The fruits harvested from the plants were considered “consumer ready”. Cultivar, agricultural practices, stage of maturity at harvest, and post harvest handling practices all affect tomato quality and deterioration during storage (Trejo & Cantwell, 1996).

The “consumer ready” red, firm fruits of all the treatments were harvested at weeks 6, 12 and 16. This represented the early, middle and late stages of the harvest season. These fruits were then stored for a period of three weeks in the dark at 12ºC (see 3.2.9), after which the physical and quality parameters were measured.

### 4.7.1 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON FRUIT MASS, SIZE AND MOISTURE CONTENT DURING STORAGE

It was previously demonstrated that the average fruit mass and size of the control fruits decreased as the harvest season progressed (Figure 4.5A&B). During a three-week storage period, the fruit mass continued to decrease between ±8% and ±10% for the different stages of the harvest period (Figure 4.15A). The highest weight loss was recorded for fruits harvested during the middle (week 12) of the harvest period (Figure 4.15A).

Calcium stressed plants produced fruits with a lower average fruit mass and size compared to that of the control fruits (Figure 4.5A&B). These fruits also lost slightly more weight and showed a greater variation in the percentage weight loss during storage for the different stages of harvest (Figure 4.15A&B). The fruits harvested at week 12, similar to the control fruits, also displayed the greatest weight loss during storage (Figure 4.15B).

Fruits from calcium stressed plants treated foliarly with $CaCl_2$ solutions, singly and mixed with Pheroids, gave results similar to that of the control- and calcium stressed fruits after storage (Figure 4.15C-F). They all exhibited the same tendency of reduced fruit mass and size as the harvest season progressed (Figure 4.6). The average reduction in mass during storage was similar to that of the calcium stressed fruits (Figure 4.15A-F). No obvious alleviation of the effects a calcium deficiency had on changes in fruit mass during storage could be observed for these corrective/preventative treatments.

Treating only the fruits of calcium stressed plants with a 2% $CaCl_2$ Pheroids mixture, resulted in the smallest fruits (Figure 4.6) and yield (Figure 4.4). Also, treating these fruits with the above mentioned mixture resulted in the greatest weight loss during storage (Compare Figure 4.15G to Figure 4.15A-F).

The additional spraying of control plants with Pheroids gave results comparable to that of the control plants with regard to the decrease in fruit mass after the three-week storage period (Figure 4.15A&H).

During storage, moisture loss may be one of the factors responsible for the decrease noticed in fruit mass. Similar to the previously observed tendency for the reduction in fruit size and mass (Figure 4.6A&B), the moisture content of the control fruits also decreased as the harvest season progressed (Figure 4.8C). Storage of these fruits for three weeks resulted in a further moisture loss (Figure 4.16A).

Fruits from the calcium deficient plants also showed a similar decrease in moisture content as the season progressed for the fresh as well as the stored fruits (Figure 4.8B and Figure 4.16B). No obvious distinction could be made between the different stages of the harvest season.

The decline in moisture content during storage for the fruits of the calcium stressed plants treated with 1% and 2% $CaCl_2$, singly and mixed with Pheroids, were similar to that of the control- and calcium stressed fruits (Figure 4.16A-F). No conclusive distinctions could be made between the efficacies of these treatments to prevent moisture loss during storage. Moreover, all the fruits from these treatments showed the highest moisture loss during the middle and later stages of the harvest season (Figure 4.16C-F).

Treating only the fruits of calcium deficient plants with a 2% $CaCl_2$ Pheroids mixture, again proved to be the least successful treatment to slow down fruit deterioration. These fruits lost the most moisture during the three-week storage period (Figure 4.16G), especially for the fruits harvested during the middle and later stages of the harvest period.

Treating control plants additionally with Pheroids, neither promoted nor inhibited moisture loss, as the fruits of this treatment displayed results similar to that of the control fruits after storage (Figure 4.16A&H).

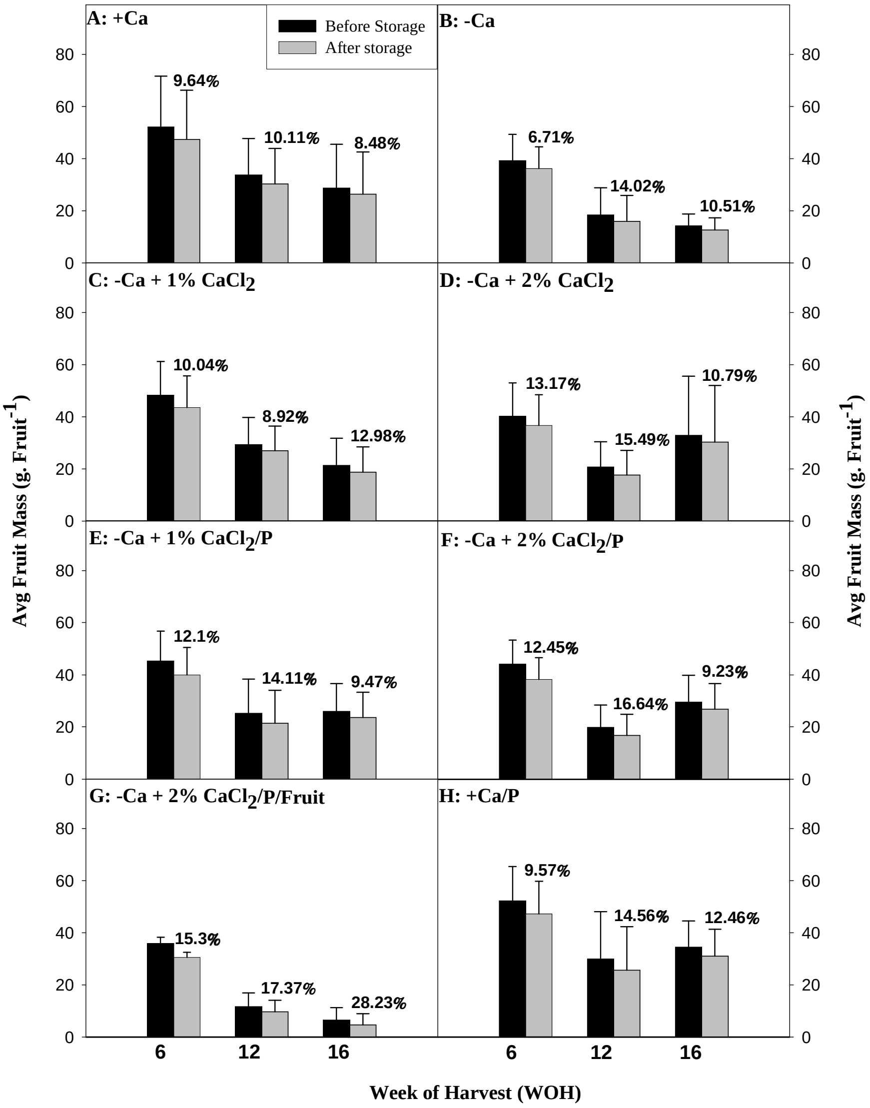


**Figure 4.15:** Changes in the average mass of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 6 (early), 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. Percentage values indicate the reduction in weight after the three-week storage period.

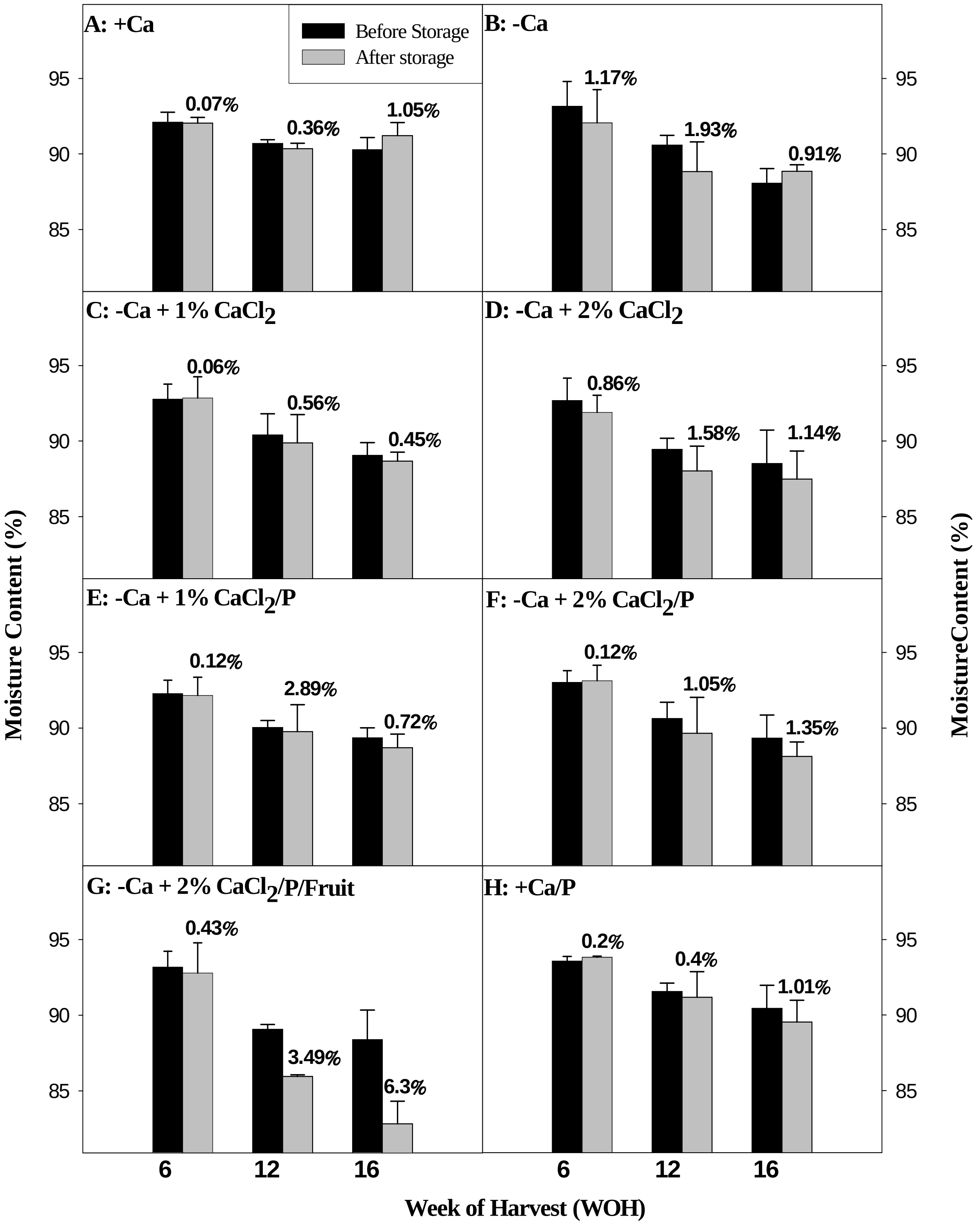


**Figure 4.16:** Changes in the average moisture content (%) of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 6 (early), 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. Percentage values indicate the change in moisture content after the three-week storage period.

### 4.7.2 EFFECT OF A CALCIUM DEFICIENCY AND THE SUBSEQUENT CORRECTIVE/PREVENTATIVE TREATMENTS ON THE BRIX INDEX, EC, pH AND LYCOPENE CONCENTRATION OF THE FRUITS DURING STORAGE

Fruit deterioration not only causes changes in fruit mass and moisture content, it also affects other quality parameters such as Brix index, electrical conductivity (EC), pH and lycopene concentration, that may affect the taste and colour of the fruits.

The Brix index of the control fruits increased as the harvest season progressed (Figure 4.8F and Figure 4.17A). Storing control fruits for three weeks resulted in a slight decrease in the Brix index of the fruits harvested during the middle (week 12) of the season, and a marked decrease for the fruits harvested during the later stages (week 16) of the season.

Fruits from the calcium deficient plants also showed the tendency of an increasing Brix index as the harvest season progressed (Figure 4.8F). However, where the Brix index of the control fruits decreased during storage, the Brix index of the calcium stressed fruits harvested during week 12 increased, while the Brix index of those harvested during week 16 remained constant (Figure 4.17B).

Treating calcium deficient plants foliarly with 1% and 2% $CaCl_2$, singly and mixed with Pheroids, resulted in increases similar to the calcium deficient fruits as the harvest season progressed. The Brix index of these fruits also showed further increases after the three-week storage period for both the middle- and late stages of the harvest season (Figure 4.17C-F).

However, spraying only the fruits of calcium stressed plants with a 2% $CaCl_2$ Pheroids mixture, reduced the Brix index of these fruits during storage (Figure 4.17G). This was similar to the results observed for the control fruits, except that the decrease in the Brix index of these fruits was much more pronounced (Figure 4.17A&G), especially for the fruits harvested during week 12.

The Brix index of the fruits from the control plants treated foliarly with Pheroids also increased as the harvest season progressed (Figure 4.8F). In contrast to the control plants, the Brix index of these fruits increased even further during storage and compared well to the increases noticed for the calcium deficient fruits and the foliage $CaCl_2$ treated plants, singly and mixed with Pheroids (Figure 4.17A-H).

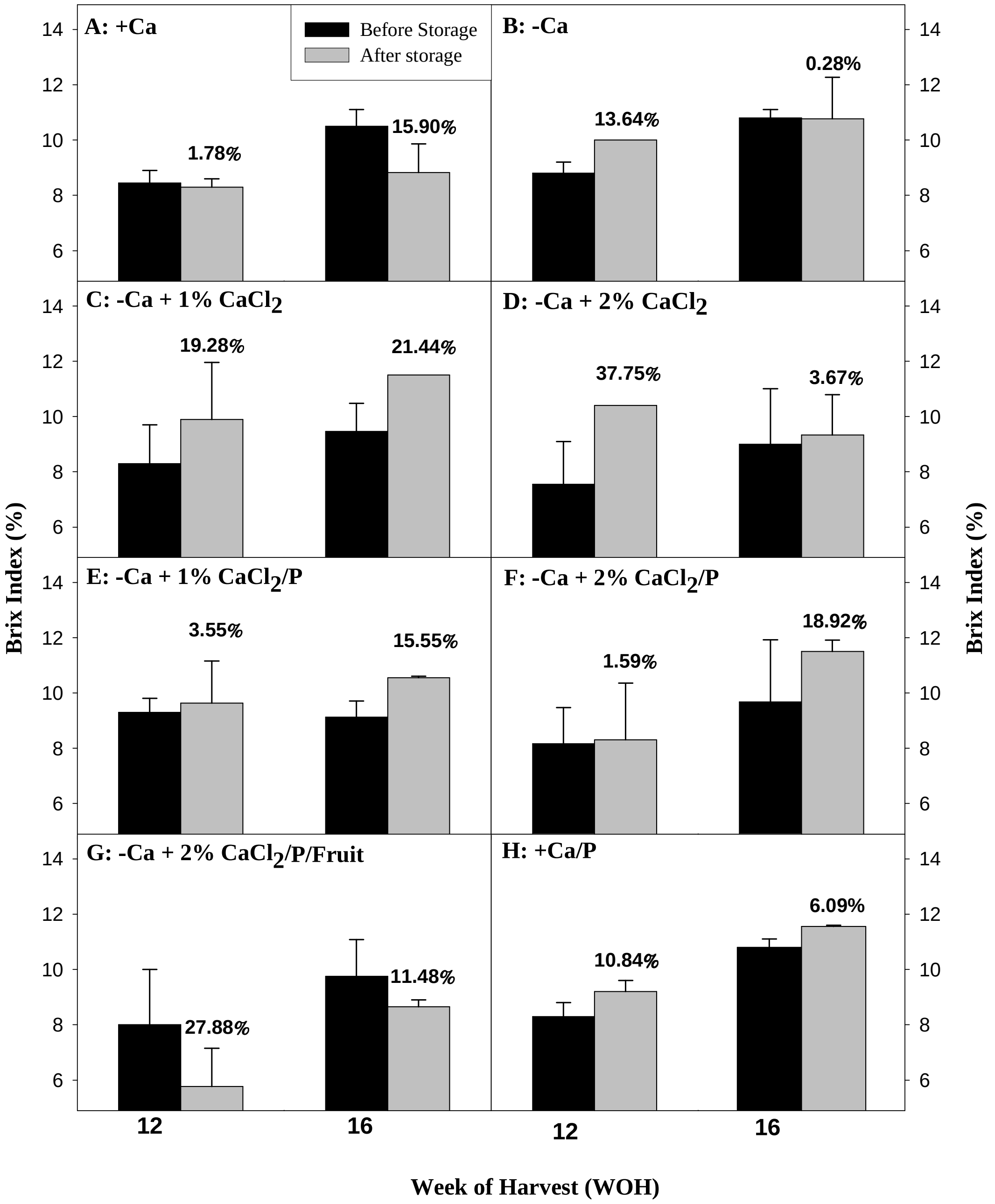


**Figure 4.17:** Changes in the Brix index of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. The percentage values indicate the changes in Brix index during the three-week storage period.

The electrical conductivity (EC) of the control fruits remained fairly constant during the harvest season (Figure 4.18A). Storage of these fruits for three weeks resulted in increased EC values for all the different stages of the harvest period. The most obvious increase in EC was measured for the fruits harvested at week 12 (middle) of the harvest period (Figure 4.18A).

Furthermore, a calcium deficiency and the subsequent corrective/preventative treatments also all resulted in increased EC values as the harvest season progressed (Figure 4.18E and Figure 4.18B-F), especially notable for fruits harvested during week 16. During the three-week storage period, the fruits from all these treatments showed a further increase in EC when harvested at weeks 6 and 12 (Figure 4.18B-F). Moreover, the increases in the EC of the calcium stressed fruits were on average nearly double that of the control fruits (Figure 4.18A&B). In addition, spraying calcium stressed plants with 1% and 2% $CaCl_2$ Pheroids mixtures, resulted in smaller increases in EC compared to the fruits of the calcium stressed plants treated with pure 1% and 2% $CaCl_2$ solutions (Figure 4.18C-F). In contrast to the fruits harvested during weeks 6 and 12, the fruits of the corrective/preventative treatments harvested during week 16, all showed a definite decrease in EC during storage (Figure 4.18C-F). These results may indicate that Pheroids, as part of a foliage $CaCl_2$ treatment, apparently prevented the marked increases in fruit EC during the early and middle stages of the harvest season, while it promoted a decrease in fruit EC in the fruits harvested during the later stages of the season.

When only the fruits of the calcium stressed plants were treated with a 2% $CaCl_2$ Pheroids mixture, a consistent increase in fruit EC of approximately 23% were measured after storage. This was similar to the results obtained for calcium stressed plants treated with 1% and 2% $CaCl_2$ Pheroids mixtures (Figure 4.18E-G).

Treatment of control plants with Pheroids decreased the EC with approximately 2% for the fruits harvested during the early (week six) and late stages (week 16) of the harvest season after the three-week storage period. Only the fruits harvested during the middle (week 12) of the season displayed an increase in the EC levels of these fruits after storage (Figure 4.18H).

Another biochemical parameter used to determine the quality (taste) of fruit, is pH. Fruit pH was not noticeably affected by control- and calcium stress conditions, as well as the stages of the harvest season, as it remained fairly constant between pH 3.8 and 4.2 (Figure 4.19). For most of the treatments, fruit pH increased only slightly during the three-week storage period. The increase was most noticeable for the fruits harvested during the middle (week 12) and late (week 16) stages of the harvest period (Figure 4.19A-H).

Lycopene is not only a measure of fruit quality, but it is also responsible for the red colour of tomato fruits (Carrari & Fernie, 2006). Fruit storage is not only associated with a loss in mass and moisture but also a loss in colour (Trejo & Cantwell, 1996; Žnidari & Požrl, 2006) and consequently, lycopene content.

The lycopene concentration of control fruits decreased as the harvest season progressed (Figure 4.12 and Figure 4.20A). After three weeks of storage in the dark, the lycopene concentration was greatly reduced, except for the fruits harvested at week 16 (Figure 4.20). The biggest reduction in lycopene was obtained for the fruits harvested in the middle (week 12) of the harvest season.

A calcium deficiency also resulted in a marked reduction in the lycopene concentration of the fruits (Figure 4.20). As with the control fruits, storage in the dark also resulted in decreased lycopene concentrations in the calcium deficient fruits. The results obtained was comparable to that of the control fruits (Figure 4.20A&B).

The lycopene concentration of fruits from calcium stressed plants treated foliarly with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, decreased similarly to that of the non-treated calcium deficient plants (Figure 4.12). Storage of these fruits all resulted in a further reduction of the lycopene concentration, especially in the fruits from plants treated with 1% $CaCl_2$ (Figure 4.20C). Mixing 1% and 2% $CaCl_2$ solutions with Pheroids, appeared to lessen the reduction in the lycopene concentration when compared to the 1% and 2% $CaCl_2$ treatments (Figure 4.20C-F). This suggested improved fruit quality in terms of lycopene concentration when Pheroids was included in the $CaCl_2$ corrective/preventative treatments.

Treating only the fruits of calcium stressed plants with a 2% $CaCl_2$ Pheroids mixture appeared to lessen the reduction in the lycopene concentration of the fruits during storage when compared to the foliage $CaCl_2$ Pheroids treatments (Figure 4.20E-G), most notably in the fruits harvested at weeks 12 and 16. This treatment even resulted in an increase in the lycopene concentration of fruits harvested and stored at the later stages of the harvest period.

Treating control plants with Pheroids also led to a reduction in lycopene content during storage (Figure 4.20H). But, on average, it was not as marked as the reduction in the lycopene concentration measured for the control fruits. Thus, Pheroids appeared to lessen lycopene degradation during storage in control fruits (Figure 4.20).

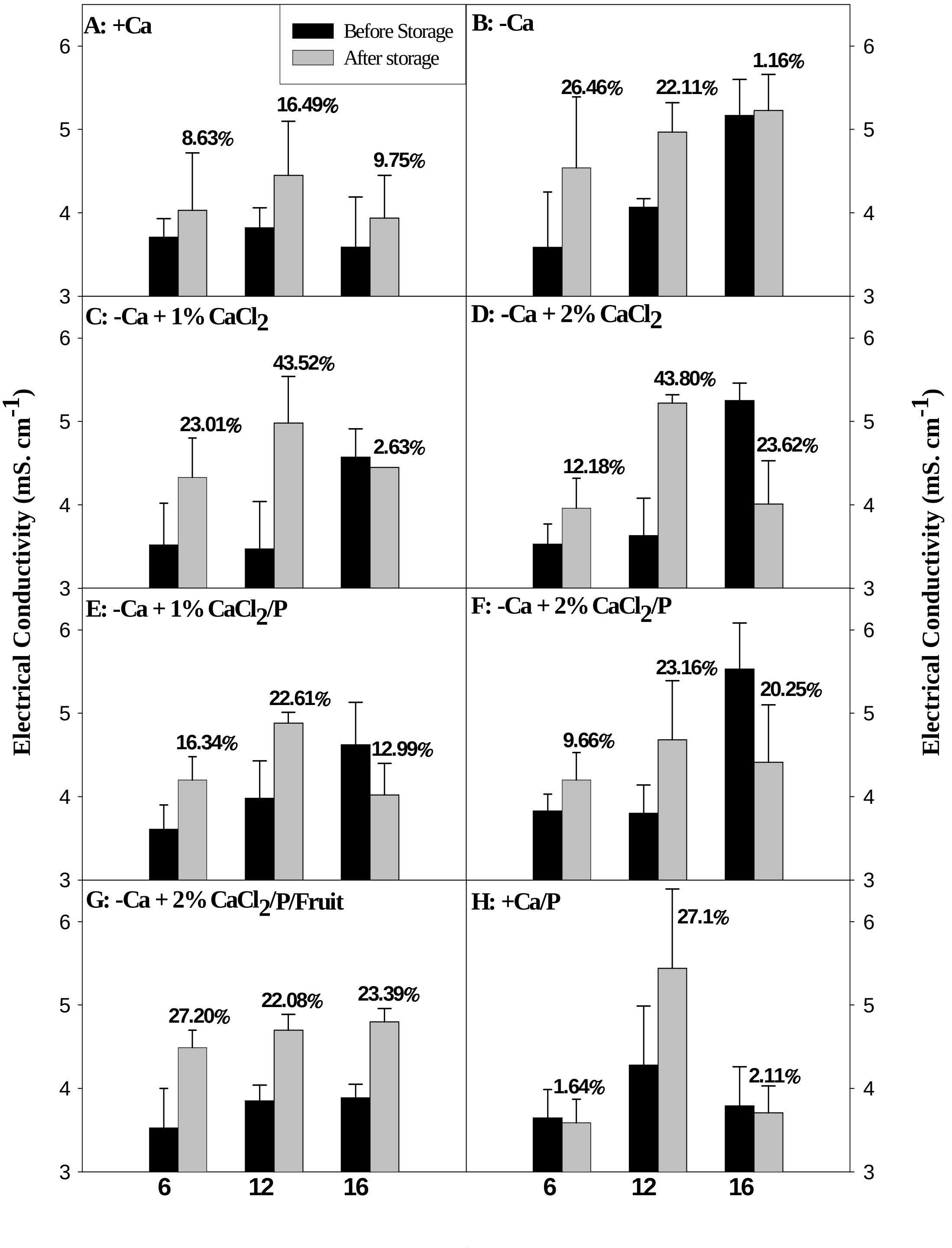


**Figure 4.18:** Changes in the electrical conductivity of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 6 (early), 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. The percentage values indicate the changes in EC during the three-week storage period.

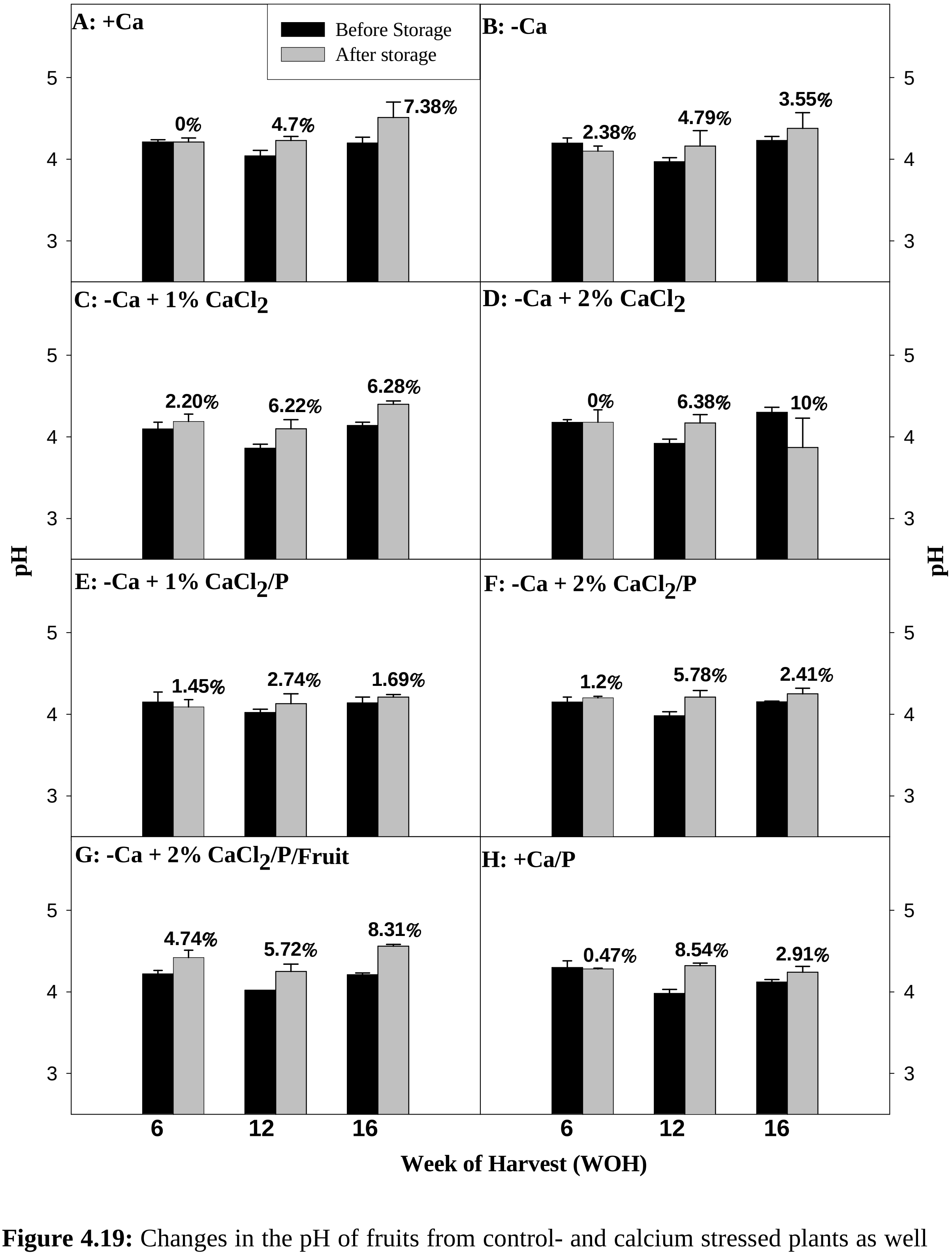


**Figure 4.19:** Changes in the pH of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 6 (early), 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. The percentage values indicate the change in fruit pH during the three-week storage period.

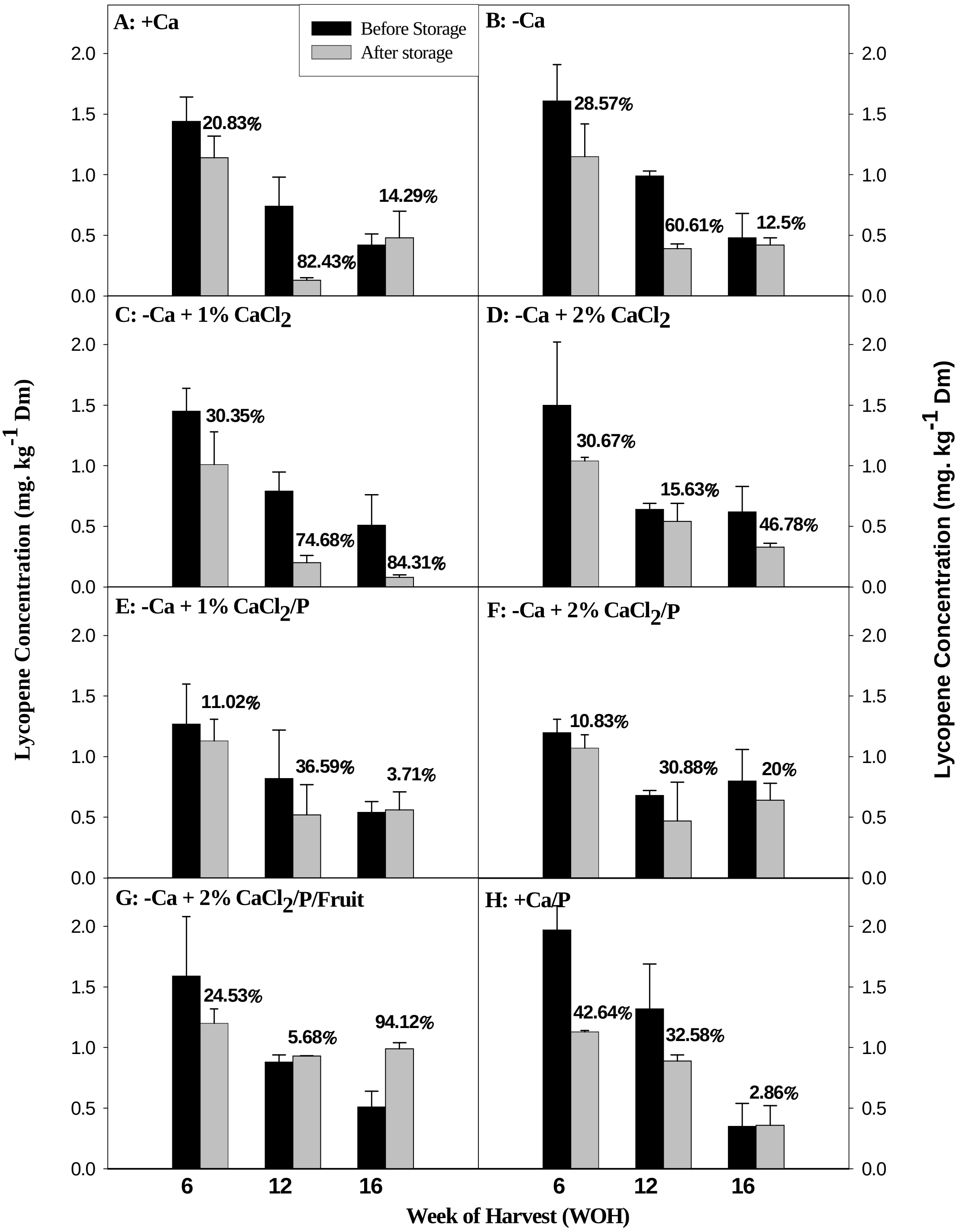


**Figure 4.20:** Changes in the lycopene concentration of fruits from control- and calcium stressed plants as well as the treated plants harvested at weeks 6 (early), 12 (middle) and 16 (late) of the harvest season and stored for three weeks under controlled conditions. The percentage values indicate the changes in the lycopene concentration during the three-week storage period.

# CHAPTER 5

# DISCUSSION

## 5.1 THE RATIONALE FOR THIS STUDY

Tomatoes are one of the most important food crops in the vegetable economy (Salunkhe *et al.*, 1974; Chapagain & Wiesman, 2004). South Africa produces enough to support an export of both fresh and processed produce. The total volume of the tomato industry in South Africa is approximately 650 000 tons to the value of R1.3 billion per annum (www.nda.agric.za/docs/Cropsestimates).

Tomato yield is affected by a variety of factors, such as the availability of water, light intensity, relative humidity, temperature, plant density, agricultural practises and the mineral composition of the soil or nutrient medium (Romer, 1993; Mahajan & Singh, 2006). Unfavourable nutritional conditions, such as a calcium deficiency, can lead to the nutritional disorder Blossom-end rot (BER). Blossom-end rot is also found in *Capsicum annuum* (pepper fruits), *Solanum melongena* (eggplants) and *Citrullus lanatus* (watermelon) plants (Taylor *et al.,* 2004; Ho & White, 2005). This disorder occurs in all the areas where these plants are grown commercially. Losses can vary from very little up to 50% to 70% of the yield, depending on the variety, method of cultivation, and environmental conditions (Taylor *et al.,* 2004).

In an effort to reduce the occurrence of BER, several practices can be used. The most common is the addition of calcium to the soil or the direct spraying of plants with additional calcium. For example, calcium deficient soils are treated several months before planting with 567 to 1134 kg $CaCO_3$. $ha^{-1}$ (http://www.ces.ncsu.edu; http://pubs.caes.uga.edu; http://vegedge.umn.edu), while established plants that display BER symptoms, can be sprayed directly with either 0.25% $CaCl_2$ or 0.5% $CaNO_3$ every seven to ten days for three or four applications (www.pubs.caes.uga.edu). Ho and White (2005) have found that where tomatoes were sprayed weekly with 0.5% $CaCl_2$ (w/v), the occurrence of BER was reduced up to 40%.

A company, Elementol (Pty) Ltd, which marketed a micro-emulsion under the name of Pheroids, claimed that it can act as a vehicle for the uptake and transport of phytologically beneficial substances over membranes. The fatty acid based emulsion apparently acts like a sponge carrying molecules across plant membranes. Pheroids also apparently stimulates plant growth in general. Elementol (Pty) Ltd provided little information on Pheroids as its patent registration is still pending.

The rationale for study was therefore to determine:

- The effect of a calcium deficiency on the growth and yield of tomatoes grown under controlled hydroponic conditions.
- The relationship between a calcium deficiency and the occurrence of BER.
- Whether externally applied calcium can reduce the symptoms and effect of a calcium deficiency and the associated occurrence of BER.
- Whether Pheroids can act as a transport vehicle (carrier molecule) for externally applied $CaCl_2$ solutions under calcium stress conditions.
- Whether Pheroids stimulate growth and yield in general and can thus be promoted as a growth stimulating substance.

## 5.2 EFFECT OF A CALCIUM DEFICIENCY ON THE VEGETATIVE AND GENERATIVE DEVELOPMENT OF TOMATO PLANTS

Tomato plants were cultivated in a greenhouse to exclude the effects of environmental conditions on vegetative growth. Temporary high light intensities were avoided by partial shading and the greenhouse temperature was regulated between 16ºC and 25ºC throughout the study. The pH of the complete- and calcium deficient Hoagland’s nutrient medium used in this study, were maintained between 5.5 and 6.5.

This study, like those by Camacho *et al.* (2002) and Tsuyuzaki *et al.* (2004), confirmed that calcium is an important inorganic nutrient needed for plant growth (Figure 4.3A&B). The growth of the young plants, both length and canopy diameter, were impeded by a calcium deficiency, which in the long term might affect the yield potential negatively.

According to Terry and Huston (1975), calcium plays an important role in the regulation of nutrient transport and photosynthetic capacity. Reduction of these processes will therefore decrease the amount of nutrients and carbohydrates available for growth and development, resulting in reduced growth potential, as was observed for the calcium stressed plants in this study.

Moreover, the growth of the calcium stressed plants might also have been hampered due to the importance of calcium for cell division (Sabba & Miller, 1993) and cell expansion (White & Broadley, 2003). In these plants the lowered $[Ca^{2+}]_{cyt}$ concentration could have reduced cell expansion by affecting the incorporation of vesicles that contain the materials and enzymes required for membrane and cell wall construction, negatively. Calcium accumulation in the vacuoles of the cells might also have decreased due to the calcium deficiency, thus decreasing cell volume and cell size (Hirschi, 2001). Thus, growth in these plants might have been reduced due to a reduction in cell number and cell size.

In addition, the integrity of the plasma membranes may be reduced in the calcium stressed plants due to a lack in calcium. In these plants less $Ca^{2+}$ is available to bind the negatively charged lipids in the plasma membrane, reducing the permeability thereof. The membranes will become leaky to charged solutes, which will cause cell death and a reduction in growth (Ho & White, 2005).

Another factor that might also have affected the vegetative growth of these plants, is reduced cell signalling and signal perception. The lack of calcium affects $[Ca^{2+}]_{cyt}$ perturbations negatively by affecting the signature of the perturbation. This may disrupt the link between developmental and/or environmental stimuli to the appropriate physiological responses.

Calcium not only affected the vegetative development of the plants, but also the generative development of the plants (Table 4.1). Generative development entails the development of the reproductive structures, which include bud-, flower- and eventually fruit formation. A calcium deficiency lengthened the time period forgoing bud development and thus delays bud development. However, a calcium deficiency had no apparent effect on the time period forgoing flower- and fruit development. Therefore, it appears that calcium is not only important for the vegetative development of plants but also for generative development.

A delay in bud development of three days, as was observed in this study (Table 4.1), may affect yield negatively to a certain extent.

## 5.3 EFFECT OF A CALCIUM DEFICIENCY AND SOME PREVENTATIVE TREATMENTS ON YIELD

In this study, a possible genotypic trait for the Floridade cultivar was observed in average fruit size and mass. It decreased for the fruits of all the treatments used in this study as the harvest season progressed (Figure 4.6). This reduction in fruit size and mass is the reason why commercial producers terminate the harvest season after a few weeks, as further harvesting present a potential financial loss (Polder *et al*., 2004). More studies that compare different determinate cultivars with regard to fruit size and mass during the harvest season are required to investigate the possibility of such a genotypic trait.

The experimental layout of this study represented approximately 43 000 plants. $ha^{-1}$. Based on this plant density, a total yield of 104 421 kg. $ha^{-1}$ (Table 4.2), with a calculated gross income of R 417 685. $ha^{-1}$ (Table 4.3), were harvested over an experimental period of 16 weeks from the control plants cultivated under controlled hydroponic nutrient conditions. The plants cultivated in a nutrient medium deficient of calcium produced smaller fruits (Figure 4.5) and subsequently a lower total yield (Table 4.2) than the control plants. This resulted in a loss of R 270 590. $ha^{-1}$ for the calcium stressed plants.

The negative effect of a calcium deficiency on yield is probably due to the necessity of calcium for normal cellular functioning. A calcium deficiency affected fruit growth and yield, possibly by affecting assimilate accumulation in the fruits. Assimilate accumulation is determined by the number of cells present in the fruits, the size of the fruit cells and the availability of assimilates (Bertin *et al.*, 2002). Thus, if a calcium deficiency affects the assimilates available for accumulation, the number of cells and/or the size of the fruit cells negatively, it will reduce fruit size and consequently yield.

The cell size of the calcium deficient plants may have been restricted by aberrant $[Ca^{2+}]_{cyt}$ signals. The latter may fail to generate the proper developmental responses that initiate cell expansion. This will restrict cell expansion, reducing cell size, and ultimately fruit size in calcium stressed plants. Cell expansion in these fruits may also have been affected adversely by a lack in hydrostatic pressure, which is necessary for normal cellular development. In calcium stressed cells, it is possible that less $Ca^{2+}$ is available for accumulation in vacuoles. Consequently, not enough hydrostatic pressure is generated to ensure normal cell expansion, thus contributing to the reduction in cell size.

A factor that may reduce the number of cells present in the calcium stressed fruits, was uncontrolled cell expansion that lead to cell death. Cell expansion is stopped when the pectins in the cell wall become progressively de-esterified and branched through the activity of pectin methylesterases and crosslinkage by $Ca^{2+}$ (Cosgrove, 2000; White & Broadley, 2003; Ho & White, 2005; Carrari & Fernie, 2006). In the calcium stressed fruits, it is possible that cell expansion cannot be stopped due to a lack in $Ca^{2+}$, which consequently results in structural weakness and ill formed cells that are prone to breakage, as was reported by Ho and White (2005).

A further factor which might have contributed to the reduction in the number of cells in the calcium stressed fruits, is the importance of calcium for membrane permeability. In the calcium stressed fruits, the plasma membranes may become leaky due to the calcium deficiency, which could lead to cell death (Ho & White, 2005) and the subsequent reduction in the number of cells present in the fruits and therefore fruit size.

A calcium deficiency might also have reduced assimilate accumulation, since fruit size is apparently linearly related to the import of sucrose into fruits (Wang *et al.*, 1993). It is possible that a calcium deficiency reduces the available assimilate by reducing the photosynthetic rate in the plants, as well as in the fruits (Rangnekar, 1975*b*; Terry & Huston, 1975). The fruits synthesize between 15% and 20% of its total required carbon via photosynthesis during the green stage (Obiadalla-Ali *et al.*, 2004), while the rest of the carbon assimilated in the fruits is imported from the green, photosynthesizing parts of the plants. Studies conducted on the chlorophyll concentration of tomato leaves, showed that after an eight to ten day calcium deficiency period, 50% of the chlorophyll content were lost and there was a 30% reduction in the photosynthetic rate (Rangnekar, 1975*b*; Terry & Huston, 1975).

Additionally, a calcium deficiency would also have reduced the translocation of assimilates to the fruits, since calcium plays a major role in the translocation of carbohydrates and proteins (Poovaiah & Leopold, 1973; Terry & Huston, 1975; Gossett *et al.,* 1977; Fuente, 1984).

In an effort to reduce the negative effect a calcium deficiency has on yield, commercial growers either treat the soil with additional calcium by adding approximately 600 to 1100 kg $CaCO_3$. $ha^{-1}$ to the soil several months before planting (http://www.ces.ncsu.edu), or they spray established plants with 0.25% $CaCl_2$ or 0.5% $CaNO_3$ every seven to ten days for three or four applications (www.pubs.caes.uga.edu). In this study, 1% and 2% $CaCl_2$ solutions, applied foliarly, were used as an external source of calcium for plants grown hydroponically in a calcium deficient Hoagland’s nutrient medium.

Applying 1% and 2% $CaCl_2$ solutions foliarly to the calcium stressed plants reduced the effect of a calcium deficiency to a certain extent, as it improved fruit mass, size and yield above that of the non-treated calcium stressed plants (Figure 4.6A&B and Figure 4.4A). However, there was little difference between the yields obtained for the plants sprayed foliarly with 1% and 2% $CaCl_2$ solutions since it resulted in yields of 67 592 kg. $ha^{-1}$ and 66 754 kg. $ha^{-1}$ respectively (Table 4.2). It appears that the additional calcium was absorbed and entered the plants to replenish the $[Ca^{2+}]_{apoplast}$ and $[Ca^{2+}]_{cyt}$ levels of the calcium stressed plants, thus possibly improving cell division, cell expansion, cell signalling (Jiang & Huang, 2001; White, 2001; Supanjani *et al.,* 2006), enzymatic functioning and (Jiang & Huang, 2001) the photosynthate/assimilate supply to the fruit cells (Poovaiah & Leopold, 1973; Terry & Huston, 1975; Rangekar, 1975*a*&*b*; Gossett *et al.,* 1977; Fuente, 1984; Skórzyńska-Polit *et al.,* 1998; Zhu *et al.,* 2004). The calcium in the solutions seemingly diffuses through the cuticle of the leaves and enters the plant and fruit cells via calcium permeable channels. However, the calcium concentration in the 1% and 2% $CaCl_2$ solutions were not sufficient to completely alleviate the effect of the calcium deficiency and to restore yield to that of the control plants. Although the 1% and 2% $CaCl_2$ treatments improved the total yield of the calcium stressed plants (44 084 kg. $ha^{-1}$) with 23 508 kg. $ha^{-1}$ and 22 670 kg . $ha^{-1}$ respectively, the yields obtained from these treatments were however, still 36 829 kg. $ha^{-1}$ and 37 667 kg. $ha^{-1}$ less than the 104 421 kg. $ha^{-1}$ harvested from the control plants (Table 4.2). The 1% and 2% $CaCl_2$ treatments resulted in gross incomes of R270 367. $ha^{-1}$ and R267 019. $ha^{-1}$ respectively, which is an improvement of R94 029. $ha^{-1}$ and R90 681. $ha^{-1}$ above that of the non-treated calcium stressed plants (Table 4.3).

To further increase the calcium concentration applied to the calcium stressed plants, the frequency of the spray applications can be increased from once every two weeks to once a week. It is suggested that the effect of weekly treatments on calcium stressed tomatoes (cv. Floridade) to reduce BER and improve yield, should be investigated. Another option is to increase the $CaCl_2$ concentration of the sprays. However, pre-test trials showed that a 4% $CaCl_2$ solution burns the vegetative- and generative parts of the plants (Results not shown). Therefore, increasing the $CaCl_2$ concentration above 2% is not a feasible option.

Based on the claims made by Elementol (Pty) Ltd that Pheroids may act as a vehicle for transporting phytologically beneficial substances over membranes, another way to increase the calcium concentration in the plants, was by "packing" the Pheroids micro-emulsion with the 1% and 2% $CaCl_2$ solutions. "Packing" Pheroids with $CaCl_2$ implies that the 1% and 2% $CaCl_2$ solutions were mixed with Pheroids in a ratio of 2 $cm^3$ Pheroids to 100 $cm^3$ $CaCl_2$ solution after which it was stirred on a mixer for 30 minutes before the mixtures were sprayed onto the plants. It was hypothesized that mixing 1% and 2% $CaCl_2$ solutions with Pheroids should improve the calcium concentration provided by the 1% and 2% $CaCl_2$ solutions by the increased absorption and transportation of calcium into the calcium stressed plants by Pheroids, resulting in higher $[Ca^{2+}]_{apoplast}$ and $[Ca^{2+}]_{cyt}$ levels than when the plants were sprayed with only the 1% and 2% $CaCl_2$ solutions. Higher levels of intracellular calcium might improve cell division, cell expansion, cell signalling (Jiang & Huang, 2001; White, 2001; Supanjani *et al.*, 2006), photosynthate/assimilate supply (Terry & Huston, 1975; Rangekar, 1975*a*&*b*; Brand & Becker, 1983; Skórzyńska-Polit *et al.*, 1998; Zhu *et al.*, 2004), enzymatic functioning (Veierskov & Meravy, 1985), antioxidant activity, guard cell tugor and stomatal opening (Jiang & Huang, 2001; White, 2001), ultimately improving cellular functioning and the overall health of the plants. It was further hypothesized that in response to the improved cellular functioning, the fruit size and mass, and consequently the yield of the calcium stressed plants treated with the $CaCl_2$ Pheroids mixtures, should also improve. However, "packing" Pheroids with 1% $CaCl_2$ resulted in a reduced yield as these plants produced 9 703 kg. $ha^{-1}$ less than the plants treated with only the 1% $CaCl_2$ solution. In contrast, mixing Pheroids with the 2% $CaCl_2$ solution increased the gross yield with 645 kg. $ha^{-1}$, representing an increase of R 5 928. $ha^{-1}$ in gross profit (Table 4.2 and Table 4.3). Thus, it may be possible that Pheroids improved the diffusion of calcium into the plants, consequently increasing the $[Ca^{2+}]_{apoplast}$ and $[Ca^{2+}]_{cyt}$ concentrations sufficiently, thus improving yield.

It is also possible that Pheroids in the $CaCl_2$ Pheroids mixtures influence the source-sink relationship by stimulating fruit growth. Heuvelink (1997) and Gary *et al*. (2003) showed that a relationship exist between the size of the fruits and the number of fruits produced. He found that the weight of individual fruits decreased as the number of fruits produced increased. In this study it was observed that mixing Pheroids with $CaCl_2$ solutions seemingly reduced fruit size (diameter) and yield (mass) when compared to the plants treated with $CaCl_2$ solutions only. However, the $CaCl_2$ Pheroids solution did exhibit a slight increase in the number (n) of fruits produced, especially in the case of the 1% $CaCl_2$ Pheroids treatment (Figure 4.4A&B and Figure 4.7A&B). The sink strength of fruits is determined by growth rate and size, which depends on the number and the size of fruit cells (Wang *et al.*, 1993; Ho, 1996). Mixing Pheroids with the $CaCl_2$ solutions might have stimulated fruit growth by increasing the assimilate supply to the cells, which will increase cellular functioning and subsequently the growth of the calcium stressed fruits, but since the calcium stressed plants were cultivated in a calcium deficient nutrient medium, fruit growth could not be maintained due to the calcium deficiency. This reduced the average fruit size and consequently yields, as was observed in this study (Figure 4.7). Thus, it appears that Pheroids may have a stimulatory effect on fruit growth, as claimed by Elementol (Pty) Ltd. The effect of the 2% $CaCl_2$ Pheroids mixture on the source-sink ratio was, however, less pronounced, possibly due to the higher calcium concentration of the mixture which might have improved cellular functioning.

In contrast to the treatments where calcium deficient plants were sprayed foliarly with the 1% and 2% $CaCl_2$ Pheroids mixtures, treating control plants with Pheroids had the opposite effect on the source-sink relationship of the plants. This treatment clearly stimulated fruit growth in terms of fruit size and mass, while it simultaneously decreased the number of fruits produced (Figure 4.4A&B and Figure 4.7A&B). This suggests that these fruits have developed a bigger sink capacity than that of the control plants, which ultimately resulted in a larger yield. Thus, treating control plants with Pheroids resulted in a total yield of 107 509 kg. $ha^{-1}$, which is 3 088 kg. $ha^{-1}$ more than that of the control plants (104 421 kg. $ha^{-1}$). This amounts to a difference of R12 349. $ha^{-1}$ in gross income between the two treatments (Table 4.3).

Pheroids, as an emulsion containing free fatty acids, might stimulate fruit growth if the fatty acid component of the emulsion is metabolized. The fatty acids are possibly broken down in the peroxisomes of the leaves via β-oxidation to acetyl-coenzyme A (acetyl-CoA), which could participate in the synthesis of sucrose in the cytosol (Anderson & Beardall, 1991; Salisbury & Ross, 1992). Sucrose may then be transported from the leaves and stems to the developing fruits increasing fruit size, since the concentration of sucrose imported into fruits is related to the size of the fruits (Wang *et al*., 1993; Islam *et al*., 1996; Obiadalla-Ali *et al*., 2004). The sink strength of these fruits would also have increased if the growth rate of the fruit cells increased (Wang *et al*., 1993; Ho, 1996). Acetyl-CoA might have affected the growth rate of fruits indirectly by acting as a substrate for the TCA cycle thereby stimulating plant- and fruit growth indirectly by increasing ATP production through cellular respiration (Anderson & Beardall, 1991; Salisbury & Ross, 1992). Moreover, acetyl-CoA is also used for the synthesis of a diverse set of phytochemicals including waxes, isoprenoids, and flavonoids in the cytosol (Salisbury & Ross, 1992), which suggests that Pheroids may stimulate plant growth in general.

It must be kept in mind that the fruits also came into contact with Pheroids during the foliage treatment of the control plants. Thus, it may be possible that the membrane permeability and strength of these fruits were improved due to contact with Pheroids. The free fatty acids in the Pheroids emulsion might strengthen and improve the plasma membrane integrity of the fruits by binding covalently to the phospholipids in the bilayer structure of the plasma membranes. This may increase the cell size of the fruits, which will also increase the sink strength of these cells, and consequently the amount of assimilates and photosynthates imported into the fruits (Wang *et al*., 1993; Islam *et al*., 1996; Obiadalla-Ali *et al*., 2004). Thus, increasing cell size by means of membrane structure may be another possibility that results in larger fruits, as was observed when control fruits were treated with Pheroids.

To determine the efficiency of the $CaCl_2$ foliage treatments on calcium stressed plants, the 2% $CaCl_2$ Pheroids foliage treatment was compared to a treatment where only the fruits of the calcium stressed plants were treated with the 2% $CaCl_2$ Pheroids mixture. It was reasoned that the 2% $CaCl_2$ Pheroids foliage treatment would give better results compared to the other foliage treatments due to the higher $CaCl_2$ concentration absorbed through the action of Pheroids. Treating only the fruits with the 2% $CaCl_2$ Pheroids mixture resulted in a low gross yield of only 36 245 kg. $ha^{-1}$, which was 31 922 kg. $ha^{-1}$ less than when the whole plant was sprayed with this mixture. This resulted in a loss of R127 968. $ha^{-1}$ in gross income. It was hypothesized that mixing Pheroids with the $CaCl_2$ solutions would stimulate fruit growth, which in turn will increase the assimilate demand of the fruits (sinks) from the vegetative parts of the plant (source). However, due to the calcium stress to which the plants were subjected to, the leaves appear to be unable to meet the demand of the fruits, since a severe calcium stress will result in aberrant cell signals, structural weakness, loss of membrane permeability, precocious cell division and expansion (Ho *et al.*, 1993; White & Broadley, 2003; Ho & White, 2005), reduced photosynthesis- (Jiang & Huang, 2001; Zhu *et al.*, 2004) and enzymatic functioning (Matsumoto *et al.*, 1980; Veierskov & Meravy, 1985; MacIntosh *et al.*, 1996; Schmitz-Eiberger *et al.*, 2002; Schmitz-Eiberger & Noga, 2003). It appeared that the inability of the leaves (source) to meet the fruits' demand finally resulted in smaller and fewer fruits (Figure 4.6A&B) and consequently a decrease in yield (Figure 4.4A&B).

It is clear that a calcium deficiency has a negative effect on yield as it reduces fruit size and subsequent yield. Applying external 1% and 2% $CaCl_2$ solutions foliarly, reduce the negative effect of a calcium deficiency and improve fruit size and yield to a certain extent. Mixing Pheroids with the 1% and 2% $CaCl_2$ solutions further enhance fruit growth and yield, which was however, still less than that of the control plants.

## 5.4 <u>EFFECT OF A CALCIUM DEFICIENCY AND THE PREVENTATIVE TREATMENTS ON FRUIT QUALITY</u>

The control plants were cultivated in a hydroponic set-up in complete nutrient conditions. It can thus be assumed that the fruits harvested from these plants represent fruits of a high quality. The fruits were allowed to develop fully on the parent plant until they were considered to be "consumer ready" before they were harvested. A number of quality parameters, namely moisture content, the dry mass: fresh mass ratio, Brix index, EC and lycopene concentration, were used to determine and quantify the quality of the fruits. Even though the fruits became smaller as the harvest season progressed (Figure 4.8A), the fruits harvested from the control plants still represented fruits of a high quality irrespective of the stage of harvest. During the early stages of the harvest period, the fruits weighed approximately 80 g and had a moisture content of ± 93% (Figure 4.8A&C). As the harvest period progressed, the average fruit weight decreased to approximately 40 g and the moisture content to approximately 90%. The dry mass remained relatively constant at approximately 5 g. fruit$^{-1}$ for the entire harvest period. However, the dry mass:fresh mass ratio of 1:15 at early harvest decreased to 1:9 during the later stages of harvest (Figure 4.8B). An increase in the EC and Brix index of the fruits from approximately 3.6 mS. cm$^{-1}$ to 5.1 mS. cm$^{-1}$ and 6.5% to 11% respectively, indicates clear metabolic changes as the harvest period progressed (Figure 4.8). Even though the fruits contain higher concentrations of assimilates during the late stages of the harvest season, the health associated properties of the fruits decreased since the lycopene concentration decreased from ± 1.5 mg. kg$^{-1}$ dry mass at early harvest to ± 0.5 mg. kg$^{-1}$ dry mass at late harvest. Amongst the carotenes, lycopene dominates (Polder *et al.*, 2004) and constitutes on average 80 to 90% of the total carotenoid content (George *et al.*, 2004). Lycopene is a strong antioxidant and serve as an intermediate for the biosynthesis of other carotenoids (Rao & Agarwal, 1999). There is a considerable body of evidence that link a high intake of tomatoes and lycopene to a reduced incidence of prostate cancer and DNA damage in white blood cells (Bramley, 2002). It was also found that lycopene is, without doubt, superior to α- and β-carotene in inhibiting cell proliferation in various epithelial cancer cell lines (Sahlin *et al.*, 2004).

Although a calcium deficiency reduced yield markedly, it had no apparent effect on most of the parameters used to determine fruit quality. Treating calcium stressed plants with 1%- and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, also had no apparent effect on fruit quality. These treatments did not improve nor decrease the quality of the fruits harvested from the calcium stressed plants, which compared well to the quality of the fruits from the control plants (Figure 4.8).

When control plants were treated additionally with Pheroids, the dry mass:fresh mass ratio (Figure 4.8B) and moisture content (Figure 4.8C) appeared to be slightly higher than that of the other treatments. This suggests that these fruits contain slightly more moisture relative to its carbon content than the fruits from the other treatments. As said before, when the plants were sprayed with Pheroids, some of it came in contact with the fruits. It may be that the fatty acid component of Pheroids was metabolized to acetyl-CoA via β-oxidation, which might increase cellular respiration, cellular functioning and plant- and fruit growth in general.

Although a calcium deficiency had no clear effect on most of the parameters used to quantify fruit quality, it improved the health benefits of the fruits by increasing the lycopene concentration in the calcium stressed fruits compared to the control fruits slightly (Figure 4.12). Treating the calcium stressed plants additionally with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, might have reduced the effect of a calcium deficiency by increasing yield, but also resulted in lowered lycopene concentrations compared to that of the non-treated calcium stressed plants (Figure 4.12). It may be possible that the fruits of the calcium stressed plants produced more lycopene as BER, the result of a calcium deficiency, is known to induce ripening (Suzuki *et al.*, 2000). Ripening is the transition from a partially photosynthetic- to a true heterotrophic metabolism by the parallel differentiation of chloroplasts into chromoplasts and the dominance of carotenoids and lycopene (Carrari & Fernie, 2006). This is a complex, genetically programmed process that culminates in colour, composition, aroma, flavour and textural changes and appears to be regulated by hormones and may be modified by genetic and environmental factors (Atherton & Rudich, 1986; White, 2002; Alexander & Grierson, 2002). In the calcium stressed fruits, the lack of calcium may have contributed to the premature differentiation of chlorophyll to chromoplasts via chlorophyll degradation. Studies have reported a 50% decrease in tomato leaf chlorophyll due to a calcium deficiency after just eight to ten days of calcium deprivation (Rangnekar, 1975*a*). Treating the calcium stressed plants with 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, apparently increased the calcium available to the fruits sufficiently to prevent the possible premature chlorophyll degradation, thus resulting in reduced lycopene accumulation compared to the calcium stressed fruits, as was noticed in this study (Figure 4.12).

In addition, treating control plants with Pheroids resulted in the highest lycopene concentration of all the treatments (Figure 4.12). It can be hypothesized that the fatty acids in the Pheroids emulsion are metabolized to acetyl-CoA via β-oxidation in the peroxisomes. Acetyl-Coenzyme A (acetyl-CoA) is a precursor molecule for the mevalonic acid (MVA) pathway (Figure 2.2) to produce cytosolic IPP (Eisenreich *et al.,* 2001; Botella-Pavia *et. al.,* 2004; Francis & Cunningham, 2002; Bramley, 2002; Yamazaki *et al.*, 2004; Ahn & Pai, 2008). Isopentenyl diphosphate (IPP) and DMAPP are condensed in their respective compartments to yield prenyl diphosphates that lead to the synthesis of isoprenoid products (Figure 2.2). Geranylgeranyl diphosphate synthase catalyze the reaction between three IPP units and one DMAPP unit to yield GGPP (Figure 2.2). Geranylgeranyl diphosphate (GGPP) is the immediate precursor for the first $C_{40}$ carotenoid, phytoene, from which lycopene is eventually synthesized (Figure 2.2; Francis & Cunningham, 2002; Bramley, 2002; Botella-Pavia *et. al.*, 2004; Ahn & Pai, 2008). However, Pheroids as part of the $CaCl_2$ Pheroids mixtures, failed to increase the fruit lycopene concentration of the calcium stressed plants. This might be due to the effect that the calcium deficiency had on growth and fruit development in general.

To summarize, it appears that fruits harvested during the early stages of the harvest season are less concentrated, but healthier in terms of lycopene content than the fruits harvested during the late stages of the harvest season. Additional treatment with Pheroids results in fruits that contain a higher moisture- and lycopene content, thus improving the health benefits of these fruits slightly. A calcium deficiency and all the subsequent preventative treatments have no clear effect on most of the quality parameters used to quantify fruit quality. A calcium deficiency does; however, seem to improve the health benefits of the fruits somewhat, as it resulted in a slightly higher lycopene concentration. The increase in the fruit lycopene concentration of the non-treated calcium stressed- and control plants sprayed with Pheroids, is not marked enough to warrant the use of these treatments to improve fruit quality for economical purposes.

## 5.5 EFFECT OF A CALCIUM DEFICIENCY AND THE PREVENTATIVE TREATMENTS ON THE OCCURRENCE OF BLOSSOM-END ROT (BER)

Blossom-end rot, as a nutritional disorder, is common in tomatoes, pepper fruits, eggplants and watermelon (Taylor *et al.,* 2004; Ho & White, 2005) and is mainly initiated by a cellular dysfunction in fruits during cell expansion upon a local transient calcium deficiency (White & Broadley, 2003). The symptoms of BER are most noticeable at the blossom-end of the fruits as discoloured necrotic tissue, because less calcium is delivered to the blossom-end of the fruits, especially during periods of rapid fruit growth (Ho & White, 2005), as the density and number of the xylem vessels decreases towards the blossom-end of the fruits during fruit expansion (Ho *et al.,* 1993; Suzuki *et al.,* 2000). All environmental and genetic factors that influence the occurrence of BER in tomatoes, either affect the rate of cell expansion, or the delivery of calcium to the young tomato fruit (Ho *et al.*, 1993). The induction of BER normally occurs one to three weeks after anthesis when a calcium deficiency is most disastrous (Sonneveld & Voogt, 1991; Ho *et al.*, 1999; Taylor *et al.*, 2004). The occurrence of BER causes substantial financial losses of up to 70% world wide depending on the cultivar, agricultural practices, environmental conditions and location (Taylor *et al.*, 2004; Ho & White, 2005; http://www.ipm.uiuc.edu/diseases). As mentioned previously, to prevent the occurrence of BER, calcium deficient soils are treated with approximately 600 to 1100 kg $CaCO_3$. $ha^{-1}$ several months before planting (http://www.ces.ncsu.edu). Alternatively, the occurrence of BER can also be reduced by spraying established plants with BER symptoms with 0.25% $CaCl_2$ or 0.5% $CaNO_3$ every seven to ten days for three to four applications (www.pubs.caes.uga.edu).

Results from this study confirmed that a calcium deficiency induces BER in tomatoes (Figure 4.13A&B and Figure 4.14). The loss of yield in the calcium stressed plants grown under hydroponic conditions was approximately 70%, which is similar to the losses reported by other authors (http://www.ipm.uiu.edu).

Calcium enters the plant by diffusing into the root cells after which it is transported by means of the symplastic- and apoplastic routes through the plant (White & Broadley, 2003). These two routes allow the roots to fulfil the calcium demand of the shoot without compromising intracellular $[Ca^{2+}]_{cyt}$ signalling (White, 2001; White & Broadley, 2003). The majority of calcium is transported via the apoplastic route through the transpiration stream in the xylem, firstly to the strongest transpiration sinks namely the older leaves, after which the remaining calcium is transported to the weaker transpiration sinks, namely the younger leaves and fruits (Pate & Hocking, 1978; Halperin *et al.*, 1997; White & Broadley, 2003; Busse & Palta; 2006). During a calcium stress, as was induced in this study, the older leaves will probably accumulate most of the calcium available to the plants. This will lead to the development of a calcium deficiency in the blossom-end of the fruits during fast growing developmental stages, resulting in the development of BER symptoms (Kuo *et al.*, 1981; Berghoef, 1986; Hernandez *et al.*, 2004). In the fruits that develop BER symptoms, the epidermis and sub-epidermal parenchyma collapse, the plasma membrane and tonoplast are disrupted, the cell wall become wavy, the mitochondria and ER brake, organelles degenerate and the plastids of the fruit cells become swollen. This will result in the formation of brown, necrotic tissue at the blossom-end of the fruits, the major symptoms of BER (Suzuki *et al.,* 2000), as was observed in the calcium stressed plants of this study (Figure 2.3).

Moreover, despite being cultivated in a complete Hoagland's nutrient medium that contains sufficient quantities of calcium, the control plants also developed BER symptoms, but to a smaller degree (Figure 4.13). This may be due to the formation of a local calcium deficiency during rapid fruit cell growth (Ehret & Ho, 1986; Marcelis & Ho, 1999), which normally occurs one to three weeks after anthesis in tomatoes (Sonneveld & Voogt, 1991; Ho *et al.,* 1999; Taylor *et al.,* 2004). There are two possible explanations for such a local cellular calcium deficiency in the control fruits. The complete nutrient medium may stimulate vegetative growth resulting in large, green leaves with a high transpiration rate. This will increase the sink strength of these vegetative parts resulting in the preferential distribution of calcium to these parts, causing a calcium deficiency in the fruits during periods of rapid cell expansion when the calcium demand in the fruits is highest (Sonneveld & Voogt, 1991; Taylor *et al.,* 2004). Alternatively, the complete nutrient medium may stimulate fruit growth to such an extent that the supply of calcium via the apoplastic- and symplastic routes cannot meet the calcium demand of the fruits during periods of rapid fruit growth (Ho *et al.,* 1993; White & Broadley 2003).

Between the control plants and the calcium stressed plants, a calcium deficiency clearly has the more noticeable effect on the occurrence of BER, as the marketable yield of the calcium stressed plants were only 28 707 kg. ha$^{-1}$ compared to the 96 354 kg. ha$^{-1}$ for the control plants (Table 4.2). Thus, a calcium deficiency resulted in a financial loss of approximately R270 000. ha$^{-1}$ (Table 4.3). This clearly indicates that a sufficient supply of calcium in the nutrient medium or soil is required to reduce the effect of BER on yield and profit margins (Figure 4.13A&B and Table 4.3).

To reduce the occurrence of BER and its effect on yield and income, additional calcium was supplied to the calcium stressed plants by spraying it foliarly with 1% and 2% $CaCl_2$ solutions. These concentrations were based on previous studies (Ho & White, 2005). It was reported that 0.5% $CaCl_2$ sprayed weekly, reduced the occurrence of BER in tomatoes up to 40% (Ho & White, 2005). Instead of spraying calcium stressed plants on a weekly basis, in this study it was sprayed once every two weeks using higher $CaCl_2$ concentrations of 1% and 2%.

The 2% $CaCl_2$ solution proved to be more successful in reducing BER (Figure 4.13) than the 1% $CaCl_2$ solution. The net income of the 2% $CaCl_2$ treatment was R228 399. $ha^{-1}$, R142 192.40. $ha^{-1}$ more than that of the calcium stressed plants. For the 1% $CaCl_2$ treatment the net income was R206 942. $ha^{-1}$, R92 114. $ha^{-1}$ more than that of the calcium stressed plants. It was postulated that the higher calcium concentration in the 2% $CaCl_2$ solution might elevate the $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{apoplast}$ levels in the plants. Thus, more calcium might have been available to the weaker sinks, namely the fruits, to reduce the occurrence of BER (Figure 4.13), more than in the case of the 1% $CaCl_2$ solution. However, this study shows that both the 1% and 2% did not provide the calcium stressed plants with enough calcium to completely reduce the occurrence of BER and improve the marketable yield to the levels observed for the control plants (Table 4.2 and Table 4.3).

The occurrence of BER may be further reduced by increasing the $CaCl_2$ concentration in the foliage treatments. However, as mentioned previously, preliminary tests have revealed that a concentration of 4% $CaCl_2$ “burned” the plants, since necrotic patches developed on the leaves (Results not shown), making this an non-viable option.

Another way to possibly raise the calcium levels in the cells, was to mix the $CaCl_2$ solutions with Pheroids and use it as foliage sprays. This was one of the principle aims of this study, namely to evaluate the potential of Pheroids to act as a vehicle for the transport of substances over membranes. It was hypothesized that the absorption of $CaCl_2$ by Pheroids might increase intracellular calcium levels by increasing the transport of calcium into the plants.

The results obtained for the 1% $CaCl_2$ Pheroids mixture (Figure 4.4A&B and Figure 4.6A&B) do not provide convincing evidence that mixing Pheroids with a 1% $CaCl_2$ solution enhance the absorption of externally applied calcium by plants and thereby reduce the occurrence of BER. It might be though that the calcium stress to which these plants were subjected to, may have been too severe for the 1% $CaCl_2$ Pheroids mixture to reduce the incidence of BER effectively.

In fact, treating calcium stressed plants foliarly with the 1% $CaCl_2$ Pheroids mixture, increased the occurrence of BER. The same phenomenon was also observed when control plants were sprayed additionally with Pheroids. This suggests that Pheroids might act as a growth stimulant, as claimed by Elementol (Pty) Ltd, since it was demonstrated previously that all environmental and genetic factors that influence the rate of cell expansion or the delivery of calcium to young tomato fruit, affect the occurrence of BER (Ho *et al.*, 1993; Marcelis & Ho, 1999). Thus, it may be argued that Pheroids, as a stimulant, might be responsible for the higher incidence of BER in both the control and 1% $CaCl_2$ Pheroids treated calcium stressed fruits. It is speculated that Pheroids in the 1% $CaCl_2$ Pheroids mixture stimulated fruit growth under the calcium stress conditions, thereby further increasing the existing calcium deficiency in the fruits by increasing the calcium demand of the fruits under calcium deficient conditions, thus increasing the incidence of BER. Similarly, the stimulation of fruit growth by Pheroids under controlled nutrient conditions will increase the calcium demand of the fruits. If the rate of calcium supply, especially during periods of rapid fruit growth, cannot meet the calcium demands of the fruits, the fruits will develop BER symptoms, even though sufficient quantities of calcium are present in the nutrient medium.

Therefore, treating calcium stressed plants with the 1% $CaCl_2$ Pheroids mixture resulted in a financial loss of R227 642. $ha^{-1}$ due to the higher incidence of BER, compared to the R178 475.80. $ha^{-1}$ lost when 1% $CaCl_2$ is applied singly. Treating the control plants with Pheroids caused a financial loss of R47 893.40. $ha^{-1}$ (Table 4.3). Thus, applying Pheroids under control or calcium stressed conditions as part of a pure Pheroids treatment or a 1% $CaCl_2$ Pheroids treatment, is counter productive and not economical as it increases the incidence of BER and decreases the marketable yield and subsequently profit margins (Table 4.3).

However, mixing Pheroids with the 2% $CaCl_2$ solution did reduce the occurrence of BER effectively (Figure 4.13A&B). The net yield obtained with the 2% $CaCl_2$ Pheroids mixture was 7 155 kg. $ha^{-1}$ more than with the 2% $CaCl_2$ solution, due to a marked reduction in the occurrence of BER (Table 4.2 and Table 4.3). Mixing Pheroids with the 2% $CaCl_2$ solution may contribute to the reduction in BER via two possible ways. Firstly, the Pheroids emulsion may be “packed” with $CaCl_2$ molecules or dissolved $Ca^{2+}$, after which the Pheroids-calcium complexes diffuse across the membranes, thus increasing the transport of additional calcium into the cells. The increased calcium levels may supply more calcium to the fruits during periods of rapid fruit growth, thereby reducing BER and increasing the marketable yield. Secondly, the fatty acid component in the Pheroids emulsion may strengthen the plasma membranes by binding to the phospholipids in the membrane structure. If the strengthened membrane remains intact and permeable under the calcium stress conditions, the occurrence of BER will probably be reduced, as loss of permeability, cell leakage and cell death are some of the fundamental causes of BER (Suzuki *et al.*, 2000; Ho & White, 2005). Pheroids may have had the same effect on the plasma membranes of the calcium stressed plants treated foliarly with the 1% $CaCl_2$ Pheroids mixture. However, because of the severity of the calcium stress and the lower calcium concentration in the 1% $CaCl_2$ Pheroids mixture, the extent to which the calcium deficiency was reduced and the membranes strengthened, was not sufficient to reduce cell leakage and loss of permeability to prevent the occurrence of BER.

To improve the efficiency of the 2% $CaCl_2$ Pheroids mixtures to reduce the incidence of BER and increase marketable yield and profits, it is suggested that the plants should be sprayed on a weekly basis rather than once every two weeks. Further studies are needed to test this and determine the costs involved of weekly applications.

The efficiency of the foliage 2% $CaCl_2$ Pheroids treatment was also compared to a treatment where only the fruits of calcium stressed plants were sprayed with this mixture. The latter treatment also effectively reduced the occurrence of BER (Figure 4.13). Direct treatment of fruits appears to increase the calcium status of the fruits more effectively than the foliage treatment. Application of the 2% $CaCl_2$ Pheroids mixture directly to the fruits might eliminate the competition for calcium between the weaker- (young leaves and fruit) and stronger transpiration sinks (mature leaves) as was observed by Taylor *et al.* (2004) in tomatoes. The rate of calcium delivery to the fruits, when applied directly, appears to be faster than when calcium had to be transported from the leaves to the fruits via the apoplastic- and symplastic routes. With this direct application and 2% $CaCl_2$ Pheroids concentration, it appeared as if the calcium may be sufficient to meet the calcium requirements of the fruits and may be readily available to the fruits during periods of rapid cell expansion, thus reducing the occurrence of BER.

However, in spite of reducing BER effectively, this treatment resulted in the second lowest net income with R131 047. $ha^{-1}$, which was just above that of the calcium deficient plants with R114 827. $ha^{-1}$, because direct treatment of the calcium stressed fruits reduced the yield by reducing average fruit size and the number of fruits produced (Figure 4.4 and Figure 4.6). Based on yield and profit margins, treating only the fruits of calcium stressed plants with a 2% $CaCl_2$ Pheroids mixture, is not a viable option to reduce the occurrence and effect of BER on marketable yield.

In conclusion, treating calcium stressed plants foliarly with a 2% $CaCl_2$ solution, is an effective way to reduce the occurrence of BER and to increase marketable yield and profit margins. Mixing Pheroids with a 2% $CaCl_2$ solution reduces the occurrence of BER and improves marketable yield even further, possibly by acting as a vehicle for transporting additional calcium into plants. Pheroids on its own stimulates fruit growth, since treating control plants with Pheroids, as well as calcium stressed plants with a 1% $CaCl_2$ Pheroids mixture, results in an increased occurrence of BER. Fruit growth in these plants is apparently stimulated to the point where the demand for calcium exceeds calcium supply by the vegetative parts of the plants, thereby promoting the development of BER.

## 5.6 THE EFFECT OF CALCIUM DEFICIENCY AND THE PREVENTATIVE TREATMENTS ON POST HARVEST FRUIT QUALITY

After a fruit is harvested from the plant, it is a self-contained support system as long as its metabolic supplies last (Laurin *et al.,* 2005). The aim of this investigation was to establish the effect of a calcium deficiency on fruit deterioration during post harvest storage and how the preventative treatments used in this study were able to prevent this.

Firm, red “consumer ready” fruits harvested at weeks 6, 12 and 16 of the harvest period, were subjected to this investigation. These harvesting weeks represented the early, middle and late stages of the harvest period. After three weeks of post harvest storage, the quality (fruit deterioration) of the fruits were measured and compared to the quality at the day of harvest.

The most noticeable and important processes related to post harvest fruit quality are changes in weight, moisture and nutritional value. Saladié *et al.* (2005) also reported changes in fruit texture, colour, taste and aroma during the post harvest period. For marketability, weight loss presents the most critical parameter during storage of tomatoes, because for the retailer, weight loss translates into a loss in profit. It also implies a shortened shelf-life and lower nutritional values for the consumers (Laurin *et al.,* 2005).

Compared to the day of harvest, the weight of the control fruits from the early, middle and late stages of harvest all decreased between 8% and 10% (Figure 4.15). However, the moisture content of these fruits remained fairly stable during storage (Figure 4.16) and varied with not more than 1%. The Brix index also decreased during storage, as much as 16% for fruits from the late stages of harvest (Figure 4.17). In contrast, EC increased between 8% and 16% for the fruits harvested at the different stages (Figure 4.18). It can be concluded that control fruits became smaller after three weeks of storage, but remained fairly firm. The fruit cells contain less soluble solids (% Brix), but a higher proportion of charged compounds per volume unit (EC). The fruits can be seen as self contained units after harvest, which metabolized reserves to maintain cellular functions, some of which is linked to processes associated with fruit deterioration such as the oxidation of phenolic substances by phenolase, respiration and the demethylation of pectic substances that lead to fruit softening (Brennan, 2005). The changes in the quality parameters of the control fruits are considered representative of fruit deterioration during storage.

Similar to the control fruits, fruits from calcium stressed plants, as well as from the preventative treatments, all lost weight during storage. The mass loss measured for all these fruits were on average slightly more than in the control fruits (Figure 4.15). During the same time, the moisture content for all these fruits also decreased on average slightly more than the control fruits (Figure 4.16). The result of this was smaller fruits, which were less firm than the control fruits. Based on this, it appeared that fruits developing under calcium deficient conditions resulted in fruits of lesser physical quality than the control fruits after storage. None of the $CaCl_2$ solutions used as preventative treatments, singly or mixed with Pheroids, could prevent the physical deterioration of the fruits.

However, a calcium deficiency, as well as the preventative foliage treatments, seemingly slowed down fruit deterioration in terms of quality during post harvest storage. In contrast to the control fruits, the Brix index of these fruits increased during this period. This quality parameter indicated that fruit deterioration is impeded in the calcium stressed fruits.

It might be that the calcium deficiency caused fruit metabolism to slow down as calcium is important for intracellular signalling (Jiang & Huang, 2001; White, 2001; Supanjani *et al.*, 2006), the transport of carbohydrates (Poovaiah & Leopold, 1973; Terry & Huston, 1975; Gossett *et al.*, 1977; Fuente, 1984) and enzymatic functioning in general (Jiang & Huang, 2001). A lowered metabolic rate, coupled to the moisture and weight loss observed in these fruits, may result in an increased Brix index due to a concentration effect.

The treatment where only the fruits of the calcium stressed plants were sprayed with the 2% $CaCl_2$ Pheroids mixture resulted in fruits with the poorest physical quality after three weeks of storage. The highest reduction in fruit mass of between 15% to 28%, coupled to a simultaneous reduction in moisture content of up to 6.3%, were measured for this treatment. Unlike the other calcium stressed plants, treated and non-treated, the Brix index decreased markedly. A possible explanation is that the direct treatment of the fruits could cause higher calcium levels inside the fruits as the fruits did not have to compete with the stronger sinks, such as the older leaves, for calcium. This resulted in increased calcium levels, which might have restored the metabolic rate. Upon harvest, the raised metabolic rate in these fruits, metabolized reserves at a higher rate during storage, hence the marked drop in the Brix index (Figure 4.17).

Treating control plants additionally with Pheroids also resulted in a similar decrease in fruit weight (Figure 4.15), moisture content (Figure 4.16) and an increase in fruit EC (Figure 4.18), resulting in similar firm, smaller fruits after storage as for the control fruits. In contrast, the Brix index of these fruits increased during storage, as was observed for the calcium stressed fruits, treated and non-treated. It may be that treatment with Pheroids increased the reserves of the fruits since Pheroids is made up of an emulsion that contains fatty acid components. This might have resulted in an increased Brix index in comparison to the control fruits. However, further in depth studies are required to fully comprehend the effect of Pheroids on fruit quality during storage.

The one quality parameter that was specifically measured because of the health benefits linked to it, was the changes in lycopene concentration during storage. The lycopene concentration of the control fruits decreased during post harvest storage in the dark, especially in the fruits harvested at week 12, which resulted in an 82% reduction in lycopene (Figure 4.20A). A study by Grumbach (1984) found that carotenoid synthesis in the dark is possible. It might be possible that the absence of light during storage, reduces the concentration of lycopene synthesized, since less lycopene is needed to protect photosynthesis against photosensitization (Rao & Agarwal, 1999). Thus, the reduced lycopene synthesis, coupled to the natural degradation of lycopene via auto-oxidation (Xianquan *et al.*, 2005), might reduce the lycopene concentration of fruits during storage.

A calcium deficiency, as well as the 1% and 2% $CaCl_2$ solutions, had no apparent effect on the lycopene concentration of fruits during post harvest storage, as a similar decrease in concentration was measured for the control fruits (Figure 4.120B-F). In contrast, the calcium stressed plants treated foliarly with the 1% and 2% $CaCl_2$ Pheroids mixtures, as well as the control plants treated additionally with Pheroids, reduced the decrease in lycopene concentrations during storage slightly. Moreover, when only the fruits of calcium stressed plants were treated directly with the 2% $CaCl_2$ Pheroids mixture, an increase in the lycopene content was observed in the fruits harvested at week 16 (Figure 4.20G). It might be possible that foliage treatment of the calcium stressed plants and fruits directly with the Pheroids mixtures, resulted in an increased supply of acetyl-CoA, since it has been hypothesized that the fatty acid component of Pheroids is metabolized in plant cells to acetyl-CoA (Salisbury & Ross, 1992). The increased supply of acetyl-CoA might stimulate lycopene synthesis during storage, thus reducing the effect of the natural degradation of lycopene via auto-oxidation. Acetyl-coenzyme A (Acetyl-CoA), as mentioned previously, is the substrate for the MVA pathway, which synthesizes GGPP, the immediate precursor for the first $C_{40}$ carotenoid and the eventual synthesis of lycopene (Eisenreich *et al.*, 2001; Francis & Cunningham, 2002; Bramley, 2002; Yamazaki *et al.*, 2004; Botella-Pavia *et. al.*, 2004; Ahn & Pai, 2008). The fruits of the plants treated foliarly with $CaCl_2$ Pheroids mixtures (Figure 4.20E&F) and the control plants treated with Pheroids, presumably did not come into contact with as much Pheroids as the fruits of the direct treatment (Figure 4.20). Therefore, less acetyl-CoA was available to these fruits to promote the synthesis of lycopene during storage and reduce the effect of lycopene degradation via auto-oxidation.

In summary, compared to the changes observed for the fruits from control plants, a calcium deficiency and the subsequent preventative treatments, have no clear effect on the deterioration of fruit quality during post harvest storage. In contrast, direct treatment of calcium stressed fruits with the 2% $CaCl_2$ Pheroids mixture, seemingly improves fruit quality in terms of the fruits' lycopene concentration, but it also causes the greatest loss in weight and moisture during storage. It is therefore recommended that this treatment should not be used commercially as it decreases the commercial value of the produce during storage.

## 5.7 CONCLUDING REMARKS

The principle aim of this study was to evaluate Pheroids as a possible vehicle for the transport of phytologically beneficial substances over plant membranes.

Pheroids was therefore used to transport additional calcium over membranes in an effort to reduce the occurrence of BER, a common nutritional disorder in tomatoes mostly linked to the availability of calcium on cellular level in the fruits.

Cultivating tomatoes (cv. Floridade) in a controlled drip hydroponic set-up, confirmed that a calcium deficiency affected both vegetative- and generative development of the plants. It also confirmed that calcium is indeed a major factor in the inducement of BER. An approximate 70% reduction in net yield and gross income due to BER was observed in the plants grown under calcium deficient conditions. Although a calcium deficiency markedly enhanced BER, it had no major impact on fruit quality as no obvious differences could be measured between the control- and calcium stressed fruits. Moreover, no obvious differences in fruit deterioration could be observed between the control- and calcium stressed fruits during post harvest storage.

The effect of a calcium deficiency on yield and the higher incidence of BER was prevented by supplying calcium stressed plants with additional calcium in the form of 1% and 2% $CaCl_2$ foliage sprays. These $CaCl_2$ sprays improved total- and marketable yield above that of the calcium deficient plants. These treatments also had little effect on fruit quality, except for lowering the lycopene content to levels comparable to that of the control fruits. Although both these treatments increased net yield and income, both failed to completely alleviate the effect of a calcium deficiency on the occurrence of BER. If the amount of additional calcium supplied to the plants could be increased, the occurrence of BER will most probably be reduced even further. Pre-test trials indicated that increasing the

$CaCl_2$ concentration to 4% burns the vegetative- and generative parts of the tomato plants, making it a non-viable option.

Mixing Pheroids with the $CaCl_2$ solutions, the principle aim of this study, is another possibility to enhance or increase the calcium concentration on cellular level.

The 2% $CaCl_2$ Pheroids mixture proved to be a very efficient treatment in reducing the occurrence of BER, thus improving total- and marketable yields. Although the occurrence of BER is not completely prevented, the net yield and income generated by this 2% $CaCl_2$ Pheroids treatment, compares favourably to that of control plants. Moreover, this mixture also had no apparent effect on fruit quality, as well as the rate of fruit deterioration during post harvest storage.

The efficiency of the 2% $CaCl_2$ Pheroids mixture as a preventative foliage spray, were subsequently compared to a treatment where only the fruits of calcium stressed plants were treated with this mixture in an effort to reduce the occurrence of BER. Treating only the fruits of the calcium stressed plants with this mixture, also reduced the occurrence of BER effectively, but a much reduced yield and net income were obtained. In fact, the net income was R125 973. $ha^{-1}$ less than with the foliage application of the 2% $CaCl_2$ Pheroids mixture. This makes this treatment where only the fruits of calcium stressed plants were treated with this mixture, ineffective, uneconomical and unpractical.

Another aim of this study was to investigate Pheroids as a potential growth promoting substance in general. This was done by spraying control plants additionally with Pheroids. It was reasoned that if Pheroids has growth promoting qualities, it may further improve the vegetative growth and yield in control plants grown under already optimum conditions. This investigation provides evidence for a stimulatory effect by Pheroids on vegetative growth, as well as on subsequent yield. A higher total yield was obtained as a result of fewer but larger fruits that formed upon treatment with Pheroids. However, treating control plants with Pheroids also increased the incidence of BER, with a subsequent reduction in net profit margins. Pheroids also marginally affected fruit quality as it resulted in more hydrated fruits with a slightly higher lycopene content. The elevation in the lycopene concentration of control fruits as a potential health benefit option, was not sufficient to warrant the use of Pheroids to improve fruit quality, as net yield was reduced due to the higher incidence of BER resulting in a profit less than that of the non-treated control plants. However, since vegetative- and fruit growth was stimulated in general by Pheroids under control conditions, it is recommended that it should be extensively tested on a variety of crops to evaluate its growth promoting potential.

In addition, the use of Pheroids under sub-optimal nutrient conditions, such as the calcium deficiency used in this study, warrants further clarification. Mixing Pheroids with the 2% $CaCl_2$ solution in an effort to alleviate the effect of a calcium deficiency on plant growth and yield, proved to be effective. This mixture increased total yield, marketable yield and markedly reduced the occurrence of BER, thus increasing profit margins. Pheroids presumably increased the transport of additional calcium into the plants. Although Pheroids in this mixture might have contributed to increased calcium concentrations in the plants, this mixture was still insufficient to completely alleviate the effect a calcium deficiency and BER had on yield. To further increase the calcium supply to the plants, it is recommended that the calcium stressed plants should be sprayed on a weekly basis with the 1% and 2% $CaCl_2$ Pheroids mixtures, instead of once every two weeks. The effect of this weekly sprays and the cost involved however, requires careful evaluation. It is also recommended that the effect of 1% and 2% $CaCl_2$ solutions, singly and mixed with Pheroids, should be tested on other tomato cultivars. Moreover, the effect of these treatments should also be tested on crops other than tomatoes that also develop BER symptoms, such as pepper fruits, eggplants and watermelon. It is further suggested that the stimulatory effect of Pheroids on vegetative growth should be tested on a variety of food crops to determine its potential as a growth stimulant in general.

In conclusion, Pheroids appears to have potential as a growth promoting substance as well as for acting as a vehicle for the transportation of phytologically beneficial substances over plasma membranes, especially in tomatoes grown under stress conditions. Taking into consideration that most crops are grown in sub-optimal nutritional- and environmental conditions, then Pheroids might have numerous potential applications in the agricultural industry.

# REFERENCES

Abushita, A.A., Hebshi, E.A., Daood, H.G & Biacs, P.A. 1997. Determination of antioxidant vitamins in tomatoes. *Journal of Food Chemistry* ***60***: 207-212

Adams, P & Ho, L.C. 1993. Effects of environment on the uptake and distribution of calcium in tomato and on the incidence of Blossom-end rot. *Plant and Soil* ***154:*** 127-132

Adams, P. 2002**.** Nutritional control in hydroponics. In: Savvas, D., Passam, H., eds. *Hydroponic production of vegetables and ornamentals*. Athens, Greece: *Embryo Publications*: 211–261.

Agarwal, S & Rao, A.V. 2000. Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association or its licensors* ***163****(6)*: 739-744

Agarwal, A., Shen, H., Agarwal, S & Rao, A.V. 2001. Lycopene content of tomato products: Its stability, bioavailability and in vivio antioxidants properties. *Journal of Medicinal Food* ***4****(1)*: 9-15

Ahn, C.S & Pai, H.S. 2008. Physiological function of IspE, a plastid MEP pathway gene for isoprenoid biosynthesis, in organelle biogenesis and cell morphogenesis in *Nicotiana benthamiana*. *Plant Molecular Biology* ***66****(5)*: 503-517

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K & Watson, J.D. 1989. Molecular Biology of the cell (2nd ed). Garland Publishing, New York.

Alexander, L & Grierson, D. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany* ***53****(377)*: 2039-2055

Anderson, J.W & Beardall, J. 1991. Molecular Activities of plant cells: An introduction to Plant Biochemistry. Blackwell Science Ltd, Oxford.

Asahira, T., Takagi, H., Tadeda, Y & Tsukamoto, Y. 1968. Studies on fruit development in tomato. II. Cytokinin activity in extracts from pollinated, auxin and gibberellin-induced parthenocarpic tomato fruits and its effect on the histology of the fruit. *Memoirs of the College of Agriculture, Kyoto University* **29**: 25-54

Atherton, J.G & Rudich, J (Eds). 1986. The tomato crop: a scientific bases for improvement, Kluwer Academic, California, USA.

Berghoef, J. 1986. Effect of calcium on tipburn of *Lilium* 'Pirate'. *Acta Horticulturae* ***177***: 433-438

Bertin, N., Gautier, H & Roche, C. 2002. Number of cells in tomato fruit depending on fruit position and source-sink balance during plant development. *Plant Growth Regulation* ***36***: 105–112.

Bewley, J.D., Banik, M., Bourgault, R., Feurtado, J.A., Toorop, P & Hilhorst, W.M. 2000. Endo-²mannanase activity increase in the skin and outer pericarp of tomato fruits during ripening. *Journal of Experimental Botany* ***51****(344)*: 529-538

Blume, B., Nürnberger, T., Nass, N & Scheel, D. 2000. Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. *The Plant Cell* ***12***: 1425-1440

Botella-Pavia, P., Besumbes, O., Phillips, M.A., Carretero-Paulet, L., Boronat, A & Rodríguez-Concepcón, M. 2004. Regulation of carotenoids biosynthesis in plants: evidence for a key role of hydroxymethylbutenyl diphosphate reductase in controlling the supply of plastidial isoprenoid precursors. *The Plant Journal* ***40***: 188-199

Bramley, P.M. 2000. Is lycopene beneficial to human health? *Phytochemistry* ***54***: 233-236

Bramley, P.M. 2002. Regulation of carotenoids formation during tomato fruit ripening and development. *Journal of Experimental Botany* ***53****(377)*: 2107-2113

Brand, J.J & Becker, D.W. 1983. Evidence for the direct roles of calcium in hpotodynthesis. *Journal of Bioenergetics and Biomembranes **16**(4)*: 239-249

Brennan, J.G. 2005. Food Processing Handbook, Miley-VCH, Germany.

Büker, M., Schünemann, D & Borchert, S. 1998. Enzymatic properties and capacities of developing tomato (*Lycopersicon esculentum L.*) fruit plastids. *Journal of Experimental Botany **49**(321)*: 681-691

Busse, J.S & Palta, J.P. 2006. Investigating the in vivo calcium transport path to developing potato tuber using 45Ca: A new concept in potato tuber calcium nutrition. *Physiologia Plantarum **128**(2)*: 313-323

Camacho, R., Malavolta, E., Guerrero-Alves, J & Camacho, T. 2002. Vegetative growth of grain sorghum in response to phosphorus nutrition. *Scientia Agricola **59**(4)*: 771-776

Carrari, F & Fernie, A.R. 2006. Metabolic regulation underlying tomato fruit development. *Journal of Experimental Botany Advance Access*: 1-15

Chapagain, P.B & Wiesman, Z. 2004. Effect of potassium magnesium chloride in the fertigation solution as partial source of potassium on growth, yield and quality of greenhouse tomato. *Scientia Horticulturae **99***: 279-288

Cholewa, E & Peterson, C.A. 2004. Evidence for symplastic involvement in the radial movement of calcium in onion roots. *Plant Physiology **134***: 1793-1802

Clarkson, D.T. 1984. Calcium transport between tissues and its distribution in the plant. *The Plant Cell & Environment **7**(6)*: 449-456

Cosgrove, D.J. 2000. Expansive growth of the plant cell walls. *Plant Physiology and Biochemistry **38**(1/2)*: 109-124

Davis, A.R., Fish, W.W & Perkins-Veazie, P. 2002. A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Journal of Postharvest Biology and Technology* ***28***: 425-430

Dietrich, P., Sanders, D & Hedrich, R. 2001. The role of ion channels in light-dependent stomatal opening. *Journal of Experimental Botany* ***52****(363)*: 1959-1967

Ehret, D.L & Ho, L.C. 1986. Translocation of calcium in relation to tomato fruit growth. *Annals of Botany* ***58***: 679-688

Eisenreich, W., Rohdich, F & Bacher, A. 2001. Deoxyxylulose phosphate pathway to terpernoids. *Trends in Plant Science* ***6***: 78-84

Evans, N.H., McAinsh, M.R & Hetherington, A.M. 2001. Calcium oscillations in higher plants. *Current Opinion in Plant Biology* ***4****:* 415–420.

Fasano, J.M., Massa, G.D & Gilroy, S. 2002. Ionic signaling in plant responses to gravity and touch. *Journal of Plant Growth Regulation* ***21***: 71–88

Faust, M., 1980. Interaction between nutrient uptake and photosynthesis. *Acta Horticulturae* ***92***: 193-200

Francis, X & Cunningham, Jr. 2002. Regulation of carotenoids synthesis and accumulation in plants. *Pure and Applied Chemistry* ***42****(8)*: 1409-1417

Fuente, F.K.D. 1984. Role of calcium in the polar secretion of indoleacetic acid. *Plant Physiology* ***76***: 342-346

Gary, C., Baldet, P., Bertin, N., Devaux, C., Tchamitchian, M & Raymond, P. 2003. Time-course of tomato whole-plant respiration and fruit and stem growth during prolonged darkness in relation to carbohydrates reserves. *Annals of Botany* ***91***: 429-438

Gautier, H., Rocci, A., Buret, M., Grassely, D., Dumas, Y & Causse, M. 2005. Effect of photoselective filters on the physical and chemical traits of vine-ripened tomato fruits. *Canadian Journal of Plant Science* ***85(2)***: 439-446

Georgeta, E., Elisabeta, M., Pavelina, T., Ecaterina, D., Areta, A. Ion, D., Antoaneta, I & Nicolae, A. 1977. Influence of some nutritional and microclimatic factors on greenhouse tomato yield and on occurrence of some physiological diseases. *Acta Horticulturae* **58**: 203-208

George, B., Kaur, C., Khurdiya, D.S & Kapoor, H.C. 2004. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Journal of Food Chemistry* ***84(1)***: 45-51

Gerster, H. 1997. The potential role of lycopene for human health. *Journal of the American College of Nutrition* ***16(2)***: 109-126

Giovannucci, E. 1999. Tomatoes, Tomato-based products, Lycopene, and Cancer: Review of the Epidemiological Literature. *Journal of the National Cancer Institute* ***91(4)***: 317-331

Gossett, D.R., Egli, D.B & Leggett, J.E. 1977. The influence of calcium deficiency on the translocation of photosynthetically fixed 14C in soybeans. *Plant and Soil* ***48***: 243-251

Grossman, Y.L & DeJong, T.M. 1995. Maximum fruit growth potential following resource limitation during peach growth. *Annals of Botany* ***75***: 561-567

Grumbach, K.H. 1984. Biosynthesis of acyclic and cyclic carotenoids in higher plants and the control by phytochrome. *Plant Physiology* ***60***: 389-394

Halperin, S.J., Kochian, J.V & Lynch, J.P. 1997. Salinity stress inhibits calcium loading into the xylem of excised barley (*Hordeum vulgare*). *New Phytologist* ***135***: 419-427

Harper, J.F. 2001. Dissecting calcium oscillators in plant cells. *Trends in Plant Science* ***6***: 395-397

Hedrich, R., Busch, H & Raschke, K. 1990. $Ca^{2+}$ and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. *The EMBO Journal* ***9***: 3889-3892

Hernandez, L., Soriano, T., Morales, M.I & Castilla, N. 2004. Row covers for quality improvement of chinese cabbage (*Brassica rapa* subsp. *Pekinensis*). *New Zealand Journal of Crop and Horticultural Science* ***34***: 379-388

Heuvelink, E. 1997. Effect of fruit load on dry matter partitioning in tomato. *Scientia Horticulturae* ***69****(1-2)*: 51-59

Hirschi, K. 2001. Vacuolar $H^+/Ca^{2+}$ transport: who's directing the traffic. *Trends in Plant Science* ***6***: 100-104

Hoagland, D.R & Arnon, D.I. 1950. The water-culture method for growing plants without soil. *Agricultural Experimental Station. Circular* ***347***: 1-32

Ho, L.C., Belda, R., Brown, M., Andrews, J & Adams, P. 1993. Uptake and transport of calcium and the possible causes of Blossom-end rot in tomato. *Journal of Experimental Botany* ***44****(259)*: 509-518

Ho, L.C., Adams, P., Li, X.Z., Shen, H., Andrews, J & Xu, Z.H. 1995. Responses of Ca-efficient and Ca-inefficient tomato cultivars to salinity in plant growth, calcium accumulation and blossom-end rot. *Journal of Horticultural Science* **70**: 909–918.

Ho, L.C. 1996. The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. *Journal of Experimental Botany* ***47***: 1230-1243

Ho, L.C., Hand, D.J & Fussell, M. 1999. Improvement of tomato quality by calcium nutrition. *Acta Horticulturae* ***481***: 463-468

Ho, L.C & White, P.J. 2005. A cellular hypothesis for the induction of Blossom-end rot in tomato fruit. *Annals of Botany* ***95****(4)*: 571-581

Ilathy, R & Hdider, C. 2007. Effect of ripening stage on lycopene content of different processing tomato cultivars grown in Tunisia. *Acta Horticulturae* ***758***: 185-190

Irving, H.R., Gehring, C.A & Praish, R.W. 1992. Changes in cytosolic pH and calcium of guard cells precede stomatal movements. *Proceedings of the National Academy of Sciences of the United States of America* ***89***: 1790-1794

Islam, M.D.S., Matsui, T & Yoshida, Y. 1996. Carbohydrate content and the activities of sucrose synthase, sucrose phosphate synthase and acid invertase in different tomato cultivars during fruit development. *Scientia Horticulture* ***65***: 125-136

Javanmardi, J & Kubota, C. 2006. Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest Biology and Technology* ***41****(2)*: 151-155

Jiang, Y & Huang, B. 2001. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *Journal of Experimental Botany* ***52****(355)*: 341-349

José, A., Franco, J.A., Bañón, S & Madrid, R. 1994. Effects of a protein hydrolysate applied by fertigation on the effectiveness of calcium as a corrector of Blossom-end rot in tomato cultivated under saline conditions. *Scientia Horticulturae* ***57***: 283-292

Kemble, J.M., Curtis, L.M & Tyson, T.W. 2000. Guide to commercial staked tomato production in Alabama, Alabama Cooperative Extension System, USA.

Kim, K.N., Cheong, Y.H., Grant, J.J., Pandey, G.K & Luan, S. 2003. CIPK$_3$, a calcium sensor-associated protein kinase that regulates absisic acid and cold signal transduction in *Arabidopsis*. *The Plant Cell* ***15***: 411-423

Knight, H., Trewavas, A.J & Knight, M.R. 1997. Calcium signaling in *Arabidopsis thaliana* responding to drought and salinity. *The Plant Journal* ***12***: 1067–1078

Kuo, C.G., Tsay, J.S., Tsai, C.L & Chen, R.J. 1981. Tipburn of chinese cabbage in relation to calcium nutrition and distribution. *Scientia Horticulturae* ***14***: 131-138

Laurin, E., Émond, J.P., Nunes, M.C.N & Brecht, J.K. 2005. Vapor pressure deficit and water loss patterns during simulated afir shipment and storage of beit alpha cucumbers. *Acta Horticulturae* ***682***:1697-1704

Lecourieux, D., Ranjeva, R & Pugin, A. 2006. Calcium in plant defence-signaling pathways. *New Phytologist* ***171****(2)*: 249-269

León, J., Rojo, E., Titarenko, E & Sánchez-Serrano, J.J. 1998. JA-dependent and -independent wound signal transduction pathways are differentially regulated by $Ca^{2+}$/calmodulin in *Arabidopsis thaliana*. *Molecular and General Genetics* **258**: 412–419

León, J., Rojo, E & Sánchez-Serrano, J.J. 2001 Wound signalling in plants. *Journal of Experimental Botany* ***52***(354): 1-9

Lin, C.H & Chen, B.H. 2005. Stability of carotenoids in tomato juice during storage. *Journal of Food Chemistry* ***90****(4)*: 837-846

MacRobbie, E.A.C. 2000. ABA activates multiple $Ca^{2+}$ fluxes in stomatal guard cells, triggering vacuolar $K^+$($Rb^+$) release. *Plant Biology* ***97****(22)*: 12361-12368

Marcelis, L.F.M & Ho, L.C. 1999. Blossom-end rot in relation to growth rate and calcium content in fruits of sweet pepper (*Capsicum annuum*). *Journal of Experimental Botany* ***50****(332)*: 357-363

Marín-Rodríguez, M.C., Orchard, J & Seymor, G.B. 2002. Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany* ***53****(377)*: 2115-2119

MacIntosh, G.C., Ulloa, R.M., Raices, M & Tellez-Inon, M.T. 1996. Changes in calcium-dependant protein kinase activity during in vitro tuberization in potato. *Plant Physiology* ***112****(4)*: 1541-1550

Mahajan, G & Singh, K.G. 2006. Responses of greenhouse tomato to irrigation and fertigation. *Agricultural Water Management **84***(1-2): 202-206

Matsumoto, H., Teraoka, K & Kawasaki, T. 1980. Repression of nitrate reductase in cucumber leaves caused by calcium deficiency. *Plant and Cell Physiology **21(1)***: 183-191

Miller, A.J & Sanders, D. 1987. Depletion of cytosolic free calcium induced by photosynthesis. *Nature **326***: 397-400

Miron, D., Petreikov, M., Carmi, N., Shen, S., Levin, I., Granot, D., Zamski, E & Schaffer, A.A. 2002. Sucrose uptake, invertase localization and gene expression on developing fruit of *Lycopersicon esculentum* and the sucrose-accumulating *Lycopersicon hirsutum. Physiologia Plantarum **115***: 35-47

Mirza, M & Chen, W. 2005. The Greenhouse Business. *Alberta Agriculture, Food and Rural Development* **4** (3-5): 2-5

Moraru, C., Logendra, L. Lee, T.C & Janes, H. 2004. Characteristics of 10 processing tomato cultivars grown hydroponically for the NASA Advanced Life Support (ALS) Program. *Journal of Food Composition and Analysis **17(2)***: 141-154

Murata, Y., Pei, Z.M., Mori, I.C & Schroeder, J. 2001. Abscisic acid activation of plasma membrane $Ca^{2+}$ channels in guard cells require cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in abi1–1 and abi2–1 protein phosphatase 2C mutants. *The Plant Cell **13(11)***: 2513-2524

Napier, D.R & Combrink, N.J.J. 2006. Aspects of calcium nutrition to limit plant physiological disorders. *Acta Horticulturae **702***: 107-116

Nukaya, A., Goto, K., Jang, H., Kano, A & Ohkawa, K. 1995*a*. Effect of K/Ca ratio in the nutrient solution on incidence of Blossom-end rot and gold specks on tomato fruit grown in rockwool. *Acta Horticulturae **396***: 123-130

Nukaya, A., Goto, K., Jang, H., Kano, A & Ohkawa, K. 1995*b*. Effect of $NH_4$-N level in the nutrient solution on the incidence of Blossom-end rot and gold specks on tomato fruit grown in rockwool. *Acta Horticulturae* ***401***: 381-388

Obiadalla-Ali, H., Fernie, A.R., Lytovchenko, A., Kossmann, J & Lloyd, J.R. 2004. Inhibition of chloroplastic fructose 1,6-biphosphatase in tomato fruits leads to decreased fruit size, but only small changes in carbohydrate metabolism. *Planta* ***215***: 533-539

Pate, J.S & Hocking, P.J. 1978. Phloem and xylem transport in the supply of minerals to a developing legume (*Lupinus albus L.*) fruit. *Annals of Botany* ***42***: 911-921

Polder, G., van der Heijden, G.W.A.M., van der Voet, H & Young, I.T. 2004. Measuring surface distribution of carotenes and chlorophyll in ripening tomatoes using spectrometry. *Postharvest Biology and Technology* ***34****(2)*: 117-129

Poovaiah, B.W & Leopold, A.C. 1973. Inhibition of abscission by calcium. *Plant Physiology* ***51***: 848-851

Prokić, L., Jovanović, Z., Stikić, R & Vuĉinić, Z. 2005. The mutual effect of extracellular $Ca^{2+}$, Abscisic Acid, and pH on the rate of stomatal closure. *Annals of New York Academy of Science* ***1048(1)***: 513516

Rains, D.W. 1972. Salt transport by plants in relation to salinity. *Annual Review of Plant Physiology* ***23***: 367-388

Ramandeep, K.T & Savage, G.P. 2004. Effect of semi-drying on the antioxidant components of tomatoes. *Food Chemistry* ***94****(1)*: 90-97

Rangnekar, P.V. 1975*a*. Effect of calcium deficiency on the translocation and utilization of C14-Photosynthate in tomato plants. *Plant and Soil* ***41****(3)*: 565-583

Rangnekar, P.V. 1975*b*. Effect of calcium deficiency on the carbon metabolism in photosynthesis and respiration of tomato leaf. *Plant and Soil* ***42***: 589-600

Rao, A.V & Agarwal, S. 1999. Role of lycopene as antioxidant carotenoid in the prevention of chronic diseases: A review. *National Research* ***19(2)***: 305-323

Raven, P.H., Everet, R.F & Eichhorn, S.E. 1992. Biology of plants. Worth Publishers, New York.

Reymond, P., Kunz, B., Paul-Pletzer, K., Grimm, R., Eckerskorn, C & Farmer, E.E. 1996. Cloning of a cDNA encoding a plasma membrane associated, uronide binding phosphoprotein with physical properties similar to viral movement proteins. *The Plant Cell* ***8***: 2265–2276

Romeis, T., Ludwig, A.A., Martin, R & Jones, J.D.G. 2001. Calcium-dependant protein kinases play an essential role in a plant defence response. *The EMBO Journal* ***20***: 5556-5567

Romer, J. 1993. Hydroponic Crop Production. Kangaroo Press, Australia.

Rosenfeld, L. 1999. Four Centuries of Clinical Chemistry. CRC Press, USA.

Sabba, R.P & Miller, J.H. 1993. Calcium delays the onset of cell division and rhizoid elongation in *gemmae* of the calcifuge fern, *Vittaria graminifolia*. *American Journal of Botany* ***80(6)***: 624-628

Sahlin, E., Savage, G.P & Lister, C.E. 2004. Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food Composition and Analysis* ***17(5)***: 653-647

Saladié, M., Rose, J.K.C & Watkins, C.B. 2005. Characterization of DFD (Delayed Fruit Deterioration): a New Tomato Mutant. *Acta Horticulturae* ***682***: 79-84

Salisbury, F.B & Ross, C.W. 1992. Plant Physiology (4th ed). Wadsworth Publishing Company, California.

Salunkhe, D.K., Jadhav, S.J & Yu, M.H. 1974. Quality and nutritional composition of tomato fruit as influenced by certain biochemical and physiological changes. *Plant Foods for Human Nutrition (Formerly Qualitas Plantarum)* ***24****(1-2)*: 85-113

Samach, A & Lotan, H. 2007. The transition to flowering in tomato. *Plant Biotechnology* ***24***: 71-82

Sanders, D., Pelloux, J., Brownlee, C & Harper, J.F. 2002. Calcium at the crossroads of signaling. *The Plant Cell* ***14***: 401-417

Saure, M.C. 2001. Blossom-end rot of tomato (*Lycopersicon esculentum* Mill.) -a calcium- or a stress-related disorder. *Scientia Horticulturae* ***90****(3-4)*: 193-208

Schmitz-Eiberger, M., Haefs, R & Noga, G. 2002. Calcium deficiency- Influence on the antioxidative defence system in tomato plants. *Journal of Plant Physiology* ***159****(7)*: 733-742

Schmitz-Eiberger, M & Noga, G. 2003. Influence of calcium deficiency on distribution and antioxidative system in tomato plants. *Acta Horticulturae* ***618***: 217-224

Schroeder, J.I., Raschke, K & Neher, E. 1987. Voltage dependence of $K^+$ channels in guard cell protoplasts. *Proceedings of the National Academy of Sciences of the United States of America* **84**: 4108-4112.

Schroeder, J.I & Hagiwara, S. 1989. Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. *Nature* ***338***: 427-430

Sharma, S.K & Le Maguer, M. 1996. Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Research International* ***29***(3-4): 309-315

Skórzyńska-Polit, E., Tukendorf, A., Selstam, E & Baszyński, T. 1998. Calcium modifies Cd effect on runner bean plants. *Enviromental and Experimental Botany* ***40***: 275-286

Sonneveld, C & Voogt, W. 1991. Effects of Ca-stress on Blossom-end rot and Mg-deficiency in rockwool grown tomatoes. *Acta Horticulturae* ***294***: 81-85

Stacewicz-Sapuntzkis, M & Bowen, P.E. 2005. Role of lycopene and tomato products on prostate health. *Biochimica et Biophysica Acta (BBA) - Biomembranes* ***1740****(2)*: 202-5.

Stahl, W & Sies, H. 1996. Perspectives in biochemistry and biophysics. *Archives of Biochemistry and Biophysics* ***336****(1)*: 1-9

Strynadka, N.C.J & James, M.N.G. 1989. Crystal structures of the helix–loop–helix calcium-binding proteins. *Annual Review of Biochemistry* ***58***: 951–998

Subrahmanyam, D & Rathore, V.S. 2005. $^{14}CO_2$ Assimilation and $^{14}$C-photosynthate translocation in different leaves of sunflower. *Photosynthetica* ***42****(2)*: 313-316

Supanjani, S., Habib, A., Mabood, F., Lee, K.D., Donnelly, D & Smith, D.L. 2006. Nod factor enhances calcium uptake by soybean. *Plant Physiology and Biochemistry* ***44***: 866-872

Suzuki, K., Takeda, H & Egawa, Y. 2000. Morphological aspect of Blossom-end Rot fruit of tomato. *Acta-Horticulturae* ***511***: 257-264

Taylor, M.D., Locascio, S.J & Alligood, M.R. 2004. Blossom-end rot incidence of tomato as affected by irrigation quantity, calcium source, and reduced potassium. *Horicultural Science* ***39****(5)*: 1110-1115

Terry, N & Huston, R.P. 1975. Effects of calcium on the photosynthesis of intact ieaves and isolated chloroplasts of sugar beets. *Plant Physiology* ***55***: 923-927

Thompson, D.S., Smith, P.W., Davies, W.J & Ho, L.C. 1999. Interactions between environment, fruit, water relations and fruit growth. *Acta Horticulturae* ***487***: 65-70

Trejo, E & Cantwell, M. 1996. Cherry tomato storage and quality evaluations. Department of Vegetable Crops, University of California Davis

Trewavas, A. 1999. Calcium makes waves. *Plant Physiology **120***: 1–6

Tsuyuzaki, S., Haraguchi, A & Kanda, F. 2004. Effects of scale-dependant factors on herbaceous vegetation patterns in a wetland, northern Japan. *Ecological Research **19**(3)*: 349-355

Veierskov, B & Meravy, L. 1985. Changes in carbohydrate composition in wheat and pea seedlings induced by calcium deficiency. *Plant Physiology **79***: 315-317

Wang, F., Sanz, A., Brenner, M. L & Smith, A. 1993. Sucrose synthase, starch accumulation, and tomato fruit sink strength. *Plant Physiology **101***: 321-327

Ward, M.R., Aslam, M & Huffaker, R.C. 1986. Enhancement of nitrate uptake and growth of barley seedlings by calcium under saline conditions. *Plant Physiology **80***: 520-524

Wertz, K., Siler, U & Goralczyl, R. 2004. Lycopene: modes of action to promote prostate health. *Archives of Biochemistry and Biophysics **430***: 127-134

White, P.J. 2000. Calcium channels in higher plants. *Biochemica et Biophysica Acta **1465***: 171-189

White, P.J. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany **52**(358)*: 891-899

White, P.J. 2002. Recent advances in fruit development and ripening: an overview. *Journal of Experimental Botany* **53***(377)*: 1995-2000

White, P.J & Broadley, M.R. 2003. Calcium in plants. *Annals of Botany **92***: 487-511

Winsor, G.W., Davies, J.N & Massey, D.M. 1959. Composition of tomato fruit. III. – Juices from whole fruit and locules at different stages of ripeness. *Journal of the Science of Food and Agriculture **13**(2)*: 108-115

Wolfe, S.L. 1993. Molecular and cellular biology. Wadsworth Publishing Company, California.

Xianquan, S., Shi, J., Kakuda, Y & Yueming, J. 2005. Stability of lycopene during food processing and storage. *Journal of Medicinal Food* ***8(4)***: 413-422

Yamazaki, Y., Kitajima, M., Arita, M., Takayama, H., Sudo, H., Yamazaki, M., Aimi, N & Saito, K. 2004. Biosynthesis of camptothecin. In silico and in vivo tracer study from [1-$^{13}$C]Glucose. *Plant Physiology* ***134***: 161-170

Zhu, X., Yang, J.Y., Yang, J.S., Liang, Y.C., Liang, Y., Lou, Y., Yang, X & Yang, X.Y. 2004. Effects of exoffenous calcium on photosynthesis and its related physiological characteristics of rice seedlings under salt stress. *Scientia Agricultura Sinica* ***37(10)***: 1497-1503

Žnidari, D & Požrl, T. 2006. Comparative study of quality changes in tomato cv. 'Malike' (*Lycopersicon esculentum Mill*.) whilst stored at different temperatures. *Acta agriculturae Slovenica* ***87***(2): 235-243

http://businessafrica.net - Cited on 30/09/07

http://www.cartage.org - Cited on 30/01/06

http://ccgb.umn.edu - Cited on 31/03/06

http://www.ces.ncsu.edu - Cited on 31/01/06

http://www.ipm.uiuc.edu/diseases - Cited on 31/01/06

http://www.nda.agric.za/docs/Cropsestimates - Cited on 19/02/07

http://pubs.caes.uga.edu - Cited on 13/03/08

http://www.uvm.edu - Cited on 31/01/06

http://vegedge.umn.edu - Cited on 13/03/08

http://en.wikipedia.org/wiki/Tomato - Cited on 14/03/08

http://www.wsutoday.wsu.edu - Cited on 03/01/06